MEASUREMENT OF SOME CHEMICALS IN RICE AND EFFECT OF EXOGENOUS SUBSTANCES UNDER THE SALINITY STRESS AT FLOWERING STAGE

By
Mr. Lu Zaw MYO

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

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โดย
Mr.Lu Zaw Myo

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรวิทยาศาสตรมหาบัณฑิต
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Title	Measurement of Some Chemicals in Rice and Effect of Exogenous Substances Under the Salinity Stress at Flowering Stage
By	Lu Zaw MYO
Field of Study	(BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
Advisor	Associate Professor PANTIPA NA CHIANGMAI , Ph.D.

Graduate School Silpakorn University in Partial Fulfillment of the Requirements for the Master of Science

.....Dean of graduate school
(Associate Professor Jurairat Nunthanid, Ph.D.)

Approved by

.....Chair person
(Associate Professor Sorapong Benchasri , Ph.D.)

.....Advisor
(Associate Professor PANTIPA NA CHIANGMAI , Ph.D.)

.....Co advisor
(Assistant Professor CHAOWANEE LAOSUTTHIPONG , Ph.D.)

.....Co advisor
(Assistant Professor Panida Duangkaew , Ph.D.)

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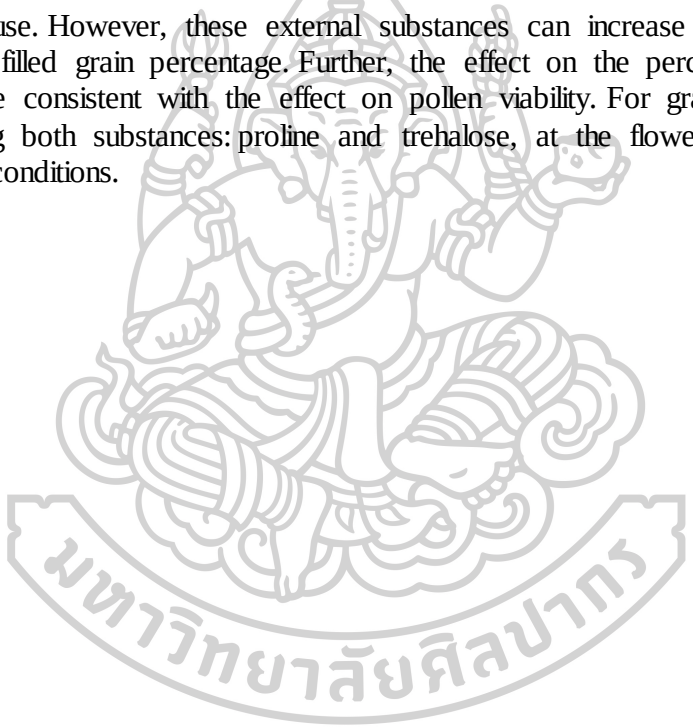
MR. LU ZAW MYO : MEASUREMENT OF SOME CHEMICALS IN RICE AND EFFECT OF EXOGENOUS SUBSTANCES UNDER THE SALINITY STRESS AT FLOWERING STAGE THESIS ADVISOR : ASSOCIATE PROFESSOR PANTIPA NA CHIANGMAI, Ph.D.

Salinity is one of the most challenging problems that adversely affects growth and development of rice. The objectives of this study are to investigate salinity stress and the effects of foliar application of proline and trehalose at flowering stage on morphological, biochemical features and proline synthesis gene in rice. The two experiments were conducted separately using different types of exogenous substances: proline and trehalose, from January to May 2020. The experiment design used 3x4x4 factorial in Completely Randomized Design (CRD) with three replications. Three types of rice varieties (factor A) including Chai Nat 1 (CNT 1), Pathum Thani 1 (PT 1) and a salt-tolerance variety from Indonesia, Inpari 35 (IN 35) were planted in 4 salinity levels (factor B) including 0, 5, 10 and 15 dS/m with 4 different levels of proline or trehalose application (factor C) including 0, 50, 100 and 150 mM. In biochemical measurement revealed that after the proline and trehalose spray, the accumulation of proline in leaf and stem, starch content both under no salinity and salinity conditions mainly depended on genetics. As for accumulation of these chemical compositions: the sugar content, proline content or starch, the increase or decrease in the leaf or the stem depends on many factors including the type of substance used (relation of the sprayed substance and the characteristics what to be measured), the change in the amount of those substances after salinity exposure (that reflects the salinity tolerance of various varieties). However, under salinity conditions (5-15 dS/m salinity), the use of external substances such as proline or trehalose in all concentrations can promote by increasing many characteristics; excluded the water content. In reproductive stage may be one stage that is tolerant of leaf dehydration when plants growing in saline soil. Nevertheless, under no salinity condition (0 dS/m), the effect of exogenous proline or trehalose was found mostly in agronomic characteristics, yield components, and yield, but rarely affected in chemical contents (proline, sugar, starch) that accumulated in plants. Assessed by the synthesis of complementary deoxyribonucleotide (cDNA), at 10 dS/m salinity, CNT 1 and PT 1 are partially able to synthesize proline automatically in leaves, although was no received external proline sprays. Nevertheless, when proline at 50-100 mM is sprayed externally, there has been an increase in the stimulation of proline synthesis in the plant. However, may have the limit of the quantity of exogenous proline be used to stimulate the synthesis of this substance inside plants. The external proline that plant was received by spraying did not increase the accumulation in the plant at 150 mM proline. For IN 35, did not respond to increase leaf proline synthesis, although has been stimulating by proline spraying. The highest salinity level (15 dS/m), exogenous proline use does not encourage the increased synthesis of this substance in the leaves in all rice varieties. Therefore, increased deposition of proline in leaves may be obtained directly from spraying.

For the chlorophyll contents, the use of proline was higher in effectiveness to increase the values more than trehalose. The results showed that Thai rice varieties

after proline spraying at high salinity (at 15 dS/m salinity) showed an increase in the accumulation of proline and sugar, although not very high, but more than in IN 35. IN 35 is resistant to salinity and to accumulate the proline in plant cells of leaves autonomic. For starch content, effect of trehalose applying showed very little change compared with proline spraying both in leaves and stems in each variety. Considering the damage on characteristics affected by salinity, the varieties have less effect or high tolerance ability to salinity is IN 35, and lower in two Thai varieties. Among Thai rice varieties, CNT 1 was affected by salinity in lower magnitude in most of the characteristics and was more sensitive by applying both proline and trehalose more than PT 1. For these reasons, CNT 1 seems higher tolerance ability to salinity more than PT 1. Since at 5 dS/m salinity, plant height and yield components were received the negative effects. Proline and trehalose showed no effect or little effect (with no significant difference) in plant height, the number of fertile tillers, and 1,000-seeds weight because these characteristics were established not consistent with the time for substance use. However, these external substances can increase the value in panicle length and filled grain percentage. Further, the effect on the percent of grain filling is likely to be consistent with the effect on pollen viability. For grain yield, to increase by applying both substances: proline and trehalose, at the flowering stage, especially at salinity conditions.



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Lu Zaw MYO

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Lists of Abbreviations

Abbreviations	Description
ASAT	Animal Sciences and Agricultural Technology
CNT 1	Chai Nat 1
PT 1	Pathun Thani 1
IN 35	Inpari 35
Pro	Proline
Tre	Trehalose
FW	Fresh weight
DW	Dry weight
C°	Degree Celsius
NaCl	Sodium chloride
Na^+	Sodium
K^+	Potassium
mm	Millimeter
Km/h	Kilometer per hour
mM	Milimolar
dS/m	Decisiemens per meter
RWC	Relatively Water content
mg	Milligrams
mg/g	Milligrams per gram
µ mole	Micro gram per gram
DMSO	Dimethyl sulfoxide
H_2SO_4	Sulphuric acid
nm	Nanometer
cDNA	Complementary deoxyribonucleic acid
RNA	Ribonucleic Acid
PCR	Polymerase chain reaction
RT-PCR	Real-time for Polymerase chain reaction
RT	Reverse Transcription
µl	Microliter
Cm	Centimeter
%	Percentage
g	Gram
I_2 - KI	Potassium Iodide
ANOVA	Analysis of Variance
DMRT	Duncan's New Multiple Range Test

CHAPTER 1

INTRODUCTION

1.1 Background and rational

Rice (*Oryza sativa* L.) is a staple food crop for more than 50% of the world's population (Abdelaziz et al., 2018). More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live. Rice accounts for 35-75% of the calories consumed by more than 3 billion Asians. It is planted on about 154 million hectares annually or on about 11% of the world's cultivated land (Khush, 2005). There are estimated 120,000 different varieties of rice in the world (Ahloowalia et al., 2004). Two major rice varieties that have been cultivated worldwide are subspecies indica and japonica (Jain et al., 2004).

In Thailand, indica rice type is mainly cultivated (Kantayos et al., 2016). Rice plays a key role in the country's trade as the staple diet and also closely related to the way of Thai life (Keyes, 2019). The world average rice consumption from 2012 to 2014 was reported to be approximately 57.2 kg per capita per year (OECD/FAO 2015). It was estimated that the amount of annual rice consumption would increase 0.2 to 1.2 times from 2015 to 2024. For Thailand, the average rice consumption from 2012 to 2014 was approximately 2.5 times higher than the world consumption, and it is expected to reach 147.2 kg per capita per year by 2024 (Hensawang & Chanpiwat, 2017).

During the past decade, approximately 85% of the rice exports have emanated from six countries, being Thailand, USA, Vietnam, India, Pakistan and China. Thailand is the largest exporter of rice and although a number of private companies manage the exports of rice from Thailand, the government still exercises some control over rice exports because of the important role that rice plays in the Thai economy (Rakotoarisoa, 2006). Thailand has been the dominant exporter accounting for 32% of total world exports followed by Vietnam with 15% in second place (Ghoshray, 2016). Thai rice be renowned for one of rice export country in the world represent as a good quality and demand of the consumer throughout the world (Calingacion et al., 2014). The signature characteristics of Thai rice are long grain, non-chalky kernels, thin-husks, well milled (Kantayos et al., 2016). Thai rice classification is deal with ecological farming system, photo-responsibility and amylose content (Kantayos et al., 2016). Four types of ecosystems for planting are including as upland rice, wetland rice, irrigated rice and deep-water rice (Bambaradeniya & Amerasinghe, 2004). While, photo-responsibility is divided into two kinds are photosensitive rice and non-photosensitive rice. For this reason, it is necessary to select the varieties for use in planting each season and improves the varieties not to be photoperiod-sensitive in Thai rice. Moreover, rice belongs to two types depending on the amylose content of

the grain. Common rice contains 15-30% amylose and 70-85% amylopectin while glutinous rice contains less than 5% of amylose and consists mostly of amylopectin (Gunaratne, 2016). Examples of rice variety that grow and consumed in Thailand, such as Chai Nat1 (CNT 1) which is a non-glutinous rice, high yielding, high nitrogen response, and photoperiod insensitive. Its grain is long slender with slight chalkiness, good milling quality, and high amylose content. (www.agris.fao.org). Another example is Pathum Thani1 (PT 1) variety. This variety is photoperiod-insensitive and is grown year-around. PT 1 famous in Thailand with unique scent, long grains, high amylose content, tend to cook firm with dry and soft texture (Kantayos et al., 2016). Salinity is a major problem in rice production along coastal areas in Indonesia. Salinity damage to the rice crop frequently happens during dry season because of sea. To overcome salinity problem in rice areas, the Indonesian Centre for Rice Research (ICRR) released in 2014 new salt-tolerant rice varieties named Inpari 35. This variety was introduced by IRRI in 2008. Inpari 35 refers to CSR90-IR-2. These released variety have hard cooked rice texture (Habibi, 2018).

In the tropical wet and dry or savanna climate like Thailand, rice can grow twice a year (Seck et al., 2012). However, water supply is the main factor for cultivation. Less than 20% of rice production area is irrigated area, which it is water supply actually usable for mainly rice cultivation in Thailand twice a year. Over 80% of rice production area in Thailand is cultivated under rain fed conditions in the wet season for once a year (Maclean et al., 2002).

The total areas of salt affected soils in Thailand are 2.302 million hectares, of which the inland saline soils are 1.904 million hectares and the rest are in the coastal areas (Arunin & Pongwichian, 2015). Management of inland salt-affected soil depends upon the degree of salinity and the prevalent local salinization processes. In general, salt-affected soils in the northeast are high in sodium and chloride contents, sandy and low in fertility, with approximately 75 % under rain-fed lowland rice cultivation (Saraphirom et al., 2013). Slightly to moderately salt affected lands are generally used for rice cultivation or other cash crops (Arunin & Pongwichian, 2015). Appropriate agronomic on-farm management was found to increase rice yield which include selected salt tolerant of rice cultivar, transplanting of older seedlings of thirty to thirty-five days, closer spacing of 15 × 20 cm, with an increased number of seedlings (6-8 seedlings/hill) and application of organic amendments such as green manure, farmyard manure or compost to increase soil fertility and improve soil physical properties (Qadir et al., 2008).

Coastal salt-affected soils are found scattered along the coast of the Southern and Eastern regions, in Thailand. These areas are subject to tidal influences and brackish or seawater intrusion. These soils are very young heavy clay or silty clay with little profile development (Gomez et al., 2002). They are very saline and most of them are flooded during spring tides only. Factors limiting plant growth include not only salinity but also potential acidity and degree of ripening of the soil (Arunin &

Pongwichian, 2015; Kumar et al., 2020). Management of coastal salt-affected soils needs to cope with the specific characteristics of the soil, crop and water regimes. Rice cultivation is common in these areas. Salt- tolerant rice varieties were introduced in these salty areas such as Lebmue Naang 111, RD 19, RD 27, KDML 105 and Hom Nai Pran. Beside rice cultivation, economic salt tolerant crops such as tomato, cabbage, sweet potato, corns, cantaloupe and taro were suggested with organic amendments and chemical fertilizer application (Arunin & Pongwichian, 2015).

Nowadays, new technologies of irrigation, cropping management and foliar application of osmoprotectants are testing to increase the crops production, such as reuse of desalinized water, deficit irrigation regulation to maintain crop yield, suitable crop water salinity model and foliar application of osmoprotectants on crops (Semida et al., 2020). Among the Thailand rice varieties, Chai Nat 1 (CNT 1) and Pathum Thani 1 (PT 1) are popular in Phetchaburi area, and this area is also facing the salinity problem. Therefore, this study was to evaluate the foliar application of osmorprotectant compounds in these popular rice varieties compare with tolerant variety from Indonesia, Inpari 35 (IN 35).

1.2 Objective

The overall objective is to examine the response of exogenously applied proline and trehalose at flowering stages on proline content and biochemical contents in plant, physiological traits, yield and yield components and proline biosynthetic genes of rice cultivated under saline condition.

CHAPTER 2

LITERATURE REVIEW

2.1 Salinity problem for crop production

The beginning of 21st century is marked by global scarcity of water resources, environmental pollution and increased salinization of soil and water. Increasing human population and reduction in land available for cultivation are two threats for agricultural sustainability (Shrivastava & Kumar, 2015). Various environmental stresses *viz.* high winds, extreme temperatures, soil salinity, drought and flood have affected the production and cultivation of agricultural crops. Among these problem, soil salinity is one of the most devastating environmental stresses, which causes major reductions in cultivated land area, crop productivity and quality (Shrivastava & Kumar, 2015)

Production under rain-fed lowland areas, a lack of rainfall often results in the absence of water in the field. Thereby, in lowland areas that contain insufficient water will cause salt particles in the soil to rise to the surface, and thereby increasing the intensity of salinity (Altieri & Koohafkan, 2008). This problem is further worsening because of climate change causing sea level rise and more frequent coastal storms incidences leading to salt intrusion in agricultural lands (McCarthy et al., 2001). Besides, poor water management practices, like poor drainage in irrigated areas, cause secondary salinization (Qureshi et al., 2008). Salt-affected areas are estimated at over 800 million hectares worldwide, which is equivalent of more than 6% of the world's total land area (Gerona et al., 2019). Based on the United States Department of Agriculture Salinity Laboratory, saline soils are defined as having an electrical conductivity (EC) of 4 dS/m [(about 40 mmol/L of sodium chloride (NaCl)] or more as a result of excess of sodium ions, with predominant anions of chloride and sulfate (Gerona et al., 2019).

In order to cope with detrimental effects of salt stress and to sustain cellular functions, plants have adopted various biochemical and molecular mechanisms (Mirza Hasanuzzaman et al., 2013). Among them, osmotic adjustment is considered to be most important mechanism, which results in increase level of organic solutes in plant cells called "osmolytes" (Chen & Jiang, 2010). These major compatible solutes; organic osmolytes, include proteins, proline, glycine betaine, proteins, polyamines and carbohydrates (sugars and starch) (Parvaiz & Satyawati, 2008). Under saline condition, these osmolytes protect the normal functioning of cell either by maintaining cellular osmotic potential and scavenging of reactive oxygen species (ROS). Higher level of ROS can seriously pose threat to normal metabolic activities of the plant through per oxidation of lipids, oxidation of proteins, nucleic acids and ultimately leads to cell death (Tabssum et al., 2019).

Saline soils are generated by geo-historical processes or human-made. Salinity is the most serious problem that reduces crop growth and development (Kantayos et al., 2016). From the report of FAO 2010, saline soil have reached a serious effect on planting area in the world with approximately 45 million ha of irrigated area are manipulated by salinity problem (Kendall & Pimentel, 1994). In Southeast Asia confront a rice production as a result of salinity problem such as in the northeastern part of Thailand where is rain fed area that face a problem with soil salinization due to underground rock salt approximately 34.18% of total area. Moreover, most of planted areas use rainwater so that affect the amount of saltiness was elevated at water deficit period (dry spell) and then decrease rice growth and yield (Kantayos et al., 2016).

In order to eliminate the severe challenges of abiotic stresses and to improve the capacity of crop plants to tolerate salt stress, several strategies have been projected (Bhatnagar-Mathur et al., 2008). Under stress condition, foliar application of proline showed improvement of the tolerance capacity of celery seedling (Chinnusamy et al., 2005) and cell culture of tobacco (Hoque et al., 2007). Furthermore, proline application improved the germination and growth rate in rape seed when exposed to high level of Na^+ and Cl^- ions (M. Ashraf & McNeilly, 2004). Similar observations by using proline application were also observed in the in wheat (Raza et al., 2007) and maize crops (Dawood et al., 2014). The exogenous foliar application of proline not only effectively regulates solute potential but also plays an important role in enhancing plant growth under stress environment (Tabssum et al., 2019).

However, the characteristics and methods used to evaluate the salt stress and be selection criteria for the salt-tolerant variety in plant is also important and varies in implementation (Arzani, 2008). Some characteristics in rice; morphological and physiological traits, were used in the current evaluation and selection of salt tolerant rice include shoot length, plant height, Na^+ and K^+ concentration in plant cell, root and shoot dry weight at the seedling stage (Chunthaburee et al., 2016). The detrimental effect of salinity on plant growth and productivity is associated with low water potential of the root medium since increasing in soil salt concentration, decreasing osmotic potential and ability of plants to take up water (Deinlein et al., 2014). Earlier studies on cereal crops was conducted at the seedling stage which suggested that salt exclusion from leaves as the most important tolerance mechanism (Munns et al., 2006). Selective ion uptake by compartmentalization and roots of harmful ions in older tissues such as older leaves and leaf sheaths reduce Na^+ accumulation and prevent its building up to toxic concentrations in photo synthetically active tissues (Gerona et al., 2019).

2.2 Rice growth stages and salinity effects

Salt-affected soil is one of the serious abiotic stresses that cause reduced plant growth, development and productivity worldwide (Barus et al.; de Oliveira et al.,

2013). During the onset and accumulation of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolism are affected (Carillo et al., 2011). During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion (Carillo et al., 2011). The osmotic effects of salinity stress can be observed immediately after salt application and are believed to continue for the duration of exposure, resulting in inhibited cell expansion and cell division, as well as stomata closure (Skirycz & Inzé, 2010). During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth (Amirjani, 2010). In fact, excess sodium and more importantly chloride has the potential to affect plant enzymes and cause cell swelling, resulting in reduced energy production and other physiological changes (Carillo et al., 2011). Ionic stress results in premature senescence of older leaves and in toxicity symptoms (chlorosis or necrosis) in mature leaves due to high Na^+ which affects plants by disrupting protein synthesis and interfering with enzyme activity (Rajendran et al., 2009). Salt stress showed rice growth rate reduction, promoted metabolic alterations, and decreased ability to uptake water and nutrients (Nounjan et al., 2012). Moreover, poor development of rice spikelets, especially inferior spikelets caused by salt stress significantly reduced rice grain yield (Gerona et al., 2019).

2.3 Rice flowering reproductive stages and salinity effects

2.3.1 Heading

After the flag leaf emerges about 18 days before heading, the panicle grows fast and moves upward in the flag leaf sheath as the internodes elongate (Moldenhauer & Slaton, 2001). About 6 days before heading (panicle exsertion), the flag leaf sheath thickens an indication that a panicle is enclosed; so called the booting stage. Elongation of the second internode from the top is completed 1 or 2 days before heading. Then, the topmost internode elongates rapidly and pushes up the panicle. As a consequence, the panicle is exserted from the flag leaf sheath. In general, panicle exsertion is fast and complete in japonica rice, but slow and incomplete in some indica rice. However, low temperatures aggravate poor panicle exertion (Liu et al., 2006). The date of heading differs not only within a plant but also among plants in the same field. It usually takes 10-14 days for all the plants in a field to complete heading. For convenience, heading date is defined as the time when 50% of the panicles have exerted (Desai et al., 2019).

2.3.2 Morphology of a spikelet

The spikelet is borne on the pedicel, a short stalk that is an extension of the panicle axis and the primary or secondary branch. There are two short rudimentary glumes at the upper end of the pedicel. A pair of sterile lemmas and the rachilla is

located between the rudimentary glumes and the spikelet. The flower is enclosed in the lemma and palea, which may be either awn or awnless (Quang & Vo, 2017). The flower consists of the pistil, stamens, and lodicules. The components of the pistil are the stigmas, styles, and ovary. The stigma is plumose, on to which pollen grains are shed. The ovary is thick, smooth, and bears two styles. There are six well-developed stamens, composed of anther and filament. Two small, oval, thick, and fleshy bodies, called the lodicules, are situated at the base of the ovary. The lodicules become distended with water and assist in separating the lemma and palea at flowering (Duan et al., 2010).

2.3.3 Anthesis

Anthesis refers to a series of events between the opening and closing of the spikelet, lasting about 1-2.5 hours (Moldenhauer & Slaton, 2001). At the beginning of anthesis, tip portions of the lemma and palea begin to open, filaments elongate, and anthers begin to exsert from the lemma and palea. As the spikelet opens wider, the tip of the stigma may become visible. The filaments elongate further to bring the anthers out of the lemma and palea. The spikelet then closes leaving the anthers outside. Anther dehiscence usually occurs just before or when the lemma and palea open; consequently, many pollen grains fall onto the stigma. For this reason, rice is a self-pollinated plant (Liu et al., 2006).

When a portion of the panicle has exserted, anthesis will occur, starting with the spikelets at the tip of upper panicle branches. Hence, the date of anthesis is the same as the date of heading. The date of anthesis of individual spikelets varies with the positions of the spikelets within the same panicle (Liu et al., 2006). Spikelets on the upper branches have anthesis earlier than those on the lower branches; within a branch, a spikelet at the tip flowers first. It takes 7-10 days for all the spikelets within the same panicle to complete anthesis; most of the spikelets complete anthesis within 5 days. Within the same field it takes 10-14 days to complete heading because panicle exsertion varies within tillers of the same plant and between plants in the same field. Hence, it takes about 15-20 days for all the spikelets of a crop to complete anthesis (Nguyen et al., 2014).

Although, previously research reported that salinity stress at 8 dS/m NaCl at flowering stage caused a reduction in the overall vigor of rice, especially in pollen germination, fertilization, and grain yield (Singh & Flowers, 2010). However, at flowering stage, a salinity level from 2.5 to 4.0 dS/m was reported seriously affected to rice at the booting stage (Grewal, 2010). This showed that the effect of salinity depends on many factors such as variety and environmental factors, including the stage of growth of rice and the length of time a plant is exposed to salinity. For this reason, even lower salinity levels than commonly reported may also affect plant growth. Salinity tolerance at the reproductive stage is important in areas where high salt stress is expected later in the season. Because at this stage, pollination and

formation of grains occur that directly contribute to economic yield (Thakur et al., 2010).

2.4 Osmoprotectants in rice to alleviate salinity stress

2.4.1 Polyamines

Polyamines are small organic compounds with two or more primary amino groups, found in all eukaryotic cells. Putrescine (Put, a diamine), spermidine (Spd, a triamine), and spermine (Spm, a tetramine) are the major polyamines found in plants involved in various processes such as cell proliferation, growth, morphogenesis, differentiation, and programmed cell death (Jastrzab et al., 2017). Polyamines occur in free or conjugated forms either with phenolic compounds or macromolecules such as proteins and nucleic acids (Walters, 2000). Polycationic nature of polyamines at physiological pH is attributed for their biological activity. Polyamines play an important role in several plant developmental processes such as cell division, embryogenesis, fruit ripening, root growth, tuber development, floral initiation, floral development, and stem elongation (Kaur-Sawhney et al., 2003).

Water-stressed finger millet (*Eleusine coracana* L. Gaertn.) plants sprayed with 0.2 mM Spd at early flowering stage, showed protection against chlorophyll degradation, and produced less electrolyte leakage, lower levels of hydrogen peroxide (H_2O_2) (Alcázar et al., 2020) Moreover, the plant that received external polyamines had caspase-like activity than unstressed plants, as well as accumulation of proline alleviating the water deficit. Another example was noted in Damask rose in which foliar applications of Spm or Spd (0.5 mM) improved relative water and chlorophyll contents, as well as stomatal conductance in plants subjected to water stress (Hassan et al., 2018).

2.4.2 Glycine betaine

Glycine betaine, a quaternary ammonium compound is widely distributed in microorganisms, higher plants and animals and one of the most common betaines found in plants. In many halotolerant plants, glycine betaine is reported to accumulate in plastids and higher levels of glycine betaine correlates with higher level of stress tolerance (Wani et al., 2013). Glycine betaine has diverse functions in plant cell such as stabilization of the quaternary structure of enzyme, proteins, and maintenance of membrane integrity under salt, cold, and heat stress (Sakamoto & Murata, 2002). The biosynthetic pathway in most plants follows the conversion of choline to glycine betaine in two oxidation steps via the intermediate betaine aldehyde. The first reaction is catalyzed by choline monooxygenase that converts choline to betaine aldehyde hydrate thus spontaneously forming betaine aldehyde which is acted upon by betaine aldehyde dehydrogenase to form glycine betaine, whereas in *Arthrobacter* spp. only one enzyme, choline oxidase is required

(Holmström et al., 2000).

Cha-um et al. (2013) investigated that the role of GlyBet (100 mM) applied exogenously in alleviating water-deficit induced stress in indica rice cv. Pathum Thani 1 (PT1). The result showed that exogenous GlyBet increased proline concentration in the leaf tissues of water deficit stressed plants depends on a degree of soil water content (SWC), especially at severe water deficit (25% SWC). Foliar GlyBet (100 mM) significantly enhanced chlorophyll a (Chl_a) and total chlorophyll (TC) content in the leaf tissues of water stressed plants (25% SWC) and stabilized total carotenoids (C_{x+c}) in 25 and 36% SWC better than those in controlled plant. A positive correlation was observed between chlorophyll a and maximum quantum yield of PSII (F_v/F_m), and TC and photon yield of PSII (photosystem).

At booting stage in rice, under severe water deficit condition, plants were ameliorated by 100 mM GlyBet foliar application. Many tissues in rice plant were maintained after 100 mM GlyBet application such as chlorophyll pigments, chlorophyll fluorescence and net photosynthetic rate, leading to them retaining overall growth performances and yield traits (Tisarum et al., 2019).

2.4.3 Mannitol

Mannitol is a hexitol sugar alcohol and widely distributed in nature including more than 100 species of vascular plants (Upadhyay et al., 2015). Mannitol is known to serve as a major carbon source in many organisms. The mannitol biosynthetic pathway in higher plants starts with the isomerization of fructose-6-phosphate to mannose-6- phosphate by mannose-6-phosphate isomerase, which is then converted to mannitol-1-phosphate by mannose-6-phosphate reductase. In the final step, mannitol-1-phosphate is acted upon by mannose-1-phosphate phosphatase to release free mannitol. In *Eschericia coli*, mannitol is catabolized by the enzyme mannitol-1- phosphate dehydrogenase in a reversible reaction whereas when expressed in transgenic tobacco it functions anabolically and synthesizes mannitol (Saxena et al., 2013). Foliar application of mannitol was effective in decreasing the adverse effects of salt stress in maize of increased biomass production under saline conditions that was associated with increased synthesis of chlorophyll contents, enhanced leaf RWCs, reduced H_2O_2 contents, increased K^+/Na^+ ratio, and increased uptake of Ca^{2+}, and nitrogen (N) (Kaya et al., 2013).

2.4.4 Inositols

Inositols and their derivatives are a functionally important class of compounds required for normal growth of cells (Michell, 2008). These inositols are cyclohexane hexitols and exist in nine isomeric forms, out of which, *myo*-inositol is the most favored form in nature. The two step inositol biosynthetic pathway is the

only *de novo* pathway for inositol synthesis and an out branch of the central glycolytic pathway (Sato & Atomi, 2011). This inositol biosynthetic pathway is highly conserved throughout the biological kingdom. Where, the rate limiting enzyme *myo*-inositol-1-phosphate synthase catalyzes the conversion of glucose-6-phosphate to *myo* -inositol-1-phosphate and subsequently *myo*-inositol-1-phosphate is converted to free *myo*-inositol by the enzyme *myo*-inositol mono phosphatase. Free inositol can be further channelized to other physiologically significant pathways and produce various inositol derivatives. These inositols are required for normal growth and development, membrane bio- genesis along with the roles of their phosphorylated derivatives as phosphorus store. It was as a secondary messenger in signal transduction pathways. In addition to this, inositol and its derivatives such as pinitol, galactinol and other raffinose series oligosaccharides have been found to act as osmoprotectants and provide protection against abiotic stresses like salt and osmotic stress (Saxena et al., 2013).

Exogenous application of an appropriate dose of Myo-inositol (1 mM) could significantly enhance drought tolerance in creeping bentgrass (Li et al., 2020). Myo-inositol induced drought tolerance could be involved in the maintenance of better water relation associated with increases in water use efficiency, the decline in chlorophyll loss for photosynthetic maintenance, and improvement in antioxidant enzymes activities superoxide dismuatase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) and gene expression contributing to less oxidative damage under drought stress (Lisar et al., 2012).

2.4.5 Trehalose

Trehalose is a non-reducing disaccharide (1,1 a -d glucopyranosyl, a-d -glucopyranoside) found in various organisms including bacteria, algae, fungi, yeast, insects, and some plants (Saxena et al., 2013). Besides being a carbohydrate reserve, trehalose protects organisms against several physical and chemical stresses (Argüelles, 2000). Trehalose is synthesized in a two steps in process in bacteria and yeast, first reaction for trehalose synthesis catalyzed by trehalose-6-phosphate synthase forming trehalose-6-phosphate from UDP-glucose and glucose-6-phosphate; in second reaction trehalose-6-phosphate phosphatase converts trehalose-6-phosphate to trehalose (Silva et al., 2005).

Trehalose is having a unique water absorption capacity, which protects the macromolecules from desiccation-induced damage. During dehydration, trehalose has been thought to replace water molecules and thereby prevent protein denaturation and membrane fusion (Hoekstra et al., 2001). It has been shown that trehalose along with other compounds like glycine betaine, proline, and mannitol are active in scavenging reactive oxygen species (both hydrogen peroxide and superoxide anion) in a concentration-dependent manner (Saxena et al., 2013).

