SOME CHEMICAL AND AGRONOMIC VALUES IN TILLERING STAGE OF RICE GROWN UNDER SALTY CONDITIONS IN THAILAND

By
Mr. Felisberto AMARAL SOARES

A Thesis Submitted in Partial Fulfillment of the Requirements
for Master of Science (BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
Graduate School, Silpakorn University
Academic Year 2021

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โดย
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Title	Some chemical and agronomic values in tillering stage of rice grown under salty conditions in Thailand
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MR. FELISBERTO AMARAL SOARES : SOME CHEMICAL AND AGRONOMIC VALUES IN TILLERING STAGE OF RICE GROWN UNDER SALTY CONDITIONS IN THAILAND THESIS ADVISOR : ASSOCIATE PROFESSOR PANTIPA NA CHIANGMAI, Ph.D.

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population, especially people in developing countries. However, salinity is one of the most common abiotic stresses causing severe reductions in plant growth and productivity of crops including rice. The objective was to determine the effect of proline and trehalose applications to alleviate salinity stress in rice plant at the tillering stage that plants received the salty conditions at the tillering stage. In each experiment was conducted in 4x4x3 factorial in Completely Randomized Design (CRD). Three factors consisted 4 levles of salinity (sodium chloride; NaCl) [0 mM (0 dS/m), 50 mM (5 dS/m), 100 mM (10 dS/m), and 150 mM (15 dS/m)], four concentrations of proline or trehalose (0 mM, 50 mM, 100 mM, and 150 mM), and three varieties of lowland rice varieties (Chai Nat; CNT 1, Pathum Thani; PT1, and Inpari 35. The result in these two experiments showed a significant negative effect of salinity level starting at 50 mM NaCl in most characteristics, but there is a slight difference between the use of the proline and trehalose. Application of external proline or trehalose started at 50 mM could promote to increase in some characteristics: including RWC, proline content, and soluble sugar in leaves in proline application and on Chlorophyll A and B contents, soluble sugar in the stem, and starch content in leaves, stem, and root in trehalose application. In addition, these characteristics were increased in the order of increasing the concentration of exogenous substances (proline and trehalose) for applying. CNT 1 and PT 1 were susceptible to salinity stress than Inpari 35. Thus, these Thai rice varieties need to be stimulated by external spraying both proline or trehalose to promote both biochemical and agronomic characteristics. effects of exogenous proline or trehalose application on rice plants in the tillering phase on plant height, the percentage of productive tillers per plant formation, and the number of seeds per panicle. However, grain yield per plant could promote only by the application of trehalose.

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Felisberto AMARAL SOARES

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1
INTRODUCTION

1.1 Background and rational

By 2050, the world population is expected to reach 9.6 billion people (Rachmani and Zulkifli, 2018). To sustainably provide sufficient food for the increasing world population, crop productivity needs to increase by ~44 million metric tons annually. This is a challenge because there is very little potential for future expansion of arable lands whilst climate predictions suggest that a larger portion of the globe will be subjected to erratic environmental conditions and abiotic stress (Eckardt, 2009; FAO, 2009, 2012; Cominelli., 2013). Two abiotic stress factors that significantly hinder world crop production are soil water deficit and salinization (Munns, 2011).

Rice is considered as the model system for monocotyledonous plants that include members of the agronomically important cereals (Lee et al., 2011). Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population, especially people in developing countries (Seck et al., 2012). Rice provides 50–80% of the calories consumed (Hossain and Fischer, 1995; Khushs, 2005). More than for consumption, rice has been considered as the single most important source of employment and income for rural people in humid and sub-humid Asia (Smith et al., 2007). Approximately 90% of the world's production and consumption of rice are in Asia (Khush, 2005). However, climate change can disrupt food availability, reduce access to food, and affect food quality. For example, projected increases in temperatures, changes in precipitation patterns, changes in extreme weather events, and reductions in water availability may all result in reduced agricultural productivity (Hatfield et al., 2011). Higher temperatures can adversely affect rice yields through two principal pathways, namely (i) high maximum temperatures that cause in combination with high humidity - spikelet sterility and adversely affect grain quality and (ii) increased nighttime temperatures that may reduce assimilate accumulation (Wassmann et al., 2009a).

Climate change will aggravate a variety of stresses for rice plants, namely heat, drought, salinity, and submergence (Wassmann et al., 2009b). Under concern due to salinity problems from climate change, Rice yield in salt-affected land is

significantly reduced with an estimation of 30–50% yield losses annually (Eynard et al., 2006). Rice is currently listed as the most salt sensitive cereal crop with a threshold of 3 dS/m for most cultivated varieties (Rao et al., 2008).

Moreover, the methods for screening plant grown under abiotic stresses are important for the success of selection in breeding program (Khush, 2005). Obtaining information of parameters changeable inside plant will help to understand both biochemical and physical processes in plant. In addition, this information also will help in planning for solving the problem of plant stress by using crop technologies. As improving yield of plants undergoing salinity stress is one of the main targets of plant production. Salinity tolerance screening that are presented the most are based on agronomical parameters such as growth, yield and yield components (Gregorio et al., 1997; Zang et al., 2002; Lee et al., 2003; Moradi and Ismail, 2007; Cha-Um et al., 2009; El-Hendawy et al., 2009). Recently, physiological parameters have also gained recognition as important selection criteria for screening salinity tolerance in plants due to the reliability of information attained (Ashraf and Harris, 2004; Munns et al., 2006; El-Hendawy et al., 2009). Few studies are reported about biochemical changes in plant under salinity stress (Oraby and Ahmad, 2012). Glycine betaine (GB) and proline are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals (Ashraf and Foolad, 2007b). Although actual roles of GB and prolines in osmotolerance plant remain controversial, both compounds are thought to have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions (Ashraf and Foolad, 2007a). Among the amino acids, proline has a fundamental role in the response of rice plants to oxidative stress. It has been shown in experiments in which exogenous proline was applied or in which the synthesis or degradation of proline was genetically manipulated (Molinari et al., 2007b).

Among various growth stages of rice, tillering stage is very important stage because rice tiller is a specialized grain-bearing branch that is formed on the unelongated basal internode and grows independently of the mother stem (culm) by means of its own adventitious roots. Rice tillering occurs in a two-stage process: the formation of an axillary bud at each leaf axil and its subsequent outgrowth (Li et al.,

2003). Thus, in this study, some chemical characteristics will be determined, and will be evaluated for salinity stress assessment together with physiological and agronomic characteristics in rice at the tillering stage.

1.2 Objective

There are plenty thoughts and ideas that will be used as the main purpose for this research is:

1. To determine some chemical values in rice plant at tillering stage under grown in salty conditions.
2. To determine the effect of salinity on yield components and yield.
3. To assess the use of some external substances for reduce the impact of stress in the tillering stage.

1.3 Hypothesis

1. Some chemicals change was observed in rice plant at tillering stage when rice was grown under salty condition.
2. Salinity stress show negative effect to yield components and yield in rice.
3. Using some external chemicals for applying in tillering stage will increase rice related growth traits and productivity.

CHAPTER 2
LITERATURE REVIEW

2.1 Salinity stress in plant

Salinity is one of the most prevalent abiotic stresses that pose severe reduction in plants' growth and productivity of crops including rice (*Oryza sativa* L). Salinity can be found in all areas such as in coastal or irrigated lands (Martínez-Atienza et al., 2007). Approximately 6% (800 million hectares) of world's total land area has been reported as salt affected (Rozema and Flowers, 2009; Guo et al., 2016). For example, in India has at least twice the productivity of rain-fed land and yields about one third of the world's consumption (Rockström et al., 2003). The irrigated land accounts for only 15% of total cultivated land in India, nevertheless, the scenario accounts for about 8.4 million ha land affected by salinity in this country. In accordance to the above facts and keeping in view the present alarming scenario, development of salt tolerant genotypes is definitely an urgent need of the hour (Ghosh et al., 2016).

The irrigated farming system provides about a third of the world's food (Munns, 2002) and it is estimated that about 20% of the irrigated area (45 million ha) is affected by salinity (Fao, 2008). Salt stress is also a problem in rain-fed agriculture, especially in coastal areas as salt water enters them during high tide. It is estimated that 2% of the rain-fed agriculture area (32 million ha) is affected by salinity (Fao, 2008). The salinity problem has been addressed through improvements in production practices and the introduction of tolerant varieties. However, the implementation of proper irrigation management in areas affected by salinity has been economically unviable and difficult to implement on a large-scale basis (Walia et al., 2005).

Salinity, mainly contributed by accumulation of salts over time in arid and semiarid regions, salts from oceans brought in by wind and rain, and weathering of the rocks (Rengasamy, 2005). Sodium chloride (NaCl) is usually considered as the major soluble salt in saline soils encountered by plants (Munns and Tester, 2008a). The responses of rice to salt stress could involve the regulation of membrane integrity, ionic compartmentation, osmotic adjustment and accumulation of macromolecules (Hu et al., 2012).

Several salt-tolerant rice varieties were recently released with the potential to increase and sustain rice productivity in salt-affected areas (Hairmansis et al., 2017). This study developed approaches to characterize and quantify tolerance in these varieties. (Yichie et al., 2018) reported at a high salinity level of 12 dS/m, no significant differences in biomass were observed among the contrasting rice genotypes. This result indicated that even the tolerant genotype was overwhelmed due to both osmotic stresses. However, at the higher accumulation of salt in plant tissues, causing toxicity and internal dehydration. Finally, it cannot tolerate and affected by salty not different from non-tolerant varieties. The responses of leaf gas exchange variables were fitted using both piecewise linear and logistic functions under salinity stress (Sitaramam, 2006). The logistic equation allowed better comparison among genotypes. Moreover, providing a simplistic quantification approach for their tolerance is providing parameters that quantified both tolerance and resilience of a genotype (Radanielson et al., 2018b). For the tolerant genotype; BRRI Dhan47, a 50% loss in leaf conductance did not occur until a salinity level of 11.08 dS/m, moreover, BRRI Dhan47 adjusted its stomatal conductance at a lower level than the other genotypes (1.04 dS/m). The transpiration and net photosynthesis rates of BRRI Dhan47 decreased to 50% also was observed. However, only at salinity levels of 14.30 and 16.15 dS/m, respectively that found the reduction in these physiology in BRRI Dhan47 (Radanielson et al., 2018a). The slope of this reduction was statistically similar to that of IR64 and IR29, thereby suggesting a window for further improvement in this trait to future enhance salinity tolerance (Salwan et al., 2019).

Osmotic stress and ion toxicity in plant are effects caused by salinity. Osmotic stress is the result of the salt in the growth solution reducing the capacity of the plant to absorb water. While ion toxicity is caused by an excessive amount of salts entering the transpiration flow and damaging leaf cells (García Morales et al., 2012). Reduced growth and photosynthesis are the main effects of salt stress (Munns et al., 2006). The ability of plants to grow under salinity is a feature that determines crop distribution and productivity in many areas; therefore, it is important to understand the mechanisms that confer tolerance in saline environments (Pattanagul and Thitisaksakul, 2008).

2.2 Salinity stress in rice

Salinity is one of the major impediments to increasing production in rice growing areas worldwide being secondary only to drought (Flowers and Yeo, 1995). Rice threshold for salt stress is 3 dS/m, with a 12% reduction in yield, per dS/m, beyond this value (Maas and Grieve, 1990), which makes rice categorized as a salt-sensitive crop (Maas and Hoffman, 1977; Chinnuamy et al., 2005). Rice sensitivity to salt varieties depended on the growth stages (Lutts et al., 1995; Khan et al., 1997; Shannon et al., 1998; Zeng and Shannon, 2000a). During germination in rice, is more tolerant to salt than during other growth stages (Zeng, 2004; Khan et al., 2016). However, it became sensitive at seedling stage in rice (Lutts et al., 1995). It recovers very susceptible during the reproductive phase (Zeng and Shannon, 2000a; Zeng, 2004). At a vegetative stage in rice, there were various results from studies in this phase that found both a relative tolerance (Zeng and Shannon, 2000) and most sensitive to salinity (Zeng et al., 2004).

In rice, salinity affects yield components such as panicle length, spikelet number per panicle, and grain yield, and it also delays panicle emergence and flowering (Khatun and Flowers, 1995; Zeng and Shannon, 2000). Moreover, the percentage of seed set and hence rice yield is clearly reduced by salinity mainly due to lower pollen viability (Khatun and Flowers, 1995).

Salinity imposes two main stresses on plant tissues: an osmotic stress due to the relatively high solute concentration in the soil. Moreover, ion-specific stresses resulting mostly from altered ratios of potassium and sodium ions (K^+/Na^+) and Na^+ and chloride (Cl^-) concentrations, that are adverse to plant physiology (Wang et al., 2007). After rice exposure to salt, the osmotic stress causes an immediate slowdown of transpiration and leaf expansion, which is usually followed by partial recovery (Tavakkoli et al., 2010). Meanwhile, transport restrict the amount of Na^+ entering the roots and also pump out much of what succeeds in getting in. Other transporters remove Na^+ from the transpiration stream as it flows to the shoot. After some time, the remaining Na^+ that reaches the fully expanded leaf blades slowly accumulates until the leaves prematurely start to senesce and die. This process may be delayed to various extents by Na^+ removal from the cytosol and sequestration in vacuoles (Negrão et al., 2011). Eventually, however, the decline in photosynthetic surface

greatly reduces plant growth and, in severe cases, will lead to plant death. The molecular basis of ionic cytotoxicity is the replacement of K^+ (plant macronutrient) by Na^+ in biochemical reactions. The conformational changes and loss of protein function when Na^+ and Cl^- ions penetrate and undergo hydration also disrupt non-covalent interactions between amino acids (Chinnusamy et al., 2005).

All these striking changes also lead to oxidative stress (Zhu, 2001). At the cellular level, the mechanisms of salinity tolerance involve all strategies able to reduce Na^+ absorption, to increase its pumping out from the cell or to compartmentalize it in the vacuole. These response is avoiding accumulation at toxic levels in the cytosol or in organelles like the chloroplast (Tavakkoli et al., 2010). To improve salinity tolerance in breeding programs, high-throughput screening methods and plant genetic variability must be available. Together with a better comprehension of the tolerance physiology and its genetic control have been concerned. In the past few years, several reviews on salinity tolerance (Xiong et al., 2002; Zhu, 2001, 2002b, 2004; Mahajan and Tuteja, 2005; Mahajan et al., 2008; Munns and Tester, 2008; Turkan and Demiral, 2009) and abiotic stress on rice (Sahi et al., 2006; Gaoet et al., 2007, 2008) have been published, synthesizing new discoveries at the molecular and physiological levels.

However, recent developments achieved with the new tools nowadays available has resulted in a knowledge outburst that must be explored and integrated for a better understanding of rice behavior under salinity. Moreover, all those new tools have been used for the development of more efficient strategies to achieve tolerance in plant. In this review, the revisit the current knowledge of rice physiological response to salt stress and discussing the tolerance mechanism identified. In addition, this study is paying special attention to the most important and/or interesting candidate genes involved in such mechanisms. Since the capacity to perceive and respond to salt stress has long been described as a quantitative genetic trait. It is also covering the current knowledge on quantitative trait loci (QTLs) linked to salt stress responses in rice, as well as on association studies conducted. Furthermore, many researches present an overview of recent information about tolerant varieties that can be used in breeding programs across the world. Ultimately, the research will provide researchers and breeders with an integrated view of the most

recent data available for improvement of salinity tolerance in one of the most important crops for mankind, rice (Arzani and Ashraf, 2016). In general, rice shows variability in its sensitivity to excessive salinity at different genetic and stages of growth (Bai et al., 2006).

2.3 Salinity tolerance breeding in plant

To date, salinity tolerance strategies in plant species have utilized three major approaches: (i) conventional breeding, (ii) marker assisted selection and (iii) genetic engineering (Shahbaz and Ashraf, 2013). Of these, genetic engineering displays great potential and has become a powerful tool in plant breeding programs. It allows the introduction of select gene(s) without affecting the desirable characteristics of an elite genotype (Bhatnagar-Mathur et al., 2008).

Genetic engineering for salinity tolerance in plants has focused on genes that encode compatible organic solutes, antioxidants [detoxification of reactive oxygen species (ROS)], ion transport, heat-shock and late embryogenesis abundant proteins (Ashraf et al., 2008). Despite some promising reports, the development of cultivars with enhanced salinity tolerance using a transgenic approach is awaiting further investigation. Currently people are able to produce transgenic crops with enhanced salinity tolerance that survive in the glasshouse. However, once applied these transgenic plants in the field the tolerance are fails due to combined stresses; salinity is commonly associated with drought or temperature stress (Mittler, 2006).

One approach with prospective application for the generation of the “next frontier of crop plants” with broad-spectrum tolerance is the exogenous expression of anti-apoptotic genes that suppress innate programmed cell death (PCD) pathways (Hoang et al., 2016). Programmed cell death or simply “the decision of whether a given cell should live or die” is essential for all multicellular (Metazoan) organisms (Williams and Dickman, 2008). Under several stimuli, this decision is dependent on the battle between anti-apoptotic and pro-apoptotic (pro-death) proteins and signal transduction pathways (Li and Dickman, 2004; Williams and Dickman, 2008; Williams et al., 2014).

Previous studies have assessed the applicability of anti-apoptotic genes for “broad stress tolerance,” however, these have focused primarily on model crops (Dickman et al., 2001; Doukhanina et al., 2006; Wang et al., 2009). The most frequent

causal mutations of the domestication phenotypes are nonsense mutations, such as premature truncation of ORFs through induced frame shifts, introduction of stop codons, changes in splicing signals, or AA changes leading to a loss of function of the protein (Nogué et al., 2016). For example, the transition from the prostrate growth of ancestral wild rice to the erect growth of rice cultivars, which was one of the critical events in rice domestication, was the result of the selection of a single mutation in the *PROG1* (*PROSTRATE GROWTH 1*) gene. This mutation produces an AA substitution in the zinc-finger nuclear transcription factor encoded by *PROG1* leading to the loss of function (Jin et al., 2008; Tan et al., 2008).

2.4 Other technologies that reduce salinity effects in rice plant at tillering stages and the effects of salinity

Tillering is an important stage in rice, the tiller number per plant determines the panicle number; which is a key component of grain yield, could record in this stage (Yan et al., 1998). High tiller numbers are often the goal for genetic improvement and breeding in rice, which seek to maximize the crop yield. Tillering of rice is a variable trait that changes over time. During changes in tillering, several characteristic biological features play important roles, and these characteristics could be optimized to manage rice production and improve its genetic foundation. For example, parameters such as the optimum tillering time provide useful information about rice production management. However, to optimize these traits the associated genes must be identified, and little is currently known about the genes that influence these parameters. To address this gap, in knowledge research used functional mapping to identify quantitative trait loci (QTLs) that influence rice tillering ability (Ma et al., 2002). Over the past several decades, several studies have attempted to identify the genetic mechanism(s) of rice tillering (Liu et al., 2010a).

Development of tillers plays an important role for getting high yields of rice by increasing number of panicles per plant (Zhou et al., 2019). The tiller develops from an axillary bud which differentiated on an axil of a leaf of rice plants (Shao et al., 2019). Number of tillers per plant depends on the differentiation of axillary buds (referred to as tiller bud's hereafter) at leaf axils and the succeeding development of the tiller buds (Zhuang et al., 2019). In other words, a leaf of a tiller develops synchronously with the development of a definite leaf of the main stem and those of

other tillers (Zadoks et al., 1974). Many studies carried out a series of experiments for the purpose of finding general rules involved in the processes of differentiation and development of tiller buds (Hanada, 1982). Tiller primordium was formed from many processes: a tiller bud differentiated firstly into a tiller bud which have a young leaf and a leaf primordium (I), followed by two young leaves and a leaf primordium (II), three young leaves and a leaf primordium (III), four young leaves and a leaf primordium (IV), then the first leaf exceeded chlorophyll (V), finally extended out the sheath of mother stem (VI) and became a tiller. In these processes, the turning stage from III to IV was the most sensitive to environment, i. e. environment sensitive stage (ESS). When some treatments, such as deep-water irrigation, were given to the plants in this time, the growth of the tiller buds which was at ESS would be inhibited, and the tiller would be controlled. Because a tiller develops synchronously with the development of a definite leaf of the main stem, the nodal position of the tiller bud that was at ESS might be determined according to the plant age. This rule could be used as a diagnosing index to control ineffective tiller in rice plant (Yue-fang et al., 1994). The vegetative growth phase is characterized by active tillering, a gradual increase in plant height and leaf emergence at regular intervals. The length of this phase primarily determines the growth duration of cultivars. Some very-early-maturing cultivars have a shortened vegetative growth phase, while others have both shortened vegetative and reproductive growth phases.

Tillering in rice is an important agronomic trait for grain production and also a model system for the study of branching in monocotyledonous plants (Li et al., 2003). Rice tiller is a specialized grain-bearing branch that is formed on the not elongated basal internode and grows independently of the mother stem (culm) by means of its own adventitious roots (PAUL, 2018). Rice tillering occurs in a two-stage process: the formation of an axillary bud at each leaf axil and its subsequent out growth (Chen et al., 2018).

Although the morphology and histology and some mutants of rice tillering have been well described. The molecular mechanism of rice tillering remains to be elucidated (Li et al., 2003). The isolation and characterization of *MONOCULM 1* (*MOC1*), a gene that is important in the control of rice tillering was studied (Xu et al., 2012). The *moc1* mutant plants have only a main culm without any tillers owing to a

defect in the formation of tiller buds. *MOC1* encodes a putative GRAS family nuclear protein that is expressed mainly in the axillary buds and functions to initiate axillary buds and to promote their outgrowth (Li et al., 2003).

However, the grain yield of semi dwarf rice has not increased significantly since the 1970s with the absence of any genotypic modification of plant type in ethylene gene (Flinn et al., 1982; Kropff et al., 1994). Recently, it has been per plant and a large number of high-density grains per panicle should be developed in rice (Nakano et al., 2012) Development of tillers in rice is a synchronous with the primary tillers producing more good quality grains compared to the secondary or tertiary tillers that initiate later (Vergara et al., 1990). The numbers of vascular bundles in rice tillers decrease acropetally from the primary to the tertiary tillers (Hayashi, 1976). Then, for late-initiated tillers, they may not have an adequate number of vascular bundles to sustain the growth of the spikelets on their panicle (Kim and Vergara, 1991). Poor vascularization reduces the supply of assimilates and hormones from the source leaves to the panicle, and thereby, significantly restricts spikelet development (Mohapatra et al., 2011). A tiller gains photosynthetic independence after emergence from the preceding leaf sheath (Toriba et al., 2019). It then transports assimilate to the sink organs growing at the apex, but at the same time, it competes with other tillers for light and nutrients (Mohapatra and Kariali, 2008).

In rice, the manipulation of tiller number is important for grain yield, but the physiological basis of the regulation of tiller growth remains unclear (Luo et al., 2012). In general, large tillers result in higher sink: source ratio, spikelet number, proportion of filled grains, leaf area per tiller and sink capacity (Efisue et al., 2014). In a field study, a low-tillering/large-panicles rice genotype was reported to have an 8% yield advantage over a profuse-tillering genotype when both the genotypes were grown under high nitrogen conditions (Kim and Vergara, 1991). In maize and sorghum, the increase in yield potential resulted from increases in sink size and a decrease in tiller number (Khush, 1996).

CHAPTER 3
MATERIALS AND METHODS

3.1 Experiments, materials and treatments

Weather parameter during the investigation in Hua-Him Prachuap Khiri Khan province

Month	Maximum temperature (ºC)	Minimum temperature (ºC)	Rainfall (mm)	Relative humidity (%)	Wind speed (km/h)
2019					
December	28	22	0.1	67	15
2020					
January	29	23	3.4	67	11.2
February	29	23	0.9	67	12.8
March	31	25	7.7	69	16.5
April	32	27	65.9	69	15
May	33	28	91.8	69	13.8

Source, https://www.accuweather.com/em/en/th/hua-hin Prachuap Khiri khan /3200001/weather-forecast/320001

Soil physical and chemical properties

Some physical and chemical properties of soil sampling were measured before the study begins. Soil properties include soil texture, which is Sandy loam with 70.97% sand, 20.43% silt, and 8.60% clay, and soil organic matter (OM), which is 0.93 % (determined by the Walkley and Black method). Soil pH (measured in 1:1 soil and water solution ratio using a pH meter) is 6.39. The electrical conductivity of the saturated soil extract (ECe) is 0.92 dS/m, and the sodium adsorption ratio (sodium and calcium in saturated soil extract) (SAR) is 0.22. Chemical nutrient available properties in soil were determined such as phosphorus (P) (determined by Bray II extraction) (5.18 mg/kg), potassium (K) (83.09 mg/kg), calcium (Ca) (88.31 mg/kg), and magnesium (Mg) (69.98 mg/mg). Electrical conductivity ($EC_{1:5}$) (soil: water ratio at 1:5) was evaluated again after receiving water salinity following each treatment of NaCl concentration. The results showed that $EC_{1:5}$ was 0.02, 4.81, 9.84, and 14.39 dS/m for 0, 50, 100, and 150 mM NaCl, respectively.

This study consists of two experiments as follows:

Experiment I: Applying proline to alleviate salty stress in rice plant at tillering stage

Experiment II: Applying trehalose to alleviate salty stress in rice plant at tillering stage

Both experiment I and II will use the same materials and methods: rice varieties, and chemicals and their measurements in laboratory, except for the exogenous substances for spraying in rice as proline and trehalose, respectively. For materials and methods of the two experiments are as follows:

3.1.1 Materials and treatments

Both experiment I and II were conducted in 4x4x3 factorial in Completely Randomized Design (CRD) which study in three rice varieties.

Factor 1: Salinity levels

Four levels of salinity (NaCl concentrations): 0 mM (control; 0 dS/m), 50 mM (5 dS/m), 100 mM (10 dS/m), 150 mM (15 dS/m) will use as factor 1 in both experiments: experiment 1 and 2.

Factor 2: Exogenous substances concentrations (proline or trehalose)

Proline levels (in experiment 1)

Four levels of proline concentration: 0 (control; 0 mM), 50 mM, 100 mM, and 150 mM, will use as factor 2 in Experiment I.

Trehalose levels (in experiment 2)

Four levels of trehalose concentration: 0 (control; 0 mM), 50 mM, 100 mM, and 150 mM, will use as factor 2 in Experiment II.

Factor 3: Three varieties of wetland rice seeds

1. Chai Nat 1 (CNT1) non-photosensitive variety and salt susceptible variety (Amano et al., 1993)

2. Pathum Thani 1 **(**PT1**)** Photoperiodic in sensitive salinity and aromatic, Thai rice (Cha-Am et al. 2008)

3. Inpari 35 Saline tolerance in the seedling phase at 12 dS/m stress is suitable for planting in paddy fields (Hairmansis et al., 2017)

3.1.2 Growing practice

Method of growing

1. Submerge the seeds in water during 24 hours

2. Rice seeds have been sown in the nursery for two weeks

3. Seedlings removed from the nursery have been planted three plants for each polybag

Method of watering, pour salinity and spray proline and trehalose

1. Frequency for watering twice every day
2. Frequency for salinity watering once a week
3. Frequency for spray proline and trehalose

Using the chemical concentration of proline (or trehalose) in spraying on plants only once at three weeks after transfer from the nursery to polybags in the greenhouse.

Days after planting to record data

In this experiment using an interval of three weeks, depending on each variety studied, two to three weeks (21-28 days) after emergence will apply proline or trehalose to rice tillers according to treatment and five replications at once. The data recorded were the number of leaves per plant, number of tillers per plant, plant height, and leaf symptom scores on plants. Using 48 plant samples and plant cuttings samples from each treatment, taking leaves for extraction and other plants for measurement, all data have been recorded after chemical administration. The data recorded using the parts of the plant is the relative water content, chlorophyll, proline, sugar and starch to identify the content contained in the plant.

3.2 Semi-quantitative RT-PCR

RNA extraction

Total RNA from 100 mg fresh rice seedlings were extracted using the Plant Total RNA Mini Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's protocol. Rice samples were homogenized by grinding with micropestle, added 500 µl RB Buffer and 5 µl of ß-mercaptoethanol. The sample mixtures were incubated at 60°C for 5 min and transferred to the Filter Column. Then, column was centrifuged and the clarified filtrate was collected to a new 1.5 ml centrifuge tube. Next, 250 µl absolute ethanol was applied to filtrate, followed by vigorous shaking. The mixture was transferred to RB column and centrifuged. The flow-through was discarded, and 500 µl W1 buffer was added to the RB column. After centrifuge, the RB column was washed twice with 600 µl of Wash Buffer and eluted using 50 µl of RNase-free

Water. The total extracted RNA was quantified with a Nanodrop spectrophotometer (OD260/280) prior cDNA synthesis.

RT-PCR and sequencing

The rice cDNA was synthesized from 1 μg of total RNA using iScriptTM cDNA Synthesis Kit (Bio-Rad Laboratories, USA). The reaction consists of 5x iScript Reaction Mix, iScript Reverse Transcriptase (RT), Nuclease-free water, and RNA template. After incubation, the cDNA was amplified by PCR (polymerase chain reaction). Polymerase chain reaction was done using gene specific primer (OsP5CS-F_5' TAG CAG GAC TGT TGG CAC TG 3' and OsP5CS-R_5' ACA GGT GTG CCG CTA TTT GA 3') and OsActin primer (OsActin_F 5' CAG CCA TGT CCC CAT CTA 3' and R_ 5' AGC AAG GTC GAG ACG AAG GA 3'). The PCR reaction mixtures consist of 1x Ultra-pure *Taq* PCR master mix (1 U of Ultra-pure *Taq* polymerase, 2 mM $MgCl_2$ and 200 μM of each dNTPs) (Geneaid Biotech Ltd., Taiwan), 0.8 μM of each primer, and 1 μl of cDNA template. The PCR cycle conditions were performed in the thermocycler (Biometra® T-gradient Thermoblock Thermal Cycler, Germany) with the initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. After final extension at 72°C for 7 min, the PCR products were cooled down to 20°C. The PCR products were determined on 1.5% agarose gel electrophoresis. The single DNA band was excised under UV-light and purified using the GenepHlowTM Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan). Then, the purified PCR products were sent to DNA sequencing service, which was performed in ABI Prism 3730XL DNA sequencer (U2Bio, Korea).

3.3 Chemicals measurements

a. Soluble sugar

Soluble sugar content in the leaf samples were estimated by the method of Dey (1990). Reagent preparation: (a) 90 % Ethanol, it was prepared by mixing 90 ml of pure ethanol in 10 ml of DDW, (b) 5 % phenol, it was prepared by mixing 5 ml of phenol in 95 ml of DDW (Jabeen and Baba, 2018).

b. Starch within leaf and stem

Starch deposited in granules in almost all green plants and in various type of plant tissues and organs, e.g., leaves, roots, fruits, grains, and stem. Basal portion of a harvest stem (10 cm long) will be harvest. Samples will be placed a strong squeezer. The squeeze juice will be poured into the measuring hole of a portable refractometer. Brix value will be measured. Brix value will be collected in micro-tubes all juices and tissue samples will be stored at 80°C until use. Frozen juice samples will be thawed on ice and then centrifuged at 11,000 × g for 5 min, and the resultant supernatants will be used soluble sugar assays. Frozen culms and leaf sheath will be ground under cryogenic condition using multi-bead shocker. The ground sample will be weighed (50 g) and extract twice with 1.0 ml of 80% ethanol at 80°C. After centrifugation at 11,000 × g for 5 min, the supernatant will be dried in vacuo, dissolved in distilled water, and for soluble sugars assays. The pellet will be re-suspended in distilled water and boiled more than 2 hours. The starch will be degraded into glucose by adding 0.2 volume 50 ml^{-1} glucoamylase in acetate buffer (pH = 4.5), and used for starch assay. The soluble sugars in juice and tissues will be measured using an enzymatic method with F-kit and a micro-plate spectrometer (Okamura et al., 2016).

Starch and Soluble sugar content

Dry sample **(**50 mg**),** grid, add 5 ml of 80**%** ethanol (x 2), transfer all sample to a glass test tube 15 ml (x 2), mix and heat 90 °C for 10-15 min (x 2), centrifuge 4000 rpm for 10 min, separate the supernatant in 15 ml tube (x 2), try to take out all of the liquid so total supernatant soluble sugar extract will be 10 ml.

Pellet part: for starch assay

Dry the residual pellet at 80 °C for 1 h, add 1 ml of sterile distilled water, let stand for a while, add 5 ml 0**.**005 N H_2SO_4 for acid hydrolysis, mix, incubate 90 °C 1 h, vortex for 1 min, centrifuge 4000 rpm for 10 min, transfer 0.5 ml of the supernatant to a new glass test tube, add 0.5 ml of 5% Phenol, add 2.5 ml of concentration H_2SO_4**,** mix, incubate 10 min RT, and read the absorbance at 480 nm.

Supernatant part: for soluble sugar assay

Take 0.5 ml from soluble sugar extract into a new test tube. Add 0.5 ml of 5**%** Phenol, add 2.5 ml of concentration H_2SO_4, mix, incubate 10 min RT, and read the absorbance at 480 nm.

Estimate samples: 3(v) × 4(s) × 4(c) × 3(r) = 144 samples

Standard glucose:

0.2 mg/ml glucose (mL)	H_2O (mL)	Glucose amount (µg)	
0	1	0	Add 0.5 ml of 5% Phenol
0.1	0.9	20	
0.2	0.8	40	
0.3	0.7	60	Add 2.5 ml of conc. H_2SO_4
0.4	0.6	80	
0.5	0.5	100	Mix, incubate 10 min RT,
0.6	0.4	120	and read the absorbance at
0.7	0.3	140	480 nm

0.2 mg/ml glucose = 2 mg (0.002 g) glucose dissolve in 10 mL water

c. Proline (or trehalose) concentrations in plant

The proline content in the leaves was estimated based on the method described by Bates et al. (1973). 50 mg of dried sample was ground with liquid N_2. Homogenize in 2 ml of 3**%** aqueous sulfosalicylic acid (w/v)**,** centrifuge 6000 rpm 15-20 min 4°C. Transfer 0.5 ml of the supernatant into the new tube, add 0.5 ml acid-ninhydrin and 0.5 ml of acetic acid, mix with a vortex mixer and incubate at 95°C 1 h, place on ice and add 2 ml of toluene. Mix by vortex and left to stand for 2 min**. (**now total volume is 3.5 ml in a glass test tube**),** transfer the reddish pink upper into the new clean tube, record the abs at 520 nm against a toluene blank**.**

Standard proline:

1 mM proline (µl)	3% sulfosalicylic acid (µl)	Expected Abs. 520 nm	Proline amount (µmole)	
0	500	0	0 ; 0	+0.5 ml acid-ninhydrin +0.5 ml of acetic acid
50	450	0.2xx	0.05 ; 0.575	Mix with a Vortex mixer and
100	400	0.5xx	0.1 ; 1.15	incubate at 95°C 1 h
150	350	0.8xx	0.15 ; 1.725	Place on ice and add 2 ml
200	300	1.1xx	0.2 ; 2.3	of toluene

Note: 100 mM proline = 0**.**11513 g dissolve in 10 ml sterile H_2O. For 3**%** sulfosalicylic acid = 3 g of sulfosalicylic acid dissolve in sterile water up to total

volume of 100 ml. And, acid-ninhydrin = Ninhydrin (1.25 g) dissolve in 30 ml of glacial acetic acid and then add 20 ml of 6 M phosphoric acid, head to dissolve, kept for 24 h at 4°C.h

3.4 Some physiological change (leaf, tillering ability etc.)

1. Water content

Collect 5 leaves from well-grown plants. Measure the fresh weight, and place inside plastic jars filled with water to saturate the leaves. Stored the plastic jars for 6-9 h at normal condition, Measure the turgid weight, oven-dried for 48 h at 50-60°C, and measure the dry weight.

$$\text{Relatively water content (\%)} = \frac{\text{(Fresh weight – dry weight)}}{\text{(Turgid weight – dry weight)}} \times 100$$

Estimate samples: 3(v) ×4(s) ×4(c) ×3(r) = 144 samples (Tsubo et al., 2007)

2. Chlorophyll content

Collect the plant (leaf), and measure the fresh weight. Dry sample in incubator, and measure the dry weight (50 mg) of each sample. Place the sample in vial containing 2 mL DMSO, mix by vortex 5 min and incubate at 65°C for 30 min (in the dark). Centrifuge the sample for 5 min, 3000 rpm, then, transfer the supernatant to a new vial. Transfer the supernatant 1 mL to a cuvette. Calibrate spectrophotometer using, DMSO as reference at 645 and 663 nm. Record absorbance at 645 and 663 nm. Calculate the chlorophyll content using Arnon's equation, Arnon's (1949) equations:

Chl a (g l-1) = 12.7 × A663 – 2.59 × A645, Chl b (g l-1) = 22.9 × A645 – 4.68 × A663, Tot Chl (g l-1) = 20.2 × A645 + 8.02 × A663 (Khaleghi et al., 2012).

Estimate samples: 3(v) × 4(s) × 4(c) × 3(r) × 5 = 720 samples

3.5 Score of symptoms

Modified standard evaluation score (SES) of visual salt injury at seedling stage (Gregoria et al., 1997)

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severally retarded; most leaves dry; Some plants dying	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

3.6 Agronomical trait (yield components, yield)

Agronomical trait of yield component and yield as: Measure the height of plants, counting leaf and tiller, panicle, 100 seeds weight and total grains weight per pot, productive tiller and harvest index in percentage were recorded at harvest.

$$\text{Harvest index (\%)} = \frac{\text{Dry panicle}}{\text{Dry panicle + dry seed + dry strow}} \times 100$$

Name of trait/ when to record

1. Height of plant (cm)
2. Leaf number
3. Tiller number
4. Productive tiller
5. Score symptoms
6. Seed weight
7. Weight 100 grain seeds (g)
8. Seed yield (g/pot)
9. Harvest index

3.7 Statistic (Design, replication) and program to analysis

The pots will arrange in 4x4x3 Factorial in Complete Randomize Design (CRD) with 5 replications, compose with 16 treatments, one plastic pot per one replication which three plants will planted in each plastic plot. Three varieties will study both experiment 1 (proline spraying) and experiment 2 (trehalose spraying). Data will analyze by using analysis of variance (ANOVA). Which variable show the statistic significant difference effected by treatments, then compared treatments by Duncan's New Multiple Range Test (DMRT). All parameters will have worked out using computer R program.

CHAPTER 4
RESULTS AND DISCUSSION

Experiment I: Applying proline to alleviate salty stress in rice plant at tillering stage

Effects on biochemical characteristics in rice at harvesting stage under difference of proline application in salinity condition

Soil salinity is a common limiting factor in crop production (Pessarakli and Szabolcs, 2019). Therefore, the application of external solute accompanied by low molecular weight as proline was conducted at tillering stage. More than to determine the yield, yield components, and agronomic characteristics, molecular and chemical also were recorded at harvesting stage.

In the application of these three factors; for varieties, salinity levels, and proline levels there was no combination of interactions between the factors that significantly affected the relative water content (RWC), but the three factors separately affected the water content (Table 1).

For varieties, the highest average of RWC was found in the variety IN 35 (44.59%) and CNT 1 (42.59%) and followed at PT 1 (39.50%). The results of RWC in the three varieties experienced a slight lower compared to previous studies regarding the application of exogenous proline to increase salt tolerance in rice. (Tabssum et al., 2019) reported the RWC in two rice varieties as Super Basmati and Shaheen Basmati that was applied proline were 53.10% and 55.42%, respectively. Each rice variety has different genetic characteristics, so each variety has the ability to bind water to plants for its own needs (Richards et al., 2006).

The water content in plants is an important issue. Plants that have a high ability of water retention can then be supplied to certain plant parts that need it for metabolic processes (Jackson et al., 1977). At high water content will have the ability to perform high physiological activities, while relatively low water content has low physiological activity (Kramer and Kozlowski, 1960). Such as in the process of photosynthesis, water has the role to transfer the nutrient in plants through plant cells; function as transporters (Harwati et al., 2012). Moreover, water is used to maintain turgor pressure and maintain the neutralize of temperature in plant, and as a driver of

the respiration process; supply energy and this energy is used for growth (Schulze et al., 2019).

The results of Fisher's test (F-test) on the effect of salinity showed that the salt stress with various levels affects RWC in rice. RWC was decreasing started at 50 mM NaCl, however, was significantly different from the not salinity condition (0 mM NaCl) at 100 mM and 150 mM NaCl (Table 1). At various levels of salinity causes a decrease in water content in rice plants since the tiller stage is formed (Hasanuzzaman et al., 2009). At that time salt stress began to attack plants and inhibited the activities in plant cells in sensitive plant species, one reason is the reduction of water absorbs (Hasanuzzaman et al., 2013a).

Table 1 Relative water content (RWC) (%) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	29.92 ± 6.70	28.04 ± 6.91	26.57 ± 8.26	27.40 ± 6.07	42.59 ± 15.40 a
	50	25.70 ± 4.34	33.30 ± 2.73	30.19 ± 8.78	29.57 ± 9.24	
	100	58.71 ± 7.34	54.87 ± 7.20	51.42 ± 7.26	51.31 ± 7.65	
	150	60.97 ± 5.50	58.71 ± 7.34	68.11 ± 7.64	56.77 ± 9.36	
PT 1	0	28.30 ± 7.75	25.35 ± 5.40	28.82 ± 8.66	27.08 ± 7.18	39.50 ± 14.19 b
	50	28.52 ± 7.83	30.31 ± 5.70	27.36 ± 4.31	25.36 ± 6.53	
	100	58.11 ± 7.64	45.59 ± 2.70	41.28 ± 9.11	45.70 ± 4.82	
	150	58.15 ± 7.84	57.10 ± 9.06	56.68 ± 9.43	48.33 ± 7.31	
IN 35	0	32.50 ± 8.20	29.83 ± 6.87	28.05 ± 8.36	29.41 ± 7.94	44.59 ± 15.62 a
	50	28.30 ± 4.27	38.25 ± 7.73	29.65 ± 2.75	30.67 ± 6.90	
	100	67.25 ± 6.71	59.26 ± 9.25	51.90 ± 9.24	49.39 ± 4.85	
	150	61.14 ± 3.70	60.97 ± 5.50	59.65 ± 6.11	56.91 ± 8.00	
Mean salinity		44.80 ± 17.28 A	43.46 ± 14.70 AB	40.81 ± 14.79 BC	39.82 ± 13.60 C	
Proline (mM)						
Mean Proline		0	50	100	150	
		28.44 ± 6.40 c	29.76 ± 6.20 c	52.90 ± 9.20 b	57.80 ± 6.97 a	
P-value (F-test)						
Variety (V) 0.00293**, Salinity (S) 0.01295*, Proline (P) < 2 x 10^{-16} **, V × S 0.905 NS, V x P 0.718, S x P 0.058 NS, V x S x P 0.999 NS. CV% 16.75						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Many studies resented about effect of salinity on the decreasing of RWC because the effect of dehydration in the cellular level caused the water loss, but less water absorption (Navarro-García et al., 2016). Thus, at higher transpiration, although in other conditions, it could induce water loss from plant leaves and causes a decrease in plant water content as well (Shrivastava and Kumar, 2015).

High salt concentrations cause disturbances in the homeostasis in water relations and changes in ion distribution at the cellular and whole plant levels. Then, changes in water homeostasis cause molecular damage that results in death due to cessation of plant growth. Decreased growth of plants under increased saline level conditions are some of the consequences of the physiological response and photosynthesis is the most important process to consider (Stępień and Kłbus, 2006).

In the form of ion absorption, it is a practical and cheapest form of osmotic adjustment in soil salinity conditions, in this section it will also cause problems that have an impact on decreased function in plant leaves as well as ion imbalances and existing toxicity (Bastías et al., 2004). For this reason, it is interesting to use exogenous substances to balance ions in plants under salty conditions. There are significant differences with varying levels of proline application ($P<0.05$) (Table 1). Increasing RWC was observed when the proline level increased with a significant difference. The highest RWC was found at 150 mM proline (57.80%) followed at 100 mM (52.90%) and lowest at 0 (28.44%) and 50 mM (29.76%) proline levels, respectively.

Sequential increase in RWC value from the use of exogenous proline; an osmoprotectant substance and is a proteogenic amino acid, in this study consistent with a sequential increase in the RWC value from the use of exogenous proline; osmoprotectant and proteogenic amino acids, in this study according to (Lama, 2013) who found RWC in exogenous application of proline. The increase in salt tolerance in rice through modulation of antioxidant activity was 50 mM (50.53%) from that applied proline in the vegetative stage or tillering. (Dolatabadian et al., 2008) reported the foliar proline application of 100 mM and 150 mM on rice at tillering stage was more effective in increasing plant stand formation, water retention, maintaining membrane permeability, photosynthetic pigments, tillering ability and kernel yield of rice cultivars. Exogenous proline supplementation can reduces the reduction of

photosynthetic activity and leaf water relations under salt stress in the leaves of rice plant varieties (Hayat et al., 2012). Exogenous application of proline-protected cell membranes from salt stress-induced oxidation by enhancing the activity of different antioxidant enzymes (Reddy et al., 2015). Even though plants are experiencing salt stress, with the presence of proline in plants, plants can bounce back to their own abilities and can absorb water (Lévai and Veres, 2013). To some extent, it was shown that the exogenous application of 100 mM and 150 mM proline was able to facilitate the growth of rice plants under cell suspension salt stress (Okuma et al., 2004).

Moreover, important properties indicate that proline plays an important role in plant growth and differentiation throughout the life cycle of plants in general. In the presence of proline can reduce the production of oxidative stress reduction (ROS) which has a strength that is strongly influenced by the response to stress factors in plants (Hossain et al., 2014). Proline has the ability to fulfill various functions in plants, in this case one of which is to maintain the water content of plants. because amino acids are the structural components of proteins, but they also play the role of compatible solutes under conditions of environmental stress i.e. salt (Lehmann et al., 2010).Therefore, proline significantly eliminates the harmful effects of salinity.

Chlorophyll is an intermediary for the process of photosynthesis that will occur if there is light and pigment. On the other hand, chlorophyll functions to attract electrons from sunlight for photosynthesis to occur (Waraich et al., 2012). For Chlorophyll A, it is a form that has a specific chlorophyll that is used in the process of oxygenic photosynthesis. Specifically, it is capable of absorbing most of the energy of the violet-blue and orange-red wavelengths of light, and is one of the poorest absorbers of the green and almost green parts of the spectrum (Kuehni, 2012).

In this study, individual factors: varieties, salinity level, and proline level, showed a significantly different effect on Chlorophyll A content (Table 2). For the variety factor, IN 35 showed the highest value (0.39 mg/g) in Chlorophyll A content and followed by two Thai rice varieties. Compared with Thai rice (CNT1 and PT 1), it was found that the chlorophyll A content of CNT 1 (0.34 mg/g) was higher than PT 1 (0.31 mg/g) (Table 2).

Table 2 Chlorophyll A content (mg/g) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	0.33 ± 0.03	0.31 ± 0.08	0.30 ± 0.08	0.28 ± 0.06	0.34 ± 0.06 b
	50	0.37 ± 0.02	0.36 ± 0.80	0.31 ± 0.71	0.30 ± 0.04	
	100	0.39 ± 0.01	0.42 ± 0.60	0.34 ± 0.05	0.33 ± 0.04	
	150	0.40 ± 0.05	0.37 ± 0.08	0.39 ± 0.02	0.34 ± 0.04	
PT 1	0	0.29 ± 0.02	0.29 ± 0.07	0.27 ± 0.07	0.25 ± 0.05	0.31 ± 0.06 c
	50	0.30 ± 0.07	0.33 ± 0.06	0.31 ± 0.06	0.28 ± 0.05	
	100	0.35 ± 0.06	0.37 ± 0.09	0.31 ± 0.04	0.30 ± 0.05	
	150	0.31 ± 0.00	0.31 ± 0.01	0.35 ± 0.05	0.31 ± 0.05	
IN 35	0	0.37 ± 0.06	0.33 ± 0.11	0.33 ± 0.11	0.31 ± 0.09	0.39 ± 0.09 a
	50	0.40 ± 0.00	0.39 ± 0.07	0.37 ± 0.08	0.33 ± 0.08	
	100	0.40 ± 0.03	0.48 ± 0.09	0.40 ± 0.09	0.39 ± 0.09	
	150	0.47 ± 0.10	0.44 ± 0.15	0.43 ± 0.07	0.40 ± 0.07	
Mean salinity		0.36 ± 0.06 A	0.37 ± 0.09 A	0.34 ± 0.07 AB	0.32 ± 0.07 B	
Proline (mM)						
		0	50	100	150	
Mean Proline		0.30 ± 0.07 c	0.34 ± 0.06 c	0.37 ± 0.07 b	0.38 ± 0.08 a	
P-value (F-test)						
Variety (V) 5.36 x 10^{-7**}, Salinity (S) 2.77 x 10^{-5**}, Proline (P) 3.95 x 10^{-5**}, V × S 0.989 NS, V x P 0.898 NS, S x P 0.800 NS, V x S x P 1.000 NS, CV% 19.81						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

There was no interaction between the three factors, either a combination of two or three factors (Table 2). However, the value of chlorophyll A presented in this rice variety was lower than that reported in the varieties MR211 (7.70 mg/g) and MR232 (7.39 mg/g) (Shakoor et al., 2010) at the seedling stage after 65 days of planting. Thus, the main dominating chlorophyll A content is caused by genetic factors (Msanne et al., 2012).

The increase in salinity significantly affected the decrease in chlorophyll A content. However, the salinity at 100 mM (0.36 mg/g) and 150 mM NaCl (0.32 mg/g) showed a significant decrease in chlorophyll content compared to the non-salinity

level (0 mM NaCl) (0.36 mg/g) (Table 2). The results of previous studies are biochemical and anatomical changes and yield reduction in rice (*Oryza sativa* L.) under varied salinity regimes; salinity 0 dS/m (8.99 mg/g), 4 dS/m (8.11mg/g), 8 dS/m (6.59 mg/g) and 12 dS/m (4.14 mg/g) at seedling stage 45 days after plant. The decrease in chlorophyll concentration may be due to its inhibitory effect on ion accumulation of various salts in different chlorophyll biosynthesis (Moura et al., 2010). The presence of a carrier fluid in cells that relates the strength of protein complexes, the stability and the strength of the membrane and pigments establishment affected by salinity (Musazade et al., 2018). Therefore, the loss of chlorophyll is often used as a cellular indication of the presence of salt stress or resistance to salt stress (Chen et al., 2007). The loss of these pigments provides flexibility as well as being forgotten is one of the mechanisms for adaptation in preventing damage caused by the photosynthesis process by minimizing the possibility of photoinhibition in photosystem in plants (Brestic et al., 2015).

