Using Validamycin A for Salinity Stress Mitigation in Two Rice Varieties Differing in Their Salt Tolerance Level

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WE PRESENT here the comparative protective potentiality of exogenously applied validamycin A, a potent inhibitor of trehalase as seed soaking prior to sowing in saline soil with salinity level (EC 10.06 dS/m) in mitigating NaCl toxicity and inducing salinity tolerance in two rice (*Oryza sativa* L.) cultivars (cv.), Sakha 103 (salt-sensitive) and Agami M5 (salt-tolerant). Salinity stress decreased starch and total soluble protein contents concomitantly with increasing total soluble sugars, proline and free amino acids in both cultivars. Validamycin A decreased the activity of trehalase which leads to the accumulation of trehalose in both cultivars of rice plants. Raising trehalose level in the plant tissues was accompanied by increasing the starch and protein content and appearance of new protein pattern of both cultivars. Application of validamycin A increased grain yield, 1000-grain weight and filled-grain percentage for both rice cultivars under saline field conditions. Soaking rice seeds with 30μM of validamycin A could alleviate the harmful effects of salinity stress.

Keywords: Salinity, Yield, Trehalose, Protein patterns .

Rice (*Oryza sativa* L.) is one of the most important food crops in the world and it is the staple food in most developing countries in Asia, South America and Africa (Mustafa, 2005). In areas such as Asia, Africa and Latin America where the demand for rice is a top priority, the population is expected to increase 1.5-fold by 2025 (Sasaki, 2002).

Most of the environmental constraints drastically decrease plant growth and development that lead to reduction in crop yield (Shahbaz *et al.,* 2012 and Shahbaz & Ashraf, 2013). Among abiotic threats, salinity is one of special concern. Rice is a salt-sensitive monocot (Darwish *et al.,* 2009). In particular, seedlings and the reproductive stages are very sensitive to salinity, while germination is relatively tolerant (Zheng *et al.,* 2001). Rice cultivars vary in their ability to tolerate salt stress, with both salt-tolerant and salt-sensitive (Zeng *et al.,* 2004 and Zeng, 2005).

During the onset and development of salinity stress within a plant, major processes, such as protein synthesis, energy, lipid metabolism, and photosynthesis are disrupted, ultimately resulting in the loss of plant productivity (Evelin *et al.,* 2009). Parida *et al.* (2002) reported that carbohydrates, such as sugars (glucose, fructose, sucrose and fructans) and starch accumulate under salt stress. However, it has also been reported that the sugar content increases in some genotypes of rice yet decreases in some genotypes under salinity (Alamgir & Ali, 1999). Most crop species are sensitive to salinity (ECe 1.0–1.8 dSm^{-1}), which reduce the crop productivity by 6–19% (Hussain *et al.,* 2010). Adverse effects of salinity on grain yield and yield components were also reported when the stress level reached an EC of 3.4 dS m^{-1} or higher (Nakhoda *et al.,* 2012).

The plants can avoid the damage caused by salinity through several mechanisms such as compatible solutes accumulation (Hajiboland *et al.,* 2010; Dudhane *et al.,* 2011 and Talaat & Shawky, 2013, 2014). Of these compatible solutes, trehalose, a non-reducing disaccharide of glucose, has been shown to stabilize biological structures and macromolecules (proteins and membrane lipids) in different organisms during dehydration (Ali & Ashraf, 2011).

Trehalose is present in plants in extremely small quantities, close just to detection level, but it plays a significant role in metabolic processes associated with abiotic stress tolerance (Aghdasi *et al.,* 2008; Duman *et al.,* 2010 and Luo *et al.,* 2010). There are five known trehalose biosynthetic pathways (Avonce *et al.,* 2006), but the best one is present in many bacteria and plants synthesized by the classic two-step process (Elbein *et al.,* 2003). Firstly, trehalose-6-phosphate synthase (TPS) catalyzes the synthesis of trehalose-6-phosphate (T6P) from UDP-glucose and glucose-6-phosphate. Secondly, T6P is dephosphorylated to trehalose by trehalose-6-phosphate phosphatase (TPP) (Zang *et al.,* 2011 and Rodr´ıguez-Salazar *et al.,* 2009).

