

Influence of antioxidants pre-sowing on biochemical and seed vigor of some rice (*Oryza sativa*, L.) varieties under salinity

EI-Manzlawy, Amal M. / Abeer El-Ward A. Ibrahim

Seed Technology Research Section, Agricultural Research Center

ABSTRACT

A new protein bands and Esterase banding pattern could be used as a biochemical marker for selecting salt tolerant rice cultivars to be grown under salt conditions, and, treatment rice seed with ascorbic and salicylic acids greatly alleviate the harmful effect of salt stress on rice plant. In this study, three rice cultivars (Giza 177, Sakha 103 and Sakha 104) were selected to determine the interactive effects of salinity (0.4 and 10 ds/m), ascorbic and salicylic acids on Esterase isoenzymes, pattern protein synthesis and seed vigor. Laboratory experiment was conducted at Seed Technology Research Department during 2010 year. The results revealed that one new band (Est 3 isoenzyme) was detected of both Giza 177 and Sakha 103 cultivars (untreated seed) under salt stress. Salinity treatments induced synthetic 3 and 2 new protein bands of Giza 177 and Sakha 103 (untreated seed), respectively. No new bands were obtained of Sakha 104 (untreated seed). Protein with molecular weight of 49 KD could play an important role in triggering a system to tolerate severe stress of salinity. Treatments of seeds of Giza 177 and Sakha 103 cultivars with salicylic or ascorbic acid induced one Esterase3 isoenzymes (RF 0.9). Three unique bands were observed under salinity with molecular weight 86, 93 and 132 KD of (Giza 177 + salicylic), (Sakha 103 +Ascorbic) and (Sakha 104+Ascorbic), respectively.

It could be summarized that only under saline conditions, Pre-sowing rice seed of Sakha 104 cultivar with Ascorbic produced the highest germination percentage, speed germination index, germination rate and seedling dry weight and the lowest mean germination time as well as time 50 % germinated seed compared with other two cultivars under study.

Keywords: Rice, salt tolerance, antioxidants pre-sowing treatment, protein and isoenzymes

INTRODUCTION

Soil salinity is a major constraint limiting agricultural productivity on nearly 20 % of the cultivated area and half of the irrigated area worldwide (Zhu, 2001). Plant peroxidases have been used as biochemical markers for various types of biotic and abiotic stresses due to their role in very important physiological processes (Gaspar *et al.*, 1982). The analysis of the activity of individual acid phosphates (AS) and esterase (EST) isozymes is important, because it can help to understand how each stress affects the different sub-cellular compartments (Scandalios 1993). Generally, the induction of new isoenzymes and the change in the isoenzyme profile is considered to play an important role in the cellular defense against oxidative stress, caused by salt stress. Our focus was to observe the response of isoenzymes to increasing salinity stress. The evaluation of the protein decay due to salt stress could be

a marker of the sensitivity of the concerning cultivars towards NaCl together with the activities of isozymes may be a good indicator for selecting salt tolerance.

Nowadays, 2-D polyacrylamide gel electrophoresis is a physiological, biochemical, and molecular processes as technique that used to study the molecular mechanisms of plant response to salinity (Ouerghi *et al.*2000). The levels of proteins differ in salt tolerant and salt-sensitive genotypes when they are subjected to salinity stress (Dubey and Rani, 1989). One approach to understanding the molecular basis of salinity tolerance is to identify stress induced changes in the levels of proteins, among the studies done in the effect of salt stress on protein synthesis, Osmotic a 26 KDa "stress protein" isolated from potato plants adapted to NaCl were quoted (Zhu *et al.*,1995).

Yeo *et al.*, (1990) reported that, rice is sensitive to salinity at the seedling stage and becomes tolerant at the vegetative phase and very susceptible at the reproductive phase in terms of grain yield. Khanam *et al.*, (2007) found that growth efficiency and other seedling characteristics of rice decreased as salinity levels increased. It is important to reduce the harmful effects of salinity to enhance the seed germination and seedling vigor, this can achieve through treating rice seed before sowing with some antioxidants such as salicycate, ascorbate, humic acid, tocopherol (Gossett *et al.*,1994).So, this work aimed to study the antioxidants solution and salinity stress in three Egyptian rice cultivars on the basis of the electrophoretic analysis of their protein , enzyme and seed vigor.