Other expected causes of decreased sugar content can be shrinkage of cell contents, decreased tissue development and differentiation, nutritional imbalances, damage to plant membranes and avoidance of various disrupted mechanisms that caused by salt (Farooq et al., 2009). For this reason, using external substances has been used to promote plant growth under various stresses. In this study, the overall average content of chlorophyll A at proline levels of 100 mM and 150 mM showed an increase in the positive effect, compared to the values for proline 50 mM and 0 Mm. While the results of previous studies on induction of salt tolerance in wheat (*Triticum aestivum* L) showed proline in seedling increased through the application of proline; 0 mM (1.86 mg/g), 50 mM (1.97 mg/g), and 150 mM (2.23 mg/g) at the seedling stage; three weeks after planting (Table 2).

There is a certain expectation that proline will accumulate in plant leaf tissue, however, proline can degrade as well. Increased proline biosynthesis in plants will be able to increase Chlorophyll A content in plants (Funck et al., 2010). (Tounekti et al., 2011) reported that the content of chlorophyll A in the leaves increased drastically by using 100 mM and 150 mM proline. The presence of proline is this study by foliar spraying could reduce salinity stress in rice plants and expect to increase protein synthesis, proline utilization, and protein hydrolysis (Fan et al., 2012).

Chlorophyll B acts as a pigment so that it functions to absorb energy rather than light to carry out the process of photosynthesis. Other pigments, such as carotenoids, are also present in chloroplasts and function as accessory pigments, trapping solar energy and passing it on trough to chlorophyll (Glazer, 1977; Telfer et al., 2008).

The results showed that the levels of chlorophyll B were significantly different influenced by individual factors (variety, salinity levels, and proline levels) and the interaction between varieties and proline (Table 3).

Table 3 Chlorophyll B content (mg/g) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	1.44 ± 0.46	1.26 ± 0.22	1.23 ± 0.13	0.96 ± 0.12	1.39 ± 0.39 b
	50	1.80 ± 0.19	1.27 ± 0.26	1.20 ± 0.20	1.06 ± 0.05	
	100	1.98 ± 0.22	1.44 ± 0.02	1.32 ± 0.06	1.07 ± 0.04	
	150	2.27 ± 0.10	1.70 ± 0.22	1.32 ± 0.10	1.07 ± 0.03	
PT 1	0	1.31 ± 0.40	1.15 ± 0.27	1.10 ± 0.26	0.93 ± 0.15	1.27 ± 0.34 c
	50	1.55 ± 0.11	1.70 ± 0.24	1.02 ± 0.13	0.96 ± 0.12	
	100	1.81 ± 0.18	1.33 ± 0.05	1.20 ± 0.06	0.99 ± 0.11	
	150	1.99 ± 0.11	1.60 ± 0.25	1.25 ± 0.06	1.02 ± 0.14	
IN 35	0	1.53 ± 0.50	1.35 ± 0.29	1.26 ± 0.22	1.10 ± 0.20	1.55 ± 0.48 a
	50	1.97 ± 0.43	1.43 ± 0.30	1.26 ± 0.17	1.20 ± 0.17	
	100	2.40 ± 0.48	1.54 ± 0.30	1.44 ± 0.05	1.21 ± 0.13	
	150	2.58 ± 0.35	1.82 ± 0.12	1.45 ± 0.04	1.27 ± 0.14	
Mean salinity		1.88 ± 0.47 A	1.42 ± 0.27 B	1.25 ± 0.17 C	1.07 ± 0.15 D	
Proline (mM)						
		0	50	100	150	
Mean Proline		1.21 ± 0.30 d	1.32 ± 0.35 c	1.47 ± 0.41 b	1.61 ± 0.49 a	
Salinity (mM NaCl)						
Proline (mM)		0	50	100	150	
	0	1.42 ± 0.41 d	1.25 ± 0.25 d-g	1.16 ± 0.20 e-h	0.99 ± 0.16 h	
	50	1.76 ± 0.30 c	1.30 ± 0.25 d-g	1.16 ± 0.18 e-h	1.06 ± 0.14 gh	
	100	2.05 ± 0.37 b	1.43 ± 0.10 d	1.32 ± 0.11 def	1.09 ± .13 fgh	
	150	2.28 ± 0.32 a	1.70 ± 0.20 c	1.34 ± 0.11 de	1.12 ± 0.15 e-h	
P-value (F-test)						
Variety (V) $1.43 \times 10^{-7**}$, Salinity (S) $< 2 \times 10^{-16**}$, Proline (P) $2.29 \times 10^{-11**}$, V × S 0.513 NS, V× P 0.957 NS, S × P 0.00015^{**}, V× S × P 1.000 Ns, CV % 15.62						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of

probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

IN 35 variety obtained higher chlorophyll B content (1.55 mg/g) followed by CNT 1 (1.39 mg/g), and PT 1 (1.27 mg/g). It shows the results of previous studies biochemical and anatomical changes and yield reduction in rice (*Oryza sativa* L.) under varied salinity regimes, content of chlorophyll B at seedling stage in 45 days after plant; variety Pokkali (3.62 mg/g) and MR211 (3.29 mg/g) at salinity 60 mM and 150 mM NaCl. The order of varietal values is the same between the content of chlorophyll A and B. Many factors affect the formation of chlorophyll such as plant age, leaf age, and leaf morphology, or it is said that this trait is a response according to genetics (Biber et al., 2007).

The increase in salt content resulted in a decrease in the content of Chlorophyll B. Thus, the highest value of Chlorophyll B occurred at 0 mM NaCl (1.88 mg/g) and the lowest at 150 mM NaCl (1.07 mg/g) (Table 3). Compared with research previously, namely induction of salt tolerance in wheat (*Triticum aestivum* L.) seedlings through exogenous application of proline, the results of chlorophyll B content in salt application were 0 mM (1.02 mg/g), 60 mM (0.70 mg/g) and 120 mM (0.54 mg/g) at seedling at age at three weeks after planting (Mahboob et al., 2016).

The application of exogenous proline had a positive effect on the increase in chlorophyll B in the leaves. The lowest value was observed at 0 mM proline (1.21 mg/g) and increased when proline was applied in increasing levels through this opportunity, as evidenced in previous research, namely induction of salt tolerance in wheat (*Triticum aestivum* L) seedling through exogenous application of proline; 0 mM (1.02 mg/g), 50 mM (0.93 mg/g) and 100 mM (1.37 mg/g) at seedling at age at three weeks after plant (Table 3). Many factors affect the formation of chlorophyll such as plant age, leaf age, and leaf morphology, or it is said that this trait is a response according to genetics. The increase in the content of chlorophyll b in plants has something to do with the increase in chlorophyll protein (Brestic et al., 2015).

However, there are significant differences in the interaction between salinity and proline factors. Chlorophyll B content is more sensitive to salinity stress and proline application (Table 3). The highest chlorophyll content was when rice was not

salinized during planting at all levels of proline use. This shows a clear effect of salinity on plant chlorophyll formation. While proline spraying the rice, plants had a significant effect in promoting the rice plants to increase the Chlorophyll B content. Influence of the interaction between salinity level and proline level on the Chlorophyll B, it was found that at no salinity the positive influence of increased proline level was evident. At 0 mM NaCl, the highest and lowest chlorophyll B content was found at 150 mM and 0 mM proline, respectively. However, at higher salinity levels; 50-150 mM NaCl, Chlorophyll B content increased was not statistically significant when using proline level increased. However, beneficial effects from external use of proline for rice grown in saline soil conditions were still noticeable.

Thus, it can be remarked that, salinity is very influential on the creation of chlorophyll B which is formed in plant leaves (Abdelkader et al., 2007). With this incident, there will be a decrease in glucose levels in plants, especially in leaves and a decrease in the value of chlorophyll B content, and resulted to reduction of photosynthesis (Björkman and Demmig, 1987). However, for the suppression of photosynthesis, it is always dependent on changes in gas, photosynthetic pigment, cultivar species or type, stomata and accumulation of organic and inorganic metabolites and antioxidants (Ashraf and Harris, 2013). Which, Chlorophyll B plays a role in this to help the photosynthesis process that takes place by absorbing light energy that is directly exposed to plant parts, especially sunlight from the leaves (Ruban, 2009). However, level of sensitive to salinity stress depend on plant species and varieties (genetic); plants can be classified into: salt sensitive and salt tolerant (Acosta-Motos et al., 2017).

Positive effect of proline application showed in rice plant grown either both non-salinity (0 mM proline) and salinity conditions (50-150 mM proline). Actually, proline, an amino acid, work plays an important role in plants. It has been reported the role to protect the plant from various stresses and helps the plant recover from stress more quickly (Hayat et al., 2012). In some cases, proline accumulation in leaves is a core pathway of proline metabolism, including in higher plants containing Chlorophyll A/B in Proline-Glutamate biosynthesis (Randall et al., 1985). Which, the presence of proline acts as an osmotic agent that can prevent damage to protein structure and membranes as well as protein denaturation (Smirnoff and Cumbes,

1989). (Santoro et al., 1992) presented proline is produced by cells immediately after stress occurred in plants, especially in leaves and effect to accumulate chlorophyll content. At each level of proline application resulted to the synthesis of a secondary amine group; gives a unique helical ring in its structure, to help plants growing in environments with salt conditions and cause plant stress (Damodaran and Parkin, 2017). That may be why the response to exogenous proline levels used in rice subjected to different levels of salinity resulted in different Chlorophyll B content.

Since the chlorophyll B content is almost four times higher than that of chlorophyll A, the total chlorophyll content tends to respond to either the influence of individual factors, or a combination of them with the same chlorophyll B pattern. There are significant differences that are influenced by variations in varieties and levels of salinity and proline. In addition, the interaction between salinity level and proline level showed a significant effect on total chlorophyll (Table 4). The highest total chlorophyll content was found in the Inpari 35 variety (1.93 mg/g) followed by CNT 1 (1.72 mg/g), and PT 1 (1.60 mg/g). High total chlorophyll content had a high ability to receive and absorb photosynthesis from sunlight during plant growth (Ji et al., 2012). This can reason be supported by previous research, namely exogenous application of proline improved salt tolerance in rice through modulation of antioxidant activities; Super Basmati (3.87 mg/g) and Shaheen Basmati (3.65 mg/g) and Shaheen Basmati (4.43 mg/g) in the vegetative or tillering stage at level of salinity 50 mM. Therefore, the selection of salt-tolerant cultivars is one of the most common and effective ways to increase the stability of crop productivity (Farooq and Azam, 2006).

In salinity, increasing salinity levels contributed greatly to the decrease in total chlorophyll content in plants. As obtained in this study, each salinity level in the control 0 mM (2.25 mg/g) increased, the concentration was 50 mM (1.79 mg/g) to 100 mM (1.58 mg/g) and up to 150 mM 1.38 mg/g). These results compare with previous studies are expression levels of some starch metabolism related genes in flag leaf of two contrasting rice genotypes exposed to salt stress; total chlorophyll content in rice leaf at salinity 0 mM (1,445.7 µg g^{-1}) and 150 mM (1,572.2 µg g^{-1}) at putting stage (Boriboonkaset et al., 2012). In addition, salinity stress is associated with osmotic effects, ion toxicity, and nutrient imbalances that cause a decrease in

photosynthetic efficiency as well as a decrease in total chlorophyll content (Parihar et al., 2015). Salinity stress causes a decrease in ion concentration and water potential in leaves will potentially accumulate chlorophyll (James et al., 2002). Osmotic tolerance can be achieved either by absorption of ions from the medium, or by the synthesis and accumulation of organic solutes (Herbert et al., 2015). On regulation by biosynthesis and catabolism as well as intercellular transport of each other to different cell compartments, of course all depend on intracellular levels rather than proline (Szabados and Savouré, 2010). On this occasion for higher plants that produce chlorophyll total content (T), proline biosynthesis moves and occurs via the glutamate or ornithine pathway (Da Rocha et al., 2012). The first involves the synthesis of proline from glutamic acid as well as via the pyrroline-5-carboxylate (P5C) intermediate in the cytoplasm or chloroplast which is a key biochemical pathway under conditions of physiological nature and under nitrogen deficiency or osmotic stress (Kishor et al., 2005). However, a significant increase in salt tolerance was also found to some extent in cells, in some parts of plant species, classified as high and rich in total chlorophyll content.

Table 4 Total chlorophyll (T) content (mg/g) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	1.76 ± 0.51	1.56 ± 0.56	1.46 ± 0.24	1.22 ± 0.15	1.72 ± 0.42 b
	50	2.14 ± 0.21	1.68 ± 0.26	1.52 ± 0.23	1.34 ± 0.07	
	100	2.28 ± 0.25	1.90 ± 0.07	1.63 ± 0.10	1.33 ± 0.16	
	150	2.65 ± 0.08	2.02 ± 0.16	1.70 ± 0 12	1.41 ± 0.06	
PT 1	0	1.60 ± 0.43	1.45 ± 0.27	1.29 ± 0.18	1.20 ± 0.15	1.60 ± 0.38 c
	50	1.94 ± 0.20	1.50 ± 0.28	1.33 ± 0.15	1.24 ± 0.13	
	100	2.24 ± 0.26	1.71 ± 0.05	1.56 ± 0.12	1.34 ± 0.02	
	150	2.31 ± 0.13	1.94 ± 0.15	1.62 ± 0.11	1.33 ± 0.14	
IN 35	0	1.90 ± 0.55	1.70 ± 0.37	0.57 ± 0.26	1.40 ± 0.20	1.93 ± 0.50 a
	50	2.35 ± 0.43	1.81 ± 0.32	1.62 ± 0.22	1.50 ± 0.12	
	100	2.76 ± 0.45	1.97 ± 0.20	1.84 ± 0.12	1.60 ± 0.04	
	150	3.04 ± 0.25	2.26 ± 0.03	1.90 ± 0.09	1.67 ± 0.08	
Mean salinity		2.25 ± 0.50 A	1.79 ± 0.30 B	1.58 ± 0.22 C	1.38 ± 0.17 D	
Proline (mM)						
Mean Proline		0	50	100	150	
		1.51 ± 0.34 d	1.66 ± 0.38 c	1.85 ± 0.44 b	1.98 ± 0.51 a	
Salinity (mM NaCl)						
Proline (mM)		0	50	100	150	
	0	1.75 ± 0.45 ef	1.56 ± 0.29 fgh	1.44 ± 0.23 ghi	1.27 ± 0.17 i	
	50	2.14 ± 0.32 c	1.66 ± 0.28 efg	1.49 ± 0.22 ghi	1.36 ± 0.15 hi	
	100	2.43 ± 0.38 b	1.86 ± 0.16 de	1.67 ± 0.16 efg	1.43 ± 0.15 ghi	
	150	2.67 ± 0.35 a	2.07 ± 0.18 cd	1.73 ± 0.15 ef	1.47 ± 0.17 ghi	
P-value (F-test)						
VC7:I30ariety (V) 2.32 x 10-9**, Salinity (S) < 2 x 10-16**, Proline (P) 1.66 x 10-13 **, V × S 0.617 NS, V× P 0.874 NS, S × P 0.0017 **, V × S × P 1.000 NS, CV% 13,28						

Note**:** CV = Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c…**)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Both processes occur, but the degree to which one process dominates the other depends on the individual plant species and on the level of salinity (Flowers and Flowers, 2005). For this reason, the use of external substances including proline is an alternative way to reduce damage caused by salinity stress. In this case, in a previous study regarding the exogenous application of proline improved salt tolerance in rice through modulation of antioxidant activities, 50 mM proline contained total

chlorophyll (6.69 mg/g) is the highest compared to other proline content at a salinity level of 150 mM at vegetative stage.

For example, rice revealed that increased levels of free proline were found in the leaves of salt stress-resistant plants compared to other types of non-stress calls (Chunthaburee et al., 2014). Similarly, salt-adapted rice plants have significantly higher proline concentrations compared to other salt-sensitive crops (Ashraf and Foolad, 2007b). Thus, proline accumulation also occurs in plants that respond to many stresses. Intracellular proline content in plants is regulated from biosynthesis, catabolism and through transport between cells and different cell compartments. Moreover, with all these cell forms that accumulated proline under stresses, there is great potential for the formation of sufficient chlorophyll by plant processes through photosynthesis (Meena et al., 2019a). For example, water-deficient rice plants accumulate proline in the leaves which is the accumulation of chlorophyll from the photosynthesis process (Hayat et al., 2012). Which is associated with an increase in the content of precursors for proline biosynthesis, including glutamic acid, ornithine and arginine. Therefore, applied plant leaves with compatible solutes such as proline is considered the most effective for reducing various forms of salt stress damage (Chen and Murata, 2002). Furthermore, proline application increased leaf growth rate and general plant chlorophyll formation as well as inhibited vegetative growth of rice when exposed to high levels of Na^+ and Cl^- ions (El Moukhtari et al., 2020).

In the application of these three factors; variety, salinity level, and proline level, there was no significant interaction between any factors that affected the proline content in rice plants. However, each factor separately affected the proline content (Table 5). In three rice varieties, IN 35 (6.95 µmol/g) showed higher proline content more than Thai rice varieties. CNT 1 (6.42 µmol/g) had higher proline content more than PT 1 (6.09 µmol/g). Rice plants have the power in a certain ability to deviate and accumulate proline content in plants naturally, depending on the conditions or environments that are affecting each variety. There is evidence linking changes in proline content to transport-related processes derived from development as well as stress-related studies. However, when compared with previous studies regarding induction of salt tolerance in wheat (*Triticum aestivum* L.) seedling through exogenous of application proline, the Khirman variety (10.33 µmol/g) and ESW 9525

(9.77 µmol/g) at three weeks old seedling stage at NaCl 60 mM and 120 mM, and proline 50 mM to 150 mM (Dien et al., 2019).

Whereas, salinity resulted in a decrease in the proline content of plants in order of increasing salinity. The highest value was observed at plants grown in salinity level at 0 mM NaCl (7.91 µmol/g), lowest at 150 mM NaCl (5.14 µmol/g) (Table 5). Although the increased proline accumulates in rice is one mechanism that responds to salinity stress in rice genetic. The previous study with the title induction of salt tolerance in wheat (*Triticum aestivum* L.) seedling through exogenous of application proline, NaCl 0 mM (5.16 µmol/g), NaCl 60 mM (6.65 µmol/g), NaCl 120 mM (8.54 µmol/g), at seedling stage, at three weeks after plant (Khan et al., 2009; Mahboob et al., 2016). Salinity causes a decrease in certain parts of plant morphology and physiology and plant development, because certain cells are disturbed, eventually the process becomes inhibited (Lisar et al., 2012). Moreover, application of salinity reduces the ability of rice plants to absorb and store higher amounts of proline in the leaves (Demiral and Türkan, 2006; Wassmann et al., 2009a).

For proline concentration applying (50-150 mM proline), the increase of proline content more than control treatment (0 mM proline) (5.63 µmol/g) was found in all concentration levels. Although, no significant difference of these values among treatments at use proline in different levels (50-150 mM proline) (6.57-6.90 µmol/g), the value increased when the level of proline application increased (Table 5). However, for this research can be compared with previous studies; induction of salt tolerance in wheat (*Triticum aestivum* L.) seedling through exogenous of application proline, proline at seedling stage three weeks after plant; Proline 0 mM (8.54 µmol/g), 50 mM (8.84 µmol/g) dan 150 mM (10.33 µmol/g) (Khan et al., 2009; Mujtaba et al., 2016).

Table 5 Proline content (µmole/g) (± standard error) in plant of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	6.89 ± 0.51	5.92 ± 0.76	5.01 ± 0.86	4.25 ± 1.24	6.42 ± 1.29 b
	50	7.58 ± 0.34	6.60 ± 0.70	6.23 ± 0.89	5.37 ± 0.66	
	100	7.70 ± 0.46	7.26 ± 0.55	6.49 ± 1.12	5.42 ± 0.64	
	150	8.83 ± 0.62	7.13 ± 0.24	6.63 ± 0.91	5.35 ± 0.41	
PT 1	0	6.61 ± 0.45	5.89 ± 0.88	4.56 ± 0.78	4.09 ± 0.44	6.09 ± 1.30 c
	50	7.40 ± 0.33	6.43 ± 0.49	6.07 ± 0.59	5.20 ± 1.13	
	100	7.41 ± 0.60	6.95 ± 1.21	6.26 ± 1.22	5.08 ± 0.24	
	150	8.61 ± 0.98	6.18 ± 0.65	5.88 ± 0.20	4.82 ± 0.31	
IN 35	0	7.52 ± 0.55	6.27 ± 0.32	5.56 ± 0.59	5.06 ± 0.68	6.95 ± 1.44 a
	50	8.22 ± 0.97	7.55 ± 1.44	6.77 ± 1.64	5.41 ± 0.57	
	100	8.56 ± 0.60	7.87 ± 1.41	7.00 ± 1.55	5.92 ± 0.34	
	150	9.55 ± 0.38	7.53 ± 0.11	6.67 ± 0.12	5.66 ± 0.70	
Mean salinity		7.91 ± 0.97 A	6.80 ± 0.96 B	6.09 ± 1.08 C	5.14 ± 0.77 D	
Proline (mM)						
Mean Proline		0	50	100	150	
		5.63 ± 1.20 b	6.57 ± 1.21 a	6.83 ± 1.27 a	6.90 ± 1.51 a	
Variety (V) 3.66 x 10^{-6**}, Salinity (S) < 2 x 10^{-16**}, Proline (P) 5.24 x 10^{-10**}, V × S 0.992 NS, V× P 0.971 NS, S× P 0.122 NS, V× S x P 1.000 NS, CV% 12.144						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Salinity stress causes osmotic stress and an imbalance of contained ions, in addition to creating positive oxidative stress (Teakle and Tyerman, 2010). Proline acts, as an appropriate helper in stabilizing and enhancing stress tolerance in plants, which should be further investigated in relation to the mechanisms involved (Chen et al., 2019). Due to the ability of plants to resist and block salt toxins, the presence of some external chemicals such as introducing proline in salty conditions can increase the ability of salt tolerance by eliminating the effect of excessive ion concentration on plants (Parida and Jha, 2010). This study demonstrated that proline utilization in plants i.e., the best possible accumulation of proline in plants, is provided by protein

degradation in the proline-rich stores of pigments imported into leaves forming sites for proline drift (Raymond and Smirnoff, 2002). Similarly, proline accumulation in certain plants was not only associated with increased gene P5CS expression, suggesting a contribution from naturally occurring proline transport processes in plants by receiving proline from external (Stines et al., 1999). The provision of proline stabilized the condition of rice plants growing in salty conditions, which may be caused by an increase in plant water absorption. Relatively high levels of proline are required for plant growth and cellular function (Dubey and Pessarakli, 2001). Plants respond to various harmful salt stresses by accumulating certain specific substances, the main of which are amino acids. Less than 5% of the total plant amino acids under stress-free conditions are provided by proline. In general, proline is present to stabilize the growth of plants experiencing salt stress (Meena et al., 2019b).

In many plants under various forms of stress, concentrations increase up to 80% of the amino acid pool. Occasional abiotic stresses such as drought and salinity cause significant worldwide crop losses (Gill and Tuteja, 2010).

(Verslues and Sharp, 1999) reported that proline deposition in rice leaves at low stress potential is not achieved by biosynthesis but by increasing proline content. However, no significant difference in proline content is affected by interactions between any two or three factors. The insignificance of any interaction between these factors (varieties, salinity level, and proline level) indicates that the response to the proline content of each rice variety was influenced by either salinity level or exogenous proline level in the same direction and quantity.

The results of the analysis of variance showed that all individual factors (varieties, salinity levels, and proline levels) and interaction between varieties and proline levels had a very significant effect on the soluble sugar content in plant leaves (Table 6). In order of decreasing values of soluble sugar content in leaves showed when plants received the level of salinity increased, which the highest was found at control treatment at 0 mM NaCl (48.19 mg/g) and lowest in 150 mM NaCl (33.38 mg/g). Therefore, from the three factors, the results of the research can be compared with previous studies; induction of salt tolerance in wheat seedling (*Triticum aestivum* L.) through exogenous proline application; sugar content in leaves data collection at seedling stage 3 weeks after planting; variety ESW 95, salinity 120 mM $NaCl^{+}$ at

proline 0 mM (8.97 mg/g), salinity 120 mM $NaCl^+$ at proline 50 mM (9.52 mg/g) and salinity 120 mM $NaCl^+$ at proline 100 mM (12.09 mg/g) (Khan et al., 2009).

The soluble sugar content in plant leaves was highest on Inpari 35 (44.14 mg/g) varieties fallow by CNT 1 (40.96 mg/g) and the lowest in PT 1 (36.34 mg/g) (Table 6). The highest soluble sugar content affected by the application of proline was found at 150 mM proline (46.03 mg/g), followed at 50 mM (39.56 mg/g) and 100 mM (40.95 mg/g) proline, and lowest at 0 mM proline (35.37 mg/g) (Table 6). Meanwhile, the results of these studies can be compared with previous studies on the induction of salt tolerance in wheat seedling (*Triticum aestivum* L.) through exogenous proline application; sugar content in leaves data collection at seedling stage 3 weeks after planting, sugar content in Khirman variety (9.16 mg/g) and ESW 9525 (12.09 mg/g) in salinity level of 60 mM and 120 mM NaCl (Yoshida and Ahn, 1968). Significant differences affected by the interaction between varieties and proline application in different levels showed the highest values at all proline levels in IN 35. However, CNT 1 had higher proline content in leaves more than PT 1 when external proline was applied to rice plants at 50-150 mM proline. However, in this study, increasing in soluble sugar content was induced by salinity increased in rice leave, although not applied the external proline. However, this occurrence was observed only in PT 1 at 150 mM NaCl (45.30 mg/g); no salinity as 40.15 mg/g (Table 6). This may indicate an attempt by the rice variety PT 1 to maintain its physiology to survive in salinity conditions.

These results represented the clearly negative effect of salinity stress. However, using external proline for application provided more accumulate of soluble sugar content in rice leaves. However, the response to exogenous substances is still influenced by plants genetics. For plants that are subject to salinity, the dehydration effect of the plant is due to a decrease in the plant's ability to absorb water. The states that plant that experiencing salt poisoning can be recognized from the reduced content of soluble sugars in plant leaves (Rengel and Römheld, 2000). This is partly due to reduced photosynthetic supply during the formation of source-to-sink soluble sugars content that accumulate in plant leaves during growth (Von Schaewen et al., 1990). Thus, sugar accumulation in plants is considered to be one of the most important consequences for osmotic adjustment under salinity pressure (Bornet et al., 2007).

Glucose that has been used by plants is formed for energy and to make various other substances such as cellulose and starch (Dhepe and Fukuoka, 2007). Moreover, carbohydrates establishment can act also as reductive oxygen species (ROS) scavengers and contribute to increased stabilization of plant membranes

Table 6 Soluble sugar content (mg/g) in leaves of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	43.54 ± 2.93	39.23 ± 4.29	33.73 ± 5.73	28.42 ± 2.82	40.96 ± 8.10 b
	50	47.67 ± 4.36	42.30 ± 3.67	39.63 ± 7.52	31.65 ± 5.36	
	100	51.12 ± 3.53	38.73 ± 1.64	36.86 ± 2.09	31.20 ± 3.80	
	150	55.49 ± 5.37	46.03 ± 1.93	44.73 ± 1.65	44.98 ± 5.01	
PT 1	0	40.16 ± 8.47	36.01 ± 6.48	23.97 ± 2.90	45.30 ± 1.90	36.34 ± 7.14 c
	50	45.58 ± 9.18	36.60 ± 5.55	27.79 ± 1.57	47.84 ± 2.85	
	100	44.39 ± 4.25	37.90 ±2.12	32.40 ± 2.18	52.53 ± 2.24	
	150	43.60 ± 4.25	41.27 ± 3.84	32.63 ± 4.28	61.11 ± 3.83	
IN 35	0	45.30 ± 1.90	39.57 ± 4.44	36.12 ± 3.30	28.25 ± 2.20	44.14 ± 8.40 a
	50	47.84 ± 2.86	43.82 ± 1.18	42.40 ± 1.25	34.65 ± 6.10	
	100	52.53 ± 2.24	47.60 ± 2.94	44.45 ± 2.23	38.17 ± 5.56	
	150	61.11 ± 3.83	54.07 ± 7.55	45.07 ± 3.06	45.25 ± 2.92	
Mean salinity		48.19 ± 7.05 A	41.93 ± 6.20 B	38.51 ± 5.71 C	33.28 ± 7.22 D	
Proline (mM)						
		0	50	100	150	
Mean Proline		35.37 ± 7.46 c	39.56 ± 7.57 b	40.95 ± 7.27 b	46.03 ± 8.244 a	
Proline (mM)						
Variety (V)		0	50	100	150	
	CNT 1	36.23 ± 6.90 ef	40.31 ± 7.61 cd	39.48 ± 7.99 cde	47.81 ± 5.72 b	
	PT 1	32.56 ± 8.21 f	36.20 ± 8.38 ef	37.68 ± 5.21 de	38.91 ± 5.34 cde	
	IN 35	37.31 ± 6.97 de	42.18 ± 5.81 c	45.69 ± 6.21 b	51.37 ± 8.07 a	
P-value (F-test)						
Variety (V) 2.58 x 10^{-13**}, Salinity (S) < 2 x 10^{-16**}, Proline (P) 3.83 x 10^{-16**}, V × S 0.940 NS, V× P 0.016*, S× P 0.610 NS, V× S × P 0.818 NS, CV% 10.626						

Note**:** CV = Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c…**)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Protective mechanism against water deficit is the accumulation of some osmoprotectant including proline (as amino acids) and soluble sugars (Sing et al.,

2015; Fàbregas and Fernie, 2019). It can be said that the dissolved sugar content in plant leaves has a central role in the structure and metabolism of plants at the cellular level and throughout the plant organism. Sucrose is the most abundant sugar in plants, occurs in plants with a strong ability to accumulate sugar in their leaves, and has a function and form in which carbohydrates are transported from one plant organ to another.

For sugar accumulation, the structures formed in response to salinity stress are also quite well documented (Porcel et al., 2012).

The importance of soluble sugar, complexes in the formation of metabolism in plants is recognized as products derived from hydraulic processes, substrates in biosynthetic processes, energy production as well as certain sugars and signaling systems (Reddy et al., 2004). It has recently been claimed that, under conditions of salinity stress, even sugar fluxes may act as signals for metabolic regulation (Masoudi-Sadaghiani et al., 2011). That is the ability of genes in plants that are functionally associated with various abiotic stresses in accumulating the formation of soluble sugars in plant leaves (Gunde-Cimerman et al., 2009).

For administration of external proline application can form soluble sugar content in plant leaves, because proline has the power to accumulate and function as an osmotic agent. Moreover, proline is also associated with protective action in plants under severe stress conditions, and is required for plant growth and function (Heuer, 2010). Results of this study, there was an accumulation of dissolved sugars in the leaves. Similarly, with an increased leaf proline content after plants were treated with proline (Ahmed et al., 2011). Proline can increase the sugar content in rice leaves significantly, to certain cells undergo healing, and eventually, the process becomes smooth (Mostajeran and Rahimi-Eichi, 2009).

There are significant differences in soluble sugar content in the stem that are only influenced by the level of salinity. No significant difference was found in the other two factors; varieties and proline. Moreover, for the interaction of factors, there was also no significant difference (Table 7). The three rice varieties contained soluble sugars in plant stems, namely at IN 35 (34.88 mg/g), PT (34.26 mg/g) and CNT 1(34.18 mg/g). These results can be compared with previous studies, namely the accumulation process of carbohydrate in rice varieties in relation to their response to

nitrogen in the tropics; IR8 (66 g/m^2), Peta (72 g/m^2), Bengawan (105 g/m^2) dan Chianuog 242 (24 g/m2) soluble sugar content in plant stem at flowering stage (Yoshida and Ahn, 1968). For salinity levels, the highest soluble sugar content in the rice stem was detected at 0 mM NaCl (40.23 mg/g), when the salinity level increase resulted to the values decreased, continuously.

In this study, it can be concluded that in each plant genetic characteristics clearly affect the content of soluble sugar only in plant leaves (Table 6-7). For plants that contain soluble sugar, to support their strong absorption of important elements in the form of nutrients in the soil (García-Jiménez et al., 2017). The existence of an osmotic adjustment is one way for the relationship to maintain water under osmotic pressure. In order to survive and cope with salt stress conditions, in plant parts, the soluble sugar content is one of the hallmarks of the strong showing a number of physiological and biochemical adaptations (de Oliveira et al., 2013). More than soluble sugar, other osmotically active molecules/ions (including sugar alcohols, proline, proline betaine, glycine betaine, glycerol, mannitol, sorbitol, organic acids, calcium, potassium, chloride ions, abscisic acid) are so important to support plants to survive under abiotic stresses (Joseph et al., 2015).

The results of ANOVA on dissolved sugar content in rice stalks grown under salinity stress were shown in (Table 7). There was a very significant difference in dissolved sugar content in rice stems which was influenced by salinity (Table 7). The highest dissolved sugar content in rice plants at 0 mM salinity (control) (40.23 mg/g) and decrease periodically according to the addition of salinity levels, increasing salinity from 50 mM (34.12 mg/g) to 100 mM (32.90 mg/g) and up to 150 mM (30.51 mg/g) can caused a decrease in dissolved sugar levels in rice stems lower than the normal condition of 0 mM. So as for the previous research, namely regarding the salinity reduces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants; rice variety CSR-1 soluble sugar content in shoot seedling stage at 0 dS/m salinity (control) (10.0 mg/g), 7 dS/m (12.0 mg/g) and14 dS/m (14.0 mg/g)(Dubey and Singh, 1999).

The negative effect of salinity on some plant parts is mainly due to the content of Na^+ and Cl^- ions in the plant and these ions produce conditions that are decisive for the survival of the plant and its parts by blocking several different plant mechanisms.

However, this ion storage depends on the stage of plant growth especially leaves, stems and roots, genetic character and environmental factors such as temperature, relative humidity and light intensity. The presence of excessive amounts of salt in the soil results in stunted growth cultivation and reduces the ability of plants to provide less soluble sugar in plant stems, limiting economic result and even causing plants to die (TAFFOUO et al., 2017).

Table 7 Soluble sugar content (mg/g) in stem of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	33.05 ± 3.96	37.45 ± 9.70	27.68 ± 1.78	27.31 ± 3.05	34.18 ± 7.05
	50	39.72 ± 6.08	32.86 ± 6.10	30.03 ± 5.41	29.52 ± 4.53	
	100	38.58 ± 9.75	33.79 ± 2.74	34.30 ± 3.33	31.51 ± 8.82	
	150	46.55 ± 7.53	40.06 ± 4.40	34.25 ± 3.31	30.27 ± 6.1635	
PT 1	0	35.21 ± 8.96	31.45 ± 4.70	32.54 ± 3.16	29.12 ± 4.50	34.26 ± 6.72
	50	38.58 ± 9.43	34.53 ± 1.85	34.45 ± 9.06	32.14 ± 4.97	
	100	41.37 ± 10.07	30.48 ± 5.10	34.63 ± 6.96	30.96 ± 3.48	
	150	43.94 ± 5.81	35.33 ± 4.62	30.20 ± 4.94	29.29 ± 4.70	
IN 35	0	39.32 ± 11.12	32.82 ± 5.88	34.19 ± 4.20	30.31 ± 5.23	34.88 ± 6.60
	50	39.49 ± 6.84	33.33 ± 4.08	34.53 ± 2.75	33.11 ± 8.34	
	100	38.35 ± 4.05	34.25 ± 1.21	31.45 ± 5.59	32.82 ± 4.52	
	150	48.70 ± 6.60	33.05 ± 3.21	32.60 ± 4.27	29.74 ± 4.28	
Mean salinity		40.23 ± 7.78 A	34.12 ± 4.81 B	32.90 ± 4.96 BC	30.51 ± 4.82 C	
Proline (mM)						
Mean Proline		0	50	100	150	
		32.54 ± 6.24	34.36 ± 6.10	34.71 ± 6.17	36.16 ± 7.96	
P-value (F-test)						
Variety (V) 0.821, Salinity (S) 3.65 x 10^{-9***}, Proline (P) 0.085, V × S 0.689 NS, V× P 0.701 NS, S× P 0.192						

Note**:** CV = Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c…**)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Protective mechanism against water deficit is the accumulation of some osmoprotectant including proline (as amino acids) and soluble sugars (Jaleel et al., 2007; Fabregas and Fernie, 2019). It can be said that the dissolved sugar content in plant leaves has a central role in the structure and metabolism of plants at the cellular

level and throughout the plant organism. Sucrose is the most abundant sugar in plants, occurs in plants with a strong ability to accumulate sugar in their leaves, and has a function and form in which carbohydrates are transported from one plant organ to another.

For exogenous application proline levels, it did not show significant results on the soluble sugar content in plant stems (Table 7). However, proline levels statistically tended to give different results, namely proline levels of 50 mM (34.36mg/g), 100 mM (34.71 mg/g) and 150 mM (36.16 mg/g) resulted in higher soluble sugar content in plant stems than the control application of proline 0 mM (32.54 mg/g). Therefore, previous studies have been carried out on increasing salt tolerance with NaCl 0.3 dS/m, 0.6 dS/m and 1.2 dS/m in plants with the application of exogenous amino acids; proline levels at 0 mM (control) (60.3 mg/g), 0.3 mM (57.7 mg/g), 0.6 mM (53.5 mg/g) and 1.2 mM (33, 4 mg/g) (Abd El-Samad et al., 2011). Co-influence with other environmental or agricultural practice factors with salinity stress in the field; for example, water management, drought, and wind that sometimes occur either directly or unintentionally can reduce the content of soluble sugar in rice plants (Pask et al., 2012). Many studies said that the proline content had a role and function in providing a strong influence and increasing the process of forming dissolved sugar levels in rice during the planting period (Ansari et al., 2019; Jouve et al., 2004). However, proline has been reported to reduce salt stress by repairing some parts of the rice plant damaged by high levels of salt stress during growth (Khedr et al., 2003). The application of proline to rice plants, can be used as an additional tolerance to the environment, it assumes that the accumulation of dissolved sugars in plant stems is an indicator for a particular crop. Each genotype that is able to withstand biotic and abiotic stresses so that it can survive, provides an opportunity for plants to store soluble content such as sugar in the stems of rice plants (Koyro et al., 2012). Proline which has compatible properties under environmental stress with dependence on plants and sugar accumulation in stems and leaves. Since this character can be the main source of sugar formed in plants under stress conditions, higher tolerance has a significant positive correlation. With the content of proline and soluble sugars found in plants, especially in the leaves and stems (Mostajeran and Rahimi-Eichi, 2009). The presence of proline in plants is a healer in plants under various stresses, thus

helping plants to be able to carry out normal activities such as accessing water absorption, photosynthesis, air and other related elements important in plant growth (Ahmad, 2014). The major role of proline accumulation is to minimize environmental stress on plants especially on salinity. Proline can act as an osmotic regulator, as a protector against enzyme denaturation, in stabilizing some macromolecules or molecular assemblies, as a reservoir source in nitrogen and carbon radical scavengers (Ahmad et al., 2020).

The soluble sugar content in plant root at the tillering stage was a highly significant difference ($P \leq 0.01$) affected by varieties and salinity level factors, but no significant difference affected by proline levels ($P > 0.05$) (Table 8). There was no statistically significant difference ($P > 0.05$) in soluble sugar content in plant root for any interaction between either two or three factors (Table 8).

The highest root soluble sugar content was IN 35 (41.83 mg/g) and followed by CNT 1 (38.57 mg/g) and PT 1 (37.10 mg/g). The reduction in soluble sugar content in plant root was found when the level of salinity increase; highest value at non-salinity stress (0 mM NaCl) (43.52 mg/g), and lower values at 100 mM (37.26 mg/g) and 150 mM (34.99 mg/g). In all the results when compared with a previous study conducted a research entitled the salinity reduces NaCl 7 dS/m and 14 dS/m the accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants, soluble sugar content in the root of seedling stage variety CSR-1 (7.0 mg/g), CSR-3 (7.0 mg/g), Ratna (10.0 mg/g) and Jaya (7.0 mg/g) (Zhou et al., 2020). In general, whole rice plants are sensitive to salt, however, the changeable biochemical contents depended on the trait, plant part, and salt concentration (Subbarao and Johansen, 1999). An accumulation of dissolved sugar content in plant roots under saline conditions is known as tolerance ability to salinity (Patade et al., 2011). Sucrose is the primary product in photosynthesis, and transport assimilated carbon between the source (sites of synthesis) to the sink (sites of use or storage) (Loescher and Everard, 2017). Rooting in plants need sucrose as a carbohydrate source for support energy demands that rooting ability can promote plant ability to withstand under abiotic stress.

Table 8 Soluble sugar content (mg/g) in root of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	42.75 ± 0.79	38.93 ± 1.17	36.61 ± 1.07	33.58 ± 5.27	38.57 ± 6.51 b
	50	38.63 ± 2.77	46.90 ± 3.66	36.73 ± 8.78	35.60 ± 6.46	
	100	44.34 ± 3.49	42.98 ± 9.27	32.32 ± 4.27	34.90 ± 6.98	
	150	44.41 ± 9.80	41.12 ± 2.70	34.64 ± 6.56	32.68 ± 5.36	
PT 1	0	41.50 ± 0.60	36.74 ± 1.20	37.11 ± 3.70	27.51 ± 1.06	37.10 ± 5.82 b
	50	41.68 ± 3.61	38.40 ± 9.11	32.40 ± 3.96	32.38 ± 6.30	
	100	42.84 ± 2.75	35.36 ± 5.41	36.13 ± 3.77	32.86 ± 5.47	
	150	43.75 ± 3.74	37.57 ± 6.48	42.11 ± 4.53	35.18 ± 3.10	
IN 35	0	44.17 ± 1.45	39.29 ± 2.06	39.17 ± 8.64	33.93 ± 3.60	41.83 ± 6.05 a
	50	40.18 ± 0.31	41.40 ± 3.60	38.33 ± 2.33	40.30 ± 4.06	
	100	44.70 ± 2.43	46.05 ± 8.40	41.14 ± 5.14	43.73 ± 7.13	
	150	53.33 ± 6.43	45.93 ± 8.70	40.40 ± 2.60	37.22 ± 1.70	
Mean salinity		43.52 ± 4.87 A	40.89 ± 6.20 B	37.26 ± 5.25 C	34.99 ± 5.80 C	
Proline (mM)						
Mean Proline		0	50	100	150	
		37.61 ± 5.23	38.58 ± 5.84	39.78 ± 6.86	40.70 ± 7.31	
P-value (F-test)						
Variety (V) 5.89 x 10^{-5} **, Salinity (S) 4.59 x 10^{-10} **, Proline (P) 0.062 NS, V × S 0.246 NS, V× P 0.360 NS, S× P 0.221 NS, V x S x P 0.765 NS, CV% 13.04						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

In plants that tolerate salts such as IN 35 more than the two Thai rice: CNT 1 and PT 1, perhaps due to innate capacity and the presence of genes that are more tolerant to stress (Kumari et al., 2009). However, many reasons are considered to interpret and differentiate tolerance associated with physiological and various forms of growth mechanisms of rice varieties (Yeo and Flowers, 1986).

The results of this study showed that with increasing salinity, the dissolved sugar content in rice roots ranged from 50 mM (40.890 mg/g) to 100 Mm (37.26 mg/g) and 150 mM NaCl (40.70 mg/g) (Table 8). Each level of salinity greatly affects the solubility content of plant roots, namely at a salinity of 50 mM to 150 mM NaCl drastically reduces sugar content in rice plant roots compared to previous studies on

the salinity reduces the accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants, soluble sugar content at the root of the seedling stage; 0 dS/m salinity (control) (5.0 mg/g), 7 dS/m (5.2 mg/g) and 14 dS/m (7.0 mg/g).

Salt stress conditions need to be treated with toxins so as not to interfere with the dissolved sugar content in plant roots, reducing the number of physiological and biochemical adaptations. This has involved the assemblage of various relatively osmotically active molecules/ions with soluble properties. The content of Na^+ stuck in the roots as well as other parts of the plant and the Cl^- formed in the youngest leaves is the most damaging to the plant (Tavakkoli et al., 2010). The rate of carbon assimilation decreases rapidly due to salinity. This resulting effect inhibits plant activity processes and decreases photosynthesis, sugar accumulation, and carbon loss with increased respiration, resulting in less carbohydrate accumulation (Everard et al., 1994). Thus, synthesized organic solutes are reached and compatible with osmotic adjustments or on organic cell accumulation (Meikle et al., 1988). Overall, plants adapt to salt stress while increasing the biosynthesis of secondary metabolites, such as soluble solids, amino acids, proteins, sugars and other organic acids (Fougere et al., 1991).

Although the use of proline had a positive effect on the sugar content of plant leaves (Table 6), in-plant stem (Table 7) and root parts (Table 8) had no statistical effect. Nevertheless, the use of controlled substances to offer benefits to productivity by promotes better health than rice plants under non-supplementing (Kishor et al., 2005; Hayat et al., 2010). In this study, at 150 mM proline had higher soluble sugar content though was not significant with other proline levels (Table 8).

There was a significant difference in starch in the leaves of rice plants influenced by individual factors, namely varieties ($P \leq 0.05$) and salinity level ($P \leq 0.01$). However, for the proline application, no significant difference was observed in the starch content in plant leaves affected by this factor (Table 9). No significant difference in starch accumulation in rice leaves was affected by any interaction between factors; either by two or three factors (Table 9).

IN 35 (39.40 mg/g) and CNT 1 (38.30 mg/g) showed higher starch content in rice leaves compared to PT 1 (35.99 mg/g). From the results of the starch content in

the leaves, compared with previous studies, biochemical and anatomical changes and reductions in yields in rice (*Oryza sativa* L.) under varying salinity regimes. The accumulation process of carbohydrate in rice varieties in relation to their response to nitrogen in the tropics, the activity for starch content at the flowering stage, variety IR8 (61 g/m^2), Chianuog 242 (11 g/m^2) (Hakim et al., 2014). Different genotypes may respond both in biochemical and physical expresses in each environmental stress are different, is called as the adaptive ability (Reiss and Hetherington, 2009; Utama et al., 2018b). This explanation is in accordance with (Utama et al., 2018b) which states that one of the environmental stresses is salinity, which has the ability to adapt well to the individual physical characteristics of each variety. This variation in behavioral growth is certainly caused by genetics in accordance with adjustments to certain environments (Reiss and Hetherington, 2009). Plants that experience water shortages have the potential for the formation of salinity to the formation of less starch content in leaves than plants that grow normally (Jouyban, 2012). Lack of water contributes to salt which causes a very significant decrease in starch and even causes plant death (Parida and Das, 2005). The response of plants experiencing a lack of water and the addition of salt to stress can be in the form of changes at the cellular and molecular levels which are indicated by decreased growth rates, reduced starch in plant leaves (Reddy et al., 2004). The presence of starch accumulates in the leaves as a form of carbon stock, which is temporarily available and is the main component of starch in plant growth that accumulates in the leaves, while sucrose is transported to certain organs that are used by plants (Hennion et al., 2019). Another step involved in photosynthetic production is of course catalyzed by the formation of sucrose phosphate synthase (SPS) which converts hexose phosphate to sucrose (Stitt et al., 1988).

In addition, the effect of salinity was confirmed by the effect of salinity on the statistically significant difference in leaf starch content values (Table 9). Increasing the salinity level will decrease the corresponding leaf starch. Compared with the control treatment at 0 mM NaCl with the highest salinity at 150 mM NaCl there was a decrease of about 5%. Thus, as for previous research on the effect of potassium application on wheat (*Triticum aestivum* L.) cultivars grown under salinity stress, activities on starch content analysis at the vegetative stage 65 days after planting;

salinity 0 mM NaCl (145.25 mg/g), 40 mM (137.68 mg/g), 80 mM (126.44 mg/g) and 120 mM NaCl (110.24 mg/g) (El-Lethy et al., 2013). The difference in starch in the leaves is caused by the assimilation of the supply to the plant parts, namely the leaves, due to the difference in the partition of the source and sink.

In general, this happens because the source of photosynthesis plants of different varieties can experience different stresses as well. Varieties under stress will absorb less photosynthesis and less starch in leaves than certain parts that are not stressed (Dien et al., 2019). Finally, the proportional partitions between photosynthetic and reproductive parts under stress conditions are more pronounced. This will allow to assess some of the effects of different varieties from growing under unsuitable conditions such as salinity. In addition, plant activity will undergo various metabolic changes, including a decrease in the rate of photosynthesis, an increase in the rate of respiration, changes in the composition of amino acids, as well as a decrease in sugar, starch, and water content of plant tissues (Graham and Patterson, 1982). Furthermore, soil salinity can change and inhibit all parts of the observed plant growth and development parameters and their contents also reported that increasing salinity caused a decrease in starch content in plant leaves (Egamberdieva and Lugtenberg, 2014).

