EFFECT OF PROLINE ON RICE SEED GERMINATION (*ORYZA SATIVA* L.) UNDER SALTY CONDITION

By
Mr. Leandro CARVALHO RODRIGUES PEREIRA

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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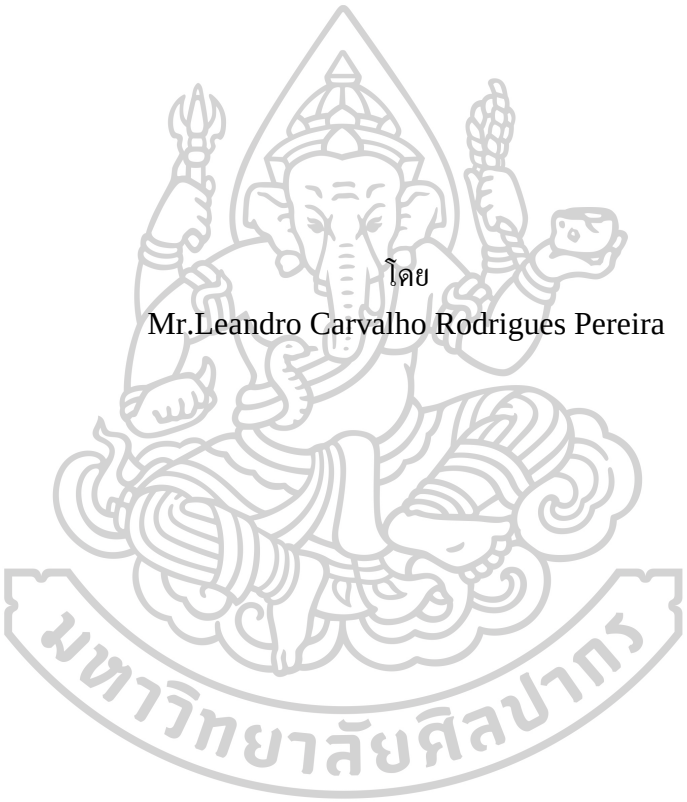

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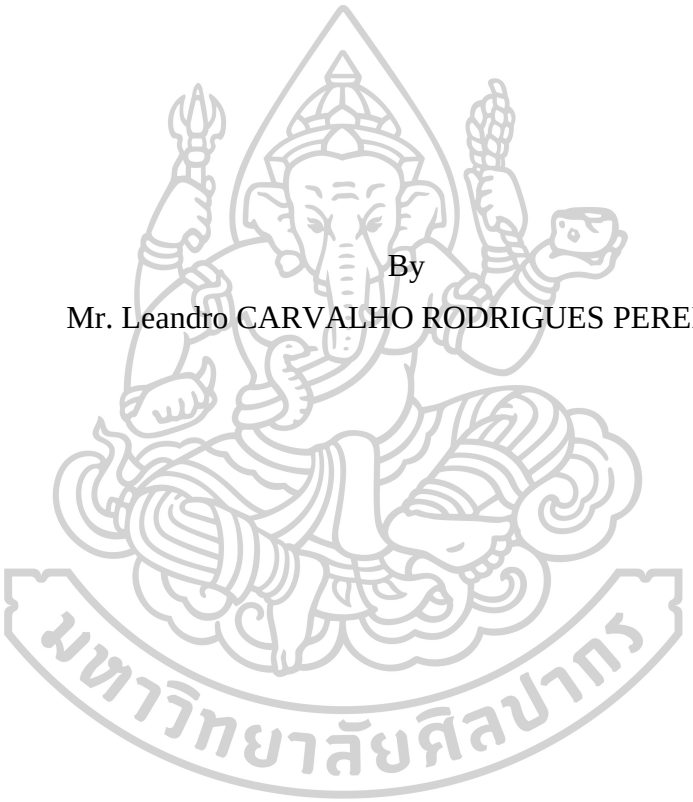

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Title	Effect of Proline on Rice Seed Germination (*Oryza sativa* L.) Under Salty Condition
By	Leandro CARVALHO RODRIGUES PEREIRA
Field of Study	(BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
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MR. LEANDRO CARVALHO RODRIGUES PEREIRA : EFFECT OF PROLINE ON RICE SEED GERMINATION (*ORYZA SATIVA* L.) UNDER SALTY CONDITION THESIS ADVISOR : ASSOCIATE PROFESSOR PANTIPA NA CHIANGMAI, Ph.D.

Salinity is a major problem both in present and has been forecast in the future on plant production. The objectives of this study are to investigate salinity stress at different levels and the effects of using external proline at germinating and seedling stages on many characteristics; both morphological, biochemical, and proline accumulation by gene synthesis in rice. Four experiments were conducted separately by using Completely Randomized Design (CRD) with three replications. Three rice varieties were used in these experiments including Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1), and Indonesia rice variety 'Inpari 35' (IN 35). These experiments assessed the effect of four levels of salinity (0, 50, 100, and 150 mM sodium chloride; NaCl) and the influence of exogenous proline use (0, 50, 100, and 150 mM proline). The results showed that salinity affects characteristics of both at the germination and seedling stage. Moreover, the salinity at levels 100 and 150 mM NaCl had a significant impact on the reduction of various characteristics of rice seedlings. At the germination stage, a low proline level at 50 mM could promote rice characteristics, and the concentration of proline should increase to 100 mM when rice is grown under salinity stress at 150 mM NaCl. However, at the seedling stage, the effect of proline was not found both on the relative water content and the content in many types of chlorophyll, and the total chlorophyll. Consistent between these results with proline accumulation from OsP5Cs1 gene expression was observed. Proline accumulation in rice varieties was observed in higher salinity levels at 100 mM and 150 mM NaCl and spraying at 100 mM and 150 mM proline.

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CHAPTER I

INTRODUCTION

Background and rational

Today's agriculture faces a daunting task of ensuring food security to the increasing human population on this planet (FAO, 2009). A great proportion (more than 60%) of this population depends on rice (*Oryza sativa* L.) as their staple food. As a food crop, it forms the staple food of more than three billion people accounting for about 50-80% of their daily calorie intake. Rice yields about one third of the total carbohydrate source (Ghosh et al., 2016b). It contributes up to 20 % of the calories consumed by human nutrition worldwide (Muthayya et al., 2014). Therefore, rice production must increase during the coming time in order to keep pace with increasing world population. During 2011-2012, rice production in the world was 718.345 million tons and it was cultivated over an area of 163.463 million hectares (Abdullah et al., 2013). Importance of rice in agricultural crops cannot be ignored as it is the staple food for more than fifty percent population of the world and a big source to cope with the food security issues of the world. So population surpassed 7 billion in late 2011; population is expected to reach 9.3 billion by 2050, and per-capita demand is likely to increase along with income growth (Khush, 2004). Across the scientific literature, global food demand is predicted to increase; 35% to 56% and 100% between 2010 and 2050 (van Dijk et al., 2021). At the same time, growth in food production is slowing (Fischer et al., 2014). Looking towards 2030, to meet the demand for grain and to feed a growing population on the available arable land, it is suggested that annual crop production should be increased to around 580 Mt and that yield should increase by at least 2% annually (Fan et al., 2011). Increasing regional production is already complicated by increasing competition for land resources by non-agricultural sectors and by the deterioration of agri-environments and water resources (Aggarwal et al., 2004). Asia is known as the main rice producer in the world by yielding more than 650 million tons (90% of total rice yield worldwide) grown in 145 million ha land (Das et al., 2015). For this important or rice, the Asian cultivated rice is the first fully sequenced crop genome and is a model crop species.

Regardless of which model is correct, the fixation of common domestication alleles in the divergent genomes of cultivars could have been driven by a combined force of artificial and natural selections. Domestication alleles are under strong human selection, while most of the domesticated genome is under selection of environmental conditions to which the wild progenitor was adapted (Sang & Ge, 2007). Although the rice is important, yield growth rates of rice have slowed down notably in many countries and for major commodities. It dropped production from 3.2 percent per year in 1960 to 1.5 percent in 2000 (Alexandratos & Bruinsma, 2012). When, the aggregate income elasticity of demand for global rice consumption is expected to rise from the 441 million metric tons (mmt) consumed in 2010 to about 450 mmt in 2020, before declining to just 360 mmt in 2050. So, climate change really does seem to be upon us, with greatly increased uncertainty about weather patterns and corresponding increases in instability of production. As noted above, instability is a real problem for food security (Timmer, 2010). Therefore, consumption equals production, this means that global production in 2050 must be 60 percent higher than in 2005/2007 (Alexandratos & Bruinsma, 2012). About 85% of the 5.2 million ha of rice land in Northeast region Thailand are under rainfed conditions with a single crop per year and low agricultural productivity (average paddy yield is 1.8 t ha^{-1}). This is mainly the result of the combined effects of low water-holding, infertile coarse-textured soils, and erratic rainfall distribution (Jintrawet, 1995; Somrith, 1997). Notwithstanding, 25% of the households living in this most populated region of the kingdom in Thailand are still engaged in the rainfed lowland rice (RLR) production (OAE, 2005). Cash incomes generated from rainfed lowland rice RLR production are inadequate to meet farmers their basic needs, leading to a relatively high rate of poverty in this region (Naivinit et al., 2010).

Important abiotic stresses such as high salinity, drought, cold, and heat affect plants for survival, biomass production, and yields of staple food crops reaching 70% (Mantri et al., 2012). The effect of salinity (NaCl) on plants is determined not in terms of viability, but in terms of its effect on vegetative growth (Safdar et al., 2019). However, plants have affected by salt stress in two main ways: osmotic stress and ionic toxicity, which affects all major plant processes, including photosynthesis, cellular metabolism, and plant nutrition (Parihar et al., 2015).

In rice, salinity alters the subcellular architect causing plasmolysis, increased cytoplasmic vesiculation, and damage to all types of membranes in the cell (Mishra et al., 2020). Therefore, sustain approaches should be undertaken to minimize the detrimental effects of climate changes on rice plant (Raza et al., 2019). The farmers when rice stand out and lower growing, their fix to promote plant growth is to supplement fertilizer but the problem found is that plants may not be able to use the nutrients from fertilizers. However, it is added more problem such as high cost. In salinity condition, although put high fertilizer, plant cannot up take the nutrient. It is like drough condition, so high cost but low yield and the remain of fertilizer cannot absorbs that it will loss underground of the water it is increase the pollution. So, it is still increasing the problem of climate change because the pollution increased. For this reason, in order to apply any external factors to solve the problem, the physiological problems of plants in those stressful conditions must be understood first. Likewise, when using exogenous plant phytoprotectance agents such as proline, it is necessary to study the physiological effects of salinity factor first.

Objectives

The objectives of this research are to investigate the effect of salinity stress in rice germination and growth at seeding and seedling stages and to investigate effect of proline to alleviate this stress in those stages.

Hypothesis

1. Different levels of salinity affect the germination and growth of seedling differently.
2. The use of proline can cause changes in both physical, physiological and chemical characteristics at the germination and growth of rice seedling when the rice grown in salty condition.
3. Using level concentration proline applying in rice germination and seedling stage will alleviate salinity stress.

CHAPTER II
LITERATURE REVIEW

2.1 Salinity Stress

Rice is an important crop accounting for food security of over half the world population; rice is the dominant irrigated crop, accounting for approximately 30 percent of the total irrigated area (Barker et al., 1999). Abiotic stress refers to suboptimal climatic and/or edaphic conditions that adversely affect cellular homeostasis and that ultimately impair growth and fitness (Mickelbart et al., 2015). Water-deficit and salinity are the major abiotic factors that affect rice productivity worldwide. Abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth and development (Vibhuti et al., 2015). Rice germplasm exhibit variability in their response to these abiotic stresses. Some genotypes possess ability to tolerate extreme drought and salinity stresses, whereas many of them are highly susceptible. This phenotypic variability may be attributed to genetic and epigenetic variations, and different regulatory architecture among them. The study of tolerance/response mechanisms to abiotic stresses has been intensively worked out based on genomics, transcriptomics and proteomics analyses (Garg et al., 2015).

Among the various factors limiting rice yield, salinity is one of the oldest and most serious environmental problems in the world (Joseph et al., 2010). Environmental factors contribute to over 70% of crop yield losses worldwide. Of these drought and salinity, they are the most significant causes of crop yield reduction (Isayenkov & Maathuis, 2019). Soil salinity is one of the key environmental factors that limit crop growth and agricultural productivity throughout the world (Shrivastava & Kumar, 2015).

Salinity affects rice growth in all stages starting from germination to maturity (Khan et al., 2016b). Several physiological pathways, i.e., physiological processes such as photosynthesis, respiration, nitrogen fixation and carbohydrate metabolism have been observed to be affected by high salinity (Soussi et al., 1998; Sudhir & Murthy, 2004a). For starch, it serves as a carbon and energy source for seed germination, seedling establishment and plant growth. Moreover, it is a dominant

factor in crop yield (Chen et al., 2008). Starch is one product in plant affected by salinity stress (Thitisaksakul et al., 2015). The role of an agronomist is, therefore, to manipulate the crop in order to counteract the influence of salt stress, and boost performance even under saline conditions (Shah, 2007). Salinity creates a dilemma for plants; increased levels of inorganic minerals in the environment create osmotic and water stress (Isayenkov & Maathuis, 2019). Seeds and young seedlings are frequently confronted by much higher salinities than vigorously growing plants because germination usually occurs in surface soils which accumulate soluble salts as a result of evaporation and capillary rise of water (Almansouri et al., 2001).

Symptoms of salt stress included a reduction in growth, whitish leaf tip, leaf rolling, drying of leaves and reduction of height seedling in salinized condition. Salinity causes complex interactions among different morphological, physiological and biochemical processes. Salinity may cause oxidative stress due to highly producing of reactive oxygen species (ROS) leading to alteration plant metabolism (Minh et al., 2016). Root and shoot dry weight and biomass were reduced in rice seedling in salinity stress (Hossain et al., 2015). However, soil salinity, particularly due to sodium chloride (NaCl), can be considered as the single most widespread soil toxicity problem that global rice production faces at present (Jamil et al., 2012b).

For salinity, it affects all stages of the growth and development of rice plant and the crop. Salinity sensitivity of rice was studied to determine salinity effects on seedlings and yield components (Zeng & Shannon, 2000b). Salinity affects the rice crop by reducing plant growth measured in terms of biomass production or plant height, thousand grain weight and number of spikes, ultimately leading to grain yield reduction (Reynolds et al., 1999). Salt stress is also known to affect the quality and composition of rice grain by hampering the physiobiochemical processes during the grain filling stage (Razzaq et al., 2020). However, the response to salinity varies with growth stages in plant, concentration of salinity chemicals, and duration of exposure to plant. Increased salinity has diverse effects on the physiology of plants grown in saline conditions and in response to major factors like osmotic stress, ion-specificity, nutritional and hormonal imbalance, and oxidative damage (Hasanuzzaman et al., 2013). Responses to salinity varies with growth stages, concentration and duration of exposure at to salt, high salinity treatment, it caused a decrease in growth rate in all

rice the varieties tested (Dionisio-Sese & Tobita, 1998). If an excessive amount of salt enters the plant, the concentration of salt eventually rises to a toxic level in older transpiring leaves causing premature senescence and reduces the photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002). More than affected to productivity, soil salinity also affected to has always been a global threat to rice production. Salinity affects not only the total yield of rice, but its nutrient quality in rice grain as well (Gupta et al., 2019). Soil salinity causes a decline in plant growth and productivity by inhibiting physiological processes, especially photosynthesis (Sudhir & Murthy, 2004b). However, Salinity affects plant growth during developmental stages and the sensitivity to crops varies from one growth stage to another in rice. In addition, production of rice under saline condition is under pressure because salinity may cause plant demise, growth, and development (Nozulaidi et al., 2015).

Seed germination is the most important stage in a plants life cycle. Water, air, temperature and light are all essential for the seed germination (Brock et al., 1989; Ghosh et al., 2016a). The initial effect of drought on the plants is the poor germination and impaired seedling establishment (Fahad et al., 2017). For many plants, salt stress is more inhibitory during seed germination than at any other stage of growth (Khan et al., 1997a). More than drough stress, seedling stage in rice are affected by salt stress, similar is as the most critical stage for these stresses (Khoshsokhan et al., 2011; Sarker et al., 2014). At lower levels of salinity, it delay germination, whereas higher levels, the final percentage of germinated seeds was reduced (Sedghi et al., 2010). The high osmolarity in the leaf tissues derived from salt stress directly affecting chlorophyll degradation, leading to its application as an efficient indicator of salt-tolerance or salt-sensitivity [reported in rice var. Khao Dawk Mali (KDML105) and Pathum Thani 1 (PT 1)] (Cha-um et al., 2007). For soil salinity, it is usually causes stunting or even death of seedlings (Dionisio-Sese & Tobita, 1998). It could say that salt stress leads to suppression of plant growth and development at all growth stages. However, depending upon plant species, certain stages such as germination, seedling or flowering stage could be the most critical stages for salts stress (Khoshsokhan et al., 2011; Sarker et al., 2014). Why the study does in seedling stage is important in plant? Because seedling stage is early stage to determine the survival in plant. During

submergences, two important factors influencing rice plant survival are limitations; resulted by gas diffusion under water, and reduced irradiance that impair photosynthesis and efficient utilization of carbohydrates (Ram et al., 2002). However, Salt stress can affect germination and seedling growth either by creating an osmotic pressure (OP) that avert of the water absorption or by the toxic effect of NaCl ions on seed germination (Akbarimoghaddam et al., 2011). Thus, one of the major environmental stress factors which adversely affect on uniform germination is salinity in arid and semi-arid regions (Kumar et al., 2019).

2.2 Chemical substances

Enhanced proline biosynthesis from glutamate was reported is the main contributor for proline accumulation under stress conditions. Which, proline biosynthesis is essentially regulated at the transcription level of Δ^1 -pyrroline-5-carboxylate synthetase 1 (P5CS1) encoding the rate-limiting enzyme of the pathway (Naghshbandi et al., 2019).

Proline biosynthesis occurs in the cytosol and in the plastids (like chloroplasts in green tissues) while proline degradation takes place in mitochondria (Trovato et al., 2019). Proline was reported can help and encourage plant growth because it can add protein in the plant cells to survive; response to a wide range of biotic and abiotic stresses (Verbruggen & Hermans, 2008). Proline is the most common endogenous osmolyte accumulated under various abiotic stresses including salinity (El Moukhtari et al., 2020). Therefore, several factors that indicate accumulation occurs in the proline such as ultraviolet radiation, exposed heavy metals, low temperatures, salinity and air deficits. In addition proline acts as the molecular chaperons it is able to maintain the protein integrity and enhancing the activities of different enzymes (Hayat et al., 2012). When applied as an exogenous compound to crops, proline can improve salt tolerance (Chaum & Kirdmanee, 2010). Proline accumulation primarily occurs in response to stresses that cause dehydration of the plant tissue such as drought (low water potential), salinity and freezing (Verslues et al., 2006). Therefore, Proline accumulation has been suggested to contribute to stress tolerance in many ways. It is generally stated that most of the proline that accumulates during hyperosmotic stress arises from increased glutamate synthesis (Verslues & Sharma, 2010). In addition,

high and low levels of proline in plant tissue are used to evaluate the tolerance level of varieties to stress (Qayyum et al., 2011). However, exogenous proline inhibited stomatal opening in *Vicia faba* whereas other amino acids such as histidine, methionine, aspartic acid, glutamic acid, asparagine, and glutamine promoted stomatal opening (Hayat et al., 2012). In a study was shown that proline applied exogenously at a low concentration (e.g., 30 mM) ameliorated the adverse effects of salinity on early seedling growth in rice, whereas at higher concentrations (40–50 mM) proline resulted in toxic effects and poor plant growth (Hayat et al., 2012). Similarly, in salt stressed *Oryza sativa*, while low concentrations (20–30 mM) of proline were effective in mitigating the adverse effect of 100 mM NaCl on growth, higher concentrations of proline (40 to 50 mM) resulted in growth reduction (Roy et al., 2019a). In addition, in plants, intracellular proline levels have been found to increase by >100-fold during stress (Liang et al., 2013). The role of proline as a free radical scavenger is more important in alleviating stress than its role as a simple osmolyte (Hayat et al., 2012). Therefore, one of the first factors shown to affect proline accumulation is the intensity and duration of light exposure (Ashrafijou et al., 2010). Thus, shoots exposed to levels of 150 mM NaCl produced more proline compared to controls, but the levels of other osmolytes (glycine betaine, choline) did not increase (Zhou et al., 2019). Feedback inhibition of P5CS (the enzyme controlling the rate-limiting step in proline biosynthesis) occurs at proline levels of about 10 mM, yet proline concentrations of up to 150 mM have been reported in leaves of plants undergoing water deficit (Taylor, 1996). Application of 1 mM proline alleviates the negative effect of 400 mM NaCl, but 100 mM proline did not have a significant effect (El Moukhtari et al., 2020).

2.3 Rice growth and development stages

Growth cycle of the rice plant is divided into three stages. These stages are designated as vegetative, reproductive and spikelet filling or ripening (Moldenhauer et al., 2003). Yield potential of rice is formed or defined during these growth stages (Fageria, 2007). The life cycle of rice cultivars in Arkansas ranges from 105 to 145 days from germination to maturity, depending on the variety and the environment (Moldenhauer & Slaton, 2001). If incorporated into existing systems, a crop growth

staging system based on plant morphogenesis, with each stage differentiated from another dichotomously, would facilitate consistent crop growth staging (Counce et al., 2000).

2.3.1 Germination

Seed germination occurs when the seed coat has imbibed adequate water to become soft and elastic. The coleorhiza (the sheath covering the radicle or embryonic primary root) elongates slightly, emerging through the seed coat, allowing the radicle to break through the coleorhiza and become anchored in the soil (Moldenhauer & Slaton, 2001). Seed dormancy and germination, and the onset of inflorescence development typically delimit these three phases. In each phase, numerous events occur sequentially (Itoh et al., 2005). Germination commences with the uptake of water by the dry seed-imbibition-and is completed when a part of the embryo, usually the radicle, extends to penetrate the structures that surround it (Bewley, 1997). Rice seed germination starts after about 4 days will begin with the breakdown of carbohydrates marked by the breakdown of starch reserves contained in endosperm (Murata et al., 1968).

Salt tolerance of rice at the seed germination stage is one of the major determinants for the stable stand establishment in salinity soil (Wang et al., 2011). It is convenient to treat separately the period from imbibition of the seed until seedling emergence from the soil. Although in rice production, it should be emphasized that leaf initiation and tiller development start after the seed is imbibed, proceeds without interruption as the seed emerges from the soil is very important issue (Kirby & Appleyard, 1987).

2.3.2 Vegetative phase

The vegetative growth phase is characterized by active tillering, a gradual increase in plant height and leaf emergence at regular intervals (Fageria, 2007). 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 (Moldenhauer & Slaton, 2001). In almost all plant species, two phase changes are recognized easily by the distinct morphological changes. Morphological changing is occur in plants

from the embryonic phase to the juvenile vegetative phase and from the adult vegetative phase to the reproductive phase (Itoh et al., 1998). The life cycle of higher plants has three mutually distinct developmental stages: the embryogenetic, vegetative, and reproductive stages. The vegetative stage can be further divided into the juvenile and adult phases, which are distinguished by many morphological and physiological (Tanaka et al., 2011).

2.3.3 Reproductive phase

In rice upon transition to reproductive phase, the vegetative apical meristem transforms to an inflorescence meristem. The latter terminates after making six to eight primary branch meristems. Primary branches produce two to four secondary branch meristems and terminate in a spikelet. Secondary branches also produce few spikelets. The branched inflorescence thus generated is called a panicle (Rao et al., 2008). The mature inflorescence of rice has ten or more primary branches that bear approximately 150 spikelets. Two types of inflorescence meristem are recognized: rachis and branch meristems. The rachis meristem forms bracts and branches as lateral organs, and finally aborts (Itoh et al., 2005). Likewise, in the mature inflorescence, a small vestigial protrusion at the base of the highest primary branch was observed, but cannot find a terminal flower of the rachis. Thus in rice, rachis meristem is assumed to abort at an early stage after producing ten or more primary branch primordial (Ikeda et al., 2004). In the reproductive growth stage panicle development takes place, booting and flowering are part of the reproductive growth stage. Panicle size or spikelets per panicle are determined in the reproductive growth stage. Spikelet size or weight is determined during the spikelet filling growth stage (Fageria, 2007).