It has been suggested that genetically engineered trehalose accumulation in crop plants could improve their tolerance to drought and salinity (Lo´pez *et al.,* 2009). However, limited amounts of trehalose were found to accumulate, likely because of the ubiquitous presence of the enzyme trehalase (TRE) in plants (Muller *et al.,* 2001). Trehalase (EC 3.2.1.28), the only enzyme capable of hydrolyzing trehalose to glucose, has been mainly found in the plant fraction, but it is also found in relatively low levels in bacteroids (Lo´pez *et al.,* 2009).

Hence, it should be possible to direct increased trehalose accumulation by down regulating plant trehalase activity or by expressing the trehalose biosynthetic genes under tissue- or stress-specific regulation (Penna, 2003).

Inhibition of trehalase activity in nodules of *Glycine max* (Lopez *et al.,* 2008a) and *Lotus japonicus* (Lopez *et al.,* 2006) by validamycin A, a potent trehalase inhibitor, appears to affect the level of other non-structural carbohydrates and induce the trehalose accumulation, indicating the role of

trehalase as the main enzyme responsible for the control of trehalose content in nodules of legume (Lopez *et al.,* 2008b).Validamycin A ($C_{20}H_{35}NO_{13}$) is a specific competitive inhibitor of trehalase. Treatment with validamycin A raised trehalose in plant tissue (Ahmed *et al.,* 2013).

The objective of this study is to provide a method and composition to enhance the productivity and growth of rice plants growing in saline soil.

Materials and Methods

Sustainable developing is the target which could be achieved through; concentrating the research programs on producing high yielding cultivars under salt stress conditions. Therfore, two field trials were conducted at El-Serw Agricultural Research Station, Agricultural Research Center, which located in North East of the Nile Delta (Damietta Governorate, North Egypt) during 2011 and 2012 summer seasons.

Soil characteristics and experimental design

The experimental field is characterized with a heavy clay salty affected soil and salty underground water because of the influence of the Mediterranean Sea. Soil analysis was carried out according to Black *et al*. (1965) and Jackson (1973). Soil characteristics were: sand clay loamy in texture, sand 51.6%, silt 20.9%, clay 28.5%, pH 8.2, electrical conductivity (EC) $10.06dSm^{-1}$, organic matter 1.25%, SP 80.0, anionic content (HCO_3^- 2.75, Cl^- 65.0, $SO4^=$ 52.25 meq\L), cationic content (Ca^{++} 36.5, Mg^{++} 19.05, Na^+ 62.68, K^+ 1.77meq\L), mineral content (N 134.99, K 819.8, P 10.30, Cu 13.40, Fe 101.80, Mn 17.60, Zn 2.31 mg\kg).

Plant and chemical materials

The seeds of the two rice cultivars (Sakha 103 and Agami M5) were obtained from Agricultural Research Centre, Ministry of Agriculture, Egypt. Validamycin A was provided by Qianjiang Biochem. Co. Ltd., China and other chemicals were purchased from Sigma and Fisher group

The seeds of two rice cultivars were surface sterilized with a 5% sodium hypochlorite solution for 5 min. After washing several times with distilled water, seeds were imbibed in beaker containing distilled water in a culture room at 28 ± 2 °C for 24h. After imbibition, the seeds of each genotype were divided into two groups, seeds of the first group were left without any treatment (control), and the second group was soaked in 30μM validamycin A for 8 h. Seeds of the two cultivars were separately sown in the mentioned experimental site at two successive seasons.

The experiment was laid out in a randomized complete block design with three replications. The net plot size was 10 m². Sowing date was April 25 in both

seasons, with plant spacing of 20 x 20 cm. The transplantation of rice was done on May19 and 13 while harvesting was on October 16 and 9 in the 1st and 2nd seasons, respectively.

Chemical analysis

At heading stage, plant samples were randomly taken and transferred to the lab to determine the total soluble sugars, trehalose, starch content, nitrogenous constituents (total free amino acids, proline and total soluble proteins) and protein electrophoresis patterns.