Table 9 Starch content (mg/g) in leaves of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	39.93 ± 4.90	34.25 ± 5.64	35.27 ± 8.50	37.47 ± 6.31	38.30 ± 4.9 a
	50	41.18 ± 4.47	39.36 ± 4.34	40.86 ± 7.07	35.91 ± 4.00	
	100	38.03 ± 1.07	40.97 ± 8.45	36.67 ± 3.67	36.47 ± 2.98	
	150	42.45 ± 2.02	38.82 ± 6.60	38.51 ± 4.18	36.31 ± 3.10	
PT 1	0	39.84 ± 1.70	31.67 ± 5.57	33.44 ± 2.70	29.14 ± 4.52	35.99 ± 5.96 b
	50	39.40 ± 3.00	38.39 ± 9.91	32.63 ± 7.72	33.66 ± 4.79	
	100	37.50 ± 2.47	37.42 ± 7.43	40.43 ± 1.45	32.70 ± 4.80	
	150	39.57 ± 9.87	37.58 ± 5.74	37.20 ± 8.74	35.34 ± 5.60	
IN 35	0	39.16 ± 1.26	36.62 ± 8.03	38.12 ± 2.02	40.68 ± 1.94	39.40 ± 5.84 a
	50	41.13 ± 6.62	41.83 ± 2.04	35.43 ± 9.30	33.87 ± 2.22	
	100	39.90 ± 6.40	42.15 ± 5.98	37.70 ± 3.09	34.95 ± 6.06	
	150	47.96 ± 2.07	40.16 ± 4.50	42.74 ± 9.83	38.86 ± 8.51	
Mean salinity		40.43 ± 4.61 A	38.27 ± 6.15 AB	37.42 ± 6.25 B	35.47 ± 5.01 B	
Proline (mM)						
		0	50	100	150	
Mean Proline		36.30 ± 5.41	37.80 ± 5.94	37.86 ± 4.87	39.63 ±6.32	
P-value (F-test)						
Variety (V) 0.013*, Salinity (S) 0.0041**, Proline (P) 0.108 NS, V x S 0.988 NS, V x P 0.759 NS, S x P 0.553 NS, V x S x P 0.964 NS, CV% 14.95						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

In this study, the reduction of starch content in leaves was measured in plants grown under salinity, with a continuous decrease when the level of salinity increase. Similarly, (Jouyban, 2012) explained plants that experience water shortages caused by salinity stress have the potential of less starch accumulation in leaves than plants that grow normally, and cause plant death finally (Reddy et al., 2004; Parida and Das, 2005; Engamberdieva and Lugtenberg, 2014). Which, the presence of starch accumulates in the leaves as a form of carbon stock in plants. Starch is temporarily available and is the main component of starch, while sucrose is transported to certain organs that are used by plants (Hennion et al., 2019). Deceased in starch in plant leaves at salinity stress may involve the process of catalyzing by the formation of

sucrose phosphate synthase (SPS) which converts hexose phosphate to sucrose (Stitt et al., 1988).

In statistical analysis, the proline content factor also did not show a significant difference to the starch content in plant leaves (Table 9). Proline levels did not give significant results for the four levels used, but at the time of administration of proline levels; control proline concentration at 0 mM to 150 mM still increased the leaf starch content, which was 3% higher than the control treatment 0 mM proline. However, compared to previous studies that improve the effect of exogenously applied proline on seed composition, seed oil quality and antioxidant activity of corn oil (*Zea mays* L.) under drought stress; application of proline at the seedling stage soluble sugar content (control) 0 mM (56.67 mg/g) and 30 mM (61.33 mg/g) (Ali et al., 2013).

Proline accumulation is a well-known plant metabolic response to drought, salinity, and other stresses (Siddique et al., 2018). As proline enables osmotic adjustment, stabilizes various protein structures and cell membranes acts as a protective agent for enzymes and is a scavenger against free radicals and antioxidants (Molinari et al., 2007a). Proline accumulation is generally an indicator of leaf dehydration and is associated with stress susceptibility (Dien et al., 2019). However, a statistically significant effect of external proline application was not found in the accumulation of starch in leaves in this study (Table 9).

The soluble sugar content of different plant parts was affected, causing the value to decrease as the salinity level increased (Table 6-8). However, the plant part was sensitive with showed a significant difference affected by applying external proline for higher accumulation of soluble sugar content as is leaves. Although both leaves and stems are directly contacted with proline substances. Therefore, the role of soluble sugar to support plants withstand and tolerance under salinity, firstly parts of the plant was responding is leaves.

Starch content in the stem of the root had significant differences affected by individual factors including varieties ($P \leq 0.05$) and salinity level ($P \leq 0.01$); excluded proline level ($P > 0.05$) (Table 10). No significant difference ($P < 0.05$) in starch content in the stem was observed to be affected by the interaction between any two and three factors (Table 10). Effect of different rice varieties showed that the highest starch content in stems was IN 35 (39.94 mg/g) and followed by CNT 1 (38.01 mg/g) and

PT 1 (36.57 mg/g), respectively. It can be compared with previous study, salinity 7 dS/m and 14 dS/m inducing the accumulation of soluble sugars and alters the activities of metabolic enzymes in rice plants; The activity starch content at seedling stage in 20 days after planting CSR-1 (157 mg/g), CSR-3 (127 mg/g).

Table 10 Starch content (mg/g) in stem of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CN T 1	0	42.25 ± 2.02	32.95 ± 6.80	35.44 ± 8.17	32.51 ± 6.02	38.01 ± 7.00 ab
	50	42.33 ± 9.16	36.63 ± 2.75	34.40 ± 6.82	32.52 ± 6.84	
	100	40.16 ± 9.43	40.04 ± 6.95	37.80 ± 9.74	38.16 ± 7.50	
	150	47.61 ± 4.56	44.05 ±3.30	34.86 ± 3.50	36.40 ± 6.35	
PT 1	0	41.67 ± 2.73	37.41 ± 7.75	32.75 ± 1.90	30.07 ± 5.02	36.57 ± 5.84 b
	50	43.33 ± 6.01	34.14 ± 3.70	34.03 ± 4.87	34.06 ± 3.87	
	100	43.40 ± 4.45	36.80 ± 3.71	36.30 ± 4.97	33.74 ± 5.82	
	150	41.78 ± 8.84	36.70 ± 6.80	34.28 ± 4.29	34.76 ± 4.95	
IN 35	0	40.64 ± 3.17	39.90 ± 7.80	41.22 ± 7.40	34.44 ± 4. 82	39.94 ± 6.62 a
	50	44.56 ± 7.45	37.71 ± 5.74	38.72 ± 5.44	34.54 ± 4.20	
	100	44.73 ± 3.20	44.51 ± 7.74	38.44 ± 5.90	34.37 ± 8.81	
	150	47.74 ± 6.37	45.30 ± 1.52	35.60 ± 3.85	36.70 ± 8.74	
Mean salinity		43.35 ± 5.63 A	38.84 ± 6.19 B	36.15 ± 5.46 BC	34.36 ± 5.60 C	
Proline (mM)						
		0	50	100	150	
Mean Proline		36.77 ± 6.31	37.25 ± 6.29	39.04 ± 6.71	39.65 ± 6.94	
P-value (F-test)						
Variety (V) 0.0271*, Salinity (S) 4.27 x 10^{-8}**, Proline (P) 0.138 NS, V × S 0.918 NS, V× P 0.948 NS, S× P 0.785 NS, V× S × P 0.986 NS, CV% 15.85						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

In plants, salinity induces starch degradation and sugar accumulation, which grows and forms rice varieties with increased accumulation of starch phosphorylase, sucrose phosphate synthase and decreased invertase activity. Furthermore, each rice genotype that experienced salt tolerance and sensitivity was different in each of these enzyme activities under salinity (Kumar et al., 2009). (Ghassemi-Golezani et al., 2011c) suggested for any plant species that has the ability to reject some degree of

salinity, depending on some metacentric or diploid chromosomes. Other reasons are also considered to distinguish several forms of tolerance of physiological associations and growth mechanisms for each rice variety plant (Ashraf and Harris, 2013). Rice cultivars widely respond to salt stress, ranging from highly sensitive to salt tolerant (Do et al., 2014; Zeng et al., 2020). Current investigations were carried out to examine changes in starch and sugar content, and related activities of the metabolic enzyme's sucrose phosphate synthase, acid invertase, and starch phosphorylase in one salt-tolerant rice cultivar and two salt-sensitive rice cultivars treated with salt during field growth.

The contents of starch content found in the leaves corresponded to those found in the stems; in both the quantities found and the order of the highest to lowest varieties and the order of the highest to lowest varieties (Table 9-10). Likewise result found in leaves, was found that the increased salinity resulted in a significant decrease in starch accumulation in the stem, respectively; with the highest values in the unsalted rice treatment at 0 mM NaCl (43.35 mg/g) (Table 10). The decrease in starch content, when grown under saline condition, showed that the stem part decreased more rapidly than the leaf part (Table 9-10). However, as for previous studies regarding salinity inducing the accumulation of soluble sugars and alters the activities of metabolic enzymes in rice plants; the activity at seedling stage in 20 days after starch content at 0 dS/m (control) (117 mg/g), 7 dS/m (150 mg/g) and 14 dS/m (154 mg/g) (Dubey and Singh, 1999; Singh et al., 2006). The cause of the decrease in starch in leaves is strongly influenced by the level of salinity according to each level in soil conditions. In order for plants to survive under salt stress, it is certainly necessary to establish ion homeostasis which is the most important requirement. Ionic properties and homeostasis have special meaning and consideration, because plants grow in salinity conditions, the concentration of Na^+ and Cl^- below the soil is strongly influenced by uptake into plants (Tavakkoli et al., 2010). Thus, the selective salt tolerance of the three rice genotypes has been associated with the ability to form starch in certain parts of the plant to partition Na^+ and Cl^-, to certain parts of the plant in leaves and midribs to protect against damage from ions. It may be the reason that the leaf part is so important to photosynthesis that it affects plant growth under stressful conditions due to various factors. Therefore, the preservation of leaf growth

tissue for plants is important. While the influence of external proline in plant stems for increasing starch, content is not as clear as soluble sugar accumulation. Although the starch content was continued to increase when external proline was applied increasing levels.

(Xu et al., 2015) also found that starch content in stems of different rice varieties changed differently under drought stress conditions. Thus, the reason for the accumulation of starch content under drought stress is not clear. In salinity stress can cause water deficiency, and increasing salt levels resulted in the starch in plant stems decreasing (Tozlu et al., 2000).

The level of proline factor did not show a significant difference in starch content in rice stalks (Table 10). Although there was no statistical difference between proline levels, the starch content values increased in stems according to the increase in proline content from 50 mM, 100 mM and to 150 mM indicating an increase in values according to proline content. However, this research was compared with previous studies, namely; the changes induced in the physiological, biochemical and anatomical characteristics of *Vicia faba* by the exogenous application of proline under seawater stress. Results were obtained at proline levels at 0 mM (control) (15.31 mg/g), 25 mM (17.88 mg/g) and 50 mM (16.55 mg/g). Proline accumulation occurs in plants in response to various stresses, one of which is salt. For example, it causes a water deficit in rice plants thereby inhibiting nutrient flow to the plant and starch accumulation in the stem which is associated with an increase in the content of precursors for proline biosynthesis, including glutamic acid, ornithine and arginine (Ozturk et al., 2021). Proline applied to crops in general and rice in particular, in addition to the tolerance applied to the environment, it is assumed that starch accumulation in the stems of plants is an indicator for a particular crop (Kafi et al., 2003). Proline is applied to crops in general and rice in particular, for increasing tolerance abiity in plants to various environmental stresses. It is assumed that starch accumulation in the stems of plants is an indicator for a particular crop (Kafi et al., 2003; Krishnan et al., 2011). Where with, the ability of proline is to prevent damage to the structure of proteins, starch, and membranes as well as protein denaturation (Arshad et al., 2017; Bandurska, 2000). Proline that accumulates in plants and functions as an osmotic or reservoir agent and is also associated with several

protective actions under conditions of severe stress (Arshad et al., 2017). Proline is produced by cells as soon as possible after cells are stressed and will function to protect plasma membranes and proteins from cells (Bandurska, 2000) In tolerant plant varietal species, certain substances are produced to regulate metabolism in plants, such as the accumulation of proline and internal starch (Ahmad et al., 2020). However, the use of external substances has also been studied in such a way and is found to play a very important positive role in plants as well (Rostami and Azhdarpoor, 2019).

Moreover, proline that accumulates in plants and functions as an osmotic or reservoir agent and is also associated with several protective actions under conditions of severe stress (Ain-Lhout et al., 2001).

Similar in starch accumulation in statistical analysis on the effect of these three factors (varieties, salinity level, and proline level) and interaction between them in root and either leaves or stem of rice (Table 11). Statistically significant difference on the starch content was analyzed as affected by different varieties and salinity level factors. The highest starch content was found in IN 35 (41.95 mg/g), and followed by CNT 1 (39.58 mg/g) and PT 1 (36.58 mg/g). Compared to previous studies on salinity level 7 dS/m and 14 dS/m which induces the accumulation of soluble sugars and changes the activity of metabolic enzymes in rice, namely CSR-1 (75 mg/g) and CSR-3 (76 mg/g) starch content in root at seedling stage (Dubey and Singh, 1999). The starch content in plant roots is the most important factor in supporting crop yields. However, the root content in plants was reduced due to salinity stress.

In tolerant rice varieties grown under salt stress, plants will form physiological mechanisms that change tolerance and osmotic adjustment, tissue water loss, ion and starch absorption (Shahid et al., 2020). In addition, plants that are resistant to salinity stress are able to have strategies to receive sufficient sunlight and carry out photosynthesis in plants to support their growth. This is because the decrease in starch formation in plant roots is caused by decreased water absorption, sodium and chloride poisoning in rice plant cells and reduced photosynthesis (Djanaguiraman et al., 2006). The Inpari 35 variety produces large amounts of starch, both from genetic factors in plants and other factors that are formed (Susiyanti et al., 2020).

For the salinity effect, the continuous reduction was observed when plants obtained higher salinity levels; with no significant difference between 100 and 150 mM NaCl. However, no significant difference between each proline level to the starch content that accumulated inside plants. Though, higher values were found at higher proline application levels.

Table 11 Starch content (mg/g) in root of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	41.88 ± 2.15	39.24 ± 5.30	34.60 ± 3.42	31.33 ± 4.11	39.58 ± 6.62 b
	50	42.81 ± 7.14	37.65 ±1,93	32.07 ± 6.31	33.37 ± 6.53	
	100	44.11 ± 8.40	40.47 ± 4.65	36.77 ± 6.97	34.18 ± 5.04	
	150	44.70 ± 6.64	41.30 ± 5.90	33,84 ± 9.44	32.91 ± 5.87	
PT 1	0	39.45 ± 3.41	38.78 ± 4.81	36.25 ± 5.74	30.72 ± 4.67	36.58 ± 5.97 b
	50	40.82 ± 5.03	36.95 ± 7.50	33.13 ± 4.32	31.23 ± 5.84	
	100	43.53 ± 9.34	37.41 ± 5.16	33.99 ± 1.86	34.09 ± 5.84	
	150	43.39 ± 4.53	40.53 ± 2.25	33.55 ± 4.87	31.50 ± 4.24	
IN 35	0	42.27 ± 6.36	39.45 ± 7.01	34.72 ± 4.56	38.20 ± 5.94	41.95 ± 7.29 a
	50	45.65 ± 4.60	41.94 ± 2.50	39.27 ± 1.38	38.73 ± 7.03	
	100	46.65 ± 8.44	42.65 ± 9.37	39.76 ± 6.67	39.43 ± 4.24	
	150	57.62 ± 8.83	43.06 ± 6.94	40.38 ± 8.80	37.44 ± 7.09	
Mean salinity		44.40 ± 7.08 A	39.95 ± 5.13 B	36.03 ± 5.61 C	34.43 ± 5.60 C	
Proline (mM)						
		0	50	100	150	
Mean Proline		37.57 ± 5.46	37.80 ± 6.33	39.42 ± 6.92	40.01 ± 8.84	
P-value (F-test)						
Variety (V) 4.95 x 10^{-5**}, Salinity (S) 3.67 x 10^{-10**}, Proline (P) 0.233 NS, V × S 0.947 NS, V× P 0.922 NS, S x P 0.769 NS, V x S x P 0.998 NS, CV% 15.22						

Note: CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

In addition, the effect of salinity was confirmed by the effect of salinity on the statistically significant difference in starch content in plant roots (Table 11). Increasing salinity can reduce the starch content in plant roots. For each increase in salinity levels can reduce starch in plant roots according to the NaCl content from 50 mM (39.95 mg/g) to 100 mM (36.03 mg/g) and 150 mM (mg/g) causing a greater

decrease in starch. However, compared to the previous study salinity induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants, the seedling stage of starch content in roots was at salinity 0 dS/m (75 mg/g) and at 14 dS/m (68 mg/g) (Gadallah, 1999).

Decreased starch content that accumulates in plant roots can caused by decreased water absorption, sodium and chloride poisoning in rice plant cells, and reduced photosynthesis (Djanaguiraman et al., 2006; Asrar et al., 2017). Among these plant parts (leaves, stem, and root), the reduction in starch accumulation was found in root highest, and followed in stem and leaves parts, respectively (Table 9-11). These events may be related to the plant's attempts to maintain each tissue normalizing under stressful conditions.

The effect of salinity levels on starch content in plant roots which among the four salinity levels varies from the highest to the lowest value according to the salinity level. Thus, starch content in plant roots, an important component in plants, clearly affects starch formation even at low concentrations. In general, the concentration due to an increase in NaCl is contained and dissolved in the soil (Rengasamy, 2010). In general, the Na^+ trapped in the roots as a barrier to starch and other parts of the plant and the Cl^- formed in the youngest leaves were the most damaging to the plant (Rengasamy, 2010; Tavakkoli et al., 2010) . However, there are many cereal crops in the crop, Na^+ being the main cause of specific ion damage. The ion compartment, translation, transport expenditure and absorption are controlled, as a precaution against ionic toxicity. The rate of carbon assimilation decreases rapidly due to the presence of salinity, inhibits the process of plant activity and decreases photosynthesis, carbon loss and increased respiration, resulting in less starch accumulation (Asrar et al., 2017).

As for certain exceptions, salt can occur in the roots of rice plants which directly reduces starch in the roots and prevents the movement of ions into the air of the plant. This mechanism occurs in some glycophytes and most halophytes (salt tolerant) (Munns and Tester, 2008b). The development of salt vesicles in the epidermis can directly accelerate the release of salt, preventing the accumulation of salt in various plant organs such as roots, stems and leaves. While an example of a more exclusive mechanism is the accumulation of salt in the higher roots which can

reduce the flow of water from the soil to certain parts of the plant and also reduce the starch that accumulates in the plant roots.

In statistical analysis, the proline content factor also did not show a significant difference to the starch content in the roots (Table 11). Proline levels did not give significant results for the four levels used, even when proline was applied; at the level of 50 mM proline gave higher starch yield than the control treatment (0 mM proline). From the results of the study, proline significantly affected the agronomic characteristics that emerged from the vegetative phase, and the starch content in plant roots. The effect also depends on the timing of the use of external proline given only at the tiller stage. Whereas ultimately for proline via deamination of ornithine and is thought to act mainly under adequate conditions and capabilities than elemental, nitrogen associated with salt tolerant cultivars (Arbona et al., 2013). This is called indirect evidence of the hypothesis that the presumed glutamate-like proline pathway occurs in plants in the starch content of roots (Baslam et al., 2021). The overall response of plants to osmotic stress consists of the accumulation of osmolytes in several compatible ones, one of which is proline. The property of proline is to act, as an appropriate adjuvant in stabilizing any increase in stress tolerance in plants in accumulating starch, which should be further investigated on the mechanisms involved (Zhao et al., 2020). Therefore, further use of proline in the starch accumulation phase may have a more pronounced effect on yield.

With various accumulations according to several compatible osmolytes in the cytosol, to contribute some cytosolic osmotic potential and vacuoles are formed stably. In addition to having compatibility and osmolyte, it can also have enzymes that function as stabilizers, membranes and forming subcellular structures (Boudko, 2010). Proline has been selected and proposed to act vigorously as a scavenger against reactive oxygen species (ROS) by converting it in a suitable and stable form for each plant species from growth to end (Fang and Xiong, 2015).

Semi-quantitative RT-PCR of *OsP5Cs1* gene in rice at tiller stage under difference of proline application in salinity condition

The *OsP5Cs1* and Actin primers were used to amplified by semi-quantitative RT-PCR. The gel electrophoresis results are shown in Figure 1-4. PCR products were 325 base pairs (*OsP5Cs1*) and 70 base pairs Actin.

In order to analyze whether any enzymes in the proline synthesis pathway after investigation were upregulated after application of multiple proline levels at the tillering stage, thus leading to higher proline accumulation, *OsP5Cs1* transcript levels were investigated by RT-PCR.

Gel Electrophoresis

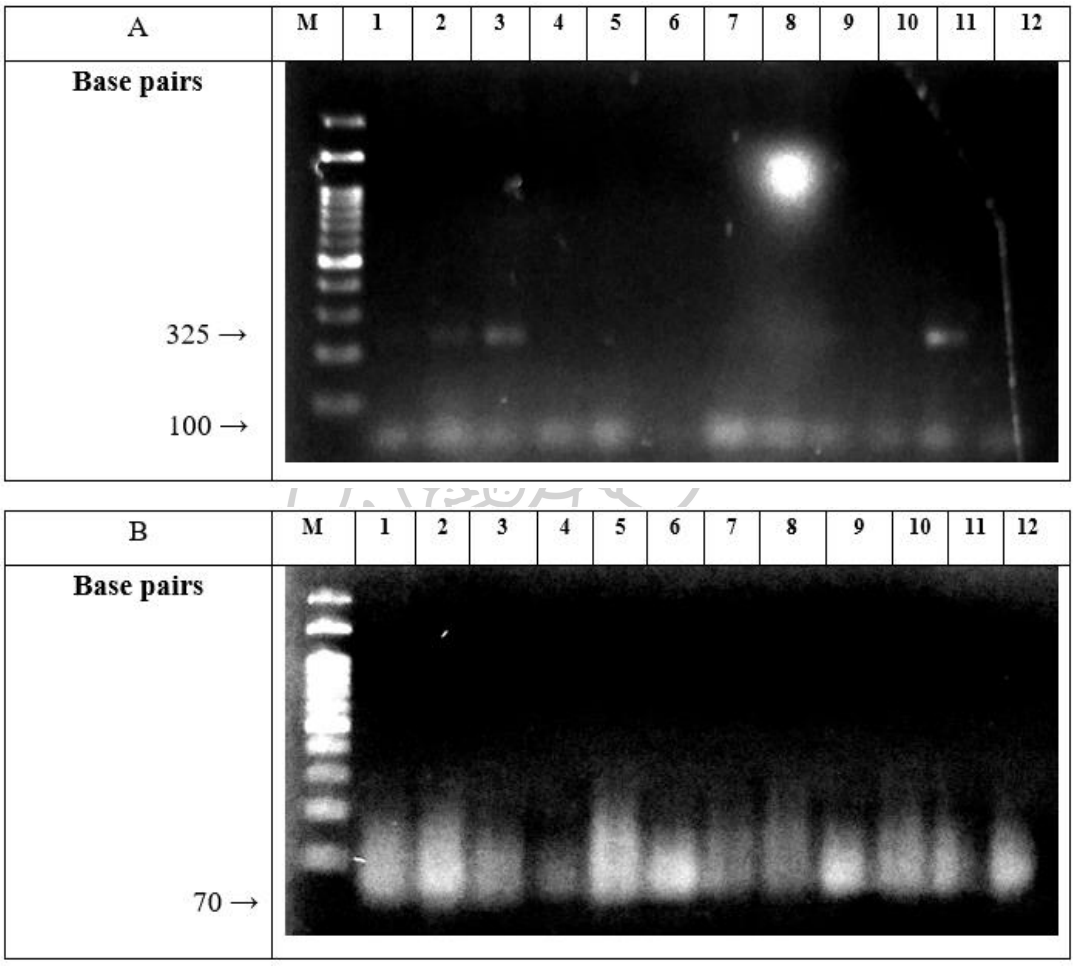

Figure 1 Gel Electrophoresis of P5Cs1 (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under 0 mM sodium chloride (NaCl), Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 = V1P2, 4 = V1P3, 5 = V2P0, 6 = V2P1, 7 = V2P2, 8 = V2P3, 9 = V3P0, 10 = V3P1, 11 = V3P2, 12 = V3P3) Note; V1= Chai Nat 1, V2= Pathum Thani 1, V3= Inpari-35, P0= control, P1= 50 mM of proline, P2= 100 mM of proline, P3= 150 mM of proline

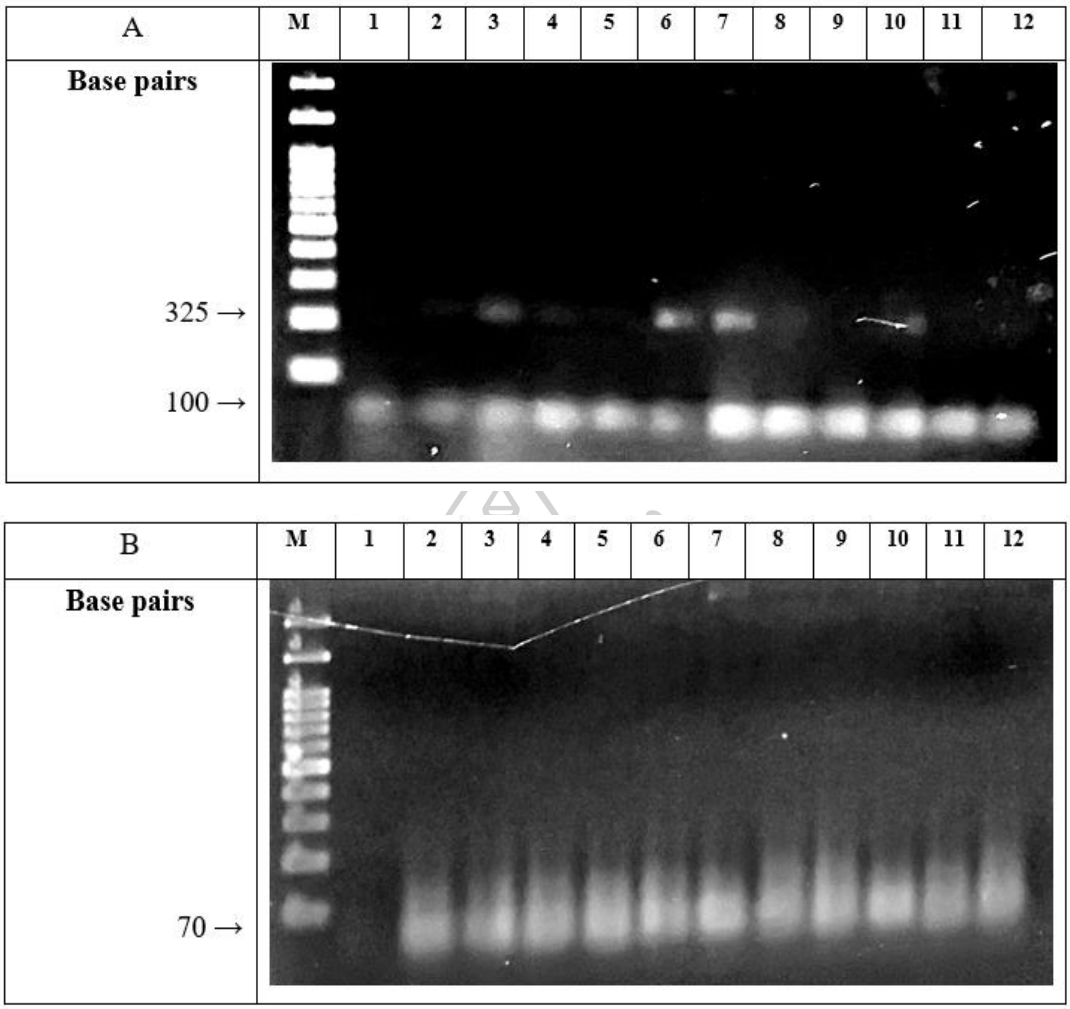

Figure 2 Gel Electrophoresis of P5Cs1 (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under 50 mM sodium chloride (NaCl), Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 = V1P2, 4 = V1P3, 5 = V2P0, 6 = V2P1, 7 = V2P2, 8 = V2P3, 9 = V3P0, 10 = V3P1, 11 = V3P2, 12 = V3P3) Note; V1= Chai Nat 1, V2= Pathum Thani 1, V3= Inpari-35, P0= control, P1= 50 mM of proline, P2= 100 mM of proline, P3= 150 mM of proline

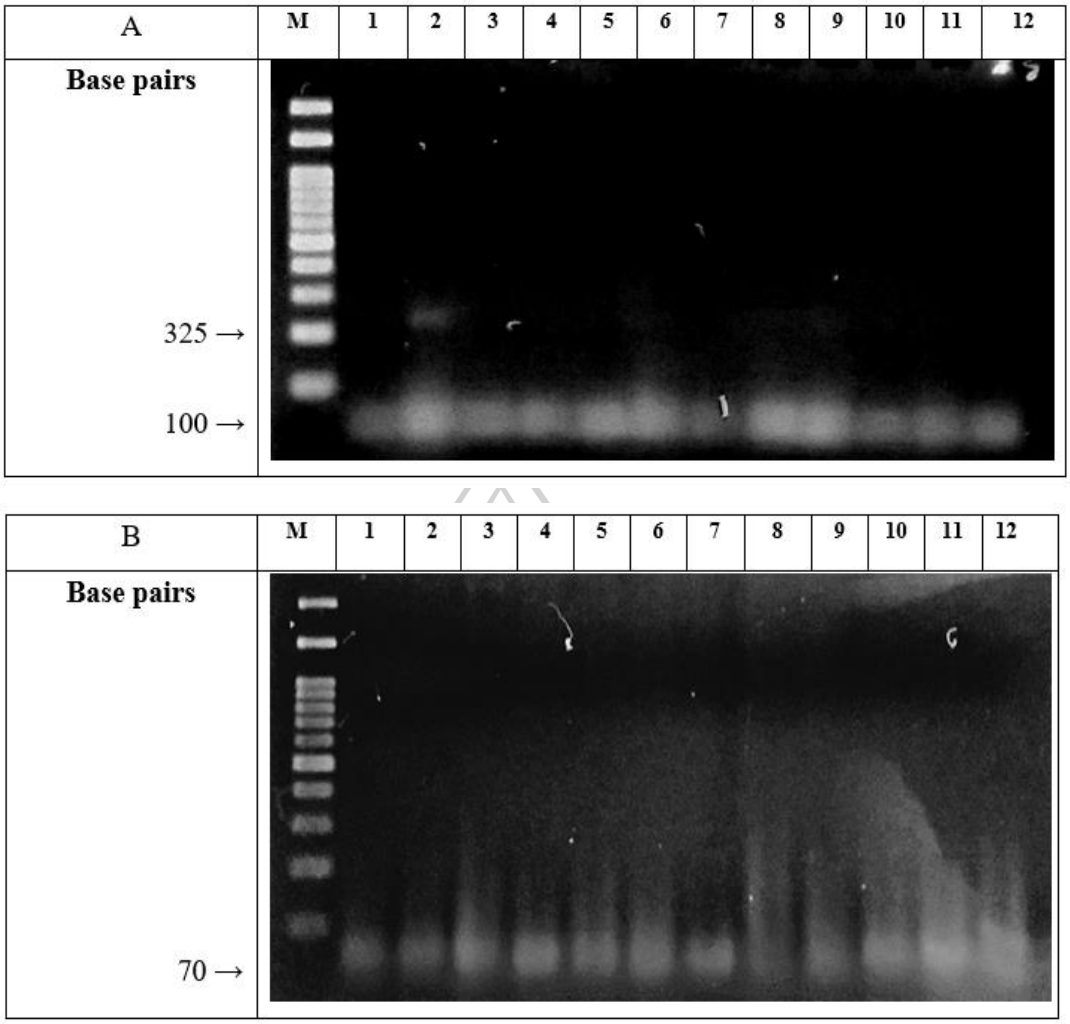

Figure 3 Gel Electrophoresis of P5Cs1 (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under 100 mM sodium chloride (NaCl), Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 = V1P2, 4 = V1P3, 5 = V2P0, 6 = V2P1, 7 = V2P2, 8 = V2P3, 9 = V3P0, 10 = V3P1, 11 = V3P2, 12 = V3P3) Note; V1= Chai Nat 1, V2= Pathum Thani 1, V3= Inpari-35, P0= control, P1= 50 mM of proline, P2= 100 mM of proline, P3= 150 mM of proline

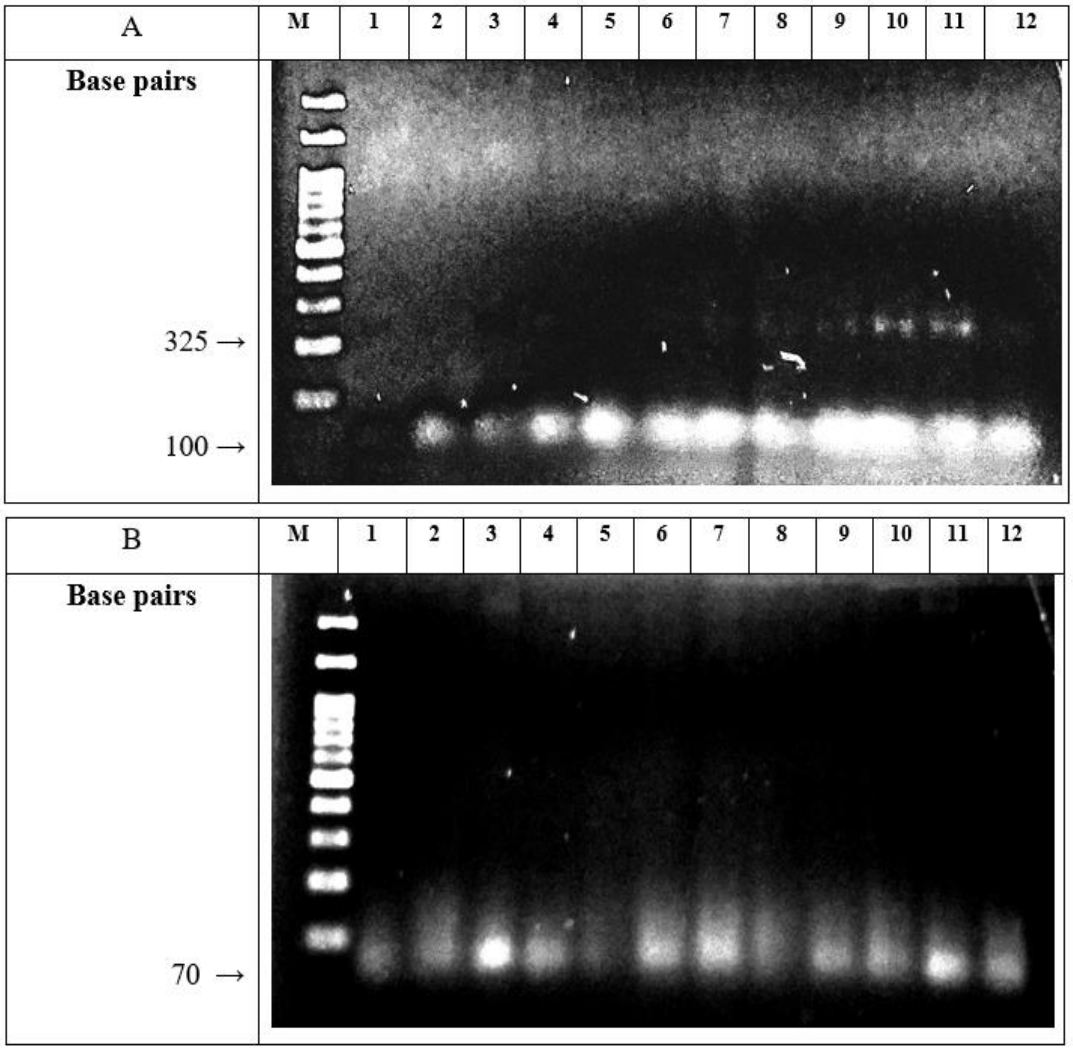

Figure 4 Gel Electrophoresis of P5Cs1 (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under 150 mM sodium chloride (NaCl), Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 = V1P2, 4 = V1P3, 5 = V2P0, 6 = V2P1, 7 = V2P2, 8 = V2P3, 9 = V3P0, 10 = V3P1, 11 = V3P2, 12 = V3P3 Note; V1= Chai Nat 1, V2= Pathum Thani 1, V3= Inpari-35, P0= control, P1= 50 mM of proline, P2= 100 mM of proline, P3= 150 mM of proline

Proline content in plant had significant effect by proline application level with increased values when the proline level increased (Table 5). No significant difference in interaction between salinity level and proline level, with proline application can induce the higher proline content inside plants in all salinity levels (Table 5). Although the transcriptional expression of *OsP5Cs1* in all varieties in different salinity levels were not clear (Figure 1-4). cDNA content was found at 50-150 mM

proline application in all salinity levels; although the presence of cDNA content was clear only in some varieties in different salinity levels.

It was observed that the proline accumulation in the rice plant increased with the increasing amount of external proline spraying. While the salinity increased at 100 mM NaCl, there was relatively little, and very little increase of proline content at level 150 mM NaCl in all rice varieties. This indicates that proline accumulation in plants after spraying by exogenous proline was not influenced solely by spraying. However, it was likely dependent on the synthesis of this substance inside the plant. Therefore, this is confirmed with cDNA synthesis that showed in Figure 1-4.

The proline synthesis of Thai rice varieties (CNT 1 and PT 1) was observed at various salinity levels but was quite evident at 0-50 mM NaCl with spraying by proline at 50-100 mM levels. For IN 35, proline synthesis was observed even though the salinity level was high (at 150 mM NaCl), especially when the rice plants were sprayed with proline at 50-100 mM. This indicates that naturally proline accumulation in the plant even when planted is grown under non-salinity conditions (Table 5), but additional proline accumulation occurred when proline was sprayed. Thus, spray proline from the outside, which might be a stimulus proline synthesis (Figure 1-4). This event is found in both Thai rice varieties (CNT 1 and PT 1) and Indonesian rice varieties (IN 35).

Proline synthesis was evident in CNT 1 and PT 1 that had been sprayed externally with proline at a salinity level of 0-50 mM NaCl. Except at salinity level 100 mM, proline synthesis was found in CNT 1 sprayed with exogenous proline at 50 mM, while at high salinity 150, PT 1 had proline synthesis, but in small amounts. For IN 35, proline was clearly synthesized at a salinity level of 150 mM NaCl, both with and without external proline spraying, but was evident when sprayed at 10–100.

This indicates that at a non-or low-level salinity (0-50 mM proline), Thai rice varieties (CNT 1 and PT 1) were able to synthesize proline inside the plant, especially when stimulated by external proline spray at an appropriate concentration of 50-100 mM proline. In contrast, IN 35 was slightly stimulated to synthesize proline at non-salinity (0 mM) to high salinity levels (100 mM NaCl). But, when the salinity level was increased to 150 mM NaCl, proline was found to synthesize the proline both with or without exogenous proline application.

Thus, it can be briefly concluded that Thai rice varieties need to be stimulated by external spraying the proline to promote proline synthesis. However, results were seen when the salinity level was not too high or had no salinity stress (0-50 mM NaCl). This may be a feature of the cultivar that is not very resistant to high salinity.

Contrastly, IN 35 had less proline synthesis in the plant by exogenous proline stimulation when there was no salinity (0 mM NaCl). But, when plants are affected by salinity, there is natural stimulation of its genetic to synthesize more proline. And at high salinity (150 mM NaCl), when IN 35 was co-stimulated by exogenous proline spraying, increased proline synthesis was observed. These may be characteristics of expression in salinity-resistant varieties.

Plenty studies have shown that salt stress triggers the induction of genes involved in proline biosynthetic activity, leading to proline accumulation (Armengaud et al., 2004; Nam, 2013; Nam et al., 2013; Nguyen, 2013). According to immobilize the function of *P5CS* in Arabiopsis thaliana demonstrated a key role for this enzyme in salt tolerance in plants because p5cs1 is hypersensitive to salt (Székely et al., 2008). There are two proline pathways for synthesis in plants viz. the glutamate pathway and the orinitin pathway. In the glutamate pathway it accounts for the accumulation of major prolines during osmotic stress such as salts. Proline is also synthesized from glutamic acid via the '-pyrroline-5-carboxylate (*P5C*) pathway (Verbruggen and Hermans, 2008). (Nounjan et al., 2012) have shown that the application of exogenous proline significantly and more efficiently can increase the expression of *P5CS* and *P5CR* in salt-stressed in *Oryza sativa*. These findings may also strengthen the argument that proline synthesis from glutamate, and not just its presence, is important in countering the effects of hyperosmotic stress (Mansour et al., 2017). It is possible that proline is an osmotic signal and regulator of various activities, in the formation of growth and development in plants (Maghsoud et al., 2014).

Rice varieties that receive exogenous proline application will provide precursors to support demand; increased production of secondary metabolites, and nucleotide synthesis that accompanies the rate of plant cell division. Which, it is accelerated after rice plants are released from stress, when oxidized proline tends to play an important role and energy source for ADP phosphorylation (Kavi Kishor and

Sreenivasulu, 2014). Another physiological reponse can directly involve with the response to stress, but in which proline metabolism is able to decipher and increase the expression of P5CS and P5CR genes that play a role in regulating rice biosynthesis under salt stress (Araujo et al., 2015).

Effects on agronomic characteristics in rice in the first to fourth weeks after salinity (after transplanting) at the beginning of tillering stage

The effect of different salinity levels on yield components of three rice varieties was evaluated on four agronomic characteristics; at one week after transplanting or three weeks after planting (Table 12). Four measured characteristics, namely number of leaves per plant, number of tillers per plant, plant height and leaf symptom scoring affected by salinity stress, were significantly affected by rice variety. However, salinity level and interaction between variety and salinity did not result in significant difference in agronomic characteristics (except effect of salinity level on leaf symptom scoring) (Table 1

2). The highest of number of leaves per plant and plant height was found in Inpari 35. The highest number of tillers per plant was observed in PT 1. The lower on three characteristics; number of leaves per plant, number of tillers per plant and plant height were recorded in CNT 1. For leaf symptom scoring, higher score was detected in Thai rice; PT 1 and CNT 1.

When the percentage of decreasing or increasing (values in parenthesis), compared with the normal condition (0 dS/m salinity) was calculated at week 1 since found tillering establishment (Table 12). Increasing the salinity was affected differently in varieties and characteristics. No reduction percentage was observed in the number of tillers per plant in all varieties; IN 35, PT 1, and CNT 1, in which positive values were presented. Although tiller numbers showed increasing trend at higher salinity stress, effective or non-effective tillers could not be detected at the start of tillering stage. Many characteristics of Thai rice varieties were affected by salinity, demonstrating as reduction percentage in the number of leaves per plant and plant height, and positive percentage in leaf symptom scoring.

No significant difference affected by salinity was found in three characteristics at one week after transplanting; the number of leaves per plant, number of tillers per plant, and plant height (Table 12). Very similar actual values in salinity

levels and low percentages either positive or negative percentages; compared with actual value at 0 dS/m in each characteristic in any variety. However, only Inpari 35 had positive change percentage (compared with normal condition at 0 mM NaCl) under increasing salinity stress for number of leaves per plant and number of tillers per plant. Although, leaf symptom scoring had a positive change percentage under increasing salinity level also. This result in Inpari 35 could mean this variety was affected by salinity as well, but was still able to tolerate and establish stems and leaves; this was only observed early in the exposure to salinity within one week after transplanting.

A significant difference effected by salinity at one week after transplant was found in leaf symptom scores ($P<0.01$) (Table 12). At higher salinity levels (100 mM and 150 mM NaCl), it showed either higher actual scores or higher positive percentages, compared with control condition (at 0 mM NaCl). For result in leaf symptom score, really showed an effect by salinity stress. Although, not much to decreasing on other agronomic traits recorded in the first week after transplanting. The symptom of leaf such as yellowing leaves, pale and burn-like appearance at the tips of plant leaves was reported as the results of salinity stress (McCauley et al., 2009b). These leaf symptoms can affect plant physiology, reducing the effectiveness of leaf's sunlight absorption and photosynthesis. The presence of these symptoms on leaves can also negatively impact the number of leaves, the size of leaves, withering and scorch in plants face with stress for a longer time (Tatagiba et al.,016; Hong et al., 2018). After that, the plant stems can become stunted, leading to death. In addition, other plant parts that interfere with carrying out activities in the transportation process and metabolism that make plant can't run normally (Tatagiba et al., 2016).

The influence of leaf formation, plant height, number of tillers and other agronomic properties occurs at the vegetative and reproductive stages (Hussain et al., 2019). Elias et al. (2020) reported that salt stress can cause symptoms in plants as reduced leaf numbers, decreased plant height, and reduced formation number of tillers (Elias et al., 2020). Moreover, some plants can also demonstrate stunted growth, chlorosis, interveinal chlorosis, and necrosis during salt stress (Acosta-Motos et al., 2017).

Table 12 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 1 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	14.0 ± 1.2	3.2 ± 0.4	55.7 ± 2.1	1.2 ± 0.4
50 mM (% NC)	14.8 ± 1.1 (+5.71)	3.6 ± 0.6 (+12.50)	55.7 ± 5.2 (0.00)	1.4 ± 0.6 (+16.67)
100 mM (% NC)	14.6 ± 1.7 (+4.29)	3.8 ± 0.4 (+18.7)	53.8 ± 5.6 (-3.41)	2.0 ± 0.0 (+66.67)
150 mM (% NC)	14.4 ± 1.5 (+2.86)	3.4 ± 0.6 (+6.25)	52.1 ± 3.9 (-6.46)	2.4 ± 0.6 (+100)
Mean (IN 35)	14.4 ± 1.3 a	3.5 ± 0.5 b	54.3 ± 4.3 a	1.8 ± 0.6 b
PT 1				
0 mM (NC)	13.4 ± 1.7	3.4 ± 0.6	48.9 ± 4.5	1.4 ± 0.9
50 mM (% NC)	12.8 ± 1.3 (-4.48)	3.8 ± 0.4 (+11.76)	48.2 ± 5.3 (-1.43)	1.8 ± 0.4 (+28.57)
100 mM (% NC)	14.6 ± 1.8 (+8.96)	3.6 ± 0.6 (+5.88)	47.3 ± 3.1 (-3.27)	2.4 ± 0.6 (+71.43)
150 mM (% NC)	11.8 ± 1.5 (-11.94)	3.6 ± 0.6 (+5.88)	47.9 ± 4.4 (-2.04)	3.0 ± 0.0 (+114.29)
Mean (PT 1)	12.6 ± 1.6 b	3.6 ± 0.5 a	48.1 ± 4.1 b	2.2 ± 0.8 a
CNT 1				
0 mM (NC)	13.8 ± 1.8	4.2 ± 0.8	50.1± 2.3	1.2 ± 0.4
50 mM (% NC)	13.6 ± 2.0 (-1.45)	4.2 ± 0.4 (0.00)	48.9 ± 3.0 (-2.40)	2.0 ± 0.7 (+66.67)
100 mM (% NC)	12.8 ± 1.1 (-7.25)	4.2 ± 0.4 (0.00)	48.1 ± 2.3 (-3.99)	2.8 ± 0.4 (+133.33)
150 mM (% NC)	12.6 ± 1.7 (-8.70)	4.4 ± 0.9 (+4.76)	48.0 ± 1.3 (-4.19)	2.8 ± 0.4 (+133.33)
Mean (CNT 1)	13.2 + 1.6 b	4.2 ± 0.6 b	48.8 ± 2.3 b	2.2 ± 0.8 a
Over all mean	13.4	3.8	50.4	2.03
0 mM (NC)	13.7 ± 1.5	3.6 ± 0.7	51.6 ± 4.2	1.3 ± 0.6 C
50 mM (% NC)	13.7 ± 1.6 (0.00)	3.9 ± 0.5 (+8.33)	51.0 ± 5.6 (-1.16)	1.7 ± 0.6 B (+30.77)
100 mM (% NC)	13.3 ± 1.8 (-2.92)	3.9 ± 0.5 (+8.33)	49.7 ± 4.7 (-3.68)	2.4 ± 0.5 A (+84.62)
150 mM (% NC)	12.9 ± 1.8 (-5.84)	3.9 ± 0.8 (+8.33)	49.3 ± 3.8 (-4.46)	2.7 ± 0.5 A (+107.69)
P value (Varieties)	0.0016^{**}	0.00024^{**}	$4.43 \times 10^{-6**}$	0.0153^{*}
P value (Salinity)	0.423 NS	0.547 NS	0.340 NS	$9.7 \times 10^{-10**}$
P value (Salinity:Variety)	0.821 NS	0.864 NS	0.976 NS	0.628 NS
CV (%)	11.55	15.25	7.6	25.41

Note: NC = normal condition, CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

There were significant differences with varies significant level ($P \leq 0.05$ and $P \leq 0.01$) effected by each factor; in the level of salinity and varieties factors, observed all characteristics shown in Table 13. For the combination of these two factors; varieties and salinity levels, there is no interaction between these factors. The highest results on the three-character measurement variables which include; The number of

leaves, tillers and plant height were obtained in Inpari 35 varieties compared to PT 1 and CNT 1.

Meanwhile, in both Thai rice varieties, leaf symptom scores were higher than the introduced varieties. For salinity levels, there were significant differences on all characteristics presented in Table 13.

The highest number of leaves and number of tillers per plant, and plant height were indicated by the level of salinity at 0 mM or control treatment. However, at the control treatment, it was not significant difference with at 50 mM NaCl on the number of leaves per plant and plant height. In contrast, since salinity at 50 mM NaCl affected the number of tillers per plant in two weeks after transplanting. The negative effect of salinity at three agronomic characteristics (no. leaves and no. tillers per plant, and plant height) was observed with negative percentage compared to control treatment (at 0 mM NaCl) for different salinity levels; the highest negative values was found at 150 and 100 mM NaCl, respectively. Likewise, an increase in salinity led to an increase in leaf symptom score, respectively, compared to the control treatment at 0 mM NaCl. The highest value on leaf symptom scoring affected by salinity was detected in PT1 and CNT1, respectively, and lowest at IN 35.

The absence of interaction between the two main factors; varieties and salinity levels for all of these characteristics makes it possible to clearly summarize the influence of each factor.