MATERIALS AND METHODS

The Laboratory experiments were conducted in 2010 year at Seed Technology Research Department, Agricultural Research Center, Egypt, to study the effect of antioxidants pre-sowing seed treatment of three rice cultivars namely Giza 177, Sakha 103 and Sakha 104 on seed vigor under salinity stress (10 dS/m) and isoenzymes, electrophoretic protein pattern. Each experiment was arranged in a completely randomized design with four replicates. Rice seed samples were obtained from the Central Administration of Seed Certification (CASC). Seeds were previously disinfected by immersion in 5% NaOCl (sodium hypochloride solution) for 5 min. to avoid fungal invasion, then washed three times with sterilized distilled water and soaked for 24 h in antioxidants solutions i. e. ascoric acid (100 ppm.), salicylic acid (100 ppm.), and control (distilled water). The ratio of seed weight to solution volume was 1: 5(gm L⁻¹).

Germination tests were carried out in sterilized Perti dishes (150 × 15 mm) covered at the bottom with two sheets of Whatman No.1 filter paper that had been autoclaved. Each dish included 50 seeds and moistened with 10 ml of salinity solutions (Tap water 0.4 dS/m and 10 dS/m) and incubated growth chamber at 25 ± 2 °C and germination was observed daily to study the following characters:

Seed vigor traits:

1- Germination percentage (G %): It was calculated by counting only normal seedlings 14 days after planting according to (ISTA rules, 1999).

2- Speed germination index (SGI): It was calculated as described in the Association of Official Seed Analysis (AOSA, 1983) by following formula:

$$SGI = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

The seeds were considered germinated when the radicle was at least 2 mm. long.

3- Germination rate (GR): was defined according to the following formula of Bartlett, (1937).

$$GR = \frac{a + (a + b) + (a + b + c) \dots (a + b + c + m)}{n(a + b + c + m)}$$

Where a, b, c are No. of seedlings in the first, second and third counts, n is the number of counts.

4- The time to get 50 % germination (T 50 %) was calculated according to the following formula of Coolbar *et al.*, (1984).

$$T\ 50\ \% = t_i + \frac{(N/2 - n_i)(t_j - t_i)}{N_j - N_i}$$

Where N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

5- Mean germination time (MGT): It was calculated based on the following equation of Ellis and Roberts (1981).

$$MGT = \frac{\sum Dn}{\sum n}$$

Where (n) is the number of seeds, which were germinated on day, D is number of days counted from the beginning of germination.

6- Seedling dry weight (gm): Ten normal seedlings 14 days after planting were dried and weighed according to Krishnasamy and Seshu (1990). All obtained data of characters were subjected to the statistical analysis according to the technique of analysis of variance (ANOVA) of completely randomized design, as described by Gomez and Gomez (1984).

Biochemical traits:

1- Isozymes electrophoresis:

Native polyacrylamide gel electrophoresis (Native-PAGE) technique was used to characterize the isozyme fingerprints of rice genotypes such as esterase (Est) according to Jonathon and Wendel (1990), Isozyme fractionation was performed on vertical slab (19.8 cm × 26.8 cm × 0.2 cm) using gel labconco electrophoresis apparatus. Leaf samples were taken from plants 15 days after planting.

2-SDS-PAGE (protein electrophoresis):

Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) procedure was carried out according to Laemmli (1970). Protein bands were visualized by staining the gel with 0.25% coomassie brilliant blue R-250. protein band sizes were determined by comparisons with the high molecular

weight protein marker. Leaf samples were taken from plants 15 days after planting.