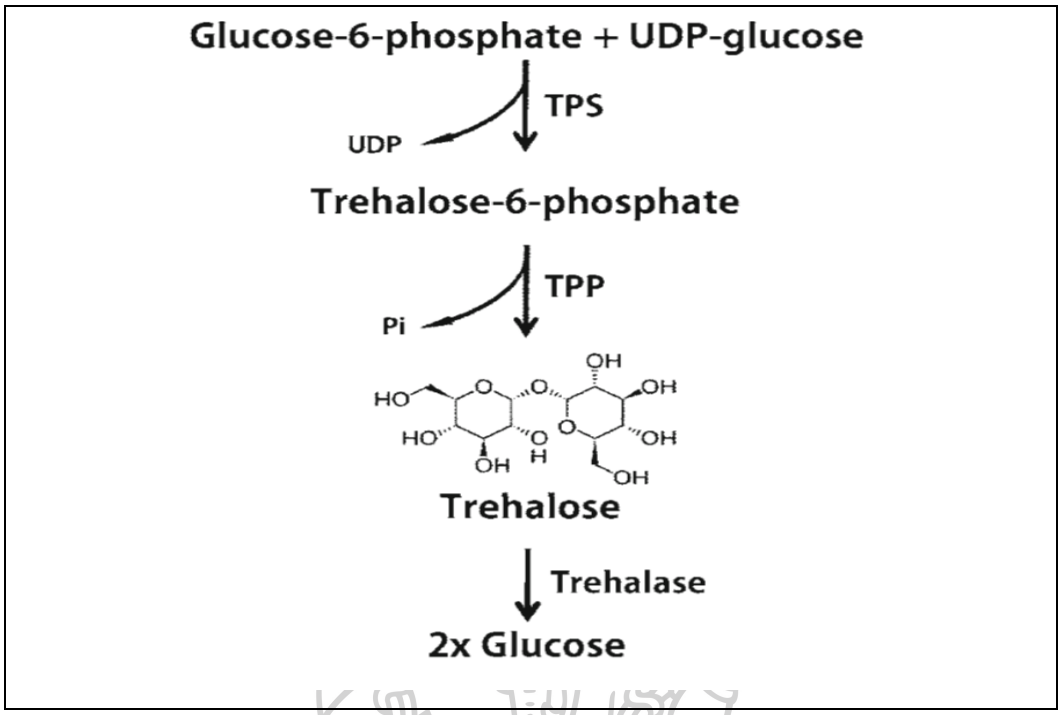


Figure 1 Trehalose biosynthesis pathway (Saxena et al., 2013)

Soil salinity presents an increasing threat to agriculture, and trehalose influences many processes that provide an advantage for plant survival under salt stress (Lunn et al., 2014). Low to moderate levels of exogenous trehalose reduces Na^+ accumulation, whereas higher levels prevent NaCl-induced loss of chlorophyll in leaves and preserve root integrity (Lunn et al., 2014). Trehalose also accumulates in a range of wheat cultivars under salt stress, potentially due to enhanced trehalose-6-phosphate synthase (TPS) activity (El-Bashiti et al., 2005). In rice, *OsTPP1* was transiently induced during salt stress, similar to the response upon chilling stress (Pramanik & Imai, 2005; Shima et al., 2007). In *Medicago truncatula*, trehalase expression is down regulated under salt stress (López et al., 2008). This allows trehalose accumulation, consistent with a role for this disaccharide as a protective agent against salt stress. In contrast, in a closely related species, alfalfa (*Medicago sativa*), the role of trehalose in osmoregulation has been questioned, as its concentration does not increase substantially upon salt stress (López et al., 2008). Even though there were large relative increases in trehalose content, absolute levels were still very low and probably had little direct protective effect against salt stress in alfalfa.

Abdallah et al. (2016) reported that soaking rice seeds with 25 mM of trehalose (Tre) could alleviate the harmful effects of salinity stress of 60 mM NaCl treatment to induce salinity stress. The activities of superoxide dismuatase (SOD), catalase (CAT) and peroxidase (POX) were increased with increasing salinity level.

Moreover, a higher solute concentration contributing to osmotic adjustment and the higher antioxidant enzymes activity were observed in shoots of salinity treated in rice. Abdallah et al. (2016) also reported that the mitigating effect of exogenous trehalose (10 mM) was evident during the recovery period by increasing the potential for growth recovery and the effect was more pronounced in the salt-sensitive cultivar, which was related to the reduction in Na^+ to K^+ ratio under 200 mM NaCl.

2.4.6 Proline

Proline, amino acid, is one of the most common compatible osmolyte with high water solubility and stable conformation (Matysik et al., 2002). It is an essential component of cellular and metabolic events and also responsible for osmotic adjustment in cell (Khan et al., 2020). Apart from plants, the accumulation of proline has been observed in bacteria, protozoa, algae, and marine invertebrates. In plants, the biosynthesis of proline can occur via glutamate or ornithine pathway (Hmida-Sayari et al., 2005).

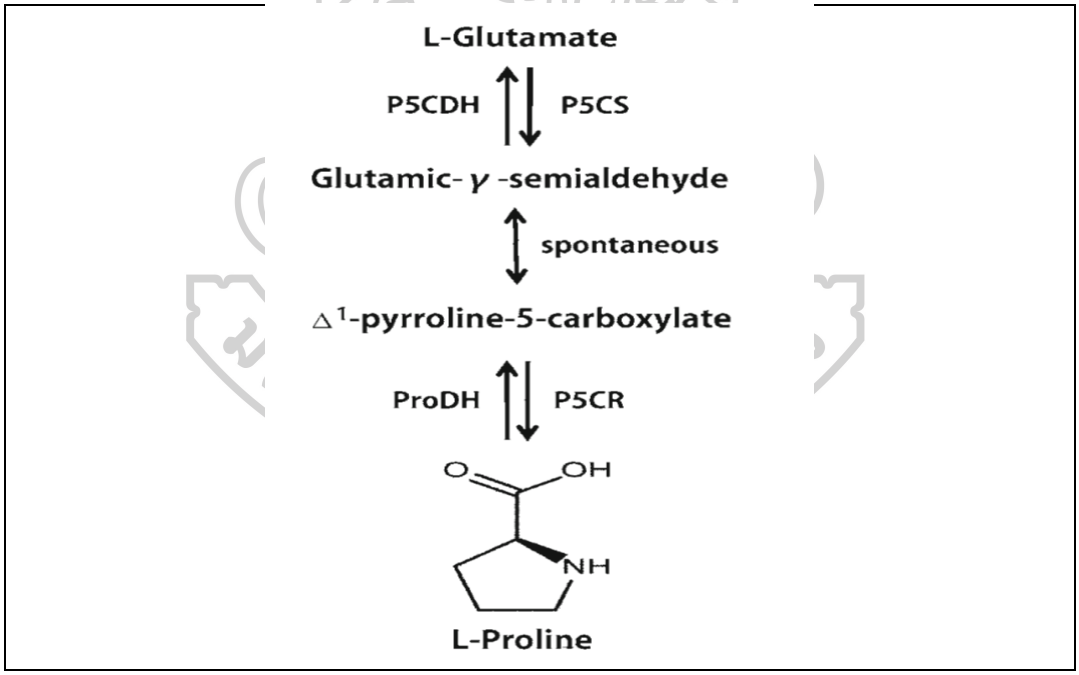


Figure 2 Proline biosynthesis pathway (Saxena et al., 2013)

Glutamate is the primary precursor for proline synthesis in osmotically stressed out and nitrogen deficient cells. While at higher levels of available nitrogen, the ornithine pathway is followed biosynthetic pathway from glutamate to proline involves two important enzymes l-D^1 -pyrroline-5-carboxylate synthetase (P5CS) and l-D^1-pyrroline-5-carboxylate reductase (P5CR) (Lehmann et al., 2010). Firstly,

glutamate is converted to glutamic-g-semialdehyde (GSA) and L-D[1]-pyrroline-5-carboxylate (P5C) by the action of P5CS, and then P5CR catalyzes the conversion of P5C to l- proline (Saxena et al., 2013).

The level of proline in plants is controlled by degradation or metabolism of proline, where ProDH (proline dehydrogenase) oxidizes proline to P5C in plant mitochondria and finally P5C dehydrogenase converts P5C to l –glutamate. In normal conditions, this oxidation pathway is followed whereas; under salt and water stress such proline degradation pathway is inhibited, as a result proline level increases (Saxena et al., 2013).

2.4.6.1 Exogenous proline application and metabolism under salt stress

Many studies showed that salt stress triggers the induction of genes involved in proline biosynthesis, which leads to proline accumulation (El Moukhtari et al., 2020). And also knocking out the function of P5CS in *A. thaliana* indicates a key role for this enzyme in plant salt tolerance because the p5cs1 plants are hypersensitive to salt. Exogenous application of proline can effectively improve tolerance of plants to salt stress through the regulation of endogenous proline metabolism, partly achieved through differential expression of specific proline-related genes. For example, application of proline on *Z. mays* foliar resulted in reduction of P5CS activity and PDH increasing under salt stress (Iqbal et al., 2014). Similar results in salt stressed *Sorghum bicolor* were reported more recently (Iqbal et al., 2014). Adding exogenous proline led to a d ecrease in P5CS activity in both stressed and unstressed *Eurya emarginata*, but to an increase in PDH activity only in unstressed plants (El Moukhtari et al., 2020). Under salt stress, *Triticum aestivum* seed priming with exogenous proline significantly decreased the content of proline and P5C with a reduction in the activity of P5CS, while PDH activity was significantly increased (Shakeri et al., 2019).

2.4.6.2 Effects of proline treatment on plant growth and biomass under salt stress

It is well documented that certain concentrations of exogenous proline regulate different aspects of plant growth and development under salt stress including rises in biomass and productivity (Mbarki et al., 2018). Addition of exogenous proline improved the growth of calli from two *Medicago sativa* cultivars upon salt stress, but dry weight and proline contents between the two were different with a better salt tolerance correlated with higher proline accumulation (Campanelli et al., 2013). Noreen and Ashraf (2008) tested the effects of 30 and 60 mM proline applied as a foliar spray to *Helianthus annuus*, concentrations that induced tolerance to 60 and 120 mM NaCl. They found that exogenous proline mitigates the salt stress

effects on plant growth as proven by longer shoots and roots, and greater fresh and dry weights of shoots and roots, and this positive effect was more pronounced at the lower proline concentration (30 mM). Similarly, Wani et al. (2016) reported that a foliar spray of 20 mM proline alleviates the negative effects of salt stress on *Brassica juncea* by increasing lengths and fresh and dry masses of both shoots and roots, and the area of leaves. In addition, exogenous proline supply significantly increased plant height and number of roots in salt stressed *O. sativa* (C.-Y. Teh et al., 2016). Likewise, application of proline increased dry mass of leaves and roots and their soluble protein contents in salt stressed *Z. mays* (Perveen & Nazir, 2018). In some cases, exogenous proline stimulates yield under salt stress. Exogenous proline was increased fresh and dry biomasses, grain yield and 1,000-grain weight of salt-stressed *T. aestivum* (Rady et al., 2019). In salt-stressed *Z. mays*, foliar-applied proline increased the number of seeds per plant, total grain weight and the 100-grain weight (El Moukhtari et al., 2020). In general, exogenous application of proline increased plant growth and productivity under salt-induced stress but the underlying mechanisms, probably linked to some hormonal regulation, still remain elusive.

2.4.6.3 Exogenous proline influences plant water relations under salt stress

Many researches have documented how exogenous proline substantially alleviates salt stress by increasing leaf water potential, water content and restoring water use efficiency. Wani et al. (2016) noted that in *Brassica juncea*, the leaf water potential was reduced under salt stress, but 20 mM proline applied as a foliar spray completely reversed the loss in water potential. Similarly, Huang et al. (2009) demonstrated that, under saline conditions, exogenous proline could alleviate the growth inhibition of salt-sensitive *Cucumis sativus*, and this was accompanied with leaves having higher water content. Studying salt-stressed *O. europaea* plants, El Moukhtari et al. (2020) found that the relative water content is 1.05 and 1.09-fold higher under 25 and 50 mM of exogenous proline, respectively, than in the absence of proline. In the same way, Zheng et al. (2015) observed that 20 mM exogenous proline significantly alleviated the negative effects of 200 mM NaCl and raised the leaf water content in *Eurya emarginata*. Zheng et al. (2015) have suggested that the increase in water content and water potential of leaves in response to exogenous proline under salt stress could be because the proline triggers the accumulation of some organic and inorganic compounds such as proline, glycine betaine, soluble sugars and K^+ that help plants adjust their cellular osmotic potential and hence maintain higher water content.

2.4.6.4 Proline treatment mediates reduction in ion toxicity due to salt stress

High salt concentrations increase Na^+ and Cl^- contents in plants

and decrease the abundance of other cations such as K^+ and Ca^{2+}, which leads to mineral nutrient imbalance reported by Tavakkoli et al. (2010). Indeed, under salty conditions, sustaining ion homeostasis is one of the adaptive strategies that tolerant plants use to cope with salt stress. Tavakkoli et al. (2010) found that these strategies may help the plant to prevent potentially toxic effects of the build-up of ions like Na^+ and Cl^- that cause various types of damage to lipids, proteins and nucleic acids. Abdelhamid et al. (2013) mentioned that application of 5mM proline in a foliar spray decreased Na^+ content and increased K^+/Na^+ ratio in *P. vulgaris*. More recently, El Moukhtari et al. (2020) reported that external application of proline decreased both Na^+ and Cl^- contents, but increased the K^+ content and the K^+/Na^+ ratio in salt-stressed *Z. mays*. Bhusan et al. (2016b) reported that comparison to salt-stressed plants, exogenous proline application increased the K^+/Na^+ ratio in *O. sativa* under 100 mM NaCl.

Bhusan et al. (2016a) demonstrated that foliar application (50 mM) of proline at seedling and vegetative stages showed a significant increase in growth, and grain and straw yields accompanied with increased K^+/Na^+ ratio and nutrient uptake of rice varieties under salinity condition (Ehsan-Ul-Haq et al., 2009). Proline (50 mM) sprayed at active vegetative stage under (25 mM) under NaCl confers tolerance to salinity in rice by increasing nutrient uptake, maintaining higher K^+/Na^+ ratio and probably increasing antioxidant defense systems. Mostofa et al. (2015) mentioned that during salt stress and recovery, Pro (10 mM) supplement promoted APX activity in the salt-tolerant rice and enhanced ascorbate peroxidase (APX) as well as catalase (CAT) and peroxidase (POX) in the salt-sensitive rice.

CHAPTER 3

METHODOLOGY

3.1 Materials and treatments, experiment design and planting practices

3.1.1 Materials, experimental design and treatments

The two experiments were conducted on an open field to assess the effect of two organic osmolytes include proline and trehalose on rice cultivars at Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT Campus, Thailand from January 2020 to May 2020. The maximum and minimum temperature was 31°C and 25°C, total rainfall was 169.7 mm and relative humidity and wind speed was 68% and 13.7 km/h, respectively during the experimental period (Appendix 1). In each experiment, the experiment design was 3x4x4 factorial in Completely Randomize Design (CRD) with three replications. Factor A was three rice varieties which two were Thai rice varieties namely Chai Nat 1 (CNT 1) and Pathum Thani 1 (PT 1) and Inpari 35 (IN 35) salt tolerance variety from Indonesia. Factor B was four levels of salinity: 0 (control), 5, 10, 15 dS/m. Factor C were four levels of proline and trehalose: 0 (control), 50, 100, 150 mM, which were analyzed separately between these substances.

3.1.2 Planting practices

Before the experiment, all three rice varieties (CNT 1, PT 1 and IN 35) were tested for the percentage of germination, seed viability and seed vigor to confirm viability and quality of seeds. The soil is sandy loam having pH 6.39, electrical conductivity (EC) 0.92 dS/m, organic matter content 0.93% and sodium absorption ratio (SAR) 0.22. Rice seeds were soaked overnight and pre-germinated at 30^{o}C for 48 hours, the germinated seed were sown in nursery trays which were filled with fertilize soil for 14 days. Five seedlings were transplanted in a round shape plastic pot (32 cm diameter x 30 cm height) filled with nutrient-rich soil (5 plants per pot, and one pot per treatment) (Appendix 2). The pots were kept in field and tap water was applied at the frequency of two-day interval. The seedlings started to receive salt stress according to each treatment condition at 30 days old until harvesting. The salt stress treatments were done by the addition of 150 ml sodium chloride (NaCl) salt solution in each pot once a week (0 dS/m (water only), 5 dS/m (2.92g of NaCl/L of water), 10 dS/m (5.84g of NaCl/L of water), and 15 dS/m (8.76g of NaCl/L of water) (Appendix 4). At one week before flowering stage, 100 mL of desired concentration (0, 50, 100 and 150 mM) of proline or trehalose were foliar-applied by spraying with

sprayer in each pot according to treatment conditions (Appendix 4).

3.2 Data collection

Data for water content, soluble sugar, chlorophyll concentration, starch and proline contents contain within the stem and leaf, pollen viability and proline synthesis gene samples were collected after 5 days of either proline or trehalose foliar application. Relative water contents as physiological trait was studied in each experiment.

3.2.1 Measurements of relative water content

Relative water content (RWC) was estimated at the same time as midday LWP (Leaf Water Potential) determination in the three water stress conditions by the method described by Barrs and Weatherley (1962). Five leaf samples were collected from each treatment. The leaf samples were weighed to determine fresh weight then submersed in water for 6-9 hours and weighted to record the turgid weight. Leaf samples were then oven-dried at 50-60°C for 24 h and measured the dry weight (Sibounheuang et al., 2006) (Appendix 5). Relative water content was calculated as follows:

$$\text{Relative water content (\%)} = \frac{\text{(Fresh weight - Dry weight)}}{\text{(Turgid weight - Dry weight)}} \times 100$$

3.2.2 Measurement of chlorophyll content

The samples were prepared for measurement of chlorophyll concentration according to the method of Zervoudakis and George (2014). Leave samples collected at the reproductive stage (the first, second and third leaves from the top) of each treatment were used for the determination of chlorophyll concentration. Dried leave (50 mg) was ground and placed in a glass vial containing 5 mL of DMSO (dimethyl sulfoxide). Then, the samples were incubated at 65°C for 30 min and subjected to centrifugation at 3000 rpm for 5 minutes. DMSO extract containing chlorophyll from leaves was then measured for absorption spectra at 645 and 663 nm using a UV spectrophotometer (Appendix 6). Chlorophyll concentration was calculated by the following formula (Morgado et al., 2011).

$$\text{Chl a (mg/g)} = [12.7(A663) - 2.69(A645)]V/(1000 \times W)$$

$$\text{Chl b (mg/g)} = [22.9(A645) - 4.68(A633)]\ V/\ (1000 \times W)$$

$$\text{Tot Chl (mg/g)} = [20.2(A645) + 8.02(A663)]\ V/\ (1000 \times W)$$

When V= Volume of solvent

W= Dry weight of sample

3.2.3 Measurement of proline accumulation in stem and leaf

The proline content was estimated based on the method described by Bates et al. (1973) with some modifications. Briefly, 50 mg of dry sample was ground with the solution compose with N_2 and 2 ml of 3% sulfosalicylic acid. The mixture was centrifuged at 4,000 rpm for 10 minutes. Take 1 ml of the filtered mixture in a test-tube, and then added 1 ml of acid- ninhydrin and 1 ml of glacial acetic acid. The mixture was mixed with a vortex mixer and boiled at 95℃ for 1 h. The mixture was then frozen in ice about 5 minutes and was combined with 2 ml of toluene, mixed, and then was left to stand at room temperature for 2 min. Absorbance of the reddish pink up- per phase was recorded at 520 nm against a toluene blank (Appendix 8). The corresponding concentration was determined against a standard curve prepared by using a proline solution. The amount of proline was expressed as (μ mole/g) dw^{-1}.

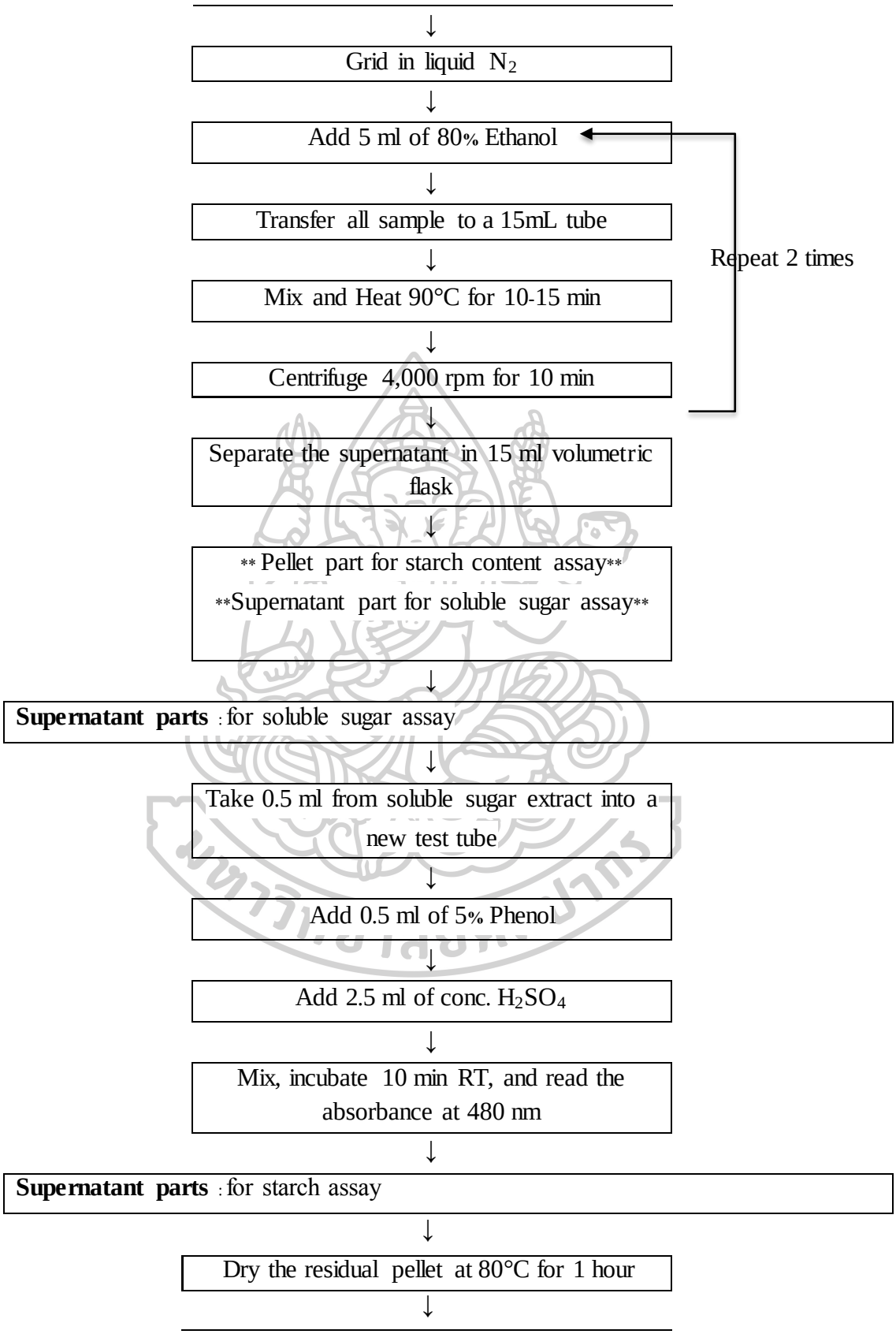
3.4 2Measurement of soluble sugar and starch in the leaves and stems

Soluble sugar and starch in the leaves and stem of rice at reproductive stage were prepared and determined according to the method of Chaw and Landhause (2004) with some modifications.

For starch assay: 50mg of dried sample was grinded, then 5 ml of 80% ethanal was added. Samples were heated at 90°C for 10-15 minutes and centrifuge at 4,000 rpm for 10 minutes. The extract was then decanted and the residues were re-extracted for 2 times. Final volume was made up to 5 ml by adding with 80% ethanal and kept for soluble sugar assay. For starch assay, the residual pellet was dried at 80°C for 1 hour. After that, 5 ml of 0.005 N H_2SO_4 was added for acid hydrolysis and mixed thoroughly, and incubated at 90°C for 1 hour. This sample was centrifuged at 4,000 rpm for 10 minutes. Then, 0.5 ml of the supernatant was transferred to a new glass test tube and added 0.5 ml of 5% phenol and mixed thoroughly. After that, 2.5 ml of analytical grade sulfuric acid was then added to it and mixed thoroughly by vertical agitation with a glass rod. For exothermic reaction, the test tube was cooled at room temperature. Absorbance was recorded at 480 nm on Spectro SC spectrophotometer (Appendix 11).

For soluble sugar assay, 0.5 ml of sample was taken into a new test tube contained 10 ml of extract solution. Adding 0.5 ml of 5% phenol in sample and mixed thoroughly. After that, 2.5 ml of analytical grade sulfuric acid was then added to it and mixed thoroughly by vertical agitation with a glass rod. For exothermic reaction the test tube was cooled at room temperature. Absorbance was recorded at 480 nm (Appendix 9). The corresponding concentration will be determined against a standard curve prepared by using a glucose solution. The amount of sugar was expressed as mg g^{-1} dw^{-1}.

Dry sample (50 mg)


Grid in liquid N_2
Add 5 ml of 80% Ethanol
Transfer all sample to a 15mL tube
Repeat 2 times
Mix and Heat 90°C for 10-15 min
Centrifuge 4,000 rpm for 10 min
Separate the supernatant in 15 ml volumetric flask
** Pellet part for starch content assay**
Supernatant part for soluble sugar assay
Supernatant parts : for soluble sugar assay
Take 0.5 ml from soluble sugar extract into a new test tube
Add 0.5 ml of 5% Phenol
Add 2.5 ml of conc. H_2SO_4
Mix, incubate 10 min RT, and read the absorbance at 480 nm
Supernatant parts : for starch assay
Dry the residual pellet at 80°C for 1 hour

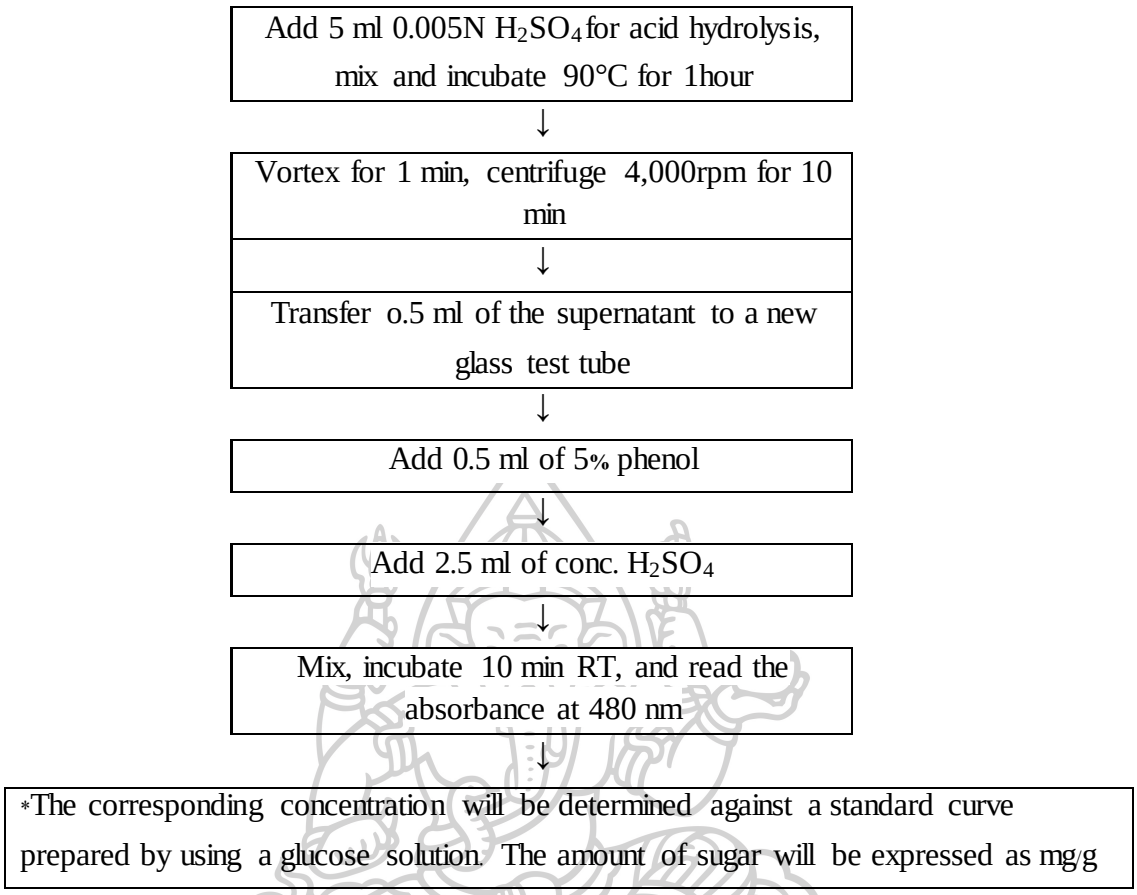


Figure 3 Diagram of starch and soluble sugar analysis method

3.2.5 Determination a key role in proline synthesis gene expression levels (*P5Cs1*) by Real-time Polymerase Chain Reaction (RT-PCR)

3.2.5.1 Ribonucleotide acid (RNA) extraction

Total RNA from 100 mg fresh rice leaves at the flowering stage; collected at five days after proline spraying, 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.

3.2.5.1 Real-time Polymerase Chain Reaction (RT-PCR)

Complementary deoxyribonucleic acid (cDNA) of rice was synthesized from 1 µg of total RNA using iScriptTM cDNA Synthesis Kit (Bio-Rad Laboratories, USA). The reaction consists of 5x iScript Reaction Mix, iScript Reverse Transcriptase (RT), Nuclease-free water, and RNA template. After incubation, the cDNA was amplified by PCR (polymerase chain reaction). Polymerase chain reaction was done using gene specific primer (p5cs F_5' TAG CAG GAC TGT TGG CAC TG 3' and R_5' ACA GGT GTG CCG CTA TTT GA 3') and OsActin primer (OsActin_F 5' CAG CCA TGT CCC CAT CTA 3' and R_ 5' AGC AAG GTC GAG ACG AAG GA 3').

The PCR reaction mixtures consist of 1x Ultra-pure Taq PCR master mix (1 U of Ultra-pure Taq polymerase, 2 mM $MgCl_2$ and 200 µM of each dNTPs) (Geneaid Biotech Ltd., Taiwan), 0.8 µM of each primer, and 1 µl of cDNA template. The PCR cycle conditions were performed in the thermocycler (Biometra® T-gradient Thermoblock Thermal Cycler, Germany) with the initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. After final extension at 72°C for 7 min, the PCR products were cooled down to 20°C. The PCR products were determined on 1.5% agarose gel electrophoresis. The single DNA band was excised under UV-light and purified using the GenepHlowTM Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan). Then, the purified PCR products were sent to DNA sequencing service, which was performed in ABI Prism 3730XL DNA sequencer (U2Bio, Korea) (Appendix 12).