Table 13 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 2 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	17.77 ± 1.2	5.42 ± 0.4	60.87 ± 0.3	1.6 ± 0.9
50 mM (% NC)	17.38 ± 0.9 (-2.20)	5.12 ± 0.2 (-5.54)	61.85 ± 4.1 (+1.63)	2.0 ± 0.0 (+25.00)
100 mM (% NC)	16.53 ± 1.1 (-6.98)	4.95 ± 0.5 (-8.67)	58.50 ± 3.9 (-4.71)	2.2 ± 0.5 (+37.50)
150 mM (% NC)	15.85 ± 1.2 (-10.80)	4.57 ± 0.5 (-15.68)	57.28 ± 3.1 (-5.89)	2.8 ± 0.5 (+75.00)
Mean (IN 35)	16.88 ± 1.3 a	5.01 ± 0.5 a	59.62 ± 3.5 a	2.15 ± 0.7 b
PT 1				
0 mM (NC)	16.72 ± 1.2	4.78 ± 0.3	55.64 ± 3.2	2.0 ± 1.0
50 mM (% NC)	16.28 ± 1.2 (-2.63)	4.42 ± 0.4 (-7.53)	53.91 ± 4.5 (-3.11)	2.8 ± 0.5 (+40.00)
100 mM (% NC)	15.22 ± 1.4 (-8.97)	4.03 ± 0.5 (-15.70)	53.36 ± 3.2 (-4.10)	3.0 ± 0.0 (+50.00)
150 mM (% NC)	14.68 ± 1.3 (-12.20)	3.77 ± 0.3 (-21.13)	52.77 ± 2.7 (-5.16)	3.4 ± 0.6 (+70.00)
Mean (PT1)	15.73 ± 1.4 b	4.25 ± 0.5 c	53.92 ± 3.4 b	2.80 ± 0.77 a
CNT 1				
0 mM (NC)	17.5 ± 1.1	5.0 ± 0.2	55.5 ± 1.0	1.8 ± 0.8
50 mM (% NC)	16.8 ± 1.2 (-8.57)	4.7 ± 0.4 (-6.00)	55.0 ± 3.4 (-0.90)	2.4 ± 0.6 (+33.33)
100 mM (% NC)	15.9 ± 1.2 (-14.28)	4.3 ± 0.4 (-14.00)	54.5 ± 2.9 (-1.80)	3.0 ± 0.0 (+66.66)
150 mM (% NC)	15.4 ± 1.3 (-12.00)	4.0 ± 0.4 (-20.00)	53.1 ± 1.8 (-4.32)	2.8 ± 0.5 (+55.55)
Mean (CNT 1)	16.4± 1.4 ab	4.5 ± 0.5 b	54.5 ± 2.5 b	2.5 ± 0.7 ab
Over all mean	16.3	4.6	56	2.5
0 mM (NC)	17.3 ± 1.2 A	5.1 ± 0.4 A	57.3 ± 3.1 A	1.8 ± 0.9 C
50 mM (% NC)	16.8 ± 1.1 A (-2.90)	4.7 ± 0.4 B (-7.84)	56.9 ± 5.2 A (-0.70)	2.4 ± 0.5 B (+33.33)
100 mM (% NC)	15.9 ± 1.3 B (-8.10)	4.4 ± 0.6 C (-13.73)	55.5 ± 3.9 AB (-3.14)	2.7 ± 0.5 AB (+50.00)
150 mM (% NC)	15.3 ± 1.3 B (-11.56)	4.1 ± 0.5 D (-19.61)	54.4 ± 3.2 A (-5.06)	3.0 ± 0.5 A (+66.66)
P value (Varieties)	0.01334^{*}	$2.07 \times 10^{-7**}$	$3.42 \times 10^{-7**}$	0.0031^{**}
P value (Salinity)	0.000113^{**}	$5.54 \times 10^{-8**}$	0.045^{*}	$4.11 \times 10^{-6**}$
P value (Salinity: Variety)	0.910 NS	0.986 NS	0.879 NS	0.767 NS
CV (%)	7.31	8.19	5.5	22.96

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

When assessing the salinity tolerance of rice varieties at two weeks post-transplanting, it was found that the variety that showed the higher tolerance and effort to maintain their agronomic characteristic was IN 35. Of cause, Thai rice is less tolerant of salinity when considering the above condition. Nevertheless, when considering the details of each characteristic, it was found that CNT1 was more salt tolerant than PT1.

This study could sort characteristics affected from salinity by highest to lowest: leaf symptom scoring, number of tillers per plant, number of leaves per plant, and plant height; determined by the direction and magnitude of the change in percentage with the control treatment at 0 mM NaCl. While the cultivation of rice at the tillering stage that received the salinity for two weeks continues, it was found that the salinity at 50 mM NaCl had already affected the characteristics, and the highest affect was observed at 150 and 100 mM NaCl, respectively. These results indicate that prolonged exposure of salinity stress will affect all agronomic characteristics in rice; both in tolerant or non-tolerant varieties, even at low salinity levels. These observations were based on assessing the effects of salinity on characteristics in weeks one and two of exposure salinity; or number of weeks after transplanting. Thus, at two weeks after exposure salinity may is the time that plant begins to weaken, and makes the ability of plant cells even more damaged and dies (Abdelaal et al., 2020). The duration that plants experiencing salinity stress is also an important consideration for management (Eisa et al., 2012b).

The difference in salinity expression of rice varieties was explained from their respective abilities such as the ability to maintain osmotic pressure, one mechanism affected by salinity stress.

Rice variety ‘IN 35’ showed the higher tolerance ability for salinity more than others; PT 1 and CNT1 in this study, it was report has the ability to survive and grow in certain places that have high salinity stress in the field as well (Sembiring et al., 2020). In this case it is seen the early symptom observed in leaves blade more than number of leaves which the leaves of the plant appear stress, the leaves turn yellow, the tips dry up, the edges of the leaves look like they are burnt and even some of the leaves begin to die and dry up (Mondal et al., 2020). Cause of salinity to membrane damage in plants. Membrane permeability and lipid peroxidation, significantly with salinity increase stress, and this oxidative damage was, membrane damage caused by different abiotic stresses including salinity is mostly mediated through membrane lipid peroxidation (Eraslan et al., 2008). Due to the excess accumulation of Na and / or iodine can be fatal in some parts of the rice plant, especially in the leaves so that the leaves experience various forms of stress that arise such as yellowing and to death slowly (Ghasemi-Omran et al., 2021).

For others characteristics; number of tillers per plant, number of leaves per plant, and plant height, the effect of salinity stress is related to the availability of nutrients contained both from plants and in the soil, including the photosynthesis process. Salt stress, the various initial steps in the diagnosis of nutrient deficiency, namely to explain the symptoms and function of each nutrient associated with plants (Hatibu, 2018a). Stunting is a common symptom of many nutrient deficiencies due to their different roles in plants (Hajiboland, 2018). For example, when the nutrients involved in plant functions such as plant leaves become pale, leaves is smaller but possibly a little thinker than normal plant, reducing for photosynthesis, and protein production is lacking, plant growth is usually slow and the plants are small (Elemike et al., 2019). The effects on the mechanics of those plants will affect tiller and leaf establishment, and plant tissue formation.

Salinity also has a stress effect on plants, which causes nutrient imbalance. NaCl treatment can induce potassium (K) deficiency and increase sodium (Na), calcium (Ca), magnesium (Mg), and chloride (Cl) in rice plants (Chrysargyris et al., 2019). This imbalance of salt associated with redox system can cause oxidation damage, especially on nucleic acids, fats and proteins. In starting of salinity stress, plant might response by releasing several antioxidant enzymes and osmoprotectant molecules to protect their tissue from the toxins and oxidative residues. However, after that, if the effect of salinity is longer and greater, the plant's ability to respond to stress tolerance in plants is reduced (Sairam et al., 2002). Thus, it was found that all salinity levels had a negative effect on all characteristics in the week 2, after transplanting.

Moreover, rice susceptible to salinity, an abiotic factor, can become less resistant to other biotic stressors, such as diseases and insects. This is obviously due to the deterioration of plant health under stress condition, however might be variety specific (Quais et al., 2020). Low concentration of salinity stress was reported to stimulate plant growth, but high concentration can severely decrease plant’s physiological characteristics (Warne et al., 1990; Yu et al., 2018). It had been found in many plants that were stressed to high salt concentration is greatly affected by physiological and biochemical processes in plant and will have decreased yield production and eventual death (D'antonio and Meyerson, 2002).

Response from genetic diversity; according to each variety showed the different effects from salinity levels. Nevertheless, it has been stated that the components of growth and production in each variety do not depend only on genetic traits (Basu et al., 2016). There may be some genetic and environmental interactions for some of the factors not evaluated here. Or even the factors that were assessed in this study that but did not have a clear effect, such as genetics and salinity levels.

The ability of the IN 35 variety tends to be more adaptive on some agronomic growth traits at vegetative stage than two Thai varieties. This is according to explain by (Utama et al., 2018a) which states that plants are tolerant of environmental stress, namely salinity has the ability to adapt both the physical properties of each individual variety. The varies in growth behavior is certainly due to genetic according to their abilities under specific environment (Letort et al., 2008).

Similar results of salinity levels studies were provided to all three lowland rice varieties during one week and two weeks after transplanting (Table 13-14). At three weeks after transplanting, all characteristics were statistically significantly affected by the main factors i.e., variety and salinity levels (Table 14). But it was non-significant difference due to the interaction between those factors (Table 14). But the difference is that three weeks after transplanting, all of the factors that were statistically significant would have highly significance difference at $P \leq 0.01$ (Table 14).

For variety factor, IN 35 showed the highest value with statistical differences in three parameters of agronomic characteristics: such as number of leaves per plant, number of tillers per plant, and plant height, and lower on score on leaves symptom affected by salinity stress (Table 14). In comparison among Thai rice (PT 1 and CNT 1), it was found that CNT 1 was less damaged due to salinity when considering the difference of mean comparison of characteristics such as number of tillers per plant and leaf symptom scoring; higher and lower values, respectively compared with PT 1 (Table 14). Nevertheless, for each component produced by each plant in production, it is highly dependent on the genetics of a particular variety (Donald, 1968; Sagare et al., 2020). For this reason, the percentage reduction compared with control condition (0 mM NaCl) was also required for consideration. In each salinity level, higher reduction with higher negative percentage was observed in PT 1 more than CNT 1.

Hence, there is a possibility that PT 1 was more susceptible to salinity stress than CNT 1 and IN 35, respectively.

For salinity levels effect, overall means of leaf symptom score at ≥100 mM NaCl showed positive percentage, compared with value at 0 mM NaCl (Table 14). Although no significant difference of interaction between variety and salinity levels, there were different responses for salinity level in each rice variety. A positive percentage, compared with the normal condition (0 mM NaCl), was found starting at the salinity level 50 mM, 100 mM, and 150 mM NaCl in PT 1, IN 35, and CNT 1 (Table 14), respectively.

At the beginning of this tillering phase, all characteristics are important because they can indicate growth which will continue to yield production. Especially the ability to produce tiller, tiller numbers per plant in rice is very important to yield component since the value of the trait could indicate the final yield (Xue et al., 2008).

Table 14 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 3 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	22.2 ± 1.8	6.2 ± 0.4	64.4 ± 1.8	1.2 ± 0.4
50 mM (% NC)	21.2 ± 1.8 (-4.50)	6.0 ± 0.0 (-3.23)	65.2 ± 3.4 (+1.24)	1.0 ± 0.0 (-16.67)
100 mM (% NC)	19.6 ± 2.2 (-11.71)	5.2 ± 0.4 (-16.13)	60.9 ± 2.7 (-5.42)	1.6 ± 0.9 (+33.33)
150 mM (% NC)	18.8 ± 1.8 (-15.32)	4.8 ± 0.4 (-22.58)	60.3 ± 1.6 (-6.37)	3.2 ± 1.1 (+166.67)
Mean (IN 35)	20.4 ± 2.2 a	5.6 ± 0.7 a	62.7 ± 3.2 a	1.8 ± 1.1 b
PT 1				
0 mM (NC)	20.2 ± 1.9	5.6 ± 0.6	60.1 ± 2.8	2.0 ± 0.7
50 mM (% NC)	19.4 ± 2.1 (-3.96)	5.0 ± 0.7 (-10.71)	57.6 ± 2.6 (-4.16)	2.2 ± 0.8 (+10.00)
100 mM (% NC)	17.6 ± 2.3 (-12.87)	4.2 ± 0.4 (-25.00)	58.0 ± 2.2 (-3.49)	2.8 ± 0.8 (+40.00)
150 mM (% NC)	16.4 ± 2.0 (-18.81)	3.8 ± 0.4 (-32.14)	56.4 ± 2.2 (-6.16)	4.2 ± 0.4 (+110.00)
Mean (PT1)	18.1 ± 2.4 b	4.6 ± 0.9 c	58.0 ± 2.6 b	2.8 ± 1.1 a
CNT 1				
0 mM (NC)	21.0 ± 1.6	5.8 ± 0.4	60.1 ± 2.0	2.0 ± 0.7
50 mM (% NC)	20.0 ± 1.6 (-4.76)	5.4 ± 0.6 (-6.90)	59.2 ± 2.7 (-1.50)	1.4 ± 0.6 (-30.00)
100 mM (% NC)	18.4 ± 10.1 (-12.38)	4.8 ± 0.4 (-17.24)	58.6 ± 2.1 (-2.50)	2.0 ± 0.7 (0.00)
150 mM (% NC)	17.4 ± 1.9 (-17.14)	4.0 ± 0.7 (-31.03)	56.3 ± 2.4 (-6.32)	3.2 ± 0.8 (+60.00)
Mean (CNT 1)	19.2 ± 2.2 b	5.0 ± 0.9 b	58.5 ± 2.6 b	2.2 ± 0.9 b
Over all mean	19.4	5.1	59.8	2.2
0 mM (NC)	21.1 ± 1.9 A	5.9 ± 0.5 A	61.5 ± 3.0 A	1.7 ± 0.7 BC
50 mM (% NC)	20.2 ± 1.9 A (-4.27)	5.5 ± 0.6 B (-6.78)	60.7 ± 4.3 AB (-1.30)	1.5 ± 0.7 C (-11.76)
100 mM (% NC)	18.5 ± 2.2 B (-12.32)	4.7 ± 0.6 C (-20.34)	59.2 ± 2.5 BC (-3.74)	2.1 ± 0.9 B (+23.53)
150 mM (% NC)	17.5 ± 2.0 B (-17.06)	4.2 ± 0.7 D (-28.81)	57.7 ± 2.7 C (-6.18)	3.5 ± 0.9 A (+105.88)
P value (Varieties)	0.0055^{**}	$3.59 \times 10^{-6**}$	0.00035^{**}	0.00014^{**}
P value (Salinity)	$167 \times 10^{-5**}$	$8.92 \times 10^{-12**}$	$1.14 \times 10^{-7**}$	$1.96 \times 10^{-10**}$
P value (Salinity:Variety)	0.910 NS	0.89 NS	0.43 NS	0.775 NS
CV (%)	9.9	9.87	4.04	32.44

Note: NC = normal condition, CV = Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Which, tiller establishment is affected by both genetic and environmental factors (Hussien et al., 2014a). Salinity has the most severe impact on plant growth and development. Salinity inhibits the activity of cells in food transportation in various parts of plant (Kumar et al., 2020). The presence of salt stress can minimize the synthesis of chlorophyll pigments and photosynthesis experiences a rate of pressure and other important processes involved in it (Desoky et al., 2020). In plants

experiencing moderate stress from high salinity levels, the salt is generally spread to several parts of the plants while causes symptoms on the leaves and reduces the number of leaves, number of tillers and plant height (Prasad et al., 2008; Hussien et al., 2014).

Salt stress is now evident and shows that the induced suppression of photosynthesis is always dependent on gas changes, photosynthetic pigments, cultivar species or types, stomata and accumulation of organic and inorganic metabolites as well as antioxidants (Zhang et al., 2020). Evidence of accumulation Na^+ and Cl^- in several tissues in leaves beyond the usual situation due to increased air loss and dehydration of the cytoplasm. The dehydration of the cytoplasm means that cells can no longer perform its metabolism normally and can ultimately reduce growth in plants (Khare et al., 2020).

Higher negative percentage of changeable values in increased salinity level; compared with the normal condition (0 mM NaCl) on the number of tillers per plant and number of leaves per plant. Which in Thai rice varieties: PT 1 and CNT 1 was observed the reduction on those traits over than IN 35. The tolerance ability of varieties is they can utilize every existing capacity to keep constant in mechanisms for normal growth and production any environment in which it grows, although that environment is dire. The varieties with high adaptability can produce better growth and more ability to adapt physiologically and morphologically than other varieties under specific stress condition (Khan et al., 2020a). In this study, these three rice varieties showed different corresponding salt tolerances at tillering stage. However, in week 2 after transplanting, salinity has shown to be very effective in influencing on all measured agronomic characteristics of these varieties (Mujeeb-Kazi et al., 2019b).

The variation in salt content which varies, its effect on all agronomic characteristics from the results of statistical analysis at four weeks after transplanting (Table 15). There are significant differences in those characteristics affected by rice varieties (Table 15). Similarly, between week 4 and other weeks, after transplanting which no interaction between two factors between salinity levels and rice varieties (Table 15). It was clear from the second to the fourth weeks after exposure the salinity that higher values on three characteristics; number of leaves, number of tillers per plant, and plant height were observed in IN 35 and lower at PT1 and CNT1,

respectively. Addition, the lower value was observed on leaf symptom scoring in IN 35, and higher in PT1 and CNT1.

Table 15 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 4 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	25.2 ± 1.5	7.0 ± 0.0	69.9± 2.6	1.0 ± 0.0
50 mM (% NC)	23.6 ± 1.5 (-6.35)	7.0 ± 0.7 (0.00)	68.3 ± 2.1 (-2.29)	2.2 ± 0.5 (+120.00)
100 mM (% NC)	22.2 ± 1.8 (-11,90)	7.0 ± 1.4 (0.00)	66.1 ± 1.7 (-5.44)	2.6 ± 0.6 (+160.00)
150 mM (% NC)	21.2 ± 1.6 (-15.87)	6.4 ± 0.9 (-8.57)	65.3 ± 1.3 (-6.60)	2.4 ± 0.9 (+140.00)
Mean (IN 35)	23.1 ± 2.1 a	6.9 ± 0.9 a	67.5 ± 2.6 a	2.1 ± 0.8 b
PT 1				
0 mM (NC)	23.0 ± 1.9	6.8 ± 0.5	65.5 ± 3.09	1.0 ± 0.0
50 mM (% NC)	21.4 ± 1.8 (-6.96)	5.6 ± 1.5 (-17.65)	63.1 ± 1.7 (-3.66)	3.0 ± 0.0 (+200.00)
100 mM (% NC)	20.6 ± 2.0 (-10.43)	6.0 ± 1.4 (-11.76)	62.7 ± 1.1 (-4.27)	3.0 ± 0.7 (+200.00)
150 mM (% NC)	18.4 ± 1.1 (-20.00)	5.2 ± 0.8 (-23.53)	62.4 ± 1.3 (-4.73)	3.6 ± 1.1 (+260.00)
Mean (PT1)	20.9 ± 2.3 c	5.9 ± 1.2 b	63.6 ± 2.1 b	2.7 ± 1.2 a
CNT 1				
0 mM (NC)	24.0 ± 1.4	7.0 ± 0.7	66.7 ± 3.0	1.0 ± 0.0
50 mM (% NC)	22.8 ± 1.5 (-5.00)	6.2 ± 0.8 (-11.43)	65.7 ± 2.7 (-1.50)	2.6 ± 0.6 (+160.00)
100 mM (% NC)	21.4 ± 1.7 (-10.83)	6.0 ± 1.2 (-14.29)	63.7 ± 1.4 (-4.50)	2.8 ± 0.5 (+180.00)
150 mM (% NC)	19.4 ± 1.1 (-19.17)	5.4 ± 1.5 (-22.68)	62.9 ± 1.3 (-5.70)	3.0 ± 0.7 (+200.00)
Mean (CNT 1)	21.90 ± 2.20 b	6.15 ± 1.18 b	64.7 ± 2.6 b	2.4 ± 0.9 ab
Over all mean	21.9	6.3	65.3	2.4
0 mM (NC)	24.1 ± 1.8 A	6. 9 ± 1.4 A	67.3 ± 3.3 A	1.0 ± 0.0 B
50 mM (% NC)	22.6 ± 1.8 B (-6.22)	6.3 ± 1.2 AB (-8.70)	66.1 ± 2.8A (-1.78)	2.6 ± 0.5 A (+160.00)
100 mM (% NC)	21.4 ± 1.8 C (-11.20)	6.3 ± 1.4 AB (-8.70)	64.1 ± 2.0 B (-4.75)	2.8 ± 0.6 A (+180.00)
150 mM (% NC)	19.7 ± 1.7 D (-18.26)	5.7 ± 1.2 B (-17.40)	63.5 ± 1.7 B (-5.65)	3.0 ± 1.0 A (+200.00)
P value (Varieties)	$1.14 \times 10^{-8**}$	0.0190^{*}	$9.46 \times 10^{-7**}$	0.0085^{**}
P value (Salinity)	0.00034^{**}	0.0205^{*}	$1.07 \times 10^{-5**}$	$1.75 \times 10^{-12**}$
P value (Salinity:Variety)	0.983 NS	0.8622 NS	0.967 NS	0.452 NS
CV (%)	7.28	16.35	3.12	24.89

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

The higher values were varying in salinity level among the number of leaves, tillers and plant height. For plant height, the reduction value; compared with 0 mM NaCl or control treatment was indicated by the level of salinity at 100 mM NaCl. Where

similarly between the number of leaves and the number of tillers per plant which changing in reduction values was recorded at 50 mM NaCl. However, clearly, different values among salinity levels were indicated on number of leaves per plant. For the foliar symptom score, higher scores compared with 0 mM NaCl were detected at the salinity level since 50 mM NaCl, however, were not significant difference among 50-100 mM NaCl (Table 15).

There was similar consistency and values of characteristics between the results of the third and fourth weeks after salting in early tillering stage (Table 14-15). It was found that the characteristics with the highest reduction compared with the control group at 0 mM NaCl were number of tillers and number of leaves per plant and their plant height, respectively (Table 15). Unlike the foliar symptoms, the fourth week salinity was significantly more severely affected than the third week; indicated by an increased percentage of foliar symptoms compared to the control treatment at 0 mM NaCl (Table 15).

The salt content affects plant growth with the content of various elements present in the plant as well as in the photosynthesis process and on the nutrients contained in the soil. Salt stress, nutrient deficiency which in diagnosis is the main step considered as a beginning effect in the plant (Hatibu, 2018b).

This can be seen from the very high tolerance of IN 35 to the effects of salinity as express in all characteristics in this study since first until fourth weeks after plant received salty. This is also supported by the theory that plants have the ability to adapt both morphologically and physiologically to abiotic stresses is called its resistance (Anjum et al., 2011). On the contrary, the sensitivity of plants to sodium chloride has been linked to the plant's inability to metabolize processes that occur in plants, especially in some parts that are essential and easily damaged due to high salinity pressure; that is beyond the ability of plants to respond (JENNINGS, 1976; Otlewska et al., 2020). Although it is the same plant, the tolerance depending on the part of certain morphology and physiology at high salinity levels (Fahad et al., 2015). However, the effect of salinity on tiller establish that will develop to produce further panicle will certainly damage the yield. This is also according to the suggestion that salt poisoning experienced by plants can be recognized as the formation of the number of rice tillers can be reduced (Läuchli and Epstein, 1990).

The detrimental effects of salinity often also depend on the stage of plant growth, which in rice plants, the tiller stage is very sensitive to salinity (Lutts et al., 1995). In general, cereal plants have decreased because in certain parts it can be reduced due to the salt content which hinders the formation of several agronomic characteristics (Wassmann et al., 2009a). However, on the side of the plant, there are certain causes that the saplings that are formed tend to be stunted, turn yellow, dry to death like burning. (Poljakoff-Mayber and Lerner, 1999).

The decrease in the absorption properties of rice varieties and the transport of Na^+ under salt stress will greatly contribute to salt tolerance in rice varieties. Inpari 35, the new rice variety in Indonesia, has been identified with a higher absorption capacity than plant characteristics and transports Na^+ from one part of the plant to another part (Zhang et al., 2018).

Effects on agronomic characteristics in rice at harvesting stage under difference of proline application in salinity condition

Soil salinity is a common limiting factor in crop production. Therefore, the application of external solute accompanied by low molecular weight as proline was conducted at tillering stage. More than to determine molecular and chemical, the yield, yield components, and agronomic characteristics also were recorded at harvesting stage. In the application of these three factors; varieties, salinity level, and proline level, no interactions of any combinations between any factors were significant different affected on plant height at harvest, but for these three factors separately affected plant height (Table 16). For varieties, overall mean other factors, plant height showed highest in the IN 35 variety, and followed by CNT 1 and PT 1, respectively. For varieties, on average of all other factors, plant height showed the highest in the IN 35 variety, followed by CNT 1 and PT 1, respectively. The IN 35 variety also had a higher plant height than previous studies on several varieties with varying levels of salinity such as; IR-64 (60.67), Inpara (46.79), IRBB-27 (73.41), Ciherang (75.35). The mean of plant height decreased, respectively, with increasing salinity levels; highest at 0 mM NaCl and lowest at 150 mM NaCl. Proline application at ≥100 mM showed higher the height of the plant, while the values between 0 mM and 50 mM were not significant difference.

There are variations in each variety that have several different genetic traits according to the type of rice plant variety in response to the growing environment, namely salty due to the treatment of the salinity level tested (Rasel et al., 2021).

Rice plants that are sensitive to sufficient salt content indicated that, when the crop is exposed to increased levels of salinity. More than cultivars that are tolerant of salinity can temporarily increase more (Cuartero et al., 2006). The characteristics of plant in which the effect of salinity was studied also influenced the expression of susceptibility or resistance to salinity. Results in week 1 to week 4 after transplantation with salinity, the plant height was least affected by salinity; compared with number of tillers and number of leaves per plant (Table 12-15). From this observation, together with the results of the study, it was found that the order of plant height of the varieties was not different from the control conditions without salinity (0 mM NaCl and at 0 mM proline); which highest is IN 35 (83.32 cm), and followed by CNT 1 (72.60 cm), and PT 1 (70.52 cm), respectively (Table 16). Therefore, may it can’t conclude from only from plant height affected by salinity whether which variety is more tolerant to salinity.

Table 16 Average plant height (cm) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	72.60 ± 10.31	64.70 ± 5.55	64.70 ± 5.65	64.02 ± 11.40	70.18 ± 9.21 b
	50	82.00 ± 3.46	71.72 ± 5.50	64.40 ± 5.28	56.26 ± 6.54	
	100	78.64 ± 5.00	71.16 ± 2.16	70.36 ± 3.00	65.12 ± 6.01	
	150	85.10 ± 5.00	75.00 ± 5.00	72.40 ± 3.36	64.80 ± 8.14	
PT 1	0	70.52 ± 8.03	65.80 ± 4.27	56.34 ± 10.61	56.20 ± 8.11	65.91 ± 9.31 c
	50	72.24 ± 8.30	68.34 ± 7.33	60.16 ± 0.11	52.86 ± 4.21	
	100	72.64 ± 4.56	73.40 ± 10.01	66.06 ± 0.45	63.04 ± 7.56	
	150	71.20 ± 13.40	69.40 ± 5.64	72.00 ± 5.70	64.40 ± 6.20	
IN 35	0	83.32 ± 8.55	76.20 ± 5.40	76.20 ± 2.40	63.40 ± 7.90	76.18 ± 11.15 a
	50	87.40 ± 6.60	78.61 ± 8.37	73.18 ± 4.33	66.74 ± 10.35	
	100	93.40 ± 15.50	76.24 ± 4.30	77.76 ± 8.02	66.26 ± 4.52	
	150	89.43 ± 8.84	79.00 ± 10.25	73.00 ± 8.37	69.21 ± 10.26	
Mean salinity		79.87 ± 11.08 A	72.46 ± 7.50 B	68.88 ± 8.30 C	62.70 ± 8.57 D	
Proline (mM)						
Mean Proline		0	50	100	150	
		67.83 ± 10.60 b	69.50 ± 1.50 b	72.84 ± 10.31 a	73.74 ± 10.23 a	
P-value (F-test)						
Variety (V) < 2 x 10^{-16**}, Salinity (S) < 2 x 10^{-16**}, Proline (P) 2.51 x 10^{-05**}, V × S 0.0983 NS, V× P 0.6383 NS, S × P 0.6510 NS, V× S × P 0.4385 NS, CV% 10.42						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

The results of ANOVA on the salinity effect showed that the stress of salt with various levels received by rice plants, especially at plant heights, was very different since at 50 mM NaCl. Salinity was assessed since at the initial tillering and impact estimated at harvesting stage. Longer salinity period provides a more pronounced effect of salinity at low concentrations. Thus, it is said that each increase in the amount of salinity will reduce the increase in plant height in the field (Razmjoo et al., 2008). Salinity significantly reduces certain parts of the plant, such as plant height and development because certain cells are disturbed, eventually the process becomes hampered (Ashraf and Wu, 1994; Latef et al., 2021). Presumably due to the effect of salinity stress which makes it difficult for plants to absorb water and the effect of

NaCl application. Consequence, inhibited the process of cell development and reduced plant height for rice varieties compared to rice plants under normal conditions (Jabeen and Baba, 2018). Why is plant height important and is it consider in combination with others characteristics? By increasing the salinity from low to moderate to a higher level, this has contributed greatly to the growth of plant height which has decreased drastically, due to salt stress, the growth of rice plants has become sluggish (Hasanuzzaman et al., 2013b). Plant height is one of the main supports in the growth of rice plants to the total yield component.

Proline is an exogenous application which under real salt conditions significantly ($p \leq 0.05$) increase plant height when supplied increase level of proline; clear results of increasing at 100 mM and 150 mM proline (Table 16).

Proline application in tillering stage improved characterized by an increase in plant height at the end of tillering stage; although was recorded at harvesting stage. This shows that if each addition of the proline level was very influential on plant height, in terms of proline 100 mM and 150 mM it is more effective, in working for the growth and development of plant height. Proline application improves rice plants grown in salty conditions which may be due to increased water uptake. However, in detecting the positive effect of proline on the salt environment, it is very important to understand the proper role of proline in rice plants, namely improving and stabilizing stress conditions of rice varieties when planting in salt conditions (Semida et al., 2021). The ability of plants to withstand some external chemicals such as introducing proline in salty conditions can improve salt tolerance by eliminating the effects of excessive ion concentration (Cherif-Silini et al., 2021).

The water-lowering potential, compatible with the accumulation of osmolytes involved in additional osmoregulation, allows water to be extracted from the environment, thereby protecting the immediate effects of water deficiency in organisms (Dikilitas, 2003). Plants can retain water if proline levels are higher. Apart from acting as an osmoprotectant, proline also functions as an energy absorber to mobilize redox potential, as a scavenger of hydroxy radicals, as a solute that protects macromolecules against denaturation, as a means of reducing acidity in cells and acts as storage (Joseph et al., 2015). Proline is the source of nitrogen and compounds for the rapid growth of rice varieties after stress (Heuer, 2010).

There was no statistically significant difference in the number of tillers per plant due to the interaction between two and three different factors (Table 17). While, the number of tillers per plant was significantly different from a single factor of two factors: varieties and salinity (Table 17). Proline concentration was the only factor that did not result in statistical differences in the number of tillers per plant (Table 17). Shows that the number of tillers was greater at the 0 mM NaCl (7.20) or control salinity level and decreased with increasing salinity; from 50 mM (6.85) to 150 mM NaCl (6.40). This is presumably due to the effect of osmotic stress which makes it difficult for plants to absorb water and the influence of excessive Na and Cl ions due to NaCl administration, so that cell division and enlargement is inhibited and the number of tillers will be reduced compared to the other rice plants under normal conditions (Menete et al., 2008; Thompson and Clewett, 2021). Of the three rice varieties tested, it was seen that the IN 35 variety produced more total tillers (7.00) than the other two varieties [PT 1 (6.73) and CNT 1 (6.70)]. These results were compared with previous studies, namely the agronomic, water and biochemical attributes of the rice genotype were influenced by differences in proline levels in saline conditions in experimental pots with results in Super Basmati (4.08) and Shaheen Basmati (4.42) varieties. However, after careful consideration, it was found that those values were close to the values at normal growing condition without salinity (at 0 mM NaCl) for each variety; although not being compared individual in each variety. Assessment of the salinity tolerance of this trait in each rice variety may need to be considered in conjunction with the productive tillers per plant characteristics. Due to the tolerant ability for salinity stress in plant should consider on physically adaptation in many characteristics (Yu et al., 2020). According to states that excess Na^+ can cause damage to plant cells. Therefore, plant growth is affected, but the severity can vary in different genetics (Serraj et al., 2011).

Table 17 Average number of tillers per plant of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	6.8 ± 0.45	6.6 ± 0.55	6.4 ± 0.55	6.2 ± 0.84	6.70 ± 0.62 b
	50	7.0 ± 0.71	6.8 ± 0.45	6.8 ± 0.45	6.4 ± 0.55	
	100	7.4 ± 0.55	6.8 ± 0.45	6.8 ± 0.45	6.4 ± 0.55	
	150	7.0 ± 1.22	6.8 ± 0.45	6.6 ± 0.55	6.4 ± 0.55	
PT 1	0	6.8 ± 0.45	6.6 ± 0.55	6.6 ± 0.55	6.0 ± 0.71	6.73 ± 0.70 b
	50	7.2 ± 0.84	6.8 ± 0.45	6.8 ± 0.45	6.6 ± 0.55	
	100	6.8 ± 1.10	7.2 ± 1.10	6.8 ± 0.45	6.4 ± 0.55	
	150	6.8 ± 0.84	6.8 ± 1.10	6.8 ± 0.45	6.6 ± 0.55	
IN 35	0	7.2 ± 0.84	7.4 ± 1.14	6.8 ± 0.44	6.4 ± 0.55	7.00 ± 0.87 a
	50	7.6 ± 1.14	6.8 ± 0.45	6.8 ± 0.45	6.8 ± 0.45	
	100	8.2 ± 1.80	6.8 ± 0.45	6.8 ± 0.45	6.6 ± 0.55	
	150	7.6 ± 1.34	6.8 ± 0.45	6.6 ± 0.55	6.8 ± 0.45	
Mean salinity		7.20 ± 1.01 A	6.85 ± 0.66 B	6.72 ± 0.45 BC	6.40 ± 0.66 C	
Proline (mM)						
Mean Proline		0	50	100	150	
		6.65 ± 0.71	6.87 ± 0.62	6.92 ± 0.87	6.80 ± 0.75	
P-value (F-test)						
Variety (V) 0.015*, Salinity (S) 1.29 x 10^{-06}**, Proline (P) 0.201 NS, V × S 0.359 NS, V× P 0.975 NS, S× P 0.907 NS, V× S × P 0.975 NS, CV% 10.56						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

The ability of plants to produce several parts of the plant, such as number of tillers or productive tillers per plant, of course, the plants need sufficient conditions of available water; water that does not contain salt or has small amount, and enough moisture for uptake from the soil (Grattan et al., 2002).

For exogenous application proline levels, although was not showed significant results, statistically proline levels tended to give different results, namely that proline levels of 50 mM, 100 mM and 150 mM produced more tillers than proline applications controls (0 mM).

Also co-influences with other factors on salinity level in the field, for example as wind, drought, and water management; irrigation that occurs either directly or accidentally and reduces the number of tillers on rice plants (BOGBENDA, 2020). (Ansari et al., 2019) said that the salinity content of NaCl has a strong effect and inhibits the process of forming rice tillers during the planting period. However, proline was reported for it could to reduce salt stress by repairs some parts of the rice plant as cells damaged of high levels of salt stress during in the field (Lee, 2013).

The number of tillers is a genetic trait and plays an important role in determining the productivity of rice plants. Plants with the ability to form a high number of tillers are estimated to have higher productivity than plants with a small number of tillers (Ajal et al., 2021). However, the tillers that were produced must be healthy and able to produce panicle of high seed weight that call as the productive tillers (Table 18). The statistical results of the test, the influence of three factors; salinity level, varieties, and proline levels, and their interaction on the number of productive tillers per plant was presented in Table 18. For number of productive tillers per plant, there was no statistically difference in the interaction between any of the two or three factors, but was found significant different affected by all three single factors.

Salinity had a negative effect on the production of productive tillers, with decreasing values in order of increasing salinity; highest value at 0 mM NaCl (5.3) and lowest value at 150 mM NaCl (2.8). Salinity stress inhibits the activity, transportation processes and plenty of nutrients that circulation from soil, and effected lack of available water (more hypertonic soils) for uptake in the plant, and continues effected the metabolism processes that acts on plant cells (Shilev, 2020). Similarly, the total number of tillers also was reported it is decreased with increasing salt stress (Gautam et al., 2021).

For the difference between the influences of the different varieties, it was found that IN 35 had the highest number of productive tillers per plant (5.0), followed by CNT 1 (3.9) and PT 1 (3.1), respectively (Table 18),

Table 18 Average number of productive tillers per plant of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	4.2 ± 1.05	3.4 ± 0.41	2.9 ± 0.48	2.2 ± 0.27	3.9 ± 1.35 b
	50	5.1 ± 1.14	4.0 ± 0.80	3.4 ± 0.69	2.5 ± 0.46	
	100	5.2 ± 0.45	4.5 ± 0.73	3.8 ± 1.12	3.0 ± 0.96	
	150	6.3 ± 1.04	4.9 ± 0.90	4.3 ± 1.51	3.2 ± 1.23	
PT 1	0	3.2 ± 0.45	2.8 ± 0.73	2.5 ± 0.48	1.6 ± 0.24	3.14 ± 1.22 c
	50	3.9 ± 0.72	3.2 ± 0.53	2.7 ± 0.70	1.8 ± 0.52	
	100	4.7 ± 0.72	3.6 ± 0.92	2.7 ± 0.92	2.3 ± 0.91	
	150	5.3 ± 0.9	4.1 ± 0.97	3.3 ± 1.45	2.3 ± 0.67	
IN 35	0	5.4 ± 0.94	4.5 ± 0.47	3.6 ± 0.34	3.1 ± 0.30	5.01 ± 1.41 a
	50	6.2 ± 0.54	5.1 ± 0.55	4.3 ± 0.77	3.4 ± 0.50	
	100	6.4 ± 1.60	5.6 ± 1.11	5.3 ± 1.2	3.9 ± 0.81	
	150	7.5 ± 0.90	6.1 ± 0.82	5.4 ± 1.08	4.5 ± 1.12	
Mean salinity		5.3 ± 1.42 A	4.3 ± 1.18 B	3.7 ± 1.28 C	2.8 ± 1.06 D	
Proline (mM)						
Mean Proline		0	50	100	150	
		3.2 ± 1.14 d	3.8 ± 1.36 c	4.2 ± 1.50 b	4.8 ± 1.71 a	
P-value (F-test)						
Variety (V) <2 x 10^{-16**}, Salinity (S)<2 x 10^{-06**}, Proline (P) <2 x 10-06 **, V × S 0.982 NS, V× P 0.954 NS, S× P 0.714 NS, V× S × P 0.999 NS, CV% 21.377						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

These results were compared with previous studies, namely the growth and production of some variety of rice at various levels of saline, with results in IRBB (5.57) and Inpara (4.89) varieties. If the high values of IN 35 on number of tillers and number of productive tillers per plant are considered both formal as a tolerability or adaptation under salinity condition more than of the other two varieties; CNT 1 and PT 1. When considering the non-proline at salinity levels at 0 mM NaCl and 150 mM NaCl, it was found that all three varieties had the following percentage reduction in the number of productive tillers per plant, i.e., IN 35, CNT 1, PT 1, were -43.38%, -

47.87%, and -49.06%, respectively. While these percentages are very close to each other, they are in order to follow the overall means of those varieties described above. Therefore, by a rough comparison, IN 35 seemed to be able to tolerate salinity better than both of the Thai rice varieties; CNT 1 and PT 1, because it had a lower reduction percentage of productive tillers value. In the ability of plants, especially in each variety to have the properties and ability to withstand and respond to all environmental influences, whether drought, excessive salt content, or excessive water release (Khaleghi et al., 2019). However, an increase in salinity levels will have an impact on all plant activities, which are associated with disruption of these activities, resulting in a decrease on agronomic characteristics such as the number of productive tillers in rice plants in this case (Menete et al., 2008). Thus, the productivity of rice tillers still depends on genetics, although growth conditions such as level of salinity is very important factor (Syed et al., 2021).

For the four proline levels, it was very significant to increase the number of productive tillers when increase concentration of proline was applied (Table 18). Various diagnoses of nutrient deficiency in plants affected by salinity can explain the function of each nutrient associated with plants (Parihar et al., 2015). Moreover, proline accumulates in plants, perhaps as a result of additional changes according to a certain value for adaptation in a plant grown under the stresses. The higher proline accumulation was caused by the increased activity that occurred in more efficient plants. Since initially the enzyme activity involved in proline biosynthesis can synergize directly with proline administration, so the presence of proline can renew and stabilize plant conditions under salt stress, helping plants to be able to deviate carbohydrates obtained from the soil and sunlight, namely through the process of photosynthesis for seed formation in rice plants (Maggio et al., 2002). On the other hand, dehydrogenase from proline functions as an enzyme for the synthesis of proline reductase and catalyzes reactions with reactants and co-enzymes that are similar, but operate in the opposite direction. In general, in some plants, proline accumulates in excess protein synthesis under osmotic pressure; it can suppress the catabolic process of proline. However, after the stress is reduced, by proline dehydrogenase also known as proline oxidase, the first enzyme in the pathway of proline degradation in plants will active (Prihastati, 2012). Thus, studying these effects of salt stress on the activity

of enzymes involved in proline metabolism can provide the valuable information about physiological significance of plants from their accumulation (Kumar et al., 2003).

All three single factors; variety, salinity level, and proline levels, influenced statistically significant differences to number of seeds per panicle and are shown in Table 8. Addition, there was no difference due to the influence of the interaction between the two factors and the three factors (Table 19).

IN 35 showed the highest value (123) with a very significant difference in the number of seeds per plant compared to the two Thai rice varieties, namely PT 1 (91.5) and CNT 1 (98.5). The result of the highest number of seeds per panicle was on the IN 35 variety compared to previous studies, namely the treatment of several rice varieties to various levels of salt. The varieties with lower seed yield were IR-64 (107.8), Ciherang (109.5) and Inpara (110.5). However, it was not significant different on number of rice seeds per plant between PT 1 and CNT 1. The number of seeds per plant is the most important factor in producing crop yield. However, the number of seeds per plant is reduced due to salinity stress. In tolerant rice variety grown under salt stress, plants will form a physiological mechanism that changes tolerance and osmotic adjustments to the loss of tissue water and absorbed ion (Shahid et al., 2020). Moreover, plants that were resistant to salinity stress have a strategy of receiving sufficient sunlight and carrying out photosynthesis in plants to support their growth. Because, decreased growth of plant caused by decreased water absorption, sodium and chloride toxicity in rice plant cells and reduced photosynthesis (Khan et al., 2021). Inpari 35 variety, it produces a large number of seeds, both from plant genetic factors and other factors (Arief et al., 2018).

The effect of the number of seeds per panicle among the four salinity levels varies from the highest to lowest values according the increased salinity. Thus, number of seeds per plant, important yield component in rice, it is clearly affected in seed formation even at low concentration. In general, the concentration due to increased NaCl is contained and dissolved in the soil (Rengasamy, 2010). Commonly, Na^+ which gets stuck in the roots and other parts of the plant and the Cl that forms in the youngest leaves is the most damaging to the plant. However, there are many cereal plants in plants, Na^+ being the main cause of specific ion damage (Irakoze et al.,

2021). The ion compartment, translate, dispense and transport absorption are controlled, so it can be alert to ionic toxicity. The rate of carbon assimilation decreases rapidly due to salinity, it inhibited plant activity processes and decrease photosynthesis, loss of carbon with increased respiration, resulting in less carbohydrate accumulation (Vanlerberghe et al., 2020).

In controlling the transport of ionic toxicity, plant mechanisms tolerate salty conditions and establish physiological, salinity tolerance such as osmotic adjustment to tissue water and ion uptake (Arif et al., 2020). One of the organs or attributes of plants to function is salt intolerant. Therefore, exhibition of the genotype is the relative salinity tolerance for the ideal plant attributes (Alam et al., 2021).

Table 19 Average number of seeds per panicle of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	100.71 ± 26.83	93.95 ± 25.68	90.03 ± 27.36	80.90 ± 17.81	98.51 ± 25.69 b
	50	105.84 ± 32.20	95.42 ± 33.59	88.90 ± 29.40	77.29 ± 16.55	
	100	109.52 ± 34.47	103.43 ± 29.03	97.52 ± 25.21	85.15 ± 13.13	
	150	119.26 ± 26.22	105.75 ± 28.11	10161 ± 23.68	88.82 ± 9.08	
PT 1	0	93.53 ± 29.01	84.91 ± 23.02	79.53 ± 18.80	76.63 ± 19.99	91.54 ± 25.02 c
	50	99.75 ± 31.31	93.52 ± 33.40	85.73 ± 26.88	73.94 ± 15.13	
	100	104.65 ± 30.78	99.11 ± 30.12	93.14 ± 24.55	81.34 ± 15.09	
	150	115.36 ± 23.09	100.96 ± 29.80	97.26 ± 24.52	85.21 ± 10.62	
IN 35	0	131.10 ± 14.91	114.44 ± 19.37	110.51 ± 17.91	87.40 ± 11.79	123.03 ± 20.18 a
	50	139.94 ± 11.92	128.53 ± 10.47	118.06 ± 11.02	103.00 ± 6.57	
	100	146.18 ± 5.45	136.82 ± 7.67	114.51 ± 19.52	94.28 ± 5.22	
	150	152.29 ± 2.67	144.23 ± 5.65	125.18 ± 9.10	111.95 ± 1.67	
Mean salinity		118.18 ± 29.30 A	108.42 ± 28.83 B	100.17 ± 24.35 C	83.99 ± 16.21 D	
Proline (mM)						
Mean Proline		0	50	100	150	
		96.14 ± 24.96 c	100.83 ± 28.77 bc	105.47 ± 27.53 ab	112.32 ± 26.27 a	
P-value (F-test)						
Variety (V) < 2 x 10^{-16**}, Salinity (S) 1.19 x 10^{-11**}, Proline (P) 0.000614**, V x S 0.596 NS, V x P 0.975 NS, S x P 0.993 NS, V x S x P 1.000 NS, CV% 21.019						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

The synthesis of organic solutes which is achieved and compatible osmotic adjustment or the accumulation of inorganic cells, internal water losses need to be considered (Abeed and Dawood, 2020). That is the reason for the application of proline to increase the tolerance of rice plant during tillering, although number of seeds per panicle was assessed in the harvesting phase.

The results showed that significant differences affected by proline levels. Which, the highest value was observed at the highest level of proline (150 mM) at 112.3 seeds per panicle, and lowest at zero of proline application (0 mM) at 96.1 seeds per panicle (Table 19). About 14.43% increased percentage compared the values between at 150 mM and 0 mM proline on number of seeds per panicle.

Although proline accumulation in the availability of rice plants occurs naturally. Sometimes, proline in plant cannot recover on its own from exposure to high levels of salt stress. In rice, proline accumulation was reported to be more salt tolerant than salt sensitive cultivars and has implications for tolerance to salinity stress. In general, proline expressed as one of the solutes is a compatible phenomenon. When water potential was decreased under abiotic stresses such as salinity, compatible osmolyte accumulation in the involvement of osmoregulation, the possibility of obtaining additional water from the environment, keeping the external influence directly on the reduced water on the organism (Ashraf and Foolad, 2007b). On the other hand, proline has an important role and function to act as an osmoprotectant, in regulating and absorbing potential redox energy as well as acting as a protection against hydroxy macromolecules and as a solute, an important means of reducing the level of acidity that occurs (Zadehbagheri et al., 2014). Proline functions as energy and a means to reduce acidity in cells, acting as a store of nitrogen and alkaline compounds for rapid plant growth after stress (Heuer, 2010). The presence of proline in plants can reduce stress due to NaCl on oxygenase and Rubisco carboxylase activities(Solomon et al., 1994). Nevertheless, the application of proline to restore plants, experiencing salinity stress is taken seriously and causes abnormal plant growth, decreased production, and even death (Hayat et al., 2012). Unlike this study that proline spray had a beneficial effect on the yield component; number of seeds per panicle.

Among the interactions between factors and influences of three single factors; varieties, salinity and proline level, only a single factor of salinity had a significant effect on the difference of 100-seeds weight at harvesting stage in rice (Table 20). Which, the highest value was found in plant grown under nil normal watering (at 0 mM NaCl), and decreased values in respectively was occurred in plants grown under increased salinity levels; at 50 mM, 100 mM, and 150 mM NaCl, respectively.

Table 20 Weight of 100 seeds (g) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	2.18 ± 0.27	1.95 ± 0.74	1.73 ± 0.69	1.97 ± .48	1.96 ± 0.67
	50	2.41 ± 0.26	1.66 ± 1.01	1.71 ± 0.77	1.63 ± 0.90	
	100	1.71 ± 1.06	2.02 ± 0.67	2.16 ± 0.15	1.10 ± 0.67	
	150	2.04 ± 0.95	1.98 ± 0.62	2.13 ± 0.45	2.10 ± 0.73	
PT 1	0	2.40 ± 0.37	1.62 ± 0.45	1.82 ± 0.70	1.83 ± 0.70	2.07 ± 0.62
	50	2.41 ± 0.32	1.98 ± 0.60	1.56 ± 1.01	2.12 ± 0.09	
	100	2.27 ± 0.77	2.35 ± 0.36	2.23 ± 0.22	2.17 ± 0.51	
	150	2.19 ± 1.01	2.35 ± 0.28	2.30 ± 0.50	1.56 ± 0.87	
IN 35	0	2.30 ± 0.84	2.23 ± 0.70	1.50 ± 0.81	1.60 ± 0.71	2.00 ± 0.76
	50	2.50 ± 0.40	1.86 ± 0.65	1.84 ± 0.64	2.05 ± 0.75	
	100	2.50 ± 0.62	2.20 ± 0.87	2.14 ± 0.67	1.87 ± 0.66	
	150	2.63 ± 0.63	1.99 ± 1.03	1.76 ± 1.11	1.16 ± 0.57	
Mean salinity		2.29 ± 0.64 A	2.01 ± 0.67 B	1.90 ± 0.67 B	1.84 ± 0.67 B	
Proline (mM)						
Mean Proline		0	50	100	150	
		1.92 ± 0.65	1.98 ± 0.68	2.13 ± 0.62	2.01 ± 0.76	
P-value (F-test)						
Variety (V) 0.594 NS, Salinity (S) 0.00202**, Proline (P) 0.376 NS, V × S 0.502 NS, V× P 0.808 NS, S× P 0.383 NS, V× S × P 0.899 NS, CV% 33.81						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Non-significant differences affected by rice varieties in this study, the overall mean in those varieties includes CNT 1, PT 1, and IN 35 at 1.96, 2.07, and 2.00 g per

100 seeds, respectively (Table 20). Compared with previous studies, namely the growth and production of several rice varieties at various levels of salt, with the results on varieties IR 64 (2.31), Ciherang (2.37), Inpari 11 (2.54), IRBB-27 (2.28) and Inpara (2.29) (Jalil et al., 2018). As with any level of proline use, the increased 100-seeds weight was not statistically significant compared to the control treatment at 0 mM proline. The problem of low-weight seed of rice when grown under salinity-affected areas is constantly evident in agriculture, and resulting in a tremendous impact on product sales.