RESULTS AND DISCUSSION

The results in Table 1 showed that 2 esterase isoenzymes (Est-1 and Est-2) were detected in rice seedlings resulted from untreated seeds and without stresses on both Giza 177 and Sakha 103 cultivars. One new band (Est3) was detected for both Giza 177 and Sakha 103 cultivars under salt stress and treated seed with antioxidants solutions. But Sakha 104 (treated and untreated seeds with antioxidants) gave three zones of activity of this isoenzymes (Est-1 , Est-2 and Est3) under salinity and control conditions. No induction of any new isoenzymes was observed in the esterase profile of Sakha 104 cultivar under salinity compared with control. These results agreed with those reported by Hassanein (1999) who stated that Salinity increases EST isozymes, the highest numbers of esterase isoenzymes were detected under the highest NaCl concentration. Rashed *et al.* (1994) observed differences in the intensities in esterase bands between salt tolerant and salt sensitive genotypes of maize and wheat. Under control conditions, electrophoretic patterns were characterized by the appearance of four main groups of esterase isozymes for both shoots and roots of maize. Whereas, Under salt stressed, 150 mM NaCl caused enhancement of the esterase isozyme bands (Amal A. Mohamed, 2005). The activity of peroxidase (POX) gradually increased in maize shoots of all treatments with increasing concentration and exposure time to salinity (Samia *et al.*, 2009).

Table 1: The Rf of esterase isozyme (EST) bands for cultivars, antioxidants and salinity.

Cultivars	Antioxidants solutions	Salinity levels	Est-1	Est-2	Est-3
Giza 177	(untreated seed)	Normal	0.6	0.8	-----
		Salinity (10 ds/m)	0.6	0.8	0.9
	Ascorbic treated seed	Normal	0.6	0.8	0.9
		Salinity (10 ds/m)	0.6	0.8	0.9
	salicylic treated seed	Normal	0.6	0.8	0.9
		Salinity (10 ds/m)	0.6	0.8	0.9
Sakha 103	(untreated seed)	Normal	0.6	0.8	-----
		Salinity (10 ds/m)	0.6	0.8	0.9
	Ascorbic treated seed	Normal	0.6	0.8	0.9
		Salinity (10 ds/m)	0.6	0.8	0.9
	salicylic treated seed	Normal	0.6	0.8	0.9
		Salinity (10 ds/m)	0.6	0.7	0.8
Sakha 104	(untreated seed)	Normal	0.7	0.8	0.9
		Salinity (10 ds/m)	0.7	0.8	0.9
	Ascorbic treated seed	Normal	0.7	0.8	0.9
		Salinity (10 ds/m)	0.7	0.8	0.9
	salicylic treated seed	Normal	0.7	0.8	0.9
		Salinity (10 ds/m)	0.7	0.8	0.8

Regarding to salinity effect on protein pattern electrophoretic, results in (Table 2) cleared that Sakha 104 cultivar (untreated seed) produced the highest pattern bands (11 bands) under salinity compared with (6 and 6 bands) for Giza 177 and Sakha 103 (untreated seed), respectively. Electrophoretic analysis of protein patterns of the Giza 177 cultivar showed five bands with molecular weights of 25, 28, 42, 95 and 98 KD and they were the most prominent in the control (without salinity). Salinity treatments resulted in the induction of 3 new bands of Giza 177 cultivar (untreated seed) with molecular weight 19, 49 and 90 KD and 2 bands of Sakha 103 cultivar (untreated seed) with molecular weight 35 and 49 KD. But, no new bands were obtained of Sakha 104 cultivar (untreated seed) in salinity conditions. No changes of bands were observed on Sakha 103 and Sakha 104 cultivars (untreated seed) in salinity or normal condition, both gave 6 and 11 bands for two cultivars, respectively. These results were similar with (Hoyos and Zhang, 2000) who found that several of new proteins which are synthesized in response to environmental stress have been reported as stress-proteins in plants. Many of these proteins were suggested to protect the cell against the adverse effect of salt stress. Kawasaki *et al.* (2001) found that protein turnover in stressed plants were observed at early time, followed by the induction of known stressed-responsive transcripts within hours, and the induction of transcripts for defense-related function later. So, it can be suggested in our work that the protein with molecular weight of 49 KD could play an important role in triggering a system to tolerate sever stress of salinity.