2.3.4 Ripening phase

Rice is primarily a self-pollinating plant. Because it is usually pollinated before the lemma and palea open to release pollen into the air, cross-pollination usually only occurs at a rate of about 1 percent (Moldenhauer & Slaton, 2001). The age of rice plants is 90-120 days, from all genotypes are in the process of flowering; towards the end of the period, all plants are in various stages of grain development and maturation. some leaves begin to age and turn yellow except for the

flag leaf which remains green. Therefore, flag leaves were collected for physiological determination when the plants were 120 days old (Kanawapee et al., 2013). It is concluded that grain-yield is impeded by high biomass; or low harvest index at flowering in direct-seeded rice, particularly in long-duration varieties (Dingkuhn et al., 1991; Nawaz et al., 2019).

2.4 Seedling emergence and development

In rice, seedling emergence occurs when the first internode called the mesocotyl has elongated and pushed the tip of the rice coleoptile (epiblast or first sheathing leaf) through the soil surface. However, the length of the mesocotyl varies with cultivars (Moldenhauer & Slaton, 2001). During the seedling stage, the secondary root system or adventitious root, does not develop properly and several unbranched roots emerge to spread in all directions from the base of the coloptoptiles which are parallel to the ground surface (Dunand & Saichuk, 2014). Etiolated rice seedlings exhibited marked morphological differences when grown in sealed containers or in containers through which air was passed continuously (Raskin & Kende, 1983). So, the germinated seedlings were counted at an interval of 24 h for 5 days and the speed of germination of seed was monitored (Mia et al., 2012). Seedling development begins when the primary leaf appears shortly after the coleoptile is exposed to light and splits open at the end (Dunand & Saichuk, 2014). Rice is one of the few plants that can germinate anaerobically through a rapid elongation of coleoptile (Magneschi & Perata, 2009). Several studies have been conducted to explain the effects of external factors such as salinity on rice seedling. From the study by (Pattanagul & Thitisaksakul, 2008), rice seedlings var. Khao Dawk Mali 105 (salt-sensitive), Luang Anan (moderately salt-tolerant) and Pokkali (salt-tolerant) were exposed to 0 mM, 50 mM, 100 mM and 150 mM sodium chloride (NaCl) for ten days. The result showed that salinity stress caused reduction in leaf relative water contents in all cultivars. For other study, seeds were placed for germination and the seedlings were allowed to grow rice seeds for ten days at NaCl concentrations of 0 mM, 50 mM, 100 mM, 150 mM and 200 mM. The result showed that NaCl decreased the germination index (GI), speed of germination, seedling height and seedling dry matter weight (Khan et al., 1997a). Comparing among rice stages, there are studies

that concluded that rice is relatively tolerant of salt stress during germination, active tillering and towards maturity, and is sensitive during the early seedling and reproductive stages (Pearson & Bernstein, 1959; Zheng et al., 2001).

2.5 Salinity and its effect to rice production

Global climate change is causing stress on the growth and development of rice plants. Therefore, a sustainable approach is needed to reduce the detrimental caused by climate change in rice plants (Wassmann et al., 2009). Hence, abiotic stresses result in more damage to growth and yield of rice plants compared to the biotic stress factor (Pareek et al., 1999). However, Accurate estimates of agricultural losses in reduced crop production and soil health in terms of agro-ecological disturbances due to abiotic stress cannot be every accurate. It is evident that these stresses affect the land area and significantly impact qualitative and quantitative losses in crop production (Dubey et al., 2019). Therefore, enzyme activity (EA) also has been regarded commonly as indicators of soil health and functionality of microbial communities (Li et al., 2020). Furthermore, microbial communities can function to modify the soil microenvironment such as light, pH, temperature and humidity, waste and root input (Surówka et al., 2020). And then plant microbiome provides fundamental support to the plants in acquiring nutrients, resisting against diseases, and tolerating abiotic stresses (Trivedi, 2021). For abiotic stress, the response can reduce or increase plant susceptibility to biotic stress caused by pests or pathogens (Iqbal et al., 2021). Environmental stresses constrain rice production, affecting about 30% of the 700 million poor in Asia alone, who live in rainfed rice-growing areas. These stresses can be caused by extreme climatic changes like drought, flooding or rising sea levels (Dar et al., 2014). Estimates of the area of salt-affected soils vary widely, ranging from 6% to 10% of earth's land area, and 77 million hectares (Mha) of irrigated lands (Eynard et al., 2005). In addition, salinity is the second major obstacle in reducing rice production after drought conditions. Thus, rice production under saline conditions is under stress because salinity can cause plant death, growth, and development by reducing yields by up to 50% (Bensidhoum et al., 2019).

After prolonged exposure to salt stress for 8-10 days, growth and water content of rice cells were progressively decreased (Summart et al., 2010). Although

environmental factors other than high salt concentration may contribute to limit plant growth and yield. The choice of the crop must take into account the specific crop tolerance to both biotic and abiotic factors. However, The selection of plant varieties that have the ability to tolerate salty conditions so that they can overcome total crop failure is one of the priority criteria (Eynard et al., 2005).

For salinity stress, it effects varied along with different growth stages in rice, thus depressed yield grain production (Sakina et al., 2016). Salinity affects both vegetative and reproductive development which has profound implications depending on whether the harvested organ is a stem, leaf, root, shoot, fruit, fiber or grain (Läuchli & Grattan, 2007). Salinity stress give effects to plant growth through the osmotic effect of salt in the growth medium and the toxic effect of salt in plants. (Rahman et al., 2016). Soil salinity, particularly due to NaCl, can be considered as the single most widespread soil toxicity problem that global rice production faces at present (Hong et al., 2007). Some toxic effects of salt stress include decreased germination and seedling growth (Ashraf, 2010; Zeng & Shannon, 2000a). Salinity caused a substantial reduction in carbon assimilation race and stomatal conductance in all cultivars (Dionisio-Sese & Tobita, 2000). Effect of salinity stress suppressed leaf expansion which ultimately reduces photosynthetic (Jamil et al., 2012b).

2.6 The solution of salinity stress for crop

Salinity is a harsh environmental factor that has the major effect on plant quantity and quality (Zhu, 2002). Thus, salt stress affects all the major processes such as germination, growth, photosynthetic pigments and photosynthesis, water relation, nutrient imbalance, oxidative stress, and yield (Parihar et al., 2015). Salinity affects the strength of the forces bringing the complex pigment protein - liquid, in the chloroplast structure (Ali et al., 2004).

Rice is highly sensitive to salinity and its tolerance varies with growth stages. For example, seed germination and seedling growth stages are very sensitive to abiotic stress (Deivanai et al., 2011). Water stress is the evident effects of salinity, therefore, the determination of water contents in plants is critical for the study of plant tolerant efficiency (Parida & Das, 2005; Qasim & Ashraf, 2006).

One of the recently gaining practices of counteracting the adverse effects of salinity on plant growth includes the implementation of salt-tolerant bacteria with natural growth promoting ability in such conditions (Etesami & Beattie, 2018). Plant growth promoting rhizobacteria, first defined (Kloepper et al., 1980). That include those bacteria, which, on inoculation into the soil, colonize the roots of plants and enhance plant growth (Chakraborty et al., 2011). So, one of the most frequently utilized is seed priming. The process of seed priming involves prior exposure to an abiotic stress, making a seed more resistant to future exposure. Seed priming stimulates the pre-germination metabolic processes and makes the seed ready for radicle protrusion. It increases the antioxidant system activity and the repair of membranes (Ibrahim, 2016).

The extent by which one mechanism affects the plant over the others depends upon many factors including the species, genotype, plant age, ionic strength and composition of the salinizing solution, and the organ in question (Läuchli & Grattan, 2007). To observe the growth of rice plants in saline soil conditions, transgenic rice plants grown in pots were watered with salt water (100 mM). Control plants started to wilt on continuous pouring with saltwater whereas transgenic plants could resist salt stress after 15 days of continuous irrigation with sodium and chloride (Prashanth et al., 2008). This result showed low tolerant on salinity stress in normal rice varieties.

Improving salt tolerance in plants was possible in different ways: direct selection of tolerant varieties of a species in saline environments or by mapping quantitative trait loci and subsequent use of selection markers or by generation of transgenic plants (Fayyaz, 2008). First of all, the solution of salinity stress should have considered on the condition to set up the salinity stress in rice and parameters for assessment. The establish the relative of osmotic versus ionic components of salt stress in rice is important issue for study (Castillo et al., 2007). For example, further studies are needed with longer stress durations to achieve a higher sodium ion (Na^+) concentration in plant tissues, and testing in several rice varieties. Several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage). Again, rice has tolerance on salinity during vegetative growth stage, becomes sensitive during pollination and fertilization. In final stage, rice becomes increasingly more tolerant at maturity (Pearson et al.,

1966;IRRI, 1967). Hence, to know the response of the rice plant to salinity as a whole, it is imperative that the effects be observed in all the various stages of its development; that is at early seedling, vegetative and reproductive stages (Chapagain et al., 2021).

Under stress conditions, plant cells have the ability to prevent water loss and to maintain the continuous growth is call tolerant ability. Moreover, tolerant plant try to reduce their osmotic potential via increasing mineral ions content and compatible solutes synthesis to better water uptake under salinity (Nemati et al., 2011). Plants commonly react to these stresses by accumulation of compatible solutes, such as proline, in cells which results in the improvement of environmental stress tolerance (Ashraf & Foolad, 2007; Hong et al., 2000; Reddy et al., 1998). These solutes can be accumulated in high concentrations without impairing plant metabolisms under the stress (Chutipaijit et al., 2009b). Proline (Pro) and Trehalose (Tre) function as compatible solutes and are upregulated in plants under abiotic stress. They play an osmoprotective role in physiological responses, enabling the plants to better tolerate the adverse effects of abiotic stress (Nounjan et al., 2012).

2.7 Using salinity tolerance varieties

The problem is exacerbated by the increase of sea water level as the impact of global climate change. High concentration of salt ion in the soil could significantly reduce rice growth and yield (Hairmansis et al., 2017). Through the transfer of genes from organisms that adapt to a stressful environment to plants is an approach in engineering plants that are resistant to stress (Purty et al., 2008). Therefore, genetic variation and differential responses to salinity stress in plants differ in stress tolerance. Hence, making it possible to identify physiological mechanisms, gene sets, and gene products that enhance stress tolerance and incorporate them in suitable species to produce salt-tolerant varieties (Gupta & Huang, 2014).

by using artificial selection and conventional breeding approaches to increase salinity tolerance in a number of potential plants, although molecular biology approaches are currently being intensively pursued to achieve this goal. (Ashraf, 2004). Likewise, using wild species and relatives of crop plants as sources of gene(s) for tolerance to several stresses is gaining importance in terms of sustainable

agriculture and for long-standing expression of tolerance to abiotic stresses (Latha et al., 2004).

The condition setting of salinity stress in rice planting was investigated (Hoang, 2015). Swelling and the destruction of the chloroplast ultrastructure including stroma (ST) and stroma lamellae (SL) in PT 1 plants under salt stress may cause photosynthetic inactivation and reduction in photosynthesis (Siringam et al., 2012). Rice plants were acclimatized from tissue culture in the glasshouse at 28/21°C day/night as described (Hoang et al., 2015). Salinity can develop naturally, but where human intervention has disturbed natural ecosystems and changed the hydrology of the landscape, the movement of salts into rivers and onto land has been accelerated. This can dramatically affect natural environment and reduce the viability of agricultural sector (Hoang et al., 2014). Salinity tolerance strategies have utilized three major approaches: (i) conventional breeding, (ii) marker assisted selection and (iii) genetic engineering (Hoang et al., 2015).

Aromatic rice varieties as “Jasmine rice” (KDML105) and Pathum Thani 1 (PT 1) are popular lowland varieties in Thailand. These varieties both have a distinctive aroma, delicate flavor, high cooking quality, long grains, high amylose content, and a soft texture. Because of this, they carry high export values (Ariyaphanphitak et al., 2005; Laohakunjit & Kerdchoechuen, 2007). However, their ability to tolerate salinity was reported to differ in these varieties. The physiological responses of KDML105 and Sangyod (SY) varieties showed the better tolerance to salinity than those of PT 1 and Black Sticky (BS) varieties (Chutipaijit et al., 2009a). In salt stress treatment, the relative water content (RWC) of rice seedlings was decreased when compared to untreated seedlings (Chutipaijit et al., 2009b). In addition, Homjan rice is a local variety that grows well in the salted rice fields near the seashore in the southern region of Thailand. This variety has been used as a resource for osmoregulation defence responses to salt-stress (Cha-um et al., 2007).

The salt tolerance ability of Pathum Thani 1 (PT 1) sensitive rice cultivar was tested on the effect of salinity stress and using exogenous sucrose for alleviation. Fourteen-day-old seedlings of PT 1, along with Homjan (HJ), salt-tolerant (positive control), were cultured in MS liquid medium supplemented with 0, 29.2, 58.4- or 116.8-mM sucrose. Then, rice plants were exposed to 0 or 342 mM NaCl. The result

showed that an osmotic potential (Ys) in the leaf tissues of rice seedlings dropped significantly when subjected to 342 mM NaCl. The increase in Ys in the leaf tissues of salt stressed seedlings directly caused damage to the ultrastructure of chloroplast organelles, as well as to photosynthetic pigments i.e. chlorophyll a, chlorophyll b and total chlorophyll (Siringam et al., 2012). Exogenous sugar application in the culture medium was directly absorbed and enriched in rice seedling. This substance (exogenous sugar) leading to soluble sugar accumulation and played a key role as osmoregulation of salt defence mechanism in rice plant.

Moreover, genetic engineering has been proved to be an efficient approach to the development of salinity-tolerant plants, and this approach will become more powerful as more candidate genes associated with salinity tolerance are identified and widely utilized (Gupta & Huang, 2014).

CHAPTER III

METHODOLOGY

3.1 Materials and treatments, experiment design and planting practices

3.1.1 Time and Place

The field trials were performed in an experimental field at Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi Information Technology (IT) campus, Phetchaburi Province, Thailand. The experiments start from January to December 2020.

3.1.2 Weather data during the investigation in Hua-Hin, Prachuap, Khiri Khan province, Thailand

Month/Year	Temperature (^{0}C)		Wind Speed (km/h)	Relative Humidity (%)	Rainfall (mm)
	Maximum	Minimum			
June 2020	31	27	12	71	4.13
July 2020	30	26	11	74	6.54

3.1.3 Genetic materials and factors

Salt tolerance studies were conducted under controlled conditions by using solution and culture in both between germination papers and pots. Three lowland rice varieties were used in this study including Inpari 35 (salt tolerant variety), Chai Nat 1 (CNT 1) (not tolerant variety), and Pathum Thani 1 (PT 1) (not tolerant variety). These varieties were selected for assessment either effect of salinity stress or effect of proline to alleviate the stress from salinity. Factors in the study include salinity levels (sodium chloride; NaCl); 0 mM (0 dS/m), 50 mM (5 dS/m), 100 mM (10 dS/m) and 150 mM (15 dS/m) NaCl, and proline levels; 0 mM, 50 mM, 100 mM and 150 mM.

3.2 Experiments, experiment design and planting

Experiment 1 : Effect of salinity concentrations on rice germination

The purpose of this experiment was to sow the seed directly into a saline planting condition; it is a stimulation of the cultivation of farmers in some areas. Experimental design used completely randomized design (CRD) for study the effect of 4 salinity levels; 0, 50, 100, and 150 mM of NaCl, with 4 replications. Independent testing was conducted in different rice varieties (CNT 1, PT 1 and Inpari 35). In this experiment, first soak the seeds of three varieties of rice for 1 min with alcohol 70% and wash used normal water after that germinate the seed. One hundred seeds were put on a paper filter wet, rolls by between paper method and arrange in a plastic box; one roll as one replication. Then, maintain the salinity in each level by spraying every day with 100 ml NaCl solution. Seven days after the observation in the experiment should collect data including; germination (score 1 is germinate and 0 is not germinate), measure shoot length (cm), roots length (cm), hair roots (density) just give score (1, 2, 3, 4 and 5) and count roots number.

Experiment 2 :Effect of seed soaking before sowing on rice germination under salt condition

The purpose of this experiment was to sow soaked seed into a saline planting condition, it is a stimulation of the cultivation of farmers in some areas, especially in saline soil stress. Soak the seeds used normal water during 24 hours after that germinated on a wet filter papers with 100 seeds, rolled by between paper method; one roll as one replication. Set up those rolls in the plastic box and keep the salinity in each level. Seven days after the transplantation to germaination paper, characterisitcs were recorded include; germination (score 1 is germinate and 0 is not germinate), measure shoot length (cm), roots length (cm), hair roots (density) just give score (1, 2, 3, 4 and 5) and count roots number.

For this experiment, the data was analysed in two proposes. The first, to analysis for comparing the effect of salinity levels. Experimental design uses CRD for study the effect of 4 salinity levels; 5, 50, 100, and 150 mM of NaCl, with 4

replications. Independent testing was conducted in different rice varieties (CNT 1, PT 1 and Inpari 35).

The second, the data was arranged as 4x2 factorials in CRD with 4 replications. Two factors were four salinity levels; 0, 50, 100, and 150 mM NaCl, and two seed soaking with water; non-soaked and soaked. Independent testing was conducted in different rice varieties (CNT 1, PT 1 and Inpari 35).

Experiment 3 :Testing effect of proline concentrations on germination in rice under salt condition.

The purpose of this study was to know the response in each rice variety to the supplementation of proline; by soaking the seed before planting under salinity conditions which conducted by using NaCl concentrations. The experiment was arranged as 4x2 factorials in CRD with 4 replications. Two factors were four proline concentrations; 0, 50, 100 and 150 mM proline, and two salinity levels; 0 and 150 mM NaCl. Independent testing was conducted in different rice varieties; CNT 1, PT 1 and Inpari 35. In step make the germinated seeds, seeds were soaking with normal water for 18 hours and soak with each proline concentration about 6 hours before sowing. Each roll of between germination paper carried 100 seeds per roll, and keep in plastic box. Subsequently, they were kept the salinity level by spraying the roll with each treatment of salinity level. In spraying ordinary water and saline solution with different frequencies according to the treatment, spraying on this germination section with 100 ml solution was done once a day. This was to keep the seeds from getting too moist.

Experiment 4: Testing effect of proline concentrations on RNA and chemicals content in rice seedling under salt condition.

In this experiment to measure chemical content of rice plant received the proline supplementation in seedling stage when it was grown under salty condition. The experiment was arranged as 4x4x3 factorials in CRD with 3 replications. Three factors consist four levels of salinity (0, 50, 100 and 150 mM NaCl), four levels of proline (0, 50, 100 and 150 mM proline) and three wetland rice varieties (CNT 1, PT 1 and Inpari 35).

Seeds from the three varieties was rinsed with tap water and soaked with nil water for 24 hours and keep moist during 24 h. After germination, the seeds were planted in a set plot in the field, each pot is planted with 10 germinated seeds; 12 pots per one replication. During the nursery stage will pour salinity or nil water after 2 weeks at two times a week in accordance with the recommended salinity concentration with 100 ml solution (58.44 g/mm). At three after planting in pots, proline solutions were sprayed depend on each treatment with 100 ml solution (115.13 g/mm). Three days after proline spraying, sample both of leaf and stem of plant; without root for ribonucleic acid (RNA) and chemical contents measurement were collected. After that, RNA and chemical analysis were conducted in laboratory.

3.3 Chemical determination

3.2.1 Chlorophyll content

The method involves the estimation of plant pigments without soften by soaking Leaves were washed with double distilled water (DDW) and chopped. 100 mg of chopped leaf material was taken in vials in triplicates and 10 ml of dimethylsulfoxide (DMSO) was added to each vial (Jabeen & Baba, 2018). 100 mg of finely chopped fresh leaves were placed in a 25 cm3 capped measuring tube containing 25 cm3 of 80 % acetone, and kept inside a refrigerator (4^{o}C) for 28 h (Kral et al., 2021). Chlorophyll amount was determined spectrophotometrically following Porra (2002) (Panda et al., 2006).

Collected samples of plant and measured the fresh weight and dry weight in the incubator. Measured the dry weight (100 mg) of each sample and placed in vial containing 10 mL DMSO and then mixed by vortex 5 min and incubated at 65°C for 30 min (in the dark). Furthermore, centrifuged the samples for 5 min at 3000 rpm, then transferred the supernatant to a new vial. Transferred 1 mL of supernatant to a cuvette, calibrated spectrophotometer by using DMSO as reference at 645 and 663 nm and recorded. Calculated the chlorophyll content by the following formula (Zhuo et al., 2021).

Chlorophyll A (mg/g) = [12.7(A663)-2.69(A645)]V/(1000xW)
Chlorophyll B (mg/g) = [22.9(A645]-4.68(A633)] V/(1000xW)
Total Chlorophyll (mg/g) = [20.2(A645) +8.02(A663)]V/(1000xW)

3.2.2 The relative water content

The relative water content (RWC) was described by Slatyer (1967) is a useful indicator of the state of water balance of a plant essentially; because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation. Thus, there are a relationship between RWC and water potential. The RWC express the water content in percent at a given time as related to the water content at full turgor. The formula of RWC in this study showed as below:

$$\text{Relatively water content \%} = \frac{\text{Fresh weight} - \text{dry weight x } 100}{\text{Turgid weight} - \text{dry weight}}$$

Estimate samples - 3(v) ×4(s) ×4(c) ×3(r) = 144 samples. Collected 5 leaves from well-grown plants and measure the fresh weight. Placed the sample inside plastic jars filled with water to saturate the leaves and then stored for 6– 9 h. After that, measure the turgid weight continue to oven-dried for 48 h at 50-60°C and measure the dry weight (Olivero Lora, 2011).

3.4 Collect data in laboratory and green house

The rice seeds germination in Experiment 1-3 was set up in the laboratory and Experiment 4 was set up in the greenhouse (seedlings stage). In Experiment 1-3, data has been collected include: germination score (0 is not germinate and 1 is germinate), measure shoot length (cm), roots length (cm); hair roots (density) just give score (1, 2, 3, 4 and 5) and count roots number. In Experiment 4, growth of seedlings was measured in terms of new leaf (count), height plant (cm) and leaf burn give the score (1, 3, 5,7and 9), count total plants/plastic pot, fresh weight (g), dry weight (g).

3.3.1 .New leave and burned leave

In measuring new leaves from rice plants was count the new leaf and can be used to measure the symptoms of the leaves from sample plants and then writing the data. The tolerance ability was scored according the standard evaluation system reported by Gregoria et al. (1997) showed (Table 1).

Table 1 Standard evaluation system of visible salt damage in rice at the seedling stage

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth with some leaves and tips whitish and rolled	Tolerant
5	Growth severely retarded with most leaves rolled and only a few elongated	Moderately tolerant
7	Complete growth arrest with most of the leaves dried and some plants dead	susceptible
9	Almost all plants dead or dying	Highly susceptible

Reference: (Gregoria et al., 1997)

3.3.2 Shoot length (cm)

To measure the shoot length of rice seedling, do it by placing the ruler on the rice paddy and pulling the shoot carefully to the tip of the leaf. Take measurements on the seventh day on germination while the seedling stage was carried out at one week intervals for a month and then writing the data.

3.3.3 Root length (cm)

Length of the roots was measured from the base of the stem until the end of root. Measurements were performed on the sample plants expressed in cm units (Delory et al., 2017). In conducting measurements of the length of the roots done on the seventh day after the observation by taking each seedling by putting it near the ruler then pull from the root base until the end of roots.