In this study, all varieties of rice were affected because salinity led to similar seed weight reduction, it is the result of the influence of heredity and environmental factors (Manela, 2015). (Mindari, 2009) indicated that one effect of NaCl on rice production is a decrease in weight of 100 grains, and the total protein content in seeds causes excess Na^+ absorption. As a result of excessive salinity, the water in plants is reduced, and if it occurs in the reproductive phase, especially during grain filling, it will have a direct effect on yield productivity (Khodabakhshi et al., 2021). Maintenance can be increased in the tissue through the process of respiration which is considered to be the main cause of decreased carbohydrate accumulation in yield of plant growth during salinity stress (Jabeen and Baba, 2018).

However, the increase in plant tolerance to salt is still difficult to understand, because the effect of salt is generally found in almost all aspects of plant physiology and biochemistry, both at the cell and plant level. Due to the function and integrity of membranes and metabolic processes in plants are disturbed, because it is caused by salinity through the osmotic effect and through toxic ions. So that, internal solutes are not balanced and the absorption of important nutrients (Ibrahim, 2016). Alternative solving in case of salinity stress for plant growth is to adopt external solutes to the plant for nutrients balance adjustment. In order to cope with salt stress, plants exhibit physiological and biochemical adaptation properties. These all involve various accumulations of osmotically active molecules or ions including soluble sugars, proline, sugar alcohols, glyceryl betaine, mannitol, glycerol, sorbitol, organic acids, calcium, potassium, chloride ions, abscisic acid and osmotine (Masoudi-Sadaghiani et al., 2011).

The presence of proline is involved in the response to many factors that arise, especially those related to the environment and one of them is salt (Filippou et al., 2014). However, proline has a role that is considered appropriate on general occasions in responding to plant stress, which is still lacking and some hypotheses suggest that the accumulation of proline is induced by stress (Ashraf and Foolad, 2007b). Thus, the use of proline must also take into account the variety's original ability to produce this compound while exposed to salinity stress.

Percentage filled grain at harvest stage of both varieties and salinity factors showed highly significant ($P<0.01$), except proline was not significant found (Table 21). Non-significant difference in filled grain percentage was observed affected by the interaction between any factors both two and three factors (Table 21). The three rice varieties that obtained the highest percentage of filled seeds were in rice variety IN 35 (43.03%) and followed by CNT 1 (39.25%) and PT 1 (34.75%), respectively. The highest percentage of grain content of the three rice varieties obtained was IN 35 (43.03%) and followed by CNT 1 (39.25%) and PT 1 (34.75%), respectively. When compared with previous studies with the title of growth and production of several rice varieties at various salinity levels, the yields on the percentage of seed content of each variety were IR 64 (51.94), Ciherang (56.05), Inpari 11 (43.16), IRBB-27 (48.94) and Inpara (52.95) (Jalil et al., 2018). In general, whole rice plants are sensitive to salt, there are certain varieties that have strong properties against high salt concentrations. There are serious concerns about the plant stands and its development and yield components are largely influenced by genetic traits and salts concentration (Hasanuzzaman et al., 2009).

Comparing in reduction percentage on percent of filled grain between the normal condition; at 0 mM NaCl and no salinity, and the overall mean in each variety, there were -7.08% in IN 35, and -9.98% in CNT 1, and -10.87% in PT 1. Tolerance of plants and salt as in Inpari 35 over than Thai rice: CNT 1 and PT 1can occur for the inherent capacity and the presence of genes that are more tolerant of stress (Abbas et al., 2013). (Ghassemi-Golezani et al., 2011a) suggested the plant species that have the capacity to reject some level of salinity, depending on the number of metacentric or diploid chromosomes. Another reason is considered to differentiate tolerance of

physiological associations and several mechanisms of rice variety plant growth (Nandi et al., 2005).

Results showed with increasing salinity, percentage of filled grain decreased, especially at 100 mM and 150 mM NaCl (Table 21). The presence of a withered seed significantly affected agricultural productivity under growing in saline soil conditions. In order for plants to survive under salt stress, it is necessary to establish ion homeostasis which is the most important requirement. This is ionic and homeostasis has a special meaning and consideration, because in salinity condition, the concentration of Na^+ and CI^- below the soil influenced seriously by uptake into plants (Bernstein, 1975).

Table 21 Percentage of filled grain in panicle of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				**Mean** Varieties
		0	50	100	150	
CNT 1	0	43.60 ± 14.74	39.99 ± 12.76	36.23 ± 9.96	31.17 ± 4.27	39.25 ± 12.01 b
	50	45.22 ± 15.33	39.43 ± 12.90	34.18 ± 11.42	32.30 ± 2.96	
	100	45.65 ± 15.80	42.04 ± 13.28	38.80 ± 11.19	32.95 ± 2.81	
	150	49.67 ± 16.21	45.64 ± 15.01	38.16 ± 11.49	32.99 ± 5.52	
PTT 1	0	38.99 ± 12.87	36.42 ± 12.93	31.46 ± 5.43	28.82 ± 3.70	34.75 ± 10.80 c
	50	39.89 ± 14.27	36.11 ± 14.10	28.52 ± 10.57	30.26 ± 3.31	
	100	40.18 ± 15.02	37.33 ± 13.98	32.44 ± 5.24	30.21 ± 3.20	
	150	42.46 ± 14.93	40.01 ± 15.60	32.67 ± 5.98	30.27 ± 5.46	
IN 35	0	46.31 ± 15.25	43.30 ± 13.14	39.53 ± 10.28	36.53 ± 7.64	43.03 ± 12.40 a
	50	48.33 ± 15.48	44.59 ± 12.95	37.35 ± 14.60	37.01 ± 6.11	
	100	48.70 ± 15.60	45.47 ± 13.58	42.60 ± 12.65	36.87 ± 4.73	
	150	51.13 ± 15.87	49.55 ± 15.06	41.50 ± 15.01	39.76 ± 5.55	
Mean salinity		45.01 ± 14.20 A	41.66 ± 13.09 A	36.11 ± 10.58 B	33.26 ± 5.49 B	
Proline (mM)						
Mean Proline		0	50	100	150	
		37.70 ± 11.16	37.76 ± 12.31	39.43 ± 11.89	41.15 ± 13.24	
P-value (F-test)						
Variety (V) 8.68 x 10^{-5**}, Salinity (S) 2.81 x 10^{-7**}, Proline (P) 0.329 NS, V × S 0.997 NS, V× P 1.000 NS, S× P 0.998 NS, V× S × P 1.000 NS, CV% 30.31						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Thus, selectively salt tolerance of the three rice genotypes has been attributed to their respective ability to partition Na^+ and Cl^- to specific parts of the plant in the leaf and sheath to protect the damage from these ions in high concentration, and has ability to uptake K^+ into growing parts (Rahnama et al., 2011). Salt properties that can renew the condition of plant, through its toxic effects. The main cause of salt in plants is that it can change plant conditions from normal turned to stress. This occurs in physiological disorders which include membranes, decreased mineral nutrition, impaired reaction oxygen species (ROS) detoxification ability, altered antioxidant enzymes, decreased photosynthetic activity and photosynthetic pigment biosynthesis (Cakmak, 2000). However, soil salinity levels that are toxic to genetically/diverse plant species, are not easy to predict but most salt concentrations that are high or excessive for plants can lead to reduced capacity and all agronomic properties of plants (Soltabayeva et al., 2021).

The proline content factor did not show a significant difference in the percentage of filled seeds in Table 10. The addition of proline content was 0 mM up to 150 mM proline showed the increased value of percentage of filled grain was 3.45%.

Again, in summarizing the effects of exogenous proline application on rice plants in the tillering phase on the yield component only within each panicle, it was found that the use of proline had a positive effect on the characterization of the number of seeds per panicle. But they did not have a statistical influence on the percentage of filled seeds and 100 seed weight. It is reasonable because the use of external proline was carried out during tillering that is related in panicle formation and determining the number of seeds per panicle (Li et al., 2016). While successful fertilizing will occur at the flowering phase, and starch accumulation are related to the seed replenishment phase in the reproductive stage of rice While (Hu et al., 2019).

The ability of quality and sturdy plants for certain conservation is carried out, so that the plants become strong. It is under controlled use of substances to offer the advantage for plants productivity under good health-promoting constituents (Halpin, 2005). The overall plant adaptation experienced according to salt stress while increasing the biosynthesis of secondary metabolites, such as dissolved solids, amino acids, proteins, sugars and organic acids (Fougere et al., 1991), undergo an action that

acts as an osmoregulators in stabilizing turgor in plants that are subjected to salt stress (Seifikalhor et al., 2019).

There are several free amino acids in plants in the stress-free condition that proline has available (Han et al., 2021). However, in plant faced with various stresses, the nature of responding to various stresses by embracing certain specifics by the metabolic, which is very well known is the amino acid in general as well as proline in particular (Arya et al., 2019). In tolerance or resistant plant species or varieties, substances are produced to adjust the metabolize inside of the plant, such as case of internal proline accumulation (Kordrostami and Rabiei, 2019). Nevertheless, external substances use has also been studies and found to play an important positive role in plants as well (Rostami and Azhdarpoor, 2019).

Although accumulation of proline at higher rates has been reported to increase seed germination, tiller and panicle formation, percentage of fill seeds of rice and other cereals (Salahvarzi et al., 2021), the growth phase in which proline is used should also be considered.

In cereal plants, as in other crops, increased production of grain and/or seeds is continuously associated with various endo and exogenous factors. Among them, the various morpho-physiological means and mechanisms that are interrelated and contribute to an increase in allocation of biomass (plant assimilation) for the reproduction of plant parts are very important.

The results of the F-test on the factors both in individual factor and in combination in interaction effect on harvest index (in percentage) in rice was shown in Table 22. There was a highly significant difference on harvest index affected by individual factors: varieties, salinity levels, and proline levels. However, no significant difference was observed in any interaction between factors. IN 35 obtained a higher harvest index value (31.98%) compared to other varieties; CNT 1 (28.03%) and PT 1 (22.83%), respectively. From these results, compared with previous studies on the effect of salt stress after final boot stage on yield and antioxidant capacity in pigmented rice grains and reduction of salt yield with exogenous spermidine, the yield index produced in varieties is as follows; KKU-LR-039 (+33%), Niewdam Gs.no.00621 (+14%), Pokkali (+8%), KKU-LLR-012 (+8%) (Chunthaburee et al., 2015).

Harvest index or HI is calculated based on the ratio between the grain yield and the total above-ground biomass. Although these characteristics, HI, is specific to a particular plant genetics. Higher or lower changeable in HI, especially in cereal crops, showed the success of productivity per plant species or variety growth ratio (Farokhzadeh et al., 2020). HI, this value reflects the plant's ability to generate value and profits, especially in grains per vegetative par: leaves and stems (Slafer et al., 2021). For this reason, either effect of salinity or the use of external additives may affect plant growth, but will it affect the grain yield ratio or not, evaluation is necessary.

Table 22 Harvest index (in percentage) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	29.80 ± 4.21	27.43 ± 3.92	25.27 ± 3.51	22.21 ± 3.15	28.03 ± 4.72 b
	50	28.05 ± 5.53	28.43 ± 3.92	26.27 ± 3.51	23.61 ± 3.70	
	100	32.65 ± 3.64	30.43 ± 3.92	28.07 ± 3.25	24.61 ± 3.86	
	150	34.69 ± 3.66	31.83 ± 3.73	28.47 ± 3.32	26.61 ± 3.38	
PTT 1	0	24.20 ± 3.63	21.54 ± 3.70	19.92 ± 3.70	17.16 ± 3.71	22.83 ± 4.54 c
	50	24.40 ± 3.64	22.54 ± 3.70	20.92 ± 3.70	18.58 ± 3.71	
	100	27.20 ± 2.95	24.54 ± 3.19	22.92 ± 3.34	19.38 ± 5.51	
	150	29.64 ± 3.10	26.14 ± 2.70	33.95 ± 3.35	21.75 ± 3.54	
IN 35	0	33.20 ± 2.49	27.52 ± 3.74	27.92 ± 1.84	23.37 ± 1.68	31.98 ± 5.81 a
	50	32.22 ± 6.72	33.52 ± 3.61	29.34 ± 2.30	25.77 ± 2.88	
	100	39.82 ± 4.91	36.32 ± 3.21	32.14 ± 2.97	28.17 ± 1.99	
	150	41.16 ± 4.31	38.16 ± 2.92	33.65 ± 3.02	29.37 ± 1.63	
Mean salinity		31.42 ± 6.43 A	29.03 ± 5.96 B	26.57 ± 4.98 C	23.42 ± 4.72 D	
Proline (mM)						
Mean Proline		0	50	100	150	
		24.99 ± 5.24 c	26.14 ± 5.64 c	28.85 ± 6.50 b	30.45 ± 6.28 a	
P-value (F-test)						
Variety (V) < 2 x 10^{-16**}, Salinity (S) < 2 x 10^{-16**}, Proline (P) < 2.54 x 10^{-15**}, V × S 0.497 NS, V× P 0.262 NS, S× P 0.489 NS, V× S × P 1.000 NS, CV% 13.06						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Considering the HI values (in percentage) of these varieties grown under normal condition at 0 mM NaCl and 0 mM proline, it was found that IN 35 (33.20%) was still the variety with higher on HI than both Thai varieties: CNT 1(29.80%) and PT 1(24.20%). However, all HI values in these varieties affected by salinity, assessed by the reduction of HI percentage in overall means. This showed that each variety had a greater reduction in grain yield than the stem and leaf parts.

In addition, the effect of salinity was confirmed by the influence of salinity on the statistically significant difference of the HI value (Table 22). Increasing the salinity will decrease the HI accordingly. Compared at the control treatment at 0 mM NaCl with the highest salinity at 150 mM NaCl, there was about 8% reduction.

The difference in harvest index is due to the assimilation of supply to the part of the plant, namely seeds, due to different source and sink partitioning. This can happen, because in general the sources of plant photosynthesis in different varieties can experience different pressures. Varieties that are under stress will absorb less photosynthesis than certain parts that are not stressed (Tester and Davenport, 2003). Finally, the proportional partition between photosynthetic and reproductive parts under stressful conditions is highly apparent. These will make it possible to assess the effects of various varieties from growing under unsuitable condition such as salinity. It can be said that the difference in the HI value in plants in various varieties is caused by the ability of plants to adapt, to different stress conditions (Venkateswarlu and Visperas, 1987). Plants that are deficient or intolerant of salinity experience various structural changes in their ultra-cells, namely swelling of the mitochondria and golgi bodies, an increase in the number of endoplasmic reticulum and damage to chloroplasts (Miyake et al., 1984). In addition, plants will experience various changes in metabolic activity, including a decrease in the rate of photosynthesis, an increase in the respiration rate, a change in the composition of amino acids, and at the same time a decrease in sugar and starch levels in plants. plant tissue plant (Impa et al., 2019). Furthermore, soil salinity can change and inhibit all parameters of observed plant growth and development (de Oliveira et al., 2013; Mola et al., 2021; Xu et al., 2021) also reported that in salinity levels increased, caused reduce the result of HI.

In osmotic regulation, the process of forming the accumulated material from the process of photosynthesis occurs through the movement of solutes called

osmolytes, which affect the movement of water between cells; to increase the standard of osmotic tolerance is one of the mechanisms involved in salt tolerance (Kirst, 1990). In general, the genotypes/species that respond to salt exposure or drought stress are adjusted for increased osmosis, changes in cell wall elasticity, decreased saturation/dry weight ratio and increased percentage of water apoplastic, which minimizes the effect of salinity levels by retaining leaves (Sanders and Arndt, 2012). The rate of osmotic adjustment can also be affected by some of the speed at which pressure is applied, and the genetic of those plant species or the age of the corresponding plant, among other factors (Hsiao et al., 1976). Exceptions to salt can occur in the roots of rice plants, thus preventing the movement of ions into the plant air. This mechanism occurs in some glycophytes and in most halophytes (salt tolerant) (Zhang and Shi, 2013). The development of salt vesicles in the epidermis can accelerate salt excretion, preventing salt buildup in various plant organs such as stems and leaves. Meanwhile, an example of the exclusion mechanism is the accumulation of salt on old leaves which causes death and loss of these organs which affects the harvest index (Kumar, 2020).

The proline factor is very significant to the HI percentage. The increase in HI value from the control treatment (0 mM proline) was observed at 100 mM proline and increased with the amount of proline used (Table 22). Actually, in higher plant groups, biosynthesis of the affected proline can be continued, either via the glutamate or ornithine pathways. The former converts glutamate to proline, in a two-step pathway and in the most important biochemistry is considered a physiological condition, and under nitrogen deficiency or Osmotic pressure is the pressure caused by water at different concentrations due to the dilution of water by dissolved molecules (solute), notably salts and nutrients.

Whereas the ultimate destination to proline via ornithine deamination and is thought to act mainly under conditions of adequate capability than elemental, nitrogen associated with salt tolerant cultivars (Wang et al., 2019). This is called indirect evidence of the glutamate-like proline pathway hypothesis occurring in plants (Forde and Lea, 2007). The overall plant response to osmotic stress consists of the accumulation of several compatible osmolytes, one of which is proline. Proline is thought to have the ability and protect plant cells from damage that caused by salt and

any environmental stress (Trovato et al., 2008). Molecular basis is an effort of salt resistance of various types and models formed in the halophyte species *Thellungella halophila*, and found a more detailed relationship between osmotolerant, increased levels of proline, and tight control in Na^+ absorption (Kant, 2006). Since some of the acquired toxic ions, especially Na^+ and Cl^-, are more, likely to accumulate in cells when subjected to salt stress, it is more important for plant cells to ensure, and maintain low levels of toxic Na^+ ions. The Na^+ compartment is supported by streams that act as specific transporters, such as the antiporter on the plasma membrane gen essential for salt tolerant in tomato plant (SOS1) Na^+/H^+ (Shi et al., 2002).

With the accumulation of several compatible osmolytes in the cytosol, to balance the osmotic potential of the cytosol and vacuoles stably. Besides having compatibility and osmolyte, it also has enzymes that function as stabilizers, membranes and subcellular structures (Kandpal and Rao, 1985) Proline has been selected and proposed to act as a scavenger against reactive oxygen species (ROS) by making a change with whatever form of stirring can be stabilized with the latter (Floyd et al., 2002). Moreover, according to reported by (Hare and Cress, 1997) that the amount of proline is produced in large quantities, with the aim of having the ability to respond to the greatest amount of stress and to function as an osmolite and be released with ROS. It is said that the proline in mitochondria can provide a redox-related support system and stabilize certain specific metabolic pathways during and after osmotic stress in plants.

For yield performance, only highly significant difference affected by salinity level; no significant difference was found both in other individual factor or in combination between any factors (Table 23). The three rice varieties had grain yield per plant were 4.00 g in IN 35, 3.60 g in CNT 1 and 4.07 g in PT 1. Although IN 35 exhibits characteristics indicating that it is more tolerant to salinity than the Thai varieties (CNT 1 and PT 1), the grain yield is not very high compared to Thai rice.

In this study it can be concluded that plant genetic traits can affect grain yield. On this occasion, both PT 1 obtained the same maximum grain yield as IN 35 while the lowest was at variety CNT 1; despite the fact that CNT 1 seems to be more tolerance than PT 1. In grain production, the yield of grain in each variety depends on its performance and potential as well as the genetics of a particular species. For plants

that have the production of each variety, plant is used by their ability adaption to the environment and the place where they are grown (Egea et al., 2017). From these results, it is possible to develop rice varieties for planting in salinity areas in Thailand. However, the use of the main genetic base of high-yielding Thai rice with good adaptability in domestic growing conditions for parent for breeding is very necessary. Considerations must be mad on both the salt tolerance and the yielding ability of the variety. Plants that are declared to be adaptable are those that have a unique response ability, that is, plants that try to attack under any circumstances, anytime and anywhere. Plants adapt morphologically and physiologically that often occur in plants in general and can be achieved naturally even though the plant materials used are the same (Ulukan, 2008).

Salinity factor, the results showed that the level of salinity had a highly significant effect on grain yields of rice plants (Table 23). The yield of grain at 0 mM (control) and 50 mM salinity levels did not differ from the results shown, the addition of salinity levels from 100 mM to 150 mM resulted in a decrease in grain yield in accordance with the increase in salinity levels. The level of salinity is assumed to affect and reduce grain yield according to the concentration absorbed in soil (Radanielson et al., 2018a). There are various effects which are thought to inhibit severe salt concentration on soil fertility. Which, may be due to different competition for carbohydrate supply between vegetative growth and limited supply to all parts of the plant, as well as reducing pollen viability under stress conditions. (Maakip et al., 2017). The grain yield decreased in rice varieties due to an increase in the level of salt stress which was also reported by (Li et al., 2019).

In statistical analysis, the proline level factor did not show a significant difference to the grain yield as well (Table 23). Proline levels did not provide significant results for the four levels used, although at the proline application; at level ≥50 mM proline gave higher grain yields than the control treatment (0 mM proline). From the results, proline had a significant effect on agronomic characteristics that appeared since vegetative stage, and the number of seeds per panicle. Those effects also depended on the timing of the external proline use given only at tillering stage. Proline applied to the vegetative or tiller stages of rice plants reduces some of the unexpected effects of saline stress. Hayat et al., (2013) observed that exogenous

proline applied to plants at the vegetative stage greatly reduced and damaged salt stress in wheat. Notwithstanding, the effects of proline application seem to remain very litter in the grain yield.

Table 23 Grain yield (g per plant) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of proline (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	3.14 ± 0.85	3.20 ± 1.65	2.56 ± 0.84	3.18 ± 1.27	3.60 ± 1.70
	50	4.57 ± 1.47	3.93 ± 3.11	3.32 ± 1.78	3.28 ± 2.50	
	100	3.54 ± 2.05	4.14 ± 2.00	4.33 ± 1.57	4.06 ± 1.65	
	150	4.00 ± 2.26	3.33 ± 1.62	3.62 ± 0.91	3.32 ±1.63	
PT 1	0	4.52 ±0.65	2.84 ± 0.86	3.31 ± 1.73	3.17 ± 1.54	4.07 ± 1.81
	50	5.30 ±2.04	4.11 ± 1.70	2.90 ± 2.03	3.65 ± 0.84	
	100	4.67 ±1.81	4.40 ±1.63	5.39 ± 1.87	3.92 ± 0.86	
	150	4.05 ± 2.53	5.67 ± 1.50	4.64 ± 2.91	2.60 ± 1.48	
IN 35	0	5.06 ± 3.26	5.76 ± 3.17	2.45 ± 1.23	2.44 ± 1.01	4.00 ± 2.31
	50	4.55 ± 2.54	3.31 ± 1.38	2.95 ± 1.14	3.83 ± 2.31	
	100	5.34 ± 2.15	4.13 ± 1.85	4.05 ±1.75	3.53 ± 2.06	
	150	6.44 ± 1.75	5.27 ± 3.50	2.50 ±1.43	2.38 ± 1.30	
Mean salinity		4.60 ± 2.05 A	4.17 ± 2.16 AB	3.50 ± 1.77 BC	3.28 ± 1.56 C	
Proline (mM)						
Mean Proline		0	50	100	150	
		3.47 ± 1.86	3.81 ± 1.94	4.30 ± 1.72	4.00 ± 2.33	
P-value (F-test)						
Variety (V) 0.228, Salinity (S) 0.00056**, Proline (P) 0.122 NS, V × S 0.125 NS, V× P 0.907 NS, S× P 0.473 NS, V× S × P 0.673 NS, CV% 48.81						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Osmotic stress and an imbalance of contained ion are giving rise to positive oxidative stress. Proline acts, as an appropriate helper in stabilizing and increasing stress tolerance in plants, which should be further investigated in relation to the mechanisms involved (Xu and Xue, 2019). Therefore, the further use of proline in the reproductive phase may have a more pronounced effect on the yield. Previous

researchers proved that the application of proline improved several plant characteristics at the vegetative stage, which includes the number of tillers, panicle length, and seed yield as well as the number of fertile tillers, the weight of a thousand grains and the length of the panicle at the productive stage, (Deivanai et al., 2011).

Experiment II: Applying trehalose to alleviate salty stress in rice plant at tillering stage

Effects on biochemical characteristics in rice at chemical difference of trehalose application in salinity condition

Soil salinity is a common limiting factor in crop production. Therefore, the application of external solute accompanied by low molecular weight as trehalose was conducted at tillering stage. More than to determine molecular and chemical, the yield, yield components, and agronomic characteristics also were recorded at harvesting stage.

For three factors in the studies; varieties, salinity level, and trehalose level had significant differences in relative water content (RWC) affected by individual factors. However, no significant difference on RWC affected by interactions between any factors (Table 24). The highest average of RWC was found in the IN 35 (55.53 %), followed by CNT 1 (52.61%) and the lowest at PT 1 (49.15%). However, the results of varieties that have relatively consistent water content compared to previous studies regarding the application of exogenous proline to increase salt tolerance in rice by modulating the antioxidant activity of Super Basmati (53.10 %) and Shaheen Basmati (55.42 %) varieties at seedling stage, respectively (Tabssum et al., 2018; Wang et al., 2020). However, plants that have the ability to accumulate water are of course for the needs and processes of their metabolic activities, so that water can be supplied to certain parts of the plant that need it. This is very important in the physiology of plants that contain water. Water is a growth that plays a role in the development of every plant (Jackson et al., 1977). Water is the main constituent in the development of protoplasm. Plants that are able to absorb water in a high capacity have the opportunity to absorb nutrients from soil and the results of photosynthesis process, distributing these absorbed elements to all parts of certain plants. The results of Fisher's test (Test F) on the effect of salinity showed that the salt stress with various levels received by rice plants, especially at the relative water content was very

different and started at 50 mM NaCl and was more severe at 150 mM NaCl (Table 24). At various levels of salinity causes a decrease in water content in plants since the beginning of tiller formation (Zeng and Shannon, 2000b). Salt stress begins to attack and inhibit sensitive plant parts, such as plant cells, thereby reducing the need for water absorbed by plants (Ahanger et al., 2017b). Highest RWC percentage was found at 0 mM NaCl (55.76 %) and 50 mM NaCl (55.21 %), and lower at 100 mM NaCl (50.03 %) and 150 mM NaCl (47.40 %). RWC percentage was continuously increasing when trehalose level for application increased, however, no significant difference of values between 100 mM and 150 mM trehalose. Compare to previous study trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems; the activities were in seedling stage and RWC at control salinity level (0 mM NaCl) (99.20 %), 150 Mm NaCl (84.2%) and 250 mM NaCl (73.91%).

Water is an important factor in plant physiology because water is used for processes of metabolic activities (Jackson et al., 1977; Ehrler et al., 1978). Plants that are able to absorb water in a high capacity will have the opportunity to absorb nutrients from the soil. Also, water has the role to distribute the results of the substance of the photosynthesis process to all plant parts (Ehrler et al., 1978; Yan et al., 2013b) Moreover, water has the role to maintain cell turgidity and opening stomata in leaves (Sanders and Arndt, 2012). Various levels of salinity have caused a decrease in water content in plants since the beginning of tiller formation (Zeng and Shannon, 2000b). Then, salt stress resulted to attack and inhibit sensitive plant parts, such as plant cells (Ahanger et al., 2017a). Salt concentration causes disruption of homeostasis in water relations and changes in the distribution of ions in various cellular systems throughout the plant (Van Breusegem et al., 2001a). Decreased plants growth under salinity increased is one of the consequences of the most important process in plants (Yan et al., 2013b; Yang et al., 2020). Salt stress can inhibit photosynthetic activity by reducing water potential. So, the main goal of salt tolerance is to increase the efficiency of various water uses under salinity (Adolf et al., 2013).

Greater dehydration at higher concentrations of NaCl application due to increased plant cellular water loss (Ahanger et al., 2017b). The most brutal factor is salinity, thus limiting the space for plants to absorb and store water within the plants

themselves, as most plants are sensitive to salinity, mainly due to the high concentration of salt in the soil (Hasanuzzaman et al., 2013b). Allegedly, the effect of salinity stress is caused by the difficulty of water absorption and the effect of NaCl application. As a result, it inhibits the development process in cells and reduces the water content of rice varieties for rice varieties compared to rice plants under normal conditions (Fukao and Bailey-Serres, 2008). With increasing salt concentration, it causes disruption of homeostasis in water relations and changes in the distribution of ions in various cellular systems throughout the plant. Then, changes in water homeostasis also cause damage to molecules that result in death due to the cessation of plant growth and development (Van Breusegem et al., 2001b). Decreased plant growth and increased salinity conditions are some of the consequences of the physiological response and photosynthesis is the most important process to consider in plants (Yan et al., 2013a). In the form of ion absorption, through petrified water which is the most practical and cheapest form of osmotic adjustment in soil salinity conditions, in this section it will cause problems that have an impact on decreased function in plant leaves as well as ion imbalance and toxic conditions (Magand et al., 2020). Salt stress can inhibit photosynthetic activity by reducing water potential. So, the main goal of salt tolerance is to increase the efficiency of various water uses under salinity (Yang et al., 2020). For this change allows plants to reduce water loss to plants i.e. leaves, by opening their stomata at night, thereby reducing transpiration water loss under various prolonged salinity conditions (Ball and Farquhar, 1984).

Table 24 Relative water content (RWC) (%) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	53.67 ± 7.30	50.83 ± 4.76	47.10 ± 8.07	46.38 ± 3.94	51.61 ± 6.18 b
	50	52.35 ± 9.50	49.35 ± 3.93	49.71 ± 1.52	46.41 ± 1.56	
	100	60.84 ± 3.20	51.28 ± 3.22	48.51 ± 6.24	51.78 ± 2.75	
	150	56.00 ± 8.55	59.40 ± 4.62	53.14 ± 4.35	47.02 ± 6.64	
PT 1	0	51.75 ± 7.25	50.23 ± 6.65	47.44 ± 3.05	42.28 ± 3.23	49.15 ± 7.10 c
	50	52.17 ± 8.73	54.01 ± 4.08	45.90 ± 1.38	40.54 ± 3.95	
	100	53.88 ± 8.72	53.19 ± 7.98	50.38 ± 6.36	45.70 ± 5.33	
	150	56.10 ± 4.97	54.14 ± 3.13	45.25 ± 10.66	43.52 ± 8.96	
IN 35	0	53.95 ± 5.28	58.20 ± 2.58	51.77 ± 2.72	48.17 ± 5,23	55.53 ± 5.97 a
	50	58.27 ± 8.97	58.93 ± 3.58	54.49 ± 4.44	52.61 ± 3.13	
	100	58.80 ± 8.81	59.30 ± 5.82	51.80 ± 5.16	52.70 ± 5.13	
	150	62.34 ± 4.80	60.63 ± 5.23	54.91 ± 3.61	51.64 ± 7.53	
Mean salinity		55.76 ± 7.00 A	55.21 ± 5.52 A	50.03 ± 5.52 B	47.40 ± 5.87 B	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		50.06 ± 6.01 b	51.56 ± 6.75 ab	53.09 ± 6.75 a	53.68 ± 7.91 a	
P-value (F-test)						
Variety (V) 2.90 x 10^{-6} **, Salinity (S) 3.97 x 10^{-9} **, Trehalose (T) 0.0446*, V × S 0.874 NS, V × T 0.923 NS, S × T 0.914 NS, V× S × T 0.989 NS, CV% 11.192						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

The results showed that the highest value was observed at the highest RWC on plants received trehalose level at 150 mM (53.68 %) and the lowest at zero trehalose application (0 mM) (50.06 %). The increasing about 3.62% compared to values between 150 mM and 0 mM trehalose application in RWC in plants. Comparing with previous study trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems; the activities were in seedling stage and RWC at trehalose control 0 mM NaCl (99.20 %), 10 mM NaCl (98.13%) (Mostofa et al., 2015a).

Trehalose is involved in various responses to salinity and cold and in the regulation of stomatal conductance and water use efficiency. This is an important

means to reduce the level of acidity that sometimes occurs at certain times (Khan et al., 2020b). Trehalose serves as a means and energy to reduce acidity in certain plant cells, acting as a storage of nitrogen compounds for rapid plant growth after stress occurs (Elbein et al., 2003).

In principle, the most important function of trehalose is as an osmoprotectant; in regulating and absorbing redox potential energy in acting as a shield against hydroxy macromolecules and solutes through the water. Trehalose serves as a means and energy to reduce acidity in certain plant cells, acting as storage of nitrogen compounds for rapid plant growth after stress occurs (Elbein et al., 2003; Khan et al., 2020b). In this perspective, the presence of trehalose facilitates metabolic processes, regarding the formation of the relative storage of water content in rice plants. On the other hand, increased activity at the trehalose level could enhance the mechanism of action in plants as efficiently as possible in contributing to salt-induced oxidative stress tolerance (Kosar et al., 2019).

Compare the use of external substances (proline and trehalose) based on the ability to increase the water content (RWC) in rice plants compared to those that did not use those external substances. It was found that spraying the rice plants with proline could increase the value more than the use of trehalose (Table 1 and 24).

For Chlorophyll A content in plant leaves, there was highly significant difference affected by individual factors including varieties, salinity levels and trehalose level. However, no significant difference in Chlorophyll A content affected by interaction of any factors either two or three factors (Table 25). Different varieties showed highest Chlorophyll A content in IN 35 (0.32 µmol/g), followed by two Thai rice varieties: CNT 1 (0.31 µmol/g) and PT 1 (0.30 µmol/g). However, these results were compared with previous studies regarding the effect of salinity and the reducing role of gibberellic acid (GA3) to improve the morphological, physiological and yield characteristics of rice varieties. This activity is at the seedling stage; MR219 (0.49 mg/g) and Pokkali (0.43 mg/g) varieties (Misratia et al., 2013). The increase in chlorophyll A is caused by the ability of each genetic trait to receive and accumulate any energy received from sunlight (Landi et al., 2020). (Hawkesford et al., 2012). In addition, there is an opportunity for the formation of sugars and starch obtained in the leaves, to be distributed to certain plant parts that need them, the formation of growth

according to the function and part of each plant part (Crumpton-Taylor et al., 2013). The decrease in the concentration of chlorophyll A received by plants, probably due to its inhibitory effect on the accumulation of ions of various salts, in different and frequent chlorophyll biosynthesis (Shakya et al., 2008). From this point of view, the addition of trehalose facilitates metabolic processes, regarding the formation of chlorophyll content A in plant leaves. On the other hand, increased activity at trehalose levels can increase the step, mechanism of action in plants as efficiently as possible thereby contributing to tolerance to salt-induced oxidative stress (Kosar et al., 2019). A negative impact on Chlorophyll A content was observed in plants grown under salinity and the reduction was detected at 50 mM - 150 mM NaCl; although not much of value increasing. The application of trehalose had a positive effect to induce Chlorophyll A increase.

For the salinity factor, the results showed that the chlorophyll A content in plant leaves decreased depending on the increase in salt content (Table 25). The content of chlorophyll A in plant leaves began to decrease at a salinity level of 50 mM NaCl (0.31 mg/g) until it reached 150 mM NaCl (0.30 mg/g) (Table 25). However, the salinity level affected the decrease in chlorophyll A content in plant leaves significantly since 50 mM and 100 mM NaCl (0.30 mg/g). There is a slight difference in the effect of salinity on the content of chlorophyll A in the leaves of rice plants. The effect is more pronounced and faster for the reduction of accumulated chlorophyll A content at the salinity level at 50 mM NaCl, salinity creates osmotic and through toxic ions. Therefore, compared with previous studies, the effect of salinity and alleviating role of gibberellic acid (GA3) for improving the morphological, physiological and yield traits of rice varieties; salinity application at seedling stage, salinity (NaCl) at 0 mM (control) (1.78 mg/g), 50 mM (1.70 mg/g), 100 mM (1.37mg/g), 150 mM (1.29 mg/g) and 200 mM (1.08 mg/g) (Misratia et al., 2013). Thus, internal solutes are not balanced and absorption of essential nutrients also disrupts various functions and integrity of membranes and metabolic processes for plants (Maas and Nieman, 1978). Salt stress usually causes oxidative stress through the production of ROS. Any increase in ROS can trigger lipid peroxidation which is considered a potential biomarker of salt-induced oxidative damage to membranes,

leading to electrolyte leakage, loss of membrane permeability, and dysfunction of membrane proteins and ion channels (Maas and Nieman, 1978).

Table 25 Chlorophyll A content (µmol/g) in plant leaves of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.02	0.29 ± 0.01	0.31 ± 0.01 b
	50	0.32 ± 0.01	0.31 ± 0.00	0.30 ± 0.02	0.30 ± 0.02	
	100	0.32 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.31 ± 0.02	
	150	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	
PT 1	0	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.02	0.28 ± 0.02	0.30 ± 0.02 b
	50	0.32 ± 0.02	0.30 ± 0.00	0.30 ± 0.01	0.30 ± 0.02	
	100	0.32 ± 0.02	0.30 ± 0.00	0.30 ± 0.01	0.30 ± 0.02	
	150	0.32 ± 0.02	0.31± 0.00	0.31 ± 0.01	0.31 ± 0.02	
IN 35	0	0.31± 0.00	0.31 ± 0.00	0.31 ± 0.01	0.30 ± 0.00	0.32 ± 0.20 a
	50	0.32 ± 0.01	0.33 ± 0.04	0.31 ± 0.01	0.31 ± 0.02	
	100	0.33 ± 0.02	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.03	
	150	0.33 ± 0.02	0.34 ± 0.03	0.33 ± 0.02	0.32 ± 0.03	
Mean salinity		0.317 ± 0.01 A	0.311 ± 0.02 AB	0.305 ± 0.02 B	0.304 ± 0.02 B	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		0.304 ± 0.01 c	0.305 ± 0.02 b	0.312 ± 0.02 ab	0.317 ± 0.02 a	
P-value (F-test)						
Variety (V) 1.12 x 10^{-6**}, Salinity (S) 0.0024**, Trehalose (T) 2.12 x 10^{-6**}, V × S 0.853 NS, V × T 1.000 NS, S × T 0.998 NS, V× S × T 1.000, CV% 5.136						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Although salinity affected Chlorophyll A content in rice leaves in this study, it was slightly difference in the effect of different salinity levels. (Moradi and Ismail, 2007) reported that chlorophyll was more sensitive to salt stress. Thus, loss of chlorophyll in leaves is often used as a cellular indication of the presence of salt stress or resistance to salt stress. The loss of these pigments provides flexibility and is one of the adaptation mechanisms in preventing damage due to photosynthesis, by minimizing the possibility of photoinhibition in plant photosystems (Goh et al., 2012;

Brestic et al., 2015). The decrease in chlorophyll content, in rice, leaves grown at high salinity levels hence reduced light interception (Shabala et al., 1998).

In statistical analysis, for levels of trehalose factor also showed significant differences in chlorophyll A content in plant leaves (Table 25). The level of trehalose can give significant results for the four levels used. In this case, it can be seen that for every addition of trehalose content from 50 mM (0.31 mg/g) to 100 mM (0.31 mg/g), the chlorophyll A content in plant leaves increased although the difference was not significantly different. but increasing the concentration up to 150 mM (0.32 mg/g) can give the result that the grain weight is higher than the three concentrations of trehalose application. However, the results of the study compared trehalose as an osmoprotectant for corn under salinity-induced stress; the application trehalose at seedling stage, with the result of chlorophyll content in trehalose control 0 mM (21.28 mg/g) and 10 mM (24.87 mg/g).

The higher accumulation rate of trehalose was due to increased plant activity which was more efficient related to the formation of chlorophyll A content in plant leaves. Because initially the activity of enzymes involved in trehalose biosynthesis can synergize directly with trehalose administration, so the presence of trehalose can create conducive and stable plant conditions under salt stress (Dirk et al., 2020). The function of trehalose is as a means and energy to reduce acidity in certain plant cells, acting as a storage of nitrogen compounds for plant growth in collecting chlorophyll A content in leaves, which takes place quickly after stress (Sadak, 2019). Trehalose is present in plants to minimize NaCl stress with various activities on carboxylase oxygenase (Redillas et al., 2012). The application of trehalose to restore plants is considered seriously experiencing salinity stress and causes abnormal plant growth, decreased chlorophyll content in leaves, and even death (Hasanuzzaman et al., 2013b). In contrast to this study, trehalose spray had a beneficial effect on the formation of chlorophyll A content in leaves as well as yield components.

The content of Chlorophyll B in plant leaves was significantly different ($P \leq 0.01$) influenced by individual factors; variety, salinity level, and trehalose level (Table 26). However, there was no significant difference in the Chlorophyll B content in the leaves which was influenced by the interaction between two or three factors (Table 26).

Table 26 Chlorophyll B content (µmol/g) in plant leaves of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	1.25 ± 0.12	1.12 ± 0.11	1.06 ± 0.15	1.02 ± 0.10	1.21 ± 0.20 b
	50	1.28 ± 0.06	1.23 ± 0.10	1.12 ± 0.09	1.01 ± 0.14	
	100	1.54 ± 0.24	1.25 ± 0.08	1.06 ± 0.13	1.14 ± 0.15	
	150	1.53 ± 0.07	1.20 ± 0.07	1.24 ± 0.08	1.27 ± 0.11	
PT 1	0	1.20 ± 0.08	1.07 ± 0.12	1.03 ± 0.14	0.98 ± 0.09	1.16 ± 0.16 b
	50	1.24 ± 0.09	1.21 ± 0.10	1.09 ± 0.13	0.98 ± 0.20	
	100	1.40 ± 0.07	1.23 ± 0.09	1.06 ± 0.11	1.09 ± 0.11	
	150	1.47 ± 0.08	1.20 ± 0.09	1.20 ± 0.04	1.21 ± 0.10	
IN 35	0	1.27 ± 0.11	1.17 ± 0.11	1.18 ± 0.10	1.11 ± 0.05	1.26 ± 0.17 a
	50	1.31 ± 0.04	1.22 ± 0.17	1.26 ± 0.24	1.07 ± 0.11	
	100	1.48 ± 0.29	1.30 ± 1.13	1.17 ± 0.10	1.29 ± 0.17	
	150	1.52 ± 0.27	1.31 ± 0.18	1.30 ± 0.09	1.22 ± 0.07	
Mean salinity		1.37 ± 0.17 A	1.21 ± 0.12 A	1.15 ± 0.14 BC	1.12 ± 0.15 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		1.12 ± 0.13 b	1.17 ± 0.15 b	1.25 ± 0.20 a	1.31 ± 0.16 a	
P-value (F-test)						
Variety (V) 0.0019**, Salinity (S) 5.78 x 10^{-13} **, Trehalose (T) 1.02 x 10^{-7}**, V × S 0.877 NS, V× T 0.994 NS, S × T 0.060 NS, V× S × T 1.000 NS, CV% 10.765						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

The three rice varieties that obtained the highest Chlorophyll B content were IN 35 (1.26 µmol/g) followed by CNT 1 (1.21 µmol/g) and PT 1 (1.16 µmol/g) (Table 26). However, these results were compared with previous studies regarding the effect of salinity levels 50 mM, 100 mM, 150 mM and 200 mM NaCl and the reducing role of gibberellic acid (GA3) to improve the morphological, physiological and yield characteristics of rice varieties, at seedling stage with chlorophyll B content in leaves for each variety, namely MR219 (1.71 mg/g) and Pokkali variety (2.18 mg/g) (Hakim et al., 2010). The content of Chlorophyll B in the leaves is very helpful in the process of photosynthesis with the ability to absorb high-energy light. It is green in color and mainly absorbs blue light. Chlorophyll B is more soluble when compared to

chlorophyll A in polar solvents considered to have a carbonyl group (Indrasti et al., 2018). Plants contain Chlorophyll B, which means they have the ability to absorb blue light directly from the sun. The main role of Chlorophyll B is to broaden the absorption spectrum of organisms in plant leaves. The increase in Chlorophyll B is an adaptation to shade, as it allows plants to absorb a wider range of light wavelengths (Pattanayak et al., 2005). Chlorophyll B transfers the extra energy it absorbs to Chlorophyll A (Björn et al., 2009). In this form, Chlorophyll B coexists in plants with an estimated 3:1 ratio to the dominant chlorophyll A (Ting et al., 2002)

For chlorophyll content, it is caused by several internal factors which include; plant genetic, plant age, leaf age, leaf morphology and genetic factors (Yadav et al., 2020). An increase in chlorophyll B content in plants is associated with an increase in chlorophyll protein (Pattanayak et al., 2005).

The content of chlorophyll B in the leaves began to decrease at a salinity level of 50 mM NaCl (1.21 mg/g) until it reached 150 mM NaCl (1.12 mg/g) (Table 26). However, the salinity level affected the decrease in the chlorophyll B content in the leaves clearly since 100 mM NaCl. There is a slight difference in the effect of salinity on the relative water content and chlorophyll A content in leaves. The effect was more pronounced and faster for the reduction of the relative accumulation of water in the leaves at a salinity level of 50 mM NaCl. Therefore, compared with previous studies, the effect of salinity and alleviating role of gibberellic acid (GA3) for improving the morphological, physiological and yield traits of rice varieties; salinity application at seedling stage, salinity level (NaCl) at 0 mM for chlorophyll B content (control) (5.99 mg/g), 50 mM (5.13 mg/g), 100 mM (4.17 mg/g), 150 mM (1.94 mg/g) and 200 mM (1.44 mg/g) (Ashrafuzzaman et al., 2009; Misratia et al., 2013). Although both types of chlorophyll (Chlorophyll A and B) are affected by the increase in salinity, the decrease in content appears to be more pronounced in Chlorophyll B than in Chlorophyll A (Table 25-26). However, the same results for the salinity effect on two types of chlorophyll were that the negative salinity levels were found since at the 50 mM NaCl onwards. Salt stress is now evident and shows that the suppression of induced photosynthesis. Photosynthesis ability is always dependent on changes in gas, photosynthetic pigment, cultivar species or type, stomata, and accumulation of organic and inorganic metabolites and antioxidants (Ashraf and Harris, 2013).

Evidence of accumulation of Na^+ and Cl^- in some leaf tissues is unusual due to increased air loss and cytoplasmic dehydration. Cytoplasmic dehydration causes cells to no longer carry out their normal metabolism, ultimately reducing plant growth and Chlorophyll B content in leaves (Netondo et al., 2004; Reddy et al., 2004), and hence reduced light interception (Azooz et al., 2015). Therefore, under salt stress, chlorophyll loss can be associated with photoinhibition or ROS formation (Goh et al., 2012).

Osmotic adjustment in salt-stressed plants occurs in the presence of accumulation of high concentrations of either inorganic ions or certain low molecular weight organic solutes. Whereas, because both play important roles in higher plants growing under saline conditions, their relative contribution varies between species, between cultivars, and even between different compartments within the same plant (Flowers and Yeo, 1989).

The trehalose content factor showed significant differences in Chlorophyll B content in plant leaves start at 100 mM (Table 26). While the changing in increasing direction on Chlorophyll A content was observed since at 50 mM trehalose (Table 25). The addition of trehalose 0 mM (1.12 mg/g) to 50 mM (1.17 mg/g) showed the same chlorophyll B content and did not differ in the leaves, but increasing the trehalose content up to 100 mM (1.25 mg) and 150 mM (1.31 mg/g) gave higher yields and significantly different from the two low trehalose levels. However, for the two high trehalose levels, the value of the chlorophyll B content in the leaves was not different or the same. In a previous study comparing trehalose as an osmoprotectant for maize under salinity pressure; application of trehalose at the seedling stage, with the results of chlorophyll content in the control trehalose 0 mM (12.26 mg/g) and 10 mM (13.03 mg/g). Although Chlorophyll B appeared to be more susceptible to salinity than Chlorophyll A. Chlorophyll B did not respond rapidly to the use of external trehalose to mitigate the effects of salinity. Trehalose restores the condition of rice varieties that are stressed salt. This is to be able to deviate from the formation of carbohydrates obtained from the soil and sunlight, which is a source of energy (Wassmann et al., 2009a). Trehalose in plants classified as high levels is stated to contain chlorophyll B which is formed in plant leaves and is synthesized in various parts of plants like leaves (Lunn et al., 2014).

For total chlorophyll content in plant leaves, the results on the effect of any factor and interaction between those factors; varieties, salinity levels, and trehalose levels, were similar with the occur in both Chlorophyll A and B. There was no combination interaction between any factor either two or three factors. However, highly significantly affected by the total chlorophyll content (Table 27).

For varieties, the highest total chlorophyll content in plant leaves was found in the IN 35 variety (1.52 µmol/g), followed by CNT 1 (1.46 µmol/g) and PT 1 (1.41 µmol/g). The results of total chlorophyll content (T) levels were compared with previous studies, namely; the effect of salinity levels; 50 mM, 100 mM, 150 mM and 200 mM NaCl and the role of reducing gibberellic acid (GA3) on the improvement of morphology, physiology and yield of rice varieties at seedling stage MR219 (2.20 mg/g) and Pokkali (2.61 mg/g) (Kazemi and Eskandari, 2011). Plants have higher chlorophyll content in leaves, it is able to carry out photosynthesis. On account of plants produces enough food and energy needed for growth and cellular respiration (Volkov, 2006). Response to different levels of both salinity and trehalose use of total chlorophyll consistent results to Chlorophyll B; for the presence of the larger proportion than Chlorophyll A in total chlorophyll. Chlorophyll total (T) is the most abundant pigment formed in plants, especially in leaves and has Mg^{2+} ions (Papenbrock et al., 2000). The degradation of chlorophyll that occurs during the ripening period is the most miraculous process in which the green color completely disappears or fades (Lewis, 1996). Including massive degradation of chlorophyll. The chlorophyll decomposition process is a multi-step, perfectly controlled process handled by six chlorophyll catabolic enzymes and metal chelating agents (MCS) (LENG, 2008). The final catabolites of chlorophyll degradation are transported via specific transporters to the central vacuole. Due to the presence of a chlorophyll molecule which has strong light-absorbing properties, it can act as a phototoxin at cellular potential during high light intensity (Hörtensteiner and Kräutler, 2011). Excessive availability of the means of photosynthesis can transfer energy to oxygen, which in turn results in the normal production of reactive oxygen species in the leaves (Logan, 2008).