Tolerant cultivars always maintained higher levels of total soluble proteins and total amino acids in salt stressed seedlings compared to susceptible cultivars (Dubey and Rani, 1989). Ashraf and O'Leary (1999) reported a decline in certain proteins in NaCl-sensitive cultivar (cv. potohar) of wheat as compared to tolerant cultivars LU265 and Kharchia. In the present study, the rice cultivar Sakha 104 which is claimed to be salt-tolerant appears to be the least affected by salinity with regard to seed vigor and protein pattern. Similar results were obtained by Badr *et al.* (1998) on the effect of NaCl treatments on protein expression of wheat cultivars Sakha 8 (salt tolerance), they reported that salinity treatment on this cultivar at plumule stage appearance showed no effect on SDS-PAGE patterns. Hoda Barakat (2003) demonstrated that slight modifications in new polypeptide patterns are observed and of wheat cultivar Sakha 69 (3 bands) compared with Sids 1 (4 bands) and Gemmiza 9 (6 bands) cultivars.

Hukam Gehlot *et al.* (2005) reported that proteins induced by NaCl-treatment were one polypeptide of 65.0 kD in salt-tolerant RT-46 sesame cultivar at 50 mM NaCl, and three polypeptides of 65.0, 62.0, 19.5 kD in salt-sensitive RT-125 sesame cultivar at 30 and 50 mM NaCl and two 62.0, 45.0 kD in salt-toler RT-54 sesame cultivar at 30 and 50 mM NaCl. The increases in the amount of protein(s) may be involved in a defense mechanism in species towards NaCl salinity, the decreases in the amount of the same protein(s) may not be enough to cope with NaCl salinity. In addition, the decrement and loss of the protein may lead to an increase in sensitivity of a genotype towards NaCl salinity (Yildiz and Terzi, 2008). In addition, salt

stress promotes a complete loss of present proteins and the synthesis of newly formed proteins (Yildiz, 2007). Zorb *et al.* (2004) detected three groups of differentially regulated proteins in roots and shoots under salt stress: A) proteins which are involved in protein biosynthesis and protein modifications by Kinase, (B) enzymes of the carbon metabolism and (C) enzymes of the nitrogen metabolism.

Table (2): SDS-PAGE of total proteins extracted of cultivars, antioxidants and salinity.

Number	Molecular weight kD	Giza 177						Sakha 103						Sakha 104							
		Control		Ascorbic		salicylic		control		Ascorbic		salicylic		control		Ascorbic		salicylic			
		Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)	Normal	Salinity (10 ds /m)		
1	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
2	98	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-
3	95	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
4	93	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
5	90	-	+	-	+	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+
6	89	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	86	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	79	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
9	69	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
10	65	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+
11	62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
12	54	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
13	49	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+
15	42	+	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	+	+	-	-
16	39	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-
17	35	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	+
18	32	-	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+	+
19	28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	19	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
Total		5	6	5	7	5	7	6	6	6	9	6	6	11	11	11	16	11	11	11	11

+ = present bands - = absent bands
 +* = new bands resulted in salinity or antioxidants

With respect to antioxidants effect on protein pattern and isoenzymes, results are presented in Table 1 and 2 .Treatments seed of Giza 177 and Sakha 103 cultivars with salicylic or ascorbic acid induced one Esterase3 isoenzyme with (Rf 0.9). Likewise, antioxidants solutions induced the appearance of some new protein bands in seedling of the three rice cultivar. Giza 177 cultivar seed treated with ascorbic and salicylic resulted in

the induction of 7 bands compared with 6 bands of untreated seed under salinity condition. Treated seed of Sakha 103 and Sakha 104 cultivars with ascorbic produced new bands only in salinity stress which were 3 and 5 new bands for two cultivars ,respectively. Three unique bands were observed under salinity with molecular weight 86, 93 and 132 KD of (Giza 177 + salicylic), (Sakha 103 +Ascorbic) and (Sakha 104+Ascorbic), respectively.