Yield and yield attributes

At harvest time, three replicates with one m^2 area of each unit area were harvested and sub samples of ten plant's shoots were taken randomly to determine the yield and yield attributes. Plant height, number of productive tillers, panicle length, number of grains/panicle, number of fertile and infertile flowers on the main spike, filled grain percentage, 1000-grain weight and grain yield were determined.

Total soluble sugars (TSS)

Total soluble sugars (TSS) were extracted according to Homme *et al.* (1992) and were estimated by anthrone-sulphuric acid method of Yemm & Willis (1954) using 0.2% anthrone in concentrated H_2SO_4 as reagent.

Estimation of trehalose

Trehalose content was extracted according to the method described by Lynch *et al.* (2010). For trehalose quantitation, the anthrone reaction was used based on Umbreit *et al.* (1972).

Starch content

Determination of starch content was carried out according to the method of Rose *et al.* (1991). The absorbance was measured at 625 nm using Spekol Spectrocolourimeter VEB Carl Zeiss.

Free amino acids

Free amino acid content was extracted (Vartanain *et al.*, 1992) and determined with the ninhydrin reagent method (Yemm & Cocking, 1955). Total free amino acids were calculated from a standard curve prepared against glycine (0–100 mg).

Proline

Proline was assayed according to the method described by Bates *et al.* (1973). A standard curve was obtained using a known concentration of authentic proline.

Total Protein

Total protein concentration of the supernatant was determined according to the method described by Bradford (1976) with bovine serum albumin as a standard.

SDS-PAGE analysis of protein

Separation of proteins was performed using Sodium Dodicy lSulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), according to the method of Laemmli (1970).

Statistical analysis

The obtained data were statistically analyzed using the one-way analysis of variance as described by Snedecor & Cochran (1969). Means were compared by LSD at 5 % using SPSS program version16.

Results

Grain yield and yield attributes

Yield performance at reproductive stage was assessed at electrical conductivity (EC) 10.06 dSm^{-1} saline field conditions. This stress level ensured a proper comparison in yield parameters between control (untreated) and Validamycin A treated plants. Grain yield, in terms of total seeds weight per plant and per hectare were used as the salient parameter for comparing the salinity tolerance and yield performance of treated plants with validamycin A at maturity (harvest) stage. Other yield related traits (tillers number, panicles number, panicle length, spikelets number, spikelets fertility/plant and 1000-grain weight) are provided as support of better performance of the plant treated with validamycin A under saline field conditions.

Data in Table 1 showed that the tolerance (Agami M5) cultivar is significantly higher plant height and yield components compared to the sensitive (Sakha 103) one. Treatment with validamycin A, gave more grain yield and its components in Agami M5 cv. than that in Sakha 103 one. The magnitude of the increase in plant height was higher in Agami cultivar.

TABLE 1. Effect of validamycin A (30μM) on grain yield and yield attributes of rice cultivars under saline field conditions. (Combined analysis of the two seasons) .

Cultivar	Treatments	Tiller No/ plant	Panicle No/ plant	Panicle length (cm)	Spikelet No/ plant	Spikelet fertility (%)	1000-grain wt (g)	Grain-yield g/plant	Plant height (cm)
Sakha 103	Control	6c	5d	13.4c	90.0d	11.5d	9.4c	2.2b	66c
	30μVal.	9b	12b	19. 4b	205.3b	88.4b	18.9b	6.4a	81b
Agami M5	Control	10b	9c	14.2c	113.0c	22.3c	8.0c	1.8b	86b
	30μVal.	13a	17a	27.9a	319.5a	98.0a	22.3a	7.2a	135a
LSD at 5%		2.45	2.22	3.14	10.25	7.89	3.42	1.25	1.25

Values followed by different letters are significantly different at = 0.05 level .

Total soluble sugars, starch and trehalose

The effect of validamycin A on the total soluble sugars, starch and trehalose of both rice cultivars under saline field conditions are shown in Table 2. As with other cellular constituents, starch and sugar levels are affected by stress. In validamycin A treated plants, starch and sugars contents were significantly increased as compared with non-treated plants. Under saline field conditions, treatment with validamycin A caused 1.5-fold and 1.3-fold increases than untreated in the total sugar content at Sakha103 and AgamiM5, respectively. While, starch content, had 1.8-fold and 1.7-fold increases in Sakha103 and Agami M5, respectively than the untreated control under saline field conditions (Table 2).

