Research Article

Exogenous trehalose application in rice to mitigate saline stress at the tillering stage[1]

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ABSTRACT

Rice (*Oryza sativa* L.) production is globally impacted by salinity stress, since it is a salt-sensitive plant species. This study aimed to determine the effect of exogenous trehalose to reduce the salinity stress at the tillering stage in three lowland rice varieties: Chai Nat 1 (CNT1), Pathum Thani 1 (PT1) and Inpari 35 (IN35). Salinity stress was induced by watering the plants with four concentrations (0, 50, 100 and 150 mM) of sodium chloride (NaCl). Thereafter, exogenous trehalose with the same concentration was applied through foliar spray to reduce the salinity stress. The induced salinity in the rice plants affected various physiological parameters, such as relative water content, chlorophyll content and chlorophyll a/b ratio. Salinity also affected the levels of soluble sugar, starch content and other eight agronomic traits. At the concentration of 50 mM, the impact of trehalose was significantly observed on the physiological, biochemical and other agronomic traits of the plant. However, the 100-grain weight of the rice did not improve with the use of trehalose, what may have been influenced by the duration of the trehalose exposure during the tillering stage. The physiological, biochemical (excluding starch content) and agronomical traits of the rice plants also varied with the varieties. The salt-tolerant variety (IN35) showed a higher content of relative water (12.98 %), chlorophyll (8.33 %), soluble sugars (12.25 %), reproductive tillers per plant (12.4 %), grains per panicle (18.81 %), 100-grain weight (10.71 %), percentage of filled grains per panicle (22.39 %) and grain yield per plant (23.49 %), in comparison to CNT1 and PT1.

KEYWORDS: *Oryza sativa* L., abiotic stress, lowland rice.

RESUMO

Aplicação exógena de trealose em arroz para mitigação do estresse salino na fase de perfilhamento

A produção de arroz (*Oryza sativa* L.) é afetada globalmente pelo estresse salino, por ser uma espécie sensível ao sal. Objetivou-se determinar o efeito exógeno de trealose na redução do estresse salino na fase de perfilhamento em três variedades de arroz de várzea: Chai Nat 1 (CNT1), Pathum Thani 1 (PT1) e Inpari 35 (IN35). O estresse salino foi induzido pela irrigação das plantas com quatro concentrações (0; 50; 100; e 150 mM) de cloreto de sódio (NaCl). Em seguida, a aplicação exógena de trealose na mesma concentração foi efetuada via pulverização foliar, para reduzir o estresse salino. A salinidade induzida nas plantas de arroz afetou vários parâmetros fisiológicos, como teor relativo de água, teor de clorofila e razão clorofila a/b. A salinidade também afetou os teores de açúcar solúvel e de amido e outras oito características agronômicas. Na concentração de 50 mM, observou-se impacto significativo da trealose nas características fisiológicas, bioquímicas e outras características agronômicas da planta. No entanto, o peso de 100 grãos de arroz não melhorou com o uso de trealose, o que pode ter sido influenciado pela duração da exposição à trealose durante a fase de perfilhamento. As características fisiológicas, bioquímicas (exeto o teor de amido) e agronômicas das plantas de arroz também variaram com as variedades. A variedade tolerante ao sal (IN35) apresentou maior teor relativo de água (12,98 %), clorofila (8,33 %), açúcares solúveis (12,25 %), perfilhos reprodutivos por planta (12,4 %), grãos por panícula (18,81 %), peso de 100 grãos (10,71 %), porcentagem de grãos cheios por panícula (22,39 %) e rendimento de grãos por planta (23,49 %), em comparação com CNT1 e PT1.

PALAVRAS-CHAVE: *Oryza sativa* L., estresse abiótico, arroz de várzea.