3.3.4 Hairy roots (density scores)

Hair roots (density) was observed that hair roots and just give score (1, 2, 3, 4 and 5) it was mean that 1 =20%, 2=40%, 3=60%, 4=80% and 5= 90-100%. With the primary root produces 40%, 60%, and 80% longer hair and also the root hair density on different root types are similar (Nestler et al., 2016).

3.3.5 Plants height (cm)

Plant height measurements were carried out before pouring the second time salinity on the plants; this is done by placing a ruler near the base of the plants then pulling the leaves to the tips of the leaves.

3.3.6 Number of leaves

In calculating, the number of leaves in plants carried out simultaneously with the measurement of plant height by counting the leaves that exists in each plant and then record data.

3.3.7 Fresh weight and dry weight (g)

For measurement of fresh weight and dry weight was done after all the plants were taken. Then, weighed by taking samples of existing plants and then weighing with analytical scales that have been provided in the laboratory. For dry weight, it carried out after samples plants were put into a hit oven for 48 h at a temperature of 70^{o}C then weighed each sample plants and then record data.

3.5 Ribonucleic acid (RNA) assessing

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.

3.6 RT-PCR

The rice cDNA was synthesized from 1 µg of total RNA using iScript™ 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). This study was done using gene specific primer OsP5CS1_F:5'-AAGGTGGGCACTGCAGTTGT-3 and OsP5CS1_R:5'- CCTTAACCT GCTCGCACAGA-3'.

The PCR reaction mixtures consist of 1x Ultra-pure Taq PCR master mix (1 U of Ultra-pure Taq polymerase, 2 mM MgCl2 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.

3.7 Statistical analysis

The analysis of all experiments (Experiment 1-4), use analysis of variance testing according to the expimental designed. For significant different at 5%, Duncan's Multiple Range Test (DMRT) will be used to compare the mean value among treatments. All data were analyzed by R-Program (Team, 2017).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect of salinity concentrations on rice germination

4.1.1 Effect of salinity level in CNT 1

The results of statistical analysis of CNT 1 showed salinity was non-significant different effect on percent germination both at 9 and 16 days after sowing between germination papers (Table 1). Percent of germination ranged between 86.5-92 % and between 88.5-90.5 % at 9 and 16 days after sowing, respectively. However, high concentration of salt was reported to reduce the water potential in the medium, which hinders water absorption by germinating seeds, and thus reduces germination (Patanè et al., 2013).

On both 9 and 16 days after sowing, other seedling characteristics, excepted root length, showed significant difference between salinity levels (Table 1). The shoot length of control treatment (0 mM and 50 mM) had higher values than the seedling in treatments of 100 mM and 150 mM NaCl on both 9 and 16 days after sowing. This result showed adverse effect of salinity on the growth of rice seedling. Seedling shoot is the important plant tissue in this stage because it directly affect photosynthesis ability (Sharma et al., 2020a). Root and shoot length provides an important clue to the response of plants to salt stress (Ratnakar & Rai, 2013).

For root-related characteristics, only hairy root density (scoring and root number per seedling were found to be different between salinity levels (Table 1). There was non-significance between 0 mM and 50 mM NaCl for hairy root (density score) and between 0 mM –100 mM NaCl for root number at 9 days after sowing. Seedling vigor was non-significance between 0 mM-100 mM at 9 days and between 0 mM-50 mM at 16 days after sowing (Table 2). The vigor index of the seedling was used to assess both the germination ability and the growth performance of the seedling in unsuitable growing conditions, or used as phytotoxicity index from various toxic stresses (Zhao et al., 2016). However, the formula of seedling vigor index may differ between literature, employing different characteristics into

calculation. Some studies use only the length of shoot, while the others combine shoot length together with the root length to multiply with germination percentage (Hossain et al., 2006; Zhao et al., 2016). Both root length and shoot length in rice were decreased when grown under salinity stress as well (Kakar et al., 2019b). For this reason, both root length and shoot length was used for calculation of the seedling vigor index in this study.

Table 2 The effect of **s**alinity levels on some characteristics of rice seedlings in variety **'**Chai Nat 1**'** **(**CNT 1**):** germination **(%)**, shoot length **(**cm**)**, root length **(**cm**)**, hairy root **(**density**)**, root number and seedlings vigor index at 9 and 16 days after sowing

At 9 days after sowing

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hairy Root (density)	Root Number	Seedlings vigor index
0 mM	91.5 ± 0.7	1.75 ± 0.08 ab	3.84 ± 0.77	3.18 ± 0.27 a	3.47 ± 0.75 a	512 ± 41 ab
50 mM	92.0 ± 4.2	2.21 ± 0.14 a	5.53 ± 0.77	3.00 ± 0.04 a	4.10 ± 0.18 a	710 ± 25 a
100 mM	89.5 ± 2.1	1.52 ± 0.36 b	5.01 ± 0.83	1.99 ± 0.07 b	2.73 ± 0.59 a	586 ± 60 a
150 mM	86.5 ± 0.7	0.74 ± 0.13 c	2.72 ± 0.37	1.09 ± 0.02 c	1.17 ± 0.13 b	299 ± 20 c
Mean	89.9	1.55	4.28	2.31	2.87	526.7
P-value, F-test	0.24 ns	0.0089 **	0.054 ns	0.000356 **	0.0152 *	0.0279 *
CV (%)	2.7	13.24	16.57	6.1	17.16	15.11

At 16 days after sowing

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hairy Root (density)	Root Number	Seedlings vigor index
0 mM	90.5 ± 3.5	3.35 ± 0.07 a	6.25 ± 0.49	3.40 ± 0.42 a	4.40 ± 0.42 a	873 ± 36 ab
50 mM	90.5 ± 4.9	3.55 ± 0.92 a	6.15 ± 1.06	2.05 ± 0.35 b	2.95 ± 0.92 b	874 ± 14 a
100 mM	87.5 ± 2.1	2.00 ± 0.14 b	6.20 ± 0.42	1.70 ± 0.14 b	2.20 ± 0.00 bc	718 ± 30 b
150 mM	88.5 ± 2.1	1.25 ± 0.07 b	4.45 ± 0.49	1.80 ± 0.14 b	1.35 ± 0.21c	506 ± 22 c
Mean	89.3	2.54	5.76	2.24	2.73	742.9
P-value, F-test	0.767 ns	0.0209 *	0.133 ns	0.013 *	0.0168 *	0.0075 **
CV (%)	3.8	18.43	11.63	13.13	19	7.5

Note : CV = coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, ns =non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability

Differently, the varies concentration of NaCl showed different effect on these characteristics: hairy root (density) score, root number seedling vigor index at 9 and 16 days after sowing. Thus, the duration of salinity stress also influences the effect on seedling performance, since the severe effect was observed at a longer period of salinity stress exposure. Effect of period that plant faced the salinity stress also has been reported in rice seedlings (Negrão et al., 2017). The higher negative effect in plant growth characteristics caused by NaCl accumulation and more toxic occurrence was observed in plant experienced the salinity stress in a long period. The roots are the first tissue affected by salinity, resulting in inhibition of nutrient uptake. The secondary effects are on physiology and morphology of seedling, which can be utilized for stress evaluation (Kakar et al., 2019a). Longer period of salinity stress can reduce plant growth and formation of root and hairy root (Chandra et al., 2007). Reduced root hair density can indicate the reduction in root length, and diameter of individual root hairs; that greatly determines the total root surface area.

Root length is the only parameter that was not significant different between salinity levels, both at 9 and 16 days after sowing (Table 2). Actually, the response of root length was reported under abiotic stress conditions including drought and salinity stresses. Both drought and salinity stresses are effects to limit water and nutrient available in plants. For example, plants tend to respond to drought stress by extending the root length, especially when plants experience insufficient water supply or excessive transpiration (Anjum et al., 2011). However, the response of the plant shows a tolerance that is limited under the stress levels the plant can tolerate. The susceptibility of plants to abiotic stress; drought, or salinity stresses, varies in dependence on stress degree, accompanying stress factors, plant species, and their developmental stages (Demirevska et al., 2009; Anjum et al., 2011). Salt stress affects plant physiology at both whole plant and cellular levels through osmotic and ionic stress, resulting in inefficient use of water by root system (Sudhir & Murthy, 2004a). Other processes involving plant growth, such as photosynthesis, ion regulation and water relations, are certainly influenced by salinity stress (Shaheen et al., 2013). It could be said that CNT 1 can exhibit salinity tolerance in response to maintaining root length under stress; to maintain the water and food availability of the roots. For this reason, using only root length may not be able to effectively identify the effect of

salinity stress condition on plant. Still, other measured characteristics of rice seedling in this study showed reduction according to salinity stress on both at 9 and 16 days after sowing. Hence, the salt stress response of rice seedling needs to be observed from multiple parameters. Despite the non-significant result for the root length in CNT 1, rice is generally regard as an especially salt-sensitive crop by various studies (Shereen et al., 2005).

4.1.2 Effect of salinity level in PT 1

PT 1 showed no significant difference on germination percentage effected by salinity levels both at 9 days (ranged between 94.5-97.5 %) and 16 days (ranged between 93.5-98.5%) after sowing in between germination papers (Table 3).

Significant difference was observed on shoot length both at 9 and 16 days after sowing (Table 3). However, the severe effect of salinity stress seems found at 150 mM NaCl at 9 and 16 days after sowing. The effects of salinity on plants are caused by the osmotic effect and resulted to lowering water potential, and reducing water uptake by the root at high concentrations of salt are accumulated in soil. Furthermore, salinity reduces photosynthetic rate and photosynthetic pigments leading to a reduction in the plant growth and survival (Jamil et al., 2012a; Khunpona et al., 2017).

For root-related characteristics showed varies between two dates; at 9 and 16 days after sowing. Which, root length and root number per seedling was significant difference only at 9 days after sowing (Table 3). Decreased root length at higher salinity stress was observed at 150 mM NaCl. For root length, it is one of the most important characters for salt stress because roots are in contact with soil and absorb water from soil (Kaya et al., 2003). Seedling vigor index showed similar effect of water salinity to root length and shoot length at 9 and 16 days after sowing, respectively (Table 2). The results were consistent for both periods: 9 and 16 days after sowing; the highest water salinity (150 mM NaCl) affected the vigor index of seedling, compared to nil water at 0 mM NaCl during the germination.

There was no significant difference effected by salinity level on hairy root (density) score both at 9 and 16 days after sowing (Table 3). However, at 9 days after sowing, the root length and root number were decreasing while the concentration of

NaCl was increasing (Table 2). The response observed on seedling characteristics seems difference between CNT 1 and PT 1 may dominate by influence of plant genetic. High salt concentrations cause various events that negatively effect on plant growth such as an impact inhibition of enzymatic activities (Gengmao et al., 2015). At high Na^+ concentrations in the cortical cells and cortical cell walls of root can result in a decrease in cell turgor and root growth (Munns, 2002). That, it is reflect the point of strong stress for root characteristics.

Table 3 Effect of salinity levels on some characteristics of rice seedlings in variety 'Pathum Thani 1' (PT 1) :germination (%), shoot length (cm), root length (cm), hairy root (density), root number and seedlings vigor index at 9 and 16 days after sowing

At 9 days after sowing

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hairy Root (density)	Root Number	Seedlings vigor index
0 mM	97.5 ± 0.7	2.70 ± 0.00 a	4.95 ± 0.35 a	2.70 ± 0.42	3.70 ± 0.56 a	693 ± 31 a
50 mM	96.0 ± 1.4	1.90 ± 0.14 b	3.95 ± 0.35 ab	2.75 ± 0.35	2.90 ± 0.42 ab	557 ± 14 a
100 mM	96.0 ± 0.0	1.65 ± 0.49 b	4.90 ±0.99 a	2.35 ± 0.21	2.60 ± 0.14 b	637 ± 72 a
150 mM	94.5 ± 0.7	0.65 ± 0.07 c	2.65 ± 0.21 b	2.10 ± 0.28	1.45 ± 0.21 c	304 ± 70 b
Mean	96	1.73	4.11	2.48	2.66	545.5
P-value, F-test	0.107 ns	0.00646 **	0.0424 *	0.295 ns	0.0173 *	0.0315 *
CV (%)	0.9	15.06	13.73	13.25	14.11	14.93

16 days after sowing

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hairy Root (density)	Root Number	Seedlings vigor index
0 mM	98.5 ± 2.1	3.10 ± 0.42 a	5.10 ± 0.99	2.70 ± 0.14	2.75 ± 0.35	805 ± 61 a
50 mM	97.5 ± 0.7	3.15 ± 0.64 a	6.45 ± 0.35	2.20 ± 0.42	2.20 ± 0.42	937 ± 42 a
100 mM	96.5 ± 2.1	3.10 ± 0.00 a	6.25 ± 0.64	2.35 ± 0.07	2.50 ± 0.42	901 ± 23 a
150 mM	93.5 ± 4.9	1.33 ± 0.10 b	4.05 ± 0.36	2.20 ± 0.15	2.15 ± 0.07	503 ± 40 b
Mean	96.5	2.67	5.46	2.36	2.4	786.6
P-value, F-test	0.447 ns	0.022 *	0.0572 ns	0.259 ns	0.395 ns	0.0160 *
CV (%)	3.02	14.44	11.71	10.09	14.58	9.86

Note: CV = coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, ns = non significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

4.1.3 Effect of salinity level in Inpari 35

Inpari 35 showed significant difference in four characteristics at 9 days after sowing, namely shoot length, hairy root (density) score, root number, and seedling vigor index (Table 4 upper). Germination percentage and root length were not significant different between salinity levels, with range values between 97-985 % and 3.25-5 cm, respectively. Inpari 35 was reported as more tolerant of salinity stress at seedling stage (Subekti et al., 2020a).

At 9 days after sowing, all of the significant different characteristics are affected by increasing salinity levels. All of these characteristics; excepted seedling vigor, showed decreasing values since 100 mM, for seedling vigor index showed reducing on value at 150 mM. at 9 days after sowing but was not significantly different among concentrations of NaCl at 16 days after sowing .Increased salinity can lead to clogging of soil. Which, at high salt levels potentially disturb the roots in nutrient uptake and damage the soil structure (Subekti et al., 2020b). Nevertheless, when considering the mean of six characteristics at 16 days after sowing, only hariy root density showed significant difference affected by salinity level. The reduction of hairy root density was clearly reduction at 50 mM NaCl. For this reason, seed vigor index was not significant different because this characteristic came from the multiply between germination percentage and the value resulting from the addition between shoot and root length.

However, the high variance in this study period (at 16 days after sowing) may result in the mean of treatments were not significant different even though many of the traits found to differ among values including shoot length, root length, root number and seedlings vigor index (Table 4 lower).

In this experiment first soak the seeds with normal water only for 1 min, however all rice varieties were found the germination in high percentages in all salinity levels (Table 2-4). It might be concluded that in viable and healthy seeds, although sowing in salty stress condition, it stills germination. However, plant survial under salinity stress not only obserb on percent of germination, the characteritics that makes plant can absorb or uptake the nutrient and for phytosisthetic ability should to concern in next step. However, plants' survival under salinity stresses not only

observe on the percent of germination, the characteristics that make plant can absorb or uptake the nutrient and for photosynthetic ability should to concern in next step as well. Thus, the length of the shoot and the root characteristics which relate to photosynthesis and water and nutrient absorption need to be evaluated.

Table 4 The effect of salinity levels on some characteristics of rice seedlings in variety ‘Inpari 35 :’germination (%), shoot length (cm), root length (cm), hairy root (density), root number and seedlings vigor index at 9 and 16 days after sowing

At 9 days after sowing

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hairy Root (density)	Root Number	Seedlings vigor index
0 mM	98.5 ± 0.7	3.50 ± 0.00 a	4.85 ± 0.21	3.15 ± 0.21 a	3.75 ± 0.78 a	780 ± 29 a
50 mM	98.0 ± 0.0	3.10 ± 0.42 a	4.60 ± 0.42	2.90 ± 0.14 a	3.45 ± 0.21 a	740 ± 34 a
100 mM	98.0 ± 00	2.25 ± 0.35 b	5.00 ± 0.99	2.35 ± 0.07 b	2.05 ± 0.07 b	717 ± 72 a
150 mM	97.0 ± 0.0	1.25 ± 0.07 c	3.25 ± 0.07	2.05 ± 0.07 b	1.65 ± 0.35 b	422 ± 20 b
Mean	97.9	2.53	4.43	2.61	2.73	664.8
P-value, F-test	0.053 ns	0.004 **	0.098 ns	0.004 **	0.022 *	0.046 *
CV (%)	0.36	11.03	12.43	5.25	16.21	13.2

At 16 days after sowing

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hairy Root (density)	Root Number	Seedlings vigor index
0 mM	98.5 ± 0.7	4.35 ± 0.64	7.25 ± 1.20	3.65 ± 0.21 a	3.85 ± 0.78	1134 ± 102
50 mM	97.5 ± 0.7	3.75 ± 0.35	6.25 ± 0.21	2.90 ± 0.28 b	2.90 ± 0.57	970 ± 29
100 mM	97.0 ± 0.0	3.15 ± 0.64	6.30 ± 1.27	2.65 ± 0.21 b	3.20 ± 0.42	928 ± 87
150 mM	97.0 ± 0.0	2.30 ± 0.57	4.45 ± 0.35	2.50 ± 0.14 b	2.20 ± 0.28	663 ± 46
Mean	97.5	3.39	6.06	2.93	3.04	923.9
P-value, F-test	0.107 ns	0.080 ns	0.135 ns	0.021 *	0.147 ns	0.124 ns
CV (%)	0.51	16.54	14.83	7.45	17.92	15.76

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, ns = non-significant difference at 0.05 level of probability, Different lower-case letter (a, b, c) = significance at 0.05 level of probability.

All germination characteristics of rice seedling of CNT 1, PT 1, and Inpari 35 in this study demonstrated the negative effect of salinity stress, except germination percentage. Salinity has been reported to negatively affect rice seed germination from the effect of osmotic stress and ionic toxicity on seeds (Tahjib-Ul-Arif et al., 2018). Na^+ and Cl^- are the two ions most frequently implicated with toxicity in plants, because both are highly water soluble, readily taken-up, and transported to the shoots in the transpiration stream (Rahman & MacNee, 2000). This can result in limitation of water absorption and germination process (Zhang et al., 2010). Seed priming is a common seed treatment to reduce the time between seed sowing and seedling emergence and increase the synchronization of emergence (Parera & Cantliffe, 1994). Priming treatments not only improve the germination rate and time, but also enhance the seedling vigour. These tests in CNT 1, PT 1, and Inpari 35 were not significantly different in germination percentage, although soaking the seed only a short time at 1 min. Thus, for living seed, having water even in salty conditions, seed can germinate, but surviving in salty conditions is important. Nevertheless, short period of seedling germination and homogenous emergence of seedling can effectively help young crops compete with weed in the Field (Farooq et al., 2019). The only non-significant difference was observed in germination percentage of all rice varieties namely variety CNT 1 mean value 89.9 %, PT 1 (96 %) and Inpari 35 (97.9 %) on germination. This parameter is likely less affected by salinity stress. While, the effects of salinity can be found even in young seedling(Jalil et al., 2018). This experiment could demonstrate a way to support rice growth in saline soil, especially when sowing is the main planting technique in the field. Generally, sowing introduces germinating seeds into large rice field, hence the growth of seedling experienced saline condition can be difficult to manage afterward. Soaking rice seeds with IAA solution can solve such problem since the seedling are pre-treated before sowing, ensuring the maximum growth in stress condition. For other traits relate to seedling growth; both above- and underground parts showed effect by salinity level increasing. However, CNT 1 and Inpari 35 seem more tolerance ability from salinity than PT 1 because most of the traits were decreased at 100 mM NaCl. While NaCl at 50 mM was recorded in PT 1 showed decreasing values in most of the traits.

4.2 Experiment 2 :Effect of seed soaking before sowing on rice germination under salt condition

I. Analysis for comparing the effect of salinity levels

The objective experiment two was the soaking the seed for 24 hours before sowing seeds because nowaday rice sowing is used by manual labor and the use of machinery to save labor instread of transplanting. Soaking the seeds with normal water first may help prepare them for germination instead of having to face germination under saline conditons in field.

4.2.1 Effect of salinity level in CNT 1 (Chai Nat 1)

The result of the CNI 1 statistical analysis showed that the salinity had no significant effect on the percent of germination at 7 days after planting (Table 4). On the other hand, the average percentage of germination is (91.4%). Susceptibility of rice to alinity stress varies depended on many variables such as varieties of rice, charaterisitcs and the stage of plant development (Zeng et al., 2001). Significant difference was observed on shoot length, root length, hairy root, root number and vigor index in CNT 1 variety. Although between 5 caracteristic has diferent results affected by different levels of salinity. There was a significant decrease in value at 100 mM NaCl, except shoot length was found at 150 mM NaCl (Table 5). Compared these results in CNT 1 with testing of not soaking the seeds with normal water before sowing. At about one week after sowing, the seedling of soaking the seed with normal water for 24 hours (at 100 mM NaCl) showed effected of salinity stress was observed at a higher level than not soaking seed at (150 mM NaCl) (Table 2 and 5). However, not much change in the effect of salinity level on two root traits (Table 2 and 5). The higher values of seedling vigor index were observed in all treatments of salinity levels compared with not soaking the seed. Therefore, seed soaking even did not increase germination but increased shoot growth, meaning increased photosynthesitc capacity.

About one week after sowing the seeds, it was found that pre-soaking the seeds by normal water results in an increase in seedling characteristics including shoot length, root length, and seedling vigor index; compared to not seed soaking overnight (Table 2 and Table 5). However, experiment 1 and 2, the change in salinity level

effected decreased values in the root characteristics was not greatly difference (effected by seed soaking). Meaning, the soaking may only clearly promote growth of seedling for a short time. In other words, finding other methods to promote seedling growth or mitigate salinity damage in the field is important to find futher. At low concentrations, salt suppresses plant growth and at higher concentration can cause death (Peel et al., 2004). Some toxic effects of salt stress include decreased germination and seedling growth (Jamil et al., 2012b). Growth suppression is generally related to the total concentration of soluble salts or osmotic potential of the root media (Maas, 1993). Hence, hairy root cultures can reach very high densities and can produce significant levels of secondary metabolites of plant (Souret et al., 2003). However, in CNT 1, all three characteristics including root length, hairy root (density), and root number showed decreasing value; compared with control treatment, start at 100 mM NaCl.