3.2.6 Assessment of pollen number and pollen viability

Khatun and Flowers (1995) was suggested to determine pollen number and percent of viability. The potassium iodide (I_2-KI) method was used for this study (Sarhadi, 2012). Panicles were collected randomly during heading stage, then, spikelet samples at before flowering phases were placed in vials with 70% ethanol and stored at 4°C. Under normal weather conditions in the tropics, most rice varieties (*O. sativa*) begin anthesis at about 8:00 and end at about 13:00 hours. When temperatures are low, anthesis may start late in the morning and continue into the late afternoon. The spikelets were dissected to expose the anthers, which were then crushed thoroughly to release the pollen and stained with 1% I_2-KI solution. Pollen were then mounted on slides and viewed under a microscope. Pollen grains stained black were considered viable, and those stained yellow or light colored were counted as sterile. Pollen viability was calculated by dividing the number of fertile pollen grains by the total number and presented as percentage (Sarhadi, 2012) (Appendix 13).

3.2.7 Determination of plant height, yield and yield components

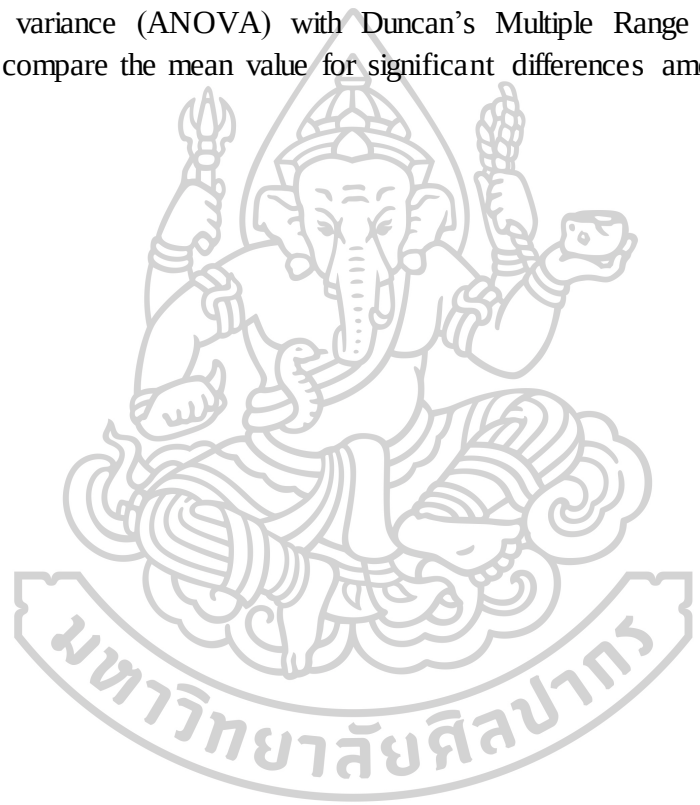
For morphological parameter, data were collected at maturity stage.

Plant height was measured from the ground level to the top of the plant from all plants in each treatment. The yield per plant and yield components including number of fertile tillers, panicle length, filled grain percentage and 1,000-seed weight were measured. Panicle length was measured from the neck node to the tip of the panicle.

From one hill in each treatment, counting of filled and unfilled grain from each of the panicle from harvest plant calculated the percentage of filled grain. For 1,000 seed weight and yield per plant, they were measured at 14% moisture content.

3.3 Statistical analysis

Statistical analyses were performed with the R program (R Core Team, 2017). Analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) was executed to compare the mean value for significant differences among treatments.



CHAPTER 4

RESULTS

Soil salinity is one of the most serious problems on planting areas, which cause an obstructive impact on crop production around the world. This crisis attracts many scientists to work towards overcoming this obstruction. Spraying of the osmo-protectant is one strategy to reduce the stress from salinity in plants. In this study, proline and trehalose were selected and test for osmoprotectant efficacy in three rice varieties planted in different salinity level at the flowering stage. Some chemical contents, physical properties, and gene expression were evaluated after proline or trehalose supplication.

The result of proline or trehalose application on physical and chemical characteristics in rice plant at reproductive stage showed in the following sections.

4.1 Effect of proline and trehalose on biochemical characters

4.1.1 Effect on water content in leaf

Table 1 Means of water content in leaf (%) (± standard error) of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	74 ± 2.0	75 ± 3.5	84 ± 5.2	84 ± 3.8	78.95 ± 3.9b
	50	78 ± 6.1	77 ± 4.6	81 ± 1.2	78 ± 4.4	
	100	82 ± 3.8	73 ± 3.3	80 ± 3.2	80 ± 3.7	
	150	81 ± 2.9	78 ± 4.4	81 ± 5.3	78 ± 4.5	
PT 1	0	86 ± 4.9	89 ± 2.3	85 ± 5.3	84 ± 4.0	85.87 ± 4.4a
	50	83 ± 4.7	87 ± 4.4	84 ± 5.7	88 ± 4.2	
	100	88 ± 2.6	91 ± 2.8	82 ± 6.6	82 ± 4.1	
	150	90 ± 5.6	87 ± 5.0	85 ± 3.9	84 ± 4.3	
IN 35	0	73 ± 2.2	79 ± 4.9	72 ± 2.0	82 ± 5.2	75.27 ± 4.2c
	50	76 ± 3.4	67 ± 4.1	80 ± 3.2	82 ± 5.7	
	100	70 ± 5.5	73 ± 5.7	75 ± 5.5	71 ± 4.3	
	150	74 ± 3.0	74 ± 5.5	81 ± 3.5	74 ± 3.3	
Mean Salinity		79.44±3.9	79.19±4.2	80.92±4.2	80.58±4.3	
		Proline (mM)				
		0	50	100	150	
Mean Proline		80.55±3.0	80.08±4.7	78.90±4.3	80.58±3.8	
P-value (F-test)						
Variety (V) =2.07 $\times 10^{-9}$**, Salinity (S) = 0.713 NS, Proline (P) = 0.760 NS, V × S = 0.208 NS, V× P = 0.961 NS, S× P = 0.726 NS, V× S × P = 0.870 NS, CV% = 9.3						

Note: CNT 1 = Chai Nat 1, PT1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** means significant difference at 0.01 levels of probability,
NS means non-significant difference at 0.05 level of probability.
Different lower-case letters (a, b, c) mean significant difference at 0.05 level of probability

Table 2 Means of water content (%) (±standard error) in leaf of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	78±0.7	79±1.2	70±4.6	76±3.1	76.91±3.5b
	50	80±5.6	83±3.2	66±3.8	72±3.8	
	100	84±1.9	75±2.6	70±5.8	77±5.2	
	150	86±2.2	74±3.2	72±5.5	82±3.8	
PT 1	0	94±0.7	95±0.3	93±0.0	95±0.0	93.37±1.0a
	50	94±0.3	93±1.0	93±0.3	94±1.2	
	100	93±2.2	91±1.2	94±0.6	92±3.2	
	150	93±1.8	94±0.9	93±1.3	93±0.9	
IN 35	0	70±1.3	75±4.3	74±4.0	79±4.7	73.83±3.3c
	50	74±4.1	74±1.7	75±5.7	81±4.3	
	100	74±4.9	73±1.9	71 ± 1.6	73±4.4	
	150	73±2.2	73±4.0	76 ± 2.1	69±1.7	
Mean salinity		82.63±2.3	81.61±2.1	79.50±3.0	81.75±3.0	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		81.44±2.1	81.58±2.9	81.02±3.0	81.44±2.5	
		Salinity dS/m				
Varieties		0	5	10	15	
CNT 1		82.0±2.6b	77.7±2.5bc	71.4±4.9e	76.5±4.0cd	
PT 1		93.3±1.2a	93.3±0.9a	93.2±0.6a	93.5±1.3a	
IN 35		72.5±3.1de	73.3±3.0ce	73.8±3.4ce	75.1±3.8ce	
P-value (F-test)						
Variety (V) = 2×10^{-16}**, Salinity (S) = 0.0974 NS, Trehalose (T) = 0.9755 NS, V × S = 0.0096**, V× T = 0.3078 NS, S × T = 0.4805 NS, V× S × T = 0.3395 NS, CV% = 6.6						

Note: CNT 1 = Chai Nat 1, PT1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
** Means significant difference at 0.01 levels of probability,
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c…) mean significant difference at 0.05 level of probability

Water content is the appropriate measure of water in the plant to indicate the status of water in the cell whether lacking or sufficient. Problems of plants growing in saline soils are similar to those in dehydrated areas. Salinity affects the plant's ability to absorb water from the soil. Therefore, the percentage of the water content indicates the conditions that the growing plant is facing.

After proline application, the results showed that water contents were significantly affected only by rice variety factor ($P<0.01$). There was no significant

effect of salinity and proline factors, neither interaction between factors (Table 1). Average means of varieties showed the highest water content was observed in PT 1, followed by CNT 1 and IN 35, respectively. Under salinity and proline condition, water content water content was not different 79.44-80.58% and 80.55-80.58%, respectively. For the influence of proline spray on the water content, there was no significant difference affected by spraying proline in different concentration. The average of means of 0 mM -150 mM proline treatment showed a range between 78.90-80.58% (Table 1).

Similar to proline experiment, for trehalose spraying, the results revealed that water content was highly affected by varieties factor ($P<0.01$) (Table 2). Average means of varieties showed the highest water content was observed in PT 1 and followed by CNT 1 and IN 35. In addition to variety factor, the effect of interaction between varieties and salinity factor on water content was also observed. In varieties and salinity interaction, PT 1 showed high water content at all salinity levels. However, in CNT 1, low salt levels showed higher water content compared to high salt levels ($P<0.01$). In IN 35, although water content in leaves was low at all salinity levels, these values were relatively similar. It was shown that the rice varieties, which leaf water content were clearly affected by salinity was CNT 1.

4.1.2 Effect on chlorophyll content in leaf

Chlorophyll is vital for photosynthesis as it helps to channel the energy of sunlight into chemical energy. With photosynthesis, chlorophyll absorbs energy and then transforms water and carbon dioxide into oxygen and carbohydrates (Nelson & Junge, 2015). For this reason, the study of post-use effects of organic osmolytes: proline and trehalose, on chlorophyll content help predict the potential benefits on photosynthesis ability of plants.

After foliar spraying the proline, chlorophyll contents were determined and presented in Table 3-5. The results of chlorophyll a content demonstrated a highly significant different ($P<0.01$) by proline factor but was not significant in varieties and salinity factor (Table 3). The application of proline at 50, 100, and 150 mM showed a significant increase of the chlorophyll a content compared with normal condition (0.25, 0.29, 0.31 and 0.30 mg/g) (Table 3). Chlorophyll b content study revealed that varieties and proline factor had a highly significant effect on chlorophyll b content. In addition, interactions between the varieties and salinity ($P<0.01$) and between the varieties and proline ($P<0.05$) also showed significant effected on the content of chlorophyll b (Table 4).

The highest chlorophyll b was recorded in PT. In proline factor, the higher the application of proline leads to the increase of the chlorophyll b content. The highest chlorophyll b was observed in 100 mM (3.54 mg/g) and 150 mM proline (3.70 mg/g). In varieties and salinity interaction, chlorophyll b content in these three rice varieties was slight change in decreasing when the salinity increased. Contrary to the results

was found by the influence of the interaction between two factors, varieties and proline factors. The content of chlorophyll b increased markedly with the use of 100 mM and 150 mM proline. The varieties that showed a predominantly favorable response to apply of proline were CNT 1, while the remaining two varieties responded subordinately (Table 4).

Table 3 Means of chlorophyll a content (mg/g) (±standard error) in leaf of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	0.27±0.02	0.29±0.04	0.20±0.05	0.21±0.03	0.30±0.04
	50	0.32±0.07	0.30±0.02	0.31±0.09	0.26±0.03	
	100	0.37±0.07	0.30±0.01	0.36±0.06	0.35±0.01	
	150	0.31±0.02	0.30±0.03	0.32±0.02	0.30±0.01	
PT 1	0	0.28±0.03	0.26±0.01	0.27±0.10	0.19±0.08	0.29±0.04
	50	0.35±0.06	0.30±0.04	0.32±0.03	0.26±0.05	
	100	0.34±0.04	0.29±0.03	0.31±0.04	0.28±0.02	
	150	0.28±0.02	0.26±0.04	0.37±0.02	0.33±0.01	
IN 35	0	0.29±0.01	0.21±0.02	0.23±0.04	0.30±0.01	0.28±0.03
	50	0.24±0.05	0.25±0.01	0.26±0.01	0.32±0.02	
	100	0.34±0.04	0.27±0.02	0.28±0.03	0.25±0.07	
	150	0.28±0.04	0.28±0.03	0.32±0.04	0.28±0.03	
Mean salinity		0.31±0.04	0.28±0.02	0.30±0.04	0.28±0.03	
		Proline (mM)				
		0	50	100	150	
Mean Proline		0.25±0.04b	0.29±0.04a	0.31±0.03a	0.30±0.02a	
P-value (F-test)						
Variety (V) = 0.2402 NS, Salinity (S) = 0.1701 NS, Proline (P) = 0.0023**, V × S = 0.5607 NS, V× P = 0.6071 NS, S× P = 0.7175 NS, V× S × P = 0.7914 NS, CV% = 24.2						

Note: CNT 1 = Chai Nat 1, PT1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.

It can be said that chlorophyll b content in leaves is controlled by genetic, and the degree of response to proline applying depended on genetic as well. From the results, surely, the use of proline can increases the content of chlorophyll b, but requires a relatively higher level of use 100 mM proline. Although CNT 1 is not a very high content of chlorophyll b when it has grown under non-salty condition. At a high proline level for application, the amount of chlorophyll b in CNT 1 was observed

rapidly and over other varieties. In addition, salinity had litter effect on chlorophyll b content.

Table 4 Means of chlorophyll b content (mg/g) (±standard error) in leaf of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	3.59±0.28	3.43±0.19	2.79±0.31	2.55±0.23	3.37±0.30b
	50	3.15±0.20	3.29±0.55	2.35±0.34	3.03±0.39	
	100	3.48±0.40	3.24±0.44	3.35±0.40	4.15±0.04	
	150	3.96±0.24	3.62±0.45	4.01±0.28	3.97±0.11	
PT 1	0	3.56±0.14	3.28±0.11	3.79±0.05	3.51±0.09	3.68±0.19a
	50	3.47±0.33	3.65±0.09	3.89±0.20	3.13±0.41	
	100	3.92±0.32	3.71±0.07	3.94±0.23	3.77±0.47	
	150	3.75±0.17	3.43±0.24	4.26±0.01	3.86±0.11	
IN 35	0	3.77±0.20	2.41±0.14	3.09±0.11	3.16±0.21	3.25±0.18b
	50	3.37±0.07	2.87±0.10	3.18±0.11	3.47±0.29	
	100	3.40±0.31	2.88±0.14	3.04±0.30	3.60±0.29	
	150	3.13±0.08	3.47±0.29	3.60±0.18	3.34±0.10	
Mean Salinity		3.56±0.23	3.27±0.23	3.44±0.21	3.46±0.23	
		Proline (mM)				
		0	50	100	150	
Mean Proline		3.24±0.17b	3.25±0.26b	3.54±0.28a	3.70±0.19a	
		Salinity dS/m				
		0	5	10	15	
CNT 1		3.54±0.28bd	3.40±0.41bd	3.12±0.33de	3.42±0.19bd	
PT 1		3.68±0.24ab	3.52±0.13bd	3.97±0.12a	3.57±0.27bc	
IN 35		3.47±0.16bd	2.91±0.17e	3.23±0.17ce	3.39±0.22bd	
		Proline (mM)				
		0	50	100	150	
CN 1		3.08±0.34cd	2.9±0.40d	3.58±0.37ab	3.89±0.27a	
PT 1		3.54±0.14ab	2.54±0.30ab	3.83±0.27a	3.82±0.22a	
IN 35		3.11±0.33cd	3.27±0.22bd	3.23±0.29bd	3.39±0.19bc	
P-value (F-test)						
Variety (V) = 2.48×10^{-5}**, Salinity (S) = 0.0600 NS, Proline (P) = 2.90×10^{-5}**, V × S = 0.0177*, V× P = 0.0424*, S× P = 0.0676 NS, V× S × P = 0.1792 NS, CV% = 13						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels
* Means significant difference at 0.05 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) mean significant difference at 0.05 level of probability

The results displayed that total chlorophyll were highly significant affected by varieties and proline factor at P< 0.01 level and also significant in salinity factor and

the interaction between varieties and salinity at P< 0.05 level (Table 5). The highest chlorophyll t was recorded in PT 1, and between CNT 1 and IN 35 was not statistically different. In salinity factor, the highest total chlorophyll content was in normal condition (0 dS/m) and there were not statistically different under 10 and 15 dS/m but lowest content at 5 dS/m of salinity level. In proline factor, the higher the application of proline was increased the total chlorophyll content compared with not use proline. So, the highest chlorophyll t was observed in 100 and 150 mM (proline) and the lowest were at 0 mM proline. In varieties and salinity interaction, total chlorophyll content showed a little change according to different salinity levels in every variety. Seems the highest value in each variety was observed at 0 dS/m or not salty, which highest was PT 1. However, at the difference among these varieties was not found at 15 dS/m (Table 5).

Table 5 Means of total chlorophyll content (mg/g) (±standard error) in leaf of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean varieties
		0	5	10	15	
CNT 1	0	3.86±0.30	3.72±0.21	2.99±0.37	2.76±0.25	3.67±0.31b
	50	3.46±0.16	3.60±0.57	2.66±0.28	3.29±0.42	
	100	3.85±0.46	3.54±0.45	3.71±0.37	4.50±0.04	
	150	4.26±0.26	3.92±0.48	4.32±0.30	4.27±0.12	
PT 1	0	3.84±0.17	3.55±0.10	4.06±0.15	3.69±0.08	3.97±0.21a
	50	3.82±0.37	3.95±0.12	4.20±0.23	3.39±0.45	
	100	4.26±0.32	4.00±0.09	4.25±0.27	4.05±0.48	
	150	4.03±0.17	3.69±0.28	4.63±0.02	4.18±0.11	
IN 35	0	4.07±0.21	2.62±0.17	3.32±0.15	3.46±0.20	3.52±0.19b
	50	3.81±0.02	3.11±0.09	3.44±0.12	3.79±0.28	
	100	3.74±0.26	3.14±0.12	3.31±0.32	3.85±0.33	
	150	3.41±0.09	3.75±0.31	3.92±0.21	3.62±0.13	
Mean salinity		3.87±0.23a	3.55±0.25b	3.73±0.23ab	3.74±0.24ab	
		Proline (mM)				
		0	50	100	150	
Mean Proline		3.50±0.20b	3.54±0.26b	3.85±0.29a	4.00±0.21a	
		Salinity dS/m				
Varieties		0	5	10	15	
CN 1		3.86±0.29bc	3.69±0.43bc	3.42±0.33cd	3.70±0.21bc	
PT 1		3.99±0.26ab	3.80±0.15bc	4.29±0.17a	3.83±0.28bc	
IN 35		3.76±0.15bc	3.16±0.17d	3.50±0.20cd	3.68±0.23bc	
P-value (F-test)						
Variety (V) = 3.83×10^{-5}**, Salinity (S) = 0.0456*, Proline (P) = 1.54×10^{-5}**, V × S = 0.0161*, V× P = 0.0595 NS, S× P = 0.0711 NS, V× S × P = 0.1908 NS, CV% = 12.7						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,

** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c…) mean significant difference at 0.05 level of probability

After the trehalose spray, the results of chlorophyll a content indicated highly significant in varieties factor (P< 0.01) and also significant in trehalose factor (P< 0.05). In varieties factor, the highest chlorophyll a level was recorded in Inpari 35 and PT 1, and the lower was found in CNT 1. In trehalose factor, chlorophyll a content was higher since at 100 mM trehalose, while at 50 mM and control (0 mM) of trehalose were the lowest (Table 6).

Table 6 Means of chlorophyll a (mg/g) (±standard error) in leaf of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	0.25±0.03	0.28±0.03	0.28±0.03	0.23±0.02	0.27±0.04b
	50	0.29±0.06	0.23±0.02	0.28±0.03	0.33±0.03	
	100	0.25±0.02	0.26±0.04	0.31±0.04	0.31±0.07	
	150	0.26±0.07	0.27±0.04	0.30±0.01	0.25±0.04	
PT 1	0	0.33±0.03	0.27±0.00	0.27±0.03	0.24±0.03	0.31±0.02a
	50	0.26±0.04	0.27±0.03	0.32±0.00	0.34±0.01	
	100	0.32±0.02	0.30±0.02	0.36±0.01	0.32±0.03	
	150	0.30±0.03	0.33±0.03	0.33±0.03	0.32±0.00	
IN 35	0	0.35±0.03	0.33±0.05	0.30±0.04	0.28±0.02	0.32±0.03a
	50	0.26±0.02	0.30±0.02	0.32±0.02	0.25±0.01	
	100	0.42±0.04	0.31±0.03	0.37±0.03	0.34±0.04	
	150	0.34±0.00	0.31±0.03	0.29±0.03	0.35±0.07	
Mean salinity		0.30±0.03	0.29±0.03	0.31±0.03	0.30±0.03	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		0.28±0.03b	0.29±0.02b	0.32±0.03a	0.30±0.03ab	
P-value (F-test)						
Variety (V) = 0.0008**, Salinity (S) = 0.4609 NS, Trehalose (T) = 0.0257 *, V × S = 0.7113 NS, V× T = 0.2648 NS, S × T = 0.2829 NS, V× S × T = 0.6054 NS, CV% = 19.4						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels
* Means significant difference at 0.05 levels of probability
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c…) mean significant difference at 0.05 level of probability

The effects on chlorophyll a content in rice leaves were both different and similar when compared with proline and trehalose sprays **(**Table 3 and Table 6**).** The difference was that there was a difference in the chlorophyll a content among three

rice varieties when sprayed with trehalose. The thing that is a similarity between spraying with both agents is both of them can also increase the chlorophyll a content.

Table 7 Means of chlorophyll b (mg/g) (±standard error) in leaf of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	2.75±0.33	2.73±0.10	2.95±0.23	2.60±0.27	2.80±0.22b
	50	2.72±0.13	2.69±0.26	3.16±0.33	2.94±0.12	
	100	2.45±0.06	2.55±0.21	2.87±0.12	2.49±0.30	
	150	2.88±0.04	2.98±0.48	3.34±0.05	2.81±0.34	
PT 1	0	3.51±0.31	2.96±0.20	2.97±0.39	2.78±0.08	3.26±0.22a
	50	3.09±0.09	2.50±0.12	3.41±0.05	3.69±0.20	
	100	3.47±0.30	3.37±0.28	3.69±0.04	3.57±0.36	
	150	3.08±0.36	3.59±0.45	3.02±0.19	3.47±0.11	
IN 35	0	3.86±0.08	3.68±0.34	3.21±0.39	2.89±0.17	3.35±0.26a
	50	2.97±0.12	3.42±0.23	3.59±0.39	2.93±0.13	
	100	3.86±0.10	3.37±0.36	3.50±0.37	3.14±0.43	
	150	3.86±0.12	3.43±0.39	2.85±0.12	3.27±0.39	
Mean Salinity		3.20±0.18	3.11±0.29	3.21±0.22	3.05±0.24	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		3.07±0.24	3.09±0.18	3.19±0.24	3.20±0.26	
		Salinity dS/m				
		0	5	10	15	
CNT 1		2.70±0.16c	2.74±0.24c	3.08±0.18bc	2.70±0.26c	
PT 1		3.29±0.27ab	3.10±0.26bc	3.27±0.17ab	3.38±0.19ab	
IN 35		3.60±0.10a	3.48±0.33ab	3.29±0.32ab	3.06±0.28bc	
P-value (F-test)						
Variety (V) = 7×10^{-8}**, Salinity (S) = 0.3888 NS, Trehalose (T) = 0.5239 NS, V × S = 0.0324*, V× T = 0.0770 NS, S× T = 0.0698 NS, V× S × T = 0.6663 NS, CV% = 14.6						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels
* Means significant difference at 0.05 levels of probability,
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) mean significant difference at 0.05 level of probability

After foliar application of trehalose, the results of chlorophyll b content were highly significantly affected by varieties factor ($P< 0.01$) and also significant in the interaction between varieties and salinity ($P< 0.05$) (Table 7). The higher chlorophyll b content was in IN 35 and PT 1, and the lowest was CNT 1. Chlorophyll b content was increased according to the increasing level of trehalose application. There was no significant difference affected by trehalose application. For the interaction of varieties and salinity level, a few changes at different salinity levels were found in each rice

variety. However, the highest of chlorophyll b was observed at 0 dS/m salinity or non-salty condition.

The results of total chlorophyll were significant difference affected by varieties factor and the interaction between rice varieties and salinity levels in significant level at 0.01 and 0.05, respectively (Table 8). The response to total chlorophyll content was not different from the response of chlorophyll b when rice varieties were exposed to different salinity or trehalose levels (Table 7-8).

Table 8 Means of total chlorophyll (mg/g) (±standard error) in leaf of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	2.99±0.36	3.01±0.12	3.22±0.26	2.83±0.29	3.08±0.24b
	50	3.01±0.09	2.92±0.28	3.44±0.35	3.27±0.15	
	100	2.70±0.08	2.81± 0.14	3.17±0.15	2.79±0.27	
	150	3.14±0.20	3.25±0.52	3.64±0.06	3.06±0.38	
PT 1	0	3.84±0.34	3.22±0.20	3.23±0.42	3.02±0.10	3.57±0.24a
	50	3.35±0.13	2.76±0.15	3.73±0.05	4.03±0.21	
	100	3.79±0.32	3.67±0.30	4.05±0.04	3.89±0.39	
	150	3.38±0.39	3.92±0.48	3.35±0.17	3.80±0.11	
IN 35	0	4.21±0.08	4.01±0.38	3.51±0.43	3.17±0.19	3.67±0.28a
	50	3.23±0.14	3.71±0.22	3.91±0.37	3.19±0.14	
	100	4.26±0.13	3.68±0.39	3.86±0.39	3.48±0.40	
	150	4.04±0.12	3.74±0.42	3.14±0.15	3.61±0.46	
Mean salinity		3.50±0.20	3.39±0.31	3.52±0.21	3.34±0.26	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		3.36±0.26	3.38±0.19	3.51±0.26	3.51±0.29	
		Salinity dS/m				
		0	5	10	15	
CNT 1		2.96±0.18c	3.00±0.29c	3.37±0.20bc	2.99±0.27c	
PT 1		3.59±0.30ab	3.40±0.28bc	3.59±0.17ab	3.68±0.20ab	
IN 35		3.94±0.12a	3.79±0.35ab	3.60±0.34ab	3.36±0.30bd	
P-value (F-test)						
Variety (V) = 5.53 $\times 10^{-7}$**, Salinity (S) = 0.3853 NS, Trehalose (T) = 0.3926 NS, V × S = 0.0402*, V× T = 0.0827 NS, S× T = 0.0578 NS, V× S × T = 0.6185NS, CV% = 14.2						

Note: CNT1 = Chai Nat 1, PT1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels of probability, NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) mean significant difference at 0.05 level of probability

4.1.3 Effect on proline content in leaf and stem

Proline is an amino acid and plays an important role in plants. It protects the plants from various stresses and also helps plants to recover from stress more rapidly.

Table 9 Means of proline content in leaf (µmole/g) (±standard error) of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	1.42±0.13	1.64±0.41	1.42±0.24	1.36±0.18	1.69±0.33b
	50	1.75±0.22	1.09±0.14	1.80±0.46	1.16±0.21	
	100	1.68±0.39	2.36±0.65	1.92±0.39	1.54±0.40	
	150	2.01±0.30	2.52±0.44	1.68±0.34	1.77±0.29	
PT 1	0	2.20±0.24	1.47±0.34	0.94±0.21	1.31±0.26	1.54±0.31b
	50	1.93±0.36	1.76±0.48	1.36±0.11	1.71±0.47	
	100	1.66±0.18	1.55±0.43	1.66±0.49	0.92±0.18	
	150	1.80±0.08	1.68±0.42	1.41±0.38	1.33±0.29	
IN 35	0	1.74±0.29	1.58±0.13	1.68±0.30	2.32±0.06	2.12±0.35a
	50	1.88±0.19	1.90±0.32	2.85±0.40	1.61±0.24	
	100	2.81±1.02	1.91±0.56	2.26±0.35	1.76±0.31	
	150	1.80±0.35	2.78±0.50	2.84±0.12	2.24±0.38	
Mean Salinity		1.89±0.31	1.85±0.40	1.82±0.32	1.59 ±0.27	
		Proline (mM)				
		0	50	100	150	
Mean Proline		1.59±0.23	1.73±0.30	1.84±0.45	1.99±0.32	
P-value (F-test)						
Variety (V) = 5.94×10^{-5}** , Salinity (S) = 0.1760 NS, Proline (P) = 0.0599 NS, V × S = 0.2567 NS, V× P = 0.4390 NS, S× P = 0.3204 NS, V× S × P = 0.5344 NS, CV% = 35.4						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c…) mean significant difference at 0.05 level of probability

After the foliar application of proline, the results showed that proline accumulation in leaf and stem (µmole/g) was highly significant only in varieties factor with significant at 0.01 levels. There were no interactions insignificant difference between these factors affected on proline accumulation both in leaves and stem (Table 9-10). The maximum proline accumulation in leaf was recorded in IN 35 followed by CNT 1 and PT 1. For three rice varieties, proline accumulation was the same trend under salinity condition which increased the salinity level, decreased the proline content [in CNT 1 as 1.71-1.45 µmole/g at 0-15 dS/m (decreased at -15.20%) in leaf

and as 1.30-1.08 µmole/g) at 0-15 dS/m (decreased at -16.92%) in stem, in PT 1, as1.89-1.31 µmole/g at 0-15 dS/m (decreased at -30.68%) in leaf and as 1.10-1.03 µmole/g (decreased at -6.36%) in stem in PT 1]. Under salinity condition (0-15 dS/m) of IN35 was 2.05-1.98 µmole/g (decreased at -3.41%) in leaf and as 1.7-1.51 µmole/g (decreased at -11.17%) in stem. Proline accumulation increased with the increase the application of proline at 0-150 mM, increased +36.30% in leaf and +4.83% in stem in CNT 1. PT 1 also increased +4.72% in leaf and +27.64% stem. In IN 35 increased +33.88% in leaf and +20.27% in stem, respectively. Under salinity condition, decreasing percentage was highest in PT 1 and increasing percentage was highest in IN 35 under foliar spray of proline in three rice varieties.

Although there were no significant in salinity and proline factor but the results suggested that the high salinity level decreased the proline accumulation in leaves while the high proline level increased the proline content in leaf (Table 9).

The highest proline accumulation in the stem was observed in IN 35, followed by CNT 1 and PT 1, respectively. For salinity level, although the decreasing of proline accumulation in the stem when salinity level increased, but with a non-significant difference. Opposite with proline factor, proline accumulation in stem seems higher at higher concentration of proline application; it also does not significantly different (Table 10).

It was observed that the change in the proline content in the stem was in the same direction as that found in the leaves. Nevertheless, the change of proline accumulation has occurred in a smaller change in stem compared to the leaves (Table 9-10).

After trehalose application, the results revealed that proline accumulation in leaf and stem of dry tissue were highly significantly affected by varieties factor. For proline content in leaf, the maximum accumulation was in IN 35 followed by PT 1 and CNT 1 (Table 11). However, in stem, the highest proline content was found in IN 35 and PT 1, and lower in CNT 1 (Table 12). Although there was no significant difference in trehalose factor, proline accumulation increased both in leaf and stem when the level of trehalose for application increased (Table 11 and 12). Highly significant difference on proline content was affected by salinity factor only in stem part (Table 11-12).