INTRODUCTION

Soil salinity is a problem caused by sea level rise, climate change, improper agriculture practices such as the excessive use of fertilizers and use of saline water for irrigation (Silva et al. 2019). Hence, the most affected agricultural regions due to soil salinity are the coastal areas. Additionally, dry and semi-arid regions make up about 7 % of the agricultural areas impacted by salinity (Hashem

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et al. 2018). Crop output in agricultural regions is considerably more impacted by the challenges of water scarcity and soil salinity.

Although rice is the primary food crop grown globally, it is also the one most sensitive to salinity (Al-Tamimi et al. 2021). The short and long-term negative effects of salinity stress on rice are well noted. The impact of salt stress on rice crop development varies depending on the variety (Rad et al. 2012). At the stage of seedling, early vegetative and panicle initiation, rice plants have been reported more sensitive to salinity stress than the reproductive, panicle emergence and ripening stages (Rad et al. 2012, Kakar et al. 2019). Salt stress at the tillering stage decreases the number of fertile tillers, what leads to a drop in yield (Dramalis et al. 2021).

Eco-friendly and cost-effective alternatives should be discovered to reduce the damage caused by salinity in rice and other crops. The current research focuses on the components in rice plants that are produced to withstand and thrive under salinity stress.

Trehalose is a non-reducing disaccharide that accumulates inside the plants and acts as a osmolyte during drought stress (Kosar et al. 2019). Exogenous trehalose application to the plant species at the seedling stage induces salinity tolerance by increasing the photosynthetic pigments (chlorophyll a and b and carotenoid), carbohydrate (sucrose, fructose and trehalose), proline and other osmotic substances (Abdallah et al. 2016, Yang et al. 2022). Trehalose is more effective in inducing salinity tolerance if compared to other sugars because it can protect proteins and cell bilayers from denaturation and degradation (Jain & Roy 2009, Vinciguerra et al. 2022). Moreover, it is easily available due to its use in food, biopharmaceuticals and cosmetics industries.

Thus, this study aimed to determine the effect of exogenous trehalose to reduce the salinity stress in rice plants at the tillering stage, as well as to evaluate their physiological, biochemical and agronomic traits.

MATERIAL AND METHODS

A pot experiment was conducted in a net house at the Silpakorn University, in Phetchaburi, Thailand (12.65245 N; 99.88186 E), from January to May 2020. The trial area has a tropical climate characterized by high temperatures and low rainfall during the study months.

Three lowland rice varieties [Inpari 35 (IN35), Pathum Thani 1 (PT1) and Chai Nat (CNT1)] were used in this study. IN35 is a salt-tolerant variety from Indonesia (Sembiring et al. 2020), PT1 is a salt-sensitive variety from Thailand and CN1 is a non-aromatic variety from Thailand.

A sandy loam soil (70.97 % of sand, 20.43 % of silt, 8.60 % of clay and 0.93 % of organic matter) was used to grow all the rice varieties. The soil pH, electrical conductivity and sodium adsorption ratio were 6.39, 0.92 dS m^{-1} and 0.22, respectively. There were 5.18 mg kg^{-1} of phosphorus (P), 83.09 mg kg^{-1} of potassium (K), 88.31 mg kg^{-1} of calcium (Ca) and 69.98 mg kg^{-1} of magnesium (Mg) present in the soil.

Salinity stress was simulated using four concentrations of sodium chloride (NaCl) tested by electrical conductivity ($EC_{1:5}$) (soil: water ratio at 1:5) as 0 mM (0 dS m^{-1}), 50 mM (5 dS m^{-1}), 100 mM (10 dS m^{-1}) and 150 mM (15 dS m^{-1}). Exogenous trehalose was prepared in four concentrations viz, 0, 50, 100 and 150 mM.

Unhusked seeds were submerged in water for 24 hours, after which they were planted in the nursery for two weeks. Three seedlings were placed in each 9 × 18-inch size polybag and transplanted to the soil.