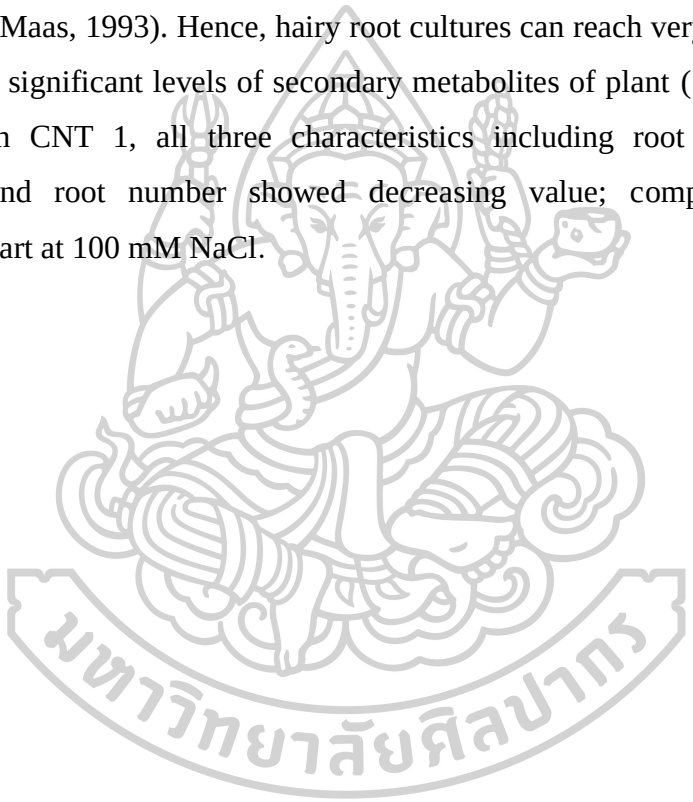

Table 5 The effect of salinity levels on some characteristics of rice seedlings in variety 'Chai Nat 1' (CNT 1): Germination (%), shoot length (cm), root length (cm), hairy root (density), root number and seedling vigor index at 7 days after sowing

Salinity	Germination (%)	Shoot Length(cm)	Root Length (cm)	Hair Root (density)	Root number	Seedlings vigor index
0 mM	92.8 ± 6.8	4.53 ± 0.72 a	7.20 ± 0.54 b	3.40 ± 0.34 a	3.65 ± 0.47 a	1,095 ± 170 ab
50 mM	91.8 ± 5.5	4.28 ± 0.43 a	9.85 ± 1.53 a	2.90 ± 0.33 a	3.78 ± 0.52 a	1,300 ± 240 a
100 mM	91.5 ± 3.1	3.88 ± 0.75 a	7.15 ± 0.87 b	2.15 ± 0.42 b	2.65 ± 0.34 b	1,008 ± 149 b
150 mM	89.8 ± 7.5	2.43 ± 0.28 b	4.18 ± 0.10 c	2.30 ± 0.20 b	2.63 ± 0.56 b	594 ± 45 c
Mean	91.4	3.78	7.09	2.69	3.18	999.4
P-value, F-test	0.911 ns	0.001 **	1.77×10^{-5} **	0.0006 **	0.006 **	0.0005**
CV	6.51	15.38	12.99	12.30	15.04	16.65

Note: CV = Coefficient of variation, *, = ** significant difference at 0.05 and 0.01 levels of probability, ns = non-significant difference at 0.05 level of probability, different lower-case letters (a, b, c) = significance at 0.05 level of probability.

4.2.2 Effect of salinity level in PT 1 (Pathum Thani 1)

The results of the analysis showed that the salinity stress was not significantly affected in the percentage of PT 1 seed germination. There was no dissimilarity in percent germination between soaking for 24 hours (Table 6) and not soaking the seed before sowing. For other traits, after 7 days of sowing, showed that increasing the salinity level will reduce the values in all traits.

However, the benefits result from soaking the seed was a higher level of salinity at starting in reducing of value was found in three traits include shoot length, root length and root number (Table 3 and 6). In Table 6, although decreased value in seedling vigor index; in treatment of seed soaked with normal water, was found at 100 mM NaCl. While this event was found at 150 mM NaCl when seeds were not soaked water overnight before plant. However, the higher values of seedling vigor index were calculated in all treatments of salinity levels compared with not soaking the seed. Moreover, about one week after sowing the seeds, it was found that pre-soaking the seeds by normal water results in an increase in seedling characteristics including shoot length, root length, root number and seedling vigor index; compared to not seed soaking overnight (Table 3 and Table 6).

The result of the transpiration flux needed to maintain plant water status and transpiration is the result of salt translocation to the roots and to the shoots so that it can cause toxic levels of ion accumulation in the shoots. (Luyckx et al., 2021). Furthermore, excess soluble salts in the root zone reduce the plant available water (Munns, 2005). In particular, changes in root system were found to be inconspicuous for variations at various salinity levels relative to shoot length (Table 3 and 6). This may be because roots are the first part to be affected by salinity as well as being an adaptive system to protect the plant. There are several changes in the root part when affected by saline soil or drought, such as changes in root length, etc (Sánchez-Blanco et al., 2019). However, the growing of stressed plants is often restricted by the capability of roots to take out water from the soil and transport it to the shoot (Franco et al., 2011). Occasionally cause a decrease in the root to shoot ratio in plants submitted to water stress (Acosta-Motos et al., 2017).

Table 6 The effect of salinity levels on some characteristics of rice seedlings in variety 'Pathum Thani 1' (PT 1): germination (%), shoot length (cm), root length (cm), hairy root (density) and root number at 7 days after sowing.

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hair Root (density)	Root number	Seedlings vigor index
0 mM	98.8 ± 1.26	5.03 ± 0.22 a	7.28 ± 0.90 b	3.75 ± 0.41 a	3.58 ± 0.62 a	1,216 ± 97 b
50 mM	98.0 ± 1.41	5.38 ± 0.49 a	10.48 ± 1.56 a	3.23 ± 0.52 ab	4.38 ± 1.15 a	1,554 ± 211 a
100 mM	98.3 ± 1.71	3.60 ± 0.18 b	6.78 ± 1.21 b	2.88 ± 0.40 bc	3.35 ± 0.26 a	1,017 ± 125 b
150 mM	95.3 ± 2.75	2.25 ± 0.37 c	3.23 ± 0.21 c	2.55 ± 0.24 c	2.18 ± 0.36 b	522 ± 36 c
Mean	97.6	4.06	6.94	3.10	3.37	1077
P-value, F-test	0.085 ns	6.58×10^{-8} **	7.91×10^{-6} **	0.008 **	0.006 **	1.21×10^{-6} **
CV	1.92	8.35	15.72	13.09	20.48	12.35

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, ns =non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

4.2.3 Effect of salinity level in IN 35 (Inpari 35)

The observed 7 days after germination. Inpari 35 showed very little change which compared to the two Thai varieties; CNT 1 and PT 1 inresponse to soaking the seed overnight before sowing; observed from most characteristics (Table 7). Unless it was found that overnight seed soaking with normal water resulted in an increase in level of salinity effected on root number of seedlings (Table 7). Morever, all treatments had approximately twice value of seedling vigor index (Table 7). Compared to not seed soaking overnight (Table 4). Overall, about one week after sowing the seeds, it was found that pre-soaking the seeds by normal water results in an increase in seedling parameters including shoot length, root length, root number and seedling vigor index; compared to not seed soaking overnight (Table 4 and Table 7).

Because salt spoilage depends on many variables such as species, variety, growth stage, environmental factors, and salt properties (Safdar et al., 2019). Thus, it is difficult to solve this problem by individual methods. Furthermore, althought many studies reported that rice varieties are tolerant to salt during germination, germination is delayed by salinity (Khan et al., 1997b). The change in some characteristics that appears is thought to be since of the capability of plants to adapt which is influenced by genetics and the environment. Therefore, assessing the effects of salinity under mitigation methods may be comlex. Plants that experience salinity stress (NaCl) adapt by showing its hindered seedling growth, indicated that injury is due to the excessive sodium (Na^+) and chloride (Cl^-) uptake (Gregoria et al., 1997; Alam et al., 2021). Althouth a possible alternative is the introduction of crop species/cultivars capable of tolerating the higher soil salinities with moderate economic yield (Hu & Schmidhalter, 2004). Using adopted practices that support early growth in preparation for seedlings before facing abiotic stress in field is another important way for growing rice under limited condition.

Table 7 The effect of salinity levels on some characteristics of rice seedlings in variety 'Inpari 35': germination (%), shoot length (cm), root length (cm), hairy root (density), root number and seedling vigor index at 7 days after sowing.

Salinity	Germination (%)	Shoot Length (cm)	Root Length (cm)	Hair Root (density)	Root Number	Seedlings vigor index
0 mM	99.0 ± 0.8	5.88 ± 0.66 a	7.93 ± 0.30 a	3.88 ± 0.15 a	5.33 ± 1.17 a	1,364 ± 92 a
50 mM	98.8 ± 1.5	5.90 ± 0.47 a	8.43 ± 0.88 a	3.45 ± 0.31 b	4.68 ± 0.43 a	1,416 ± 83 a
100 mM	98.0 ± 1.8	4.63 ± 0.41 b	6.80 ± 0.55 b	3.10 ± 0.24 bc	4.18 ± 0.97 a	1,118 ± 89 b
150 mM	97.0 ± 1.4	3.63 ± 0.46 c	4.60 ± 0.41 c	2.75 ± 0.29 c	2.90 ± 0.24 b	796 ± 88 c
Mean	98.2	5.01	6.94	3.29	4.27	1173
P-value, F-test	0.249 ns	8.3×10^{-5} **	3.31×10^{-6} **	0.0003 **	0.007 **	1.33×10^{-6} **
CV	1.46	10.17	8.29	7.78	18.77	7.51

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, ns = non- significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability

Analysis for comparing the effect of soaking and salinity levels

Soaking is better than non-soaking because it is easy method make the seeds uniform and can induce the germinated but some farmers not practice because farmers used the machine. In normal practice of the farmers when rice stand out and lower growing, they put or supplement fertilizer to promote but when the people put the fertilizer still increase problem of high cost and climate change (pollution), it is not sustainability.

More than the present separately between soaking and non-soaking the seed before sowing, these results showed analysis in factorial in CRD to compare between soaking and non-soaking the seeds in three varieties (Table 9-10).

In CNT 1, The results of statistical analysis showed either soaking or salinity level were not significantly affected GP (%), and was not found the interaction between these factors; soaking and levels of salinity (Table 8). SL showed decreasing values when the salinity level was increased, and soaking the seeds with normal water overnight was higher on SL compared to non soaking the seeds. There was non interaction between soaking the seeds and salinity levels in SL.

High salt concentrations cause negative effect on plant growth, such as inhibition of enzymatic activities (Gengmao et al., 2015). SL of soaked rice seeds was higher values in all salinity levels compared to non-soaked seeds in CNT 1. Hence, soaking could prevent significant reduction of seedling's SL in salinity environment . Moreove, the soaking the seed is simply to practice by farmers. The compromised SL can further affect photosynthesis ability (Sharma et al., 2020b), and the consequent water management by farmers.

For root-related characteristics, all factors (salinity level and soaking the seeds) and interaction between them, were significant differences in two characteristics including RL and hairy root (density) (Table 8). Soaked the seeds by the normal water overnight had the benefit to increase both RL and the density of hairy roots. Consideration, overall, the decrease both in RL and hairy root density occurred at 100 mM NaCl in CNT 1. However, for those characteristics; RL and hairy root density had significant differences caused by the interaction between the factors of salinity level and soaking the seeds. For hairy root density, seed soaked with water

before sowing showed a similar value between 100 and 150 mM NaCl, but was a higher reduction in seed was not soaked at 150 mM NaCl. However, for RL traits, changing between increasing of salinity in each soaking practice was not clear, but it was clearly showing the lowest values in both practices at 150 mM NaCl.

A. Effect of non-soaked and soaked in CNT 1 (Chai Nat 1)

In addition of a higher concentration of NaCl, it will inhibit root growth so that the concentration occurs where this occurs depending on the concentration of $Ca^{+}2$"and the growth index used (Cramer, 1986). Generally, root length decreases with increasing NaCl concentration (Akbarimoghaddam et al., 2011). For RN, it was significant difference only by salinity levels which decreasing values were observed since at 100 mM NaCl.

Vigor index (VI) was the characteristic caused by the multiply between the percentage of germination and the summation between SL and RL (Table 8). Thus, the result was consistent with those traits, which seed soaked overnight by the normal water showed higher value of vigor index, and clearly deceased values were detected at 150 mM NaCl with no significant difference caused by the interaction between salinity levels and soaking practice. (Kim, 2012) reported that at high soil salinity levels, it is interfered seed germination and plant growth, so that, the osmotic gradient is weak thereby preventing water uptake, and causing nutritional stress caused by ion toxicity and nutrient imbalance during plant growth. Additional, plants that may suppress growth under saline conditions may be due to decreased water availability or increased sodium chloride toxicity associated with increased salinity (Singla, 2005). Salinity is not only affects the final soil water content, but also the rate at which plant use water (Sheldon, 2017). Moreover, salinity caused decreasing absorption of water in plant, because activities and events normally associated with germination can be either delayed and/or proceed at reduced rates (Cuartero et al., 2006). Lack of salinity and water causes a decrease in plant metabolic activity and ultimately reduces plant growth (Nawaz et al., 2010). So, salinity problem is common in arid and semi-arid regions where rainfall is insufficient to leach salts out of the root zone (Kaya, 2003).

Table 8 The effect of salinity levels on some characteristics of rice seedlings in variety ‘Chai Nat 1’ (CNT 1): germination percentage GP (%), Shoot Length SL (cm), Root Length RL (cm), Hairy Root HR (density), Root Number RN and seedling vigor index VI at one week after sowing.

Salinity (mM Nacl)	GP (%)		Mean (salinity)	SL (cm)		Mean (salinity)	RL (cm)		Mean (salinity)
	Non-Soaked	Soaked		Non-Soaked	Soaked		Non-Soaked	Soaked	
0	91.5±0.7	93.0±1.4	92.2±1.3	1.8 ± 0.1	4.6 ± 0.8	3.2 ± 1.7a	3.9 ± 0.8de	7.3 ± 0.5b	5.6 ± 2.0b
50	92.0±4.2	92.0±2.8	92.0±2.9	2.2 ± 0.1	4.3 ± 0.1	3.2 ± 1.2a	5.6 ± 0.8c	9.9 ± 0.1a	7.7 ± 2.5a
100	89.5±2.1	92.0±1.4	90.8±2.1	1.6 ± 0.4	3.9 ± 0.8	2.7 ± 1.4a	5.0 ± 0.9cd	7.2 ± 0.6b	6.1 ± 1.4b
150	86.5±0.7	90.0±4.2	88.2±3.2	0.8 ± 0.1	2.5 ± 0.4	1.6 ± 1.0b	2.8 ± 0.4e	4.2 ± 0.1cde	3.5 ± 0.8c
Mean (Soaking)	89.9±2.9	91.8±2.4		1.6 ± 0.6b	3.8 ± 1.0a		4.3 ± 1.3b	7.1 ± 2.2a	
Overall mean	90.8			2.67			5.69		
CV (%)	2.85			16.2			10.24		
P-value (F-test)									
Soaking	0.185 (NS)			$7.13x10^{-6**}$			$1.10x10^{-5**}$		
Salinity	0.192 (NS)			0.002**			$5.28x10^{-5**}$		
Soaking x Salinity	0.802 (NS)			0.388(NS)			0.032*		

Note :CV =coefficient of variation. *, = **significant difference at 0.05 and 0.01 levels of probability. NS =non-significant difference at 0.05 level of probability. Different lower-case letters (a, b, c) significance at 0.05 level of probability.

B .Effect of non-soaked and soaked in PT 1 (Pathum Thani 1)

Results analysis indicated significant differences between non-soaking and soaking in different salinity levels for all characteristics including GP, SL, RL, HR, RN and seedling vigor index characteristics (Table 9).

However, for seed soaking, it was found to promote an increase in all characterisitcs, especially SL and VI of seedling, about twofold. The effect of salinity levels found a significant difference in all characteristics, except GP (Table 9). These characteristics showed affected by increasing the salinity level. Which, HR received the affected of salinity since at 50 mM NaCl, and other root characteristics and SL showed negative effect since at 100 mM NaCl. Compared to CNT 1, PT 1 is slightly more scusceptible to salinity.

Three from six characteristics; SL, RL, and VI had significant differences in the interaction between salinity levels and soaking the seeds (Table 8). When considering the interactions between the two factors for this PT 1, there was little difference in the effect of different salinity levels on soaking or without soaking the seeds. However, both soaked and unsoaked seeds showed a clear reduction of values at 150 mM NaCl. In other words, at 150 mM NaCl is level of salinity can cause more serious crop damage than rice can withstand. In addition, soaking had a greater role in all characterisitcs in PTT1 compared non-soaked seeds in each salinity levels. Actually, rice plant is considered as moderately sensitive to saline condition (Lutts et al., 1996a; Gregorio, 1997). So that the main reason for germination failure was the inhibition of seed water uptake due to a high salt concentration. Whereas, others have suggested that germination of rice was affected by salt toxicity (Akbarimoghaddam et al., 2011). So, soaking the seeds before sowing will help mitigate the effects of seed to absorb water due to saline soil problems .Morethan at germination and seedling, salinity affects rice growth in other growth stages until maturity (Nozulaidi et al., 2015). The influence of salinity stress appears as a result of the link between plant physiology and molecular responses (Hu & Schmidhalter, 2005;Isayenkov, 2019). Beside that osmotic inhibition is the result of the salt present in the soil solution which decrease the ability of the plant to take up water and leads to slower growth (Pattanagul & Thitisaksakul, 2008). This means that even the small amount of water

that the seed absorbs can promote germination. But, ultimately the survival of saline soil conditions depends on the strength of the seedling and the longer solution at different stages of the plant growth. Nevertheless, higher salt concentrations were reported decrease the percentage of germinated rice seeds (Laghmouchi et al., 2017). Moreover, salinity slows emergence and if stress is severe enough, plant stand formation can be reduced (Aslam et al., 2017). However, considering all the assessed characteristics, salinity remains a significant problem affecting rice seedlings and likely affecting further growth like other reports (Tripathi et al., 2021). Thereby, high concentrations of salt such as 150 mM NaCl was reported effect in the germination percentage and delay of germination time in rice (Kaveh et al., 2011).

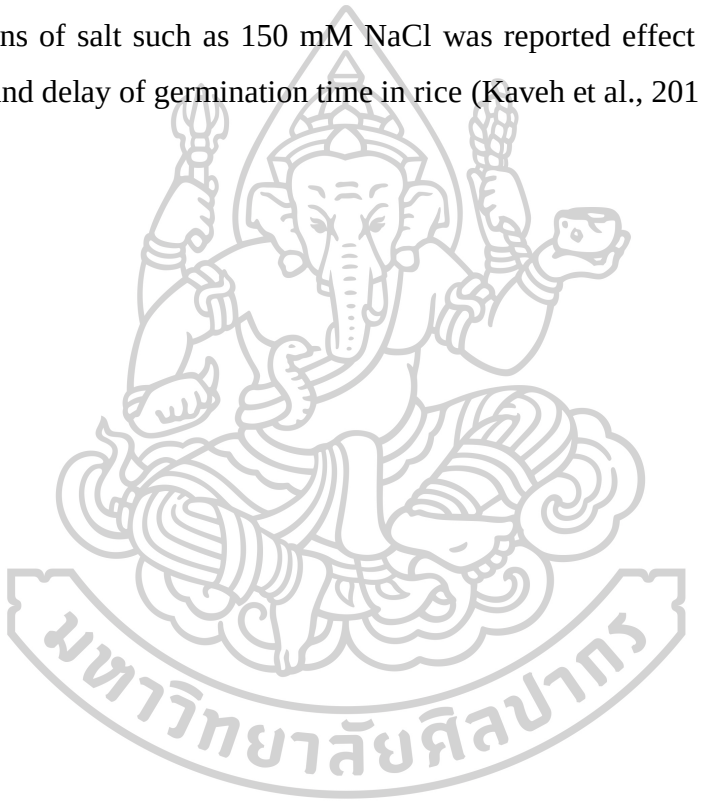

Table 9 The effect of salinity levels on some characteristics of rice seedlings in variety 'Phatum Thani 1 ('PT 1'): germination percentage GP (%), Shoot Length SL (cm), Root Length RL (cm), Hairy Root HR (density), root number (RN) and seedling vigor index (VI) at one week after sowing.

Salinity (mM NaCl)	GP (%)		Mean (Salinity)	SL (cm)		Mean (Salinity)	RL (cm)		Mean (Salinity)
	Non-soaked	Soaked		Non-soaked	Soaked		Non-soaked	Soaked	
0	91.5 ± 2.1	99.0 ± 1.4	95.2 ± 4.6	2.7 ± 0.1c	5.1 ± 0.2a	3.9 ± 1.4a	4.9 ± 0.4c	7.3 ± 0.4b	6.1 ± 1.4b
50	95.5 ± 0.7	98.5 ± 0.7	97.0 ± 1.8	1.9 ± 0.1c	5.4 ± 0.5a	3.6 ± 2.0a	4.0 ± 0.4cd	10.5 ± 0.0a	7.2 ± 3.8a
100	95.5 ± 0.7	98.5 ± 2.1	97.0 ± 2.2	1.7 ± 0.5d	3.6 ± 0.2b	2.6 ± 1.1b	5.0 ± 0.9c	6.8 ± 0.4b	5.9 ± 1.2b
150	93.0 ± 2.8	95.5 ± 0.7	94.2 ± 2.2	0.7 ± 0.01e	2.3 ± 0.4cd	1.5 ± 1.0c	2.6 ± 0.1e	3.3 ± 0.1de	2.9 ± 0.4c
Mean (Soaking)	93.9 ± 2.3b	97.9± 1.8a		1.7 ± 0.8b	4.1 ± 1.4a		4.1 ± 1.1b	7.0 ± 2.8a	
Overall mean	95.87				2.88			5.53	
CV (%)		1.69			10.53			7.68	
P-value (F-test)									
Soaking		0.00114**			3.10×10^{-7}**			1.00×10^{-6}**	
Salinity		0.107 (NS)			1.32×10^{-5}**			3.82×10^{-6}**	
Soaking x Salinity		0.179 (NS)			0.0117*			5.62×10^{-5}**	

Salinity (mM NaCl)	HR (density)		Mean (Salinity)	RN		Mean (Salinity)	VI (%)		Mean (Salinity)
	Non-soaked	Soaked		Non-soaked	Soaked		Non-soaked	Soaked	
0	2.4 ± 0.4	3.8 ± 0.1	3.1 ± 0.8a	3.6 ± 0.6	3.6 ± 0.4	3.6 ± 0.4a	693 ± 63d	1,217 ± 66b	955 ± 307a
50	2.5 ± 0.4	3.3 ± 0.2	2.9 ± 0.5ab	2.4 ± 1.1	4.4 ± 0.8	3.4 ± 1.4a	557 ± 37de	1,554 ± 63a	1,055 ± 577a
100	2.1 ± 0.1	2.9 ± 0.1	2.5 ± 0.5b	2.5 ± 0.3	3.3 ± 0.3	2.9 ± 0.5a	627 ± 145de	1,017 ± 48c	822 ± 242b
150	1.4 ± 0.1	2.6 ± 0.2	2.0 ± 0.7c	1.2 ± 0.1	2.2 ± 0.3	1.7 ± 0.6b	305 ± 14f	522 ± 40e	413 ± 128c
Mean (Soaking)	2.1 ± 0.5b	3.1 ± 0.5a		2.4 ± 1.1b	3.4 ± 0.9a		545 ± 169b	1,077 ± 402a	
Overall mean	2.6			2.89			811		
CV (%)	9.58			19.86			8.58		
P-value (F-test)									
Soaking	$3.65 \times 10^{-5**}$			0.0106*			$3.33 \times 10^{-7**}$		
Salinity	0.00117**			0.0061**			$5.60 \times 10^{-6**}$		
Soaking x Salinity	0.36578(NS)			0.1984(NS)			0.000272**		

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS = non-significant difference at 0.05 level of probability, different lower-case letters (a, b, c) significance at 0.05 level of probability

C .Effect of non-soaked and soaked in IN 35 (Inpari 35)

In Inpari 35, most traits except GP and HR (density) were higher values when soaked before planting such as SL, RL, RN and VI of seedling (Table 10). Priming could arouse a range of metabolic activities and biochemical changes in the seed required for initiating the germination process i.e., breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibition and enzymes activation (Dawood, 2018). Priming seeds is to facilitate the absorption of more water due to the increased elasticity of the cell wall and the development of a stronger and efficient root system (Goodhead, 2019). The results of statistical analysis on several growth characteristics of the Inpari 35 variety are SL, RL, HR, RN, and VI of seedling, showed significant differences between salinity level treatments except for GP (Table 10). For salinity levels showed reducing of value in those traits are different. All of those traits showed the reduciton of values at 100 mM NaCl. Salinity affects plants in distinct ways like osmotic effects, specific-ion toxicity, and/or nutritional disorders (Läuchli & Epstein, 1990). However, decrease in cell division and elongation translate into slower leaf appearance and size. Plants that are severely salt-stressed often spread visual injury due to excessive salt uptake (Riaz et al., 2019).