Proline content in stem, the reduction of proline content was observed in plants growing at higher salinity levels (Table 12). Proline accumulation decreased with the increase the salinity level 0-15 dS/m in three rice varieties. Proline content decreased -12.15% in leaf and -19.58 in stem (CNT 1). PT 1 also decreased -1.85% in leaf and -8.08% in stem. IN 35 decreased – 0.99% in leaf and -24.64% in stem under salinity condition. Proline content increased with the increase the application of trehalose 0 mM-150 mM in tested varieties. Proline accumulation increased +21.16 in leaf and +13.33% in stem (CNT 1). PT 1 also increased +1.85% in leaf and +7.01% in stem. IN 35 also increased +15.15 in leaf and +5.60% in stem, respectively.

Table 10 Means of proline content in stem (µmole/g) (±standard error) of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	1.15±0.29	1.40±0.13	1.28±0.20	1.16±0.47	1.34±0.19b
	50	1.21±0.43	1.62±0.04	1.20±0.21	0.88±0.11	
	100	1.51±0.28	1.87±0.15	1.88±0.03	1.14±0.25	
	150	1.34±0.17	1.27±0.19	1.45±0.01	1.16±0.15	
PT 1	0	1.15±0.07	1.16±0.17	1.10±0.14	0.89±0.29	1.12±0.20c
	50	1.14±0.19	1.12±0.30	1.04±0.16	1.51±0.39	
	100	1.00±0.15	1.19±0.13	0.93±0.03	0.81±0.05	
	150	1.13±0.23	1.62±0.20	1.27±0.31	0.92±0.33	
IN 35	0	1.40±0.20	1.47±0.44	1.39±0.45	1.48±0.34	1.64±0.29a
	50	2.12±0.32	1.45±0.29	2.05±0.44	1.23±0.28	
	100	1.48±0.13	1.80±0.20	1.60±0.32	1.84±0.38	
	150	1.90±0.21	1.87±0.19	1.62±0.14	1.49±0.36	
Mean Salinity		1.38±0.21	1.49±0.19	1.40±0.23	1.21±0.30	
		Proline (mM)				
		0	50	100	150	
Mean Proline		1.25±0.27	1.38±0.27	1.42±0.18	1.42±0.21	
P-value (F-test)						
Variety (V) = 5.68×10^{-6}**, Salinity (S) = 0.106 NS, Proline (P) = 0.398 NS, V × S = 0.825 NS, V× P = 0.363 NS, S× P = 0.988 NS, V× S × P = 0.663 NS, CV% = 35						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c…) mean significant difference at 0.05 level of probability

To explain in the percentage of induction or reduction of proline content both in leaf and stem. Under the non-salty conditions, a rice variety CNT 1 was 1.71 and 2.04 µmole/g at 0 and 150 mM trehalose, respectively (increased at +19.30%). PT 1 was 1.43 and 1.70 µmole/g at 0 and 150 mM trehalose, respectively (increased at +18.88%) were less accumulation of proline in leaf compared to IN 35. IN 35 was 1.61 and 2.53 µmole/g at 0 and 150 mM trehalose, respectively (increased at +57.14%) (Table 11). All three varieties had higher accumulations when sprayed with trehalose than when sprayed with proline (Table 9 and 11). In non-stress plants, trehalose (sugar) applying showed an increase in proline content (amino acid) in leaves (Table 11).

Table 11 Means of proline content in leaf (µmole/g) (±standard error) of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	1.71±0.23	1.45±0.24	1.06±0.05	1.28±0.43	1.56±0.24b
	50	1.79±0.32	1.37±0.20	1.23±0.30	1.79±0.32	
	100	1.70±0.18	1.12±0.12	2.15±0.32	1.57±0.26	
	150	2.04±0.23	1.09±0.08	1.81±0.36	1.72±0.16	
PT 1	0	1.43±0.2.9	1.54±0.38	1.64±0.31	1.40±0.41	1.66±0.32b
	50	1.74±0.42	1.98±0.47	1.02±0.04	1.56±0.35	
	100	1.62±0.14	1.99±0.41	1.66±0.28	2.05±0.19	
	150	1.70±0.37	2.11±0.26	1.54±0.41	1.60±0.42	
IN 35	0	1.61±0.42	1.98±0.36	2.31±0.40	2.03±0.34	2.01±0.33a
	50	1.81±0.35	1.81±0.33	1.75±0.40	1.92±0.06	
	100	2.01±0.19	2.07±0.33	1.76±0.24	1.59±0.33	
	150	2.53±0.29	2.74±0.27	1.78±0.26	2.50±0.29	
Mean Salinity		1.80±0.29	1.77±0.29	1.64±0.28	1.75±0.30	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		1.62±0.32	1.65±0.30	1.77±0.25	1.93±0.28	
P-value (F-test)						
Variety (V) = 0.0001 **, Salinity (S) = 0.6008 NS, Trehalose (T) = 0.0611 NS, V × S = 0.1242 NS, V× T = 0.4257 NS, S× T = 0.8003 NS, V× S × T = 0.4852 NS, CV% = 30.5						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels of probability, NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) means significant difference at 0.05 level of probability

Whereas in the non-trehalose supplements, when the three rice varieties were subjected to growth under the increasing of salinity, only IN 35 had an increase in proline content accumulated in leaves (was 1.61 and 2.03 µmole/g at 0 and 15 dS/m, respectively or increased at +26.09%); which has an increase similar to that present in Table 9 (at +33.33%). While, there was a significant reduction in proline content in leaves in both Thai rice varieties as CNT 1 (was 1.71 and 1.28 µmole/g at 0 and 15 dS/m, respectively or decreased at -25.15%) and PT 1 (was 1.43 and 1.40 µmole/g at 0 dS/m and 15 dS/m, respectively or decreased at -2.10%) (Table 11). Although the effect (negative percentage) on salinity was not different from previous studies in CNT (-4.23%) and PT 1 (-40.45%) shown in Table 9. However, in this study, the degree of salinity impact of the two Thai rice varieties was different, with CNT 1 being more affected than PT 1 (Table 11).

Table 12 Means of proline content in stem (µmole/g) (±standard error) of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	1.00±0.11	0.70±0.19	0.73±0.12	0.57±0.10	0.82±0.15b
	50	1.02±0.17	0.73±0.25	0.75±0.11	0.68±0.05	
	100	0.95±0.13	0.82±0.17	0.81±0.03	0.88±0.17	
	150	0.92±0.12	0.73±0.24	0.78±0.13	1.00±0.37	
PT 1	0	1.34±0.04	0.90±0.03	1.14±0.17	1.18±0.06	1.21±0.21a
	50	1.53±0.18	1.02±0.17	0.97±0.27	1.21±0.12	
	100	1.09±0.31	1.42±0.30	1.15±0.12	1.63±0.63	
	150	1.07±0.21	1.45±0.17	0.92±0.22	1.45±0.30	
IN 35	0	1.71±0.20	0.92±0.22	0.91±0.32	0.75±0.34	1.11±0.28a
	50	1.41±0.80	1.06±0.09	0.84±0.08	0.91±0.21	
	100	1.59±0.32	1.08±0.35	1.14±0.38	1.30±0.13	
	150	1.42±0.29	0.93±0.22	0.76±0.23	1.04±0.24	
Mean Salinity		1.26±0.24a	0.98±0.20b	0.91±0.18b	1.05±0.23ab	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		0.98±0.16	1.01±0.21	1.16±0.26	1.04±0.23	
P-value (F-test)						
Variety (V) = 8.8 $\times 10^{-5}$**, Salinity (S) = 0.0094**, Trehalose (T) = 0.3916 NS, V × S = 0.2683 NS, V× T = 0.9875 NS, S × T = 0.6540 NS, V× S × T = 0.9993 NS, CV% = 42						

Note: CNT 1 = Chai Nat 1, PT1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels of probability, NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) mean significant difference at 0.05 level of probability

4.1.4 Effect on sugar content in leaf and stem

In plants, energy and carbon requirement for various processes of growth and development is met through sugars. Sugars synthesized in photosynthesis are transported to sink tissues, and channeled to respiration or converted into storage compounds (lipids, starch, protein, sucrose, fructose). After the proline spray, the results demonstrated sugar content in leaf was significant at $P< 0.05$ level and stem was highly significant in varieties factor at $P< 0.01$ level (Table 13 and 14).

The highest sugar content in leaf was found in PT 1, IN 35 and the lowest in CNT 1. In salinity factor was not a specific trend, however, at salinity conditions, it showed the sugar content more than at no salinity. For the proline factor, increased application of proline resulted in gradually decreasing the sugar content with a non-significant difference (Table 13).

Table 13 Means of sugar content in leaf (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	36.5±3.3	43.8±5.0	36.6±1.6	48.0±7.2	40.48±4.6b
	50	35.1±3.8	40.7±2.9	41.7±5.5	47.2±2.2	
	100	40.3±7.9	39.6±4.5	35.5±6.1	42.2±3.3	
	150	37.9±6.7	36.6±2.5	43.5±5.6	42.5±5.1	
PT 1	0	43.4±3.3	44.1±6.7	40.4±4.1	47.7±1.0	45.58±5.3a
	50	45.9±2.7	48.2±5.4	52.3±5.8	47.7±6.7	
	100	43.0±5.2	47.1±8.5	58.3±7.7	37.3±4.7	
	150	42.1±3.7	47.6±6.2	44.4±0.7	39.9±3.0	
IN 35	0	50.6±1.3	51.7±1.5	53.7±6.0	46.1±3.0	44.54±4.2a
	50	31.9±7.0	43.4±3.7	48.6±2.5	44.4±2.3	
	100	43.4±3.3	44.2±5.1	34.3±5.8	50.9±7.9	
	150	47.5±5.2	48.1±5.2	43.7±5.8	43.7±1.3	
Mean Salinity		41.46±4.8	44.57±4.8	43.69±4.8	44.41±4.4	
		Proline (mM)				
		0	50	100	150	
Mean Proline		45.5±4.0	43.5±4.6	42.8±5.8	42.1±4.2	
P-value (F-test)						
Variety (V) = 0.031*, Salinity (S) = 0.514 NS, Proline (P) = 0.495 NS, V × S = 0.499 NS, V× P = 0.404 NS, S× P = 0.807 NS, V× S × P = 0.514 NS, CV% = 22						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
* Means significant difference at 0.05 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c…) means significant difference at 0.05 level of probability

In stem, the maximum sugar content was recorded in CNT 1 and followed by PT 1 and IN 35. Although, there was non-significant in salinity factor, increased the applying of salinity level decreased the sugar content in stem (Table 14). These happening on sugar content in the leaf part were different from what happens in the stem part. Response on salinity stress for sugar accumulation in the leaf was increasing, but in the stem was decreasing. However, the things that happen similarly in leaf and stem parts were that the application for proline not significantly affected sugar content, although seem to decrease that value (Table 13 and 14). Overall mean, it could to say that in PT 1 had more sugar content in leaf 45.58 mg/g while in CNT 1 was 84.11 mg/g in stem determined this trait at flowering stage in rice (Table 13 and 14).

Table 14 Means of sugar content in stem (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	75.0±4.9	93.5±2.7	94.6±11.0	85.5± 12.6	84.11±10.4a
	50	79.9±12.0	95.8±1.6	83.0±4.6	82.9±12.0	
	100	77.7±12.2	78.5±13.4	88.8±17.8	82.9±10.2	
	150	74.0±3.1	79.9±16.4	88.0±16.7	85.8±13.5	
PT 1	0	92.9±10.0	68.0±6.9	68.3±13.2	75.5±13.1	71.60±11.6b
	50	76.0±13.7	69.9±13.5	66.0±7.7	70.1±9.9	
	100	76.8±12.2	53.9±5.6	62.0±16.0	69.8±3.6	
	150	77.7±13.9	75.8±13.1	75.9±16.9	67.1±15.7	
IN 35	0	63.7±15.1	70.7±21.1	67.4±14.6	53.7±8.9	63.64±11.1b
	50	77.6±14.3	66.0±11.5	65.6±14.2	53.3±6.4	
	100	58.2±7.8	72.6±13.2	58.8±3.7	63.5±10.9	
	150	74.1±17.1	70.0±8.9	51.2±5.5	52.0±4.3	
Mean Salinity		75.29±11.3	74.55±10.7	72.45±11.8	70.17±10.3	
		Proline (mM)				
		0	50	100	150	
Mean Proline		75.72±11.2	73.83±10.1	70.27±10.6	72.64±12.3	
P-value (F-test)						
Variety (V) = 2.41 $\times 10^{-6}$**, Salinity (S) = 0.722 NS, Proline (P) = 0.727 NS, V × S = 0.241 NS, V× P = 0.973 NS, S× P = 0.998 NS, V× S × P = 0.993 NS, CV% = 28.3						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) means significant difference at 0.05 level of probability

A comparison of the sugar content in percentage change was performed as well as the proline content in the leaf or stem sections. At the no stress from salinity at 0 dS/m, less change in sugar content was observed in all rice varieties received proline at 150 mM by spray. The results showed the changing percentage value of sugar content in CNT 1 (was 36.5 and 37.9 mg/g at 0 and 150 mM proline, respectively or increase at +3.84 %) and PT1 (was 43.4 and 42.1 mg/g at 0 and 150mM proline, respectively or decrease at -3.00 %) were lowest when compared to IN 35 (was 50.6 and 47.5 mg/g at 0 and 150 mM proline, respectively or decrease at -6.13 %) (Table 13).

Table 15 Means of sugar content in leaf (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	48.3±3.6	63.5±5.8	56.2±0.2	75.0±3.9	61.46±4.9a
	50	70.5±8.0	61.1±2.8	62.3±2.4	66.2±7.0	
	100	57.0±5.4	59.5±6.3	57.2±6.4	58.9±4.8	
	150	65.9±5.1	66.5±6.2	61.7±6.1	53.5±4.4	
PT 1	0	51.2±1.0	59.7±4.1	57.1±4.6	55.0±4.2	58.61±4.3ab
	50	68.2±2.3	64.9±6.4	59.1±4.5	61.6±5.6	
	100	54.9±4.5	57.4±5.4	59.6±3.5	52.5±4.4	
	150	64.8±4.0	61.5±7.4	50.3±3.2	60.0±3.3	
IN 35	0	52.2±3.0	53.6±6.2	58.7±6.2	47.1±4.1	54.51±3.7b
	50	55.3±1.5	50.5±5.1	52.5±5.5	53.8±0.5	
	100	58.1±3.6	50.7±2.1	53.6±2.7	65.5±2.8	
	150	50.4±3.0	50.1±5.5	56.4±7.0	67.1±1.3	
Mean Salinity		58.06±3.8	58.25±5.3	57.06±4.3	59.67±3.9	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		56.45±3.9	60.50±4.3	57.07±4.4	59.00±4.7	
P-value (F-test)						
Variety (V) = 0.0056**, Salinity (S) = 0.7431 NS, Trehalose (T) = 0.3050 NS, V × S = 0.5836 NS, V× T = 0.5035 NS, S× T = 0.6414 NS, V× S × T = 0.2475 NS, CV% = 17.2						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels of probability, NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) means significant difference at 0.05 level of probability

After foliar application of trehalose, the results exhibited sugar content in leaf and stem (mg/g) was highly significant in varieties factor at P< 0.01 level. The highest sugar content was found in CNT 1, followed by PT 1, and the lowest in IN 35. Although there were not significant in proline factor, the sugar content in leaf was lower in the normal condition (0 mM trehalose) than compared with the application of trehalose (50-150 mM trehalose); excluding on sugar content in the stem at 150 mM trehalose application (Table 15 and 16).

Table 16 Means of sugar content in stem (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CN 1	0	86.6±6.4	88.8±15.1	87.1±9.4	78.8±9.6	86.14±10.7a
	50	78.5±22.7	77.5±12.2	93.9±10.6	90.1±11.7	
	100	93.4±10.7	83.4±15.0	94.7±1.9	95.8±8.0	
	150	88.0±11.8	82.3±7.6	66.9±7.9	92.6±10.9	
PT 1	0	96.0±11.5	75.3±8.0	74.7±11.3	77.9±5.9	77.54±8.9b
	50	92.7±2.1	81.8±8.6	71.8±16.9	92.6±8.5	
	100	73.9±14.2	85.6±2.8	76.9±21.2	73.3±7.1	
	150	69.8±9.6	61.9±2.6	72.3±4.9	63.1±7.3	
IN 35	0	80.3±3.5	54.2±4.3	45.5±10.6	52.9±14.9	59.34±8.4c
	50	69.4±4.1	68.5±14.5	48.2±5.7	62.8±8.7	
	100	66.5±12.3	65.0±12.6	57.5±7.1	61.0±13.1	
	150	52.9±3.3	59.3±3.8	48.8±2.7	56.6±13.1	
Mean Salinity		79.08±9.3	73.63±8.9	69.85±9.2	74.79±9.9	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		74.93±9.2	77.31±10.5	77.23±10.5	67.86±7.1	
P-value (F-test)						
Variety (V) = 4.16×10^{-10}**, Salinity (S) = 0.1993 NS, Trehalose (T) = 0.0967 NS, V × S = 0.7393 NS, V × T = 0.7512 NS, S × T = 0.8566 NS, V× S × T = 0.8776 NS, CV% = 24						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation, ** Means significant difference at 0.01 levels of probability, NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) mean significant difference at 0.05 level of probability

4.1.5 Effect on starch content in leaf and stem

After the proline spray, the results displayed that starch content in leaf was not significant in main factors and the interaction between of them. However, in proline factor, increase the foliar application of proline, trended to increase the starch content in leaf (Table 17). Similarly, the results of starch content in leaf were non-significant affected by both varieties and salinity factors (at P> 0.05 level). Also, non-significant differences were affected by varieties and salinity interaction (at P> 0.05 level) (Table 17). However, varieites factor and interaction between varieties and salinity showed significant differences in starch content in stem (Table 18). In stem, the highest starch content was recorded in IN 35 followed by PT 1 and CNT 1. The interaction between varieties and salinity factor, considering the mean of each salinity level of each rice cultivar, it was found that CNT 1 tended to experience decreased starch accumulation when the salinity level increased. While the remaining two varieties of rice, PT 1 and IN 35, starch accumulation tended to increase.

Table 17 Means of starch content in leaf (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	6.4±0.5	7.2±1.2	7.7±2.0	5.0 0.1	7.46±1.6
	50	11.9±5.9	7.0±0.6	7.7±1.6	7.9±1.5	
	100	10.7±3.4	5.4±1.0	5.2±0.8	5.8±0.5	
	150	10.6±1.7	6.4±1.4	10.0±2.7	4.5±0.6	
PT 1	0	5.6±0.5	7.9±2.6	9.2±1.2	6.0±1.3	9.07±2.9
	50	7.9±3.3	6.7±2.2	7.4±1.1	7.5±1.8	
	100	13.0±7.9	6.7±2.3	16.0±6.2	11.9±3.1	
	150	17.4±6.1	7.1±2.0	7.4±1.8	7.4±3.5	
IN 35	0	10.7±1.9	11.3±3.7	7.9±1.2	8.5±2.2	8.93±1.5
	50	8.7±0.8	7.7±0.9	8.6±1.9	9.2±0.7	
	100	8.9±1.2	8.2±2.7	9.7±1.3	7.7±1.7	
	150	8.8±1.0	8.1±2.0	9.3±0.1	9.4±0.6	
Mean Salinity		10.05±2.9	7.50±1.8	8.84 ±1.8	7.57±1.5	
		Proline (mM)				
		0	50	100	150	
Mean Proline		7.7±1.5	8.2±1.9	9.0±2.6	8.9±1.9	
P-value (F-test)						
Variety (V) = 0.1548 NS, Salinity (S) = 0.0542 NS, Proline (P) = 0.5926 NS, V × S = 0.7503 NS, V× P = 0.1830 NS, S × P = 0.6198 NS, V× S × P = 0.7979 NS, CV% = 52						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
NS means non-significant difference at 0.05 level of probability.

After foliar application of trehalose, the results indicated that starch accumulation in leaf of dry weight showed non-significant at P> 0.05 level for all factors both in individual and interaction among these factors (Table 19). However, starch accumulation in stem of dry tissue was shown highly significant (P< 0.01) affected by varieties factor (Table 20). The maximum starch accumulation was observed in IN 35 and followed by CN 1 and PT 1. But the results were not regular trend the accumulation of starch in leaf and stem.

Although the trehalose factor was a non-significant factor on starch content accumulated in the stem, the application of trehalose trended to increase the starch content compared with the normal condition (0 mM trehalose) (Table 20). Therefore, trehalose spraying did not promote increased starch accumulation in both leaves and stems for testing in all three rice varieties. In addition, spraying of trehalose in conditions where the plants were experiencing saline soils (15 dS/m) showed very little change in the starch content (both in leaves and stems in each variety) compared with at 0 mM salinity (Table 19-20).

Table 18 Means of starch content in stem (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	70.6±3.8	41.2±9.2	54.6±3.8	40.8±8.0	54.7±6.6b
	50	82.0±7.6	45.6 ± 3.7	59.3±9.1	64.0±12.1	
	100	49.5±7.7	44.7±6.2	42.5±0.9	54.8±6.1	
	150	48.8±10.2	50.9±1.3	47.8±3.3	77.9±13.2	
PT 1	0	46.6±5.0	53.8±9.1	55.1±4.2	63.5±7.5	57.24±8.8b
	50	51.0±1.1	53.9±8.6	67.7±8.6	61.0±9.7	
	100	63.5±12.5	62.5±8.8	63.6±12.3	63.3±12.6	
	150	52.5±10.1	53.5±10.6	58.5±12.5	46.1±7.3	
IN 35	0	56.2±10.2	57.8±13.5	58.5±16.2	62.4±14.8	65.44±10.2a
	50	70.7±7.0	69.1±8.6	71.5±2.6	72.6±9.1	
	100	63.3±14.5	71.3±11.8	67.5±6.7	77.8±6.6	
	150	60.4±6.3	73.0±18.6	51.1±6.1	63.4±11.3	
Mean salinity		58.3±8.0	58.3±9.2	60.8±7.2	60.0±9.9	
		Proline (mM)				
		0	50	100	150	
Mean Proline		56.4±8.8	62.9±7.3	57.7±8.9	59.3±9.2	
		Salinity dS/m				
Varieties		0	5	10	15	
CNT 1		62.7±7.3ab	45.5±5.1c	51.0±4.3bc	59.4±9.8abc	
PT 1		53.3±7.2bc	55.8±9.3abc	61.2±9.3ab	58.4±9.3abc	
IN 35		58.7±9.4abc	70.9±13.5a	70.0±7.9a	61.9±10.4ab	
P-value (F-test)						
Variety (V) = 0.0048**, Salinity (S) = 0.8182 NS, Proline (P) = 0.3617 NS, V × S = 0.0423*, V× P = 0.3493 NS, S× P = 0.9634 NS, V× S × P = 0.5537 NS, CV% = 27						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
** Means significant difference at 0.01 levels
* Means significant difference at 0.05 levels of probability, respectively
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c…) mean significant difference at 0.05 level of probability

Positive change was observed in IN 35 (59.2 and 62.1 mg/g at 15 dS/m salinity, respectively or increase at +4.90%), but negative change in PT 1 (62.2 and 58.6 mg/g at 15 dS/m salinity, respectively or decrease at -5.79%) and CNT 1 (56.2 and 54.6 mg/g at 15 dS/m salinity, respectively or decrease at -2.85%) on starch content in stem under the application of trehalose (150 mM trehalose) (Table 20).

Table 19 Means of starch content in leaf (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	8.7±1.0	11.0±3.5	8.6±1.1	12.2±2.6	10.01±2.0
	50	10.5±1.2	9.0±1.2	16.6±6.8	14.1±4.0	
	100	11.0±3.6	9.0±1.5	8.3±0.3	7.0±0.7	
	150	8.5±0.7	7.4±0.9	8.8±1.1	9.4±2.1	
PT 1	0	12.7±2.2	10.8±2.3	8.8±1.8	12.4±4.0	10.92±2.4
	50	7.6±1.2	10.8±0.2	11.1±3.6	9.2±1.4	
	100	7.4±1.5	10.0±1.9	10.6±2.6	9.4±1.5	
	150	9.1±1.7	11.6±2.5	18.5±6.6	14.6±3.9	
IN 35	0	11.5±2.3	11.1±2.3	10.2±2.5	10.3±1.3	11.0±1.9
	50	11.8±2.4	11.5±2.4	10.8±2.0	11.6±1.2	
	100	11.9±2.2	15.0±4.5	9.6±1.4	9.6±1.2	
	150	9.8±0.5	10.9±1.6	10.3±1.9	10.2±0.8	
Mean Salinity		10.06±1.7	10.68±2.1	11.01±2.6	10.83±2.1	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		10.71±2.2	11.22±2.3	9.89±1.9	10.74±2.0	
P-value (F-test)						
Variety (V) = 0.475 NS, Salinity (S) = 0.810 NS, Trehalose (T) = 0.636 NS, V × S = 0.611 NS, V × T = 0.075 NS, S × T = 0.541 NS, V× S × T = 0.915 NS, CV% = 41						

Note: CNT 1 = Chai Nat 1, PT1 = Pathum Thani 1, IN 35= Inpari 35
CV= Coefficient of variation,
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) means significant difference at 0.05 level of probability

Table 20 Means of starch content in stem (mg/g) (±standard error) of three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	48.9±10.4	54.5±7.1	54.2±7.5	56.2±6.6	55.24±7.9b
	50	44.6±4.3	67.5±10.1	47.4±2.8	49.0±5.6	
	100	63.2±19.2	67.5±12.0	53.0±2.8	56.0±8.0	
	150	60.8±7.8	50.8±8.4	55.8±11.8	54.6±2.0	
PT 1	0	55.0±6.4	61.5±9.7	54.3±1.3	62.2±4.6	59.88±6.8ab
	50	48.9±2.7	65.9±4.6	57.6±6.0	69.6±10.9	
	100	70.2±10.3	63.9±5.6	58.9±11.3	52.7±5.8	
	150	55.6±2.9	59.6±9.6	63.3±7.2	58.6±9.8	
IN 35	0	55.0±3.9	69.3±7.5	62.0±5.3	59.2±8.1	64.61±7.2a
	50	57.8±7.9	67.6±8.5	69.9±8.6	71.5±8.9	
	100	57.3±3.6	77.6±8.5	66.2±6.5	50.1±6.4	
	150	61.1±6.1	71.0±12.9	76.2±7.8	62.1±5.3	
Mean Salinity		56.52±7.1	64.74±8.7	59.89±6.6	58.48±6.8	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		57.69±6.5	59.75±6.7	61.38±8.3	60.79±7.6	
P-value (F-test)						
Variety (V) = 0.0056**, Salinity (S) = 0.0818 NS, Trehalose (T) = 0.6883 NS, V × S = 0.6908 NS, V × T = 0.8311 NS, S × T = 0.3142 NS, V× S × T = 0.9827 NS, CV% = 23						

Note: CN1 = Chai Nat 1, PT1 = Pathum Thani 1, IN 35 = Inpari 35
CV = Coefficient of variation, ** Means significant difference at 0.01 levels of probability, NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b) means significant difference at 0.05 level of probability

4.2 Semi-Quantitative RT-PCR

Total RNA from 3 varieties, 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 amplify by semi-quantitative RT-PCR. The gel electrophoresis results are shown in Figure 1-4. PCR products were 325 base pairs (*OsP5Cs1*) and 70 base pairs Actin.

In order to investigate if any of the enzymes in the proline synthesis pathway was upregulated after proline application at flowering stage, and so could have led to the higher accumulation of proline, the level of *OsP5Cs1* transcripts was investigated by RT-PCR. Salt stress was observed to induce *OsP5Cs1* transcript expression and, moreover, exogenous proline application additionally further upregulated the *OsP5Cs1* transcript levels in CNT 1, PT 1 and IN 35 varieties at flowering stage under salinity condition (Figure 1-4). However, 0 mM and 150 mM of proline in PT 1 and 50 mM of proline in IN 35 under normal salinity condition (0 dS/m) was not

clearly observed *OsP5Cs1* in Figure 4. The presence of this ambiguous expression in cDNA product; in these presented results was not consistent with the amount of proline analyzed using chemical analysis in leaves at the same age post-spraying of proline (Table 9).

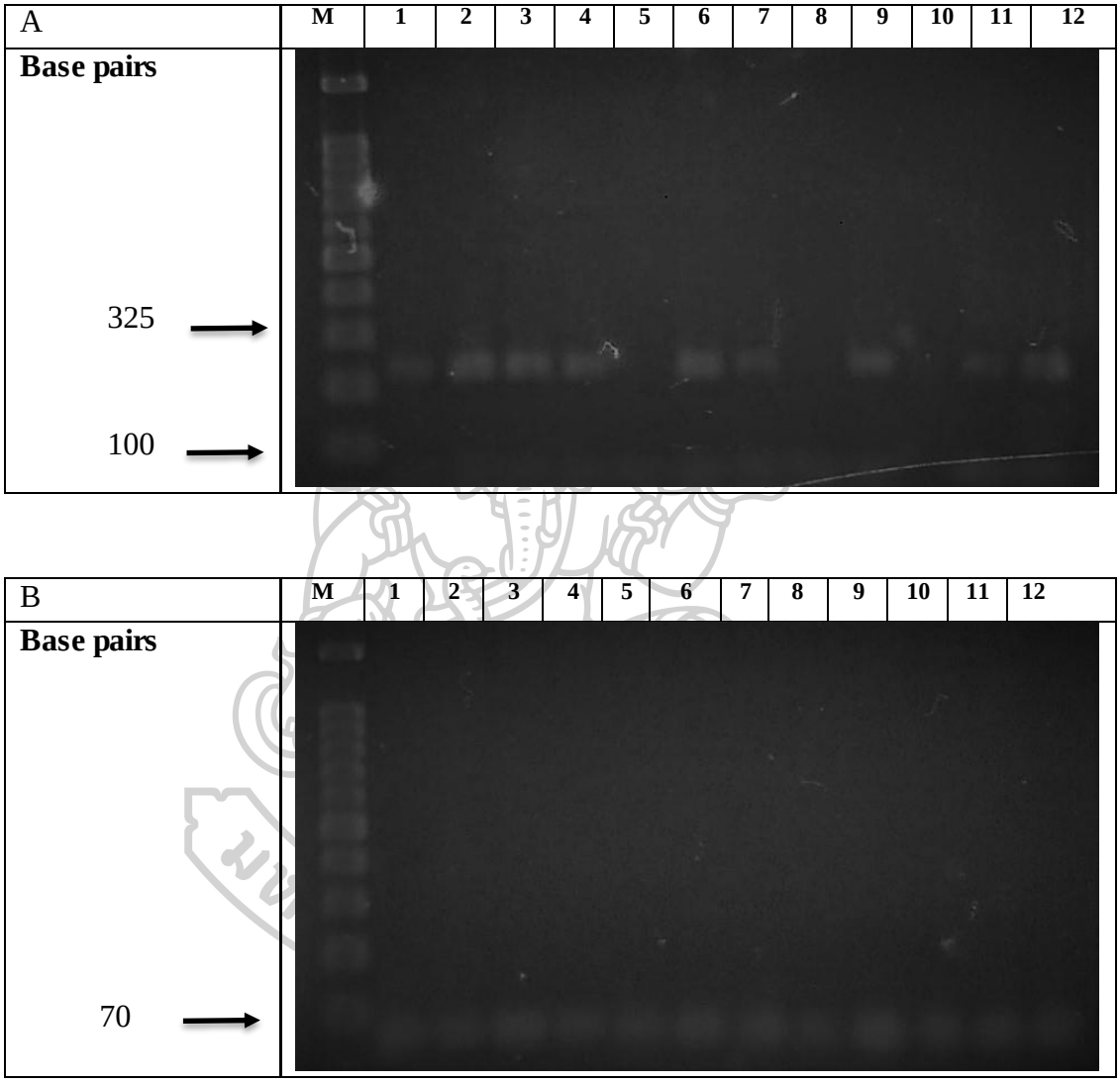


Figure 4 Gel Electrophoresis of *P5Cs1* (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under normal condition (0dS/m), Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 = V1P2, 4 = V1P3, 5 = V2P0, 6 = V2P1, 7 = V2P2, 8 = V2P3, 9 = 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

In 5 dS/m of salinity condition, 100 mM of proline was lost in PT 1 variety (Figure 5). Although it could not to clear-cut for use to discuss with chemical content

in the leaves; at 5 dS/m with 100 mM proline spays in PT 1, proline in leaves showed quite lower (1.55 µmole/g) than other concentrations of proline application in PT 1; exclude at 0 mM proline (1.47 µmole/g) (Table 9). All base pairs were recorded in 10 dS/m and 15 dS/m salinity level (Figure 6 and 7). Obtaining a cDNA analysis result showing clear RNA expression at both of these high salinity levels: 10 and 15 dS/m in all varieties is useful for discussion in conjunction with chemical analysis (Table 9). Even at low concentrations of proline deposition in the leaves of PT 1 at 0 mM (0.94 µmole/g) and 100 mM (0.92 µmole/g) proline spraying at the respective two salinity levels, 10 and 15dS/m, respectively, could estimate the expression by the bio-molecular method.