TABLE 2. Effect of validamycin A (30μM) on the contents of starch, total soluble sugars, total protein, total free amino acids and proline in the plant tissue of two rice cultivars under saline field conditions .

Cultivar	Treatments	Soluble sugar content (mg/g glucose DW)	Starch (mg/g glucose DW)	Trehalose (mg/g glucose DW)	Soluble protein content (mg/g DW protein)	Total free amino acids (mg/g glycine DW)	Proline content (μmol/g.DW)
Sakha 103	Control	43.4c	105.6c	0.4d	55.2c	20.5a	2.04a
	30μVal.	64.2b	194.3b	1.3c	90.4b	18.4b	1.80b
Agami M5	Control	62.3b	191.5b	2.0b	83.5b	20.7a	1.50c
	30μVal.	77.5a	316.3a	4.7a	103.5a	14.5c	0.90d
LSD at 5%		5.38	10.68	0.625	8.54	2.14	0.22

However under control (saline field conditions), Agami cultivar showed a higher trehalose content than Sakha103 (Table 2) by 4.3 folds increase. A significant increase in trehalose content was observed in both rice cultivar primed by validamycin A which increased by 2.7 and 2.8-folds, when compared to the un- treated control in Sakha103 and Agami cultivars, respectively.

Total leaf protein, free amino acids, and proline

Protein content was increased in treated plants than in the controls in both rice cultivars (Table 2). The maximum increase, recorded in Sakha103, was1.64-fold higher than that in the controls (from 55.2 to 90.4 mg/g DW), while in Agami M5, was 1.24-folds higher than that in the controls (from 83.5 to 103.5 mg/g DW). Total protein contents decreased in both cultivars

when compared with validamycin treated plants. This decrease may have resulted from an adverse effect of NaCl on protein synthesis or proteolysis.

The pool of free amino acids increased in the control plants when compared with the treated plants with validamycin A in both cultivars under saline field conditions. Thus, the increase in the total amino-acid pool and the decrease in protein content reflect the mode of adjustment to salinity stress by rice plant under saline field conditions.

Total amino acids in Sakha 103 cultivar decreased slightly, whereas their decrease was steady in Agami M5. The content measured at Agami M5 was 1.43-fold lower than the control (Table 2).

Under saline field conditions, the proline content was strongly increased in Sakha103 cultivar than Agami M5, but the level was more than that measured in response to validamycin A treated plants in both cultivars (Table 2).

*Changes in protein electrophoresis in harvested seeds*
In the present work (Table 3 and Fig. 1), three types of modifications were observed in the protein patterns of both rice cultivars seeds, some protein bands were disappeared, other proteins were selectively increased and synthesis of new set of protein was induced. Some of these responses were observed in both tolerance and sensitive salt cultivars under saline field conditions, while others were induced by either salinity with or without validamycin A treatment in both cultivars. A set of 23 protein bands of molecular weight ranged between 170.74 and 11.12 kDa were observed in both rice cultivars seeds.

The protein patterns of rice seeds under saline field conditions comprise five major bands (common bands) having molecular weights of (99.46, 40.74, 26.27, 12.61 and 11.5 kDa). Two protein bands of molecular weight (99.46 and 11.5) are specific for Agami M5 cultivar. Moreover one protein band of molecular weight (26.27kDa) was present in both cultivars of untreated plants, while another one band of molecular weight (40.74 kDa) was present in both cultivars treated with validamycin under saline field conditions. Either one protein band of molecular weight (12.61 kDa) was de novo synthesized in Agami M5 seeds treated with validamycin A under saline field conditions.

The results of SDS-PAGE analysis could reveal two different genetic mechanisms, *i.e.,* salt stress resulted in the over expression of some gene and/or de novo induction of gene expression. The total number of bands was affected under saline field conditions and plant treated with validamycin A in both cultivars, but some bands appeared and the other bands disappeared.

TABLE 3. Effect of validamycin A on the protein patterns (separated by SDS-PAGE) in seeds of two rice cultivars under saline field conditions.