In addition, the first osmotic effect reduces the plant's ability to absorb water. This has an effect on water stress and shows a small genotypic variation so that at the beginning of the decrease in leaf growth there is a gradual recovery of growth rate until a normal state is reached, depending on the salt concentration outside the roots (Cramer et al., 2007). Thereby, there is a reduction in the supply of photosynthate to the plant, affecting the overall carbon balance necessary to sustain growth (Munns, 2002). The decrease in the early growth of salt-susceptible and salt-resistant plants was caused by the osmotic effect of salt in the medium outside the roots. In contrast, salt-sensitive species or genotypes differ from salt-tolerant species or genotypes due to their disability to avoid salt from accumulating in leaves that are being transformed to toxic levels (Radi, 2013). Consideration, the fact that different characteristics of rice seedlings were susceptible to different levels of salinity reflected the effects of different uptake both water and nutrient in underground plant part (and photosynthesis rates) in above ground plant part. Nethetheless, in finally, ultimately affects of salinity occurs the whole plant. For resilient traits and have the

adaptation for the stress, the effects are more difficult to assess and a reduction in traits at high salinity levels is often observed, such as RL in this study (Ismail et al., 2007). Only observation at the results in this study, it seems that Inpari 35 has similar salinity tolerance with CNT 1. However, another consideration must be given to study at the greenhouse level. In Inpari 35, only RL showed significant difference effected by interaction between soaking the seeds and salinity levels (Table 10). RL was significantly reduced at 150 mM and 100 mM for seeds that were not soaked and soaked the seeds prior to sowing. That is, the advantage of soaking the seeds with water decreases rapidly when the rice takes root under saline soil conditions.

Table 10 The effect of salinity levels on some characteristics of rice seedlings in variety ‘Inpari 35’ (‘IN 35): germination percentage GP %), shoot length (SL cm), root length (RL cm), hairy root (HR density), root number (RN) and seedling vigor index (VI) at one week after sowing.

Salinity (mM NaCl)	**GP (%)**		**Mean (Salinity)**	**SL (cm)**		**Mean (Salinity)**	**RL (cm)**		**Mean (Salinity)**
	Non-soaked	Soaked		Non-soaked	Soaked		Non-soaked	Soaked	
0	93.5 ± 9.2	99.5 ± 0.7	96.5 ± 6.4	3.5 ± 0.0	5.9 ± 0.6	4.7 ± 1.4a	3.9 ± 0.2c	7.9 ± 0.3ab	6.4 ± 1.8a
50	96.5 ± 2.1	99.0 ± 1.4	97.8 ± 2.1	3.1 ± 0.4	5.9 ± 0.4	4.5 ± 1.7a	4.6 ± 0.4c	8.4 ± 0.7a	6.5 ± 2.2a
100	99.0 ± 1.4	98.0 ± 0.0	98.5 ± 1.0	2.3 ± 0.4	4.7 ± 0.5	3.5 ± 1.4b	5.0 ± 1.0c	6.8 ± 0.6b	5.9 ± 1.2a
150	94.5 ± 5.0	97.5 ± 0.7	96.0 ± 3.4	1.3 ± 0.1	3.7 ± 0.5	2.5 ± 1.4c	3.3 ± 0.1d	4.6 ± 0.4c	3.9 ± 0.8b
Mean (Soaking)	95.9 ± 4.6	98.5 ± 1.1		2.5 ± 1.0b	5.0 ± 1.1a		4.4 ± 0.9b	6.9 ± 1.6a	
Overall mean	97.19			3.77			5.67		
CV (%)	3.96			11.04			9.32		
P-value (F-test)									
Soaking	0.210 (NS)			$2.2 \times 10^{-6**}$			$3.33 \times 10^{-5**}$		
Salinity	0.788 (NS)			0.00022**			0.00039**		
Soaking xSalinity	0.659 (NS)			0.857(NS)			0.036 *		

Salinity (mM NaCl)	HR (density)		Mean (Salinity)	RN		Mean (Salinity)	VI (%)		Mean (Salinity)
	Non-soaked	Soaked		Non-soaked	Soaked		Non-soaked	Soaked	
0	2.8 ± 0.0	3.9 ± 0.1	3.4 ± 0.6ab	3.8 ± 0.8	5.4 ± 1.3	4.6 ± 1.3a	780 ± 58	1364 ± 97	1,072 ± 34a
50	3.8 ± 1.0	3.4 ± 0.3	3.6 ± 0.6b	3.5 ± 0.2	4.7 ± 0.2	4.1 ± 0.7ab	740 ± 68	1,416 ± 49	1,078 ± 393a
100	2.2 ± 0.9	3.1 ± 0.3	2.6 ± 0.8bc	2.1 ± 0.1	4.2 ± 0.6	3.1 ± 1.3bc	717 ± 146	1,118 ± 107	918 ± 254b
150	2.1 ± 0.1	2.8 ± 0.4	2.4 ± 0.4c	1.7 ± 0.4	3.0 ± 0.1	2.3 ± 0.8c	422 ± 40	796 ± 76	609 ± 221c
Mean (Soaking)	2.7 ± 0.9	3.3 ± 0.5		2.7 ± 1.0b	4.3 ± 1.1a		665 ± 166b	1173 ± 270a	
Overall mean	3.00			3.506			919		
CV (%)	17.28			17.32			9.4		
P-value (F-test)									
Soaking	0.057			0.000883**			2.49×10^{-6}**		
Salinity	0.035*			0.003494**			0.000181 **		
Soaking x Salinity	0.243(NS)			0.6966(NS)			0.1071(NS)		

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS = non-significant difference at 0.05 level of probability, Different lower-case letters (a.b, c) = significance at 0.05 level of probability

4.3 Experiment 3 :Testing effect of proline concentrations on germination in rice under salt condition.

In germination stage, this study was to know the ability to find out each seed of three varieties through the testing of the sodium chloride concentration (NaCl) of each level of supplementing with proline concentration. The results in this study showed in Table 11-13.

The results of the analysis effect of proline levels on some characteristics showed in Table 11. There were significant differences effected by salinity in all chareacteristics: germination percentage, shoot length, root length, hairy root, root number, and seedling vigor index. The salinity factor clearly affected the decrease of these characterisitcs. For the proline factor, there were three characteristics that showed the significantly different effects by proline as shoot length, root length and seedling vigor index. Proline spraying at low concentration of 50 mM had the best overall effect on those three characteristics. However, there were two characteristics that had the statistically significant difference of interaction between proline application and salinity levels including root number and seedling vigor index. The results were similar for those two traits: in the absence of salinity, no use of proline gave the highest trait values. If in the case of rice-growing under salinity conditions, the use of proline can increase the values both on root number and vigor index. In saline conditions, root number characteristics can be increased at all levels of proline use; available at 50-150 mM with no significant difference. For the vigor index of seedlings when rice plants were exposed to salinity, values were the highest when proline sprayed at 50-100 mM.

Under stress condition, exogenous proline application was reporte to improve tolerance of somatic embryos (Saranga et al., 1992). Plants commonly react to these stresses by accumulation of compatible solutes, such as proline, in cells which results in the improvement of environmental stress tolerance (Chutipaijit et al., 2009a). For this reason, under stress conditions, plant cells have the ability to prevent water loss and to maintain the continuous growth. These solutes can be accumulated in high concentrations without impairing plant metabolisms. Over accumulation of these osmolytes may help plants to tolerate against stress by improving their ability to

maintain osmotic balance within the cell (Hare et al., 1998). Due to, the maintenance of turgor by osmotic adjustment is an importance of physiological adaptations for minimizing the detrimental effects of salt stress (Chen & Jiang, 2010). For this reason, the favorable cause of seed priming with proline on various properties is more pronounced under salinity than under normal conditions (Singh et al., 2018).

Table 11 The effect of proline levels on some characteristics of rice seedlings in variety 'Chai Nat 1('CNT 1'): germination percentage (GP %), shoot length (SL cm), root length (RL cm), hairy root (HR density), root number (RN) and seedling vigor index (VI) at one week after sowing.

Proline (mM)	GP (%)		Mean Proline	SL (cm)		Mean Proline	RL (cm)		Mean Proline
	Salinity (NaCl)			Salinity (NaCl)			Salinity (NaCl)		
	0 (mM)	150 (mM)		0 (mM)	150 (mM)		0 (mM)	150 (mM)	
0	99.3 ± 0.5	97.5 ± 1.7	98.4 ± 1.5	4.1 ± 0.7	3.4 ± 0.2	3.7 ± 0.6 a	4.4 ± 0.9	2.8 ± 0.6	3.6 ± 1.1 ab
50	99.5 ± 0.6	98.5 ± 1.3	99.0 ± 1.1	3.6 ± 0.2	3.5 ± 0.3	3.6 ± 0.3 a	4.3 ± 0.7	3.8 ± 0.4	4.0 ± 0.6 a
100	99.0 ± 0.8	98.5 ± 0.5	98.8 ± 0.7	3.8 ± 0.3	3.6 ± 0.4	3.7 ± 0.4 a	3.3 ± 0.6	2.9 ± 0.4	3.1 ± 0.5 b
150	98.8 ± 0.5	98.0 ± 0.8	98.4 ± 0.7	3.2 ± 0.4	3.2 ± 0.3	3.2 ± 0.3 b	3.8 ± 0.5	2.8 ± 0.6	3.3 ± 0.7 b
Mean Salinity	99.1 ± 0.6 a	98.1 ± 1.1 b		3.7 ± 0.5a	3.4 ± 0.3b		3.9 ± 0.8 a	3.1 ± 0.6 b	
overall mean		98.63			3.54			3.51	
P-value (Salinity)		0.0064**			0.0479*			0.00056**	
P-value (Proline)		0.48674(NS)			0.0193*			0.035522*	
P-value (Salinity:Proline)		0.58997(NS)			0.2652(NS)			0.206197(NS)	
CV (%)		1.0			10.40			17.71	

Proline (mM)	HR (density)		Mean Proline	RN		Mean Proline	VI (%)		Mean Proline
	Salinity (NaCl)			Salinity (NaCl)			Salinity (NaCl)		
	0 (mM)	150 (mM)		0 (mM)	150 (mM)		0 (mM)	150 (mM)	
0	4.5 ± 0.7	3.1 ± 0.6	3.8 ± 1.0	5.5 ± 1.3 a	2.8 ± 0.7 c	4.1 ± 1.8	839 ± 73 a	598 ± 51 de	719 ± 141 ab
50	3.7 ± 0.2	3.4 ± 0.5	3.6 ± 0.4	4.3 ± 0.6 ab	3.2 ± 0.5 bc	3.7 ± 0.8	787 ± 60 ab	721 ± 45 bc	754 ± 60 a
100	3.4 ± 0.6	2.9 ± 0.8	3.2 ± 0.7	3.8 ± 0.7 bc	3.3 ± 1.0 bc	3.6 ± 0.9	707 ± 40 bc	638 ± 53 cde	673 ± 57 bc
150	3.2 ± 0.3	3.1 ± 0.3	3.1 ± 0.3	3.2 ± 0.7 bc	3.3 ± 0.7 bc	3.3 ± 0.6	687 ± 83 cd	583 ± 69 e	635 ± 90 c
Mean Salinity	3.7 ± 0.7 a	3.1 ± 0.5 b		4.2 ± 1.2 a	3.1 ± 0.7 b		755 ± 86 a	635 ± 74 b	
overall mean		3.41			3.67			695	
P-value (Salinity)		0.00627 **			0.00146**			9.22×10^{-6} **	
P-value (Proline)		0.0603 (NS)			0.26508(NS)			0.00369**	
(Salinity:Proline)		0.1210 (NS)			0.01367*			0.02614*	
CV (%)		15.89			22.50			8.74	

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS = non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) =significance at 0.05 level of probability.

The results of the analysis showed that there was no significant difference affected by interaction; combination between the salinity levels and proline concentrations, but salinity and proline had a significant effect separately on characteristics (Table 12).

Like CNT 1, it was found that PT 1 was affected by salinity causing all characteristics with a statistically significant reduction. For proline factor, there were 4 in 6 characteristics significant different affected by this factor including shoot length, root length, hairy root and seedling vigor index. All root-related traits; excluding shoot length, proline used at level 50 mM caused these traits to be the highest values. For shoot length, it had the highest value at the application of proline at 100 mM. No statistical significance was found due to interaction between salinity levels and proline levels, especially those traits in which statistical differences were found affected by individual factors. This means that those traits have a response to proline used and to salinity levels in accordance with the observed means.

From the results of the study in both two Thai rice varieties: CNT 1 and PT 1, it can be said that the high salinity at 150 mM NaCl affects all characteristics of rice in the seedling stag. However, despite the high levels of salinity at 150 mM NaCl in the seedling stage, the use of proline was more effective than no application. The use of a low concentration of proline had a greater effect on the root traits than on the shoots. However, growing in a high saline condition does not mean that high proline level use will benefit rice growth.

That exogenous proline application effectively regulates osmotic potential and plays a vital role in sustaining plant growth under osmotic stress (Deivanai et al., 2011). Exogenous proline also alleviates salt stress by improving antioxidant activities and reducing Na^+ and Cl^- uptake, and translocation while enhancing (K^+ potassium ion) assimilation by plants (Kaya et al., 2007).

The vulnerability of rice seeds to an increase in NaCl concentration drastically affected on many characteistics was reported such as germination (%), root and shoot length (mm), chlorophyll content, and protein content (Deivanai et al., 2011). Caused by the reduction of photosynthesis that affected by all kinds of stress, including salinity (Mohamed & Gomaa, 2012). Salinity interferes with the availability of carbon, hence, causing damage to cellular organelles (Liu et al., 2019b). Exogenous

application including proline has ameliorated the negative effect of salt stress by regulating cellular osmotic balance (Hu et al., 2012). Proline was also reported to contribute to photosynthesis improvement by protecting RuBisCo activity and mitochondrial electron transport chain (El Moukhtari et al., 2020). Likewise, proline catabolism was provided energy to the bacteroids during biological nitrogen fixation (Kim & Nam, 2013).

In this study, the root length variable showed the correlation between the salinity level and the proline concentration. For plant root, it plays an important role in water and nutrient transport from the soil to support plant growth. However, root growth is significantly affected by environmental stimuli (Canarini et al., 2019). The effect of NaCl on changes in proline levels in the roots and root growth of rice seeds. Under salinity stress, although inhibit root growth, increasing proline accumulation in the roots was reported for increasing the tolerant ability to stress (Lin & Kao, 1996). Proline anabolism allows plants to adjust their osmotic homeostasis which helps restore plant water content especially under osmotic pressure (Shafi et al., 2019b). Therefore, proline also plays an important role as a modulator of cell division, especially in the zone of root elongation (Biancucci et al., 2015).

There was significant difference between the proline concentrations effect on many roots related characteristics (Table 12). Although proline is synthesized and accumulates in the leaves, it is transferred to the roots, where it is degraded provides energy and ingredient for sustainable root growth (Trovato et al., 2019). Thus, proline not only acts as an osmotolerant, it also acts as a source of nutrition (Blumwald & Grover, 2006). That, proline function to protect plants from drought and salinity stress (Seki et al., 2002).

Root traits are likely to be important in salinity stress tolerance in environments where soil salinity increases with root depth (Harris et al., 2010). So that, in response to specific salts associated with early-stage salinity stress possibly in root tissues. Due to, at high Na^+ concentrations in cortical cells and cortical cell walls may result in decreased cell turgor and root growth (Shelden et al., 2013). Moreover, in this study, is the seedling stage, ensuring that the primary effect of NaCl would be to the roots (Shelden et al., 2013). In addition, the osmotic effect due to salinity was the main inhibitory factor that reduces seed germination (Vibhuti et al., 2015).

However, in this study, seeds were soaked with the normal water for 18 hours before sowing, and then they have received the salinity. Thus, the germination percentage was not affected by salinity in this study.

Table 12 The effect of proline levels on some characteristics of rice seedlings in variety 'Pathum Thani' (PT) germination percentage (GP) (%), shoot length (SL) (cm), root length (RL) (cm), hairy root (HR) (density), root number (RN) and seedling vigor index (VI) at one week after sowing.

Proline (mM)	**GP (%)**		**Mean Proline**	**SL (cm)**		**Mean Proline**	**RL (cm)**		**Mean Proline**
	Salinity (NaCl)			**Salinity (NaCl)**			**Salinity (NaCl)**		
	0 (mM)	**150 (mM)**		**0 (mM)**	**150 (mM)**		**0 (mM)**	**150 (mM)**	
0	100.0 ± 0.0	98.8 ± 0.5	99.4 ± 0.7	4.8 ± 0.2	3.8 ± 0.1	4.3 ± 0.5 ab	5.9 ± 0.4	5.7 ± 1.1	5.8 ± 0.7 b
50	100.0 ± 0.0	98.5 ± 1.0	99.3 ± 1.0	4.4 ± 0.3	3.7 ± 0.2	4.1 ± 0.4 bc	7.4 ± 0.5	6.6 ± 0.2	7.0 ± 0.5 a
100	100.0 ± 0.0	98.0 ± 0.8	99.0 ± 1.2	4.6 ± 0.3	4.1 ± 0.2	4.3 ± 0.3 a	6.6 ± 0.4	5.3 ± 0.4	5.9 ± 0.8 b
150	99.8 ± 0.5	98.0 ± 1.4	98.9 ± 1.4	4.1 ± 0.1	3.8 ± 0.3	3.9 ± 0.3 c	6.7 ± 0.7	5.0 ± 0.9	5.9 ± 1.1 b
Mean Salinity	99.9 ± 0.3 a	98.3 ± 1.0 b		4.5 ± 0.3 a	3.8 ± 0.3 b		6.6 ± 0.7 a	5.7 ± 0.9 b	
overall mean	99.13			4.15			6.14		
P-value (Salinity)	1.39×10^{-6} **			1.11×10^{-7} **			0.000203 **		
P-value (Proline)	0.506			0.00598 **			0.003669 **		
(Salinity:Proline)	0.754			0.06023 (NS)			0.0991 (NS)		
CV (%)	0.73			5.67			10.40		

Proline (mM)	HR		Mean Proline	RN		Mean Proline	VI (%)		Mean Proline
	Salinity (NaCl)			Salinity (NaCl)			Salinity (NaCl)		
	0 (mM)	150 (mM)		0 (mM)	150 (mM)		0 (mM)	150 (mM)	
0	3.1 ± 0.4	3.1 ± 0.3	3.1 ± 0.3 b	6.8 ± 0.1	5.5 ± 0.2	6.2 ± 0.7	1,064 ± 35	943 ± 107	1,004 ± 98 b
50	4.1 ± 0.3	3.6 ± 0.6	3.9 ± 0.5 a	6.5 ± 0.2	4.9 ± 1.2	5.7 ± 1.2	1,175 ± 84	1,015 ± 10	1,095 ± 102 a
100	3.6 ± 0.5	3.3 ± 0.5	3.4 ± 0.5 b	4.4 ± 2.3	5.1 ± 1.2	4.8 ± 1.8	1,117 ± 60	914 ± 45	1,015 ± 119 b
150	3.9 ± 0.5	2.7 ± 0.4	3.3 ± 0.8 b	6.4 ± 0.2	4.3 ± 1.1	5.3 ± 1.3	1,078 ± 88	858 ± 82	968 ± 142 b
Mean Salinity	3.7 ± 0.5 a	3.2 ± 0.6 b		6.0 ± 1.4 a	5.0 ± 1.0 b		1,109 ± 77 a	932 ± 87 b	
overall mean	3.41			5.49			1,020		
P-value (Salinity)	0.00371 **			0.0133 *			2.78×10^{-7} **		
P-value (Proline)	0.01165 *			0.0980 (NS)			0.0112 *		
(Salinity:Proline)	0.093 (NS)			0.0871 (NS)			0.5085		
CV (%)	13.24			19.88			6.93		

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability NS =non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

In Inpari 35, four in six characteristics showed significant differences affected by increasing salinity include GP, SL, RN, and VI of the seedlings. Higher values in all these characteristics were observed in non-salty conditions (Tablet 13). For the proline effect, only two characteristics had significant differences affected by proline concentrations including GP and SL, while root-related characteristics were not found a significant effect (Tablet 13). Significant difference affected from the interaction between salinity levels and proline levels was observed in three characteristics including GP, SL, and VI (Tablet 13). Thus, both GP and SL were two characteristics that were sensitively affected by the application both of salinity and proline in this study.

The use of proline could promote various characteristics, but the suitable concentration for each characteristic uses it in different concentrations. In the absence of salinity effects, proline is best used at approximately 50 mM, but in the case of salinity during seedling growth, the use of proline must be increased to 100 mM. Soaking with exogenous proline was reported could improve the germination status of rice under salt stress (Hua-long et al., 2014). However, with reduced germination percentage and root length, probably due to the toxicity of sodium chloride ions and negative effects on cell membranes. Root length decreases with an increased salinity level (Farooq et al., 2015). Hence, that the accumulation of proline and/or the upregulation of proline biosynthesis genes in plant can improve seed germination rates under the stresses (Dar et al., 2016a). Protein hydrolysis under salt-stressed plants is associated with increased PRO content (Sitohy et al., 2020). So plant cells accumulate proline as an osmoprotectant to maintain osmotic stability and prevent damage under salt stress, exhibiting high proline accumulation (Al-Saady et al., 2012). However, salt-resistant cultivated rice accumulated less free proline than those that are salt-sensitive (Lutts et al., 1996). In salt-sensitive plant, many plants tend to accumulate proline as a defense mechanism against osmotic challenge by acting as a compatible solute (Momayezi et al., 2009). In addition, amylase is a key enzyme that plays an important role in hydrolyzing seed starch reserves thereby supplying sugar to the growing embryo (Nawaz et al., 2013).

Table 13 The effect of proline levels on some characteristics of rice seedlings in variety ‘Inpari 35’ (IN 35): germination percentage (GP) (%), shoot length (SL) (cm), root length (RL) (cm), hairy root (HR) (density), root number (RN) and seedling vigor index (VI) at one week after sowing.