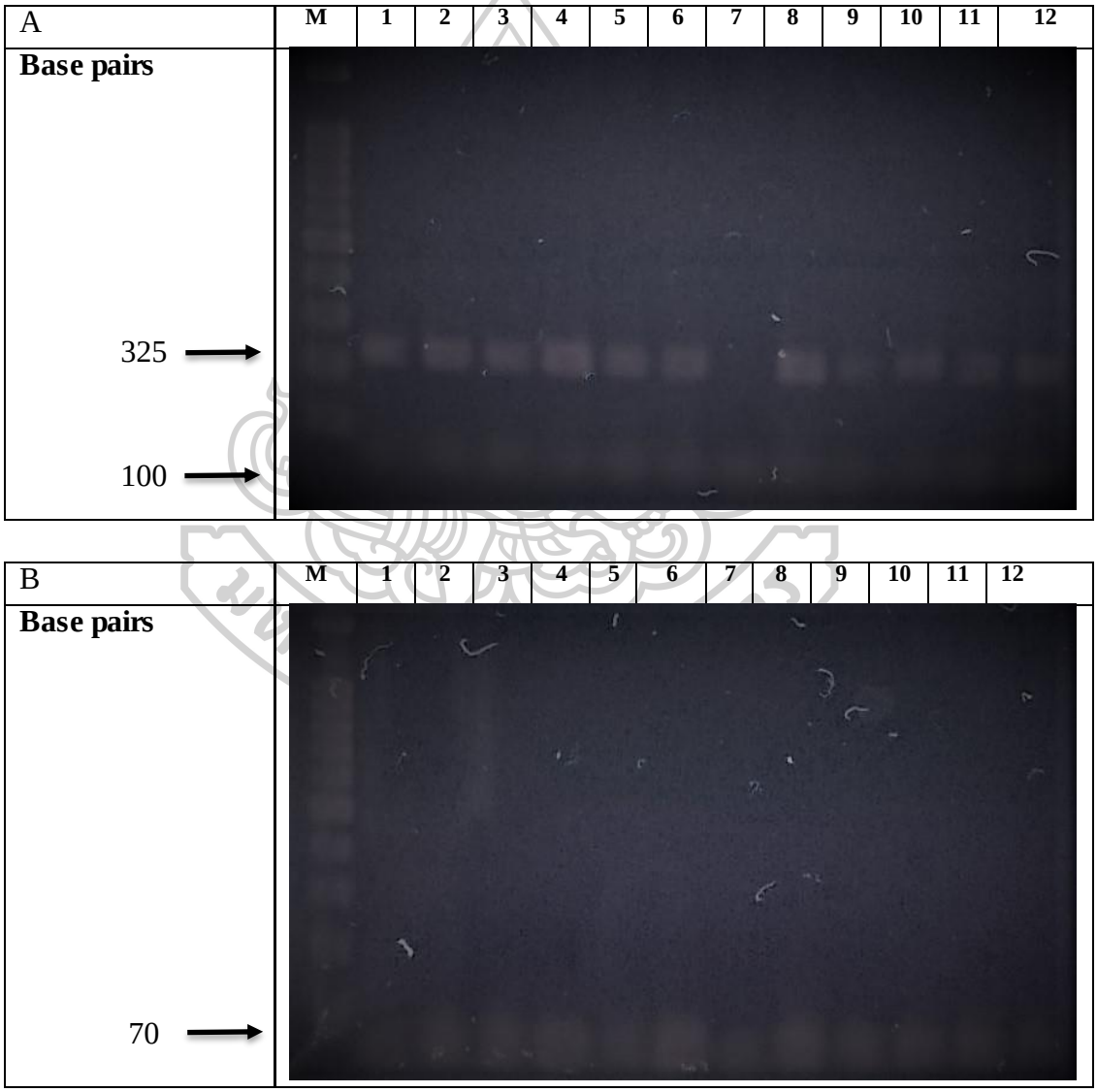


Figure 5 Gel Electrophoresis of *P5Cs1* (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under 5dS/m condition, Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 =

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 responses of the three rice varieties grown in salinity at 15 dS/m to polyline synthesis in leaves when external proline sprayed at different levels resulted in the same direction as at the salinity level 10 dS/m (Figure 6 and 7). While, proline deposition in leaves found to be higher at the spraying of proline at 150 mM; compared to other concentrations (except for some concentrations). It is possible that when plants are exposed to high salinity (15 dS/m), but proline production in stems is limited by individual genetic capabilities. Therefore, exogenous proline accumulation in leaves may increase an accumulation in order to for durability and reducing stress on cells in plants.

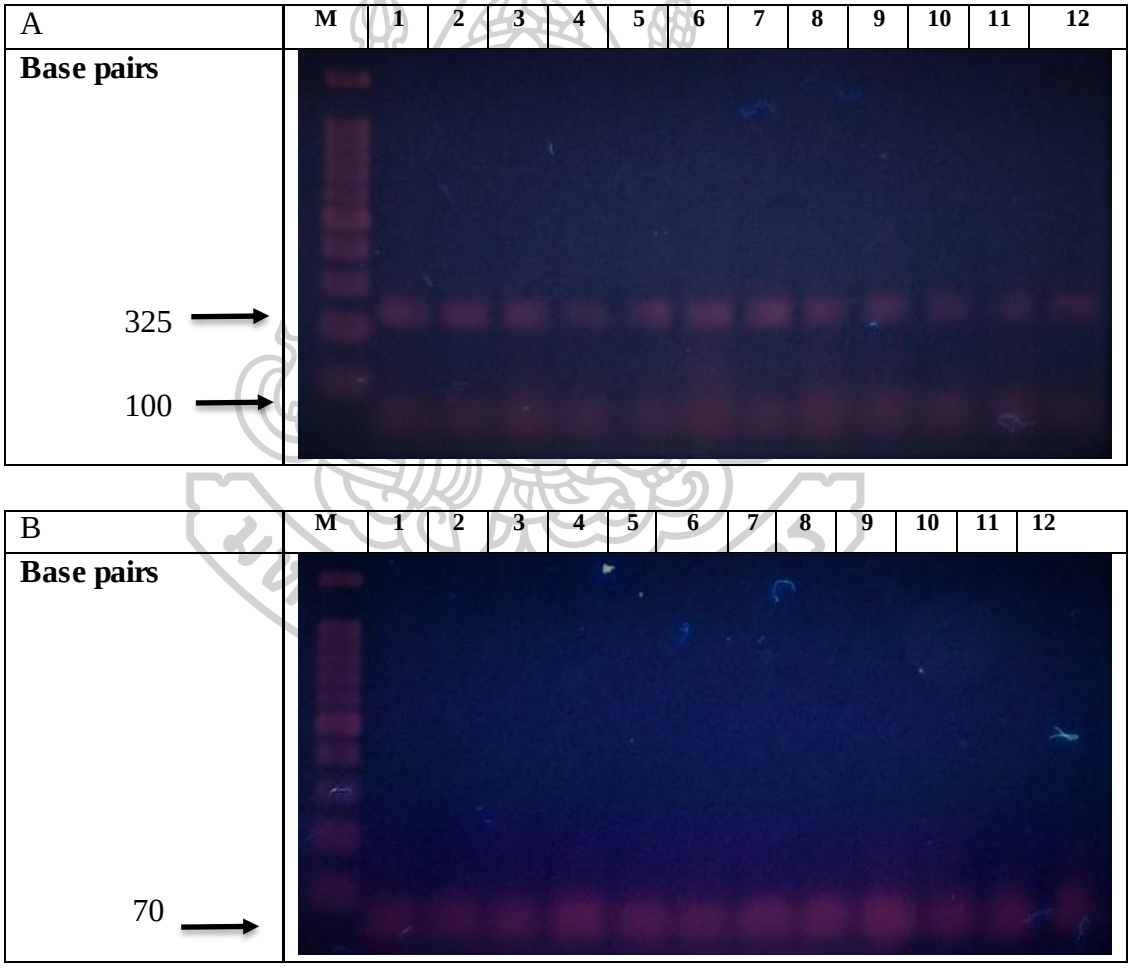


Figure 6 Gel Electrophoresis of *P5Cs1* (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under 10dS/m condition, Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 = V1P2, 4 = V1P3, 5 = V2P0, 6 = V2P1, 7 = V2P2, 8 = V2P3, 9 = V3P0, 10 = V3P1,

11 = V3P2, 12 = V3P3) Note; V1= Chai Nat 1, V2= Pathum Thani 1, V3= Inpari 35, P0= control, P1= 50 mM of proline, P2= 100 mM of proline, P3= 150 mM of proline

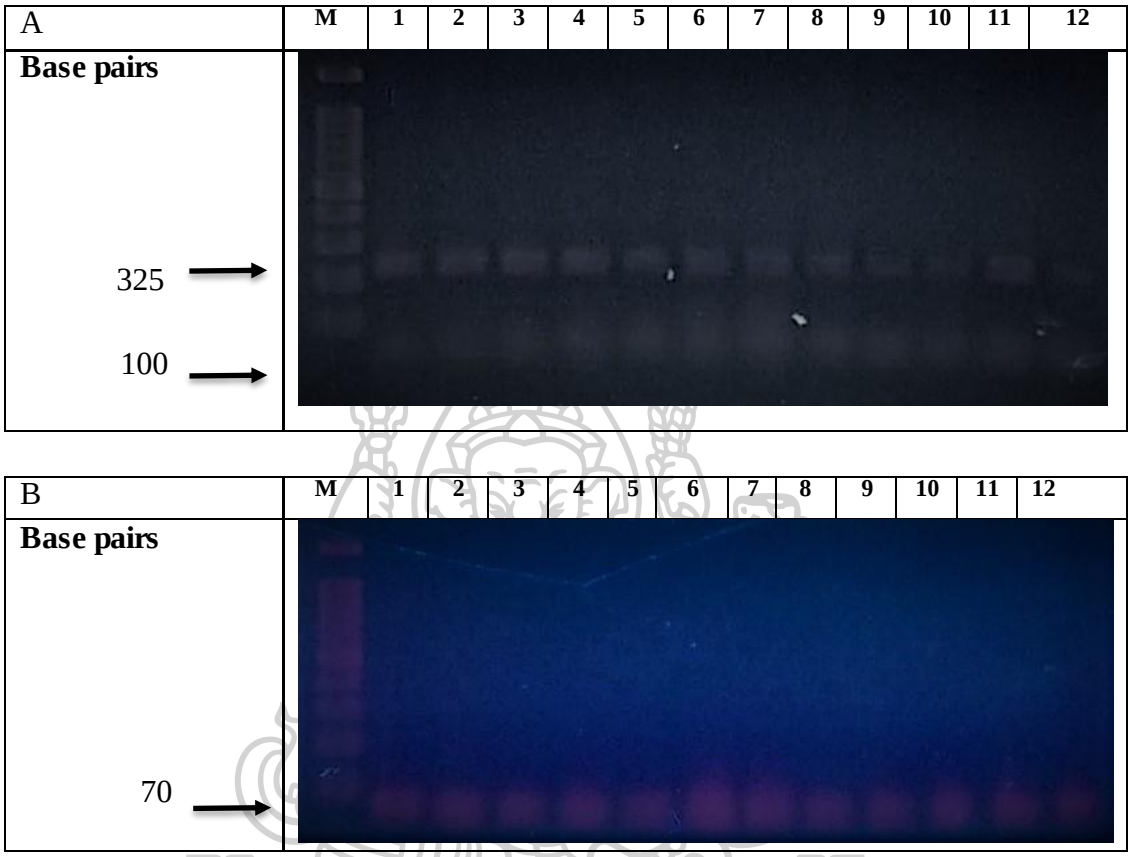


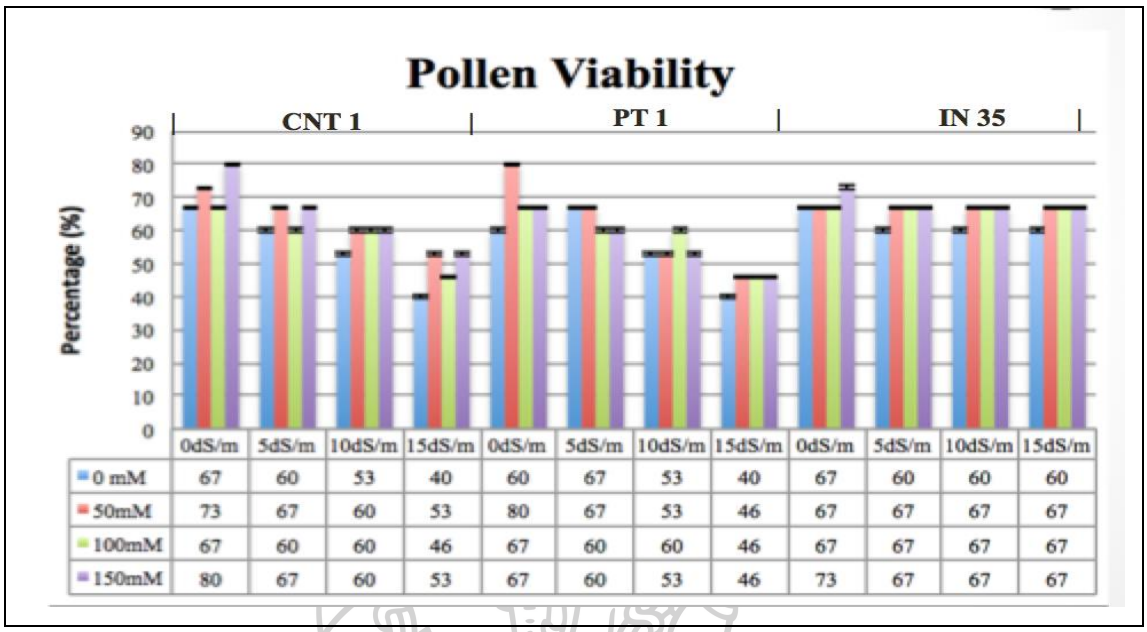
Figure 7 Gel Electrophoresis of *P5Cs1* (A) (Lane M = 100 bp DNA marker) the application of proline and (B) Actin gene (Lane M = 100 bp DNA marker) under 15dS/m condition, Lane number 1-12 = PCR products from 1 = V1P0, 2 = V1P1, 3 = V1P2, 4 = V1P3, 5 = V2P0, 6 = V2P1, 7 = V2P2, 8 = V2P3, 9 = V3P0, 10 = 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

4.3 Effect of proline and trehalose on agronomic characters

4.3.1 Effect of salinity and external substances on pollen viability

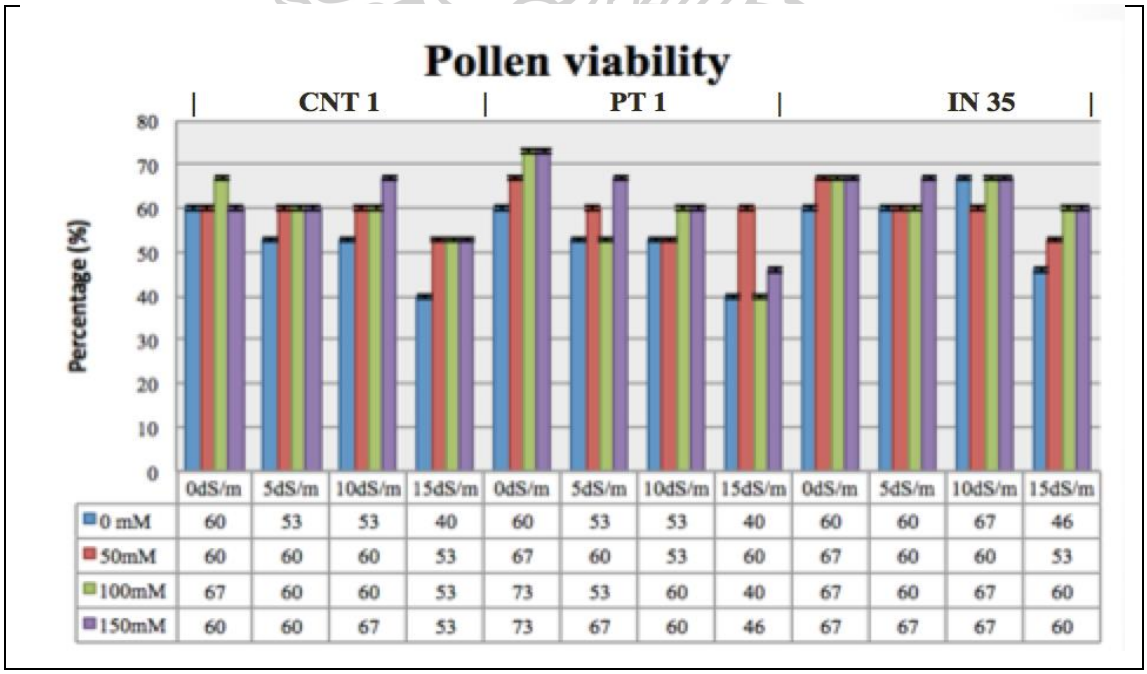
For this study, it was not a statistical test, but rather an average taken into account to determine the trend of salinity effects and the potential effects of external substances use. The results showed that salt stress reduced pollen viability, but the varying extents depended on the varieties (Figure 8). The result showed that pollen viability percentage was highest in normal condition at 0 dS/m salinity and the lowest in 15 dS/m salinity: in CNT 1 (was 67% and 40 %, respectively or decrease at -27 %), PT 1 (was 60 % and 40 %, respectively or decrease at -20 %) and IN 35 (was

67 % and 60 %, respectively or decrease at -7%), at not use proline for spraying. The benefit of the use of proline begins at the lowest concentration of 50 mM.



	0dS/m	5dS/m	10dS/m	15dS/m	0dS/m	5dS/m	10dS/m	15dS/m	0dS/m	5dS/m	10dS/m	15dS/m
0 mM	67	60	53	40	60	67	53	40	67	60	60	60
50mM	73	67	60	53	80	67	53	46	67	67	67	67
100mM	67	60	60	46	67	60	60	46	67	67	67	67
150mM	80	67	60	53	67	60	53	46	73	67	67	67

Figure 8 Pollen viability percentage (%) of three rice varieties in four levels of proline foliar application under different level of salinity stress condition at flowering stage



	0dS/m	5dS/m	10dS/m	15dS/m	0dS/m	5dS/m	10dS/m	15dS/m	0dS/m	5dS/m	10dS/m	15dS/m
0 mM	60	53	53	40	60	53	53	40	60	60	67	46
50mM	60	60	60	53	67	60	53	60	67	60	60	53
100mM	67	60	60	53	73	53	60	40	67	60	67	60
150mM	60	60	67	53	73	67	60	46	67	67	67	60

Figure 9 Pollen viability percentage (%) of three rice varieties in four levels of trehalose foliar application under different level of salinity stress condition at flowering stage

Similar between the effects of proline and trehalose, and salinity to pollen viability percentages, that it is varying extent depended on the varieties. The pollen viability percentage was highest in non-salty condition (0 dS/m) and the lowest in 15 dS/m at salinity in all varieties (Figure 9). So, the increase the salinity level, and the decreased the pollen viability percentage, compared with no salinity (0 dS/m). For external substances application, the benefit of the use of proline and trehalose begins at the lowest concentration of 50 mM (Figure 8-9).

4.3.2 Effect of salinity and external substances on plant height

Plant height is an important parameter although it is not the yield component character.

Table 21 Means of plant height (cm) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	95.3±0.88	93.0±1.15	92.6±0.88	90.0±1.53	93.3±1.11a
	50	96.0±0.58	93.6±1.33	93.3±0.88	91.6±0.67	
	100	95.0±0.58	93.0±1.15	93.3±0.88	91.6±1.20	
	150	95.3±0.67	94.0±1.15	93.6±0.88	92.0±1.53	
PT 1	0	92.6±1.45	91.3±1.20	88.0±1.53	86.0±1.53	90.2±1.43b
	50	92.6±2.03	91.6±1.45	88.3±1.20	86.6±1.33	
	100	92.3±1.45	90.0±2.65	89.0±3.21	87.6±1.20	
	150	92.3±0.33	89.3±1.76	89.0±2.52	88.0±1.15	
IN 35	0	91.6±1.86	90.3±1.20	89.0±1.73	87.0±1.53	90.2±1.58b
	50	92.3±1.45	90.3±3.18	90.3±2.52	87.0±2.08	
	100	92.6±2.33	90.6±2.40	91.6±1.45	88.0±2.08	
	150	92.6±2.60	91.0 ±1.53	91.0±2.08	88.6±1.07	
Mean Salinity		93.4±1.40a	91.5±1.19b	90.7±1.38b	88.6±2.53c	
		Proline (mM)				
		0	50	100	150	
Mean Proline		90.5±1.40	91.1±1.35	91.2±1.45	91.4±1.20	
P-value (F-test)						
Variety (V) = 1.05 x 10^{-8**}, Salinity (S) = 1.06 x 10^{-8**}, Proline (P) = 0.642 NS, V × S = 0.850 NS, V× P = 0.980 NS, S × P = 0.984 NS, V× S × P = 0.1000 NS, CV% = 3.17						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation,
** means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

The results revealed that plant height was highly significant different in

varieties factor and salinity factor. Highest plant height was found in CNT 1 (93.3 cm) followed by PT 1 (90.2 cm) and IN 35 (90.2 cm). In salinity factor, plant height was decreased with increased in salinity level. The highest plant height was found in no salinity stress at 0 dS/m (93.4 cm) and the lowest was recorded in 15 dS/m (88.6 cm) (Table 21). In each rice varieties, proline application could increase the height of the rice plant with minimal value at 10 dS/m and above salinity (Table 21).

In experiment II for trehalose testing, the plant height was highly significant different in varieties factor and salinity factor. The highest plant height found in CNT 1 (93.2 cm) followed by PT 1 (92.1 cm) and IN 35 (90.7 cm). Plant height was decreased with increased in salinity level. The highest plant height (95.3 cm) was found in control treatment (0 dS/m) and the lowest (88.1 cm) was recorded in 15 dS/m at salinity level (Table 22). In CNT 1 and IN 35, trehalose application could increase the height of the rice plant with minimal value at 15 dS/m salinity (Table 22).

Table 22 Means of plant height (cm) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	95.3±1.33	94.0±1.53	92.3±2.33	90.6±1.45	93.2±1.66a
	50	95.6±2.03	94.0±0.58	91.6±2.33	91.3±1.45	
	100	96.0±1.73	94.3±1.86	92.3±2.40	91.6±1.20	
	150	95.6±1.86	94.6±1.45	91.6±1.86	91.3±0.88	
PT 1	0	94.3±1.76	90.3±0.67	89.0±2.08	86.3±1.45	92.1±1.49b
	50	95.6±0.67	90.6±2.91	89.3±1.20	88.6±1.33	
	100	95.3 ±1.45	90.6±2.91	89.0±1.53	88.6±1.45	
	150	93.6 ±1.86	91.6±1.33	90.3±1.45	88.3±0.88	
IN 35	0	95.0±1.15	93.3±1.76	90.0±3.31	87.0±2.52	90.7±1.94b
	50	96.3±1.45	93.0±2.65	91.3±2.33	89.3±1.76	
	100	96.0±0.58	94.0±1.00	91.6±2.91	88.3±3.28	
	150	95.6±1.20	93.0 ±3.79	91.3±2.19	88.3±1.20	
Mean Salinity		95.3±1.42a	92.8±1.32b	90.8±2.24b	88.1±1.81c	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		91.4±1.42	92.2±1.38	92.3±1.25	92.1±1.64	
P-value (F-test)						
Variety (V) = 0.00106**, Salinity (S) = 7.47 x 10^{-12}**, Trehalose (T) = 0.6655 NS, V × S = 0.6182 NS, V× T = 0.9978 NS, S × T = 0.9988 NS, V× S × T = 0.1000 NS, CV% = 3.52						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35

CV = Coefficient of variation,

** Means significant difference at 0.01 levels of probability, respectively.

NS means non-significant difference at 0.05 level of probability.

Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

4.3.3 Effect of salinity and external substances on fertile tiller number

The results demonstrated that the number of fertile tillers was highly significant different in varieties factor and salinity factor. The maximum number of fertile tillers was found in IN 35 (10.4) followed by PT 1 (9) and CNT 1 (7.3) in varieties factor. In salinity factor, the number of fertile tillers decreased with increased in salinity level. The highest number of fertile tillers was found in control treatment (at 0 dS/m) (10.2) and the lowest (7.9) was recorded in 15 dS/m (Table 23).

Although not statistically significant due to the influence of interaction between factors, when considering each rice variety, it was found that PT 1 had high number of fertile tillers per plant similar to that of IN 35; under the salinity-free condition (0 dS/m). However, start at 5 dS/m, all three varieties had a considerable reduction in the number of fertile tillers per plant in high value compared with no salinity condition (0 dS/m) (Table 23).

Table 23 Means of fertile tiller numbers (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	8±0.3	7±0.3	7±0.0	7 ± 0.3	7.3±0.25c
	50	9±0.3	7±0.3	7±0.0	7 ± 0.3	
	100	8±0.3	7±0.3	7±0.0	6 ± 0.3	
	150	9±0.3	8±0.3	7±0.3	6 ± 0.0	
PT 1	0	11±0.6	9±0.6	9±0.6	8 ± 0.7	9.0±0.60b
	50	10±0.6	9±0.7	8±0.3	8 ± 0.3	
	100	11±0.3	9±0.3	9±0.0	8±0.0	
	150	10±0.3	9±0.6	9±0.3	7±0.3	
IN 35	0	12±0.0	10±0.3	10±0.7	10±0.9	10.4±0.47a
	50	12±0.0	10±0.3	10±0.3	10±0.6	
	100	11±0.6	10±0.6	10±0.7	10±0.3	
	150	11±0.7	10±0.7	10±0.9	10±0.3	
Mean Salinity		10.2±0.30a	8.9±0.41b	8.5±0.41c	7.9±0.63d	
		Proline (mM)				
		0	50	100	150	
Mean Proline		8.9±0.30	8.9±0.50	8.9±0.41	8.8±0.44	
P-value (F-test)						
Variety (V) = 2 x 10^{-16**}, Salinity (S) = 2 x 10^{-16**}, Proline (P) = 0.860 NS, V × S = 0.338 NS, V× P = 0.588 NS, S × P = 0.904 NS, V× S × P = 0.965 NS, CV% = 8.8						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35

CV = Coefficient of variation,

** Means significant difference at 0.01 levels of probability, respectively.

NS means non-significant difference at 0.05 level of probability.

Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

For trehalose application, the highest number of fertile tillers was found in IN 35 (9.8) followed by PT 1 (8.5) and CNT 1 (8.0) with significant differences affected by varieties factor (Table 24). In salinity factor, the number of fertile tillers decreased with an increase in salinity level with a highly significant difference. However, the interaction between varieties and salinity had a highly significant difference, the highest number of fertile tillers was found in the control treatment (at 0 dS/m), and the lowest was recorded in 15 dS/m in all rice varieties, but with different degrees of reduction (Table 24). A higher reduction value after plants were exposed to salinity levels was observed in CNT 1 and PT 1, and lower in IN 35 (Table 24).

Table 24 Means of fertile tiller number per plant (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	10±0.6	9±0.3	8±0.3	6±0.6	8.0±0.46c
	50	10±0.3	8±0.9	7±0.3	6±0.7	
	100	10±0.6	9±0.3	7±0.3	6±0.3	
	150	10±0.6	8±0.3	7±0.3	6±0.1	
PT 1	0	11±0.3	9±0.3	8±0.1	8±0.3	8.5±0.25b
	50	10±0.3	8±0.3	9±0.3	7±0.7	
	100	10±0.7	9±0.3	7±0.6	7±0.3	
	150	11±0.9	9±0.9	8±0.1	7±0.3	
IN 35	0	10±0.9	10±0.3	9±0.1	9±0.3	9.8±0.39a
	50	11±0.6	10±0.3	10±0.3	10±0.3	
	100	11±0.6	9±0.3	10±0.7	10±0.1	
	150	11±0.7	9±0.6	9±0.3	9±0.3	
Mean Salinity		10.4±0.60a	8.8±0.33b	8.2±0.11c	7.6±0.41d	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		8.8±0.60	8.8±0.41	8.8±0.61	8.6±0.71	
		Salinity dS/m				
Varieties		0	5	10	15	
CNT 1		9.9±0.46bc	8.5±0.55d	7.2±0.39ef	6.1±0.31g	
PT 1		10.5±0.25ab	8.5±0.42d	7.9±0.48de	7.0±0.52f	
IN 35		10.9±0.39a	9.4±0.39c	9.4±0.39c	9.5±0.48c	
P-value (F-test)						
Variety (V) = 2 x 10^{-16**}, Salinity (S) = 2 x 10^{-16**}, Trehalose (T) = 0.858 NS, V × S = 4.54 x 10^{-6**}, V × T = 0.748 NS, S × T = 0.631 NS, V× S × T = 0.940 NS, CV% = 9.4						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35

CV = Coefficient of variation,

** Means significant difference at 0.01 levels of probability, respectively.

NS means non-significant difference at 0.05 level of probability.

Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

4.3.4 Effect of salinity and external substances on panicle length

After the foliar application of proline, the result exhibited that the panicle length was highly significant influenced by varieties, salinity and proline factors (Table 25). The longest panicle length was observed in Thai rice varieties: CNT 1 (24.1 cm) and PT 1 (23.8 cm), and lower in IN 35 (21.8 cm) varieties. In salinity factor, panicle length was a remarkable decreased with increased in salinity level. The highest panicle length number was found in control treatment at 0 dS/m (23.9 cm) and the lowest was recorded in 15 dS/m salinity (22.6 cm). In proline factor, the panicle length was markedly increased with increased with the foliar application of proline. The longest panicle length was observed in 150 mM of proline level (23.7 cm) and the shortest in control treatment (23 cm) (Table 25).