The results of Fisher's test (F test) on the effect of salinity showed that the salt stress with various levels received by rice plants, especially the chlorophyll (T)

content in plant leaves was very different because at 50 mM NaCl (1.40 mg/g). Salinity is assessed from the beginning of tillers and the impact is estimated at the harvesting stage. A longer salinity period gives a more pronounced salinity effect at lower concentrations. The results of total chlorophyll content (T) levels were compared with previous studies, Trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems, the activity was in seedling stage at level of salinity (NaCl) for chlorophyll (T) content; control treatment (0 mM) (1.55 mg/g), 150 mM (1.29 mg/g) and 250 mM (0.96 mg/g) (Hasanuzzaman et al., 2012; Mostofa and Fujita, 2013).

Table 27 Total chlorophyll content (µmol/g) in plant leaves of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	1.50 ± 0.13	1.35 ± 0.13	1.29 ± 0.16	1.24 ± 0.12	1.46 ± 0.20 b
	50	1.54 ± 0.06	1.48 ± 0.11	1.36 ± 0.11	1.24 ± 0.16	
	100	1.82 ± 0.26	1.50 ± 0.09	1.30 ± 0.13	1.38 ± 0.17	
	150	1.81 ± 0.07	1.45 ± 0.08	1.49 ± 0.09	1.53 ± 0.13	
PT 1	0	1.45 ± 0.10	1.30 ± 0.14	1.25 ± 0.15	1.20 ± 0.10	1.41 ± 0.18 b
	50	1.50 ± 0.09	1.46 ± 0.11	1.33 ± 0.14	1.20 ± 0.23	
	100	1.66 ± 0.08	1.48 ± 0.10	1.29 ± 0.12	1.33 ± 0.13	
	150	1.75 ± 0.08	1.44 ± 0.10	1.45 ± 0.50	1.46 ± 0.12	
IN 35	0	1.53 ± 0.12	1.42 ± 0.12	1.43 ± 0.12	1.35 ± 0.06	1.52 ± 0.19 a
	50	1.58 ± 0.04	1.49 ± 0.17	1.52 ± 0.27	1.31 ± 0.12	
	100	1.76 ± 0.31	1.56 ± 0.15	1.43 ± 0.10	1.55 ± 0.20	
	150	1.81 ± 0.29	1.59 ± 0.18	1.56 ± 0.09	1.48 ± 0.09	
Mean salinity		1.64 ± 0.19 A	1.40 ± 0.13 B	1.39 ± 0.15 C	1.36 ± 0.17 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		1.36 ± 0.15 a	1.41 ± 0.17 a	1.50 ± 0.22 b	1.57 ± 0.17 b	
P-value (F-test)						
Variety (V) 0.00083**, Salinity (S) 2.94 x 10^{-13**}, Trehalose (T) 3.76 x 10^{-8**}, V × S 0.874 NS, V× T 0.994 NS, S × T 0.065 NS, V× S × T 1.000 NS, CV% 9.814						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Salinity is assessed from the beginning of tillers and the impact is estimated at the harvesting stage. A longer salinity period gives a more pronounced salinity effect at lower concentrations. In this study, the decrease of total content was observed at 50 NaCl onwards. For trehalose level, the increase of total chlorophyll was found at 50 mM trehalose onwards (Table 27). Therefore, the influence of salinity stress which makes it difficult for plants to absorb water and reduce photosynthesize ability that one caused is by the reduction of chlorophyll content (Ashraf and Harris, 2013).

The effect that is considered the main constraint is salinity on plant growth and development (Ghosh et al,. 2011; Arif et al., 2020). Salinity related to the osmotic effect, ionic toxicity, and nutrient imbalance lead to a decrease in photosynthetic efficiency for plants in the formation of chlorophyll types and other physiological disorders (Parihar et al., 2015). Salt causes a decrease in ion concentration and water potential in leaves that have the potential to accumulate chlorophyll (Liu and Shi, 2010; Sharma et al., 2020). Chlorophyll was inhibited and a decrease in the formation of the enzyme rubisco, occurred when the plant was water-deficient (Salvucci and Crafts-Brandner, 2004).

This study showed a significant difference influenced by the concentration of trehalose administration (Table 27). Higher concentrations of chlorophyll total (T) in plant leaves were observed at 100 mM (1.50 mg/g) and 150 mM (1.57 mg/g) trehalose, and were followed by lower concentrations at 50 mM (1.41 mg/g) and 0 mM (1.36 mm) trehalose (Table 27). However, it was found that trehalose helps the formation of chlorophyll total (T) content in plant leaves. for the two high trehalose levels, the value of the chlorophyll B content in the leaves was not different or the same. For the results of study compared trehalose as an osmoprotectant for corn under (-0.2 MPa) level of salinity-induced stress; the application trehalose at seedling stage, with the result of chlorophyll total (T) content in trehalose control 0 mM (1.55 mg/g) and 10 mM (1.53 mg/g) (Zeid, 2009).

The use of trehalose was carried out at the tillering stage. Trehalose was proven to have a positive effect on increasing the chlorophyll content of plant leaves in this study. According to trehalose, it plays an important physiological role as protection against abiotic stress in a large number of organisms (including bacteria, yeasts, and plants) that accumulate chlorophyll in plant leaves (Almeida et al., 2007).

Trehalose may work through its ability to scavenge various reactive species, providing protection for protein synthesis machinery and chlorophyll creation in plants (Luo et al., 2010). Trehalose has the added advantage of being a signaling molecule and a contained antioxidant, and also acts in genes involved in detoxification and the response to stress (Bae et al., 2005; Abdallah et al., 2016).

The ANOVA results showed that there were significant differences in the soluble sugar content of plant leaves which were influenced by individual factors, including rice varieties and salinity levels. However, there was no interaction between two or three factors with significant differences in this characteristic (Table 28).

IN 35 was higher on plant leaf sugar content (47.33 mg/g) than the two Thai rice varieties; CNT 1 (43.53 mg/g) and PT 1 (42.21 mg/g) (Table 28). The results of this study can be compared with previous studies, namely: effect of potassium application on wheat (*Triticum aestivum* L.) cultivars grown under salinity stress for salinity, this activity was conducted at seedling stage, soluble sugar content in leaf of variety Gemiza 9 (17.07 mg/g) and Sakha 93 (19.86 mg/g), for salinity parts control (0 mM) (15.49 mg/g), 40 mM (17.58 mg/g), 80 mM (19.69 mg/g) and 120 mM (21.09 mg/g) (El-Lethy et al., 2013). The ability of genes in plants that are functionally associated with various abiotic stresses in accumulating the formation of soluble sugars in plant leaves. In general, the genes present in salinity-regulated plants indicate that each stress will have several stress pathways. For certain properties by rice plants that can provide space for sugar to dissolve, as for parts that have special properties to withstand external pressure that sometimes appears suddenly (Hoseney and Rogers, 1990; McCully, 2001). Sucrose is the most abundant sugar in plants, especially in accumulation, occurs in plants with a strong ability to accumulate sugar in their leaves, and has a function and form in which carbohydrates are transported from one plant organ to certain plant parts (Van den Ende and Peshev, 2013).

For every increase in dissolved sugar content in plant leaves, three cultivars of rice varieties experienced salinity stress. For sugar accumulation in plants, the structures formed in response to salinity stress are also quite well documented (Roychoudhury et al., 2011). The importance of soluble complex sugars in the formation of metabolic processes in rice plants is recognized as products derived from

hydraulic processes, substrates in biosynthetic processes, energy production as well as certain sugars and signaling systems (W. Patrick et al., 2013).

For salinity, the highest soluble sugar content (52.52 mg/g) in plant leaves at 0 mM NaCl and the value decreased followed by an increase in salinity level; started to decrease at 50 mM NaCl (Table 28). For salinity, the highest dissolved sugar content (52.52 mg/g) was in plant leaves at 0 mM NaCl and the value decreased followed by an increase in salinity levels; started to decrease at 50 mM NaCl (Table 28). However, these results were compared with previous studies, namely about improvement of plants salt tolerance by exogenous application of amino acids. At salinity levels (control) 0 dS/m, 0.3 dS/m, 0.6 dS/m and 12 dS/m were 44.9 mg/g, 41.2 mg/g, 37.6 mg/g and 27.9 mg/g, respectively (Abd El-Samad et al., 2011).

The content of soluble sugar is very important, it is considered to integrate with every part of the plant, which is a storehouse of sugar formation (Meena et al., 2016; Paul, 2016). The importance of soluble complex sugars in the formation of metabolic processes in rice plants is recognized in many biochemical processes. They are products derived from hydraulic processes, substrates in biosynthetic processes, energy production as well as certain sugars and signaling systems (W. Patrick et al., 2013). Sugar has a function and form in which carbohydrates are transported from one plant organ to certain plant parts (Keunen et al., 2013; Van den Ende and Peshev, 2013). Moreover, soluble sugar has sufficient ability and function as an osmoprotectant against each characteristic, stabilizes various cell membranes, and maintains turgor pressure that occurs due to environmental pressure (Iqbal, 2018). It can be said that sugar content in plant leaves has a central role in plant structure and metabolism at the cellular level in all plant organisms (Rosa et al., 2009).

For every increase in dissolved sugar content in plant leaves, three cultivars of rice varieties experienced salinity stress. For sugar accumulation in plants, the structures formed in response to salinity stress are also quite well documented (Roychoudhury et al., 2011). Salt-induced stress has a toxic effect on plants and cause changes in metabolic processes; such as loss of chloroplast activity, decreased photosynthetic rate, and increased photorespiration rate which leads to an increase in leaf sugar content of reactive oxygen species (ROS) (Hasanuzzaman et al., 2013b).

Although the application of trehalose was reported to increase the sugar content in the leaves of rice plants under salty conditions which may be caused by increased water absorption, chlorophyll, and increased plant stabilization (Asaf et al., 2017; Sadak, 2019). In this study, trehalose application was not significantly affected the changeable soluble sugar content (Table 28). (Figueroa and Lunn, 2016) commented that the sugar content in the leaves of plants is still quite naturally available. If trehalose is added with a small concentration to plants, it is not able to increase the sugar content in the plant leaves. Increasing the concentration of trehalose from 100 mM to 150 mM tend to be higher than the two slightly lower concentrations, however, there were not significant differnece. Increasing the concentration of trehalose from 100 mM to 150 mM tends to be higher than the two slightly lower concentrations, however, there was no significant difference.

Table 28 Soluble sugar content (mg/g) in plant leaves of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	47.77 ± 3.31	46.06 ± 3.32	44.04 ± 2.23	35.72 ± 5.86	43.53 ± 5.62 b
	50	48.50 ± 1.95	43.80 ± 2.64	42.51 ± 1.20	36.45 ± 5.85	
	100	50.46 ± 3.65	44.77 ± 1.43	43.49 ± 0.55	37.92 ± 4.97	
	150	50.40 ± 3.23	45.63 ± 1.58	43.00 ± 3.86	36.02 ± 6.41	
PT 1	0	47.69 ± 4.67	43.06 ± 3.06	42.66 ± 0.91	36.02 ± 5.41	42.21 ± 7.34 b
	50	47.03 ± 3.28	42.76 ± 3.14	40.30 ± 3.05	34.74 ± 6.47	
	100	51.62 ± 6.59	44.53 ± 4.48	37.98 ± 6.17	35.90 ± 6.07	
	150	53.64 ± 5.69	45.81 ± 4.06	39.64 ± 5.72	32.30 ± 9.44	
IN 35	0	52.97 ± 5.55	52.16 ± 7.51	41.67 ± 3.37	41.28 ± 1.47	47.33 ± 7.87 a
	50	54.50 ± 6.52	48.87 ± 4.83	43.42 ± 1.94	39.03 ± 4.05	
	100	54.20 ± 5.62	49.24 ± 5.05	45.81 ± 3.39	38.22 ± 5.17	
	150	59.76 ± 10.34	51.20 ± 6.13	47.03 ± 4.57	38.04 ± 5.27	
Mean salinity		51.52 ± 5.80 A	46.49 ± 4.68 B	42.63 ± 3.82 C	36.80 ± 5.30 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		44.23 ± 6.37	43.50 ± 6.43	44.51 ± 7.10	45.20 ± 9.15	
P-value (F-test)						
Variety (V) 3.48 x 10^{-6**}, Salinity (S) < 2 x 10^{-16**}, Trehalose (T) 0.516 NS, V × S 0.635 NS, V× T 0.987 NS, S× T 0.666 NS, V× S × T 0.992 NS, CV% 10.951						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability,

Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Soluble sugar content formed in plant leaves from 100 mM (44.51 mg/g) to 150 mM (42.63 mg/g) showed a positive effect, compared to values under normal conditions of 50 mM (43.50 mg/g) and (control) 0 mM (44.23 mg/g) in (Table 28). Sugar content in plant leaves decreased positively in the presence of trehalose 50 mM compared and control (0 mM) was not compared with trehalose levels shown at 100 mM and 150 mM, where sugar content in plant leaves increased higher for both trehalose concentrations. The trehalose content of 50 mM showed that the sugar content in the leaves was almost the same as the control (0 mM). There was compared with the previous research about enhancement the performance of cowpea plants grown under drought conditions via trehalose application; trehalose application in seedling stage 60 days after sowing and analysis soluble sugar content, at 0 µM (control) (46 mg/g), 100 µM (55.63 mg/g) and 500 µM (61.74 mg/g) (Khater et al., 2018). Compared to others, sugars can be explained by some of their more unique physical properties. Which, includes high hydrophilicity, chemistry, and the absence of internal hydrogen formation, by which trehalose is able to stabilize proteins (Shtark et al., 2010).

Trehalose functions for plants in stabilizing physical conditions, especially plant leaves. Which, trehalose has the role for photosynthesis and establishes chlorophyll by adding a green color, and the opportunity to accumulate sugar (Shahidi and Zhong, 2010). The occurrence of a sharp decrease in the sugar content in the leaves during the growth process in this study, indicates that the dissolved sugar in the rice leaves undergoes catabolism during the leaf formation process, which can provide energy and structural substances for growth and development in plants, especially leaves (Singh et al., 2017). Trehalose has the added advantage of acting as an antioxidant and signaling molecule. Trehalose acts as elicitor of genes involved in detoxification and stress response in plants (Fernandez et al., 2010). Trehalose itself is eventually hydrolyzed by trehalase to form two glucose molecules (Elbein et al., 2003). Trehalose has also been shown to be efficient in stabilizing dehydrating enzymes, proteins, and membrane lipids, as well as protecting important biological

structures from damage during salt stress, supporting the creation of sugars and starches in certain plants (Redillas et al., 2012).

However, compared with the effect of proline application, exogenous proline was clearly affected to promote the accumulated soluble sugar content in leaf more than external trehalose (Table 6 and 28).

Statistical analysis showed that soluble sugar content in rice stalks was significantly different ($P \leq 0.01$) influenced by individual factors; rice varieties, salinity level, and trehalose level (Table 29). However, there was no significant difference in the soluble sugar content in the stems which was affected by the interaction between two and three factors (Table 29).

The three rice varieties that obtained the highest stem soluble sugar content were IN 35 (43.34 mg/g) and CNT 1 (43.15 mg/g), and followed by PT 1 (40.11 mg/g), (Table 29). However, compared to previous studies, namely salinity induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants; this activity was in seedling stage, soluble sugar content of variety CSR-1(14.0 mg/g), CSR-3 (17 mg/g), Ratna (17.5 mg/g) and Jaya (30 mg/g) at salinity levels 7 dS/m and 14 dS/m (Dubey and Singh, 1999; Sharma et al., 2012). In rice plants with certain components in plant parts (roots, stems, and leaves) such as chlorophyll, sugar, starch, and other elements that naturally occur in plants so as to arrange growth and development while in the field (Venkateswarlu and Visperas, 1987; Reynolds et al., 2012). Although rice plants are considered sensitive to salt, there are certain varieties that are still resistant to some concentrations of salt (Quinet et al., 2010). Cellulose, which is considered pure, can be obtained by regularly separating it from a mixture containing fat, pectin, lignin, and so on. Meanwhile, cellulose in rice stems can be converted into glucose as well (Oraby et al., 2007; Sun, 2010). For plant characters, some are sensitive to environmental stresses and some are stress-resistant because of their genetic characteristics (Jones and Qualset, 1984). In each species, plants that are able to respond to high salinity do not depend on the number of diploid or metacentric chromosomes (Shah et al., 1987). Another reason is considered able to distinguish various physiological tolerances and several growth mechanisms of rice varieties (Boote et al., 2001).

The effect of osmotic stress is thought to complicate water absorption in plants, as well as the effect of Na^+ and Cl^- ions due to excessive NaCl administration. Results of salinity are inhibiting cell enlargement and reducing sugar content in stems compared to other rice plants under normal conditions (Kronzucker and Britto, 2011). Similarly, the negative effect of salinity at 50 mM -150 mM NaCl was found on decreased the soluble sugar content in rice stem (Table 29).

For the effect of trehalose, the application showed a positive effect on increase soluble sugar content at 50-150 mM trehalose (Table 29). The ability of plants to survive under the stress condition after using some external chemicals; such as promote better tolerance ability in plants grown under excessive ion concentrations (Hajibagheri et al., 1989; Flowers and Yeo, 1989b). The efficiency of trehalose, due to its physicochemical properties for proteins, dehydrating enzymes, and lipid membranes, as well as for protection against damage during salt stress (Ramadan et al., 2019; Sadak, 2019). Trehalose acts as an elicitor gene in detoxification and resistance to stress (Ingram and Bartels, 1996). In addition, trehalose was reported to have the possibility of increased water absorption, chlorophyll content, and increased stabilization in plants (AbdElgawad et al., 2014). Account of trehalose is an exogenous source of sugar and nitrogen as well as compounds for the rapid growth of rice varieties after received the stress (Kosar et al., 2019). For this reason, trehalose is used under proper supervision under control to offer various benefits to plant productivity under constituents that can promote better health (Oleńska et al., 2020).

Table 29 Soluble sugar content (mg/g) in plant stem of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	41.00 ± 5.50	40.17 ± 5.35	39.37 ± 4.88	37.54 ± 4.43	43.15 ± 7.76 a
	50	45.67 ± 5.82	40.00 ± 5.78	38.65 ± 5.06	38.07 ± 4.86	
	100	51.40 ± 6.14	46.32 ± 5.03	39.36 ± 5.07	38.71 ± 5.27	
	150	59.82 ± 7.37	53.27 ± 6.07	41.17 ± 4.97	39.88 ± 4.63	
PT 1	0	37.54 ± 4.43	39.65 ± 4.26	37.78 ± 3.00	36.66 ± 2.90	40.11 ± 5.86 b
	50	38.07 ± 4.86	41.34 ± 3.35	37.55 ± 3.97	37.31 ± 4.15	
	100	38.71 ± 5.27	48.36 ± 5.46	40.58 ± 3.07	38.25 ± 4.26	
	150	39.88 ± 4.63	53.57 ± 5.50	43.86 ± 4.02	39.77 ± 4.53	
IN 35	0	43.16 ± 6.70	41.52 ± 5.73	40.35 ± 5.36	36.84 ± 3.60	43.34 ± 5.70 a
	50	45.85 ± 4.70	41.52 ± 6.57	40.41 ± 6.07	40.06 ± 6.28	
	100	49.88 ± 4.71	45.80 ± 4.01	42.40 ± 4.83	41.46 ± 4.78	
	150	52.05 ± 4.87	45.32 ± 4.52	44.80 ± 4.91	41.99 ± 4.11	
Mean salinity		47.65 ± 7.38 A	42.81 ± 5.90 B	39.87 ± 4.53 C	38.47 ± 4.36 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		39.05 ± 4.60c	40.26 ± 4.60b	43.35 ± 6.15a	46.14 ± 7.90a	
P-value (F-test)						
Variety (V) 0.00245**, Salinity (S) 1.06 x 10^{-11**}, Trehalose (T) 4.86 x 10^{-8**}, V × S 0.714 NS, V× T 0.873 NS, S× T 0.054 NS, V× S × T 0.995 NS, CV% 11.753						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

In this study, increasing in soluble sugar content was induced by salinity increased in rice stem, although not applied the external trehalose. However, this occurrence was observed only in PT 1 at 50 mM NaCl (39.65 mg/g) onwards; no salinity as 37.54 mg/g (Table 29). This, indicate the natural response in rice plant grown under the stress condition. Salt stress can generally be fully adopted at the same time for the biosynthesis of secondary metabolism. Which, substances are significantly increased in plants such as soluble solids, sugars, amino acids, proteins, and organic acids (Pourali et al., 2009; Meza et al., 2020). Nevertheless, the uncoordinated response of soluble sugar accumulation to increased salinity of these

three rice varieties was found only in stems but not in leaves (in leaves of all three rice varieties, the value was reduced) (Table 28-29).

The results of this study confirm the results for previous trials (Table 6) that showed that this rice variety had a salinity response to increased soluble sugar accumulation in rice parts. That may indicate the salinity tolerance of PT 1 compared to the original cultivar potential (at 0 mM NaCl).

The soluble sugar content in the roots of rice plants at the tillering stage, both varieties and salinity factors were very significant ($P<0.01$); except for trehalose which was not significant (Table 30). No significant difference in soluble sugar content in rice roots was observed to be affected by the interaction between two and three factors (Table 30).

The three rice varieties that obtained the highest root soluble sugar content were IN 35 (44.96 mg/g) followed by CNT 1 (41.19 mg/g), and the lowest was PT 1 (40 mg/g). The results of this study can be compared with previous studies, namely: Overall, these results were compared with a previous study about salinity levels 7 dS/m and 14 dS/m induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants, this activity on the seedling stage of rice CSR-1 (7.0 mg/g), CSR-3 (7.0 mg/g), Ratna (10.0 mg/g) and Jaya (7.0 mg/g) (Sharma et al., 2012). (Ghassemi-Golezani et al., 2011b) suggested that each plant species was able to reject some level of salinity, depending on the number of metacentric or diploid chromosomes. In general, whole rice plants are sensitive to salt, there are certain varieties that have strong properties against high salt concentrations. Therefore, the accumulation of soluble sugar content in rice roots may be an indicator under stressful conditions such as salinity, which varies in different rice varieties (Fageria and Baligar, 2008). Which, the ability to carry out plant growth activities such as the accumulation of essential substances in plant parts under saline conditions, this is known as tolerance to salinity (Läuchli and Epstein, 1990). Considering this concept, IN 35 is more resistant to salinity than the two Thai rice varieties. This is due to innate capacity and genes in each variety resistant to salt stress (Boriboonkaset et al., 2013). An(Fageria and Baligar, 2008)other reason considered to interpret and differentiate tolerance is related to physiological and shape mechanisms on the growth of rice varieties (Wissuwa et al., 2006).

However, clearly a negative effect of salinity level of soluble sugar content in the root. The highest soluble sugar content in plant roots at 0 mM NaCl was 49.23 mg/g and this value decreased followed by an increase in salinity; started to decrease at 50 mM NaCl to 100 150 mM NaCl, continuously (Table 30).

Table 30 Soluble sugar content (mg/g) in plant root of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	45.56 ± 3.42	43.80 ± 3.43	41.71 ± 2.31	33.11 ± 6.05	41.19 ± 5.81 b
	50	46.32 ± 2.09	41.45 ± 2.72	40.13 ± 1.24	33.87 ± 6.04	
	100	48.34 ± 3.78	42.46 ± 1.48	41.14 ± 0.57	35.39 ± 5.14	
	150	48.28 ± 3.34	43.35 ± 1.63	40.63 ± 3.98	33.43 ± 6.63	
PT 1	0	45.18 ± 4.84	40.70 ± 3.15	4029 ± 0.93	33.43 ± 5.60	40.00 ± 7.25 b
	50	44.80 ± 3.40	40.38 ± 3.24	37.85 ± 3.16	32.10 ± 6.68	
	100	49.54 ± 6.81	42.21 ± 4.63	35.45 ± 6.38	33.30 ± 6.27	
	150	51.63 ± 5.88	43.54 ± 4.20	37.16 ± 5.91	32.42 ± 7.39	
IN 35	0	50.93 ± 5.74	50.10 ± 7.76	39.25 ± 3.52	38.86 ± 1.52	44.96 ± 7.79 a
	50	52.51± 6.74	46.70 ± 4.99	41.07 ± 2.01	36.53 ± 4.18	
	100	52.20 ± 5.81	47.08 ± 5.22	43.54 ± 3.50	35.70 ± 5.34	
	150	55.42 ± 8.56	49.10 ± 6.34	44.80 ± 4.72	35.51 ± 5.45	
Mean salinity		49.23 ± 5.51 A	44.24 ± 4.84 B	40.25 ± 3.95 C	34.47 ± 5.10 D	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		41.91 ± 6.58	41.14 ± 6.64	42.94 ± 7.33	42.94 ± 8.54	
P-value (F-test)						
Variety (V) 5.99 x 10^{-6**}, Salinity (S) < 2 x 10^{-16**}, Trehalose (T) 0.474 NS, V × S 0.636 NS, V× T 0.995 NS, S× T 0.869 NS, V× S × T 0.992 NS, CV% 11.530						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

There was the result in previous study about salinity effect on salinity induces accumulation of soluble sugars and alters the activity of sugar metabolizing enzymes in rice plants, at seedling stage in salinity 0 dS/m (5.0 mg/g), 7 dS/m (5.2 mg/g) and 14 dS/m (7.0 mg/g) (Dubey and Singh, 1999; Singh, 2018). Salt stress conditions are treated with poison, so as not to interfere with the dissolved sugar content in plant roots, reducing the number of physiological and morphological adaptations (Nawaz et

al., 2010). It involves the collection of several active molecules or ions that are relatively osmotically soluble and naturally. In general, the concentration of NaCl has increased, contained, and dissolved in the soil. The Na+ attached to the roots and other parts of the plant and the Cl formed in the youngest leaves were the most damaging to the plant (Majeed et al., 2010; Richter et al., 2019). The rate of carbon assimilation decreases rapidly due to salinity, photosynthetic activity, sugar accumulation in roots, and carbon loss with increased respiration, resulting in reduced carbohydrate accumulation (Everard et al., 1994).

The trehalose concentration factor did not show a significant difference in soluble sugar content in plant roots (Table 30). Although the addition of trehalose 0 mM to 150 mM showed an increase in root soluble sugar content of 1.03%. However, in a previous study of trehalose as osmoprotectant for maize under (-0.2 MPa) level of salinity-induced stress, the activity on the analysis of soluble sugar content at seedling stage in the application level of trehalose; control 0 mM (32.82 mg/g) and 10 mM (38.96 mg/g) (RES, 1993; Zeid, 2009). The contribution of external trehalose for plants to form sugars in the roots is quite small. This is reasonable that the external trehalose carried out during the tillering period was sent to above-ground parts to maintain the photosynthesis ability. Rice plants are able to adapt to salt stress. While occurs the salinity stress, increasing the biosynthesis of secondary metabolites; such as soluble solids, amino acids, and sugars, formed in plant roots (Ahanger et al., 2020). In addition, the presence of trehalose acts as an osmoregulatory in stabilizing turgor in rice plants experiencing salt stress (Gobbs, 2007; Hasanuzzaman et al., 2013b). However, for this trial, an automatic increase of soluble content was observed only in the plant stem, not in leaves and root parts (Table 28-30).

Statistical analysis showed that the starch content in rice leaves was significantly different ($P \leq 0.01$) influenced by individual factors; rice varieties, salinity and trehalose (Table 31). However, there was no significant difference in the rice variety factor on the starch content in plant leaves which was influenced by the interaction between two and three factors (Table 31). The three rice varieties that obtained the highest starch content were IN 35 (44.03 mg/g) followed by CNT 1 (43.81 mg/g) and PT 1 (42.37 mg/g) (Table 19). From the results of the starch content in the leaves, compared with previous studies the accumulation process of

carbohydrate in rice varieties in relation to their response to nitrogen in the tropics, this activity at flowering stage of starch content in the leaf of variety IR8 (52 g/m^2), Peta (69 g/m^2), Bengawan (77 g/m^2) and Cianung 242 (44 g/m^2), respectively according to the opinion which states that salinity is one of the environmental stresses, which has the ability to reduce the adaptability and individual physical characteristics of each rice variety (Utama-Ang et al., 2018). This variation in behavioral growth is of course accompanied by strong genetics, according to a particular environment (Sitompul and Guritno, 1995). Salinity easily accumulates in places where water is scarce. Thus, rice plants that grow in these places have the potential to experience a water deficit so that they have the opportunity to produce salt (Kijne, 2006). If the plant is experiencing salt stress, which of course will reduce the ability of the plant to absorb water, interfere with the photosynthesis process, so that it can easily reduce starch in the leaves of all plant growth (Yadav et al., 2020).

Plants that experience water shortages have the potential for salinity formation to accumulate less starch in leaves than plants that grow under normal conditions (Liu et al., 2010b). The reduced water certainly contributes greatly to salt, which can cause a very significant decrease in starch, even causing plant death (Yadav et al., 2011a). The response of plants that experienced an increase in water and salt deficiency, caused stress to experience changes at the cellular and molecular levels, which indicated a decrease in growth rate and reduced starch formation in plant leaves.

The formation of starch content in the leaves as a form of stock rather than carbon, temporarily available is the main component of starch in plant growth that accumulates in the leaves, while sucrose is transported to certain organs that can be used by plants. The steps involved in the production of photosynthesis are of course catalyzed by the formation of sucrose phosphate synthase which converts hexose phosphate to sucrose (Bahaji et al., 2015). In addition, there was also an effect of salinity which was confirmed by the effect of salinity on the statistically significant difference in the value of starch content in leaves (Table 31).

Statistical analysis showed that the starch content in rice leaves was significantly different ($P \leq 0.01$) influenced by two individual factors; salinity level and trehalose level (Table 31). However, there was no significant difference in the rice variety factor on the starch content of plant leaves, the values ranged from 42.37-

44.03 mg/g (Table 31). The results of this study can be compared to previous studies, namely; effect of potassium application on wheat (*Triticum aestivum* L.) cultivars grown under salinity stress. Starch content at salinity level 0 mM (control) (145.25 mg/g), 40 mM (137.68 mg/g), 80 mM (126.44 mg/g) and 120 mM (110 mg/g) (El-Lethy et al., 2013). Salinity is one of the environmental stresses that has the ability to reduce adaptability, individual physical characteristics of rice and salinity easily accumulates in places that lack water. Thus, rice plants that grow in these places have the potential to experience a water deficit so that they have the opportunity to produce salt (Kijne, 2006). However, decrease on both starch content (Table 31) and soluble content in leaves (Table 28) to confirm the severe impact of salinity.

The formation of starch content in the leaves as a form of stock rather than carbon, temporarily available is the main component of starch in plant growth that accumulates in the leaves. The steps involved in the production of photosynthesis are of course catalyzed by the formation of sucrose phosphate synthase which converts hexose phosphate to sucrose (Bahaji et al., 2015). In all rice varieties at 0 mM trehalose, the reduction of starch content in plant leaves was observed when salinity level increased. The decrease in the starch content in the leaves is due to the corresponding increase in the salinity level. Compared with the control treatment at 0 mM NaCl with the highest salinity at 150 mM NaCl a decrease of about 12%. In addition, sucrose is transported to certain organs that can be used by plants. However, a decrease in both starch content (Table 31) and soluble sugar content in leaves (Table 28) confirm the severe impact of salinity. Varieties that experience salt stress will do less photosynthesis and reduce starch content in leaves when compared to certain varieties that don't have experience salt stress (Chartzoulakis et al., 2006).

In statistical analysis, the trehalose content factor showed a significant difference to the starch content in plant leaves (Table 31). The starch content in leaves has increased after using trehalose for spaying; at 0 mM was lowest at 35.05 mg/g and increased value in continuous when trehalose level incrased. However, this research compares to previous study that about; enhancement the performance of cowpea plants grown under drought conditions (95 % FC) via trehalose application. Trehalose level application 0 mM (39.53 mg/g), 100 mM (47.10 mg/g) and 500 mM (52.27 mg/g) (Khater et al., 2018). For this reason, that trehalose accumulation is a well-

known plant metabolic response to drought, salinity, and other stresses (Szabados and Savouré, 2010). Trehalose allows osmotic adjustment, increases energy, stabilizes various protein structures and cell membranes, acts as a protective agent for enzymes, and is a scavenger against free radicals and antioxidants (Kosar et al., 2019). It is clear that trehalose is a non-reducing disaccharide in which the two available glucose units are linked in an a, a-1,1-glycosidic linkage (Teramoto et al., 2008). This sugar is found in various organisms, one of which is in lower and higher plants, which can function as a source of energy and carbon. Thus, trehalose is present in plants, to increase the ability to form starch content in rice plant leaves (Garg et al., 2002).

Compared between two substances, the effect of trehalose on starch accumulation in leaves was clearly with significantly different effect (Table 31) more than use the proline (Table 9).

Table 31 Starch content (mg/g) in plant leaves of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	41.26 ± 5.84	35.86 ± 2.54	33.62 ± 3.97	30.98 ± 3.21	43.81 ± 8.52
	50	49.48 ± 5.44	47.30 ± 5.54	36.66 ± 3.88	34.65 ± 1.99	
	100	53.57 ± 5.35	50.40 ± 4.36	44.20 ± 4.66	39.02 ± 1.56	
	150	55.34 ± 7.22	53.27 ± 4.27	50.06 ± 1.17	45.29 ± 1.56	
PT 1	0	40.40 ± 4.66	34.43 ± 4.66	32.82 ± 4.76	30.52 ± 3.10	42.37 ± 7.70
	50	48.33 ± 5.25	45.63 ± 5.05	42.24 ± 4.27	38.56 ± 5.05	
	100	51.27 ± 6.73	47.93 ± 4.86	42.54 ± 4.37	37.87 ± 1.64	
	150	54.94 ± 6.53	49.25 ± 5.34	41.72 ± 1.05	39.31 ± 1.47	
IN 35	0	42.01 ± 6.48	35.00 ± 3.36	33.45 ± 3.53	30.23 ± 1.56	44.03 ± 8.62
	50	50.52 ± 7.41	46.49 ± 5.38	42.53 ± 5.16	40.23 ± 5.61	
	100	53.56 ± 7.35	50.75 ± 6.65	43.85 ± 4.49	39.94 ± 3.29	
	150	56.95 ± 6.82	52.35 ± 7.76	45.17 ± 4.35	41.44 ± 2.52	
Mean salinity		49.80 ± 7.65 A	45.72 ± 7.86 B	40.75 ± 6.21 C	37.34 ± 5.25 D	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		35.05 ± 5.30 d	43.55 ± 6.62 c	46.25 ± 6.88 b	48.76 ± 7.10 a	
P-value (F-test)						
Variety (V) 0.187 NS, Salinity (S) <2 x 10^{-16**}, Trehalose (T) <2 x 10^{-16**}, V × S 0.998 NS, V× T 0.408 NS, S× T 0.800 NS, V× S × T 0.900 NS, CV% 11.026						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and

0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

For starch content in rice stems, the statistical analysis showed a significant difference ($P<0.01$) influenced by an individual of two factors; salinity level and trehalose level (Table 32). However, there was no significant difference in the rice variety factor or any interaction either between two or three factors on the starch content of plant stems (Table 32).

Again, for starch content in rice stems, the statistical analysis showed no significant difference ($P\leq0.01$) influenced by individual factors; rice varieties, salinity and trehalose (Table 32). However, there was no significant difference in the rice variety factor on the starch content of plant stems which was influenced by the interaction between two and three factors (Table 32). The three rice varieties that obtained the highest starch content were CNT 1 (45.80 mg/g) followed by IN 35 (45.23 mg/g) and PT 1 (43.56 mg/g) (Table 32). From the results of starch content in stems, compared with previous studies, salinity level 7 dS/m and 14 dS/m induce the accumulation of dissolved sugars and changes the activity of sugar metabolism enzymes in rice plants, as follows; The CSR-1 varieties contained starch (117 mg/g) and CSR-3 (130 mg/g) (Verma and Dubey, 2001). According to the opinion (Utama et al., 2018) which states that one of the environmental influences is salinity stress which is able to reduce the adaptability and individual physical characteristics of each variety.

Salinity affects plants, inducing starch degradation and sugar accumulation as well. In rice varieties with increased accumulation of starch phosphorylase, sucrose phosphate synthase and decreased invertase activity. Furthermore, individual rice genotypes experienced different tolerance and salt sensitivity for each enzyme activity under salinity. (Weisany et al., 2011) suggest that individual plant species are able to withstand several degrees of salinity, on some metacentric or diploid chromosomes. Other reasons are also considered regarding the differentiation of several forms of tolerance experienced, physiologically through growth and each mechanism existing plants. Each cultivar has a wide range of performance in responding to salt stress, ranging from tolerant to highly sensitive to salt, because changes rather than

morphology are associated with abnormalities by different metabolisms during growth and tillering stages (Munns et al., 2006). This study was conducted to determine changes in starch and sugar content, and several metabolic enzyme activities of sucrose and phosphate synthase, acid invertase, and starch phosphorylase in one salt tolerant rice cultivar and two salt sensitive rice cultivars during growth.

The results of statistical analysis showed that with increasing salt content, starch content in plant stems also decreased, especially at 50 mM followed by 100 mM and up to 150 mM NaCl (Table 32).

Table 32 Starch content (mg/g) in plant stem of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	41.53 ± 4.86	40.76 ± 4.76	39.70 ± 5.36	37.53 ± 7.08	45.80 ± 7.37
	50	49.81 ± 4.27	47.70 ± 4.56	40.41 ± 9.00	39.53 ± 9.00	
	100	54.10 ± 4.36	50.87 ± 4.56	45.17 ± 4.76	39.90 ± 1.59	
	150	55.92 ± 6.23	53.80 ± 3.27	51.16 ± 1.20	44.95 ± 0.65	
PT 1	0	41.53 ± 5.16	40.53 ± 4.66	39.41 ± 5.46	37.06 ± 7.18	43.56 ± 5.09
	50	49.16 ± 4.96	44.29 ± 1.34	42.11 ± 2.60	40.47 ± 4.56	
	100	48.11 ± 1.07	46.29 ± 0.90	42.76 ± 3.08	40.47 ± 4.56	
	150	50.70 ± 3.00	47.34 ± 0.91	45.58 ± 4.56	42.40 ± 2.79	
IN 35	0	41.94 ± 4.96	40.76 ± 5.06	39.59 ± 5.77	37.18 ± 7.38	45.23 ± 6.92
	50	49.45 ± 5.46	47.34 ± 6.37	44.64 ± 6.87	40.94 ± 7.80	
	100	51.98 ± 6.17	49.00 ± 4.96	44.23 ± 5.56	39.06 ± 2.22	
	150	55.92 ± 6.27	50.63 ± 5.96	45.40 ± 4.26	45.70 ± 0.71	
Mean salinity		49.18 ± 6.50 A	46.61 ± 5.47 B	43.35 ± 5.47 C	40.32 ± 5.28 D	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		39.80 ± 5.02 c	44.55 ± 6.35 b	46.00 ± 5.81 b	49.12 ± 5.47 a	
P-value (F-test)						
Variety (V) 0.078 NS, Salinity (S) 1.05 x 10^{-10**}, Trehalose (T) 7.89 x 10^{-11**}, V × S 0.966 NS, V× T 0.684 NS, S× T 0.653 NS, V× S × T 1.000 NS, CV% 11.145						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Thus, the decrease in starch in the stem is strongly influenced by the level of salinity, according to each concentration level obtained. However, when compared

with the previous study about nitrate reductase in *Zea mays* under salinity. Starch content in salinity level (NaCl) 0 mM (control) (2.62 mg/g) and 150 mM (19.2 mg/g) (Baki et al., 2000). Several causes in the salty condition of the plant directly reduce the starch content formed in the stems of rice plants (Zhen-hua et al., 2012; Sharma et al., 2019). Disorders of plant physiology are membranes, decreased mineral nutrition, the impaired ability of oxygen species detoxification reactions, changes in antioxidant enzymes, decreased photosynthetic activity, and biosynthesis of photosynthetic pigments (Sharma and Dubey, 2005; Sadak et al., 2020). In a previous experiment, found that under drought conditions and salt stress, the starch content in the stems was reduced and various forms in rice (Yadav et al., 2011b; Dien et al., 2019).

The levels of trehalose factor showed a very significant difference in the formation of starch in rice stalks (Table 32). The value of starch content in stems increased according to the increase in trehalose content at 50 mM trehalose onwards. However, when compared with the previous study about physiological role of trehalose on enhancing salinity tolerance of wheat plant. Starch content in stem on trehalose level (control) 0 mM (53.65 mg/g), 10 mM (61.52 mg/g), 50 mM (73.52 mg/g) (Sadak, 2019). By reason that the presence of trehalose in plants can increase energy and carbon. However, the level of trehalose can vary widely in certain cells depending on many factors; such as the stage of plant growth, nutrition, state of the organism or cell, and environmental conditions prevailing at the time of measurement (Elbein et al., 2003; Wolf et al., 2003). Therefore, trehalose is also an important substance for application to plants because it can serve as a source of carbon for synthesis and glucose for energy (Elbein et al., 2003). Another role due to trehalose is in protecting cells against oxygen radicals that help create starch content in plant stems (Jain and Roy, 2009).

Comparison between two substances, the effect of trehalose on starch accumulation in stem was clearly with significantly different effect (Table 32) more than use the proline (Table 10).

For starch content in rice roots, statistical analysis showed a significant difference ($P \leq 0.01$) which was influenced by individual factors of salinity level and trehalose level (Table 33). However, there was no significant difference in the rice variety factor in the starch content of plant roots. Non-significant differences were

influenced by the interaction between two and three factors (Table 33). In the three rice varieties, the starch content in plant root range between 40.94-42.07 mg/g, however, their values were not significantly different (Table 33). From the results of starch content in roots, when compared with previous studies, salinity induces the accumulation of dissolved sugars and changes the activity of sugar metabolism enzymes in rice plants, as follows; The CSR-1 varieties contained starch (117 mg/g) and CSR-3 (130 mg/g) (Dubey and Singh, 1999; Rosa et al., 2009). Salinity stress is one of the environmental influences that can reduce plant adaptability and individual physical characteristics of each variety (Chen and Jiang, 2010).

In rice varieties that are tolerant to salt stress conditions will form a natural physiological mechanism that changes tolerance and osmotic adjustment, tissue water loss, ion absorption and starch formation in plant roots (Shahid et al., 2020). In addition, plants that are resistant to salinity stress must have a strategy of receiving sufficient sunlight in photosynthesis and absorbing nutrients to support their growth. This is related to a decrease in starch formation in plant roots due to decreased water absorption, high sodium and chloride toxicity in rice plant cells and reduced photosynthesis (Farooq et al., 2009). This time the CNT1 variety was able to compete with IN 35 in producing the same amount of starch, this was due to genetic factors in plants and other factors that influenced it (Patindol et al., 2015).

Table 33 Starch content (mg/g) in plant root of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at tiller stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	38.89 ± 3.33	38.07 ± 3.22	36.93 ± 3.85	33.95 ± 4.60	42.07 ± 5.86
	50	45.93 ± 1.22	43.65 ±1.05	37.69 ± 7.74	36.74 ± 7.74	
	100	49.92 ± 2.00	48.97 ± 1.81	41.94 ± 1.81	37.05 ± 1.16	
	150	48.40 ± 1.62	48.27 ± 0.76	44.22 ± 2.00	42.58 ± 2.25	
PT 1	0	38.89 ± 3.64	37.81 ± 3.12	36.61 ± 3.96	34.08 ± 5.79	40.94 ± 4.64
	50	44.85 ± 1.16	41.87 ±1.30	39.63 ± 1.16	36.35 ± 3.22	
	100	46.50 ± 2.00	44.03 ± 2.00	40.22 ± 1.54	37.75 ± 3.01	
	150	48.90 ± 2.55	45.17 ± 2.00	42.63 ± 2.00	39.84 ± 1.30	
IN 35	0	39.33 ± 3.43	38.07 ± 3.54	36.80 ± 4.28	34.20 ± 6.01	42.01 ± 5.00
	50	45.55 ± 1.10	43.46 ± 2.10	41.24 ± 3.75	37.81 ± 5.68	
	100	47.51 ± 1.00	45.67 ± 1.45	42.06 ± 3.84	37.87 ± 2.20	
	150	48.78 ± 2.77	46.81 ± 1.45	44.28 ± 2.31	42.76 ± 1.16	
Mean salinity		45.29 ± 4.39 A	43.48 ± 4.17 B	40.34 ± 4.07 C	37.58 ± 4.54 D	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		36.97 ± 3.93 d	41.22 ± 4.74 c	43.30 ± 4.70 b	45.22 ± 3.32 a	
P-value (F-test)						
Variety (V) 0.157 NS, Salinity (S) $<2 \times 10^{-16**}$, Trehalose (T) $<2 \times 10^{-16**}$, V × S 0.882 NS, V× T 0.482 NS, S× T 0.316 NS, V× S × T 1.000 NS, CV% 7.701						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Salinity showed a negative effect on starch accumulation in plant roots. In this study, the highest starch content in root was measured at 0 mM NaCl, and decreased at the level of salinity increased, continuously (Table 33). Thus, salinity showed a negative impact on starch content start at 50 mM NaCl. However, when compared with the previous study about salinity induces the accumulation of dissolved sugars and changes the activity of sugar metabolism enzymes in rice plants. Starch content in root at salinity level (NaCl) 0 mM (control) (75 mg/g), 7 mM (79 mg/g) and 14 mM (68 mg/g). This is related to a decrease in starch formation in plant roots due to decreased water absorption, high sodium and chloride toxicity in rice plant cells, and reduced photosynthesis (Sharma and Dubey, 2005; Farooq et al., 2009). Salinity

levels affect the starch contained in plant roots, among the four salinity levels, the influence varies from the highest to the lowest value according to the salinity level obtained. Thus, it is the starch content in plant roots, an important component in plants, which obviously affects starch formation even at low concentrations. In general, the concentration due to an increase in NaCl can increase the content of soluble acids in the soil (Dkhil and Denden, 2010). In general, Na^+ present in the soil will be trapped in the roots of the plant so that it prevents the formation of starch in plant roots and other parts of the plant. Meanwhile, the Cl- formed in the youngest leaves was the most damaging to the plants that morning. However, there are many cereal crops in the crop, Na^+ being the main cause of specific ion damage (Karlsson, 2014). Controlled ion compartment, translation, transport outflow and absorption, as a precaution against the formation of ionic toxicity. The rate of carbon assimilation decreases more rapidly due to the presence of salinity. Salinity has strong properties in inhibiting plant activity processes and can also reduce photosynthesis, carbon loss and increase respiration and less starch accumulation in root (Parida and Das, 2005). There are certain exceptions, salt stress begins to occur in the roots of rice plants which can directly reduce starch in the roots and prevent the movement of ions into the air that move within the plant (Sharma and Dubey, 2005). There is something special about the mechanism that occurs in some glycophytes and most halophytes (salt tolerant) (Aslamsup et al., 2011). The development of salt vesicles in the epidermis can directly accelerate the release of salt, preventing the accumulation of salt in various plant organs such as roots, stems and leaves (Rewald et al., 2013). While another example of a more exclusive mechanism is the formation of salt accumulation in the higher roots which can reduce the flow of water from the soil to certain parts of the plant and also reduce the starch content that accumulates in the plant roots (Yensen and Biel, 2008).

In statistical analysis, the trehalose factor showed a significant difference in the starch content in plant roots (Table 33). The lowest starch content in rice root was 36.97% at 0 mM trehalose and a significant increase according to the level of trehalose application increased. Compare to previous study about mitigation of drought stress on Fenugreek plant by foliar application of trehalose. In trehalose level (control) 0 mM (41.64 mg/g), 100 mM (42.75 mg/g) and 150 mM (43.25 mg/g).

Trehalose accumulation is a well-known plant metabolic response to drought, salinity, and other stresses. Trehalose composes osmotic adjustment, adds energy, improves the shape of protein structure and cell membranes. Moreover, trehalose acts as a protective agent for enzymes, and is a scavenger against free radicals and antioxidants (Ma et al., 2013; Zhang et al., 2019). The overall plant response to osmotic stress consists of the accumulation of osmolytes in several compatible ones, one of which is trehalose. The property of trehalose is to act, as a restoration of energy-depleted plants due to salt stress, stabilizing any increase in stress tolerance in plants in starch formation, which should be investigated further about the mechanisms involved (Zachariassen, 1999). Plants in addition to having compatibility and osmolytes can also have enzymes that function as stabilizers, membranes, and forming subcellular structures (Slama et al., 2015; Yancey, 2020).

Comparison between two substances, the effect of trehalose on starch accumulation in root was clearly with significantly different effect (Table 33) more than use the proline (Table 11). However, considered in starch accumulate in all plant parts at tillering stages (leaves, stem, and root), trehalose application showed higher influence to increase the value more than proline application.

Effects on agronomic characteristics in rice in the first to fourth weeks after salinity (after transplanting) at the beginning of tillering stage

Evaluate four yield components and different agronomic characteristics of the three varieties affected by salinity levels; in the first week after transplanting or the third week after planting (Table 34). Measurement of four agronomic characteristics, namely number of leaves per plant, number of tillers per plant, plant height and leaf symptom score were influenced by salinity stress in two characteristics such as number of leaves per plant and leaf symptom scoring. However, significantly affected by rice varieties was observed in all characteristics. For interaction between salinity levels and varieties did not produce significant differences in all agronomic characteristics (Table 34).