Band NO.	M.W (kDa)	Lane1	Lane2	Lane3	Lane4	RF
1	170.74	1	1	1	1	0.066
2	163.75	1	1	1	1	0.08
3	116.51	1	1	1	1	0.194
4	111.75	1	1	1	1	0.208
5	99.46	1	1	0	0	0.247
6	78.33	1	1	1	1	0.327
7	60.6	1	1	1	1	0.413
8	57.26	1	1	1	1	0.432
9	45.1	1	1	1	1	0.512
10	40.74	0	1	0	1	0.546
11	34.88	1	1	1	1	0.598
12	26.27	1	0	1	0	0.693
13	25.04	1	1	1	1	0.709
14	23.81	1	1	1	1	0.726
15	20.51	1	1	1	1	0.776
16	19.72	1	1	1	1	0.789
17	18.42	1	1	1	1	0.812
18	15.12	1	1	1	1	0.878
19	13.91	1	1	1	1	0.906
20	12.61	0	1	0	0	0.939
21	12.09	1	1	1	1	0.953
22	11.5	1	1	0	0	0.970
23	11.12	1	1	1	1	0.981
Total	23	21	22	19	19	
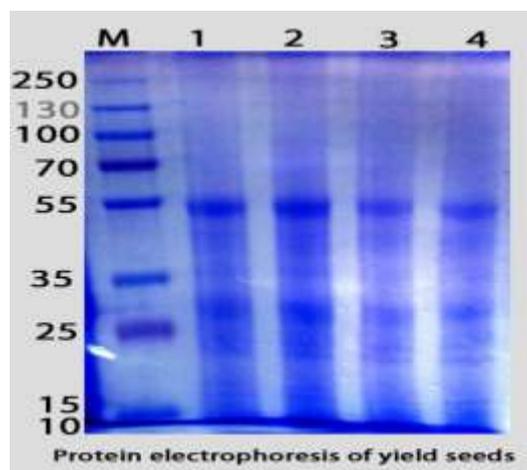
Fig.1. Electrophoretic banding patterns of two rice cultivars seeds which yielded from saline field conditions alone or in combination with validamycin A. 1-Agami M5 Control; 2- Agami treated with Val. (30µM); 3- Sakha103 Control; 4-Sakha103 treated with Val. (30µM).

Discussion

Reductions in survival and growth of rice seedlings under salt stress are the major causes of stand failure and biomass and yield reduction in salt-affected areas, as reported by Zeng & Shannon (2000). This reduction of yield and its component rated under salt stress conditions may also be attributed to low production, expansion, senescence and physiologically less active green foliage (Hasanuzzaman *et al.,* 2013).

In *Oryza sativa* varieties, grain yield, which is the ultimate product of yield components, is greatly influenced by salinity levels. This reduction in grain yield was suggested to be due to reduced number of tillers, spikes and grains per plant and individual grain weight. Murty & Murty (1982) suggested the severe inhibitory effects of salts on fertility may be due to differential competition in carbohydrate supply between vegetative growth and constrained supply of these to the developing panicles. Also, reduced viability of pollen under stress condition could result in failure of seed set (Abdullah *et al.,* 2001). Grain yield reduction of rice varieties due to salt stress is also reported earlier by Gain *et al.* (2004). Zeng & Shannon (2000) found similar observations. They further reported that spikelets per panicle were more sensitive to salt stress compared with fertility and kernel weight. This is expected when salinity was high during panicle initiation.Therefore, a well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to develop salt-tolerant crop varieties. Exploring suitable ameliorants or stress alleviant is one of the tasks of varieties.

In recent decades, xogenous protectants such as osmoprotectant (proline, glycine betaine, trehalose, etc.) have been found effective in mitigating the salt induced damage in plant (Hoque *et al.*, 2007 and Hasanuzzaman *et al.,* 2013). Trehalose can serve as a carbohydrate storage molecule as well as a transport sugar, similar to the function of sucrose (Muller *et al.,* 1999). It can also stabilize proteins and membranes of plants when exposed to stress by replacing hydrogen bonding through polar residues, preventing protein denaturation and fusion of membranes (Iturriaga *et al.,* 2009). Moreover Trehalose acts as a source of carbon and energy and as a protector against stresses (Fernandez *et al.,* 2010).