Table 25 Means of panicle length (cm) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	24.6±0.2	23.8±0.2	23.6±0.4	23.2±0.3	24.1±0.3a
	50	24.0±0.3	24.0±0.4	23.3±0.1	22.8±0.3	
	100	25.0±0.3	24.4±0.8	23.8±0.8	24.4±0.7	
	150	25.1±0.4	24.5±0.5	24.6±0.2	24.0±0.2	
PT 1	0	25.0±0.5	23.0±0.5	23.8±0.1	22.7±0.5	23.8±0.4a
	50	25.3±0.2	23.7±0.9	23.5±0.2	23.1±0.3	
	100	23.7±0.4	24.1±0.7	23.5±0.7	22.8±1	
	150	25.4±0.5	23.8±0.4	24.8±0.2	23.6±0.4	
IN 35	0	21.7±0.4	22.0±0.4	21.1±0.3	21.4±0.5	21.8±0.4b
	50	22.0±0.1	21.5±0.7	21.5±0.3	20.8±0.6	
	100	22.6±0.5	22.0±0.2	21.9±0.5	21.8±0.2	
	150	22.6±0.3	23.0±0.6	22.2±0.1	20.8±0.2	
Mean Salinity		23.9±0.4a	23.3±0.4b	23.12±0.3b	22.6±0.4c	
		Proline (mM)				
		0	50	100	150	
Mean Proline		23.0±0.4b	23.0±0.2b	23.3±0.4ab	23.7±0.4a	
P-value (F-test)						
Variety (V) = $2 \times 10^{-16**}$, Salinity (S) = $2.56 \times 10^{-8**}$, Proline (P) = 0.0004^{**}, V × S = 0.3339 NS, V× P = 0.2213 NS, S × P = 0.8138 NS, V× S × P = 0.6552 NS, CV% = 3.5						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35

CV = Coefficient of variation,

** Means significant difference at 0.01 levels of probability, respectively.

NS means non-significant difference at 0.05 level of probability.

Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

For trehalose application, the result showed that the panicle length was highly significantly influenced by varieties and salinity factors (Table 26). The longest panicle length was observed in Thai rice varieties: CNT 1 and PT 1 at 23.9 cm, and lower in IN 35 (21.6 cm) varieties (Table 26). In salinity factor, panicle length was a remarkable decrease with increase in salinity level since at 5 dS/m. However, the panicle length was not significantly different affected by trehalose levels (Table 26).

Table 26 Means of panicle length (cm) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	24.2±0.25	23,6±0.50	23.2±0.49	23.1±0.93	23.9±0.54a
	50	24.2±0.86	24.1±0.18	23.4±0.72	23.2±0.22	
	100	24.1±0.58	23.6±0.35	24.1±0.24	23.8±0.57	
	150	24.8±0.81	24.4±0.40	24.3±0.32	24.1±0.49	
PT 1	0	24.6±0.41	23.3±1.26	23.5±0.43	23.2±0.44	23.9±0.63a
	50	24.7±0.53	23.4±0.45	23.8±0.27	23.8±0.45	
	100	24.2±0.34	23.9±0.19	24.3±0.86	23.3±0.41	
	150	25.0±0.47	23.6±0.20	23.9±0.52	24.2±0.31	
IN 35	0	21.7±0.71	21.9±0.10	21.1±0.22	21.2±0.55	21.6±0.26b
	50	21.1±0.55	22.5±0.18	21.4±0.32	21.3±0.38	
	100	21.3±0.43	21.7±0.46	22.1±0.39	21.6±0.06	
	150	22.1±0.29	21.7±0.38	21.5±0.21	21.2±0.55	
Mean Salinity		23.5±0.27a	23.2±0.62ab	23.1±0.38b	22.8±0.64b	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		22.9±0.27	23.1±0.65	23.2±0.45	23.4±0.52	
P-value (F-test)						
Variety (V) = 2x 10^{-16**}, Salinity (S) = 0.0124*, Trehalose (T) = 0.0987 NS, V × S = 0.1999 NS, V× T = 0.8439 NS, S × T = 0.6229 NS, V × S × T = 0.9943 NS, CV% = 3.6						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation, ** Means significant difference at 0.01 levels,
* Means significant difference at 0.05 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

4.3.5 Effect of salinity and external substances on 1,000 seed weight

After the proline spray, the 1,000 seed weight showed that highly significant affected by varieties and salinity factors. In varieties factor, the highest 1,000 seed weight was in IN 35 (25.72 g) and CNT 1 (25.32 g), followed by PT 1 (24.64 g). In salinity factor, 1,000 seed weight decreased with increased in salinity level; compared with no salinity condition at 0 dS/m. The maximum 1,000 seed

weight was in control treatment (0 dS/m) (25.38 g) and the minimum was in 15 dS/m salinity level (25.08 g) (Table 27).

However, in this study, no significant effect of proline on 1,000 seeds weight was analyzed. Considering that at each level of salinity, the use of proline did not increase the 1,000 seed weight in all rice varieties (Table 27). Therefore, the results of the statistical analysis showed no influence of the interaction between factors; proline level and salinity level, on the increase or decrease of 1,000 seed weight.

Table 27 Means of 1,000 seed weight (g) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	25.5±0.06	25.3±0.24	25.4±0.06	25.1±0.12	25.32±0.12a
	50	25.4±0.19	25.3±0.16	25.3±0.21	25.2±0.12	
	100	25.3±0.18	25.3±0.09	25.4±0.10	25.3±0.19	
	150	25.3±0.15	25.3±0.10	25.3±0.06	25.2±0.16	
PT 1	0	24.8±0.09	24.7±0.10	24.5±0.06	24.4±0.21	24.64±0.12b
	50	24.7±0.19	24.8±0.16	24.6±0.15	24.5±0.12	
	100	24.8±0.19	24.7±0.18	24.5±0.16	24.4±0.12	
	150	24.7±0.18	24.7±0.20	24.6±0.18	24.5±0.12	
IN 35	0	26.0±0.09	25.8±0.09	25.6±0.21	25.5±0.07	25.72±0.11a
	50	25.9±0.06	25.8±0.18	25.7±0.18	25.5±2.08	
	100	25.8±0.15	25.7±0.12	25.6±0.21	25.6±0.19	
	150	25.9±0.09	25.6 ±0.19	25.7±0.21	25.5±0.21	
Mean Salinity		25.38±0.08a	25.27±0.15ab	25.19±0.11bc	25.08±0.13c	
		Proline (mM)				
		0	50	100	150	
Mean Proline		25.23±0.08	25.25±0.15	25.23±0.17	25.21±0.14	
P-value (F-test)						
Variety (V) = 2 x 10^{-16**}, Salinity (S) = 4.56 x 10^{-5**}, Proline (P) = 0.928 NS, V × S = 0.751 NS, V× P = 0.983NS, S × P = 0.970 NS, V× S × P = 1.000NS, CV% = 1.4						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

Similarly, with proline application, for trehalose application, the 1,000 seed weight showed that highly significant affected by varieties and salinity factors. In varieties factor, the highest 1,000 seed weight was in IN 35 (25.71 g) followed by CNT 1 (25.34 g) and PT 1 (24.67 g), respectively. In salinity factor, 1,000 seed

weight decreased with increase in salinity level; the negative effect of salinity was observed since at 5 dS/m compared with no salinity condition at 0 dS/m. (Table 28).

The results of the statistical analysis showed no influence of the interaction between factors; trehalose level and salinity level, on the increase or decrease of 1,000 seed weight.

Table 28 Means of 1,000 seed weight (g) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	25.5±1.33	25.3±0.16	25.2±0.18	25.1±0.06	25.34±0.11b
	50	25.4±0.24	25.4±0.20	25.3±0.06	25.4±0.06	
	100	25.5±0.16	25.3±0.16	25.4±0.10	25.3±0.16	
	150	25.5±0.19	25.3±0.06	25.3±0.10	25.2±0.06	
PT 1	0	24.9±0.06	24.7±0.16	24.5±0.22	24.4±0.17	24.67±0.15c
	50	24.7±0.07	24.6±0.09	24.7±0.09	24.5±0.12	
	100	24.7 ±0.16	24.6±0.21	24.7±0.12	24.6±0.06	
	150	24.8 ±0.12	24.7±0.14	24.7±0.18	24.5±0.12	
IN 35	0	25.9±0.10	25.8±0.12	25.5±0.07	25.4±0.21	25.71±0.12a
	50	25.8±0.24	25.8±0.15	25.7±0.14	25.4±0.12	
	100	25.9±0.18	25.8±0.15	25.6±0.21	25.5±0.09	
	150	25.8±0.12	25.8 ±0.26	25.7±0.07	25.6±0.6	
Mean Salinity		25.40±0.06a	25.27±0.14b	25.21±0.16b	25.08±0.15c	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		25.20±0.06	25.25±0.18	25.25±0.16	25.26±10.14	
P-value (F-test)						
Variety (V) = 2 x 10^{-16**}, Salinity (S) = 5.88 x 10^{-16**}, Trehalose (T) = 0.704 NS, V × S = 0.681 NS, V × T = 0.996NS, S × T = 0. 876NS, V × S × T = 0.999, CV% = 1.0						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

4.3.6 Effect of salinity and external substances on filled grain percentage

The number of filled grain per panicle is the most influential yield component, and most closely correlated with seed yield. After the proline spray, the results demonstrated that filled grain percentage were highly significant influenced by varieties factor, salinity factor, proline factor and interaction between varieties and salinity at $P<0.01$ levels (Table 29). The maximum filled grain percentage was observed in IN 35 (69.2 %) followed by CNT 1 (57.9 %) and PT 1 (50.8 %), respectively. In salinity factor, filled grain percent was markedly decreased with

increased in salinity level. The highest filled grain percentage was found in control treatment at 0 dS/m (76 %) and the lowest was recorded in 15 dS/m salinity (43.9 %). In proline factor, filled grain percent was increased with increased the foliar application of proline. The highest filled grain percentage was observed in 150 mM of proline level (61.6 %) and the lowest in control treatment at no salinity condition (0 mM proline) (57.1 %).

Although at no salinity stress (0 dS/m), there were equal values among these three rice varieties. The decreasing of filled grain percentage under the highest level of salinity stress (15 dS/m salinity) was higher in PT 1 (was 76.2% and 26.5 % at 0 dS/m and 15 dS/m, respectively or decreased at -65.22 %), and followed by CNT 1 (was 77.4 and 41.6% at 0 dS/m and 15 dS/m, respectively or decreased at -46.25 %), and the lowest in IN 35 (was 74.4 and 63.5 % at 0 dS/m and 15 dS/m, respectively or decreased at -14.65 %).

Table 29 Means of filled grain percentage (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	76.7±0.3	57.0±4.0	48.7±4.9	35.0±2.6	57.9±2.98b
	50	78.0±0.6	59.0±2.0	51.0±4.0	39.3±3.4	
	100	77.3±1.2	59.0±1.0	56.7±0.9	43.3±1.8	
	150	77.7±1.2	60.3±3.2	59.3±4.6	49.0±2.5	
PT 1	0	74.7±0.9	55.0±0.6	41.3±0.9	24.3±0.9	50.8±0.81c
	50	75.7±0.3	56.0±0.6	43.0±0.6	26.0±1.2	
	100	76.3±1.8	58.3±0.9	44.0±1.0	27.0±0.6	
	150	78.3±1.2	59.0±0.6	45.0±0.6	29.0±1.5	
IN 35	0	73.0±0.6	70.7±0.3	66.7±0.9	62.3±1.2	69.2±0.75a
	50	73.7±0.9	71.3±0.3	67.3±0.7	63.3±1.2	
	100	74.7±0.7	70.7±0.7	68.0±0.6	64.0±1.5	
	150	76.3±0.9	72.3±0.3	69.0±0.6	64.3±1.2	
Mean Salinity		76.0±0.60a	62.4±1.65b	55.0±2.22c	43.9±1.58d	
		Proline (mM)				
		0	50	100	150	
Mean Proline		57.1±0.60c	58.6±0.60b	59.9±1.21b	61.6±1.10a	
		Salinity dS/m				
		0	5	10	15	
CNT 1		77.4±2.98a	58.8±2.50f	53.9±1.21g	41.6±2.88h	
PT 1		76.2±0.81ab	57.0±0.66f	43.3±1.06h	26.5±0.97i	
IN 35		74.4±0.75b	71.2±0.77c	67.7±0.86d	63.5±0.75e	
P-value (F-test)						
Variety (V) = $2 \times 10^{-16**}$, Salinity (S) = $2 \times 10^{-16**}$, Proline (P) = $1.11 \times 10^{-7**}$, V × S = $2 \times 10^{-16**}$, V× P = 0.185 NS, S× P = 0.594 NS, V× S × P = 0.583 NS, CV% = 5.1						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) means significant difference at 0.05 level of probability

Table 30 Means of filled grain percentage (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	74.3±1.2	57.3±2.2	45.0±2.6	34.0±1.5	55.5±1.59b
	50	76.0±1.5	58.0±2.0	46.7±1.5	37.7±1.9	
	100	75.3±1.8	58.6±0.9	52.0±1.2	41.7±1.7	
	150	76.3±1.2	59.7±1.5	52.7±1.8	42.7±1.2	
PT 1	0	72.0±1.5	52.7±1.2	39.3±2.2	21.3±0.9	48.3±1.26c
	50	74.0±1.2	53.3±0.9	39.7±1.5	23.0±1.0	
	100	75.0±1.7	56.0±1.2	41.7±1.9	24.0±0.6	
	150	76.3±1.2	57.7±1.5	42.0±1.2	25.3±0.7	
IN 35	0	72.7±0.9	70.7±0.3	65.7±0.9	61.7±0.9	68.4±0.73a
	50	72.3±1.2	71.3±0.3	66.7±0.9	62.3±0.9	
	100	72.7±0.9	71.3±0.7	67.0±0.6	62.7±0.9	
	150	73.7±0.9	71.7±0.3	67.7±0.9	63.7±0.3	
Mean Salinity		74.2±1.26a	61.5±1.07b	52.2±1.41c	41.7±1.03d	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		55.6±1.36c	56.7±1.23b	58.2±1.18a	59.1±1.06a	
		Salinity dS/m				
Varieties		0	5	10	15	
CNT 1		75.5±1.4a	58.4±1.6f	49.1±1.8h	39.0±1.6i	
PT 1		74.3±1.4ab	54.9±1.2g	40.7±1.7i	23.4±0.8j	
IN 35		72.8±1.0bc	71.3±0.4c	66.8±0.8d	62.6±0.7e	
P-value (F-test)						
Variety (V) = $2 \times 10^{-16**}$, Salinity (S) = $2 \times 10^{-16**}$, Trehalose (T) = $4.43 \times 10^{-9**}$, V × S = $2 \times 10^{-16**}$, V × T = 0.0861 NS, S × T = 0.6905 NS, V × S × T = 0.7050 NS, CV% = 3.9						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) means significant difference at 0.05 level of probability

For trehalose application by foliar spraying, filled grain percentage was highly significant affected by varieties factor, salinity level, trehalose level and interaction between varieties and salinity at P<0.01 levels (Table 30). The maximum filled grain percentage was observed in IN 35 (68.4 %) followed by CNT 1 (55.5 %)

and PT 1 (48.3 %), respectively. In salinity factor, filled grain percentage was decreased with increased in salinity level. The highest filled grain percentage was found in no salinity condition (0 dS/m) (74.2 %) and the lowest was recorded in 15 dS/m salinity (41.7 %). For trehalose levels, filled grain percentage increased with increased the level of proline. The highest filled grain percentage was observed in 150 mM of trehalose level (59.1 %) and the lowest in control treatment at 0 mM of trehalose level (55.6 %). In varieties and salinity interaction, the higher the salinity level was caused the lower percent of filled grain in all varieties.

For trehalose sprays, the greatest decrease in grain filling in rice variety was PT 1 (was 74.3 and 23.4 % at 0 dS/m and 15 dS/m, respectively or decreased at -68.51 %) followed by CNT 1 (was 75.5 and 39 % at 0 dS/m and 15 dS/m, respectively or decreased at -48.34 %) and IN 35 (was 72.8 and 62.6 % at 0 dS/m and 15 dS/m, respectively or decreased at -14.01 %), respectively. Further, the effect on percent of grain filling is likely to be consistent with the effect on pollen viability enhanced by trehalose spray (Figure 6).

4.3.7 Effect of salinity and external substances on yield per plant

For grain yield per plant under proline application, the results of mean yield per plant (g) demonstrated that highly significant in varieties factor, salinity levels, proline levels and interaction between varieties and salinity at $P< 0.01$ level (Table 31).

The maximum yield per plant was assessed in IN 35 (10.18 g) followed by CNT 1 (7.91 g) and PT 1 (6.43 g), respectively. The yield per plant decreased with increased with salinity level; the highest yield was in no salinity condition (0 dS/m) (10.60 g) and the lowest yield was in 15 dS/m at salinity level (5.56 g). For proline levels, the yield increased with increased foliar application of proline. The highest yield was observed in 150 mM of proline level (8.63 g) and the lowest yield was in control condition (0 mM proline) (7.81 g).

In varieties and salinity interaction, the yield per plant decreased with an increase in salinity levels in all rice varieties; but in different magnitudes. IN 35 (11.8 and 8.6 g at 0 and 15 dS/m, respectively or decreased at -27.12 %) showed the reduction of yield in lowest, the higher reduction was observed in CNT 1 (was 10.3 and 4.7 g at 0 and 15 dS/m, respectively or decreased at -54.37 %) and PT 1 (was 9.7 and 3.3 g at 0 and 15 dS/m, respectively or decreased at -65.98 %).

In case of mean yield per plant after the trehalose spraying, the result revealed significantly high influence by all factors including varieties, salinity levels and trehalose levels at $P< 0.01$ level (Table 32). The maximum yield was in IN 35 (10.2 g) followed by CNT 1 (7.3 g) and the minimum yield was in PT 1 (6.2 g). The yield per plant decreased with increased with salinity level. The highest yield was in no salinity condition (0 dS/m) (10.6 g) and the lowest yield was in 15 dS/m at salinity level (8.2 g). In trehalose factor, the yield increased with increased foliar application

of trehalose. The highest yield was observed in 150 mM of trehalose level (8.2 g) and the lowest yield was in control condition (0 dS/m) (7.6 g).

Table 31 Means of yield per plant (g) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the proline supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages.

Varieties	Proline (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	9.8±0.3	9.0±0.5	7.4±0.4	4.5±0.3	7.91±0.39b
	50	10.5±0.1	8.4±0.2	7.9±0.2	4.4±0.1	
	100	10.0±0.3	8.9±0.2	7.9±0.3	4.7±0.2	
	150	11.1±0.4	6.9±0.5	7.9±0.1	5.3±0.2	
PT 1	0	9.5±0.3	7.6±0.2	4.6±0.3	3.1±0.3	6.43±0.27c
	50	9.7±0.3	7.7±0.3	4.9±0.3	3.1±0.2	
	100	9.6±0.1	7.9±0.3	4.9±0.3	3.4±0.2	
	150	9.9±0.1	8.2±0.4	5.2±0.1	3.6±0.3	
IN 35	0	11.0±0.5	10.1±0.1	9.4±0.1	8.2±0.2	10.18±1.9a
	50	11.7±0.3	10.5±0.1	9.8±0.1	8.2±0.1	
	100	12.6±0.4	10.8±0.2	9.6±0.3	8.5±0.3	
	150	11.7±0.3	11.1±0.4	10.0±0.3	9.6±0.1	
Mean Salinity		10.60±0.37a	9.16±0.26b	7.38±0.25c	5.56±0.25d	
		Proline (mM)				
Mean Proline		7.81±0.4c	8.02±0.3bc	8.24±0.3b	8.63±0.3a	
		Salinity dS/m				
		0	5	10	15	
CNT 1		10.3±0.39b	9.0±0.14d	7.5±0.24f	4.7±0.31g	
PT 1		9.7±0.27c	7.8±0.29f	4.8±0.21g	3.3±0.28h	
IN 35		11.8±0.19a	10.6±0.15b	9.7±0.28c	8.6±0.28e	
P-value (F-test)						
Variety (V) = $2 \times 10^{-16**}$, Salinity (S) = $2 \times 10^{-16**}$, Proline (P) = $1.07 \times 10^{-8**}$, V × S = $2 \times 10^{-16**}$, V× P = 0.418 NS, S× P = 0.495 NS, V× S × P = 0.215 NS, CV% = 5.8						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation, ** Means significant difference at 0.01 levels,
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

Table 32 Means of yield per plant (g) (±standard error) (at maturity stage) in three rice varieties grown under different salinity levels and received the trehalose supplementation (in different concentrations) by foliar spraying at 1 week before flowering stages

Varieties	Trehalose (mM)	Salinity dS/m				Mean Varieties
		0	5	10	15	
CNT 1	0	9.8±0.7	8.4±0.6	5.8±0.5	3.5±0.6	7.3±0.35b
	50	10.6±0.4	8.6±0.3	6.0±0.6	3.8±0.8	
	100	10.2±0.8	8.3±0.3	7.2±0.7	3.9±0.5	
	150	11.2±0.3	8.8±0.2	7.2±0.2	4.1±0.5	
PT 1	0	9.5±0.5	7.2±0.3	4.5±0.4	2.8±0.5	6.2±0.24c
	50	9.6±0.4	7.4±0.3	4.3±0.2	3.1±0.7	
	100	9.5±0.1	7.6±0.1	4.4±0.3	3.2±0.8	
	150	9.8±0.1	7.5±0.4	4.9±0.1	3.4±0.9	
IN 35	0	11.2±0.1	10.6±0.5	9.6±0.4	9.7±0.6	10.2±0.24a
	50	11.8±0.1	10.9±0.4	9.8±0.5	8.3±0.7	
	100	12.0±0.1	11.0±0.8	9.9±0.6	8.3±0.6	
	150	12.4±0.1	11.3±0.9	10.0±0.1	8.6±0.5	
Mean Salinity		10.6±0.25a	9.0±0.26b	8.0±0.26c	8.2±0.32d	
		Trehalose (mM)				
		0	50	100	150	
Mean Trehalose		7.6±0.25c	7.9±0.17b	8.0±0.20b	8.2±0.12a	
		Salinity dS/m				
Varieties		0	5	10	15	
CNT 1		10.4±0.35c	8.5±0.29e	6.5±0.33g	3.8±0.17i	
PT 1		9.6±0.24d	7.4±0.23f	4.5±0.18h	3.1±0.21j	
IN 35		11.8±0.24a	10.9±0.25b	9.8±0.30d	8.2±0.25e	
P-value (F-test)						
Variety (V) = 2 x 10^{-16**}, Salinity (S) = 2 x 10^{-16**}, Trehalose (T) = 2.26 x 10^{-16**}, V × S = 2 x 10^{-16**}, V× T = 0.753 NS, S × T = 0.749 NS, V × S × T = 0.819 NS, CV% = 6.3						

Note: CNT 1 = Chai Nat 1, PT 1 = Pathum Thani 1, IN 35= Inpari 35
CV = Coefficient of variation,
** Means significant difference at 0.01 levels of probability, respectively.
NS means non-significant difference at 0.05 level of probability.
Different lower case letters (a, b, c) mean significant difference at 0.05 level of probability

CHAPTER 5

DISCUSSION

5.1 Effect of proline and trehalose on biochemical characters

Salinity was reported to decrease the water reserve in leaves, the grain growth rate, and the current dry matter production (Sultana et al., 2002). Moreover, in some research papers, relatively water content was different in under normal condition and salinity stress condition. In addition, salinity caused reduction in leaf relatively water content in rice seedling. Relatively water content of treated seedling was reduced from 87-90% in the control plants to 74-77% in the stress plants; about 15 dS/m (Amirjani, 2010). Suriya-arunroj et al. (2004) mentioned that salt tolerance group of RWC was 94%, moderate tolerance group was 86% and susceptible group was 75 % under 6 dS/m salinity condition and 93% - 95% under normal condition at seedling stage in rice.

However, result in this study as shown in Table 1, the average of means in water content at salinity levels 0-15 dS/m ranged between 79.19-80.92 % with no significant difference affected by salinity levels. Moradi and Ismail (2007) reported rice grown in salty soils may be affected on leaf formation in the vegetative stage, but may not effect on water content in rice leaves at reproductive stage. Suh et al. (2010) indicated that water content in plants was reported that it is influenced by genotypes and was partially resistant at the reproductive and flowering stage,

Consistent with the results of this study, the relative water content was not significantly different although plants received the salinity at different levels. Therefore, the water content in leaves at the reproductive stage may not be a good parameter to indicate the stress from the salinity. Actually, toxins ions in the leaves such as Na^+ may interfere with phloem loading. Munns et al. (2006) mentioned that translocation of assimilate reserves was inhibited in salinized plants, independent to the leaf water potential and relative leaf water content. Moreover, Siddique et al. (2000), which reported that leaf relative water content decreased more rapidly in the salt-treated plants than untreated plants. Nevertheless, the content of water in leaves depends on the genetic; the ability of rice variety to maintain the water level in the leaves when dealing with salinity problems.

In this study, chlorophyll a, b and total chlorophyll were increased in proline factor. It could mean that increased proline in foliar application induced the increased in chlorophyll content. A strong consensus results in increasing content of chlorophyll a was found by using these two substances, proline, and trehalose. However, the effect on chlorophyll b and total chlorophyll content was caused only by proline application. Comparison between chlorophyll a and b contents, chlorophyll b is greater than chlorophyll a in all the rice genotypes. Thus, the response in the content

of total chlorophyll was similar to chlorophyll b. The two varieties were IN 35 and PT 1 seem higher than CNT 1 in chlorophyll contents **(**Table 3-8**).** The salinity may have a negative effect on the chlorophyll b content only in proline application, suggesting that genetics have a very high influence on this trait **(**Table 3-8**).** The pigments showed the variation in plant genotypes (2.67, 3.35, 3.57 and 4.01 mg/g fw^{-1}) of total chlorophyll under grown in different levels of salinity in japonica rice is reported by Wang et al. (2013).

Although the effect of salinity is to decrease the concentration of chlorophyll, it was not clear in the level of decreasing chlorophyll content in levels of salinity increased. Using exogenous proline for foliar spraying showed a significant effect to the increase in all types of chlorophyll measured at 100 mM proline and above. According to C. Y. Teh et al. (2015) chlorophyll content in leave of rice at seedling stage was reported in different values 2.7 mg/g under salinity stress (10 dS/m) and was 6.8 mg/g when applied exogenous proline at 20 mM. Tabssum et al. (2019) who found that total chlorophyll content was highest 6.19 mg/g of 50 mM of proline application and control was 5.90 mg/g in non-salinity stress (0 dS/m) and at stress condition (15 dS/m) was lowest value as 3.81 mg/g at vegetative stage in basmati rice. Moreover, Bhusan et al. (2016b) have shown that exogenous application of proline (25 and 50 mM) significantly increased chlorophyll b and total chlorophyll contents in salt-sensitive rice at 25 mM NaCl stress. Abdallah et al. (2016) reported that for exogenous application of trehalose at 25 mM by pre-soaking in rice increased in total chlorophyll content from 4.1 mg/g to 5.9 mg/g under 6 dS/m salinity condition. Moreover, Shahbaz et al. (2017) found that foliar application of trehalose (10-20 mM) significantly increased in chlorophyll content in two tested rice varieties under 15 dS/m of salinity condition. And also, Shahbaz et al. (2017) has been observed that photosynthetic pigments increased due to trehalose application rice under salt stress. Such pattern of increase in chlorophyll contents has already been observed in sugar beet, radish, and cabbage. Prosba-Bialczyk et al. (2013) mentioned that Carotenoids, likewise chlorophylls, take part in light absorption in the process of photosynthesis. Yet, apart from this fact, carotenoids belong to the non-enzymatic substances present in plants participating in the process of inactivating of the reactive oxygen forms. Higher resistance to unfavorable environmental conditions usually characterizes plants with higher amounts of carotenoids. Reduction in chlorophyll concentrations is probably due to loss of photosynthetic capacity and the inhibitory effect of the accumulated ions on the biosynthesis of the chlorophyll fractions.

Chlorophyll degradation is induced by many stresses, leading to changes of certain enzyme activities, photosynthetic electron transport, carbon metabolism, and photophosphorylation in photosynthesis. During salt stress, salt-sensitive plants clearly showed chlorophyll degradation and growth reduction. Kang et al. (2005) observed that salt-sensitive rice generally had lower chlorophyll contents than salt-

tolerant rice cultivars. Nevertheless, in this study, little negative impact on chlorophyll contents was caused by salinity level. Mitsuya et al. (2003) reported that chlorophyll content changing caused by a light-dependent reaction and not directly by accumulation of excess salt. In vice versa, increased chlorophyll due to active enzymes and compatible compounds to increase photosynthetic and translocate to roots and maintain chlorophyll levels reported by Farooq et al. (2013). These may explain the reason why the effect of salinity on the chlorophyll content was relatively small, but the influence of the high use of proline on the chlorophyll content was found in this study.

It can be summarized that the use of proline can significantly increase the content of chlorophyll, especially chlorophyll b and total chlorophyll, more than the use of trehalose**.** That may be the reason that when using proline, the foliar symptoms are less shriveled. Although, Adrees et al. (2015) who mentioned that salinity reduced chlorophyll and carotenoids contents of the leaf and cause chlorosis in many field crops such as alfalfa corn and *Triticum* species. In this study, salinity insignificantly affects chlorophyll contents. The rice genetic in different varieties influence is evident on the presence of different chlorophyll content**.**

The accumulation of proline depended on the proline synthesis in stress conditions and the received from exogenous spraying. The results demonstrated that the two Thai rice varieties (CNT 1 and PT 1) showed lower average response on proline content than the Indonesian rice variety (IN 35). However, under non-saline condition (0 dS/m), CNT 1 showed higher response to proline application on the accumulation of proline content than PT 1 and IN 35 (CNT 1; 1.42 and 2.01 µmole/g at 0 and 150 mM proline, respectively or increased at +41.55%, PT 1; 2.20 and 1.80 µmole/g at 0 and 150 mM proline, respectively or decreased at -18.18%, IN 35; 1.74 and 1.80 µmole/g at 0 and 150 mM proline, respectively or increased at +3.45%) (Table 9). These responses may relate to the salinity tolerance ability of rice varieties. Whereas when the three rice varieties were subjected to grow under the increasing of salinity without exogenous proline, only rice IN 35 had an increase in proline content accumulated in leaves (1.74 and 2.32 µmole/g at 0 and 15 dS/m, respectively or increased at +33.33%). However, a significant reduction in proline content in leaves was observed in both Thai rice varieties as CNT 1 (was 1.42 and 1.36 µmole/g at 0 and 15 dS/m, respectively or decreased at -4.23%) and PT 1 (was 2.20 and 1.31 µmole/g at 0 and 15 dS/m, respectively or decreased at -40.45%).

Consideration on the proline content accumulation of rice grown on high salinity level condition (15 dS/m) with proline supplement, the results showed that Thai rice varieties showed an increase in the accumulation of proline (CNT 1; 1.36 and 1.77 µmole/g at 0 mM and 150 mM proline, respectively or +30.15%, PT 1; 1.31 and 1.33 µmole/g at 0 mM and 150 mM proline, respectively or +1.53%). However, for IN 35 at 15 dS/m, it was found that the proline accumulation in the leaves increased spontaneously when grown under salinity conditions. The exogenous

proline did not affect the accumulation of this substance in IN 35 at 15 dS/m (2.32 and 2.24 µmole/g at 0 mM and 150 mM proline, respectively or -3.45%). So, proline accumulation in rice varieties may be dominantly controlled by genetic.

The results, it did not accumulate or slightly accumulate proline in the leaf tissues. This may mean that this substance was used in other processes, to maintain its ability to grow. Therefore, it is necessary to consider the effects of salinity and proline use on yield and yield composition. These responses behaviors in three rice varieties may demonstrate that IN 35 is resistant to salinity in response to increased proline accumulation in leaves. While the Thai rice varieties (CNT 1 and PT 1) were less resistant to salinity, they were more affected by proline deposition in the leaves. PT 1 appears to be more susceptible to salinity than CNT 1. However, the response of both Thai rice varieties; to the use of exogenous proline, must be considered in conjunction with the preservation of other agronomic characteristics of the rice under salinity conditions.