Proline (mM)	GP (%)			SL (cm)			RL (cm)		
	Salinity (NaCl)		Mean Proline	Salinity (NaCl)		Mean Proline	Salinity (NaCl)		Mean Proline
	0 (mM)	150 (mM)		0 (mM)	150 (mM)		0 (mM)	150 (mM)	
0	99.8 ± 0.5ab	98.8 ± 50 bc	99.3 ± 0.7 a	4.8 ± 0.2 b	4.6 ± 0.5 b	4.7 ± 0.4 b	7.2 ± 0.5	6.9 ± 0.3	7.0 ± 0.4
50	100.0 ± 0.0a	98.50 ± 0.6 c	99.3 ± 0.9 a	5.4 ± 0.5 a	4.7 ± 0.3 b	5.1 ± 0.5 a	7.5 ± 1.2	6.4 ± 0.2	7.0 ± 1.0
100	100.0 ± 0.0a	99.5 ± 0.6 abc	99.8 ± 0.5 a	4.8 ± 0.2 b	4.9 ± 0.1 b	4.8 ± 0.2 ab	7.4 ± 0.1	7.8 ± 0.4	7.6 ± 0.3
150	99.8 ± 0.5ab	94.8 ± 1.5 d	97.3 ± 2.9 b	5.5 ± 0.5 a	3.6 ± 0.1 c	4.5 ± 1.1 b	7.4 ± 0.6	7.0 ± 0.5	7.2 ± 0.6
Mean Salinity	99.9 ± 0.3a	97.9 ± 2.1b		5.1 ± 0.5 a	4.4 ± 0.6 b		7.4 ± 0.7	7.0 ± 0.6	
overall mean	98.88			4.78			7.2		
P-value (Salinity)	$1.45 \times 10^{-8**}$			$8.9 \times 10^{-6**}$			0.0553 (NS)		
P-value (Proline)	$5.62 \times 10^{-7**}$			0.0185^{*}			0.1332 (NS)		
(Salinity:Proline)	$2.26 \times 10^{-6**}$			$1.2 \times 10^{-5**}$			0.1186 (NS)		
CV (%)	0.69			7.13			7.80		

Proline (mM)	HR		Mean Proline	RN		Mean Proline	VI (%)		Mean Proline
	Salinity (NaCl)			Salinity (NaCl)			Salinity (NaCl)		
	0 (mM)	150 (mM)		0 (mM)	150 (mM)		0 (mM)	150 (mM)	
0	3.4 ± 0.1	3.4 ± 0.3	3.4 ± 0.2	6.4 ± 0.4	6.0 ± 0.3	6.2 ± 0.4	1,192 ± 54 abc	1,131 ± 78 bc	1162 ± 70
50	3.4 ± 0.9	3.5 ± 0.2	3.5 ± 0.6	5.8 ± 2.7	6.1 ± 0.5	6.0 ± 1.8	1,294 ± 169 a	1.096 ± 55 cd	1195 ± 157
100	3.6 ± 0.2	3.4 ± 0.0	3.5 ± 0.1	6.7 ± 0.3	6.1 ± 0.4	6.4 ± 0.5	1,217 ± 21 abc	1,258 ± 35 ab	1238 ± 34
150	3.4 ± 0.1	3.1 ± 0.2	3.3 ± 0.2	6.7 ± 0.3	4.4 ± 0.4	5.6 ± 1.3	1,291 ± 96 a	995 ± 54 d	1143 ± 174
Mean Salinity	3.4 ± 0.4	3.4 ± 0.2		6.4 ± 1.3 a	5.6 ± 0.8 b		1,249 ± 102 a	1,120 ± 110 b	
overall mean	3.40			6.0219			1,184		
P-value (Salinity)	0.601 (NS)			0.0453*			0.00018 **		
P-value (Proline)	0.704 (NS)			0.4184 (NS)			0.1359 (NS)		
(Salinity:Proline)	0.635 (NS)			0.1038 (NS)			0.0022 **		
CV (%)	10.78			16.82			6.95		

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS = non significant difference at 0.05 level of probability. Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

4.4 Experiment 4 : Testing effect of proline concentrations on rice seedling under salt condition

To obtain a definitive answer on the effects of salinity on the different rice varieties, a collaborative study; between salinity levels and three rice varieties was conducted under greenhouse conditions, presented in Table 14-16. In terms of the seedling stage analysis only observed the effect of the used of three varieties and 4 levels of salinity on plant height, number of leaves, and symptoms (Table 14-16).

Table 14 The effect of salinity levels on plant height of rice seedlings in variety Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35) at 30 days after planted.

Variable	Varieties	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
Heigh	CNT 1	34.1 ± 1.5	33.0 ± 0.4	30.6 ± 0.9	30.2 ± 0.5	32.0 ± 1.9 c
	PT 1	35.9 ± 1.2	34.0 ± 0.8	34.1 ± 1.2	33.2 ± 1.5	34.3 ± 1.5 b
	Inpari 35	38.0 ± 1.9	35.3 ± 1.3	36.0 ± 0.1	35.8 ± 1.9	36.3 ± 1.7 a
Mean Salinity		36.0 ± 2.2 a	34.1 ± 1.3 b	33.6 ± 2.5 b	33.0 ± 2.7 b	
P- Value (F-test)						
Variety (V) 3.87x10-8**, Salinity (S) 0.0002 **, V x S 0.2732 (NS)						

Note: CV= coefficient of variation, *, = * *significant difference at 0.05 and 0.01 levels of probability N S =non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

The comparison of the average salinity concentration of the three varieties showed that in the case of rice seedlings with an increase in salinity, the plant height decreased significantly but there was no interaction between the two factors in Table 14. The results showed that separately at the salinity level, Increased soil salinity adversely influences plant growth, leading to significant reductions in plant height since at 50 mM NaCl. Water is a major component of photosynthesis and other functions of plants, and its deficiency inhibits more than other environmental aspects and has the main effect of deficiency contributing more to poor stand formation and impaired seed germination (Fageria et al., 2006). In-plant growth depends on photosynthesis, therefore, environmental pressures that affect growth are mainly on photosynthesis (Hoch et al., 2001). For these reasons, increased salinity is a stringent problem and a major limiting factor for crop production around the globe (Rai, 2020). Therefore, high salinity mostly causes anatomical alterations such as reduction of somata number (Nejadhabibvash & Rezaee, 2021). In addition, high salt levels

potentially disturb the roots in nutrient uptake and damage the soil structure (Wu & Zou, 2017). The presence of salinity exerts a detrimental effect on plant growth and plant height through the low osmotic potential of the soil solution and nutrient imbalance (Syvertsen & García-Sánchez, 2014). Salinity generally reduces shoot growth of crops more than root growth, based on dry weight rather than length measurements (Hasanuzzaman et al., 2009). Furthermore, salinity is defined as the presence of an excessive concentration of dissolved salts in the soil which suppresses plant growth (Abbas et al., 2020). Hence high levels of soil salinity can significantly inhibit seed germination and seedling growth, due to the combined effect of high osmotic potential and specific ion toxicity.

Effect of different varieties was found in plant height, which the higher value was recorded in Inpari 35, and followed by PT 1 and CNT 1, respectively. No significant difference affected by between varieties and salinity levels was appeared in plant height (Table 14).

Although the plant height of Inpari 35 appeared to be the highest in the untreated with sodium chloride for planting. The two Thai rice varieties; CNT 1 and PT 1 appeared to be equally valuable when grown under non-salty conditions. Considering only the plant height, it seemed that the most salinity sensitive varieties were CNT 1, PT 1 and Inpari 35 respectively. That was different from the results were observed in laboratory testings for SL that showed that CNT 1 was more salinity tolerant than PT 1.

Salt stress has an adverse effect on plant function and metabolism severely hampers productivity (El Naim et al., 2012). Salinity has an adverse effect on seed germination of many crops by creating anosmotic potential outside the seed inhibiting the absorption of water, or by the toxic effect of Na+ and Cl- (Abbas et al., 2021). However, there are differences in toleration to salinity between species and cultivars as well as between distinct plant growth parameters tolerance (Roy et al., 2019b). The interaction between salinity and environmental factors such as soil, water, and climatic conditions depends on the ability of plants to tolerate salinity (Munir et al., 2021). Therefore, some plant species are more susceptible to salinity when grown under hot and dry conditions than under cold and humid conditions (Safdar et al., 2019). That is why there are differences in observations made between

laboratory studies (between paper testings) and greenhouse conditions in which rice is grown in the soil. This is more simulating rice cultivation in real conditions in farmers' fields.

While ideal rice tolerance range at planting time is ECe value more than 4 dS/m (about 40 mM NaCl) (Sembiring et al., 2020). Inpari 34 and Inpari 35 varieties were more tolerant of salinity stress at the seedling stage with electrical conductivity (EC) of 12dS/m (about 120 mM NaCl) (Subekti et al., 2020b). The capacity to tolerate salinity is a key factor in plant productivity (Sharma et al., 2019).

For leaf number, a significant difference was only found affected by varieties, which the higher value was found in PT 1 and Inpari 35, and lower in CNT 1. However, this finding seems not different from the result at the non-salty condition at 0 mM NaCl (Table 15). No significant difference was observed affected by salinity levels and interaction between salinity levels and varieties (Table 15). In this case, comparing of ranking among these varieties between 0 mM NaCl and overall means could help to assess the effect of salinity on individual rice varieties. Why is it important to know about the tolerance ability to salinity compared among rice varieties? Because the use of salty tolerance rice variety for planting in the salty areas should be the first suggestion to farmers. It is often included in experiments to analyze the genetics of salinity tolerance (Gregorio & Senadhira, 1993).

Table 15 The effect of salinity levels on leaf number of rice seedlings in variety Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35) at 30 days after planted.

Variable	Varieties	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
Leaf number	CNT 1	3.6 ± 0.6	3.6 ± 0.6	3.7 ± 0.7	3.4 ± 0.5	3.6 ± 0.5 b
	PT 1	4.6 ± 0.1	3.8 ± 0.4	4.1 ± 0.1	4.0 ± 0.4	4.1 ± 0.4 a
	Inpari 35	4.7 ± 0.6	4.3 ± 0.1	4.5 ± 0.2	4.3 ± 0.3	4.5 ± 0.3 a
Mean Salinity		4.3 ± 0.6	3.9 ± 0.5	4.1 ± 0.5	3.9 ± 0.5	
P- Value (F-test)						
Variety (V) $7.73x10^{-5}$**, **Salinity (S)** 0.129 (NS), **V x S** 0.833 (NS)						

Note: CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability NS=non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

Morethan leaf number, feaf senescence rate increases and the leaf's physiologically active period is shortened under salinity (Hasanuzzaman et al., 2009). Therefore, If an excessive amount of salt enters the plant, the concentration of salt eventually rises to a toxic level in older transpiring leaves, causing premature senescence and reducing the plant's photosynthetic leaf area to a level that cannot sustain growth (Munns, 2002).

The leaf symptoms in rice plants grown under different salinity levels were evaluated and were shown in Table 16. Leaf symptoms including dried leaf, leaf chlorosis, and leaf necrosis were recorded the significant differences affected by the only effect of varieties factor. There were higher symptoms on leaves were assessed in CNT 1 and lower symptoms in PT 1, and Inpari 35.

Table 16 The effect of salinity levels on leaf Symptoms of rice seedlings in variety Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35) at 30 days after planted.

Variable	Varieties	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
Leaf Symptoms	**CNT 1**	0.0 ± 0.0	0.2 ± 0.2	0.6 ± 0.5	0.7 ± 0.6	0.4 ± 0.5 a
	PT 1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0 b
	Inpari 35	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 b
Mean Salinity		0.0 ±0.0	0.1 ± 0.1	0.2 ± 0.4	0.2 ± 0.5	
P- Value (F-test)						
Variety (V)	0.00108**, **Salinity (S)** 0.16283 (NS), **V x S** 0.09705 (NS)					

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability NS=non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

Inpari 35 and PT 1 was less sensitive to salinity than CNT 1 in terms of the time of appearance: 30 days after planting, and the severity of the symptoms. Chlorosis and necrosis are well known nutrient deficiency symptoms (McCauley et al., 2009). Rice plants under salinity stress will be deficient in certain essential nutrient elements (Gregorio, 1997; Hu & Schmidhalter, 2005). Consistent results among three characteristics: plant height, leaf number, and leaf symptoms, that CNT 1 showed negative affected from salinity more than PT 1 and Inpari 35, although both leaf number and leaf symptoms weren't significant affected by salinity factors.

Salinity applied at the seedling stage frequently induces premature senescence of leaves (Saroj et al., 2018). Moreover, leaf senescence is most often quantified by decreases in protein or chlorophyll concentration (Lutts et al., 1996). Furthermore, the specific effects of salt stress on leaf senescence have been associated with accumulation of toxic ions (Na^+ and Cl^-) or depletion of K^+ and Ca^{2+} (Bansal, 2016). A decrease in Mg^{2+} absorption could also be responsible for decreased chlorophyll content (Farouk, 2011). Anywise, the response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Jamil et al., 2012b).

The combined analysis of the three rice varieties under growing at different salinity levels and the use of proline at different concentrations was investigated. For plant height at 33 days after planting, the results of the analysis showed that there was no interaction between the three factors but separately there were significant differences between the three varieties and also the level of salinity; excluding proline (Table 17). The damage to the plant height was observed at salt levels 50-150 mM NaCl, when compared to the control treatment (0 mM NaCl). As for the influence of varieties, it was found that Inpari 35 had the highest plant height, followed by PT 1 and CNT 1, respectively.

Increased levels of salinity can interfere with the absorption of nutrients by plants .Therefore, the decrease in plant height is real and shows a real effect. Plant height is measured from the soil surface to the tip of the tallest leaf and increasing salinity reduces the plant height of rice (Efisue & Dike, 2020). Soil stress inhibits plant growth and development with side effects such as osmotic stress, Na+ and Cl- toxicity, ethylene production, plasmolysis, nutritional imbalance, and photosynthetic interference (Abbaspoor et al., 2009). Exposure of plants to soil salinity rapidly reduces their growth and transpiration rates (TRs) due to the ‘osmotic component ’of salt stress (Al-Tamimi et al., 2016). Salinity reduces growth and finally causes death through osmotic, ionic, and nutritional imbalances (Nawaz et al., 2010). However, the reduction in plant height was not large compared to salinity (50-150 mM NaCl) and non-salinity (0 mM NaCl). It was found that plant height is one of the characteristics that are highly hereditary or is mainly controlled by genetics (Charlesworth & Willis, 2009).

Table 17 The effect of proline concentrations and salinity levels on plant heigh (PH)(cm) of rice seedlings in variety Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35): at 33 days after planted.

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	32.9 ± 0.8	33.9 ± 2.1	35.5 ± 2.2	36.1 ± 1.7	32.7 ± 2.2 c
	50	33.1 ± 0.6	33.9 ± 1.3	33.3 ± 1.7	33.3 ± 1.1	
	100	30.4 ± 1.6	31.2 ± 2.2	31.2 ± 1.8	32.0 ± 0.6	
	150	31.9 ± 2.6	31.0 ± 1.5	31.8 ±1.7	31.2 ± 2.9	
PT 1	0	36.4 ± 2.0	36.9 ± 1.9	35.9 ± 2.1	36.1 ± 2.4	35.0 ± 2.4 b
	50	32.0 ± 3.7	35.5 ± 1.5	35.3 ± 1.9	33.8 ± 1.5	
	100	36.4 ± 0.6	35.2 ± 1.6	34.8 ± 0.5	34.5 ± 4.3	
	150	33.4 ± 3.6	32.9 ± 0.9	35.9 ± 1.8	34.7 ± 3.6	
IN 35	0	38.7 ± 3.1	38.4 ± 1.8	39.4 ± 2.0	36.4 ± 1.6	36.1 ± 2.5 a
	50	34.6 ± 2.8	36.9 ± 2.2	36.3 ± 2.4	34.8 ± 5.0	
	100	33.5 ± 0.8	37.3 ± 1.5	35.9 ± 1.1	34.9 ± 1.4	
	150	35.0 ± 0.5	34.9 ± 3.6	35.3 ± 1.4	34.8 ± 1.4	
Mean Salinity		36.4 ± 2.5 a	34.4 ± 2.5 b	34.0 ± 2.7 b	33.6 ± 2.6 b	
Mean Proline (mM): at 0 mM = 34.0 ± 2.9, at 50 mM = 34.8 ± 2.8, 100 mM = 35.0 ± 2.6, 150 mM = 34.4 ± 2.7						
P-Value(F-test) **Variety (V)** 4.65×10^{-11} **, Salinity (S)** 9.68×10^{-7} **, Proline (P)** 0.191 (NS), **V x S** 0.247 (NS), **V x P** 0.704 (NS), **S x P** 0.840 (NS), **V:SL:P** 0.801 (NS)						

Note: CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of robability N S =non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

However, the fact that Inpari 35 had the highest mean plant height was not only the original trait but also related to its ability to withstand salinity and its response to proline at various concentrations. This makes it difficult to assess what is the primary influence on the expression of such means? The resistant varieties were those which was better able to limit the accumulation of ions in the shoots, often by the retention of ions in the roots (Colmer et al., 2006). Therefore, the stresses caused by salinity effect to the growth rate in different rice varieties was observed (Flowers & Yeo, 1981). The response of a variety to salinity is predicted as its genetic background to tolerance (Dodig et al., 2015). Hence, the effect of salinity on plant

elongation of different varieties was different, which might be due to the genetic potentiality of the varieties (Puvanitha & Mahendran, 2017).

K^+ in plant tissues evidently decreases when plants are exposed to salt stress, especially rice genotypes (Theerawitaya et al., 2020). That increasing the concentrations of salinity developed a decline in the heights of these plants (Qados, 2011). Salinity tolerance is a complex multigenic trait, both genetically and physiologically (Shabala et al., 2013). And than differences in salt-tolerance responses among rice genotypes at different growth stages (Zeng et al., 2002).

The results of statistical analysis showed that there was no interaction between the three factors in the number of leaves. However, separately there was a significant difference between salinity levels and the three varieties. While, the proline concentration had no effect on leaf number shown in Table 18.

Increasing the level of salinity concentration decreased the number of leaves since 50 mM NaCl. This effect was the same as the change in plant height at low salinity) 50 mM NaCl also affected the leaf number. Moreover, Inpari 35 was the highest number of leaves compared with the two Thai rice varieties. For Thai rice varieties, PT 1 was a higher number of leaves more than CNT 1.

Salinity affects rice growth in all stages starting from germination to maturity (Khan et al., 2016a). These salts will eventually rise to toxic levels, especially in older leaves (Gerona et al., 2019). Salinity causes dicrease total leaves area (Dolatabadian et al., 2011). However, in this study, at seedling stage, early salinity exposure affected leaf formation. At lower salinity (50 mM NaCl) affected leaf establishment. Due to, salinity can differently affect the micronutrient concentrations in plants, however, depending upon crop species and salinity level (Zayed et al., 2011). Therefore, A high salt concentration in soil solution reduces the ability of plants to acquire water, which is referred to as the osmotic or water deficit effect of salinity (Machado & Serralheiro, 2017). Hence, the number of leaves is dicrease as a result of salinity pressure inhibiting the formation of leaf primordia (HanumanthaRao et al., 2016).

The results showed that the three varieties significantly affected the number of leaves (Table 18). This shows that the Inpari 35 variety has better leaf forming than the other two varieties. Although, the reduction of characteristics under the stress is

obtained with susceptible cultivars (Kanawapee et al., 2011). It is difficult to justice higher or lower values of the average mean of leaf number under the response to salinity and proline is the tolerance or susceptibility for salinity. Because, the genetics of those varieties at non-salinity condition is the main factor for the expression of traits as well.

Moreover of reduction of leaf production, plants growing in salt infested areas may be smaller and darker blue-green in color than the normal leaves, that effect of photosynthesis was different based on light quality (Subekti et al., 2020b). In addition, loss of chlorophyll (chlorosis) increases reflectance across the visible and near-infrared spectrum and shifts the red edge (the long-wavelength edge of the chlorophyll absorption) toward shorter wavelengths (termed the “blue shift”) (Ustin et al., 2009). The increased sensitivity is mainly due to a lower ability to utilize absorbed light energy (Hoch et al., 2001). However, when photosynthesis is limited by stomatal closure, which occurs during water stress, CO_2 availability in the chloroplast is reduced, increasing the ratio of O_2/CO_2.

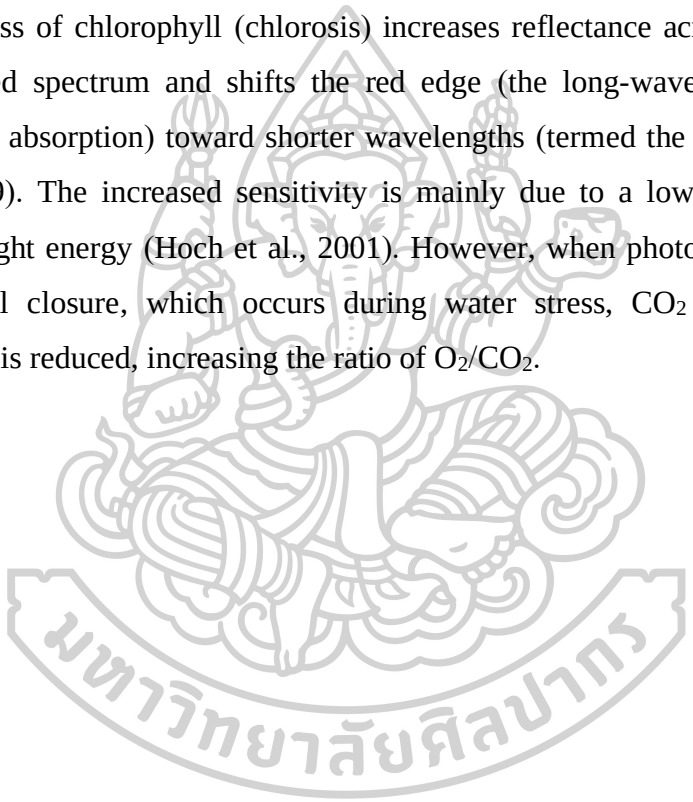

Table 18 The effect of proline concentrations and salinity levels on leaves number of rice seedlings in variety Chai Nat 1 (CNT 1), Pathum Thani 1(PT 1) and Inpari 35 (IN 35): at 33 days after planted.

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	3.8 ± 0.4	3.8 ± 0.6	3.8 ± 0.7	4.1 ± 0.1	3.8 ± 0.5 c
	50	3.9 ± 0.5	3.7 ± 0.5	3.8 ± 0.5	3.7 ± 0.4	
	100	3.5 ± 0.2	4.0 ± 0.6	4.0 ± 1.0	3.8 ± 0.4	
	150	3.9 ± 0.6	3.6 ± 0.3	3.4 ±0.4	3.6 ± 0.3	
PT 1	0	4.6 ± 0.2	4.7 ± 0.1	4.3 ± 0.1	4.7 ± 0.4	4.2 ± 0.4 b
	50	4.0 ± 0.3	4.0 ± 0.2	4.0 ± 0.7	3.8 ± 0.3	
	100	4.1 ± 0.2	4.3 ± 0.2	3.7 ± 0.1	4.5 ± 0.3	
	150	4.2 ± 0.4	3.6 ± 0.2	3.8 ± 0.4	4.2 ± 0.4	
IN 35	0	4.8 ± 0.1	4.6 ± 0.3	4.7 ± 0.01	4.9 ± 0.1	4.5 ± 0.4 a
	50	4.2 ± 0.3	4.6 ± 0.0	4.2 ± 0.4	4.3 ± 0.6	
	100	4.3 ± 0.6	4.5 ± 0.2	4.6 ± 0.3	4.5 ± 0.2	
	150	4.4 ± 0.1	4.4 ± 0.9	4.4 ± 0.3	4.0 ± 0.6	
Mean Salinity		4.4 ± 0.5 a	4.0 ± 0.4 b	4.2 ± 0.5 b	4.0 ± 0.5 b	
Mean Proline (mM): at 0 mM = 4.1 ± 0.4, at 50 mM = 4.1 ± 0.5, 100 mM = 4.1 ± 0.5, 150 mM = 4.2 ± 0.5						
P-Value(F-test)						
Variety (V) 7.27 x 10^{-12} **, **Salinity (S)** 6.57 x 10^{5}**, **Proline (P)** 0.769 (NS), **V x S** 0.531 (NS), **V x P** 0.7626 (NS), **S x P** 0.630 (NS), **V:SL:P** 0.800 (NS)						

Note :CV =coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability. NS=non-significant difference at 0.05 level of probability, Different lower case letters (a, b, c) = significance at 0.05 level of probability.

Electron flow toward oxygen thus increases, particularly through photorespiration (Busch, 2020). Salinity stress is an important characteristic when selecting a variety for salinity tolerance (Saboora et al., 2006). Indentification of genetics that is weak or tolerant to salinity is essential. Salt-sensitive genotypes expressed more nutritional imbalance while the salt tolerant varieties were able to maintain balance among the nutrients in the tissues (Hakim et al., 2014). Leaf cell growth is sensitive to saline solutes even when export and compartmentalization processes are functioning optimally (Subbarao & Johansen, 2001). Being affected by salinity and affecting plant characteristics at an early stage will ultimately affect yields.