Saline water was applied to each treatment (Moldenhauer et al. 2013) at the rate of 1 L bag^{-1} $week^{-1}$ to stimulate the soil salinity. Starting at the tillering stage (V5; collar development in leaf 5 on main stem), irrigation with salt water was continued until the harvest stage (R9; all grains have brown hull). Irrigation with water without salt content was performed twice daily with approximately 1 L $polybag^{-1}$. During the active tillering period, the trehalose treatment was started. For three weeks, 100 mL of the trehalose solution were applied to each polybag, each week.

Two days after the last week of applying trehalose, some physiological (relative water content - RWC; chlorophyll a and b contents; total chlorophyll content; chlorophyll a/b ratio) and biochemical (soluble sugars and starch contents) properties were determined from the leaves, stem and roots of the rice plants. The RWC was calculated using the following formula: RWC (%) = [(fresh weight - dry weight)/(turgid weight - dry weight)] x 100.

The chlorophyll content (a, b and total) was measured according to Khaleghi et al. (2012). The chlorophyll a/b ratio was calculated by dividing the chlorophyll a by the chlorophyll b content. The soluble sugars and starch content in the rice plants were determined according to modified methods by Chow & Landhäusser (2004).

During the harvest stage, agronomic traits such as plant height, total yield, number of tillers per plant, number of productive tillers per plant, number of grains per panicle, 100-grain weight and percentage of grains per panicle were recorded. The harvest index (HI) was also calculated after measuring the physiological, biochemical and agronomic traits, as it follows: HI (%) = (grain yield per plant/biological yield per plant) x 100.

A completely randomized design, with a 4 × 4 × 3 factorial scheme and five replications, was employed. The four NaCl concentrations, four trehalose concentrations and three genetically distinct rice varieties comprised the three factors. Analysis of variance (Anova) was used to analyze the data, with a significant difference detected at the probability of < 0.05, and then these treatments were compared using the Duncan's new multiple range test. All the RWC characteristics were analyzed by the R software version 4.0.2 (R Core Team 2020).

RESULTS AND DISCUSSION

As the concentration of NaCl increased, the relative water content (RWC) values significantly decreased. The impact on RWC values suggests that rice at the tillering stage is moderately resistant to salinity. The RWC values decreased to 8.81 and 14.12 % at the 100 and 150 mM concentrations of NaCl (Table 1). The RWC values were increased when the trehalose concentrations increased (Table 1). Trehalose acts as an osmoprotectant compound inside the plant cells and supports plant growth by improving the RWC during normal or salinity stress (Sadak 2019). For RWC, there was a non-significant interaction for NaCl × trehalose. The RWC values were significantly affected by the rice varieties and, among the three rice varieties, the highest RWC was observed for IN35 (Table 1).

The chlorophyll contents (a, b and total) and chlorophyll a/b ratio were influenced by factors such as salinity, trehalose and rice variety, excluded different varieties that did not affect the chlorophyll a/b ratio (Table 2). The IN35 variety had higher chlorophyll contents than the CNT1 and PT1 (Table 2). There was a decrease in the chlorophyll content as the concentration of NaCl was increased.

Table 1. Relative water content for the Chai Nat 1 (CNT1), Pathum Thani 1 (PT1) and Inpari 35 (IN35) lowland rice varieties under NaCl and trehalose treatments.

Relative water content (%)					
Varieties		NaCl		Trehalose	
CNT1	51.61 b	0 mM	55.76 a	0 mM	50.06 b
PT1	49.15 c	50 mM	55.21 a	50 mM	51.56 ab
IN35	55.53 a	100 mM	50.03 b	100 mM	53.09 a
		150 mM	47.40 b	150 mM	53.68 a
F-test					
Variety (V)	2.90×10^{-6}**				
NaCl (N)	3.97×10^{-9}**				
Trehalose (T)	0.0446*				
V × N	0.874^{ns}				
V × T	0.923^{ns}				
N × T	0.914^{ns}				
V× N × T	0.989^{ns}				
CV (%)	11.19				

CV: coefficient of variation; * and ** significant difference at p = 0.05 and p = 0.01, respectively; ^{ns} non-significant difference at p = 0.05. Different letters in the column for each factor show a significant difference at p = 0.05.