Low trehalose contents were obtained in transgenic tobacco, whereas no traces of trehalose were detected in the engineered potato. To improve trehalose accumulation, a potent trehalase inhibitor (validamycin A) was added to the culture medium (Almeida *et al.,* 2007). In the present work, higher trehalose levels were recorded in both rice species as a result of validamycin A treatment. This study demonstrated for the first time that trehalose accumulation is dependent on trehalase activity in higher plants (reviewed in, Almeida *et al.,* 2007). Several researchers have used validamycin A to raise the level of trehalose in plants. López *et al.* (2006 and 2009) reported that validamycin A was able to increase the level of trehalose in nodules of *Lotus japonicas* and *Medicago truncatula*. Trehalose significantly increased in rice seedling by using

validamycin A (Garg *et al.,* 2007). Validamycin A was used safely to raise the level of trehalose in plant tissues, since it proved to have no effect on growth (Müller *et al.,* 2001; Gracia *et al.,* 2005 and Qaid, 2010).

In assays on tobacco leaves, validamycin A (100μM) is sufficient to reduce > 99% of trehalase activity (Goddijn *et al.,* 1997). Comparatively, extracts prepared from flowers of Arabidopsis show a 10-fold reduction in trehalase activity in the presence of 10μM validamycin A, whereas leaf and root extracts are completely inhibited (Muller *et al.,* 2001). In *Lotus japonica* trehalase activity was reduced by 65% when plants were cultured in the presence of (30μM) validamycin A (Lopez *et al.,* 2006).

It is clear from the present work that the grain yield and yield components of both rice cultivars under saline field conditions were improved due to pre-sowing seed treatment with validamycin A.

Our results can be in agreements with some earlier studies which reported that exogenous application of osmolytes as foliar spray or pre-sowing seed treatment increased plant productivity under drought stress conditions in different crop species. For example, exogenous trehalose (30 mM) induced the expression of genes involved in detoxification and osmotic stress response within 6–12 h (Bae *et al.,* 2005). These findings suggest that exogenous trehalose acts as an elicitor of genes involved in abiotic stress responses (Paul *et al.,* 2010 and Fernandez *et al.,* 2010). Although the actual role of compatible solutes in improving grain yield is not clear, it can be suggested that the beneficial effect of compatible solutes on grain yield may have been due to their promotion effect on reproductive parts such as inflorescence meristem, flower buds, developing seeds resulting in increased number of flowers (that developed into grains) and size of grains by increasing the photosynthates translocation rate to developing reproductive organs. It is well known that trehalose is necessary for inflorescence development or normal transition to flowering (van Dijken *et al.,* 2004; Gomez *et al.,* 2006 and Paul *et al.,* 2008). In maize, over accumulation of trehalose in axillary inflorescence meristem caused large changes in inflorescence phenotype, and thus has a crucial role in cell growth and embryo development (Satoh-Nagasawa *et al.,* 2006).

Total soluble sugars, polysaccharides and total carbohydrates
The accumulation of carbohydrate plays a key role in alleviating the salinity stress, either via osmotic adjustment (Ackerson, 1985) or by conferring some desiccation resistance to plant cells (Srivastava *et al.,*1995).

The data herein obtained revealed that pre-soaking grains with validamycin A stimulated the accumulation of total soluble sugars and starch as compared with the corresponding control. Soluble sugar may play a key role in osmotic adjustment at the cellular level of plants under salt stress (Gupta & Kaur, 2005). In rice, soluble sugars content in salt-tolerant cultivar was significantly greater than in salt-sensitive one in plants exposed to salt stress (Cha-um *et al.,* 2009).

At 100 mM salt treatment, total soluble sugars in the salt-tolerant variety accumulated to a higher level than in salt susceptible (Nemati *et al.,* 2011). In the present study, the soluble sugars content in shoots of both Sakha103 (salt-sensitive) and AgamiM5 (salt tolerance) cultivars was enriched. Sowing pre-soaked rice seeds in validamycin A in saline field conditions (10.8 dS m^{-1}NaCl), leads to an increasing in starch content, especially in AgamiM5 (salt tolerance) cultivar. The increase of total soluble sugars in rice shoots (Table 2) may function as an osmotic adjustment to prevent water loss in the plant cells during salt stress (Siringam *et al.,* 2011). Trehalose, which may play a role in regulating carbohydrate allocation in plants during development, has often been proposed as acting as an osmoprotectant during periods of drought or water deficit-induced stresses (Muller *et al.,* 2001; Eastmond *et al.,* 2003 and Penna, 2003).