Proline applied exogenously at a low concentration (e.g., 30 mM) ameliorated the adverse effects of salinity on early seedling growth in rice. Whereas, at higher concentrations (40–50 mM) proline resulted in toxic effects and poor plant growth (Hayat et al., 2012). However, in this study, leaf number was not affected by proline application in different concentrations. Therefore, understanding of the role of proline accumulation in salt-tolerant rice, under salt stress, is still unclear (Negrão et al., 2011). The foliar symptoms resulting from the effects of salinity assessed are shown in Table 18. The results of the analysis showed that the level of salinity and concentration of proline showed the effect on leaf symptoms (burnt, withered, chlorosis, white tip or leaf curl) in rice at the seedling stage. However, proline concentrations did not give a significantly different effect on the leaf symptoms. CNT 1 showed higher average foliar symptoms than the other two varieties: PT 1 and Inpari 35. The level of salinity at the onset of foliar symptoms caused by salinity was at level 50 mM NaCl, and symptoms increased at level 100-150 mM NaCl. While there is a significant difference between varieties and salinity levels so that there is an interaction between these two factors (variety and salinity level). Only CNT 1 showed increased foliar symptoms at 100 mM NaCl and above, while the remaining two varieties showed no difference at all levels of salinity. Therefore, salt injury symptoms varied with concentration of salt and between cultivars. The relative salt sensitivity of cultivars was not consistent across salt levels (Maas, 2019). Although salt-affected plants were reported are stunted with dark green leaves which, in some cases, are thicker and more succulent than normal (Orak & Ates, 2005). These

observations were used to assess foliar damage in this study. Symptoms of salt stress in rice plants can cause abnormal growth, such as dried leaves at the tip and yellow symptoms in the leaves (chlorosis) (Jones Jr, 1997). Leaf injury and death is probably due to the high salt load in the leaf that exceeds the capacity of salt compartmentation in the vacuoles, causing salt to build up in the cytoplasm to toxic levels (Nawaz et al., 2010). Hence, assumed that the membrane damage caused by salt is negatively correlated with the capacity to increase enzyme activity in plants (Chen et al., 2020). Plants also show the high chlorophyll degradation symptom, chlorosis, as a common morphological and physiological characteristic in response to salt stress (Kanmani et al., 2017). In addition, ionic stress or toxic ionic effect occurs when the concentration of the salts in mature leaves reaches a toxic level. Due to the influx of large amounts of Na^+ into the plant. This resulted in increased Na^+ concentrations in the vacuole and cytoplasm leading to the interruption of metabolic processes. Consequently, the death of the cell occurred (Munns & Tester, 2008).

Rice is highly sensitive to salinity stress at seedling and reproductive stages. The symptoms of salt injury in rice are stunted growth, rolling of leaves, white tips, drying of older leaves, and grain sterility that cover both vegetative and reproductive stages. Rice plant is considered as moderately sensitive to saline condition (Joseph et al., 2010). However, under high salt stress conditions, most of the crop plants are susceptible and unable to survive (Läuchli & Epstein, 1990). For tolerance varieties, the tolerance to salinity is genetically and physiologically complicated and inherited quantitatively (Joseph et al., 2010). The salt-tolerant varieties of rice maintain a low concentration of Na^+ in their leaves than those of salt sensitive lines, when exposed to salt stress (Umego et al., 2020). Rice is considered to be very salt-tolerant during germination, but very sensitive during the early seedling stages and reproduction, and less sensitive during tillering and grain filling stage (Hossain et al., 2015). Salt stress caused both osmotic and ionic stresses on rice plants which result in plant growth reduction and premature leaf senescence (Liu et al., 2019a). Photosynthetic function and chlorophyll content were inversely proportional to salinity level (Yadav et al., 2019).

In this study at the seedling stage, the effect of exogenous proline was not affected or did not support all characteristics under stress salinity. However, proline is

the most general compatible solute that act a pivotal role in the process of osmotic adjustment in several plants (Siddique et al., 2018). Moreover, that exogenous proline had a positive concentration-dependent effect on seed germination under salt stress (El Moukhtari et al., 2020).

In summary, use proline to treat salinity in three varieties showed that the level of salinity concentration had an effect on plant height growth, leaf number, and symptoms at 30 days after planted, but at 33 days after planted applying exogenous proline was not effective on plant height growth, leaf number, and symptoms.

Table 19 The effect of proline concentrations and salinity levels on symptoms (%) of rice seedlings in variety Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35): at 33 days after planted

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	0.0 ± 0.0	0.1 ± 0.2	0.7 ± 0.7	0.7 ± 0.8	0.4 ± 0.6 a
	50	0.0 ± 0.0	0.3 ± 0.3	0.6 ± 0.8	0.9 ± 1.3	
	100	0.0 ± 0.0	0.2 ± 0.3	0.5 ± 0.9	0.5 ± 0.5	
	150	0.0 ± 0.0	0.2 ± 0.2	0.2 ± 0.03	0.7 ± 0.6	
PT 1	0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1 b
	50	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	150	0.0 ± 0.0	0.2 ± 0.3	0.0 ± 0.0	0.1 ± 0.2	
IN 35	0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0 b
	50	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	150	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
Mean Salinity		0.0 ± 0.0 b	0.1 ± 0.2 ab	0.2 ± 0.4 a	0.2 ± 0.5 a	

Mean Proline (mM): at 0 mM = 0.1 ± 0.4, at 50 mM = 0.2 ± 0.5, 100 mM = 0.1 ± 0.3, 150 mM = 0.1 ± 0.3

	Varieties (V)	Salinity (mM NaCl)			
		0	50	100	150
Vx S	CNT 1	0.0 ± 0.0 b	0.2 ± 0.2 b	0.5 ± 0.6 a	0.7 ± 0.7 a
	PT 1	0.0 ± 0.0 b	0.1 ± 0.2 b	0.0 ± 0.0 b	0.0 ± 0.1 b
	Inpari 35	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b

P-Value(F-test)

Variety (V) 5.13 x 10^{-7} **, **Salinity (S)** 0.01539*, **Proline (P)** 0.92429 (NS), **V x S** 0.00315**, **V x P** 0.91256 (NS), **S x P** 0.98174 (NS), **V:SL:P** 0.99995 (NS)

Note: CV = coefficient of variation, *, = **significant difference at 0.05 and 0.01 levels of probability. NS = non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) = significance at 0.05 level of probability.

The relative water content (RWC)

Relative water content (RWC) in rice plants was strongly influenced by salinity and varieties separately (Table 19). In this experiment, the water content in the leaves will decrease if the salinity concentration were added, but at 50 mM NaCl, the water content in the leaf relatives is still high (66.5%) was similar and not significantly different from that in controls (67%). However, this decreases with a significant differencd at 100 mM (63.8 %) and 150 mM NaCl (61.2%) than the control treatment. High concentrations of salt in solution result in increased osmotic stress, which limits water absorption by the plant and in turn, affects leaf water content, stomatal conductance, leaf growth, and photosynthesis (Mitra, 2018). herefore, the higher the salinity concentration reduced the absorption of water by the roots and the higher the transpiration by the leaves so that the water storage within the leaves decreases. Therefore, when the salt concentration increased, Na^+ concentration in the leaves increased and K^+ content decreased (Ahire et al., 2012). High salt levels can influence the balance of other ions within cells, leading to ion deficiencies (Nawaz et al., 2010). Salt stress causes various effects on plant physiology such as increased respiration rate, ion toxicity, changes in plant growth, mineral distribution, and membrane instability resulting from calcium displacement by sodium (Nawaz et al., 2010). That the relative moisture content of the leaves decreased more rapidly in the treated plants with salt than in the control plants (Kapoor & Pande, 2015). Salinity reduces the plant's ability to benefit from water and causes a decrease in plant growth and production by inhibiting plant metabolism (Munns, 2002). The translocation of salts to the roots and to the shoots is the outcome of the transpiration flow required to maintain the water status of the plant and unorganized transpiration can lead to poisoning levels of ion accumulation in the shoots (Farooq et al., 2015).

The result showed significant differences between the three varieties on relative water content, Inapri 35 (71.2 %) had the highest value and was followed by PT 1 (65.7 %) and CNT 1 (57.7 %), respectively. However, at the control condition (0 mM NaCl and 0 mM proline), it was found the values of water content in all three varieties have a sequence of such values were the same (Table 20).

Table 20 Mean of relative water content (%) of three rice varieties [Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35)] grown under different salinity levels and received the proline supplementation in different concentration

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	61.3 ± 6.0	61.8 ± 12.9	50.5 ± 10.8	46.9 ± 10.3	57.7 ± 9.2 c
	50	56.3 ± 4.0	66.6 ± 6.2	60.6 ± 7.7	59.0 ± 6.8	
	100	60.5 ± 4.8	55.8 ± 9.2	62.3 ± 3.4	52.5 ± 10.3	
	150	56.8 ± 10.8	63.5 ± 14.5	60.4 ± 6.4	47.7 ± 7.7	
PT1	0	67.9 ± 9.4	69.1 ± 1.4	62.7 ± 2.2	63.3 ± 3.6	65.7 ± 7.2 b
	50	67.6 ± 8.8	71.5 ± 7.7	66.0 ± 3.3	67.2 ± 4.4	
	100	70.9 ± 6.7	67.6 ± 10.3	59.1 ± 10.1	60.1 ± 12.2	
	150	69.7 ± 6.15	63.9 ± 4.6	66.7 ± 4.2	58.5 ± 10.1	
IN35	0	75.9 ± 3.5	66.6 ± 4.2	72.4 ± 1.3	69.2 ± 7.0	71.2 ± 6.8 a
	50	77.3 ± 4.3	68.0 ± 6.3	70.9 ± 1.6	72.1 ± 6.6	
	100	80.3 ± 4.6	70.4 ± 13.1	70.0 ± 4.3	68.4 ± 10.6	
	150	71.0 ± 6.4	73.2 ± 1.0	64.0 ± 11.9	68.9 ± 3.8	
Mean Salinity		68.0 ± 9.4 a	66.5 ± 8.6 ab	63.8 ± 8.0 bc	61.2 ± 10.8 c	
		Proline (mM)				
		0	**50**	**100**	**150**	
Mean Proline		64.0 ± 10.1	66.9 ± 7.6	64.8 ± 10.6	63.7 ± 9.7	
		P-Value(F-test)				
Variety (V) 6.56×10^{-13}**, Salinity (S)** 0.00141**, **Proline (P)** 0.27044 (NS), **V x S** 0.21056 (NS), **V x P** 0.91125 (NS), **S x P** 0.74225 (NS), **V x S x P** 0.73934 (NS)						

Note: CV = Coefficient of Variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS= non-significant difference at 0.05 level of probability, different lower-case letters (a, b, c) mean significance at 0.05 level of probability

Therefore, although salinity affected such percentage reduction, the influence of the varieties is still pronouncing. Plants respond to salinity by sequestering toxic ions in the vacuoles and accumulating compatible solutes in the cytoplasm to balance water potential decrease (Heidari, 2012). Sensitive varieties are losing vigor quickly by losing water from the stress condition. Opposite, resistant genotypes can tolerate well and survive in severely saline soils (Misratia et al., 2013). Plants have developed complex mechanisms to overcome salt stress such as osmotic adjustment which provides the means to avoid cellular dehydration that is essential for maintaining cellular activity (Boughalleb et al., 2017).

Hence, salt-tolerant plants show thickening of leaves, which can help in maintaining leaf water content and turgor (Boughalleb et al., 2017). As osmotic stress and ion toxicity are the predominant effects of salt stress. Plants have correspondingly adapted to salt stress by decreasing their susceptibility to these effects and continuously grow under salt stress conditions (Khalid et al., 2017). Neither proline nor interaction between proline and other factors (salinity or varieties) was a significant difference effected to relative water content (Table 20). It was seen that there was no relationship between the amount of proline and the percentage of moisture content, at least in the three rice genotypes. Although feasible of proline and other free amino acids was reported their ability with improving salt tolerance (Zegaoui et al., 2017).

The chlorophyll contents

Chlorophyll a is the most commonly used photosynthetic pigment and absorbs blue, red and violet wavelengths in the visible spectrum. It participates mainly in oxygenic photosynthesis in which oxygen is the main by-product of the process. All oxygenic photosynthetic organisms contain this type of chlorophyll and include almost all plants and most bacteria (Kalaji et al., 2017).

The results of the analysis indicated that there was no significant difference in any combination among treatment factors of salinity levels, proline levels, and rice varieties. In addition, proline was not significantly different effect on Chlorophyll A. However, factors individually include salinity levels and rice varieties showed a significant effect on Chlorophyll A (Table 21). Decreased chlorophyll A content was significantly different from non-salinity level (0 mM NaCl) (0.15 mg/g) at 100 mM (0.12 mg/g) and 150 mM (0.10 mg/g) NaCl. Based on result, the decrease in chlorophyll content at high salinity may be related with impaired cellular function and damage to chlorophyll due to accumulation of salt ions, especially sodium (Khoshbakht et al., 2015).

Table 21 Mean of Chlorophyll A content (mg/g) of three rice varieties [Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35)] grown under different salinity levels and received the proline supplementation in different concentration

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CN1	0	0.12 ± 0.07	0.15 ± 0.06	0.10 ± 0.03	0.09 ± 0.02	0.12 ± 0.04 b
	50	0.13 ± 0.05	0.14 ± 0.02	0.09 ± 0.03	0.11 ± 0.03	
	100	0.10 ± 0.04	0.15 ±0.01	0.12 ± 0.00	0.09 ± 0.03	
	150	0.16 ± 0.08	0.12 ± 0.03	0.11 ± 0.01	0.10 ± 0.03	
PT1	0	0.14 ± 0.10	0.16 ± 0.08	0.10 ± 0.02	0.09 ± 0.01	0.13± 0.05ab
	50	0.15 ± 0.06	0.13 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	
	100	0.16 ± 0.11	0.15 ± 0.02	0.14 ± 0.04	0.09 ± 0.01	
	150	0.16 ± 0.05	0.18 ± 0.12	0.11 ± 0.02	0.12 ± 0.03	
IN35	0	0.17 ± 0.11	0.16 ± 0.07	0.11 ±0.00	0.10 ± 0.01	0.15± 0.05 a
	50	0.18 ± 0.07	0.16 ± 0.02	0.13 ± 0.00	0.12 ± 0.02	
	100	0.18 ± 0.09	0.18 ± 0.08	0.13 ± 0.01	0.12 ± 0.03	
	150	0.18 ± 0.08	0.18 ± 0.04	0.15 ± 0.03	0.11 ± 0.01	
Mean Salinity		0.15 ± 0.07 a	0.16 ± 0.05 a	0.12 ± 0.03 b	0.10 ± 0.02 b	
		Proline (mM)				
		0	**50**	**100**	**150**	
Mean Proline		0.12 ± 0.06	0.13 ± 0.03	0.13 ± 0.05	0.14 ± 0.06	
		P-Value(F-test)				
Variety (V) 0.0133*, **Salinity (S)** 2.59 x10^{-5}**, **Proline (P)** 0.6246 (NS), **V x S** 0.9369 (NS), **V x P** 0.9981 (NS), **S x P** 0.9670 (NS), **V x S x P** 0.9946 (NS)						

Note: CV = Coefficient of Variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS= non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) significance at 0.05 level of probability

Abnormal in chlorophyll can causes disruption of photosynthesis process, so that plant growth is not optimal. Because chlorophyll and carotenoids are central to energy acquisition for green plants, and significant changes in their concentrations marked effects on the entire process of plant metabolism (Gong et al., 2018). For physiological processes, such as photosynthesis, was affected by salt stress with reduction of chlorophyll pigment and stomatal closure associated with decreased CO2 pressure and suppression of the Rubisco enzyme (Rady et al., 2019). Chlorophyll A, it is the major chlorophyll species functioning in the photosystems (Tanaka & Tanaka, 2011). The decrease in chlorophyll concentration in saline plants could be associated with an increase in the activity of the chlorophyll-degrading enzyme chlorophyllase (Fariduddin et al., 2013). Furthermore, The accumulation of ions in the leaves also affects the chlorophyll concentration (Jamil et al., 2012b).

Statistical analysis of varieties showed significant differences between rice varieties (Table 21). CNT 1 varieties tended to have lower Chlorophyll A conten (0.12 mg/g), followed by PT 1 (0.13 mg/g) when compared to Inpari 35 varieties which had higher Chlorophyll A content (0.15 mg/g). However, there was no significant difference between the two Thai rice varieties: CNT 1 and PT 1. When considering the Chlorophyll, A content at normal conditions when there was no salinity (0 mM NaCl) and no proline added (0 mM proline), it was found that Inpari 35 had the highest value compared to the two Thai rice varieties. The high content of chlorophyll content is one of the factors that promote the efficiency of photosynthesis. Thus, the decline in productivity observed for many plant species subjected to excess salinity is often associated with the reduction in photosynthesis capacity (Rawat et al., 2012). Thus, Inpari 35 stood out from the rest when considering only the content of Chlorophyll A produced by the plant.

Salinity often causes water deficiency and ion poisoning that impede plant growth by interfere physiological processes, especially photosynthesis (Safdar et al., 2019). An increase in stomatal density and a reduced stomatal area under salinity, indicating an adaptation to salt stress (Naz et al., 2010). Hence, salts are taken up by plants affect to indirectly control their growth by affecting turgor, photosynthesis, or enzyme activities. However, the accumulation of salt in old leaves may hasten leaf death (Jamil et al., 2007).

Plants tolerant to NaCl implement is one in patterns of adaptations to acclimate to salinity, to maintain normal processes including morphological, physiological, biochemical, and molecular (Hernández, 2019). In addition, salt-tolerant genotypes have induced the capability of plant protection against oxidative damage caused by salt stress such as produce antioxidant enzymes in preventing cell damage (Sevengor et al., 2011).

Chlorophyll B primarily absorbs blue light and was used to complement the absorption spectrum of chlorophyll a by extending the range of light wavelengths a photosynthetic organism is able to absorb (Schliep et al., 2013). Both of these types of chlorophyll (Chlorophyll A and B) work in concert to allow maximum absorption of light in the blue to red spectrum; however, not all photosynthetic organisms have the Chlorophyll B pigment (Croce & Van Amerongen, 2014).

The results of statistical analysis showed that there was no significant difference in Chlorophyll B caused by both combinations between any factors and individual factors; excluded salinity levels (Table 22). Chlorophyll B content was significantly reduced as the salinity level was increased, with the salinity level at 100 mM (1.40 mg/g) and 150 mM (1.30 mg/g) NaCl had values decreased compared to the non-salinity level (0 mM NaCl) (1.80 mg/g). Chlorophyll is one of the major components of chloroplasts with role in photosynthesis, and chlorophyll content was positively correlated with the rate of photosynthesis (Bettini et al., 2016). However, chlorophyll plays an important part in the light-harvesting process of photosynthesis and in reducing over energy (Sharma et al., 2020a). Hence, there was strong evidence that salt affects photosynthetic enzymes, chlorophyll, and carotenoids (Hepaksoy,2015).

The reduction in photosynthesis under salinity can explain one reason be attributed to a decrease in chlorophyll content. Salinity reduces the chlorophyll content in salt susceptible plants and increases it in salt-tolerant plants (Asch et al., 2000; Heidari, 2012). Therefore, at high salt concentrations, sodium chloride cause osmotic stress by decreasing water potential within the cells, and ionic stress due to specific inhibition of metabolic processes (Safdar et al., 2019). Reductions in photosynthesis due to salt stress, which has been attributed to decrease in stomatal and mesophyll conductance of CO2 (Khatri & Rathore, 2019). The negative effect of salinity on plant growth and water content may be due to the occurring of defense metabolism in plant cells (Çiçek & Çakirlar, 2002). Morever, chlorophyll content in many types as one of the parameters of salt tolerance in crop plants (Sairam et al., 2005).

Table 22 Mean of Chlorophyll B content (mg/g) of three rice varieties [Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35)] grown under different salinity levels and received the proline supplementation in different concentration

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CNT 1	0	1.37 ± 0.75	1.86 ± 0.89	1.14 ± 0.37	1.21 ± 0.35	1.53 ± 0.62
	50	1.49 ± 0.44	1.76 ± 0.29	1.15 ± 0.40	1.41 ± 0.32	
	100	2.39 ± 1.55	2.14 ± 0.36	1.38 ± 0.10	1.12 ± 0.47	
	150	1.79 ± 0.71	1.67 ± 0.45	1.35 ± 0.14	1.26 ± 0.45	
PT1	0	1.57 ± 0.97	2.26 ± 1.41	1.02 ± 0.39	1.13 ± 0.21	1.66 ± 0.73
	50	1.56 ± 0.36	1.67 ± 0.05	1.75 ± 0.12	1.52 ± 0.05	
	100	1.91 ± 0.94	2.14 ± 0.58	1.78 ± 0.65	1.04 ± 0.23	
	150	1.90 ± 0.45	2.43 ± 1.80	1.39 ± 0.16	1.55 ± 0.46	
IN35	0	1.80 ± 1.03	2.14 ± 1.44	1.26 ± 0.16	1.19 ± 0.23	1.73 ± 0.72
	50	1.99 ± 0.66	1.90 ± 0.44	1.44 ± 0.03	1.36 ± 0.22	
	100	1.90 ± 1.04	2.40 ± 1.48	1.41 ± 0.09	1.47 ± 0.44	
	150	2.04 ± 0.71	2.30 ± 0.93	1.74 ± 0.29	1.31 ± 0.04	
Mean Salinity		1.81 ± 0.76a	2.06 ± 0.87a	1.40 ± 0.34b	1.30 ± 0.31b	
		Proline (mM)				
		0	**50**	**100**	**150**	
Mean Proline		1.50 ± 0.79	1.58 ± 0.36	1.76 ± 0.81	1.73 ± 0.70	

P-Value(F-test)
Variety (V) 0.372 (NS), **Salinity (S)** 1.94 x 10^{-5}*, **Proline (P)** 0.343 (NS), **V x S** 0.987 (NS), **V x P** 0.989 (NS), **S x P** 0.905 (NS), **V x S x P** 0.998 (NS)

Note: CV = Coefficient of Variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS= non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) significance at 0.05 level of probability

The significant difference in total chlorophyll content was observed effect by salinity level only (Table 23). The results of this study were consistent with Chlorophyll A and B contents where salinity levels at 100 mM (1.52 mg/g) and 200 mM (1.40) NaCl resulted in a marked reduction in total chlorophyll content compared to the non-salinity level (0 mM NaCl) (1.96 mg/g). However, no influence was found due to different varieties, which was different from the individual chlorophyll types analysis. Moreover, no influence was found due to the use of proline at different concentrations on total chlorophyll content.