Chlorophylls a and b are important pigments for plant photosynthesis and primarily absorb red-orange and blue-violet light, respectively (Li et al. 2018). Chlorophyll a is a core pigment in the photosynthetic process and chlorophyll b is an accessory pigment of the light-harvest complex (Tanaka & Tanaka 2011). However, the chlorophyll content is sensitive to environmental factors such as climate, soil nutrients, phylogeny and salinity stress (Li et al. 2018).

Several additional stresses develop when plants are under salinity stress. The chlorophyll content decreases under exposure to salt stress due to oxidative stress in plants (Alharbi et al. 2022). Oxidative stress caused by reactive oxygen species accumulation in plants is a secondary stress after the ion and osmotic stresses (Yang & Guo 2018). In this study, the chlorophyll a and b contents declined as salinity increased, but the chlorophyll a/b ratio increased, indicating that the chlorophyll b content decreased more than that of the chlorophyll a. Chlorophyll b plays an important function in the light-harvest complexes. The reduction in chlorophyll b under salt stress in plants affects the stabilization of binding proteins in light-harvest complexes (Tanaka & Tanaka 2011). A higher chlorophyll a/b ratio in plants exposed to higher salinity stress suggests a higher disorder in the antenna complex in the photosystems (PSI or PSII) containing chlorophyll b than in the reaction

Table 2. Chlorophyll a, b and total, and chlorophyll a/b ratio in the Chai Nat 1 (CNT1), Pathum Thani 1 (PT1) and Inpari 35 (IN35) lowland rice varieties under NaCl and trehalose treatments.

Factors	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Chlorophyll a/b ratio
Varieties				
CNT1	0.306 Y	1.207 Y	1.456 Y	0.258
PT1	0.303 Y	1.165 Y	1.408 Y	0.264
IN35	0.320 X	1.262 X	1.522 X	0.258
NaCl (mM)				
0	0.317 a	1.373 a	1.642 a	0.234 c
50	0.312 ab	1.208 b	1.460 b	0.260 b
100	0.305 b	1.147 bc	1.391 c	0.259 ab
150	0.304 b	1.117 c	1.356 c	0.276 a
Trehalose (mM)				
0	0.297 B	1.121 B	1.358 B	0.268 A
50	0.310 C	1.170 B	1.418 B	0.269 A
100	0.313 AB	1.249 A	1.505 A	0.255 AB
150	0.318 A	1.305 A	1.569 A	0.247 B
F-test				
Variety (V)	**	**	**	ns
NaCl (N)	**	**	**	**
Trehalose (T)	**	**	**	**
V × N	ns	ns	ns	ns
V × T	ns	ns	ns	ns
N × T	ns	ns	ns	ns
V × N × T	ns	ns	ns	ns
CV (%)	5.14	10.76	9.81	11.17

CV: coefficient of variation; ** significant difference at p = 0.01; ns: non-significant difference at p = 0.05. Different letters in the column show a significant difference for each factor at p = 0.05.

centre containing chlorophyll a (Yahia et al. 2019). However, the exchange between chlorophyll a and b occurs through the chlorophyll cycle, and the chlorophyll b is synthesized through chlorophyll a (Tanaka & Tanaka 2011). Therefore, the decrease in the chlorophyll a content may also affect the chlorophyll b content of plants. The proportion of the light-harvest complex and accessory chlorophyll complex was estimated by the chlorophyll a/b ratio (Khatri & Rathore 2022). On the other hand, it has been reported that a low chlorophyll a/b ratio in plant species indicates that plants are shade tolerant (Maina & Wang 2015).