This sugar possesses the unique capacity for reversible water absorption, and appears to be superior to other sugars in protecting biological molecules from desiccation-induced damage (Rontein *et al.,* 2002). In addition, application of trehalose increased the concentrations of sucrose and fructose in leaves. Trehalose may have an indirect effect on carbohydrate metabolism by interfering with photosynthetic capacity, and the conversion and utilization of other sugars (Ranwala & Miller, 2009). Furthermore, in rice plants genetically engineered to synthesize trehalose under drought and salt stress conditions showed improved photosynthetic ability and increased concentrations of soluble sugars (Garg *et al.,* 2002). Meanwhile, plant tissues of the validamycin A-treated seeds showed a more accumulation of total soluble sugars in both stressed rice cultivars. This increment was also paralleled with increased content of starch. Interestingly, the link with starch has been extended to starch breakdown.

Recent work suggests that the induction of high starch on trehalose is due to inhibited breakdown, which is due to inhibited transcription of SEX1 and β-amylase, regulated by the transcription factor ABI4 (Ramon *et al.,* 2007). In Arabidopsis, exogenous application of trehalose induces accumulation of starch by increasing the activity of ADP-glucose pyrophosphorylase (AGPase), a major enzyme controlling starch synthesis (Wingler *et al.,* 2000).

In the present work, the starch content increased in tissues of rice by almost 45% and 40 % in Sakha103 and Agami M5, respectively, over the control in response to validamycin A treatment and consequence of trehalose accumulation. Our results are in agreement with Wingler *et al.* (2000) and Fritzius *et al.* (2001) who's reported that starch was accumulated in the shoot of Arabidopsis grown on trehalose. Bae *et al.* (2005) reported that starch was 3-folds greater in the trehalose treated samples than in the control of *Arabidopsis thaliana* seedlings.

Finally, trehalose metabolism may also act as a sugar sensor; T6P concentration is inversely related to sucrose concentration during carbon starvation and trehalose concentration has been shown to correlate with increasing sucrose concentration in sugarcane internodes during maturation (Glassop *et al.,* 2007 and Lunn *et al.,* 2006).

Proline and free amino acids

Proline is one of the so-called 'compatible compounds' that are commonly found at high concentrations when plants are exposed to salt stress and which confer them with salt stress tolerance (Wang *et al.,* 2003 and Pagter*et al.,* 2009). Proline may not only act as an osmoregulator but also play a role in the protection of enzymes and the structure of macromolecules, and as a reservoir of energy and nitrogen for utilization upon exposure to salinity (Tounektia *et al.,* 2011). Interestingly, priming of rice seeds with validamycin A decreased the concentration of this osmoticum when compared with control. Similar results were obtained by Nounjana *et al.* (2012) who reported that supplements of rice with trehalose negatively affected proline amounts in both unstressed and salt-stressed conditions resulting in a significant reduction in proline. Furthermore, exogenous trehalose also reduced proline accumulation in two maize cultivars under drought stress while increasing biomass production, improving plant water relations and some key photosynthetic attributes (Ali & Ashraf, 2011). Its appear that priming rice seeds with validamycin A for sowing in saline soil has a significant role in alleviation the salinity stress; it may be presumed, in this case, that osmoprotective effects of the accumulated trehalose reduced the need for plants to accumulate proline.