Proline accumulation decreased with the increasing the salinity stress condition (1.89-1.59 µmole/g) and accumulation increased (1.59-1.99 µmole/g) with the increasing foliar application of proline in leaf. The decreased growth may be in the presence of proline in plant growing in salinity stress is negatively correlated with descended rates of photosynthetic capacity; which is the role of plant leaves. However, both the accumulation of proline in leaves and stem, the resistant variety (IN 35) stored more than the susceptible varieties (CNT 1 and PT 1). Kibria et al. (2017) reported that the increased accumulation of proline in plants was correlated with improved salinity tolerance. Deivanai et al. (2011) also reported that salinity stress markedly increased proline accumulation in leaf tissues. This increase was significantly elevated at progressive level of salt in MR 232 and MR 220 cultivars and becomes static at higher concentration 400 mM NaCl in MR232 and control was 0.09 µmole/g and 0.55 µmole/g in under saline condition and in MR220, accumulation of proline was 0.09 µmole/g and 0.45 µmole/g under 400mM NaCl salinity condition. Wanichananan et al. (2003) who reported that although proline accumulation was minimal in control, exposure to external proline increased (0.10-0.45 µmole/g) its content drastically in leaf tissues of both the cultivars. Proline content of rice seedlings was affected by the presence of NaCl in the growth medium in 23 rice lines. The increment of NaCl concentration from 0 to 513 mM raised the proline inside the plants significantly, by more than 8-fold increase in some rice line.

Carillo et al. (2011) obsreved that proline is a proteinogenic amino acid with an exceptional conformational rigidity, essential for primary metabolism, which normally accumulates in large quantities in response to drought or salinity stress. Its accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment was reported by MFMR Ashraf and Foolad (2007). And also suggested that in the conditions where the rice is not affected by salinity, the exogenous supplement of proline cannot induce the accumulation of large amounts of

proline in some varieties. Nounjan et al. (2018) demonstrated that the decreased proline accumulation in saline soil growth conditions may reflect the decreased ability of photosynthesis in rice. Kishor et al. (2005) found that proline accumulation is due primarily to de novo synthesis associated with decreased oxidation and utilization, but increased transport processes are also likely involved. Also, proline is the rapid breakdown upon the relief of stress in plants. In case of plants may have sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages, then the proline is reduced (Carillo et al., 2011).

Proline accumulation changes in rice leaves of all three rice varieties under saline soil conditions. The results showed that Thai rice varieties after trehalose spraying at high salinity (at 15 dS/m salinity) showed an increase in the accumulation of proline, although not very high, with CNT 1 (was 1.28 and 1.72 µmole/g at 0 and 150 mM trehalose, respectively at increased at +34.38%) accumulated more than PT 1 (was 1.40 and 1.60 µmole/g at 0 and 150 mM trehalose, respectively at increased at +14.29%). However, the use of trehalose spray (+34.38%) could increase the proline content similar to that of proline spray (+30.15%) in CNT1 (Table 9 and 11). In PT 1, it was found that trehalose spraying (+14.29%) increased proline content more than sprayed proline itself (+1.53%) (Table 9 and 11). For IN 35, it was found that the proline accumulation in the leaves increased spontaneously when grown under saline conditions. The spraying of such trehalose sugar from the outside showed affect the accumulation of proline in leaf (was 2.03 and 2.50 µmole/g at 0 and 150 mM trehalose, respectively at increase at +23.15%) (Table 11) more than that sprayed by proline itself (-3.45%) (Table 9).

To summarize, the results shown in Table 9, in case of did not accumulate or slightly accumulate in the leaf part when spray the proline may mean that this substance was used in other processes for maintenance its growth ability; especially in non-stress condition. These responses behaviors in three rice varieties may demonstrate that IN 35 is resistant to salinity in response to increased proline accumulation in leaves when the stress was occurred. Two Thai rice varieties may be less tolerant to salinity; they were more affected by proline deposition in the leaves. PT 1 seems to be more affected and is more susceptible to salinity than CNT 1. In this case, the spray of trehalose or sugar for protection of its photosynthesis ability may is the priority. However, finally, the response of proline accumulation increasing was observed after applying the exogenous trehalose. The change in the proline content in the stem was in the same direction as that found in the leaves. Nevertheless, proline content accumulated in stem lower than in leaves. The noteworthy point was that the change in proline accumulation in the stems of IN 35 was decreased when the plants were grown in higher salinity conditions. Still, it maintains a higher amount of proline content in leaves under salty stress. Proline accumulation increased (1.62-1.93 µmole/g) with the increased application of trehalose under salt stress condition.

Abdallah et al. (2016) reported that soaking of rice seeds with 25 mM of trehalose (Tre) could alleviate the harmful effects of salinity stress at 60 mM NaCl treatment and the proline accumulation increased (60-122 μg/g fw^{-1}). This implied that salt in combination with trehalose had the ability to rapid increase proline at the onset of salt stress to improve plant salt tolerance. These results were in agreement with the results observed by Abdallah et al. (2016) on cultivars of canola plant. Moreover, the foliar application of trehalose (20 mM) at tillering stage in rice under salinity condition (15 dS/m) and proline content increased (0.9-1.1 μmole/g) is reported by Shahbaz et al. (2017). In addition, the accumulation of proline increased in rice at seedling stage (0.7-1.2 μmole/g), when the trehalose spray (30 μM) under salinity stress condition (10 dS/m) is demonstrated by Abdelgawad et al. (2014). The exogenous application of trehalose (10 mM) was evident during the recovery period by increasing the potential for growth recovery and the effect was more pronounced in the salt-sensitive cultivar, which was related to the reduction in Na^+ to K^+ ratio under 200 mM NaCl. The trehalose are known to function in protecting macromolecules by stabilizing protein structure and/or scavenging ROS produced under stress conditions (Slama et al., 2015).

Abiotic stresses can severely limit agricultural productivity in crop species, particularly at the reproductive stage. Joshi et al. (2020) mentioned that trehalose levels significantly affect the regulation of carbon allocation and utilization in plants, resulting in yield improvements under environmental stresses. Fernandez et al. (2010) indicated that previous reports suggest that trehalose acts as a positive regulator of stress tolerance in plants. However, its explicit function is still unclear, because of its multifaceted contributions in responses to environmental conditions. Beauzamy et al. (2014) mentioned that for proline, it is a known osmo-protectant, and plays an important role in osmotic balancing. Proline was reported its role for protection of sub-cellular structures, enzymes and in increasing cellular osmolarity (turgor pressure) that provide the turgor necessary for cell expansion under stress conditions. Chen and Jiang (2010) mentioned that proline is the key osmolyte, which helps plants to maintain cell turgor and helps to avoid salinity. The results of this study, was not obviously influenced of the use of trehalose on the proline content of rice. The increased of proline content in both leaf and stem from the spray of trehalose at increased levels that may show some relative to occur within plants between these substances.

Sugar content was significantly affected by variety factor in the two experiments. However, increasing in sugar content was observed in two Thai rice varieties when received the salinity stress and not use proline supplementation; CNT 1 was 36.5 and 48 mg/g at 0 and 15 dS/m, respectively or increase at +31.51 % and PT 1 was 43.4 and 47.7 mg/g at 0 and 15 dS/m, respectively or increase at +9.91 %. While, to the opposite for changing in sugar content was detected in IN 35 was 50.6 and 46.1 mg/g at 0 and 15 dS/m, respectively or increase at -8.89 %. At 15 dS/m

salinity, the spray of proline substance did not show a positive increasing percentage in sugar content in leaves. Decreased values of sugar content in all three varieties after spaying proline may infer that about the advantage of external proline to alleviate salinity stress. The results in the reduction of sugar content values in three rice varieties include in CNT 1 was 48 and 42.5 mg/g at 0 and 150 mM proline, respectively or decrease at -11.46%, PT 1 was 47.7 and 39.9 mg/g at 0 and 150 mM proline, respectively or decrease at -16.35%, and IN 35 was 46.1 and 43.7 mg/g at 0 and 150 mM proline, respectively or decrease at -5.21%.

To summarize, in case of no salinity stress (0 dS/m), the increase in accumulate of sugar in leaves was not found. However, proline may be an advantage to promote another metabolism for growth processes in plants. The spray of proline for protection of processes in plant cells and tissues, finally, decreasing sugar accumulation was observed. It was observed that the change in the sugar content in the stem was in the same direction as that found in the leaves (Table 13-14). Excepted, sugar content accumulated in stem in PT 1 was decreasing after plants growing under the salinity levels. However, PT 1 still maintains a higher amount of sugar content in leaves under salty stress. Kerepesi and Galiba (2000) observed that sugar content was influenced by plant genetic. Abdelgawad et al. (2014) also demonstrated that sugar content was reported different among the rice cultivars (range between 20-51 mg/g) under salinity stress condition. The connection between sugar content and tolerance ability for stresses has been reported. It was found that sugar contents of leaves decreased in tolerant genotypes of wheat under NaCl stress. Similarly, in the drought stress, the water-soluble carbohydrate was decreased in plants, which had linked to stress tolerance. Hakim et al. (2014) reported that salinity levels significantly influenced the content of reducing sugars in rice leaves. At 4 dS/m, maximum reducing sugar was found in MR52 (37.22 mg/g fw^{-1}), Pokkali (34.57 mg/g fw^{-1}) and the minimum amount were observed in IR20 (19.82 mg/g fw^{-1}). However, at 12 dS/m, the reducing sugar in leaves decreased with increasing salinity in all varieties and the highest value was observed in MR211 (21.92 mg/g fw^{-1}), while the lowest (13.81 mg/g fw^{-1}) was recorded in BRRI dhan29. Sultana et al. (1999) reported that the initial changes in osmotic potential were largely due to reducing sugars. After seed initiation, leaf mesophyll tissues are the closest source of sugars and provide current photo-assimilate for the growth and development of seeds. By analyzing the biochemical constituents of both control and stressed grains, ovary photosynthetic pigments and photo-assimilates (sugars) were less sensitive to salinity at the early stage of grain filling, but the sensitivity increased markedly when salinity level and duration were increased. Ashraf and Harris (2013) who found that reduction in photosynthetic pigments, hill reaction, carboxylase enzymes, and chlorophyll induction under saline conditions led to poor photosynthetic formation.

The presence of rice genetic (varieties) influence on the response to sugar accumulation resulted in statistically insignificant differences in mean differences due

to salinity and trehalose levels (Table 15 and 16). For this reason, although there was no statistical significance of the interaction between any of the factors, changes in the sugar content of each rice variety should be considered. For the percentage change of sugar content in leaf, at the no stress from salinity at 0 dS/m, a positive change in sugar content was observed only in Thai rice varieties received trehalose at 150 mM by spray. The results showed the positive change percentage values of sugar content in CNT 1 (was 48.3 and 65.9 mg/g at 0 and 150 mM trehalose, respectively or increase at +36.44 %) and PT 1 (was 51.2 and 64.8 mg/g at 0 and 150 mM trehalose, respectively or increase at +26.56 %), and negative change percentage value in IN 35 (was 52.2 and 51.4 mg/m at 0 and 150 mM trehalose, respectively or decrease at -1.53 %) (Table 15). The responses of all three rice varieties at no salinity stress to trehalose used (0 and 100 mM trehalose) were the same as those of proline used (0 and 100 mM proline) to sugar accumulation in leaf, which increasing value was found in Thai rice varieties. In addition, there was an increase in leaf sugar accumulation in both Thai rice varieties and a decrease in IN 35; as follows: in CNT 1 (was 48.3 and 75 mg/g at 0 and 15 dS/m, respectively or increase at +55.28 %), PT 1 (was 51.2 and 55 mg/g at 0 and 15 dS/m, respectively or increase at +7.42 %) and IN 35 (was 52.2 and 47.1 mg/g at 0 and 15 dS/m, respectively or decrease at -9.77 %) (Table 15). At the level of salinity stress, 15 dS/m salinity, the spray of trehalose substance showed a positive percentage in sugar content in leaves only in PT 1 and IN 35. The results in the reduction of sugar content values were observed in CNT 1 (was 75 and 53.5 mg/g at 0 and 150 mM trehalose, respectively, or decrease at -28.67%). While the positive percentage in sugar content in leaves was determined in PT 1 (was 55 and 60 mg/g at 0 and 150 mM trehalose, respectively or increase at +1.82%) and IN 35 (was 47.1 and 67.1 mg/g at 0 and 150 mM trehalose, respectively or increase at +42.46%) (Table 15).

Eastmond and Graham (2003) observed that trehalose-6-phosphate synthase (TPS) is required for both sucrose regulations during deposition of storage reserves and in metabolism during late-embryo development, implying that it has a role in sugar signaling. Joshi et al. (2020) reported that previous studies on rice and maize have also shown that effective partitioning and enhanced growth can be achieved through spatial and temporal regulation of enzymes in sink tissues that hydrolyse trehalose-6-phosphate (T6P). It has been demonstrated that sucrose is required for spikelet development and that it acts as a signal for preventing starvation- induced abortion. Ba et al. (2020) mentioned that enhanced production of soluble sugars may also lead to higher starch accumulation in leaves as a temporary carbon reserve and as a primary component of dry matter accumulation. Aboagla and Terada (2003) have been found that trehalose to be more effective than most sugars at increasing lipid bilayer fluidity and at preserving enzyme stability during drying. And also, Figueroa and Lunn (2016) who observed that trehalose must initiate a phase change or intercalate into a target structure in the cell to prevent it from being denatured by

NaCl. Then, suboptimal levels of the sugar would lead to parts of the target forming bonds with trehalose, whereas, other parts would be making hydrophilic contacts with water or inorganic ions. The additional disorder that this asymmetry would introduce would add to the distortion of the molecule mean to be protected. By contrast, higher trehalose concentrations would provide sufficient sugar to produce a more regular covering for the osmotically sensitive target and so reduce the damage from the stresses.

Munns and Tester (2008) reported that the differences in response that observed between lamina and sheath of leaf or between short and prolonged treatment might reflect differences in accumulation or catabolism of trehalose in different parts of the plant. To summarize, in case of no salinity stress, the increase in accumulate of sugar in leaves was found in Thai rice varieties. The responses behaviors after salinity stress occurred in three rice varieties, it might be concluding that IN 35 (reduced sugar accumulation) is more tolerant to salinity stress more than CNT 1 and PT 1 (induced sugar accumulation). In this case, the spray of trehalose for protection of processes involved the photosynthesis. For trehalose application, it was observed that the change in the sugar content in the stem was a different direction of accumulation as that found in the leaves (Table 16). Decreasing sugar content accumulation was found in stem in all rice varieties; compared between at 0 and 15 dS/m salinity. These changes were likely related to the need for sugar to maintain photosynthesis at the leaves rather than the stems.

Starch content in stem was significantly different in three tested varieties. As a result of higher sugar accumulation in CNT1 (Table 13 and 14), starch accumulation was clearly reduced both in leaf and stem when affected by salinity (Table 17 and 18). For starch content in stem part, CNT 1 was reduced in this trait when compared at 0 and 15 dS/m salinity (was 70.6 and 40.8 mg/g at 0 and 15 dS/m, respectively or decrease at -42.21 %). Opposite, the induction on starch content was observed in both PT 1 (was 46.6 and 63.5 mg/g at 0 and 15 dS/m, respectively or increase at +36.27 %) and IN 35 (was 56.2 and 62.4 mg/g at 0 and 15 dS/m, respectively or increase at +11.03 %). This may be related to the plant's ability to transform organic matter to protect the plant from damage caused by salinity. The benefit of the starch accumulation in the stem during the flowering stage is the plant transport with xylem from stem to panicle for grain filling mechanism.

In salinity-free conditions, proline applying did not significantly increase the starch accumulation in the three rice varieties. On the other hand, the use of proline when plants were grown in saline soil conditions was found to increase the starch content in the stem in CNT 1 (was 40.8 and 77.9 mg/g at 0 and 150 mM proline, respectively or increase at +90.93 %) (Table 18). This is, CNT 1 is probably not salinity-tolerant variety, but responds better to external agents such as proline than PT 1 (was 63.5 and 46.1 mg/g at 0 and 150 mM proline, respectively or decrease at -27.40 %). While in IN 35 (was 62.4 and 63.4 mg/g at 0 and 150 mM proline,

respectively or increase at +1.60 %), which tended to be more tolerant to salinity than other varieties, was less responsive to external use. Nonetheless, the results on the increase or decrease change in these three varieties were not linear, hence careful consideration of the direction and magnitude of changing on starch content was necessary; although insignificant in those interactions of factors. Starch content in the stem was higher than the leaf at the flowering stage. Lunn et al. (2014) reported that starch is a product of photosynthesis for use in plant growth, and the stem is part of the plant that stores that product or sink part in plants.

Starch accumulation in leaves was less than in stems, as was the case with studies using proline spray. The starch accumulation in stem was recorded the same trend with leaf content (Table 17-18). Nawaz and Farooq (2017) observed that in plant organ, which deposited the largest amount of starch, was kernel, followed by internode, leaf-sheath, leaf-blade, and root in that order. Both in leaves and stem, higher starch content was found in IN 35, although was significant difference only in stem (Table 17 and 18). Hirose et al. (2006) mentioned that stem (leaf sheath and culms) of rice plants accumulate high level of starch before heading, which is subsequently remobilized after heading to provide a carbon source of grain filling. In flowering stage in rice, young panicles were formed, tiller ceased and root growth was limited, but photosynthesis continued. Therefore, much transitory starch was gradually accumulated in the expanded, maturing parenchyma of leaf-sheath and stem-internode, reaching its peak before or after heading. Oh-e et al. (2007) found that a decrease of starch amount in leaf-sheath at heading is caused by death of lower and older leaves. Therefore, the developing/expanding organs of plants may cause the higher starch content of the stem at this flowering stage in rice growth (approximately 6-7 times compared to the leaf portion). Xu et al. (2021) reported that before heading, nitrogen content of shoots showed, in general, a negative correlation with starch content, probably because more carbohydrates were consumed by more growth and more respiration of plants with higher nitrogen content than plants of lower nitrogen content, resulting in less starch remaining in the former. Xu et al. (2021) also mentioned that during the ripening period, most transitory starch was mobilized and translocate to grains during about a month after heading. After that, starch was considerably re-accumulated in the basal internodes but not in leaf- sheaths.

Starch accumulation perhaps resulted from the increased activity of alkaline inverse activity, which hydrolyzes sucrose converts into simpler sugars. Starch may be synthesis from such sugar. Pattanagul and Thitisaksakul (2008) mentioned that although starch may not play a crucial role in salt tolerance mechanism, it was suggested that the ability of plant to partition sugar into starch might help to avoid metabolic alternations by lowering feedback inhibition caused by excess amount of sucrose into cytoplasm. Sadak (2019) reported that the exogenous application of trehalose (50 mM) in wheat under 6.25 dS/m salinity stress condition, starch accumulation increased from 53 mg/g to 73 mg/g under stress condition of foliar

application of trehalose. Overproduction of trehalose in various systems including plants has been reported to be beneficial for improving tolerance against salinity and drought. In addition to its role as an osmoprotectant, trehalose and its intermediate trehalose-6-phosphate (T6P) have also been reported by Redillas et al. (2012) and to enhance plant stress tolerance via sugar signaling by allocating and metabolizing carbohydrates. Garg et al. (2002) found that higher levels of soluble carbohydrates (including trehalose) under stress conditions indicate that trehalose acts as a positive regulator of genes associated with sugar sensing and carbon metabolism, as shown in previous studies. Gangola and Ramadoss (2018) mentioned that the diverse and complex network of sugar signaling has been identified as a crucial component in responses to abiotic stresses in plants.

In this study, when Thai rice plants thrive under saline conditions (at 15 dS/m), a significant factor affected by proline levels was mostly found to be genetic differences among the varieties. Exogenous proline use does not encourage the increased accumulation of this substance in the leaves, in other words, genetics influences this potential. Whereas IN35 increased an accumulation in leaves likely came from the proline substance directly sprayed to the rice. Maybe, for the salinity tolerance genetics may not respond to proline synthesis from the application of exogenous substances for stimulating.

5.2 Semi Quantitative RT PCR

For CNT 1 at 10 dS/m, RNA expression as measured by leaf cDNA content was consistent with the chemically analyzed proline content (Table 9 and Figure 6). Both chemical and cDNA analysis resulted, proline content was found higher at two concentrations of proline spray: at 50 mM (1.80 µmole/g) and 100 mM (µmole/g) proline; although there was not a significant difference. It is possible that external spraying of proline (at 50 and 100 mM proline) would have a beneficial effect on stimulating proline production in rice at 10 dS/m salinity compared to the control treatment at 0 mM proline (1.42 µmole/g). However, higher concentrations of exogenous proline (150 mM proline) did not increase the accumulation of proline in leaves (Table 9) or stems (Table 10) and did not increase the amount of cDNA in the leaves (Figure 6). Both chemical and molecular proline analysis in leaves was carried out after five days of external spraying of proline. Therefore, the maximum amount of spraying, at 150 mM proline, may not be accumulated on leaves or stems but may be used to promote the growth of rice that is experiencing salinity and affects growth.

These observations were found with the PT 1 by using different concentrations of proline sprayed at the salinity level 10 dS/m. In other words, this may be the response of Thai rice varieties at 10 dS/m salinity to the use of exogenous proline. Proline is deposited to some extent in the plant along with the plant synthesis of the proline to make the plant resistant to stress. Plants are partially able to produce proline on their own (at 0 mM proline), but when proline is sprayed externally, there

has been an increase in the stimulation of proline production in the plant, but only at the use of proline at 50-100 mM (Figure 6). Therefore, when sprayed at 150 mM proline, it was found that proline synthesis in the plant decreased (Figure 6). The reduction of proline in both the synthesis and the deposition in the leaves at 150 mM proline application were observed (Table 9 and Figure 6). Which, may mean that have the limit of the quantity of exogenous proline be used to stimulate the synthesis of this substance inside plants. At highest concentrations of proline from outside by spraying (150 mM proline), more than not increase stimulate the synthesis of proline in the plant, it also did not increase the accumulation in the plant; but may be used in other processes in the plants.

For IN35, the leaf proline accumulation from the chemical analysis was higher at concentrations between 50-150 mM proline (Table 9). This was in contrast to the cDNA quantitative analysis in which the absence of exogenous proline (0 mM) seemed the highest (Figure 6). This may reflect that IN 35, which is likely to be salinity tolerant when assessed in other agronomic characteristics, did not respond to increase leaf proline synthesis. Increasing the amount of proline in the leaves may be caused by the accumulation of exogenous proline; which, was in the same direction between the chemical values found in the leaves (Table 9) and stems (Table 10) in IN 35. Many studies showed that salt stress triggers the induction of genes involved in proline biosynthesis, which leads to proline accumulation (El Moukhtari et al., 2020). According to Shafi et al. (2019), knocking out the function of *P5Cs* in *A. thaliana* indicates a key role for this enzyme in plant salt tolerance because the *P5Cs1* plants are hypersensitive to salt. Exogenous application of proline can effectively improve tolerance of plants to salt stress through the regulation of endogenous proline metabolism, partly achieved through differential expression of specific proline-related genes. Nounjan et al. (2012) have shown that applying exogenous proline significantly increased expression of *P5Cs* and *P5CR* in salt-stressed *Oryza sativa*.

5.3 Effect of proline and trehalose on agronomic characters

For these results, the varieties most affected by salinity as assessed by the damage to pollen viability were both Thai rice varieties; CNT 1 and PT 1, and the lowest was IN 35 (Figure 8-9). The use of external proline and trehalose can increase the viability of pollen in both no salinity and salinity conditions. Salinity stress and proline affected on pollen viability percentage in all varieties. The results showed that, pollen viability percentage decreased with increased the salinity level. CNT 1 was 67% and 40% at 0 and 15 dS/m, decreased at – 40.2% and increased pollen viability percentage was 40% and 53% at 0 and 150 mM of proline under 15 dS/m saline condition at increase + 32.5% respectively. PT 1 was 60% and 40% at 0 and 15 dS/m, decreased at – 33.3% and increased the pollen viability percentage was 40% and 46% at 0 and 150 mM of proline under 15 dS/m saline condition, increased at +15% and

IN 35 was 67% and 60% at 0 and 15 dS/m, decreased at – 10.5% and increased the pollen viability percentage was 60% and 67% at 0 and 150 mM of proline under 15 dS/m saline condition, increased at +11.7% resceptively (Figure 8).

Using of trehalose spray was similar trend with the proline spray, CNT 1 was 60% and 40% at 0 and 15 dS/m, decreased at – 33.3% and increased the pollen viability percentage was 40% and 53% at 0 and 150 mM of trehalose under 15 dS/m saline condition at increase + 32.5% respectively. PT 1 was 60% and 40% at 0 and 15 dS/m, decreased at – 33.3% and increased the pollen viability percentage was 40% and 46% at 0 and 150 mM of trehalose under 15 dS/m saline condition, increased at +15% and IN 35 was 60% and 46% at 0 and 15 dS/m, decreased at – 23.3% and increased the pollen viability percentage was 46% and 60% at 0 and 150 mM of trehalose under 15 dS/m saline condition, increased at +30.4% resceptively (Figure 9). The increasing percentage was seen in all three rice varieties at 50 mM proline and trehalose. However, the effect of proline at different concentrations for application was less (Figure 8 and 9).

Abdullah et al. (2001) observed that the reduction in pollen viability will affect the percentage of success fertilization or as assessed by the percentage of filled grain, and indicated by the number of seeds per panicle that is an important yield component and finally, yielding was affected in the last. Palao et al. (2013) reported that the higher accumulation of sodium in floral parts, it has been reported to reduced plant's inflorescences, possibly caused by ionic toxicity under salinity stress and the decrease of pollen viability. Shereen et al. (2005) found that the severe inhibitory effects of salts on fertility may be due to the differential competition in carbohydrates supply between vegetative growths and constrained its distribution to the developing panicles. Whereas, other is probably linked to reduce viability of pollen under stress condition, thus resulting failure of seed set is reported by Shereen et al. (2005).

Plant height is the most important character among the morphology and physiology, which also acts as a key of shoot yield as well as total biomass production. In this study, plant height changes in all varieties under salinity stress condition**.** The results showed that at high salinity (at 15 dS/m salinity) was shortest plant height in all cultivars, CNT 1 was 95.3 cm and 90 cm at 0 and 15 dS/m, at decreased – 5.56% and PT 1 was 92.6 cm and 86.0 cm at 0 and 15 dS/m, respectively at decreased – 7.13% and IN 35 was 91.6 cm and 87 cm at 0 and 15 dS/m, at decreased – 5.02% respectively in proline experiment (Table 21). In each rice varieties, although the using of proline could increase the height of the rice plant, the increase was minimal. The plant height increase was seen when using proline on rice grown in soils with salinity at 10 dS/m and above (Table 21).

Using of trehalose spray was a little affected but high saline condition influenced on plant height in all varieties. CNT 1 was 95.3 cm and 90.6 cm at 0 and 15 dS/m, at decreased – 4.93% and PT 1 was 94.3 cm and 86.3 cm at 0 and 15 dS/m,

at decreased – 8.48% and IN 35 was 95 cm and 87 cm at 0 and 15 dS/m, at decreased – 8.42% respectively (Table 22).

This experiment showed that plant height of these rice varieties was affected by salinity since at 5 dS/m, which is in agreement with study by Hakim et al. (2014) that rice is a salinity sensitive plant species. M. Hasanuzzaman et al. (2009) has been reported that plant height decreased progressively with increase in salinity levels. The results were similar to that of proline use, it was found that the use of trehalose increased the plant height in both non-salty and salty conditions, but had little effect with no significant difference **(**Table 21-22**).** Both proline and trehalose applying showed less effect to plant height may because proline application was applied to rice plants one week before the flowering stage. Therefore, the plant height is a pre-existing characteristic that is not affected by these substances spray. Plant height in rice was reported decreased with increased salinity levels. Moreover, Siddique et al. (2015) observed that the effects of salinity is reduced grain yield under salinity condition might be due to the production of less effective tillers, lower panicle length, lower number of grains per plant, seed weight, plant height, etc. Although, Tabssum et al. (2019) published that salinity significantly decreased plant growth, the application of proline can be increased the growth and yield at seedling stage in rice. Scudiero et al. (2015) reported that from the assessment it can be said that plant height is a trait that is mainly controlled by genetics. It is characterized by relatively little effect of both salinity and proline use. Saline soil was considered as the soil with more than 4 dS/m salinity levels.

Zeng and Shannon (2000) observed that the number of fertile tillers is an important parameter among the yield component characters. Fertile tiller number also changes in all varieties under salinity stress condition but the use of proline and trehalose spray was not affected in under normal and saline condition**.** The results revealed that at high salinity (at 15 dS/m salinity) was lowest fertile tiller number in all cultivars, CNT 1 was 8 and 7 at 0 and 15 dS/m, respectively at decreased – 12.5% and PT 1 was 11 and 8 at 0 and 15 dS/m, respectively at decreased – 27.3% and IN 35 was 12 and 10 at 0 and 15 dS/m, at decreased – 16.7%, respectively in proline experiment (Table 23). However, could be said that starting at 5 dS/m salinity that all three varieties had a considerable reduction in the number of fertile tillers per plant in high value compared with no salinity condition (0 dS/m) (Table 23). Kibria et al. (2017) also reported that significant decrease in number of total tiller per hill has been reported by application of different level of salinity.

The usage of trehalose spray, CNT 1 had fertile tiller number as 10 and 6 at 0 and 15 dS/m, respectively at decreased – 40% and PT 1 was 11 and 8 at 0 and 15 dS/m, respectively at decreased – 27.3% and IN 35 was 10 and 9 at 0 and 15 dS/m, at decreased – 10% respectively (Table 24). Thus, higher tolerance to salinity on fertile tiller number per plant was IN 35; lower in two Thai rice varieties (CNT 1 and PT 1). Salinity affects the fertile tillers because rice is salinized at the beginning of the

tillering stage (at 30 days old after planting until harvesting). However, for proline or trehalose application, it was applied to rice plants one week before the flowering stage. Chakraborty et al. (2018) indicated that the number of fertile tillers is pre-existing characteristic is not affected by proline spray. Na^+ in the soil solution and the resultant reduction of K^+ and Ca^{2+} uptakes cause the inhibition of the proper functioning of cells, instability of cell membrane, and hindrance of enzymatic activity. Appearance of rice tillers can be observed since the vegetative stage. Shereen et al. (2005) reported that the number of tillers is one parameter that could affect other yield components developed during vegetative and reproductive stages, such as number of productive tillers, panicle length.

Salinity stress and proline affected on panicle length in all varieties. The results found that, panicle length decreased with increase the salinity level. CNT 1 was 23.6 cm and 23.2 cm at 0 and 15 dS/m, decreased at – 5.69% and increased panicle length was 23.2 cm and 24 cm at 0 and 150 mM of proline under 15 dS/m saline condition at increase + 3.5% respectively. PT 1 was 25 cm and 22.7 cm at 0 and 15 dS/m, decreased at – 9.2% and increased the panicle length 22.7 cm and 23.6 cm at 0 and 150 mM of proline under 15 dS/m saline condition, increased at +3.96% and IN 35 was 21.7 cm and 21.4 cm at 0 and 15 dS/m, decreased at – 1.4% and increased the panicle length was 21.4 cm and 21.8 cm at 0 and 100 mM of proline under 15 dS/m saline condition, increased at +1.90% resceptively (Table 25). The absence of a statistically significant difference between the interactions between the factors showed that the use of proline could increase the length of the panicle at different salinity levels (Table 25).