Table 23 Mean of total chlorophyll content (mg/g) of three rice varieties [Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and Inpari 35 (IN 35)] grown under different salinity levels and received the proline supplementation in different concentration

Varieties	Proline (mM)	Salinity (mM NaCl)				Mean Varieties
		0	50	100	150	
CN1	0	1.48 ± 0.82	2.00 ± 0.95	1.24 ± 0.40	1.31 ± 0.37	1.65 ± 0.64
	50	1.63 ± 0.48	1.90 ± 0.30	1.24 ± 0.43	1.51 ± 0.34	
	100	2.49 ± 1.51	2.30 ± 0.37	1.50 ± 0.10	1.21 ± 0.49	
	150	1.95 ± 0.79	1.79 ± 0.48	1.46 ± 0.14	1.35 ± 0.48	
PT1	0	1.71 ± 1.07	2.42 ± 1.49	1.12 ± 0.40	1.22 ± 0.22	1.80 ± 0.78
	50	1.71 ± 0.41	1.80 ± 0.05	1.90 ± 0.13	1.65 ± 0.07	
	100	0.06 ± 1.04	2.28 ± 0.60	1.92 ± 0.69	1.13 ± 0.23	
	150	2.06 ± 0.50	2.60 ± 1.92	1.50 ± 0.18	1.66 ± 0.48	
IN35	0	1.97 ± 1.14	2.30 ± 1.51	1.37 ± 0.17	1.29 ± 0.24	1.88 ± 0.77
	50	2.16 ± 0.72	2.06 ± 0.46	1.57 ± 0.03	1.47 ± 0.24	
	100	2.08 ± 1.13	2.58 ± 1.55	1.54 ± 0.10	1.59 ± 0.47	
	150	2.23 ± 0.79	2.48 ± 0.97	1.89 ± 0.32	1.42 ± 0.04	
Mean Salinity		1.96 ± 0.81a	2.21 ± 0.9 a	1.52 ±0.37 b	1.40 ±0.33b	
		Proline (mM)				
		0	50	100	150	
Mean Proline		1.62 ± 0.84	1.72 ± 0.39	1.89 ± 0.85	1.87 ± 0.75	
		P-Value(F-test)				
Variety (V) 0.313 (NS), **Salinity (S)** 1.69 x 10^{-5}**, **Proline (P)** 0.359 (NS), **V x S** 0.988 (NS), **V x P** 0.992 (NS), **S x P** 0.915 (NS), **V x S x P** 0.998 (NS)						

Note: CV = Coefficient of Variation, *, = **significant difference at 0.05 and 0.01 levels of probability, NS= non-significant difference at 0.05 level of probability, Different lower-case letters (a, b, c) significance at 0.05 level of probability

Salinity reduces the rate of photosynthesis and photosynthetic pigment, which causes a decrease in plant growth and survival (Mbarki et al., 2018). NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: chlorophyllase (Jamil et al., 2007). When a plant is under stress, the changes in the chlorophyll content may be small in the initial stages. However, as the salty stress level increases, the plant chlorophyll content decreases more quickly than the other pigments (Anjum et al., 2011). Salt stress affects plant physiology at the whole plant as well as cellular levels through osmotic and ionic

stress (Nawaz et al., 2010). The accumulation of ion under salinity stress is adversely affected chlorophyll concentration in leaves (Jamil et al., 2012b). Thereby, high accumulation of Na^+ and Cl^- in the leaves also reduces photosynthetic capacity and Na^+ content in the leaves of rice (Zuccarini, 2008). Increasing the accumulation of NaCl in the chloroplasts of leaves in higher plants are affecting growth rates, and is often associated with decreased photosynthetic electron transport activity (Nimir et al., 2016). Hence, chlorophyll content decreased is an indicative response across different plants subjected to salinity stress (Kibria et al., 2017).

Semi-Quantitative RT-PCR

Total RNA from 3 varieties; CNT 1, PT 1 and Inpari 35, 4 salinity levels and 4 proline levels of low rice were extracted and reverse transcribed into cDNA. 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.

To investigate whether any enzyme in the proline synthesis pathway was upregulated after proline application at the seedling stage. At non-salinity stress (0 mM NaCl), the result showed that proline accumulation was lower at 0 mM and 50 mM proline application but increased at 100 mM and 150 mM in CNT 1, the transcript levels of *OsP5Cs1* were investigated by RT -PCR. For Inapri 35, proline synthesis was low at all levels of proline use including the control treatment (0 mM proline). However, 0 mM and 50 mM proline in PT 1 and 50 mM proline in InpRI-35 under normal salinity conditions were observed to lose the OsP5Cs1 band in (Figure 1). Overall, proline accumulation in all rice varieties was low, despite external proline application when growing rice under non-salinity (Figure 1).

Gel Electrophoresis

P5Cs1 gene content in three rice varieties of foliar application of proline under normal condition (0 mM)

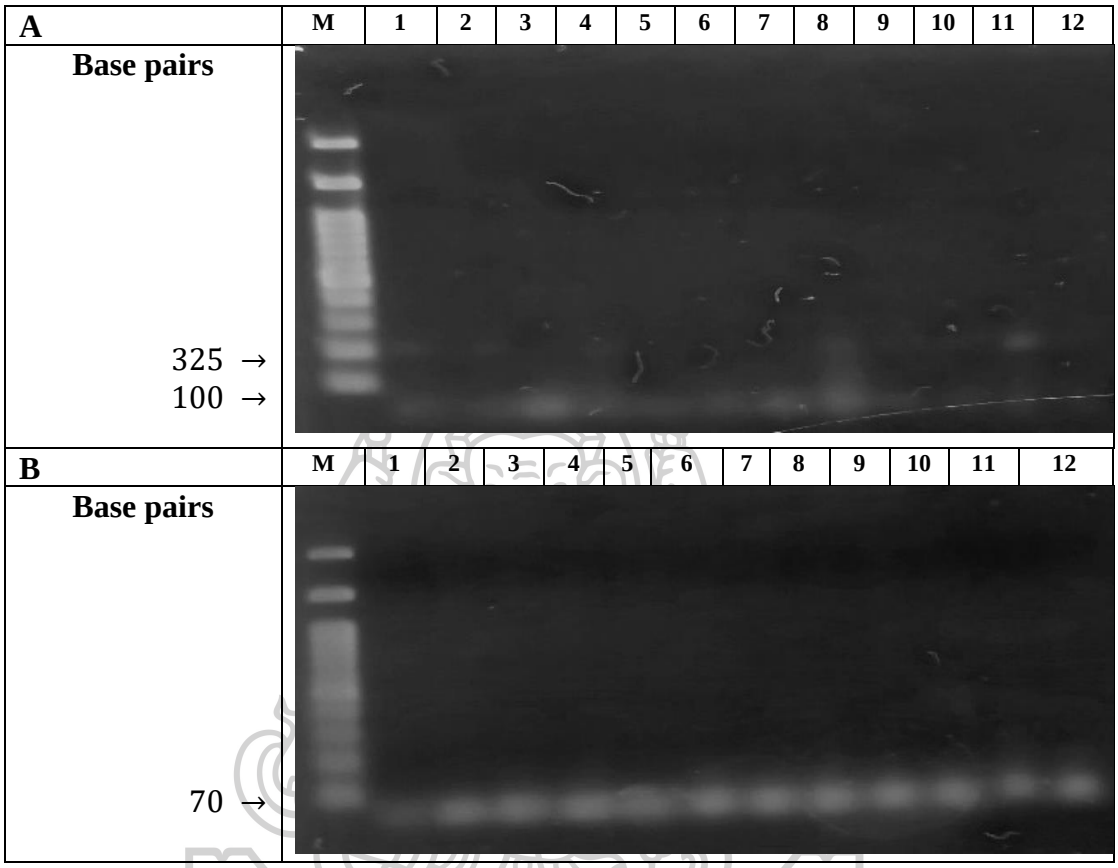

Figure 1 Gel Electrophoresis of *P5Cs1*(A) (Lane M =100 bp DNA marker) the application of proline and (B) Actin gene (Lane M =100 bp DNA marker) under normal condition (non-salinity) [0 mM sodium chloride (NaCl)], Lane number 1-12 = PCR products from 1 = V1P0, 2 =V1P1, 3 =V1P2, 4 =V1P3, 5 =V2P0, 6 =V2P1, 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

The results were similar between 0 mM and 50 mM NaCl at relatively low proline synthesis in leaves (Figure 2). Thai rice varieties (CNT 1 and PT 1) had a higher response to proline application, especially at concentrations 100 mM and 150 mM proline, than that of Inpari 35. However, at 50 mM salinity, 100 mM proline was lost in the PT 1 variety (Figure 2).

P5Cs1 gene content in three rice varieties of foliar application of proline under (50 mM)

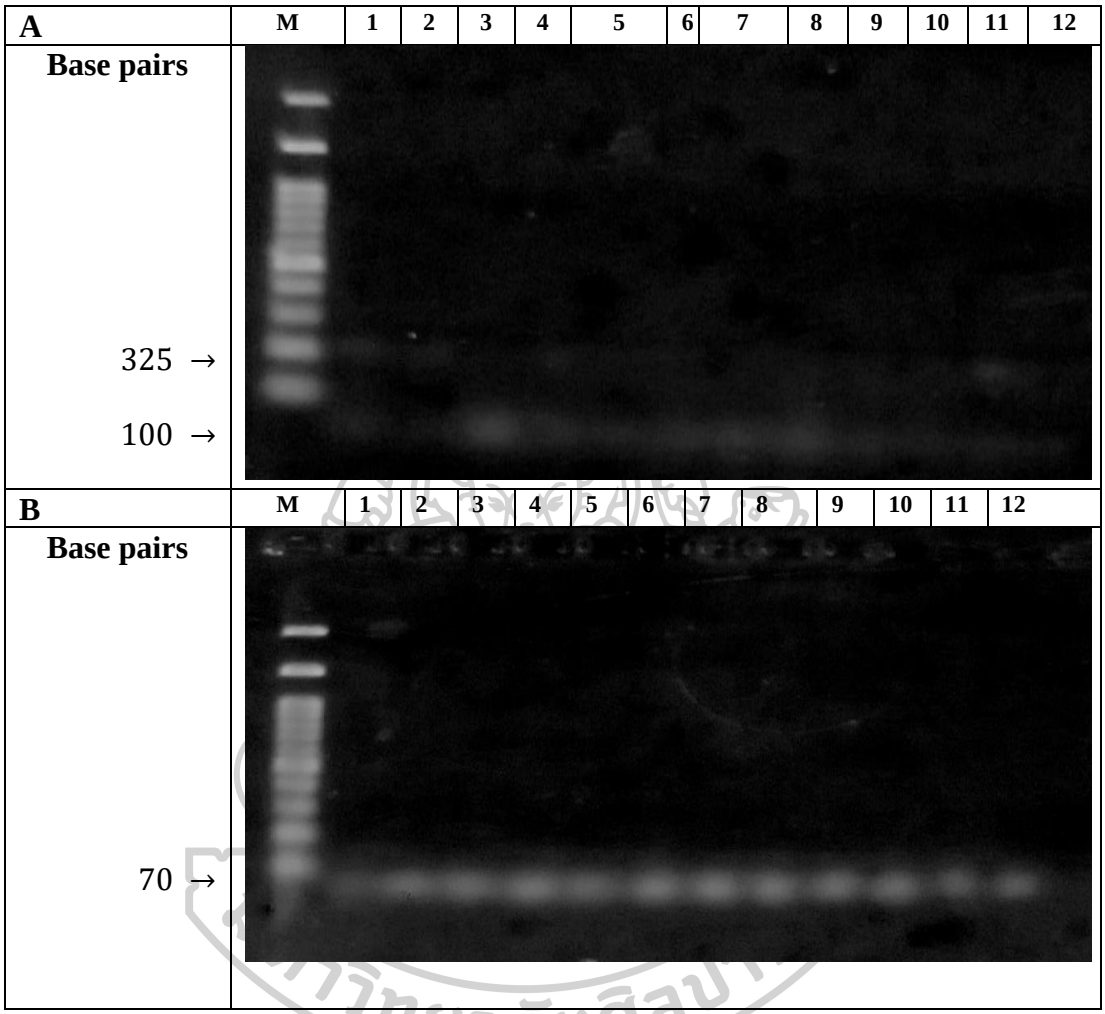

Figure 2 Gel Electrophoresis of *P5Cs1*(A) (Lane M =100 bp DNA marker) the application of proline and (B) Actin gene (Lane M =100 bp DNA marker) under 50 mM sodium chloride (NaCl), Lane number 1-12 =PCR products from (1 = V1P0, 2 = V1P1, 3 =V1P2, 4 =V1P3, 5 =V2P0, 6 =V2P1, 7 =V2P2, 8 =V2P3, 9 =V3P0, 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.

Salt stress was observed to induce the expression of the *OsP5Cs1* transcript and the application of additional exogenous proline further increased the OsP5Cs1 transcript level in the CNT 1, PT 1 and Inpari 35 varieties at the seedling stage under

salinity conditions at 100 mM and 150 mM NaCl (Figure 3-4). Inpari 35 appears to have a low proline accumulation response to proline use at lower salinity levels (0, 50, and 100 mM NaCl) (Figure 1-3). However, it was found that proline accumulation in plants was relatively high compared to Thai rice varieties (CNT 1 and PT 1) when high levels of proline were applied to rice grown under the highest salinity condition at 150 mM NaCl (Figure 4).

P5Cs1 gene content in three rice varieties of foliar application of proline under (100 mM)

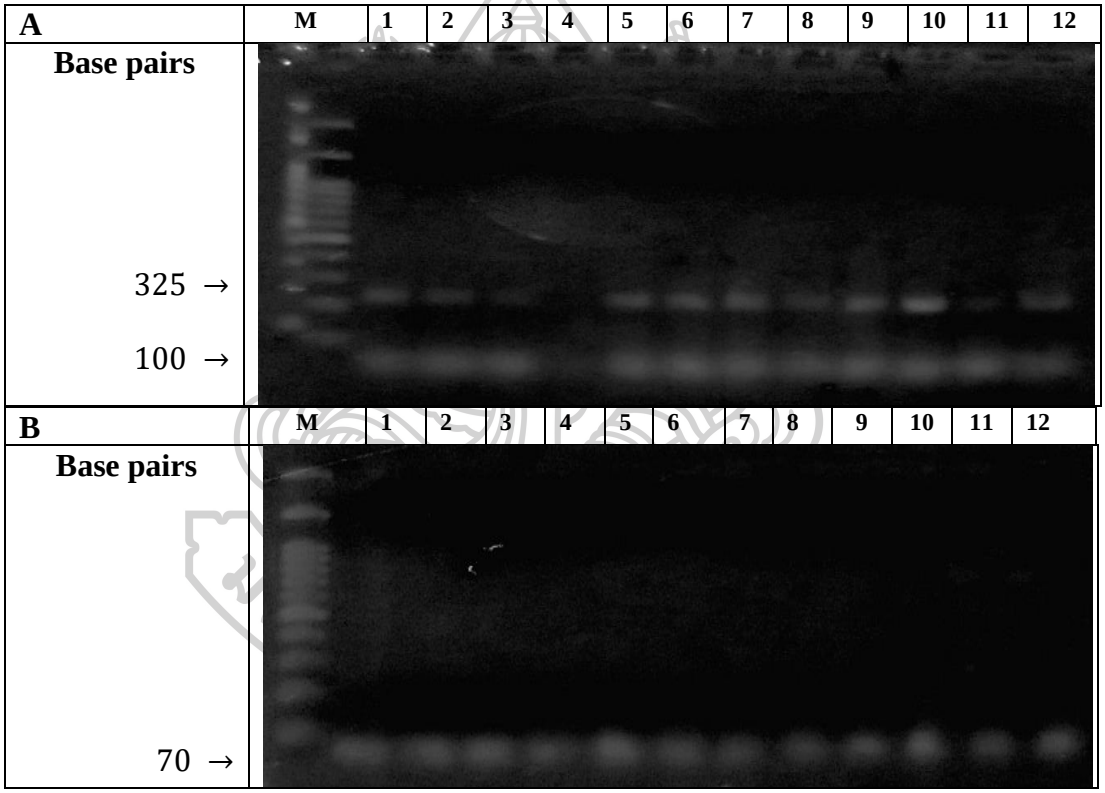

Figure 3 Gel Electrophoresis of *P5Cs1* (A) (Lane M =100 bp DNA marker) the application of proline and (B) Actin gene (Lane M =100 bp DNA marker) under 100 mM sodium chloride (NaCl), Lane number 1-12 =PCR products from (1 = V1P0, 2 = V1P1, 3 =V1P2, 4 =V1P3, 5 =V2P0, 6 =V2P1, 7 =V2P2, 8 =V2P3, 9 =V3P0, 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.

P5Cs1 gene content in three rice varieties of foliar application of proline under (150 mM)

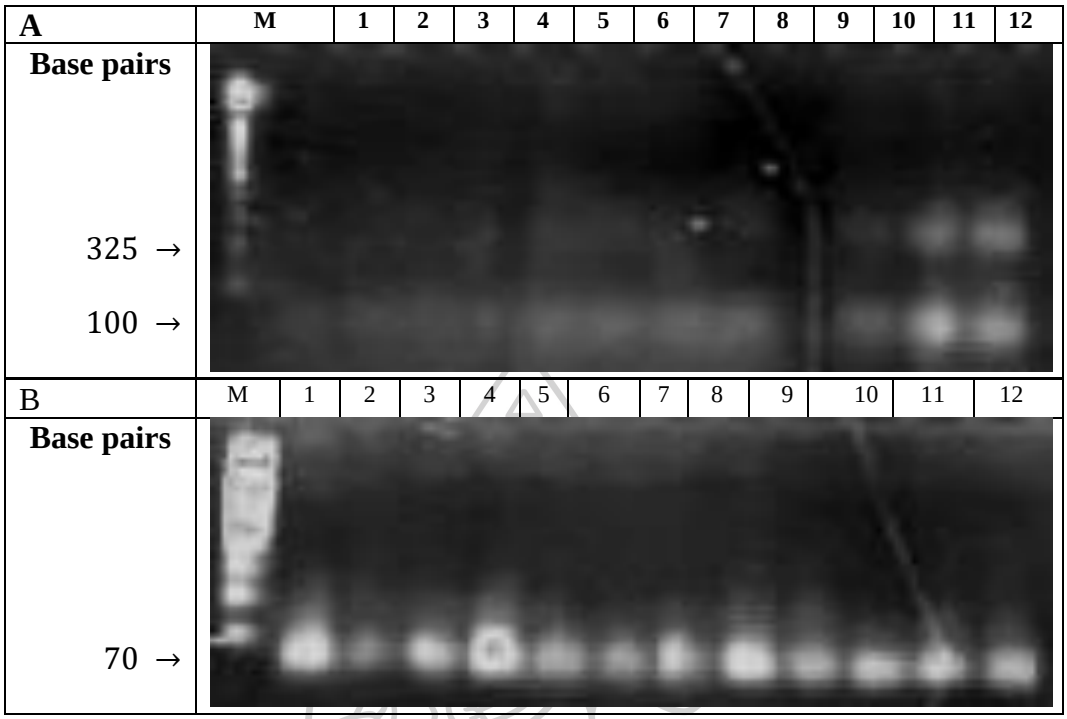

Figure 4 Gel Electrophoresis of *P5Cs1* (A) (Lane M =100 bp DNA marker) the application of proline and (B) Actin gene (Lane M =100 bp DNA marker) under 150 mM sodium chloride (NaCl), Lane number 1-12 =PCR products from (1 = V1P0, 2 = V1P1, 3 =V1P2, 4 =V1P3, 5 =V2P0, 6 =V2P1, 7 =V2P2, 8 =V2P3, 9 =V3P0, 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

After application of leaf proline, the *OsP5Cs1* gene showed at higher salinity conditions (100 and 150 mM NaCl) compared to lower salinity (50 mM NaCl) and normal conditions (0 mM NaCl). However, proline accumulation cannot be considered is a marker of salt tolerance, but its accumulates under various stress conditions such as temperature, drought, and starvation (Misra & Gupta, 2005). Indeed, proline can also act as a signaling/regulatory molecule capable of activating various responses that are components of the adaptation process (Boughalleb et al., 2017). In addition, the high and low levels of proline in plant tissue were used to evaluate the tolerance level of varieties to stress (Chunthaburee et al., 2016).

However, the exogenous response differs greatly among plant species. ie not all species are responsive to exogenous application and the effective dose varies from species to species, and also sometimes overdose can show toxic effects resulting in growth or yield reduction (Yang et al., 2019). On the other hand, the accumulation of *P5CS1* and *P5CR* in chloroplasts during salt stress conditions suggests that under such adverse conditions, glutamate-derived proline biosynthesis is increased in plastids, where photosynthesis takes place (Shafi et al., 2019a). Besides that, Proline accumulated under stress as well stores energy for survival and growth and thus helps plants to permit stressful conditions (Shrivastava & Kumar, 2015). Proline may also serve as an organic nitrogen reservoir ready to be used after stress relief to sustain both amino acid and protein synthesis (Summart et al., 2010). There is increasing evidence that proline enhances antioxidant protection mechanisms and increase stress tolerance in plants (Bhusan et al., 2016). Proline protects membranes, proteins, and enzymes from damaging interference from various stresses (Bhusan et al., 2016). Moreover, prolin juga menjaga dan melindungi terhadap stres garam melalui mempertahankan homeostasis redoks (Hossain & Fujita, 2010). Although, proline is a much-accepted positive role, the poisonous effects of proline at high concentrations can cause problems (Dar et al., 2016b).

When semi-quantitative RT-PCR analysis data were linked to the expression of other traits in greenhouse experiment from this study (Experiment 4), PT 1 seems to be more tolerant to salinity than CNT 1 in those tesing but were not different in *OsP5Cs1* gene expression. Therefore, the salinity response by proline synthesis in plants may be one of the only forming salinity stress tolerance. For Inpari 35, it showed various characteristics of tolerance from germination to the seedling stage (Experiment 1-4). Moreover, it had more proline accumulation in the leaf; the transcript levels of *OsP5Cs1* were investigated by RT-PCR was more pronounced than in Thai rice varieties when rice is grown at high salinity (100 mM and 150 mM NaCl). These results might assess the salinity tolerance in Inpari 35 was higher than two Thai rice varieties; CNT 1 and PT 1.

CHAPTER V

CONCLUSION

This study provided the testing in four experiments; to test the effect of the practice for rice planting at germination stage and seedling stage in rice both normal and salinity conditions.

For testing the effect of salinity concentrations on rice germination by between paper germination in Experiment 1, it was found that salinity affected to decrease all characteristics of seedling; excluded percent of germination and root length. Salinity at levels from 50 mM and 100 mM NaCl had a significant impact to decrease characteristics at the germination and seedling stage compared to the non-salinity level (0 mM NaCl) in PT 1 and two rice varieties; CNT 1 and Inpari 35, respectively. Seems, CNT 1 and Inpari 35 are two varieties that showed better salinity tolerance which could maintain the characteristics compared with PT 1.

Testing on the effect of seed soaking before sowing on rice germination under the salt condition in Experiment 2, it was found that most characteristics (excluded germination percentage) were affected by salinity increasing in all rice varieties. Pre-soaking the seeds by nil water results in an increase in seedling characteristics including shoot length, root length, and seedling vigor index; compared to not seed soaking in all rice varieties. Consideration, most characteristics of seedling were affected since at 100 mM NaCl and above in CNT 1. For PT 1, the hairy root received the affected salinity at 50 mM NaCl, and other root characteristics and SL showed a negative effect since at 100 mM NaCl. Compared to CNT 1, PT 1 is slightly more susceptible to salinity. In Experiment 2, only observation at the results in this study, it seems that Inpari 35 has similar salinity tolerance with CNT 1.

In Experiment 3, the use of different proline concentrations could promote various characteristics under salinity conditions (150 mM NaCl). In the absence of salinity effects (0 mM NaCl), proline is best used at approximately 50 mM, but in the case of salinity during seedling growth, the use of proline must be increased to 100

mM. A low concentration of proline had a greater effect on the root traits than on shoots.

In greenhouse testing the effect of proline concentration on rice seedling grown under salt level (150 mM NaCl), soil salinity since at 50 mM NaCl showed adversely influences plant growth, leading to significant reductions in plant height. Considering characteristics; plant height, leaf number, and leaf symptoms, it seems that the most salinity-sensitive varieties were CNT 1, PT 1, and Inpari 35 respectively. That was different from the results were observed in laboratory testings at the germination stage that CNT 1 was more salinity tolerant than PT 1. Using proline by spraying did not significantly affect the characteristics of rice at the seedling stage.

The salinity affected the relative water content at 100 mM and 150 mM NaCl by decreasing values compared with the control treatment; in all rice varieties. However, the overall means of water content were different between different rice varieties, which Inapri 35 had the highest value and was followed by PT 1 and CNT 1, respectively. Similarly, significant decreased Chlorophyll A, B, and total chlorophyll contents were observed compared with non-salinity level (0 mM NaCl) since at 100 mM NaCl. Moreover, only Chlorophyll A was found the difference in varieties which the highest was indicated in Inpari 35, and followed by PT 1 and CNT 1, respectively. However, neither proline nor interaction between proline and other factors (salinity or varieties) was a significant effect on relative water content and chlorophyll contents (Chlorophyll A, B, and total chlorophyll contents).

For semi-quantitive RT-PCR of *OsP5Cs1* gene expression, proline accumulation was higher at high salinity levels at 100 mM and 150 mM NaCl. Moreover, at those salinity levels, it was found that proline accumulation by the expression of *OsP5Cs1* gene was clearly at 100 mM and 150 mM proline for application.

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