Accumulation of proteins of molecular weights 23 and 22KDa which induced by SA had a possible role in salt adaptation and osmotic adjustment (Parida *et al.*2005). In addition , 48 KDa protein was identified as salicylic acid induced protein Kinase (SIPK) that is activated by various stress stimuli, two bands with molecular weight 48, 23 KD (induced by SA) and not found in proteins of stressed or un-stressed control, the highest POX activity was recorded in maize shoots of SA-treatments of 3 weeks (Samia *et al.*, 2009). Applied ASA caused a further increase in superoxide dismutase (SOD) activity of salt stressed plants of MH-97 wheat cultivar (salt sensitive), whereas it remained unchanged in the salt stressed plants of (salt tolerance) S-24 wheat cultivar (Khan *et al.* 2006).

Results in Tables 3, 4 and 5 cleared that Salinity (10 ds/m) make reduction in germination percentage of Giza 177, Sakha 103 and Sakha 104 cultivar seed untreated which were 39, 31 , 25 % for three cultivars, respectively. Whereas, reduction in germination percentage of these cultivars seed treated with ascorbic were, 32,24 and 16 % and seed treated with salicylic 26, 24 and 19 % for previously cultivars. Only under saline condition, Pre-sowing rice seed Sakha 104 cultivar with Ascorbic produced the highest germination percentage, speed germination index, germination rate and seedling dry weight and the lowest mean germination time as well as time 50 % germinated seed compared with other two cultivars under study. No significant differences of germination % were obtained in all seed treatments of three cultivars under normal condition (without stress). These results are confirmed with Farooq *et al.*, (2007), who reported that soaking seed with Ascorbic or salicylic acid enhanced the seed germination . Improved seedling dry weight might be due to increased cell division within the apical meristem seedling roots. Moreover, salicylate treatment maintains the IAA and Cytokinin levels, which enhances the cell division (Sakhabutdinova *et al.*, 2003) and induces an increase in the resistance of seedlings to osmotic stress (Borsani *et al.*, 2001).

Table 3: Effect of interaction between salinity, cultivars and antioxidants solutions on germination percentage % and speed germination index.

	Germination percentage %						Speed germination index (SGI)					
	Normal			Salinity (10 ds/m)			Normal			Salinity (10 ds/m)		
	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104
Control	86	87	91	52	57	68	27.3	28.4	26.6	8.5	9.1	10.9
Ascorbic	86	90	92	58	68	77	27.6	28.8	26.8	10.6	11.0	11.5

salicylic	86	87	91	64	66	73	27.8	28.8	26.8	10.9	9.5	11.3
L.S.D 5 %	7						1.6					

Table 4: Effect of interaction between salinity, cultivars and antioxidants solutions on germination rate and Time 50 % germinated seed (days).

	Germination rate						Time 50 % germinated seed (days)					
	Normal			Salinity (10 ds/m)			Normal			Salinity (10 ds/m)		
	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104
Control	0.80	0.85	0.76	0.41	0.58	0.62	4.1	3.9	4.3	5.9	5.6	5.5
Ascorbic	0.81	0.86	0.78	0.47	0.62	0.69	3.8	3.3	4.0	5.3	5.3	4.9
salicylic	0.81	0.85	0.76	0.49	0.61	0.70	3.7	3.5	3.9	5.5	5.5	5.1
L.S.D 5 %	0.07						0.3					

Table 5: Effect of interaction between salinity, cultivars and antioxidants solutions on Mean germination time (days) and Seedling dry weight (g)

	Mean germination time (days)						Seedling dry weight (g)					
	Normal			Salinity (10 ds/m)			Normal			Salinity (10 ds/m)		
	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104	Giza 177	Sakha 103	Sakha 104
Control	4.6	4.3	5.5	6.5	6.7	6.3	0.26	0.29	0.24	0.08	0.09	0.09
Ascorbic	4.6	4.1	5.3	6.1	6.3	6.0	0.28	0.34	0.27	0.13	0.15	0.12
salicylic	4.5	4.2	5.4	5.9	6.4	6.1	0.29	0.29	0.25	0.16	0.14	0.12
L.S.D 5 %	0.4						0.04					

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تأثير معاملة التقاوى بمضادات الأكسدة قبل الزراعة على حيويتها والصفات البيوكيميائية في بعض أصناف الأرز تحت الظروف الملحية.