The highest number of leaves per plant and, number of tillers and plant height was found in IN 35 and followed by CNT 1 and PT 1. In contrast, the higher leaf symptom scoring was observed in two Thai rice: CNT 1 and PT 1, and lower in IN 35. For plant height, the highest plant height was recorded in IN 35 and followed

by CNT 1 and PT 1, respectively. Decrease or increase in the percentage of certain explanations (values in brackets) was compared with normal conditions (0 mM salinity) (Table 34). Two characteristics; the number of leaves per plant and leaf symptoms, were significantly different affected by salinity levels. Which, at 0 mM NaCl showed the highest number of leaves per plant, but the lowest the leaf symptom affected by salinity. Decrease the number of leaves per plant was recorded at 50 mM NaCl and clearly affected at 150 mM NaCl. However, increased symptoms on leaves caused by the salinity effect were clearly assessed at 50 mM NaCl. Although, there were not significant difference affected by interaction between salinity levels and varieties in any characteristics,

Although there was no significant difference affected by the interaction between rice varieties and salinity levels (Table 34). Considering in detail the three rice varieties, it was found the response under salinity stress in two Thai rice varieties: CNT 1 and PT 1, was different from in IN 35. excepted in leaf symptom scoring. Under facing the salinity stress, CNT 1 and PT 1 showed plant height and the number of tillers per plant was increased, but decreasing in the number of leaves per plant was recorded since at 50 mM NaCl. While, in IN 35, it was decreased in plant height since at 50 mM NaCl. However, the number of leaves per plant was counted at 150 mM NaCl. For number of tillers per plant was increased in IN 35 when it received the salinity during planting. Different physical response in three rice varieties that occurred after transplanting in salty soil was assessed. A stunt in plant height, but it remains constant on the number of tillers per plant and number of leaves per plant was observed in IN 35. Contrast to two Thai rice varieties, CNT 1 and PT 1, there was slender plant performance which higher plant height, but the number of leaves was reduced.

Table 34 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 1 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	12.0 ± 1.0	4.2 ± 0.4	48.0 ± 2.7	1.1 ± 0.1
50 mM (% NC)	12.2 ± 0.4 (+1.67)	4.2 ± 0.4 (0.00)	44.8 ± 0.8 (-6.70)	1.4 ± 0.3 (+27.27)
100 mM (% NC)	12.0 ± 1.6 (0.00)	4.6 ± 0.5 (+9.52)	46.4 ± 3.4 (-3.33)	1.8 ± 0.4 (+63.64)
150 mM (% NC)	11.4 ± 0.9 (-5.00)	4.2 ± 0.8 (0.00)	46.6 ± 1.5 (-2.92)	2.2 ± 0.3 (+100)
Mean (IN 35)	11.9 ± 1.0 a	4.3 ± 0.6 a	46.5 ± 2.4 a	1.6 ± 0.5 b
PT 1				
0 mM (NC)	10.8 ± 1.0	3.2 ± 0.4	39.0 ± 3.5	1.2 ± 0.3
50 mM (% NC)	10.6 ± 1.5 (-1.85)	3.6 ± 0.5 (+12.50)	40.2 ± 2.9 (+3.08)	1.7 ± 0.0 (+41.67)
100 mM (% NC)	10.4 ± 1.3 (-3.70)	3.6 ± 0.5 (+12.50)	40.8 ± 1.5 (+4.62)	2.1 ± 0.3 (+75.00)
150 mM (% NC)	9.6 ± 0.5 (-11.11)	3.2 ± 0.4 (0.00)	40.8 ± 4.1 (+4.62)	2.3 ± 0.4 (+91.67)
Mean (PT1)	10.1 ± 1.1 b	3.4 ± 0.5 b	40.2 ± 3 c	1.8 ± 0.5 a
CNT 1				
0 mM (NC)	11.8 ± 1.1	3.4 ± 0.5	41.0 ± 3.5	1.1 ± 0.1
50 mM (% NC)	10.6 ± 1.1 (-10.17)	3.8 ± 0.4 (+11.76)	44.0 ± 3.2 (+7.32)	1.7 ± 0.3 (+54.55)
100 mM (% NC)	10.6 ± 1.1 (-10.17)	3.8 ± 0.4 (+11.76)	43.0 ± 3.5 (+4.88)	2.1 ± 0.3 (+90.91)
150 mM (% NC)	10.0 ± 1.4 (-15.25)	3.6 ± 0.5 (+5.88)	43.4 ± 2.9 (+4.88)	2.2 ± 0.4 (+100)
Mean (CNT 1)	10.8 ± 1.3 b	3.7 ± 0.5 b	42.9 ± 3 b	1.8 ± 0.5 a
Over all mean	11	3.78	43.16	1.72
0 mM (NC)	11.5 ± 1.0 A	3.6 ± 0.6	42.7 ± 4.8	1.1 ± 0.2 C
50 mM (% NC)	11.1 ± 1.3 AB (-3.48)	3.8 ± 0.5 (+5.56)	43.0 ± 3.1 (+0.70)	1.6 ± 0.3 B (+45.45)
100 mM (% NC)	11.0 ± 1.5 AB (-4.35)	4.0 ± 0.7 (+11.11)	43.4 ± 3.6 (+1.64)	2.0 ± 0.3 A (+81.82)
150 mM (% NC)	10. 3 ± 1.2 B (-10.43)	3.7 ± 0.7 (+2.78)	43.6 ± 3.7 (+2.11)	2.2 ± 0.4 A (+100.00)
P value (Varieties)	0.000173^{**}	$7.53 \times 10^{-6**}$	$4.25 \times 10^{-8**}$	0.0272^{*}
P value (Salinity)	0.03931^{*}	0.163 NS	0.807 NS	$2.23 \times 10^{-13**}$
P value (Salinity:Variety)	0.846 NS	0.955 NS	0.359 NS	0.912 NS
CV (%)	10.13	14.06	6.54	16.68

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Although the number of tillers showed a tendency to increase at higher salinity stress in all rice varieties, effective or ineffective tillers could not be detected in early tillers. Many characteristics of Thai rice varieties are affected by salinity. Indicated by the percentage reduction in the number of leaves per plant and the positive percentage in leaf symptom scores (Table 34). Moreover, the results obtained in the second study are consistent with the first or previous studies. Due to both the decreasing of leaves

per plant and leaf damage increasing caused by salinity, the effectiveness of photosynthesis may be affected as well; resulting in the tiller no fertility.

Only in IN 35 kept the constant number of leaves per plant until reducing the value at 150 mM NaCl. These results at Inpari 35 may mean that this variety is also affected by salinity, but is still tolerant and forms stems and leaves; this was only observed at the beginning of the salinity exposure within one week after transplanting. Leaf symptom scores strongly increased in all rice varieties indicate the effect of salinity stress and caused the reduction of effective leaf area for photosynthesis. The symptom of leaf such as yellowing leaves, pale and burn-like appearance at the tips of plant leaves was reported as the results of salinity stress (Guide, 2010; McCauley et al., 2009). These leaf symptoms can affect plant physiology, reducing the effectiveness of leaf's sunlight absorption and photosynthesis. The presence of these symptoms on leaves can also negatively impact the number of living leaves, the size of leaves, withering and scorch in plants face with stress for a longer time (Tatagiba et al., 2016; Hong et al., 2018). After that, the plant stems can become stunted, leading to death. In addition, other plant parts that interfere with carrying out activities in the transportation process and metabolism that make plant can't run normally (Tatagiba et al., 2016).

The influence of leaf formation, plant height, number of tillers and other agronomic properties occurs at the vegetative and reproductive stages (Hussain et al., 2019). Elias et al. (2020) reported that salt stress can cause symptoms in plants as reduced leaf numbers, decreased plant height, and reduced formation number of tillers (Elias et al., 2020). Moreover, some plants can also demonstrate stunted growth, chlorosis, interveinal chlorosis, and necrosis during salt stress (Acosta-Motos et al., 2017; Cantabella et al., 2017). However, the different responses in the early vegetative stage (three weeks after planting) in rice varieties in this study, are dominant by its genetics. The longer observation on plants grown under salinity stress should concern.

At two weeks after transplanting, in each factor: salinity level and variety, there is a significant difference with a significant level of variation ($P < 0.05$ and $P < 0.01$) in all characteristics shown in Table 35. However, there is no interaction between the two factors was observed in all characteristics (Table 35).

The measurement results, the highest variables in two characteristics including; The number of tillers per plant and plant height were obtained for the IN 35 variety, followed by CNT 1 and PT 1, respectively. Contrast to leaf symptom scoring, the higher this trait was observed in PT 1 and CNT 1, respectively, the lower value was IN 35. For two Thai rice varieties; CNT 1 and PT 1 showed the higher number of leaves per plant more than IN 35.

There was no interaction between salinity and varieties with significant difference in all these characteristics. However, considered the value increased or decreased in percentage compared with control treatment in each variety in characteristics shown in Table 35. All varieties showed a reduced number of leaves per plant and plant height. There were differences between these results in week 1 and the results in the same characteristics in week 2 after transplanting. Which, in week 2 after transplanting, the plant seems under the stress more than the week 1 after transplanting in salty condition. Thus, decreasing both the number of tillers per plant and plant height were observed in all rice varieties. The reduction in these characteristics was a percentage increase as the salinity level increased, which was observed in all three rice varieties. That is, these two characteristics are clearly affected by the salinity problem and vary with increasing salinity levels. For tillering, it is an important yield component of rice yield, found that at both weeks; week 1 and 2 after transplanting showed the highest overall mean values in IN 35. However, the increase in tillering within one week; compared between values in week 1 and 2 after transplanting in IN 35 was lower than that of two Thai rice. This was because there was a relatively high percentage reduction compared to the control group for IN 35 at different salinity levels at week 2 after transplanting. This appearance will also be used to assess the salinity tolerance at tillering stage in these rice varieties.

The highest number of leaves and number of tillers per plant, as well as plant height was indicated by 0 mM salinity or control treatment. However, the control treatment was not significantly different by giving any concentration of NaCl to the number of leaves per plant (Table 35). Although both of the number tiller or plant height was recorded affected by salinity at 50 mM NaCl with the decreasing on values in all rice varieties. At 50 mM NaCl seems no negative effect on the establishment of a new leaf, as shown in the percentage increase compared to the control treatment at 0

mM NaCl in all varieties. The negative affected by salinity to leave forming started at 100 mM NaCl and was the highest affected at 150 mM NaCl in all varieties. It was also noted that at the second week of transplanting in IN 35, the effect of salinity was seen at 50 mM NaCl, whereas in the first week after planting, the negative effect was at 150 mM NaCl. Opposite to two Thai rice varieties; CNT 1 and PT 1, there were reduction in number of leaves at 50 mM and 100 mM NaCl in first and second week after transplanting, respectively (Table 34-35). Effect of high salinity causes leaf development, cell elongation nutrient uptake. Salinity in general affects the decline in plant growth due to reduced leaf area and the number of leaves formed. Salinity also affects the roots, which shortens the roots and makes the roots thinner (Neumann et al., 1994). (Pangaribuan, 2001) reported that high salt stress would cause the process of respiration and photosynthesis to become unbalanced. If the respiration process becomes greater than photosynthesis, then the dry weight of the plant is reduced. Crop production is greatly affected by salinity stress, such as in paddy fields, in grasslands and trees by interfering with nitrogen uptake, reducing growth and also stopping plant reproduction. Some ions (especially chloride) are toxic to plants and as the concentration of these ions increases, plants become poisoned and die (Alam, 1999).

Nevertheless, leaf symptom scoring caused by salinity levels in each variety should assess together with the number of leaves forming. Though, the increasing of the leaf was formed at 50 mM NaCl in all rice varieties. The symptom on leaves also was scored increase since at 50 mM NaCl. This means the plant is affected by salinity since at 50 mM NaCl. But, at low stress of salinity, leaf damage is affected before the formation of leaves is reduced. The observed foliar symptoms were consistent with all traits in all cultivars with increased damage as salinity increased. Moreover, the mean leaf count of Inpari 35 was the highest and the least damage on the leaf was found, while the other two varieties had less leaf count, but more damage.

Table 35 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 2 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	15.0 ± 0.5	5.1 ± 0.4	57.3 ± 2.5	1.7 ± 0.5
50 mM (% NC)	15.7 ± 2.0 (+4.67)	4.6 ± 0.3 (-9.81)	55.4 ± 1.9 (-3.32)	2.0 ± 0.3 (+17.65)
100 mM (% NC)	14.3 ± 1.0 (-4.67)	4.3 ± 0.2 (-15.70)	52.6 ± 1.0 (-8.20)	2.2 ± 0.4 (+29.41)
150 mM (% NC)	12.6 ± 0.5 (-16.00)	4.0 ± 0.1 (-21.60)	49.9 ± 3.4 (-12.91)	2.4 ± 0.3 (+41.18)
Mean (IN 35)	14.4 ± 1.5 a	4.5 ± 0.5 a	53.8 ± 3.6 a	2.1 ±0.4 c
PT 1				
0 mM (NC)	13.0 ± 1.0	4.5 ± 0.4	50.5 ± 2.5	2.2 ± 0.5
50 mM (% NC)	13.3 ± 1.4 (+2.31)	4.0 ± 0.3 (-11.11)	48.2 ± 2.5 (-3.98)	2.8 ± 0.2 (+27.27)
100 mM (% NC)	12.5 ± 0.7 (-3.85)	3.9 ± 0.3 (-13.33)	46.7 ± 1.4 (-6.97)	2.9 ± 0.3 (+31.82)
150 mM (% NC)	10.9 ± 0.3 (-16.15)	3.7 ± 0.3 (-17.78)	43.8 ± 2.5 (-13.27)	3.3 ± 0.2 (+50.00)
Mean (PT1)	12.4 ± 1.3 b	4.0 ± 0.4 c	47.3 ± 3.2 c	2.8 ± 0.5 a
CNT 1				
0 mM (NC)	14.6 ± 1.4	4.7 ± 0.4	52.3 ± 2.1	1.9 ± 0.5
50 mM (% NC)	15.2 ± 2.2 (+4.11)	4.3 ± 0.2 (-8.51)	50.2 ± 1.6 (-4.02)	2.4 ± 0.2 (+26.32)
100 mM (% NC)	13.4 ± 1.3 (-8.85)	4.1 ± 0.2 (-12.77)	48.9 ± 1.6 (-6.50)	2.5 ± 0.3 (+31.60)
150 mM (% NC)	11.5 ± 0.7 (-21.23)	3.9 ± 0.2 (-17.02)	46.6 ± 1.8 (-10.90)	2.6 ± 0.4 (+36.84)
Mean (CNT 1)	13.7 ± 2.0 b	4.2 ± 0.4 b	49.4 ± 2.7 b	2.4 ± 0.5 b
Over all mean	13.5	4.24	50.18	2.41
0 mM (NC)	14.2 ± 1.3 AB	4.7 ± 0.5 A	53.4 ± 3.7 A	1.9 ± 0.6 C
50 mM (% NC)	14.7 ± 2.1 A (+3.52)	4.3 ± 0.3 B (-8.51)	51.3 ± 3.7 B (-3.93)	2.4 ± 0.4 B (+26.32)
100 mM (% NC)	13.4 ± 1.2 B (-5.63)	4.1 ± 0.3 B (-12.77)	49.4 ± 2.8 C (-7.50)	2.5 ± 0.4 AB (+31.58)
150 mM (% NC)	11.7 ± 0.9 C (-17.61)	3.9 ± 0.2 C (-17.02)	46.7 ± 3.6 D (-12.55)	2.8 ± 0.5 A (+47.37)
P value (Varieties)	$4.88 \times 10^{-8**}$	$1.65 \times 10^{-5**}$	$3.82 \times 10^{-12**}$	$3.38 \times 10^{-6**}$
P value (Salinity)	$2.20 \times 10^{-5**}$	$1.09 \times 10^{-9**}$	$4.13 \times 10^{-10**}$	$5.19 \times 10^{-6**}$
P value (Salinity:Variety)	0.925 NS	0.980 NS	0.959 NS	0.948 NS
CV (%)	9.03	6.85	4.3	16.1

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

The occurrence in the second week after transplanting could be summarized that this IN 35 appeared to be more affected by salinity causing the reduction of tiller forming than the other two varieties. However, the potential of the original in IN 35 with a high number of tillers per plant kept the highest value in the overall mean. Moreover, Inpari 35 has more leaves per plant, but fewer foliar symptoms compared to two Thai rice varieties. While, between the Thai rice varieties; CNT 1 and PT 1,

they appear to be somewhat similar responses to salinity levels on most of the characteristics. Except that the PT 1 has higher damage on leaves on average more than CNT 1 under salinity stress. While the effects of salinity on plant height in all three rice varieties were similar.

At the third week after transplanting, no interaction between the two main factors on each characteristic. That allows clearly the influence of these two factors, both factors of variety and salinity separately (Table 36). At two weeks post-planting, it was found that the Inpari 35 variety started to be tolerant of salinity, with a higher rating for maintaining its agronomic characteristics more than others varieties. In three weeks after transplanting, similar results have been observed. To consider in detail each characteristic, it was found that the IN 35 was higher in all characteristics than CNT 1 and PT 1. Between Thai rice varieties, CNT 1 showed a higher number of tillers per plant and plant height more than PT1. In contrast, to leave symptom scoring, the higher value was recorded in PT 1, followed by CNT 1 and IN 35, respectively. However, the number of leaves per plant was not significantly different between CNT 1 and PT 1. There was a significant difference effect by salinity level in all characteristics (Table 36). A decreased value was observed started at 50 mM NaCl and decreased values will increase according to the salinity increased. However, only the number of leaves per plant was clearly different between values at 50 mM and 100 mM NaCl. Therefore, the characteristics appear to be consistent with salinity affecting both tillers and leaf formation, and plant height development. Salinity also affects foliar symptoms that affect photosynthetic efficiency (Francois and Maas, 1999).

Although there was no significant difference in the interaction between factors; salinity levels and varieties. Changing in percentage compared to control treatment in each variety in each characteristic has been calculated for analysis (Table 36). In all the characteristics in each variety (excepted leaf symptom scoring in Inpari 35 at 50 mM-100 mM NaCl), the effect of salinity could be identified from values that were lowest at 0 mM NaCl to highest at 150 mM NaCl. Comparing with control treatment, in all characteristics and in each variety at third week after transplanting, the decreasing percentage was observed since at 50 mM NaCl.

At a plant age of three weeks after transplanting, rice at the tillering stage received salinity, it was found that salinity effect starts at 50 mM NaCl; had affected

all characteristics. In accordance with the existing results, in this case, it can be shown that prolonged exposure to salinity stress will affect all agronomic characteristics; tolerant and intolerant varieties, even at the lowest salinity levels. The effect of salinity based on observations found through several characteristic assessments in the first and second weeks of exposure to salinity; plants when they begin to weaken two weeks after exposure to salinity or within a few weeks after planting. This can make plants with cell capabilities begin to break down and even die (Abdelaal et al., 2020)). The most important thing about its management is the length of time the plants experience salinity stress (Eisa et al., 2012a).

The effect of salinity as a percentage was relatively stable between the second and third weeks after transplant for two characteristics: leaf number per plant and plant height (Table 35-36). The other two characteristics, namely tiller number per plant and scoring of foliar symptoms, were affected increasing by salinity stress; in the third week compared to the second week after transplant, especially in Thai rice varieties, CNT 1 and PT 1. More than 50 percent of decreasing value was found in leaf symptom scoring in CNT 1 and PT 1 at 150 mM NaCl this week (Table 15).

Through the expression of differences in salinity and rice varieties explained about the ability of each to maintain osmotic pressure, salinity stress is one of the mechanisms that affect plant growth and development. On this occasion, the rice variety 'Inpari 35' demonstrated its ability to tolerate the highest salinity when compared to the other two varieties; PT 1 and CNT 1 also have reports on their ability to survive and grow in certain places and have high salt stress conditions in the field (de Oliveira et al., 2013; Sembiring et al., 2020). It was observed and seen that, the symptoms that appeared on the plants began to stress and some leaves with a greater number of yellowing, dry ends, leaf edges look like burning and some even start to die and dry up (McCauley et al., 2009a; Mondal et al., 2020). Salinity causes more membrane damage in plants. On membrane permeability and lipid peroxidation, with a significant increase in salinity stress, and this oxidative damage, abiotic stress can cause damage to different membranes including some of the most mediated through membrane lipid peroxidation (Eraslan et al., 2008; Gunes et al., 2008). The presence of fatal consequences due to excess accumulation of sodium and iodine in certain parts of the rice plant, especially for the leaves, can indicate that some leaves are

starting to experience stress that appears such as turning yellow until they die slowly (El-Ramady et al., 2014; Ghasemi-Omran et al., 2021). Various salt stresses, the first step in diagnosing deficiencies of several important nutrients, are about specific explanations for the symptoms and functions of each nutrient which are then directly related to the interests of plant growth (Hatibu, 2018a). As one example, when there are nutrients and visible functional things that occur in plants such as plant leaves turning pale, leaf size is smaller but can be considered less than normal plants, photosynthesis is reduced, and protein production is less, plants that grow are usually slow and dwarf plants (Elemike et al., 2019). The mechanism in the formation of plants that will affect the formation of tillers and leaves, as well as the formation of plant tissue. The effect of salinity on plants has a very strong effect on certain characteristics and nutritional imbalances.

The presence of factors used in NaCl treatment can induce deficiency of certain elements such as; potassium (K) and increase sodium (Na), calcium (Ca), magnesium (Mg), and also chloride (Cl) in the state of rice plants (Chrysargyris et al., 2019). Cause oxidative damage, especially in nucleic acids, fats and proteins. is that which arises with the salt imbalance associated with the presence of a redox system. In the early stages when salinity stress occurs, with the plant's ability to respond and the ability to tolerate the stress; to release certain antioxidant enzymes, and osmoprotectant molecules to filter and protect plant tissues from toxins and oxidative residues. However, after that, if salt exposure is further increased, there is still an opportunity to respond to salt stress tolerance through decreased capacity in plants (Parida and Das, 2005; Ahmad et al., 2019). Thus, it has been found that from all levels of salinity very negative effect on all agronomic characteristics in the second week, after transplanting. In addition, some varieties are easily affected by exposure to salinity, and other abiotic factors that are considered less resistant to certain abiotic stresses, such as insects and disease. Opposite, in some varieties, have resistance to stress conditions that can reduce plant health specifically (Quais et al., 2020).

It has been reported that low concentrations of salt stress stimulate plant growth and development, but at the highest concentrations it can reduce plant capacity, especially for certain physiological characteristics (Warne et al., 1990; Yu et al., 2018). Many plants have been found through various methods to explore salt

stress with high concentrations. It can be said that salinity affects plant growth through certain physiological and biochemical processes and will reduce production yields and ultimately cause death (D'antonio and Meyerson, 2002). However, each variety shows different results and does not match the characteristics that are influenced by different levels of salinity, this is because after all it depends on the response of genetic diversity (De Leon et al., 2015; Agustian, 2020). There are certain possibilities according to some genetic interactions and some environmental factors which are not evaluated directly here. Or as for certain factors that are assessed in this study but do not have clarity other than influences, such as genetics and salinity levels.

IN 35 is a variety that is capable and tends to be more adaptive in several parameters compared to the agronomic characteristics of plants at the vegetative stage when compared to the two Thai varieties. States that the tolerance of certain plants to environmental stresses, for example as salinity, the tolerant variety shows very in adaptable to several physical characteristics than susceptible variety according to certain environment (Sitompul and Guritno, 1995; Singh et al., 2009).

The appearance in the third week after transplanting could be summarized that this IN 35 appeared to be affected by salinity causing the reduction number of tillers per plant (in percentage value) to be similar to a second week after transplanting. However, in CNT 1 and PT 1, the decreasing percentage in each salinity level in this characteristic showed higher at third weeks more than at two weeks after transplanting. Moreover, the potential of the original in IN 35 with a high number of tillers per plant than other varieties, thus, it kept the highest value in the overall means.

Table 36 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 3 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	18.2 ± 0.7	6.8 ± 0.6	59.7 ± 4.6	2.2 ± 0.5
50 mM (% NC)	16.8 ± 0.6 (-7.69)	6.4 ± 0.5 (-5.90)	58.3 ± 1.1 (-2.35)	2.0 ± 0.2 (-9.09)
100 mM (% NC)	16.2 ± 0.7 (-10.99)	6.0 ± 0.5 (-11.76)	57.2 ± 1.4 (-4.19)	2.2 ± 0.5 (0.00)
150 mM (% NC)	15.2 ± 1.0 (-16.48)	5.5 ± 0.4 (-19.12)	54.6 ± 2.0 (-8.54)	2.7 ± 0.5 (+22.73)
Mean (IN 35)	16.6 ± 1.3 a	6.2 ± 0.7 a	57.4 ± 3.1 a	2.1 ± 0.6 b
PT 1				
0 mM (NC)	16.8 ± 0.7	6.2 ± 0.4	54.0 ± 5.6	2.0 ± 0.6
50 mM (% NC)	16.5 ± 1.1 (-1.79)	5.4 ± 0.5 (-12.90)	53.5 ± 2.0 (-0.93)	2.4 ± 0.3 (+20.00)
100 mM (% NC)	15.3 ± 0.4 (-8.93)	5.2 ± 0.6 (-16.13)	50.8 ± 4.8 (-5.93)	2.7 ± 0.7 (+35.00)
150 mM (% NC)	14.3 ± 0.9 (-14.88)	4.7 ± 0.5 (-24.20)	47.1 ± 3.3 (-12.78)	3.2 ± 0.7 (+60.00)
Mean (PT1)	15.7 ± 1.3 b	5.4 ± 0.7 c	51.3 ± 4.7 c	2.6 ± 0.7 a
CNT 1				
0 mM (NC)	17.4 ± 0.6	6.4 ± 0.4	57.7 ± 3.6	1.9 ± 0.5
50 mM (% NC)	16.4± 0.6 (-5.75)	5.8 ± 0.4 (-9.40)	55.1 ± 4.4 (-4.51)	2.2 ± 0.2 (+15.79)
100 mM (% NC)	15.5 ± 0.4 (-10.92)	5.6 ± 0.5 (-12.50)	52.1 ± 4.8 (-9.71)	2.6 ± 0.5 (+36.84)
150 mM (% NC)	14.9 ± 0.8 (-14.37)	5.1 ± 0.6 (-20.31)	49.3 ± 5.4 (-14.37)	3.0 ± 0.6 (+57.90)
Mean (CNT 1)	16.1 ± 1.3 b	5.7 ± 0.6 b	53.5 ± 5.3 b	2.4 ± 0.6 ab
Over all mean	16.13	5.76	54.1	2.37
0 mM (NC)	17.5 ± 1.0 A	6.5 ± 0.5 A	57.1 ± 4.9 A	1.9 ± 0.5 C
50 mM (% NC)	16.6 ± 0.7 B (-5.14)	5.9 ± 0.6 B (-9.23)	55.6 ± 3.3 AB (-2.63)	2.2 ± 0.3 BC +15.80)
100 mM (% NC)	15.7 ± 1.0 C (-10.29)	5.6 ± 0.6 B (-13.85)	53.4 ± 4.7 B (-6.48)	2.5 ± 0.6 B (+31.60)
150 mM (% NC)	14.8 ± 1.0 D (-15.43)	5.1 ± 0.6 C (-21.54)	50.3 ± 4.8 C (-11.91)	2.9 ± 0.6 A (+52.63)
P value (Varieties)	0.00609^{**}	$3.33 \times 10^{-5**}$	$4.05 \times 10^{-5**}$	0.0297^{*}
P value (Salinity)	$5.34 \times 10^{-11**}$	$8.13 \times 10^{-8**}$	$1.08 \times 10^{-4**}$	$3.54 \times 10^{-6**}$
P value (Salinity:Variety)	0.82 NS	0.99 NS	0.94 NS	0.990 NS
CV (%)	5.08	8.96	7.18	21.67

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

Similar to week 2 after transplanting, IN 35 has more leaves per plant, but fewer foliar symptoms compared to two Thai rice varieties assessed in percentage reduction in each salinity level. While, between the Thai rice varieties; CNT 1 and PT 1, appear to be similar responses to salinity levels on most of the characteristics. Except that the PT 1 has higher damage on leaves on average more than CNT 1 under salinity stress. While the effects of salinity on plant height in all three rice varieties

were similar, although, in overall means in plant height, the highest observed in IN 35. Foliar damage percentage (leaf symptom scoring) was found in increasing in CNT 1 and PT 1 in week 3 compared to week 2 after transplanting. While there was a similar reduction percentage in the number of leaves per plant in all varieties in week 3 after transplanting.

In the early stages of rice tiller formation, the agronomic characteristics of plant play an important role in plant growth and sustainable production. One part of the plant that can contribute to production yields such as tillers production, because of the characteristic values that can be indicate yield and final production (Bashir et al., 2010). Where, genetic and environmental factors greatly affect the formation of new tillers from rice plants (Hussien et al., 2014b).

Salinity greatly inhibits all cell activities in food transportation and metabolism processes in all parts of plant (Safdar et al., 2019). For other characteristics including number of leaves per plant, plant height and the healthy leaves, they are relating to photosynthetic ability. However, in salt stress condition, it will be able to reduce the synthesis of chlorophyll pigments and also photosynthesis will experience a pressure rate and various other important processes are involved in it (Najar et al., 2019). For certain plants that are experiencing salinity stress from the highest level, in general salt begins to spread in various directions and other plant parts, causing leaf symptoms to reduce the number of leaves, number of tillers and plant height during the growing period (Prasad et al., 2008; Hussien et al., 2014). It has been proven that salt stress and causes of suppression of induced photosynthesis often experience dependence according to changes in the gas formed, photosynthetic pigments, species or specific cultivars, stomata and metabolic accumulations that are organic and inorganic as well as some chemical elements is categorize as antioxidants (Ahmed et al., 2009).

There is evidence of unusual accumulation of Na^+ and Cl^- in some plant leaf tissues and due to increased air loss and dehydration in the stomata. In the leaf stomata, dehydration occurs which causes the cells to no longer function, and carry out normal metabolism which in turn to reduce growth and development in plants (de Oliveira et al., 2013).

At the tiller stage at four weeks after transplanting, the three rice varieties showed their ability to tolerate salt in different. However, in varieties that have the ability to be effective, it will be kept the constant on agronomic characteristics until entering to the next stage (Mujeeb-Kazi et al., 2019a). At the age of four weeks after planting, the effect of various variations in salt content, statistical analysis showed the agronomic characteristics of rice plants that were actually exposed to salt levels and varieties (Table 16).

In the varietal factor, there are significant differences in several agronomic characteristics which are the observed parameters in Table 37. Which the higher on characteristics was detected in IN 35, followed by CNT 1 and PT 1, respectively. There is a new IN 35 variety from Indonesia, which has been identified as having a higher absorption capacity, especially for plant characteristics and the transport of Na^+ from one part of the plant to another (Tester and Davenport, 2003).

For salinity level, is clearly affected by salinity levels, excepted on number of leaves per plant and number of tillers per plant at 50 mM and 100 mM NaCl. Similarly, between the fourth week and several weeks after planting, there was no interaction between the two combined factors, namely; between salinity level and rice variety.

Similar to three weeks after transplanting, all characteristics showed underwent a change in the reduction value obtained was 50 mM NaCl. Although no interaction between salinity level and varieties, the values that have a negative percentage change because they are in accordance with the increase in salinity levels compared with normal conditions (0 mM NaCl), were considered on all characteristics (Table 37). In two Thai rice varieties, namely: CNT 1 and PT 1 the trait decreased in percentage more than IN 35; especially in two characteristics such as number of tillers per plant and leaf symptom scoring. These occurrences may assume that the higher tolerance ability was found in IN 35, than Thai rice varieties.

Table 37 Effect of different salinity levels (0 mM, 50 mM, 100 mM and 150 mM NaCl) on number of leaves per plant, number of tillers per plant, plant height and leaf symptom score of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)], at week 4 after transplanting

Variety/Salinity	Characteristic values (% NC)			
	Number of leaves per plant	Number of tillers per plant	Plant height (cm)	Leaf symptom scoring
IN 35				
0 mM (NC)	22.4 ± 2.0	7.8 ± 0.6	78.2 ± 5.0	1.5 ± 0.4
50 mM (% NC)	21.1 ± 1.7 (-5.80)	7.7 ± 0.7 (-1.28)	75.1 ± 2.0 (-3.96)	2.0 ± 0.5 (+33.33)
100 mM (% NC)	20.3 ± 1.5 (-9.38)	7.2 ± 0.5 (-7.79)	72.2 ± 2.0 (-7.67)	2.5 ± 0.4 (+66.67)
150 mM (% NC)	19.5 ± 1.2 (-12.95)	6.8 ± 0.8 (-12.82)	69.8 ± 2.1 (-10.74)	2.7 ± 0.4 (+80.00)
Mean (IN 35)	20.8 ± 1.9 a	7.4 ± 0.7 a	73.8 ± 4.3 a	2.2 ± 0.6 b
PT 1				
0 mM (NC)	21.0 ± 1.4	7.1 ± 1.1	74.0 ± 3.9	1.7 ± 0.6
50 mM (% NC)	20.0 ± 0.3 (-4.76)	7.1 ± 0.9 (0.00)	70.1 ± 1.1 (-5.27)	2.7 ± 0.3 (+58.82)
100 mM (% NC)	19.3 ± 0.9 (-8.09)	6.3 ± 1.0 (-11.27)	66.6 ± 1.2 (-10.00)	3.1 ± 0.4 (+82.35)
150 mM (% NC)	18.2 ± 0.3 (-13.36)	5.8 ± 1.2 (-18.31)	66.3 ± 2.3 (-10.41)	3.6 ± 0.6 (+111.76)
Mean (PT1)	19.6 ± 1.5 b	6.6 ± 1.1 b	69.2 ± 4.0 c	2.8 ± 0.9 a
CNT 1				
0 mM (NC)	21.7 ± 1.7	7.4 ± 0.5	76.0 ± 3.9	1.4 ± 0.3
50 mM (% NC)	20.1 ± 1.5 (-7.37)	7.3 ± 0.5 (-1.36)	72.5 ± 2.3 (-4.61)	2.4 ± 0.4 (+71.43)
100 mM (% NC)	19.7 ± 1.2 (-9.22)	6.8 ± 0.6 (-8.11)	70.1 ± 1.1 (-7.76)	2.9 ± 0.4 (+107.14)
150 mM (% NC)	18.8 ± 1.0 (-13.36)	3.3 ± 1.2 (-14.86)	67.6 ± 2.1 (-11.05)	3.3 ± 0.5 (+135.71)
Mean (CNT 1)	20.1 ± 1.6 ab	7.0 ± 0.8 ab	71.5 ± 4.0 b	2.5 ± 0.8 a
Over all mean	20.18	6.96	71.52	2.5
0 mM (NC)	21.7 ± 1.7 A	7.4 ± 0.8 A	76.1 ± 4.4 A	1.5 ± 0.4 D
50 mM (% NC)	20.4 ± 1.6 B (-5.99)	7.3 ± 0.7 AB (-1.35)	72.6 ± 2.7 B (-4.60)	2.4 ± 0.5 C (+60.00)
100 mM (% NC)	19.8 ± 1.2 BC (-9.22)	6.8 ± 0.8 BC (-8.11)	69.6 ± 2.7 C (-8.54)	2.8 ± 0.5 B (+86.67)
150 mM (% NC)	18.9 ± 1.7 C (-12.90)	6.3 ± 0.9 C (-14.86)	67.9 ± 2.5 C (-10.78)	3.2 ± 0.6 A (+113.33)
P value (Varieties)	0.0296^{*}	0.00136^{**}	$2.30 \times 10^{-10**}$	0.00058^{**}
P value (Salinity)	$1.63 \times 10^{-5**}$	0.01941^{*}	$1.24 \times 10^{-5**}$	$1.93 \times 10^{-12**}$
P value (Salinity:ariety)	0.99 NS	0.99 NS	0.98 NS	0.771 NS
CV (%)	7	12.09	3.8	18.52

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

In rice varieties that have tolerance capabilities, in general each plant can utilize its capacity to maintain its condition in growth; with its own mechanism and produce normal production from any environment. In this case, the tolerance means the ability of varieties to adapt to the environment is high both physiologically and morphologically when compared to other varieties with very weak conditions such as grown under salinity stress (Herbst, 2001).

The appearance in the fourth week after transplanting could be summarized that salinity causing the reduction number of leaves per plant and plant height (in percentage value) to be similar to a third week after transplanting. Seems to be a decrease in the percentage of reduction in the number of tillers per plant in each variety that received the salinity in different levels. Opposite, in leaf symptom scoring, over 50% reduction value was observed at 100 mM NaCl, excepted in IN 35. It can be said that in two Thai rice: CNT 1 and PT 1, the decreasing percentage in each salinity level in this characteristic showed higher at four weeks more than at three weeks after transplanting. Both the potential of the original in control treatment in IN 35; with a higher on all characteristics (number of leaves per plant, number of tillers per plant, and plant height) than other varieties, and a lower percentage in a reduction in each salinity level. Thus, it kept the highest value in the overall means in IN 35 more than CNT 1 and PT 1.

Similar to week 3 after transplanting, Inpari 35 has more leaves per plant, but fewer foliar symptoms compared to two Thai rice varieties assessed in percentage reduction in each salinity level. While, between the Thai rice varieties; CNT 1 and PT 1, appear to be similar responses to salinity levels on most of the characteristics. Although CNT 1 has higher damage on leaves on average, it showed a lower effect on the number of tillers per plant compares to PT 1 under salinity stress.

At fourth week after transplanting salinity was significantly more severely affected than other weeks; indicated that both the new tissue establishment was limited and the developed tissue was destroyed. Salt content affects plant growth because it contains various elements in plants as well as in the process of photosynthesis and in nutrients contained in the soil (Hatibu, 2018a).

However, this also supported by a theory which suggests that, plants have the ability to adapt both morphologically and physiologically to abiotic stresses called resistance (Anjum et al., 2011). In other parts of the plant, its sensitivity to sodium chloride is directly related to the plant's inability to metabolize a process, which in the plant in some major vital parts, is easily damaged by salinity stress levels, which are beyond plant limits ability to respond (Otlewska et al., 2020). Although all plants have the same type, it depends on the morphology and physiology of tolerance at high salinity levels (Nawaz et al., 2010; Fahad et al., 2015).

However, from the formation of tillers to the formation of the next stage, it produces panicles which of course will interfere and even damage the results in the next stage. Thus, the tiller stage of rice is generally very sensitive to the influence of certain salt levels. the tiller that produced in this stage must be followed up to be able to form a panicle in the next stage or in the reproductive stage.

Effects on agronomic characteristics in rice at harvesting stage under difference of trehalose application in salinity condition

In the application of these three factors; varieties, salinity level, and trehalose level, only interactions of between salinity level and trehalose was significant different affected on plant height; recorded at harvesting stage. However, for these three factors separately affected plant height (Table 38). Overall means, for varieties, plant height showed the highest in the IN 35 variety (76.67 cm), followed by two Thai rice CNT 1 (74.43 cm) and PT 1 (73.90 cm). For salinity level, the highest plant height was found at 0 mM NaCl (control treatment) (83.26) and followed by 50 mM NaCl (78.23 cm), the lowest at 100 mM (70.23 cm) and 150 mM NaCl (68.28 cm). Whereas, when trehalose was applied to the rice plants, the highest plant height was observed at 100 mM and 150 mM, followed by 0 mM and 50 mM, respectively.

Interaction between salinity level and trehalose showed the highest plant height was found at no salinity (0 mM NaCl) in all trehalose concentrations. At 50 mM-150 mM NaCl, the decreased value was showed according to salinity increase. In these salinity levels (50-150 mM NaCl), the higher values were found in different concentrations of trehalose (Table 38). Thus, seems the dominant effect on plant height was the salinity effect.

Table 38 Average plant height (cm) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvesting stage by application of four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) at tillering stage (6 weeks after transplanting) when plants grown under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Variety	Trehalose (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	81.80 ± 3.10	77.14 ± 2.91	71.91 ± 1.74	67.63 ± 1.90	74.43 ± 9.92 b
	50	81.26 ± 3.01	78.45 ± 3.33	58.30 ± 2.71	67.86 ± 1.64	
	100	83.60 ± 2.00	79.60 ± 3.76	71.56 ± 2.10	67.40 ± 1.64	
	150	84.93 ± 0.90	78.20 ± 3.70	73.07 ± 3.65	68.23 ± 1.33	
PT 1	0	81.86 ± 3.04	77.83 ± 3.74	71.34 ± 1.56	68.36 ± 1.94	73.90 ±10.06 b
	50	80.01 ± 6.03	78.41 ± 3.25	57.67 ± 2.45	68.06 ±1.90	
	100	83.37 ± 1.01	78.03 ± 2.54	71.65 ± 1.60	64.73 ± 5.30	
	150	84.83 ± 1.47	76.51 ± 3.90	71.81 ± 3.26	67.90 ± 0.72	
IN 35	0	82.11 ± 2.60	76.20 ± 2.61	73.91 ± 4.20	69.74 ± 0.54	76.67 ± 6. 07 a
	50	83.34 ± 2.45	79.26 ± 3.55	71.85 ± 1.64	70.31 ± 1.30	
	100	85.45 ± 0.91	79.17 ± 3.27	74.52 ± 2.75	69.22 ± 2.10	
	150	86.50 ± 0.31	80.00 ± 3.66	75.18 ± 3.78	70.00 ± 1.55	
Mean salinity		83.26 ± 3.04 A	78.23 ± 3.23 B	70.23 ± 12.17 C	68.28 ± 2.44 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		74.98 ± 5.65 ab	72.90 ± 13.72 b	75.70 ± 7.04 a	76.43 ± 6.80 a	
Salinity (mM NaCl)						
Trehalose (mM)		0	50	100	150	
	0	81.91 ± 2.71 ab	77.06 ± 2.98 cd	72.39 ± 2.81 de	67.57 ± 1.73 ef	
	50	81.56 ± 4.08 ab	78.71 ± 3.15 b	62.60 ± 22.66 g	68.74 ± 2.06 ef	
	100	84.14 ± 1.62 a	78.93 ± 3.10 b	72.58 ± 2.48 de	67.11 ± 3.70 fg	
	150	85.41 ±1.22 a	78.23 ± 3.67 bc	73.36 ± 3 60 de	68.70 ± 1.50 ef	
P-value (F-test)						
Variety (V) 0.017*, Salinity (S) < 2 x 10^{-16}**, Trehalose (T) 0.021*, V × S 0.652 NS, V× T 0.7834 NS, S × T 0.00762**, V× S × T 0.995 NS, CV% 8.59						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Since salinity reacts at the beginning of tillers are formed and the impact is estimated at the final stage of harvest. For a longer period of salinity, will give the effect of salinity if it is more pronounced at lower concentrations. Thus, it can be said that for every increase in the amount of salinity, the increase in plant height in the field will decrease (Bernstein, 1964). Salinity can reduce the parameter of rice plant height growth significantly (Lee et al., 2003). Reduced parts of plants, such as for

plant height and development because certain cells are disturbed, eventually the process becomes hampered (Ashraf and Wu, 1994). The effect of salinity stress is thought to be due to the difficulty of absorbing water and the effect of NaCl application. As a result, it inhibits the development process in cells and reduces plant height for rice varieties compared to rice plants under normal conditions (Negrão et al., 2017).

Plant height is important and is considered in combination with other characteristics. It can be said that plant height has an important role, including traits such as; free to receive sunlight, and photosynthesizing because it is not shaded by other plants around it, providing opportunities and stem segments for the growth of the surrounding leaves. A very large contribution in decreasing plant height is through salt concentration with a gradual increase in salinity from low to medium to high which can cause slow growth of rice plants (Hasanuzzaman et al., 2013b). Plant height is one of the characteristics of plants as the main support for rice plant growth to the total yield component. Trehalose was an exogenous application only once at tiller stage; thus, less effect may be observed in this study.

The results of ANOVA showed a significant difference in the number of tillers per plant affected by individual factors include rice variety and salinity level. No interaction between any factors either two or three factors with significant differences on this characteristic (Table 39).

Effect of rice variety, IN 35 (7.59) was higher on the number of tillers per plant than two Thai rice varieties; CNT 1 (6.29) and PT 1 (6.26). There was the highest value of tiller numbers per plant at 0 mM NaCl at 8.33 tillers, and values decreased followed salinity increased; start decreasing at 50 mM NaCl (Table 39). Similar between the influence of the two factors: variety and salinity level on these both traits: plant height and the number of tillers per plant.

Although, application of trehalose was reported can increase rice plants growing in salty conditions which may be due to increased water absorption and enhancing plant stabilizing (Khan et al., 2014). In addition, the ability of plants to withstand after using some external chemical can improve the tolerance ability of plants growing under excessive ion concentration (Kerbab et al., 2021).

The decrease in water potential, compatible with the accumulation of osmolytes involved in reserve osmoregulation, allows water to be extracted from the environment. Thus, protecting the direct effects of water shortage on plant organisms is one answer to help plants to survive under this osmotic stress (Serraj and Sinclair, 2002). The effect of osmotic stress is thought to make water absorption in plants difficult to absorb, as well as the effect of Na and Cl ions due to excessive NaCl administration, cell enlargement was inhibited and the number of tillers was reduced compared to other rice plants under normal conditions (Menete et al., 2008). If the trehalose content is higher, it can retain water in the plant. Trehalose as a solute that protects macromolecules against denaturation, as a means to reduce acidity in cells and acts as a storage (Hoekstra et al., 2001). Trehalose is a source of sugar and nitrogen and compounds for rapid growth of rice varieties after stress (Mohamed et al., 2018).

Nevertheless, in detecting the positive effect of trehalose on the salt environment in this study is less effect Thus, it is very important to understand the exact role of trehalose in rice plants even though all three concentrations contribute little and do not differ from normal control conditions on promotes the tiller production, in each of the three rice varieties that have been tested, it can be seen that the IN 35 variety produced more total tillers than the other two varieties (PT 1 and CNT 1). However, after careful consideration, it was found that these values were quite different from the values under normal growing conditions without salinity (at 0 mM NaCl). For this reason, the higher on the number of tillers per plant in IN 35 may cause by its own potential and the tolerance ability under growing in salinity stress. However, it should not justice only the tiller numbers production, it is necessary to monitor the tolerance of varieties from other characteristics such as the number of productive tiller numbers as well. Due to its ability to tolerate salinity stress in plants, it is necessary to consider physical adaptation to many characteristics (Barus et al., 2015).

Table 39 Average number of tillers per plant of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvesting stage by application of four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) at tillering stage (6 weeks after transplanting) when plants grown under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	7.6 ± 2.90	5.8 ± 2.05	5.6 ± 1.52	54 ± 1.82	6.29 ± 1.85 b
	50	7.4 ± 2.70	6.6 ± 1.34	6.2 ± 1.64	4.8 ± 1.48	
	100	7.6 ± 1.14	6.2 ± 1.30	6.4 ± 1.52	4.4 ± 1.34	
	150	8.4 ± 1.14	6.8 ± 1.10	6.6 ± 0.90	4.8 ± 1.10	
PT 1	0	7.2 ± 3.35	6.4 ± 2.90	5.6 ± 2.70	5.0 ± 2.83	6.26 ± 2.25 b
	50	8.0 ± 1.60	7.8 ± 1.80	5.4 ± 2.07	5.0 ± 2.00	
	100	7.8 ± 1.10	6.6 ± 2.70	5.8 ± 2.92	4.6 ± 1.82	
	150	7.8 ± 1.92	6.8 ± 1.50	5.6 ± 0.90	4.8 ± 1.64	
IN 35	0	8.8 ± 2.68	7.4 ± 2.30	7.0 ± 2.92	6.0 ± 2.92	7.59 ± 2.46 a
	50	9.0 ± 3.00	7.2 ± 2.60	7.0 ± 1.41	5.6 ± 1.82	
	100	10.2 ± 2.68	9.0 ± 2.24	7.4 ± 1.14	5.6 ± 2.00	
	150	10.2 ± 2.17	7.8 ± 0.64	7.6 ± 0.55	5.6 ± 1.67	
Mean salinity		8.33 ± 2.32 A	7.03 ± 2.00 B	6.35 ± 1.74 B	5.13 ± 1.81 C	
Trehalose (mM)						
		0	50	100	150	
Mean Trehalose		6.50 ± 2.60	6.67 ± 2.21	6.80 ± 2.33	6.90 ± 1.96	
P-value (F-test)						
Variety (V) 2.12 x 10^{-5**}, Salinity (S) 2.09 x 10^{-14**}, Trehalose (T) 0.695 NS, V × S 0.777 NS, V × T 0.860 NS, S × T 0.956 NS, V × S × T 1.000 NS, CV% 29.94						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

According to the statement that excess Na^+ can cause damage to plant cells. It is therefore affected by plant growth, but varies genetically with different degrees of severity (Mostofa et al., 2015b).

If other environments that affect the formation of the tiller is ignored, as long as the plants were still growing under the stresses such as salinity in this case, strong impact on plant growth can be observed.

For trehalose, its efficient caused by physicochemical properties to stabilize proteins, dehydrating enzymes, and lipid membranes, as well as to protect the

structure from damage during drying stress (Sadak, 2019). The ability of trehalose has the added advantage of being a signaling molecule as well as an antioxidant. Trehalose acts as an elicitor of genes involved in detoxification and resistance to stress (Kosar et al., 2019). Additional applications increase the internal osmolyte and are suggested as an alternative approach to induce tolerance to salinity (Sadak, 2019). However, using exogenous trehalose, the frequencies for using is one criterion should to concern in addition with the concentration.

To assess the number of productive tillers per plant according to effect of genetic (varieties), salinity level, and trehalose level for application and showed the results in Table 40. Individual three factors showed significant differences that affected the number of productive tillers per plant include rice variety, salinity level, and trehalose level. For varieties, IN 35 (5.40) showed that the highest on this trait, followed by CNT 1 (4.40), and lowest in PT 1 (3.70). There was the highest number of productive tillers per plant at 0 mM NaCl (6.07), and rapidly decreasing at 50 mM NaCl (4.78). At 100 mM and 150 mM NaCl, there was a lower number of productive tillers per plant compared to other salinity, and they were significant difference in mean. The highest value of the number of traits was observed with the higher concentration of trehalose at 150 mM (4.90). While other concentrations of trehalose (0, 50, and 100 mM) were lower value (4.28-4.38) with no significant difference among them. Although, plants with the ability to form large numbers of tillers are estimated to have higher tiller productivity when compared to plants with few tillers (Yan-Ling et al., 2021). Moreover, healthy tillers can produce the fertile panicles and produce the filled seed, it is called productive tillers. However, in this case, the tiller was forming from plants growing under salinity stress. Thus, the relation between the number of tillers and the number of productive tillers per plant should be evaluated too. Comparison between the number of tillers and the number of productive tillers per plant and showed in percentages (in the parenthesis) in Table 40. The result showed that the highest percentage of forming the tiller in plants observed was IN 35 (71.15%), and followed by CNT 1 (69.95%) and PT 1 (59.10%), respectively. Evaluated from different varieties, IN 35 seems more tolerant to salinity stress than other varieties; assessed on the percentage for productive tillers production more than

others. Among Thai rice varieties, CNT 1 showed a higher percentage of productive tillers number, thus, it seems more tolerant to the stress caused by salinity than PT1.