Exogenous trehalose supplied via foliar spray might reduce salt stress by increasing the chlorophyll content, including chlorophyll a, b and total (Table 2). Supplementing the exogenous trehalose to plants under salinity stress could increase the osmotic substances, carbohydrates, K^+, adjust the K^+/Na^+ ratio and maintain the cell membrane stability, therefore reducing the stress caused by salinity (Farooq et al. 2017, Yang et al. 2022). However, different concentrations of exogenous trehalose used in plants exposed to salinity stress were reported and the results were varied. At low concentration, sodium ions accumulate in plants, while, at high concentration, the chlorophyll content remains constant in the plant parts (Yang et al. 2022). The absence of statistical significance from the NaCl × trehalose interaction in all the chlorophyll contents and chlorophyll a/b ratio suggests that the increased trehalose concentration showed beneficial effects on the chlorophyll increase at all the salinity levels, even without NaCl exposure.

The non-significant NaCl × trehalose interaction affected the soluble sugar content in all parts of the rice plant. The two studied factors do not interact with each other to affect the soluble sugar content in plants. Therefore, instead of studying the combined effect of the two factors, the focus here is on observing the individual effect of each factor on the soluble sugar content in plants. The values of the soluble sugar in this study were significantly affected due to the stress caused by salinity (Table 3). A decrease in the accumulation of the soluble sugar was observed in all parts of the rice plants, including leaves, roots and stems, when they were exposed to salinity (Table 3). Salinity stress in salt-tolerant varieties increases the accumulation of soluble sugars, which act as an osmoprotectant in plants. Soluble sugars serve as the carbon source for plant energy, regulate genes for several processes and are a signal for driving metabolisms under stress conditions (Keunen et al. 2013, Oszvald et al. 2018). Soluble sugars were also reported to have an important role in cell metabolism (Couée et al. 2006). Nevertheless, Yang et al. (2022) reported a reduction in glucose and fructose contents, while the sucrose and trehalose amounts increased when plants were exposed to salt stress. On the other hand, the use of exogenous sucrose in salt-sensitive varieties of rice plants that faced salinity stress could promote the accumulation of glucose and fructose (Siringam et al. 2012). Therefore, this may be a reason for the fact that exogenous foliar trehalose application could increase the soluble sugars content in this study (Table 3).

The application of trehalose via foliar spray is reported to support the accumulation of carbohydrates in leaves (Yang et al. 2022), but, in the present study, the trehalose application via the same method could increase the content of soluble sugars in the stem more than in leaves and roots (Table 3). Therefore, both the leaves and roots are the source of energy (nutrients, carbon and water) to drive sink growth (Walter & Schurr 2005). The soluble sugar content accumulation in all plant parts was significantly affected by the rice plant varieties: IN35 had the highest values in all plant parts, followed by CNT1 and PT1, respectively. Thus, the IN35 variety may have more salt-tolerant ability.

In this study, the starch content was non-significantly affected by the NaCl × trehalose interaction and decreased in all plant parts (leaves, stem and roots) when the rice plants suffered from salinity (Table 4). Carbohydrates might be influenced by osmotic stress in response to abiotic challenges

Table 3. Soluble sugars content in leaves, stems and roots for the Chai Nat 1 (CNT1), Pathum Thani 1 (PT1) and Inpari 35 (IN35) lowland rice varieties under NaCl and trehalose treatments.

Factors	Soluble sugars content (mg g^{-1})		
	Leaves	Stems	Roots
Varieties			
CNT1	43.53 Y	43.15 X	41.19 Y
PT1	42.21 Y	40.11 Y	40.00 Y
IN35	47.33 X	43.34 X	44.96 X
NaCl (mM)			
0	51.52 a	47.65 a	49.23 a
50	46.49 b	42.81 b	44.24 b
100	42.63 c	39.87 c	40.25 c
150	36.80 c	38.47 c	34.47 d
Trehalose (mM)			
0	44.23	39.05 C	41.91
50	43.50	40.26 C	41.14
100	44.51	43.35 B	42.20
150	45.20	46.14 A	42.94
F-test			
Variety (V)	**	**	**
NaCl (N)	**	**	**
Trehalose (T)	ns	**	ns
V × N	ns	ns	ns
V × T	ns	ns	ns
N × T	ns	ns	ns
V × N × T	ns	ns	ns
CV (%)	10.95	11.75	11.53

CV: coefficient of variation; ** significant difference at p = 0.01; ns: non-significant difference at p = 0.05. Different letters in the column show a significant difference for each factor at p = 0.05.