A gradual increase in the amino acids at high salinity level could be due to increased degradation of protein. Amino acids accumulation occurs not only under salinity but also under water stress in higher plants (Rajaravindran & Natarajan, 2012). The changes in soluble protein showed a reverse trend to that of free amino acids implying that the increase in protein content may be at the expense of amino acids and that the salinity changes influenced the inter conversion of these compounds. Protein content in the tissues of many plants declined under drought or salinity stress, because of proteolysis and decreased protein synthesis (Joshi & Misra, 2000)

Increased levels of specific amino acids have a beneficial effect during stress acclimation (Krasensky & Jonak, 2013). However, the priming of rice seeds with validamycin A increased the concentration of soluble protein; it decreased the content of free amino acids in both cultivars when compared with the untreated control. Similar results were obtained by Best *et al.* (2011) who reported that exogenous validamycin A (trehalase inhibitor) treatment increased foliar cellulose and starch content and decreased total amino acid, and nitrate content of mature tobacco plants (Best *et al.,* 2011).

Protein patterns in yielded rice seeds of two cultivars

Validamycin A and salinity stress induced a considerable variation in the protein patterns of both rice cultivars seeds. This variation has been manifested as the novel expression of some polypeptide, the absence of others and over expression of a third class polypeptides. The protein bands which have molecular weight (40.7 kDa) was de novo synthesized in salinized both rice seeds in combination with validamycin A, while the appearance of another de novo band

in Agami alone has molecular weight (12.61 kDa). It has been suggested that these proteins have an osmoprotection function or protected cellular structures. Moreover, the absence of one protein band which has molecular weight (26.27 kDa) in both cultivarsas response   to validamycin A treatment which concomitant with trehalose accumulation. This result is agree with Zeid (2009) who suggested that trehalose treatment reduced salt expression. Also, salt stress lead to a variation in gene expressions, where alterations in protein could be due to alteration in regulation of transcription, mRNA processing or due to altered the protein degradation rates (Mohammed *et al.,* 2012). Also the appearance of two bands of proteins which have molecular weights (99.46 and 11.5 kDa) in Agami cultivar alone in both treated and untreated plants with validamycin A under saline field conditions these bands may be due to salt tolerance genotypes.

Finally, trehalose as a sugar osmolyte shows the best coincidence in both thermodynamic and structural characteristic. Trehalose induces enzyme stabilization in the chemical and thermal denaturation. These changes show a good agreement with conformational studies and enzyme constriction of tertiary structure with the presence of trehalose. Thus it should be consider as an exceptional protein stabilizer (Gheibi *et al.,* 2006)

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استخدام فاليدامايسين للتخفيف من أجهاد الملوحة فى صنفين من نبات الأرز مختلفين فى مستوى تحملهم لإجهاد الملح

تهانى عباس حتحوت ، سامية محب الخلال ، زينب أحمد عبد الجواد ، إجلال محمد سيد* والشيماء زكى المقدم*

قسم النبات- كلية البنات للأداب والعلوم والتربية ـ جامعة عين شمس و *معمل التكنولوجيا الحيوية – معهد البساتين– مركز البحوث الزراعية – القاهرة – مصر .

نقدم هنا امكانية الحماية النسبية للتطبيق الخارجى للفاليدامايسن، المانع لنشاط إنزيم التريهاليز من خلال نقع البذور قبل زراعتها في التربة المالحة مع مستوى الملوحة EC 10.06 dS /m للتخفيف من سمية كلوريد الصوديوم و احداث تحمل للملوحة لصنفين من الأرز (صنف حساس للملوحة سخا ١٠٣ وصنف مقاوم للملوحة عجمى M5) . أدت الملوحة إلى انخفاض مستوى النشا و البروتين الكلي مصحوبة بزيادة السكريات الكلية القابلة للذوبان، البرولين و الأحماض الأمينية الحرة في كلا الصنفين المعاملين بالفاليداميسين و انخفاض نشاط إنزيم التريهاليز الأمر الذي يؤدي إلى تراكم التريهاليز في كلا الصنفين من نباتات الأرز . كما لوحظ مستوى تريهالوز في الأنسجة النباتية رافقه زيادة النشا و البروتين و ظهور نمط جديد من البروتين في كلا الصنفين .وأشارت النتائج إلى أن استخدام الفاليداميسين أدى إلى زيادة محصول الحبوب وزيادة وزن ١٠٠٠ حبة لصنفى الأرز ولذا فانه من الممكن نقع حبات الأرز مع ٣٠ μM من الفاليداميسين للتخفيف من الآثار الضارة لإجهاد الملوحة.