The use of trehalose spray was similar trend with the proline spray, CNT 1 was 24.2 cm and 23.1 cm at 0 and 15 dS/m, decreased at – 4.5% and increased panicle length was 23.1 cm and 24.1 cm at 0 and 150 mM of trehalose under 15 dS/m saline condition at increase + 4.3% respectively. PT 1 was 24.6 cm and 23.2 cm at 0 and 15 dS/m, decreased at – 5.7% and increased the panicle length was 23.2 cm and 24.2 cm at 0 and 150 mM of trehalose under 15 dS/m saline condition, increased at +4.3% and IN 35 was 21.7 cm and 21.2 cm at 0 and 15 dS/m, decreased at – 2.3% and increased the panicle length was 21.2 cm and 21.6 cm at 0 and 100 mM of trehalose under 15 dS/m saline condition, increased at +1.9% resceptively (Table 26). For panicle length, rice plants showed a response to the application of proline more than trehalose.

Panicle length decreased with increased salinity levels and that increased with the application of proline. Ali et al. (2004) found that salinity affects plant physiology through changes of the water and ionic status in the cells and reduced panicle length Siddique et al. (2015) also reported by similar findings that reduction in rice panicle length was observed under increased salinity level.

1,000 seed weight also changes in all varieties under salinity stress condition but the use of proline and trehalose spray was not affected in under normal and saline condition (Table 27-28). The results showed that at high salinity (at 15 dS/m salinity)

was lowest 1,000 seed weight in all varieties. CNT 1 was 25.5 g and 25.1 g at 0 and 15 dS/m, respectively at decreased – 1.6% and PT 1 was 24.8 g and 24.4 g at 0 and 15 dS/m, respectively at decreased – 1.6% and IN 35 was 26 g and 25.5 g at 0 and 15 dS/m, at decreased – 1.9% respectively in proline experiment (Table 27).

The use of trehalose spray, CNT 1 was 25.5 g and 25.1 g at 0 and 15 dS/m, respectively at decreased – 1.6% and PT 1 was 24.9 and 24.4 at 0 and 15 dS/m, respectively at decreased – 2% and IN 35 was 25.9 g and 25.4 g at 0 and 15 dS/m, at decreased – 1.9% respectively (Table 28). Reduction of 1,000-seed weight under salinity condition might be due to lower amounts of assimilate translocation from leaf to grain. Mahmood et al. (2009) explained that 1,000-grain weight decreased with increased salinity levels. The results of this study were similar to the response of rice from proline spraying. Where, the frequency of use of the substance use is still too low and does not correspond to the trait to be assessed: such as 1,000 seed weight. Fageria (2007) reported that seed weight was found to be stable (highly heritable within cultivar) under various stressful conditions, compared to other yield components, such as plant height, active tillering and seed per panicle. The results of this study were similar to the response of rice from proline spraying. Where, the frequency of use of these substances (proline and trehalose) use is still too low and does not correspond to the trait to be assessed: such as 1,000 seed weight. However, no effects of use of trehalose that directly promote on either 1,000 seed weight (Table 28) or panicle length (Table 26).

Presumably salinity reduces the contents of photosynthetic pigments and soluble proteins in the ovaries. This change might cause the decline of ovary photosynthesis leading to poor sugar production in the ovaries. A reduced number of fertile florets and a lower rate of assimilate translocation from shoot to panicles are responsible for the lower dry weight of panicles and grain. This might be attributable to the rapid reduction in leaf photosynthesis, which is related to the decrease in photosynthetic pigments. Therefore, translocation of assimilates from stem to grain is the main source as well as limiting factors for growth and development of seeds. Maas et al. (1986) obesrved that salinity in reproductive growth stage decreases weight of 100 seeds weight in rice. De Lacerda et al. (2003) also reported that this might be due to lower accumulation of carbohydrates and other food materials due to salt stress, thus, 1,000-grain weight decreases with increase in levels of salinity. In this study, the using of proline promoted the increase in the panicle length, but no effect on the 1,000-grain weight. Nevertheless, salinity had a clear effect on the grain weight decreased. It can be explained that only spray of proline or trehalose; one week after flowering, may affect the panicle length (Table 25), but not extend to the accumulation of weight within the grain (Table 27-28).

Salinity stress and proline affected on filled grain percentage in all varieties. The results showed that, filled grain percentage decreased with increased the salinity level. CNT 1 was 76.7% and 35% at 0 and 15 dS/m, decreased at –54.4% and

increased filled grain percentage was 35% and 49% at 0 and 150 mM of proline under 15 dS/m saline condition at increase + 40% respectively. PT 1 was 74.7% and 24.3% at 0 and 15 dS/m, decreased at – 67.5% and increased the filled grain percentage was 24.3% and 29% at 0 and 150 mM of proline under 15 dS/m saline condition, increased at +19.3% and IN 35 was 73% and 62.3% at 0 and 15 dS/m, decreased at – 14.7% and increased the filled grain percentage was 62.3% and 64.3% at 0 and 150 mM of proline under 15 dS/m saline condition, increased at +3.2% resceptively (Table 29). In varieties and salinity interaction, the higher the salinity level was caused the decreasing percent of filled grain in all varieties, but in different of magnitudes. However, this is another characteristic feature that can be used to assess the tolerance of varieties to salinity.

Using of trehalose spray was similar trend with the proline spray, CNT 1 was 74.3% and 34% at 0 and 15 dS/m, decreased at – 54.2% and increased the filled grain percentage was 34% and 42.7% at 0 and 150 mM of trehalose under 15 dS/m saline condition at increase + 25.6% respectively. PT 1 was 72% and 21.3% at 0 and 15 dS/m, decreased at – 70.4% and increased the filled grain percentage was 21.3% and 25.3% at 0 and 150 mM of trehalose under 15 dS/m saline condition, increased at +18.8% and IN 35 was 72.7% and 61.7% at 0 and 15 dS/m, decreased at – 15.1% and increased the filled grain percentage was 61.7% and 63.7% at 0 and 150 mM of trehalose under 15 dS/m saline condition, increased at +3.2% resceptively (Table 30).

In this study, the use of external proline and trehalose can increase the filled grain percentage that may also be associated with an increase in the viability of pollen; in both no salinity and salinity conditions when this substance was used. Moreover, the increasing percentage in filled grain was seen at 50 mM proline, similar to the effect on pollen viability (Figure 8 and Table 29). In addition, under salinity condition increased the salinity level decrease the filled grain percentage. Sterility and reduction in seed sets were primarily affected by salinity stress. Many mechanisms of plants were reported affected by salinity stress, such as reduced translocation of soluble carbohydrates to primary and secondary spikelets. Moreover, the accumulation of more sodium and less potassium in all floral parts in rice also was found. Abdullah et al. (2001) reported that the physiological inhibition of the specific activity of starch synthesis during development of rice grains can be a factor to reduced seed set at salinity stress. Aref and Rad (2012) mentioned that the number of filled grains per panicle was decreased with the increase of soil salinity levels also was reported in many studies. Increased number of empty grains might be a result of assimilates shortage during grain filling, brought about by early leaf senescence caused in this case by salinity. Sterility and reduction in seed set were primarily due to reduced translocation of soluble carbohydrates to primary and secondary spikelets. Moreover, Abdullah et al. (2001) reported that the accumulation of more sodium and less potassium in all floral parts and inhibition of the specific activity of starch synthetase in developing rice grains, thus reducing seed set. Islam et al. (2018) also

reported that reducing seed set in the panicle, possibly as a consequence of decreased pollen viability, which is greatly influenced by the ionic toxicity under salinity.

Similarly, the result of the reduction in filled grain percentage both in proline and trehalose spraying. That, the magnitude of reduction in plants received salinity levels increased was lowest in IN 35 followed by CNT 1 and highest in PT 1 (Table 29-30). An important problem of rice cultivation in saline soils is grain withered, with can be caused by fertilizing failure. Even the fertilized rice seeds are found; to the lighter seed weight of rice seeds are observed. Considering this important characteristic; filled grain percentage, it could be seen that IN 35 is likely to be more tolerant to salinity than Thai rice varieties: CNT 1 and PT 1. So, CNT 1 was more tolerant to salinity stress than PT 1 (Table 29-30).

Salinity stress and proline influenced on yield per plant in all varieties. The results showed that, yield per plant decreased with increased the salinity level. CNT 1 was 9.8 g and 4.5 g at 0 and 15 dS/m, decreased at – 54% and increased yield per plant was 4.5 g and 5.3 g at 0 and 150 mM of proline under 15 dS/m saline condition at increase + 17.8% respectively. PT 1 was 9.5 g and 3.1 g at 0 and 15 dS/m, decreased at – 67.3% and increased the yield per plant was 3.1 g and 3.6 g at 0 and 150 mM of proline under 15 dS/m saline condition, increased at +16.1% and IN 35 was 11 g and 8.2 g at 0 and 15 dS/m, decreased at – 25.4% and increased the yield per plant was 8.2 g and 9.6 g at 0 and 150 mM of proline under 15 dS/m saline condition, increased at +17% resceptively (Table 31). From these observed results in yield components and yield per plant, varieties with the highest salinity tolerance were assessed as IN 35, followed by CNT 1, while PT 1 was the most susceptible to salinity. The absence of statistical significance between the use of proline and any of the factors; in terms of interaction, it showed that all rice varieties benefited from proline spray by increasing grain yield per plant.

The use of trehalose spray was similar trend with the proline spray, CNT 1 was 9.8 g and 3.5 g at 0 and 15 dS/m, decreased at – 64.2% and increased the yield per plant was 3.5 g and 4.1 g at 0 and 150 mM of trehalose under 15 dS/m saline condition at increase + 17.1% respectively. PT 1 was 9.5 g and 2.8 g at 0 and 15 dS/m, decreased at – 70.5% and increased the yield per plant was 2.8 g and 3.4 g at 0 and 150 mM of trehalose under 15 dS/m saline condition, increased at +21.4% and IN 35 was 11.2 g and 9.7 g at 0 and 15 dS/m, decreased at – 13.4% and increased the yield per plant was 9.6 g and 10 g at 0 and 150 mM of trehalose under 10 dS/m saline condition, increased at +4.2% resceptively (Table 32). However, in yield per plant, interaction with significant differences between varieties and salinity was observed. The yield per plant decreased with an increase in salinity levels in all rice varieties; but in different magnitudes. Same as results of the effect of proline applying, IN 35 showed less reduction on yield per plant than CNT 1 and PT 1, respectively. No interaction between trehalose with any factors means the effectiveness of trehalose to increase the grain yield in all varieties in all salinity levels.

In general, three major processes operate simultaneously during grain filling: photosynthesis, translocation of photosynthetic to the grain, and grain growth. In analyzing the reduction in grain dry matter due to stress, it is necessary to identify which of these processes is the limiting factor. The reduction in dry matter at dough stage might be through inhibition of current photo-assimilation, because salinity reduces the contents of photosynthetic pigments and soluble proteins in the ovaries; this change might cause the decline of ovary photosynthesis leading to the poor sugar production in the ovaries. Islam et al. (2018) reported that a reduced number of fertile florets and a lower rate of assimilate translocation from shoot to panicles are responsible for the lower dry weight of panicles and grain. This might be attributable to the rapid reduction in leaf photosynthesis, which is related to the decrease in photosynthetic pigments. Therefore, translocation of assimilates from stem to grain is the main source as well as limiting factor for growth and development of grain. Zeng and Shannon (2000) reported that filled grain per panicle in rice decreased by salinity. Abdullah et al. (2001) found that the reduced spikelet fertility might be due to failure of grain formation in rice grain, which could be caused by lack of pollen viability and reduced seed set.

Aref (2013) estimated a yield loss in rice of 50% with an EC of around 7.4 dS/m. (Fraga et al., 2010) demonstreated that the salinity of soil solution from 1.9 dS/m is already sufficient to significantly reduce the seedlings biomass and an EC of 3.4 dS m compromises their survival. Jouyban (2012) reported that crop yield reductions in salt-affected soils result primarily from alteration of various metabolic processes in plants under salt stress. Siddique et al. (2015) observed that proline application is to improve the growth and yield of salt-sensitive rice but not of salt-tolerant rice at 50 mM NaCl stress.

Munns et al. (2006) reported that the magnitude of salt induced yield losses could not be attributed to single factor. Different physiological, biochemical factors at different stages of rice plants may be involved. Any factor may affect some mechanisms (before flowering) of sodium uptake through root properties and its subsequent distribution in different vegetative and floral parts; especially in leaves. Where it causes leaf mortality, thereby reduces transportation of total assimilates to the growing and productive parts. Nozulaidi et al. (2015) demonstrated that about 50 percent of yield was loss with an electric conductivity (EC) of around 6.65-7.4 dS/m. It could be said that, increasing salinity level in soil could reduce rice yield. However, Zeng et al. (2001) found that the degree of susceptibility for salinity stress may be inconsistent between rice varieties. Moradi and Ismail (2007) has been reported rice as being salt-sensitive in both its vegetative and reproductive stages.

CHAPTER 6

CONCLUSION

This study provided salinity to rice from the first thirty days after planting to harvest. The results showed that salinity affected all characteristics: water content, physiological traits, and agronomic characteristics, except chlorophyll content in leaves. However, under salinity conditions (5-15 dS/m salinity), the use of external substances such as proline or trehalose in all concentrations can promote by increasing many characteristics; excluded the water content. In reproductive stage may be one stage that is tolerant of leaf dehydration when plants growing in saline soil. Nevertheless, under no salinity condition (0 dS/m), the effect of exogenous proline or trehalose was found mostly in agronomic characteristics, yield components, and yield, but rarely affected in chemical contents (proline, sugar, starch) that accumulated in plants. Nevertheless, the response on characteristics, it mainly controlled by genetics, thus, the negative effect of salinity levels or the effect from using exogenous proline or trehalose; either at salinity conditions or no salinity, varied in magnitude in each variety.

Considering the damage on characteristics affected by salinity, the varieties have less effect or high tolerance ability to salinity is IN 35, and lower in two Thai varieties. Actually, PT 1 showed better performance on many characteristics. However, CNT 1 was affected by salinity in lower magnitude in most of the characteristics and was more sensitive by applying both proline and trehalose more than PT 1. For these reasons, in Thai rice varieties, CNT 1 seems higher tolerance ability to salinity more than PT 1. Due to applying both proline and trehalose only one time at one week before the flowering stage, the influence of these substances seems observed in characteristics that establish during or after applying these substances. However, the effect of external proline or trehalose, not extended to other characteristics that build far from the time after when these substances were used.

As for accumulation of these chemical compositions: the sugar content, proline content or starch, the increase or decrease in the leaf or the stem depends on many factors including the type of substance used (relation of the sprayed substance and the characteristics what to be measured), the change in the amount of those substances after salinity exposure (that reflects the salinity tolerance of various varieties).

For the chlorophyll contents, the use of proline was higher in effectiveness to increase the values more than trehalose. The results showed that Thai rice varieties after proline spraying at high salinity (at 15 dS/m salinity) showed an increase in the accumulation of this substance, although not very high, but more than in IN 35. One

reason to explain this result in IN 35, it was found that the proline accumulation in the leaves increased automatically when grown under saline conditions.

The results, it does not accumulate or accumulate very little proline in the leaf tissues, although after proline sprays. This may mean that this substance was used in other processes to maintain its ability to grow. These responses behaviors in three rice varieties may demonstrate that IN 35 is resistant to salinity to accumulate the proline in plant cells of leaves autonomic. While, the rice varieties were less tolerant to salinity, such as CNT 1 and PT 1, they were more affected by proline deposition in the leaves when received the proline application. Nevertheless, the change of proline accumulation has occurred in a smaller change in stem compared to the leaves. In CNT 1 and PT 1, at higher salinity level (10 dS/m), plants are partially able to produce proline on their own (at 0 mM proline). However, when proline is sprayed externally, there has been an increase in the stimulation of proline synthesis in the plant; detected by cDNA synthesis, but only at the use of proline at 50-100 mM. Therefore, when sprayed external proline at the highest concentration (150 mM proline), it was found that proline synthesis and accumulation in the plant decreased. Which, may mean that have the limit of the quantity of exogenous proline be used to stimulate the synthesis of this substance inside plants. External proline that plant received by spraying did not increase the accumulation in the plant at 150 mM proline, but this substance may be used in other processes in the plants. For IN 35, which is likely to be salinity tolerant when assessed in other agronomic characteristics, did not respond to increase leaf proline synthesis, although has been stimulating by proline spraying. Thus, the increasing the amount of proline that accumulated in the leaves may be caused by exogenous proline accumulation. However, at the highest salinity level (15 dS/m), exogenous proline use does not encourage the increased synthesis of this substance in the leaves in all rice varieties, in other words, genetics influences this potential. Therefore, increased deposition of proline in leaves may be obtained directly from spraying.

In case of no salinity stress, the increase in accumulate of sugar in leaves was found in Thai rice varieties. It may be concluding that IN 35 (reduced sugar accumulation) is more tolerant to salinity stress more than CNT 1 and PT 1 (induced sugar accumulation). In this case, the spray of trehalose for protection of processes involved the photosynthesis. For trehalose application, it was observed that the change in the sugar content in the stem was a different direction of accumulation as that found in the leaves. Decreasing sugar content accumulation was found in stem in all rice varieties; compared between at 0 and 15 dS/m salinity. These changes were likely related to the need for sugar to maintain photosynthesis at the leaves rather than the stem.

Similarly, the use of proline when plants were grown in saline soil conditions was found to increase the starch content in the stem in CNT 1. CNT 1 is probably not salinity-tolerant varieties, but it responds better to external agents such as proline than

PT 1. While lower changing was observed in IN 35, tended this variety to be more tolerant to salinity than other varieties, was less responsive to external use. Effect of trehalose applying showed very little change compared with proline in the starch content of both leaves and stems in each variety

Pollen viability was affected in decrease value by salinity in all rice varieties. Which, the varieties most affected by salinity were both Thai rice varieties; CNT 1 and PT 1, and the lowest was IN 35. The use of external proline and trehalose can increase the viability of pollen in both no salinity and salinity conditions; although not test the statistic. The increasing percentage was seen in all three rice varieties at 50 mM of these substances and above. At the normal condition (no salinity, 0 dS/m), PT 1 and IN 35 had a high number of fertile tillers. However, at 15 dS/m salinity, PT 1 had a considerable reduction in the number of fertile tillers per plant in high value compared with CNT 1 and IN 35. Salinity affects the fertile tillers because rice is salinized at the beginning of the tillering stage (at 30 days old after planting until harvesting). Thus, for proline application, it was applied to rice plants one week before the flowering stage. Therefore, the number of fertile tillers is the pre-existing characteristic that is not affected by proline spray. Since at 5 dS/m salinity, plant height and yield components were received the negative effects. Thai rice was decreasing in most of the characteristics more than IN 35, among Thai rice varieties; PT 1 seems more affected by salinity than CNT 1. Proline and trehalose showed, no effect or little effect (with no significant difference) in plant height, the number of fertile tillers, and 1,000-grain weight because these characteristics were established not consistent with the time for substance use. However, these external substances can increase the value in panicle length and filled grain percentage. Further, the effect on the percent of grain filling is likely to be consistent with the effect on pollen viability. In comparision between the use of proline and trehalose, the result of proline spray is better performance in biochemical and agronomic characters than the trehalose.

Finally, yield could induce to increase by applying both proline and trehalose at the reproductive stage, especially at salinity conditions. This experiment should find out next research to confirm these research finding.

Appendix

Appendix 1 Weather parameter during the investigation in Hua-Hin, Prachuap Khiri Khan Province

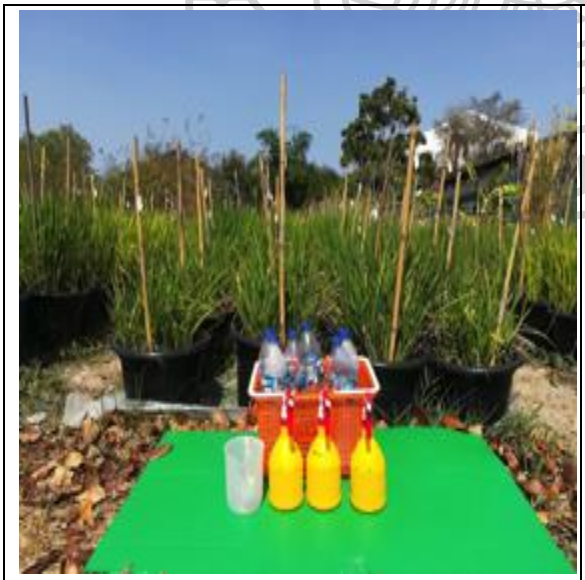
Month	Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (mm)	Relative humidity (%)	Wind speed (km/h)
2020					
January	29	23	3.4	67	11.2
February	29	23	0.9	67	12.8
March	31	25	7.7	69	16.5
April	32	27	65.9	69	15
May	33	28	91.8	69	13.8

Source;https://www.accuweather.com/en/th/hua-hin prachuap khiri khan/320001/ weather-forecast /320001

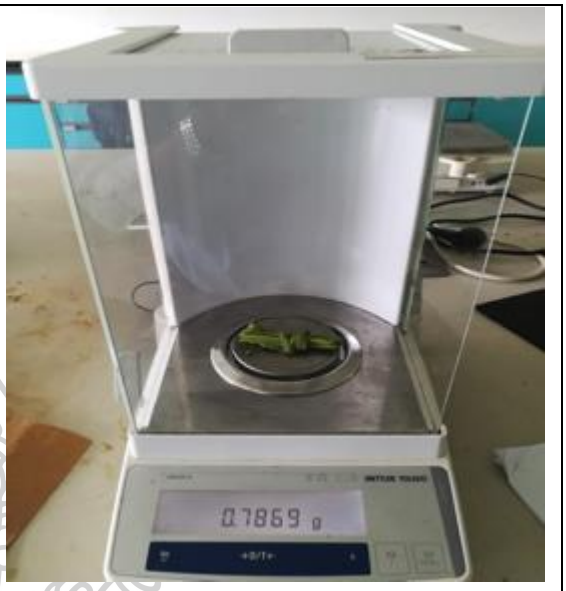
Appendix 2 Seedling and experimental layout of the two experiments

Appendix 3 Preparation of salt solution and pouring the different level of salinity water into plant

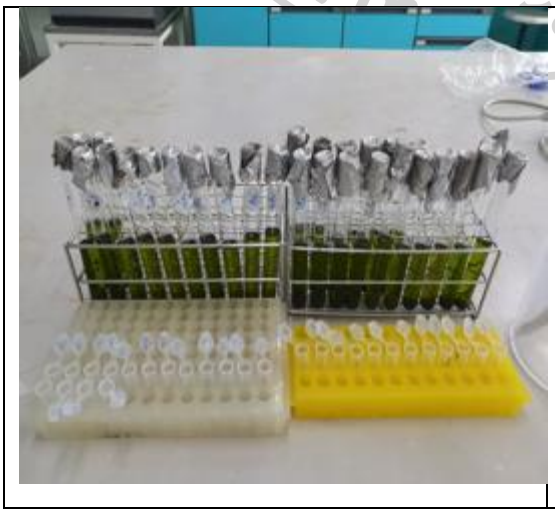
Appendix 4 Preparation and foliar application of proline and trehalose into plants



Appendix 5 Measurement of fresh weight, turgid weight and dry weight of leaf sample



Appendix 6 Measurement of chlorophyll determination in leaf by using the dimethyl sulfoxide (DMSO) solution



Appendix 7 Standard curve for proline

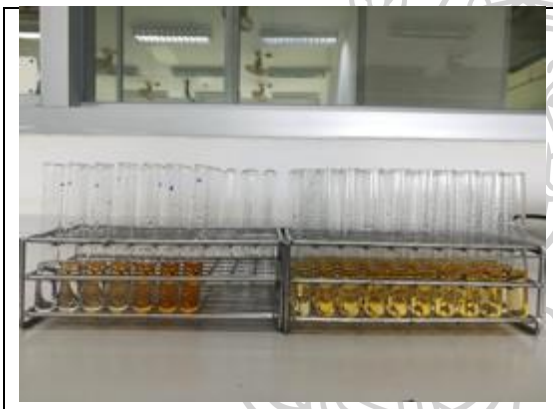
100 mM proline (mL)	3% sulfosalicylic acid (mL)	Proline amount (µmole)
0.0	1.0	0
0.2	0.8	20
0.4	0.6	40
0.6	0.4	60
0.8	0.2	80
1.0	0.0	100

Appendix 8 Measurements of proline from dry tissue and determined against the proline standard curve

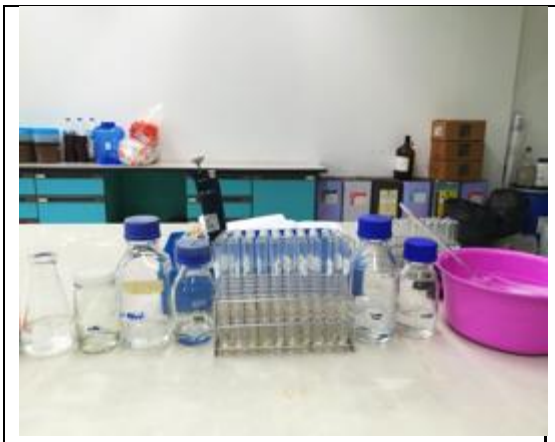
Appendix 9 Standard curve for sugar and starch

0.2 mg/ml glucose (mL)	H_2O (mL)	Glucose amount(µg)
0.0	1.0	0
0.1	0.9	20
0.2	0.8	40
0.3	0.7	60
0.4	0.6	80
0.5	0.5	100
0.7	0.3	140

Appendix 10 Measurement of sugar from dry tissue and the standard curve

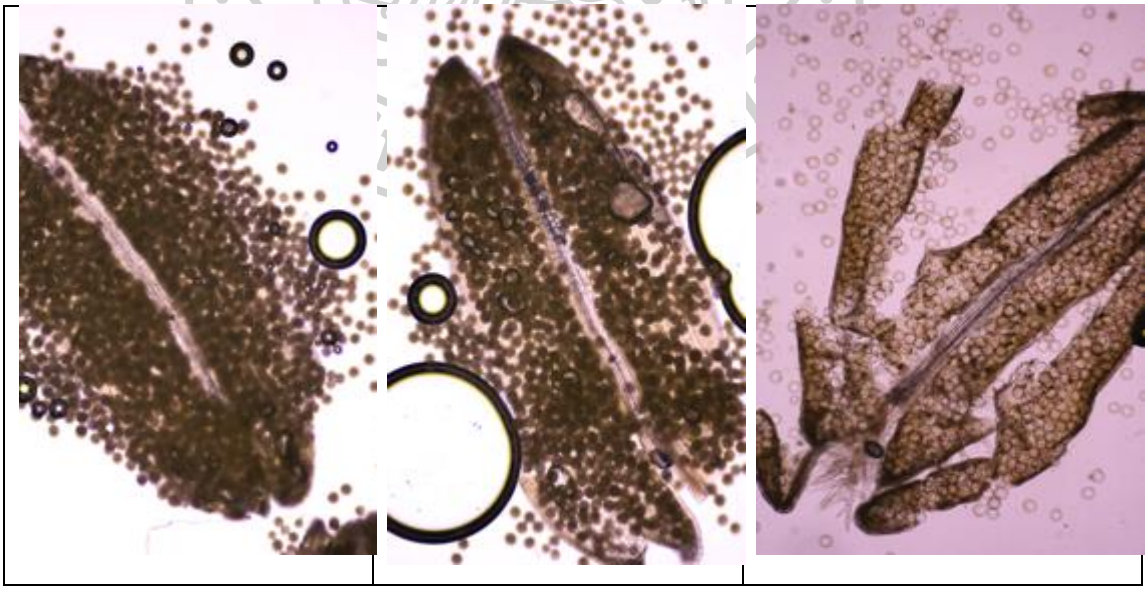


Appendix 11 Measurement of starch from dry tissue

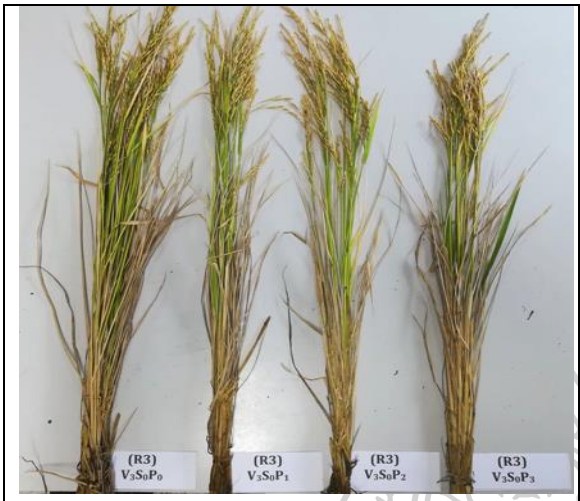


Appendix 12 Preparation of complementary deoxyribonucleic acid **(**cDNA**)** and agarose gel electrophoresis

Appendix 13 Pollen viability test by using the potassium iodide solution



Appendix 14 Measurement of plant height and counting of fertile tiller number



Appendix 15 Measurement of panicle length and weighting of 1000 seed weight

Appendix 16 Counting of filled grain percent and weighting the yield per plant

Appendix 17 Turnitin percentage of thesis book

Thesis

ORIGINALITY REPORT

32%	27%	24%	14%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

Appendix 18
Research publication

RSU 6th International Research Conference 2021 https://rsucon.rsu.ac.th/proceedings
30 APRIL 2021

Effects of Water Salinity on Yield and Yield Components of Three Rice Varieties

Lu Zaw Myo[1*], Pantipa Na Chiangmai[1], Chaowanee Laosutthipong[1], Panida Duangkaew[1], Soranot Chotnipat[1] and Nurhidayati[2]

[1]Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, IT Campus, Phetchaburi, Thailand[2]Department of Agrotechnology, Faculty of Agriculture, University of Islam Malang, Malang, East Java, Indonesia

[*]Corresponding author, E-mail: luzawmyo@yau.edu.mm

Abstract

This study aims to determine the effects of different levels of water salinity on the growth and yield of three rice varieties. The experiment was conducted on an open field from January to May 2020. The experiment design used 4x3 factorial in Completely Randomized Design (CRD) with three replications. Factor A treatments were four levels of water salinity: 0 (control), 5, 10, and 15 dS/m. Factor B treatments were three rice varieties, of which two were Thai namely Chai Nat 1 (CNT 1) and Pathum Thani 1 (PTT 1) and the other was a salt-tolerance variety from Indonesia; Inpari-35 (IN 35). The results showed that increased water salinity level could decrease all yield and yield components, both assessed from average means and percent reduced values as compared with 0 dS/m (control). The number of fertile tillers, the percentage of filled grain, and the yield per plant are severely affected by the salinity stress from 5 dS/m onward. Thai rice varieties (CNT 1 and PTT 1) showed higher reduction values in all parameters (excepted 1000 seed weight) as compared with Inpari 35. Although both CNT 1 and PTT 1 varieties showed different susceptibility to water salinity levels on different yield components, PTT 1 was more adversely affected at all water salinity stress levels, especially on the number of fertile tillers, filled grain percent, and yield per plant.

Keywords: salty tolerance, rice growth, rice yield

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มหาวิทยาลัยศิลปากร

VITA

NAME	Lu Zaw Myo
DATE OF BIRTH	31 July 1984
PLACE OF BIRTH	Phyu, Bago Regional Division, Myanmar
INSTITUTIONS ATTENDED	Yezin Agricultural University
HOME ADDRESS	Yezin Agricultural University, Zayyarthri Township, Nay Pyi Taw, Myanmar
PUBLICATION	Lu Zaw Myo, Pantipa Na Chiangmai, Chaowanee Laosutthipong, Panida Duangkaew, Soranot Chotnipat and Nurhidayati. Effects of Water Salinity on Yield and Yield Components of Three Rice Varieties. Proceedings of RSU International Research Conference (2021). Page 574-583.