Table 4. Starch content in leaves, stems and roots for the Chai Nat 1 (CNT1), Pathum Thani 1 (PT1) and Inpari 35 (IN35) lowland rice varieties under NaCl and trehalose treatments.

Factors	Starch content (mg g^{-1})		
	Leaves	Stems	Roots
Varieties			
CNT1	43.81	45.80	42.07
PT1	42.37	43.56	40.94
IN35	44.03	45.23	42.01
NaCl (mM)			
0	49.80 a	49.18 a	45.29 a
50	45.72 b	46.61 b	43.48 b
100	40.75 c	43.35 c	40.34 c
150	37.34 d	40.32 d	37.58 d
Trehalose (mM)			
0	35.05 D	39.79 C	36.97 D
50	43.55 C	44.55 B	41.22 C
100	46.25 B	45.99 B	43.30 B
150	48.76 A	49.12 A	45.22 A
F-test			
Variety (V)	ns	ns	ns
NaCl (N)	**	**	**
Trehalose (T)	**	**	ns
V × N	ns	ns	ns
V × T	ns	ns	ns
N × T	ns	ns	ns
V × N × T	ns	ns	ns
CV (%)	11.03	11.14	7.70

CV: coefficient of variation; ** significant difference at p = 0.01; ns: non-significant difference at p = 0.05. Different letters in the column show a significant difference for each factor at p = 0.05.

in both the source and sink parts of photosynthesis (mobilization and utilization) (Henry et al. 2015). Salinity stress altered the metabolism in plants, including biosynthesis, deterioration of starch and sugars. The activity of enzymes involved in the degradation of starch, such as amylase, hexokinase and glucose-6-phosphate dehydrogenase, was reported to increase in leaves. A reduction in the starch content was caused by increasing the energy required to keep plants alive under salinity stress (Ahmad et al. 2017).

Using exogenous trehalose as an osmoprotectant compound could induce the accumulation of starch content in all the plant parts (Table 4). Trehalose exogenous applied to wheat plants also increased the accumulation of starch and other carbohydrate constituents, such as sucrose, glucose, trehalose and total soluble sugars in the leaves of plants irrigated either with normal or salty water (Sadak 2019). Trehalose controls the metabolism of carbohydrates, causing an accumulation of starch in leaves to increase the carbon supply as a defence strategy against abiotic stress (Mishra & Prakash 2010, Sadak 2019). However, the rice varieties did not differ significantly in starch accumulation in different parts of the plant (Table 4).

In this study, the salinity stress affected both the physiological (RWC and chlorophyll contents) and biochemical (soluble sugars and starch content) properties of the rice plant (Tables 1-4), being these characteristics related to plant metabolism and photosynthetic process. In addition, the salinity stress also interrupted some carbohydrates transformation and translocation, therefore causing a significant reduction in agronomic traits such as yield and other yield components (Razzaq et al. 2020). All the eight agronomic traits in this study were significantly affected by salinity stress with decreased values starting at 50 mM of NaCl (5 dS m^{-1}) (Table 5).

The ability to form productive tillers of rice is an important parameter for evaluating the rice yield; however, it is affected by salinity (Aref & Rad 2012). In this study, the tillering capacity and productive tiller formation per plant decreased when the plants were exposed to salinity (Table 5), although, under non-salinity conditions, the percentage of productive

Table 5. Agronomic traits for the Chai Nat 1 (CNT1), Pathum Thani 1 (PT1) and Inpari 35 (IN35) lowland rice varieties under NaCl and trehalose treatments.