Moreover, in plants where non-stress from salinity showed highest on productive tillers number, it also found higher in percentage to produced productive tillers as well (Table 40). The percentages in the production of productive tillers were reducing at salinity level increase, respectively. Therefore, the stress caused by salinity affected infertile tillers and surely will cause a reduction of rice yield at harvest time. While increasing the amount of trehalose was found to increase the percentage of productive tillers per plant formation. Which, the highest percentage of productive tillers were found at 150 mM trehalose application (Table 40).

Table 40 Average number of productive tillers per plant of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] and percentage compared to the number of tillers per plant (in parenthesis) at harvest stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	5.8 ± 0.84	4.8 ± 0.84	3.8 ± 0.84	2.8 ± 0.84	4.40 ± 1.40 b -69.95%
	50	5.4 ± 1.34	4.6 ± 1.14	3.8 ± 0.84	3.0 ± 1.00	
	100	5.8 ± 0.84	4.4 ± 0.55	3.8 ± 0.83	2.8 ± 1.30	
	150	6.2 ± 1.64	5.4 ± 1.14	4.0 ± 0.71	3.6 ± 0. 55	
PT 1	0	5.2 ± 0.84	3.8 ± 0.84	3.0 ± 1.00	1.8 ± 0.84	3.70 ± 1.37 c -59.1%
	50	5.0 ± 1.00	4.0 ± 0.71	3.0 ± 0.71	2.8 ± 0.84	
	100	5.0 ± 0.71	3.4 ± 0.55	3.2 ± 0.84	2.6 ± 1.14	
	150	5.8 ± 0.84	4.6 ± 0.90	3.0 ± 0.71	2.4 ± 1.14	
IN 35	0	6.6 ± 1.14	5.6 ± 1.14	5.0 ± 1.00	4.0 ± 0.71	5.40 ± 1.50 a -71.15%
	50	6.8 ± 1.30	5.4 ±1.14	4.8 ± 0.84	4.0 ± 1.00	
	100	7.2 ± 0.84	5.2 ± 0.83	4.2 ± 0.84	4.8 ± 0.84	
	150	7.4 ± 1.82	6.2 ± 1.10	5.2 ± 0.84	4.6 ± 0.55	
Mean salinity		6.07 ± 1.31 A -72.87%	4.78 ± 1.15 B -67.99%	4.00 ± 1.07 C -62.99%	3.20 ± 1.14 D -62.38%	
Trehalose (mM)						
		0	50	100	150	
Mean Trehalose		4.35 ± 1.58 b -66.92%	4.38 ± 1.46 b -65.67%	4.28 ± 1.51 b -62.94%	4.90 ± 1.70 a -71.01%	
P-value (F-test)						
Variety (V) < 2 x 10^{-16**}, Salinity (S) < 2 x 10^{-16**}, Trehalose (T) 0.0039**, V × S 0.9828 NS, V× T 0.9595 NS, S × T 0.7762 NS, V× S × T 0.9961, CV% 21.57						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

To increase the number of productive tillers, it had a negative effect caused by salinity. For activities experiencing salinity stress, the nutrients contained in the soil are transport processes that are widely circulated, causing a shortage of available water, plant absorption capacity, circulation processes rather than metabolism that is working in plant cells (Chavarria and dos Santos, 2012).

Likewise, increased salt stress decreased the number of tillers (Khursheed et al.; Yaghoubian et al., 2012). For different varieties that can be influenced by different factors, it was found that, Inpari 35 obtained the highest number of productive tillers, followed by CNT 1 and PT 1, respectively. Each variety has a special ability where a plant has strong properties to withstand several environmental influences, both to excessive salt content and drought and excess water, which are factors that often occur in certain places (Syvertsen and Garcia-Sanchez, 2014). However, salinity can cause all plant activities to be disrupted, resulting in a decrease in productivity rather than plant agronomy characteristics, such as the number of productive tillers. Salinity can occur anytime and anywhere in a place if it is likely to give rise to salt (Hillel, 2000). Thus, rice tiller productivity is highly dependent on genetics, although growth conditions such as salinity are very important factors (Singh et al., 2009).

For the four levels of trehalose, the increase in the number of productive tillers was very significant with the addition of trehalose concentration. Various diagnoses of nutrient deficiency in plants affected by salinity can explain the respective functions of the nutrients associated with plants (Liang et al., 2017). In addition, trehalose accumulates in the state of the plant, perhaps as a result of additional changes in value and adaptation to certain plants that grow and develop under stress (Pilon-Smits et al., 1998). The accumulation of higher levels of trehalose occurs due to increased activity that occurs in more efficient plants. Because initially the activity of enzymes involved in the biosynthesis of trehalose can synergize directly with the administration of trehalose, so the presence of trehalose can renew and stabilize plant conditions under salt stress (Thammahong et al., 2017). Trehalose helps this rice variety to be able to deviate from carbohydrates obtained from the soil and sunlight which are a source of energy. The source of the preparation of certain materials is through the process of photosynthesis for the formation of seeds in rice plants (Xu et

al., 2007). In general, for certain plants, trehalose accumulates in protein synthesis under osmotic pressure; suppress the trehalose catabolic process. However, after the stress is reduced, by trehalose dehydrogenase also known as trehalose oxidase, the first enzyme in the trehalose degradation pathway in plants is activated (Foster et al., 2003). Thus, studying the effects of salt stress on the activity of enzymes involved in trehalose metabolism may provide valuable information on the plant physiological significance of the accumulation present (Hare et al., 1998).

Trehalose can affect the growth and physiology of the three varieties of rice under normal conditions and salt stress. The unstressed salt, trehalose, is the most effective in helping to promote the growth of plant tillers, these are productive tillers. In addition, under salt stress, trehalose also exerted the most beneficial positive effect on all plant growth parameters compared to plants that did not receive trehalose. (Azooz, 2009) reported that salinity stress can cause a decrease in several growth parameters rather than plant agronomic characteristics.

On three single factors; Rice variety, salinity content, and trehalose content had a statistically significant effect on the number of seeds per panicle and are shown in Table 20. In addition, there was no difference due to the effect of the interaction between the two factors and the three factors (Table 41). IN 35 had more seeds, which showed the highest value (118.14) with a significant difference when compared to the two Thai rice varieties, namely PT 1 (99.40) and CNT 1 (107.81). So, compared with the results of previous studies with the title; growth and production of several varieties of rice (Oryza sativa L) at various levels of salinity. The results were the number of seeds per panicle for each rice variety [IR-64 (101.8), Ciherang (109.5), Inpari 11 (153.8), IRBB 27 (180,3) and Inpara (110.5)]. From that study (ChairunnisakA and SantosaB, 2018), a similar value number of seeds per panicle was found in IN 35 within this study. Thus, this characteristic seems constant and mainly controlled by genetics; although the results were reported from different countries (Indonesia and Thailand).

In contrast, a higher number of seeds per panicle was detected at a lower salinity level. For trehalose, at a higher level for application of this substance was observed the higher number of seeds per panicle. The number of seeds per plant was the most important factor in producing yields. However, due to salinity stress, it can

reduce the number of seeds planted. For rice varieties that are resistant, can grow under salt stress, plant physiological mechanisms will begin to form and undergo changes in tolerance and osmotic adjustment to the water content in the tissues and ions absorbed by plants (Arif et al., 2020). In addition, plants that have the ability and are resistant to salinity stress have a strategy to receive sufficient sunlight and carry out photosynthesis in plants to support their growth. Seeds per panicle is yield component presented at reproductive stage. However, seeds forming was occurred inside plants at the beginning of reproductive stage. Means, growth performance of plants since vegetative stages could effect on seeds forming as well.

Table 41 Average number of seeds per panicle of three rice varieties [(Inpari 35 (IN), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	106.0 ± 11.40	93.0 ± 13.04	82.6 ± 15.04	79.8 ± 20.40	107.80 ± 22.80 b
	50	124.2 ± 23.82	100.0 ± 9.64	96.8 ± 15.60	87.4 ± 9.84	
	100	130.2 ± 16.15	109.8 ± 10.94	107.0 ± 14.05	92.0 ± 16.48	
	150	141.2 ± 19.80	131.0 ± 11.34	124.4 ± 11.72	119.6 ± 12.20	
PT 1	0	90.0 ± 18.71	85.4 ±11.22	78.0 ± 16.08	68.6 ± 20.54	99.40 ± 22.20 c
	50	110.2 ± 14.89	92.6 ± 14.94	86.6 ± 13.76	77.6 ± 11.63	
	100	120.4 ± 13.09	102.4 ± 16.04	99.8 ± 16.27	89.6 ± 4.51	
	150	132.8 ± 9.09	120.4 ± 7.83	120.4 ± 10.45	115.6 ± 15.17	
IN 35	0	118.0 ± 13.17	96.6 ± 18.93	91.8 ± 28.67	87.8 ± 8.22	118.14 ± 26.94 a
	50	132.2 ± 13.42	109.8 ± 11.32	101.2 ± 21.20	90.0 ± 14.00	
	100	141.0 ± 18.34	118.6 ± 6.88	110.8 ± 18.20	108.2 ± 19.72	
	150	159.8 ± 10.78	154.4 ± 8.65	138.6 ± 29.20	131.4 ± 11.53	
Mean salinity		125.50 ± 22.80 A	109.50 ± 21.70 B	103.17 ± 24.10 B	95.63 ± 22.45 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		89.80 ± 19.95 a	100.82 ± 19.73 c	110.82 ± 19.73 b	132.47 ± 18.70 a	
P-value (F-test)						
Variety (V) 5.62 x 10^{-12**}, Salinity (S) < 2 x 10^{-16**}, Trehalose (T) < 2 x 10^{-16**}, V × S 0.871 NS, V× T 0.786 NS, S× T 0.805NS, V× S × T 1.00 NS, CV% 14.20						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Consistent analysis results between the number of seeds per panicle (Table 41) and 100 seeds weight (Table 42). Which, a significant difference was found affected

by rice variety and salinity level on these characteristics. For 100 seed weight, the highest value was found in IN 35 (2.48 g), followed by CNT 1 (2.38 g) and PT 1 (2.24 g), respectively. Moreover, reduced seed weight was shown in plants that received the stress of salinity in level increasing; the highest value was observed in the control treatment (0 mM NaCl). However, for this characteristic, no significant difference was affected by trehalose application.

In general, for this study, all rice varieties were affected by salinity which caused various seed weight losses, this was the result of the influence of heredity and environmental factors (Thitisaksakul et al., 2012). According to the opinion (Mindari, 2009) about research showing that one of the effects of NaCl on rice production is a decrease in the weight of 100 grains, and the total protein content in the seeds causes excess Na^+ absorption. As a result of excessive salinity concentrations, water in plants will decrease, and if it occurs in the reproductive phase, especially during grain filling, it will directly affect productivity rather than yield (Farooq et al., 2011).

When salinity stress requires maintenance so that, it can be increased in the tissues through the respiration process which is considered the main cause of decreased carbohydrate accumulation in plant growth yields (Barnabás et al., 2008). However, increasing plant tolerance to salt is still difficult to understand, because in each of these salt effects are generally present in almost all aspects of plant physiology and biochemistry, both at the cellular and plant levels.

The number of seeds per plant, an important yield component in rice, clearly the salinity affected to seed formation even at low concentrations (Akbar et al., 1972; Wong et al., 2010). In general, Na^+ is trapped in the roots and other parts of the plant and Cl^- is formed and accumulates in the leaf shoots of plants which are most easily damaged. However, for many crops such as cultivated cereals, Na^+ is the main cause of specific ion breakdown (Tester and Davenport, 2003). As for the ion compartment, translation, outflow transport, and absorption are controlled, to guard against ionic toxicity. The rate of carbon assimilation decreases rapidly due to salinity, inhibits the activity process in plants and decreases photosynthesis, loses carbon with increasing respiratory power, so that the accumulation of carbohydrates becomes less (Lawlor, 2002; Zlatev and Lidon, 2012). Osmotic regulation formed in the water network and absorbed ions, through the control of transported ionic toxicity. On the mechanism

that plants have in tolerance to some salty conditions and physiological formation, as for salinity tolerance in adjustments in the field (Munns and Tester, 2008b). One part of plant organs is considered as an attribute in plants that functions is in intolerance to salt. The ability of genotypes to show relative salinity tolerance for ideal plant attributes (Subbarao and Johansen, 1999). When water potential in plants decreases under abiotic stresses such as salinity, the accumulation of compatible osmolytes is involved in osmoregulation. Possibly obtaining additional water naturally from the environment, maintaining a direct additional effect on water depletion in organisms (Slama et al., 2015). It is necessary to take into account that the synthesis of dissolved organic matter is achieved the compatible osmotic adjustment or accumulation by organic cells and internal lost water must be considered (Robinson and Jones, 1986).

Table 42 Weight of 100 seeds (g) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	2.50 ± 0.10	2.37 ± 0.20	2.35 ± 0.21	2.33 ± 0.34	2.38 ± 0.22 b
	50	2.50 ± 0.21	2.33 ± 0.13	2.30 ± 0.15	2.35 ± 0.18	
	100	2.50 ± 0.20	2.40 ± 0.40	2.40 ± 0.22	2.25 ± 0.16	
	150	2.52 ± 0.33	2.51 ± 0.18	2.31 ± 0.18	2.26 ± 0.14	
PT 1	0	2.30 ± 0.20	2.10 ± 0.45	2.27 ± 0.50	1.90 ± 0.47	2.24 ± 0.33 c
	50	2.44 ± 0.20	2.31 ± 0.37	2.21 ± 0.20	2.15 ± 0.22	
	100	2.44 ±0.10	2.28 ± 0.20	2.35 ± 0.36	2.00 ± 0.30	
	150	2.46 ± 0.26	2.26 ± 0.22	2.23 ± 0.36	2.06 ± 0.37	
IN 35	0	2.52 ± 0.21	2.50 ± 0.15	2.40 ± 0.18	2.39 ± 0.40	2.48 ± 0.22 a
	50	2.57 ± 0.24	2.43 ± 0.23	2.41 ± 0.30	2.41 ± 0.23	
	100	2.60 ± 0.04	2.55 ± 0.18	2.53 ± 0.15	2.33 ± 0.36	
	150	2.65 ± 0.06	2.59 ± 0.10	2.50 ± 0.34	2.45 ± 0.15	
Mean salinity		2.50 ± 0.20 A	2.40 ± 0.27 B	2.35 ± 0.30 B	2.24 ± 0.32 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		2.32 ± 0.33	2.36 ± 0.24	2.40 ± 0.27	2.40 ± 0.28	
P-value (F-test)						
Variety (V) 5.76 x 10^{-8**}, Salinity (S) 2.84 x 10^{-6**}, Trehalose (T) 0.375 NS, V × S 0.541 NS, V× T 782 NS, S× T 0.873, V× S × T 1.00, CV% 10.97						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability,

Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

Trehalose has the most important function as an osmoprotectant; in regulating and absorbing redox potential energy in acting as a protector against hydroxy macromolecules and solutes. It is an important means of reducing acidity levels that sometimes occur at certain times (Lisar et al., 2012). Trehalose serves as a means and energy to reduce acidity in certain plant cells, acting as a store of nitrogen compounds for rapid plant growth after the stress occurred (Elbein et al., 2003).

From this perspective, the presence of trehalose facilitates metabolic processes, regarding the formation of rice seeds in the final stages. On the other hand, increased activity at trehalose levels can improve the mechanism of action in plants as efficiently as possible in contributing to tolerance to salt-induced oxidative stress (Tahjib-Ul-Arif et al., 2018). The presence of trehalose in plants can minimize NaCl stress on various carboxylase oxygenase activities (Parvaiz and Satyawati, 2008). The application of trehalose to restore plants is considered seriously subject to salinity stress and causes abnormal plant growth, decreased crop yields, and even death (Hasanuzzaman and Fujita, 2013). The goal with trehalose administration is to assist and increase the tolerance of rice plants at the time of tillering, although the number of seeds per panicle is assessed at harvest. However, it isn't significantly different effect on this trait by trehalose applying in this study.

At the harvest stage, the percentage of filled grain significantly difference ($P<0.01$) affected by individual factors; rice varieties and the salinity (Table 22). However, no significant difference in the percentage of filled grain to be affected by the interaction between two and three factors (Table 43).

The three rice varieties that obtained the highest percentage of filled grain were IN 35 (50.51%) and followed by CNT 1 (45.28%) and PT 1 (41.27%), respectively (Table 43). The results of IN 35 in this study were higher percentage when compared with previous studies. Regarding the growth and production of several rice varieties at various levels of salinity reported by (Jalil et al., 2018), the percentage yield of seed content of each variety is IR 64 (51.94), Ciherang (56.05), Inpari 11 (43.16), IRBB-27 (48.94) and Inpara (52.95).

Whole rice plants are generally sensitive to salt, however, there are certain varieties that have strong properties against high salinity levels. There needs to be serious concerns about plant stands and their development and yield components are largely influenced by genetic traits and salt concentrations (Holmberg and Bülow, 1998). Which, plant species that are able to respond to high salinity do not depend on the number of diploid or metacentric chromosomes (Ghassemi-Golezani et al., 2011c). Another reason is considered to differentiate tolerance of physiological associations and multiple growth mechanisms of rice varieties (Famoso et al., 2011).

As a very important requirement in establishing homeostasis, this is done with the aim of making plants able to survive under soil that contains salt stress. The ionic properties and homeostasis have important meanings and special considerations, because under certain conditions there is salinity, the concentration of Na^+ and CI^- under soils is critically affected by uptake into growing plants (Tavakkoli, 2011). Each plant that has the ability to tolerate salt, namely participating in Na^+ and Cl^- gets certain parts of the plant such as midribs and leaves to anticipate damage from these ions in high concentrations, the ability to absorb K^+ to the growing parts (Foy, 1984). The condition of plant is greatly influenced by the nature of salt with its toxic effect. The main thing is cause of plant changing from normal conditions to worse and stress in growth. There are disturbances that often occur and change all important properties of plants, namely physiology which includes membranes, reduced mineral nutrients, experiencing the ability to react which undergoes species detoxification by oxygen (ROS), changes in antioxidant enzymes, decreased photosynthetic activity and photosynthetic biosynthesis pigment (Choudhary11 et al., 2017). However, it is not easy to predict the salinity of toxic soil properties for each genetic from a particular plant, most of the traits of high or excessive salt concentration for plants cause a decrease in overall plant capacity and characteristics (Arzani, 2008).

For salinity level, the results showed that the percentage of filled grain in rice decreased depending on the increase in salt content (Table 43). The percentage of filling grain began to decrease at a salinity level of 50 mM NaCl until it reached 150 mM NaCl (Table 43). However, the salinity level affected the decreasing of percent of filled grain clearly since 100 mM NaCl. There is a slight difference in the effect of the salinity on 100 seed weight and percentage filled grain. The effect was more

pronounced and faster for the reduction on grain weight accumulation; 100 seed weight at the salinity level at 50 mM NaCl. Filled seeds, but the seeds are withered significantly affects yield because it is the number of seeds per plant, an important yield component in rice production. Moreover, withered seeds or low seed weight is a serious problem in farmers' fields facing salinity stress.

Salinity creates osmotic and through toxic ions. Thus, internal solutes are not balanced and the absorption of essential nutrients also disrupts the function and integrity of membranes and plant metabolic processes (Waraich et al., 2011). There is a certain way to cope through a good alternative in the case of salinity stress, in plant growth is to adopt external solutes for plants to adjust and stabilize the nutrient balance. To cope with salt stress, plants show more ideal traits in adapting both physiologically and biochemically. This may involve various accumulations of the most osmotically active molecules or ions including dissolved sugars, trehalose, sugar alcohols, glyceryl betaine, mannitol, glycerol, sorbitol, organic acids, calcium, potassium, chloride ions, abscisic acid and osmotic (Abosmaha Mohammed, 2013).

Through the presence of trehalose involved in the response to many emerging factors, especially those related to the environment and one of them is salt (Lunn et al., 2014). However, trehalose has a role that is considered appropriate under general conditions in response to stress in plants, which is still lacking and several hypotheses suggest that trehalose accumulation is caused by excessive stress (Henry et al., 2015). Thus, the use of trehalose must also take into account the ability of native plants rather than varieties to produce this compound when exposed to serious salinity stress.

The results in this study showed significant differences affected by the concentration of applying the trehalose. The higher overall means of percent of filled grain was observed at 100- and 150-mM trehalose, and followed by a lower concentration at 50- and 0-mM trehalose (Table 22). Interestingly, the use of trehalose did not show a clear influence on the increase in seed weight accumulation (100 seed weight) (Table 21). However, it was found that trehalose helps about the fulfilling in seeds. Using trehalose was conducted at tillering stage, and it showed a positive effect on increasing the number of seeds per plant (Table 20). Thus, in this case, the application of trehalose may not much involve help increase the percentage of fertilization, but help formulate the number of seeds per panicle.

This is an overall opportunity for the use of trehalose which is an external treatment at the time of tillering formation which is directly related to panicle formation and determination of the number of panicle seeds (Chen et al., 2020). For a while, it's time for fertilization to occur in the flowering phase, and begin to form starch accumulation in the preparation stage towards production at harvest time (Ren et al., 2007).

Table 43 Percentage of filled grain in panicle of three rice varieties [(Inpari 35 (IN), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	47.4 ± 12.76	42.4 ± 11.28	37.8 ± 9.26	29.0 ± 4.12	45.28 ± 12.87 b
	50	51.0 ± 12.59	46.2 ± 12.68	42.0 ± 10.42	33.0 ± 5.79	
	100	55.0 ± 13.84	49.8 ± 14.25	48.6 ±13.56	38.4 ± 8.93	
	150	52.8 ± 7.26	55.4 ± 14.96	51.8 ± 18.40	44.0 ± 3.24	
PT 1	0	44.2 ± 13.99	39.8 ± 12.93	33.4 ± 8.14	27.2 ± 8.41	41.27 ± 13.23 c
	50	45.2 ± 11.61	41.4 ± 10.95	36.0 ± 7.52	29.2 ± 7.92	
	100	49.0 ± 12.10	47.0 ± 16.46	43.4 ± 11.46	35.6 ± 9.53	
	150	49.4 ± 9.61	51.4 ± 15.84	47.0 ± 14.66	41.2 ± 19.92	
IN 35	0	53.8 ± 14.94	48.2 ± 12.64	43.2 ± 10.57	36.9 ± 9.00	50.51 ± 12.90 a
	50	59.4 ± 19.46	49.8 ± 11.52	46.8 ± 10.55	41.0 ± 9.85	
	100	58.2 ± 13.77	54.4 ± 15.70	53.0 ± 12.27	44.6 ± 7.33	
	150	55.8 ± 10.18	58.4 ± 13.46	57.4 ± 7.10	48.2 ± 10.66	
Mean salinity		51.77 ± 12.65 A	48.68 ± 13.52 AB	45.03 ± 12.53 B	37.28 ± 10.86 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		40.20 ± 12.59 a	43.42 ± 13.01 b	48.08 ± 13.20 a	51.07 ± 12.81 a	
P-value (F-test)						
Variety (V) 1.48 x 10^{-5**}, Salinity (S) 2.32 x 10^{-9**}, Trehalose (T) 6.12 x 10^{-6**}, V × S 0.999, V× T 0.996, S× T 0.870, V× S × T 1.00, CV% 26.41						

Note: NC = normal condition, CV =Coefficient of variance), *, ** = Significant difference at 0.05 and 0.01 levels of probability, respectively, NS = Non-Significant difference at 0.05 level of probability, Lower case letters (a, b, c…) = significant difference at 0.05 level of probability, Upper case letters (A, B, C) = significant difference at 0.05 level of probability.

As for the conservation of plant growth, it is necessary to pay attention to the resistance possessed by the ability of quality plants. Substances used under proper supervision are under control to offer a variety of benefits to crop productivity under constituents that can promote better health (Hewett, 2006). Salt stress generally adapts overall at the same time for the biosynthesis of secondary metabolism. Which, it has

been significantly increased in plants as for soluble solids, sugars, amino acids, proteins and organic acids (Massaretto et al., 2018). Having the ability to exert a role as the main source of osmoregulators in stabilizing turgor is certain plants experience salt stress (Slama et al., 2015).

The presence of free amino acids in each plant under stress-free conditions is available in trehalose (Genç et al., 2018). However, salt stress which is the source is starting to be exposed to plants, by this trait which is a response to various stresses that include the operation of all certain specific metabolisms, which are becoming well known are amino acids in general and trehalose in particular (Bohnert et al., 1995). For each variety that has more tolerant properties will produce certain substances in the regulation of metabolism in plants, and have the opportunity to accumulate internal trehalose (Sah et al., 2016). However, external substances have positive properties that have been studied and found to play an important role. So that, they can determine the success rate of plant growth under normal conditions (Bourgaud et al., 2001). Accumulation of trehalose at the highest rate it has been reported that an increase in seed germination, tillering, panicle formation. Moreover, the percentage filled seeds in rice and other cereals has been reported affected by the application of trehalose (Rengel and Graham, 1995) Nevertheless, the growth stage at which trehalose is used must also have a certain consideration.

In cereals, generally as in other crops, the continuous increase in grain and/or seed production can be attributed to a variety of internal and external factors. Among them, appear various parts and mechanisms rather than morpho-physiological that are interrelated and contribute to each other, in each increase allocated to biomass (plant assimilation) for the reproduction of plant parts that are considered very important.

The results of ANOVA on harvest index (HI) of rice growing under the salinity stress shown in Table 44. There is a very significant difference in the harvest index, which is influenced only by the salinity factor. There was no significant difference affected by both between two and three factors in this characteristic.

For the percent yield weight of each IN 35 variety, the HI value was 25.26%, and in two Thai rice varieties were 23.80% and 22.61% in CNT 1 and PT 1, respectively. To determine the harvest index or HI, it is necessary to calculate based on the ratio between the weight yield of grain and the total above-ground biomass.

Although for some of these characteristics, harvest index, is specific to all genetics in a particular plant. The changes that occur in the high and low HI, especially in plants classified as cereals, have shown a success ratio in productivity per species for certain plants or varieties (Richards, 2006). Harvest index, this value has reflected the ability of plants to produce significant values and benefits, especially in plant grains per vegetative leaf and stem (Rengel and Graham, 1995).

For this advantageous reason, either the effect on salinity or the use of external additives can alter and affect plant development and growth, but will affect the grain yield ratio or not, according to the evaluation that required. Considering the HI affected by salinity level, there was lowest value at 150 mM NaCl (20.47%), followed by 50 mM (24.57%) and 100 mM NaCl (22.03%). For non-salinity condition (0 mM NaCl), the highest value was observed at 28.50% of harvest index.

Table 44 Harvest index of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	26.4 ± 2.30	23.6 ± 5.94	19.6 ± 6.58	18.4 ± 7.37	23.80 ± 8.01
	50	28.2 ± 7.26	24.2 ± 6.42	21.0 ± 5.87	19.8 ± 6.61	
	100	29.2 ± 7.60	24.4 ± 5.94	23.8 ± 9.52	20.8 ± 8.23	
	150	30.2 ± 11.95	25.4 ± 12.30	24.8 ± 11.14	21.0 ± 7.18	
PT 1	0	25.6 ± 4.04	22.8 ± 5.17	19.2 ± 6.76	17.2 ± 8.67	22.61 ± 7.53
	50	26.0 ± 4.18	23.0 ± 7.68	20.6 ± 4.56	19.6 ± 6.35	
	100	28.2 ± 8.04	23.0 ± 8.69	21.2 ± 6.42	19.6 ± 4.77	
	150	28.6 ± 10.31	24.0 ± 9.08	22.4 ± 11.20	20.8 ± 10.08	
IN35	0	26.6 ± 2.30	24.0 ± 5.70	21.8 ± 6.20	19.6 ± 8.20	25.26 ± 8.70
	50	30.2 ± 8.32	25.6 ± 7.27	22.0 ± 4.95	22.4 ± 6.23	
	100	31.2 ± 8.56	25.8 ± 9.96	22.8 ± 9.60	22.8 ± 9.34	
	150	31.6 ± 11.40	29.0 ± 11.79	25.2 ± 11.90	23.6 ± 12.52	
Mean salinity		28.50 ± 7.36 A	24.57 ± 7.70 B	22.03 ± 7.70 AB	20.47 ± 7.61 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		22.07 ± 6.33	23.55 ± 6.65	24.40 ± 8.16	25.55 ± 10.50	
P-value (F-test)						
Variety (V) 0.124 NS, Salinity (S) 9.9 x 10^{-7**}, Trehalose (T) 1.000 NS, V × S 1.000 NS, V× T 1.000 NS, S× T 1.000 NS, V× S × T 1.000 NS, CV% 34.18						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability,

Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

The increase in salinity has that effect and to determine the corresponding decrease in HI. Sometimes there are differences in this harvest index due to the assimilation of supplies to some parts of the plant. which are present in seeds, due to differences in the source and sink partitions. This was found to occur, because in general those originating from photosynthetic sources in plants and in different varieties can experience different stresses (Funk et al., 2004). For varieties that have experienced stress will be able to absorb photosynthesis less when compared to certain parts that have not experienced stress (Grzybowski et al., 2021). However, the proportionate partition between the essential parts of photosynthesis and reproduction under certain conditions is very clear.

This opportunity will allow to assess the effect of different varieties from growing under unfavorable conditions such as salinity. There is a difference that the HI value for plants in various varieties is caused by plants that have adaptability to different stress conditions (Ceccarelli, 1996). In plants that are non-tolerant to salinity often experience certain structural changes, especially in ultra-cells that cause swelling of the mitochondria and golgi bodies. An increase in the number of endoplasmic reticulum and damage to chloroplasts also was observed under the salinity stress (Liu and Li, 2019). In addition, plants will always experience various changes in metabolic activity, changes in amino acid composition, and decreases in sugar and starch levels in the body and plant tissues than the plant itself (Kölling et al., 2015). Furthermore, soil salinity changes and inhibits all characteristics with certain parameters of plant growth and development that are important to observe. In osmotic regulation, various processes of material formed will accumulate from each process rather than photosynthesis. An accumulation of substances often occurs through the movement of a solute considered to be dissolved called osmolyte. Which, undergoes changes in the movement of water between cells; for standard improvement of osmotic tolerance, it is one of the mechanisms involved in salt tolerance (Nounjan et al., 2018). In general, on genotypes or species that respond to salt or drought exposure are adjusted for increased osmosis, changes in cell wall elasticity, decreased saturation or dry weight ratios and increased apoplastic

percentages of water, which can minimize the effect of maintaining salinity levels on leaves (Ashraf and McNeilly, 2004). The extent to which osmotic adjustment exists, can be influenced by the varying degrees at which pressure is applied, and plant genetics or plant age, among other factors (Yeo and Flowers, 1986).

The release of salt can occur in the roots of rice plants, thereby helping and preventing the movement of ions into the air of the plant. This mechanism occurs in some glycophytes and in most halophytes (salt tolerant) (Colmer and Flowers, 2008). The development of salt vesicles in the epidermis can accelerate the excretion of salt, preventing the accumulation of salt in various plant organs such as stems and leaves. Meanwhile, in the example of the exclusion mechanism, the accumulation of salt in old leaves will cause death and the loss of organs that affect the HI (Kumar, 2020).

In this study, the trehalose factor did not give a significant result on the percentage of HI. However, at each increasing HI value from 0 mM control treatment to 150 mM trehalose slowly increased (Table 44). In fact, in some plants with higher plant groups, the biosynthesis of the affected trehalose can be continued, either through the contained sugar pathway. The ultimate goal of trehalose is performance enhancement. The increase in plant performance under salinity caused by trehalose is likely partly due to its ability to reduce sodium accumulation, and increase potassium in plant leaves. Exogenous trehalose can cause high levels of fructose, glucose, sucrose and trehalose in salt-stressed plants during growth in the treatment field (Asaf et al., 2017).

On this occasion, it can be proposed that trehalose can act as a signal to make salt stress plants active by increasing other compatible soluble essential substances; including soluble sugars and free amino acids. These substances can control water loss, leaf gas exchange, and ion flow in early stress of salty, and occasionally occurs in plants. Application of trehalose under saline conditions can also promote the accumulation of alkaloids. The regulatory role of trehalose in promoting optimal salt tolerance in *Arabidopsis* with an exogenous concentration of 150 mM trehalose (Yang et al., 2014). A greater concentration of trehalose is supra-optimal and affects plant growth. It has been reported that trehalose has the capacity to increase plant salt tolerance in the presence of exogenous trehalose supply experiments (Chang et al., 2014) or through overexpression of linkage genes that can control trehalose synthesis

(Chen and Haddad, 2004). These results have implications for exogenous trehalose in inducing soluble sugar if it will continue to accumulate when plant growth is in salty conditions. Trehalose is increasingly playing its function as effectively as possible as a storage of carbohydrate molecules and sugar as a transport, similar to the function found in sucrose (Leslie et al., 1995). Through the results reported that, the increase in the accumulation of trehalose in rice provides a close relationship with dissolved carbohydrate levels so that it can increase photosynthetic capacity both under and without stress (Abdallah et al., 2016).

In the presence of the disaccharide trehalose plays an important physiological role against various abiotic stresses in many organisms. However, for certain plants its exact role is still unclear, although some data still show that trehalose has a full protective role during abiotic stress. In this study, the results obtained suggest the possible and regulatory role of exogenous trehalose on the metabolism of reactive oxygen species (ROS) in inducing salt tolerance in rice (Mostofa et al., 2015a).

The results of ANOVA on the factors both in individual factor and in combination in interaction effect on harvest grain yield per plant of rice was shown in (Table 45). There was a highly significant difference on grain yield affected by individual factors: rice varieties, salinity levels, and trehalose levels. However, no significant difference was observed in any interaction both between the two and three factors.

The three varieties showed significant differences in the statistical analysis of grain yield or grain weight per plant (Table 45). The highest yield was found in IN 35 (5.94 g), compared to Thai rice CNT 1 (5.43 g) and PT 1 (4.81 g), respectively. From other yield components, IN 35 seems more tolerant and showed higher on most of yield components under growing in salinity stress than Thai varieties (CNT 1 and PT 1) (Table 16, 18-22). Thus, the grain yield in IN 35 was higher than the two Thai rice varieties.

In grain production, grain yield for each variety depends on its performance and potency rather than the genetics of the species. For plants capable of producing high production, each variety is used based on its adaptability to the environment and where it grows (Abdallah et al., 2016). From these results, it is possible to selection rice varieties to be grown in salinity areas in Thailand. More than to consider on

variety can produce high yields with good adaptability to specific growing condition: salt stress (Wassmann et al., 2009b). It should be concerned in quality of rice and acceptance of consumers and the market as well.

The uniqueness of responding separately for each plant that is declared adaptive is a plant that tries to attack under any circumstances, anytime and anywhere (Born et al., 2007). For plants that are able to adapt morphologically and physiologically which often occur in various parts of plants, it can generally be achieved naturally even though the plant materials used are the same (Bradshaw, 1965). For factor salinity, the results showed that, the grain weight of rice plants was strongly influenced by various levels of salinity (Table 45). The grain yield of rice at salinity level at 0 mM NaCl (control treatment) and 50 mM NaCl did not significant different. However, salinity levels at 100 mM to 150 mM NaCl resulted clearly in a decrease in grain yield compared to control treatment.

Table 45 Grain yield (g per plant) of three rice varieties [(Inpari 35 (IN 35), Pathum Thani 1 (PT 1) and Chai Nat 1 (CNT 1)] at harvest stage by four levels of trehalose (0 mM, 50 mM, 100 mM, 150 mM) under four levels of salinity [(0 mM, 50 mM, 100 mM, 150 mM sodium chloride (NaCl)]

Varieties	Trehalose (mM)	Salinity (mM NaCl)				Mean varieties
		0	50	100	150	
CNT 1	0	5.78 ± 0.19	4.53 ± 0.55	4.40 ± 1.27	3.61 ± 0.53	5.43 ± 1.41 b
	50	6.00 ± 0.30	5.86 ± 0.53	5.14 ± 1.20	4.21 ± 0.63	
	100	6.30 ± 2.83	5.93 ± 1.34	5.62 ± 0.86	5.36 ± 1.71	
	150	6.41 ± 1.47	6.12 ± 1.41	6.07 ± 1.81	5.60 ± 1.09	
PT 1	0	5.03 ± 0.25	4.40 ± 0.60	4.21 ± 1.11	3.12 ± 0.70	4.81 ± 1.48 c
	50	5.28 ± 0.80	4.66 ± 1.67	4.49 ± 0.36	3.70 ± 050	
	100	5.71 ± 2.68	5.08 ± 2.36	5.03 ± 0.71	4.41 ± 1.62	
	150	6.00 ± 1.40	5.68 ± 2.36	5.52 ± 0.93	4.72 ± 1.55	
IN 35	0	6.05 ± 0.28	5.35 ± 0.70	4.82 ± 1.34	4.28 ± 0.75	5.94 ± 1.67 a
	50	6.46 ± 0.36	5.98 ± 0.70	6.06 ± 0.61	5.22 ± 0.50	
	100	6.76 ± 2.53	6.58 ± 0.70	6.33 ± 0.67	5.64 ± 1.42	
	150	6.92 ± 1.95	6.34 ± 4.13	6.21 ± 2.99	6.02 ± 1.44	
Mean salinity		6.06 ± 1.53 A	5.54 ± 1.74 AB	5.32 ± 1.40 B	4.65 ± 1.36 C	
Trehalose (mM)						
Mean Trehalose		0	50	100	150	
		4.63 ± 1.10 c	5.25 ± 1.08 b	5.73 ± 1.75 ab	5.97 ± 1.94 a	
P-value (F-test)						
Variety (V) 1.55 x 10^{-5**}, Salinity (S) 8.66 x 10^{-6**}, Trehalose (T) 5.63 x 10^{-6**}, V × S 1.00 NS, V× T 0.986 NS, S × T 987 NS, V× S × T 1.00 NS, CV% 27.30						

Note**:** NC = normal condition, CV =Coefficient of variance**)**, *, ** = Significant difference at 0**.**05 and 0**.**01 levels of probability, respectively, NS = Non-Significant difference at 0**.**05 level of probability, Lower case letters **(**a, b, c**…)** = significant difference at 0**.**05 level of probability, Upper case letters **(**A, B, C**)** = significant difference at 0**.**05 level of probability**.**

The level of salinity is assumed to affect and reduce grain yield according to the concentration absorbed in the soil (Meiri and Plaut, 1985). A place that has been polluted with salt is certainly bad for every plant that will grow in that place (Wu et al., 2010). Which, probably due to the different competition for each supply of carbohydrates between vegetative growth and limited supply to all parts of the plant. Reduces the plant growth performances and the viability of pollen under plant stress condition was reported (Barnabás et al., 2008). Finally, the decrease in grain yield; resulted in forming of all yield components in plant production, in rice varieties was caused by an increase in the level of salt stress (Razzaq et al., 2020)

In statistical analysis, the trehalose content factor also showed a significant difference in grain yield (Table 45). In this case, it can be seen that for each addition of trehalose content, the grain yield also increases. Thus, grain yield at 150 mM trehalose showed higher than three concentrations of trehalose application.

From the results of the study, trehalose significantly affected the agronomic characteristics that emerged from the vegetative phase to reproductive phase: the number of productive tillers per plant (Table 40), the number of seeds per panicle (Table 41), and percentage of filled grain in panicle (Table 43). The effect also depends on the timing of external use of trehalose given at the tillering stage, because trehalose has strong properties and is supported by natural sugar reserves from plants that can reduce salt stress (Hasanuzzaman et al., 2013b). So, trehalose is possible for plants to get maximum yields and supported by the genetic traits of each plant (Paul et al., 2020). Thus, the effects of trehalose application seemed to remain highly dispersed in grain yields.

The existence of the ability of trehalose to act as an appropriate helper and stabilize the situation and increase stress tolerance in plants, which is considered necessary and should be investigated further regarding the various mechanisms involved (Khoshru et al., 2020). Therefore, in the further use of trehalose, the reproductive phase may have a more pronounced effect on the results obtained. Previously, researchers have proven that the application of trehalose can improve several characteristics of plants in the vegetative phase, including the number of tillers, panicle length, and seed yield as well as the number of fertile tillers, thousand grain weight and panicle length of panicle stem at the end of productive stage (Tabssum et al., 2019).

CHAPTER 5
CONCLUSION

Experiment I: Applying proline to alleviate salty stress in rice plant at tillering stage

Biochemical characteristics in rice at the tillering stage showed affected by all factors (varieties, salinity levels, and proline application levels), however, affected different characteristics. For rice varieties, IN 35 had higher means on many characteristics over two Thai rice varieties (CNT 1 and PT 1) including RWC, Chlorophyll B, proline content in leaves, starch in stem and leaves. However, CNT 1 seems to have higher means on many characteristics more than PT 1.

The significant negative effect of salinity level increased in many characteristics; including RWC, Chlorophyll A, proline content, soluble sugar content in leaves, stem, and leaves, and starch content in leaves. Which, the level of salinity is start effect to decrease values in characteristics at 50 mM NaCl. There was a severe effect on all characteristics consequences according to the increased level of salinity.

Application of external proline started at 50 mM could promote to increase in some characteristics; including RWC, proline content, and soluble sugar. In addition, these characteristics were increased in the order of increasing the concentration of exogenous proline for applying.

In this study, increasing in soluble sugar content was induced by salinity increased in rice leaves, although not applied the external proline. This may indicate an attempt inside plants to maintain their physiology to survive in salinity conditions. However, when plants were exposed to increase salinity, their soluble sugar content is reduced.

Plant parts were positively affected by applying external proline for higher accumulation of soluble sugar content as is leaves. Although both leaves and stems are directly contacted with proline substances. Therefore, the role of soluble sugar to support plants withstand and tolerance under salinity, firstly parts of the plant was responding is leaves.

Among these plant parts (leaves, stem, and root), the reduction in starch accumulation was found in root highest, and followed in stem and leaves parts,

respectively. It may be the reason that the leaf part is so important to photosynthesis that it affects plant growth under stressful conditions due to various factors. Therefore, the preservation of leaf growth tissue for plants is important.

Proline content in plants had significantly affected by the proline application level with increased values when the proline level increased. The proline synthesis of these rice varieties was indicated that naturally proline accumulation in the plant even when planted is grown under non-salinity conditions. However, proline accumulation occurred when proline was sprayed. Thus, spray proline from the outside, which might be a stimulus proline synthesis. This indicates that at a non or low-level salinity (0-50 mM proline), Thai rice varieties (CNT 1 and PT 1) were able to synthesize proline inside the plant, especially when stimulated by external proline spray at an appropriate concentration of 50-100 mM proline. In contrast, IN 35 was slightly stimulated to synthesize proline at non-salinity (0 mM) to high salinity levels (100 mM NaCl). But, when the salinity level was increased to 150 mM NaCl, proline was found to synthesize the proline both with or without exogenous proline application.

Thus, it can be briefly concluded that two Thai rice varieties need to be stimulated by external spraying the proline to promote proline synthesis. However, results were seen when the salinity level was not too high or had no salinity stress (0-50 mM NaCl). This may be a feature of the cultivar that is not very resistant to high salinity. Contrastly, IN 35 had less proline synthesis in the plant by exogenous proline stimulation when there was no salinity (0 mM NaCl). But, when plants are affected by salinity, there is natural stimulation of its genetic to synthesize more proline. And at high salinity (150 mM NaCl), when IN 35 was co-stimulated by exogenous proline spraying, increased proline synthesis was observed. These may be characteristics of expression in salinity-resistant varieties.

At the tillering stage (first week to four weeks after planting), increasing the salinity was affected differently in varieties and characteristics (number of leaves per plant, plant height and leaf symtom scoring). Although tiller numbers showed an increasing trend at higher salinity stress, effective or non-effective tillers could not be detected at the start of the tillering stage.

Comparing different varieties, IN 35 was able to tolerate and establish stems and leaves; this was only observed less impact in the exposure to salinity within four

weeks after planting. In Contrastly, two Thai rice varieties were less tolerant of salinity compared with IN 35 when considering the agronomic characteristics within four weeks after planting. It was found that CNT1 seems more salt-tolerant than PT1.

This study could sort characteristics affected from salinity by highest to lowest: leaf symptom scoring, number of tillers per plant, number of leaves per plant, and plant height; determined by the direction and magnitude of the change in percentage with the control treatment at 0 mM NaCl. While the cultivation of rice at the tillering stage that received the salinity for two weeks continues, it was found that the salinity at 50 mM NaCl had already affected the characteristics, and the highest affect was observed at 150 and 100 mM NaCl, respectively. These results indicate that prolonged exposure of salinity stress will affect all agronomic characteristics in rice; both in tolerant or non-tolerant varieties, even at low salinity levels. These observations were based on assessing the effects of salinity on characteristics in weeks one and two of exposure salinity; or number of week after transplanting.

Longer salinity period provides a more pronounced effect of salinity on all characteristics at low concentrations at harvesting stages: number of productive tiller per plant, seeds per panicle, percent of filled grain, and 100 seeds weight.

Proline application at 100 mM and 150 mM in tillering stage is improved characterized by an increase in plant height, but did not affect to number of tillers per plant. However, rice plants exposed to proline from spraying are likely to have good health effects and increase fertility. Thus, number of productive tillers per plant and number of seed per panicle significant affected by proline application.

For the difference between the influences of the different varieties, it was found that Inpari 35 had the highest number of productive tillers per plant, followed by CNT 1 and PT 1, respectively. Therefore, tolerance of plants and salt as in IN 35 over than two Thai rice varieties: CNT 1 and PT 1.

Again, in summarizing the effects of exogenous proline application on rice plants in the tillering phase on the yield component only within each panicle, it was found that the use of proline had a positive effect on the characterization of the number of seeds per panicle. But they did not have a statistical influence on the percentage of filled seeds and 100 seed weight. It is reasonable because the use of

external proline was carried out during tillering that is related in panicle formation and determining the number of seeds per panicle.

Experiment II: Applying trehalose to alleviate salty stress in rice plant at tillering stage

Biochemical characteristics in rice at the tillering stage showed affected by all factors (varieties, salinity levels, and trehalose application levels), however, affected different characteristics. For rice varieties, IN 35 had higher means on many characteristics over two Thai rice varieties (CNT 1 and PT 1) including RWC, Chlorophyll A, Chlorophyll B, total chlorophyll, soluble sugar content in leaves and root.

The significant negative effect of salinity level increased in many characteristics; including RWC, Chlorophyll A, Chlorophyll B, soluble sugar content in leaves, stem, and root, starch content in leaves, stem, and root. Which, the level of salinity is start effect to decrease values in characteristics at 50 mM NaCl. There was a severe effect on all characteristics consequences according to the increased level of salinity. In this study, increasing in soluble sugar content was induced by salinity increased in rice stem, although not applied the external trehalose. However, this occurrence was observed only in PT 1 at 50 mM NaCl that showed its response to salinity stress.

Application of external trehalose started at 50 mM could promote to increase in some characteristics; including Chlorophyll A, Chlorophyll B, soluble sugar in the stem, starch content in leaves, stem, and root. Although Chlorophyll B appeared to be more susceptible to salinity than Chlorophyll A. Chlorophyll B did not respond rapidly to the use of external trehalose to mitigate the effects of salinity. The trehalose concentration factor did not show a significant difference in soluble sugar content in plant roots. This is reasonable that the external trehalose carried out during the tillering period was sent to above-ground parts to maintain the photosynthesis ability.

Compared with the effect of proline application, exogenous proline was clearly effective to promote the accumulated soluble sugar content in the leaf more than external trehalose. However, the effect of trehalose on starch accumulation in all plant parts: leaves, and roots, was clearly with a significantly different effect more than the use of the proline.

At the tillering stage (until four weeks after planting), increasing the salinity start at 50 mM NaCl affected all characteristics including number of leaves per plant, number of tillers per plant, plant height, and leaf symptom scoring. Decreased values on these characteristics were observed at the first week to fourth week after rice plants were exposed by salinity. However, a longer salinity period provides a more pronounced effect of salinity on all characteristics.

Comparing different varieties, IN 35 was able to tolerate and establish stems and leaves; this was only observed less impact in the exposure to salinity within tiller formation. Moreover, the potential of the original in IN 35 with a high number of tillers per plant kept the highest value in the overall mean. In addition, IN 35 has more leaves per plant, but fewer foliar symptoms compared to two Thai rice varieties. While, between the Thai rice varieties; CNT 1 and PT 1, appear to be similar responses to salinity levels on most of the characteristics. Although CNT 1 has higher damage on leaves on average, it showed a lower effect on the number of tillers per plant compares to PT 1 under salinity stress.

For agronomic characteristics in rice at the harvesting stage, salinity could be affected on characteristics at 50 mM NaCl and above concentrations (100 mM and 150 mM NaCl). These characteristics were decreased when salinity level increased such as plant height, number of tillers per plant, number of productive tillers per plant, number of seed per panicle, 100 seeds weight, percent of filled grain in panicle, harvest index, and yield per plant.

The result showed that the highest characteristics; including plant higher, number of tillers per plant, number of productive tillers per plant, number of seeds per panicle, 100 seeds weight, percent of filled grain in panicle, yield per plant, observed were IN 35, and followed by CNT 1 and PT 1, respectively. Evaluated from different varieties, IN 35 seems more tolerant to salinity stress than CNT 1 and PT 1.

Increasing the amount of trehalose for application was found to increase many characteristics measured at the harvesting stage (plant height, number of seeds per panicle, the percentage of productive tillers per plant formation, and grain yield per plant). Most of these characteristics have increased since 50 mM trehalose exposure.

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