امال محمود المنزلاوي وعيبر الورد أحمد إبراهيم
قسم بحوث تكنولوجيا البذور - مركز البحوث الزراعية

تم دراسة تأثير الملوحة (١٠ ديسيمتر/م) ومعاملة التقاوى بمضادات الأكسدة (الأسكوربيك، الساليسيليك) من خلال ٣ أصناف تختلف في درجة تحملها للملوحة (جيزة ١٧٧، سخا ١٠٣ و سخا ١٠٤) على مشابهاة إنزيم الإستيريز ، وحزم البروتين الناتجة من التفريد الكهربائي (البيكتروفوريسيس) و حيوية التقاوي. لهذا الغرض أجريت تجربة معملية بمعامل قسم بحوث تكنولوجيا البذور خلال عام ٢٠١٠ م ، في تصميم التام العشوائية في أربع مكررات و يمكن تلخيص أهم النتائج فيما يلي :

أدى انبات تقاوى الارز عند مستوى ملوحة قدرة (١٠ ديسيمتر/م) إلى ظهور مشابه ثالث جديد لإنزيم الإستيريز مع RF ٠.٩ وذلك في التقاوي صنفى جيزة ١٧٧ و سخا ١٠٣ وغير معاملة بمضادات الأكسدة مقارنة بانبات التقاوي بالمياه المقطرة، كما أدت الملوحة إلى ظهور ٢ ، ٣ حزم بروتينية جديدة في التقاوي غير المعاملة لصنفى جيزة ١٧٧، سخا ١٠٣ على الترتيب بينما لم تظهر أي حزمة بروتين جديدة من التقاوي غير المعاملة لصنف سخا ١٠٤ تحت الإجهاد الملحي. وهذه الحزمة البروتينية ذات وزن جزئى قدرة ٤٩ كيلودالتون، ويحتمل ان يكون لها دور هام في تحفيز أنظمة تجمّل الإجهاد الملحي .

أدت معاملة التقاوي لصنفى جيزة ١٧٧ و سخا ١٠٣ بمضادات الأكسدة (الأسكوربيك ، الساليسيليك) قبل الزراعة إلى ظهور مشابه ثالث جديد لإنزيم الإستيريز مع RF ٠.٩ و ذلك في الظروف الملحية والظروف غير الملحية (المياه المقطرة) ، كما ظهرت ٣ حزم بروتينية نادرة تحت ظروف الإجهاد الملحي ذات وزن جزئى ٨٦ ، ٩٣ ، و ١٣٢ كيلودالتون لـ (جيزة ١٧٧ + الساليسيليك) ، (سخا ١٠٣ + الأسكوربيك) و (سخا ١٠٤ + الأسكوربيك) على الترتيب. تحت ظروف الإجهاد الملحي أظهرت معاملة تقاوي صنف سخا ١٠٤ بالأسكوربيك أعلى القيم لصفات نسبة الإنبات ، سرعة الإنبات ، معدل الإنبات و الوزن الجاف للبادرات و أقل القيم لصفتي متوسط زمن الإنبات و الزمن اللازم لإنبات ٥٠ % من البذور مقارنة بصنفى سخا ١٠٣ و جيزة ١٧٧.

توصي الدراسة بأن مشابهاة انزيم الاستيريز و الحزم البروتينية الجديدة الناتجة عن التفريد الكهربائي (البيكتروفوريسيس) يمكن أن تستعمل في برامج التربية كوسيلة بيوكيميائية لانتخاب أصناف الأرز المتحملة لظروف الإجهاد الملحي و زراعة صنف سخا ١٠٤ و معاملة التقاوي الخاصة به بمحاليل مضادات الأكسدة تقلل من الأثر السلبي و الضار للإجهاد الملحي

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة
مركز البحوث الزراعية

أ.د / علي السعيد محمد شريف
أ.د / محمود إبراهيم العميري