Factors	PH (cm)	TP	PTP (% with TP)	NGP	100 GW (g)	FGP (%)	HI (%)	GYP (g)
Variety								
CNT1	74.43 Y	6.3 Y	4.4 Y (69.8 %)	107.8 Y	2.38 Y	45.28 Y	23.80	5.43 Y
PT1	73.90 Y	6.3 Y	3.7 Z (58.7 %)	99.4 Z	2.24 Z	41.27 Z	22.61	4.81 Z
IN35	76.67 X	7.6 X	5.4 X (71.1 %)	118.1 X	2.48 X	50.51 X	25.26	5.94 X
NaCl (mM)								
0	83.26 a	8.3 a	6.1 a (73.5 %)	125.5 a	2.50 a	51.77 a	28.50 a	6.06 a
50	78.23 b	7.0 b	4.8 b (68.6 %)	109.5 b	2.40 b	48.68 ab	24.57 b	5.54 ab
100	70.23 c	6.4 b	4.0 c (62.5 %)	103.2 b	2.35 b	45.03 b	22.03 ab	5.32 b
150	68.28 c	5.1 c	3.2 d (62.7 %)	95.6 c	2.24 c	37.28 c	20.47 c	4.65 c
Trehalose (mM)								
0	74.98 AB	6.5	4.4 B (67.7 %)	89.8 D	2.32	40.20 B	22.07	4.63 C
50	72.90 B	6.7	4.4 B (65.7 %)	100.8 C	2.36	43.42 B	23.55	5.25 B
100	75.70 A	6.8	4.3 B (63.2 %)	110.8 B	2.40	48.08 A	24.40	5.73 AB
150	76.43 A	6.9	4.9 A (71.0 %)	132.5 A	2.40	51.07 A	25.55	5.97 A
F-test								
Variety (V)	**	**	**	**	**	**	ns	**
NaCl (N)	**	**	**	**	**	**	**	**
Trehalose (T)	**	ns	**	**	ns	**	ns	**
V × N	ns	ns	ns	ns	ns	ns	ns	ns
V × T	ns	ns	ns	ns	ns	ns	ns	ns
N × T	ns	ns	ns	ns	ns	ns	ns	ns
V × N × T	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	8.59	29.94	21.57	14.70	10.97	26.41	34.18	27.30

PH: plant height; TP: number of tillers per plant; PTP: number of productive tillers per plant; NGP: number of grains per panicle; 100 GW: 100-grain weight; FGP: percentage of filled grains per panicle; HI: harvest index; GYP: grain yield per plant; CV: coefficient of variation; ** significant difference at p = 0.01; ns: non-significant difference at p = 0.05. Different letters in the column show a significant difference for each factor at p = 0.05.

tiller formation was approximately 73.5 % higher than for the control. The percentage of fertile and productive tillers decreased as salinity levels increased (Table 5). Additionally, salinity stress was reported to inhibit other agronomic traits of rice plants, including plant height, number of leaves, filled panicles, number of spikelets per panicle, percentage of filled grains per panicle and grain yield (Aref & Rad 2012, Khanam et al. 2018, Dramalis et al. 2021). Therefore, the affected agronomic, physiological and biochemical traits can be used as a criterion for selecting salinity tolerant rice varieties during breeding practices. The decreased grain yield of rice can be correlated to the reduced yield components due to the salinity effect. At 50 mM of NaCl, the grain yield per plant was reduced by approximately 8.58 % (Table 5). Therefore, the rice genotypes used in this study were moderately susceptible to salinity from the start of the tillering stage to the harvest stage.

Almost all the agronomic traits were enhanced by the use of foliar trehalose, except for the number of tillers per plant, 100-grain weight and harvest index (Table 5). However, only the number of grains produced by each panicle and grain yield per plant increased just after the application of 50 mM of trehalose (Table 5), despite the fact that both the physiological and biochemical traits in this study showed increased values after the application of the same trehalose concentration (Tables 1-4). This indicates that, in contrast to the findings of this study, several metabolic processes in plants connected to physiological and biochemical components are more likely to influence changes in agronomic traits. However, it is confirmed that the impacts on the rice yield and yield components can be reduced by using trehalose at the tillering stage when plants are grown under salinity conditions.

In addition to the harvest index, the 100-grain weight was the trait that did not change during the foliar spray of trehalose at the tillering phase (Table 5). Grain weight is the only trait that does not develop during the vegetative growth phase (overlap with the beginning of the reproductive phase or panicle initiation; PI or R0) and is very far from those stages and characterized during the grain filling phase (R6-R8 in the ripening phase) (Moldenhauer et al. 2013). As a result, it is possible that the influences of the exogenous trehalose applied during the vegetative phase did not have a favourable effect on grain weight accumulation. The number of potential grains per panicle is determined based on the physiological phases of rice growth throughout the panicle differentiation phase (PD; R1 growth stage). The number of fertilized flowers that self-pollinate and the conditions during the anthesis phase (R4 growth stage) determine the percentages of filled grains per panicle (Moldenhauer et al. 2013). Regarding plant vigour in the vegetative stage, determined from tillering and plant height, it was closely correlated to panicle initiation and development, what can be assessed from panicle size and number of spikelets (Adriani et al. 2016), as well as determined on timely and complexly flowering (Reddy et al. 2021). As a result, the exogenous trehalose application via foliar spray is very important to promote fertility, vigour, tiller formation and other yield components. The foliar application of trehalose between 50 and 150 mM plays a significant role in determining the yield ability of the rice crop. The IN35 variety was identified with enhanced agronomic traits after the application of salinity and trehalose in different concentrations (Table 5).

CONCLUSIONS

1. The treatment with 50 mM of NaCl decreased the contents of chlorophyll a, b and total by 1.58, 12.02 and 11.08 %, respectively, and increased the chlorophyll a/b ratio by 11.11 %, in comparison to the control;
2. The relative water content (RWC) decreased by 10.28 % at 100 mM of NaCl, in comparison to the control;
3. The soluble sugar and starch contents in all the plant tissues (leaf, stem and root) decreased in the range of 9.76-10.16 and 4.00-8.19 %, respectively, with an increase in salinity to 50 mM of NaCl;
4. For the agronomic traits, a reduction was observed in the number of tillers per plant (15.66 %), number of reproductive tillers per plant (21.31 %), number of grains per panicle (12.75 %) and harvest index (13.79 %), at 50 mM of NaCl. In addition, the plant height, 100-grain weight, filled grains per panicle and grain yield per plant were also reduced by 6.04, 4.00, 5.97 and 8.58 %, respectively, at 50 mM of NaCl;
5. The exogenous trehalose application via foliar spray at the tillering stage enhanced the RWC, chlorophyll a and starch content in all the

plant tissues (leaf, stem and root) by 3.00, 4.38 and 11.50-24.25 %, respectively, at 50 mM of trehalose. The contents of chlorophyll b, total chlorophyll and soluble sugar in the stems increased by 11.42, 10.82 and 11.01 %, at 100 mM. The chlorophyll a/b ratio was reduced by 4.85 %, at 100 mM;

6. A higher range of RWC (7.60-12.98 %), chlorophyll content (4.53-8.33 %) and soluble sugars (7.98-12.25 %) in all the plant tissues was observed for the IN35 variety than for CNT1 and PT1. In addition, IN35 showed higher values for agronomic traits than CNT1 and PT1, in terms of reproductive tillers per plant (1.3-12.4 %), grains per panicle (9.55-18.81 %), 100-grain weight (4.20-10.71 %), percentage of filled grains per panicle (11.55-22.39 %) and grain yield per plant (9.39-23.49 %).

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