ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Journal of Agronomy 6 (3): 433-438, 2007
ISSN 1812-5379

Relationship Between Peroxidase Activity and Salt Tolerance During Barley Seed Germination

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Abstract: The responses of some Tunisian barley cultivars to salt stress were studied. Under gradual salt stress intensity, southern cultivars showed a moderate increase in their peroxidases (POD) activities associated with an important elevation in their germination rate and relative steady root/shoot ratio especially at 3 and 6 g L^{-1} of NaCl. However, under 9 g L^{-1} salt concentration, a decrease in germination rate was observed for these cultivars. Northern cultivars registered an important increase in their peroxiadases activities under moderate salt stress concentrations (3 and 6 g L^{-1} NaCl) followed by a drastic decrease in POD activity at severe stress (9 g L^{-1} NaCl). Whatever the salt stress degree, cultivars originated from North of Tunisia, showed a decrease in their germination rate and shoot/root ratio especially for the cultivar Kalaâ. These results indicated that salt tolerance in barley might be related to maintaining or even increasing peroxidase activity when subjected to increasing salt stress.

Key words: Salt stress, barley, seed germination rate, peroxidase, tolerance

INTRODUCTION

Salinity stress has a negative impact on agricultural yield throughout the world. Salinity can seriously alter plant metabolic activities such as assimilation of mineral nutrients (Arshi *et al*., 2002, 2005; Munns *et al*., 2000) stomatal conductance (Ouerghi *et al*., 2000), mesophyll conductance (Delfine *et al*., 1998), carbon metabolism and/or efficiency of photosynthetic enzymes (Brugnoli and Bjorkman, 1992). The general response of non-halophytes to increasing salinity has been well documented (Hasegawa *et al*., 2000). High concentration of salts causes ion imbalance and hyper-osmotic stress in plants. These primary effects often lead to secondary stresses, such as oxidative stress (Zhu, 2000), due to production of activated oxygen species, which can damage DNA, proteins, chlorophylls and membrane functions (Zhu, 2001; Gomez *et al*., 1999; Hernandez *et al*., 1999). To overcome the toxicity of active oxygen species, a complex antioxidative defense system, composed of both non-enzymic and enzymic constituents, is present in all plant cells (Foyer *et al*., 1994a). In response to the increased production of oxygen radicals the capacity of the antioxidant defense system is increased (Gressel and Galun, 1994) but in most situations the response is moderate (Foyer *et al*., 1994b). The produced ROS (Reactive Oxygen Species) are suppressed to low levels by antioxidant molecules and by the enzymes that scavenge these species (Del Rýó *et al*., 2002) such as Super oxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), ascorbate peroxidase (EC 1.11.1.11) and a variety of general peroxidases (PODs; EC 1.11.1.7) that are the main enzymes involved in the detoxification of ROS (Hernandez *et al*., 1999). PODs are hemecontaining glycoproteins which participate in a great number of physiological processes, such as the biosynthesis of lignin and ethylene, defense against pathogens and wounding, auxin metabolism and stress response (Kim *et al*., 1999). This type of POD catalyses the dehydrogenation of structurally diverse phenolic and endiolic substrates by H_2O_2 and are thus often regarded as antioxidant enzymes, protecting cells from the destructive effect of derived oxygen species (Vianello *et al*., 1997). Meloni *et al*. (2003) and Sairam and Srivastava (2002) showed that changes in the activity of antioxidant enzymes in response to salinity were different in tolerant and sensitive cultivars. It was reported that plants with high levels of antioxidants, either constitutive or induced, have greater resistance to this oxidative damage (Hernandez *et al*., 1999; Meneguzzo *et al*., 1999). This was also reported by Meloni *et al*. (2003) who showed that salt-tolerant cotton cultivar may exhibit better protection against ROS by increasing the activity of antioxidant enzymes under salt stress. Under salt

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stress, Khales and Baaziz (2006) reported that tolerant *Opuntia* ecotypes exhibited high peroxidase activity compared to sensitive ones.

The scope of this study is to investigate the tolerant barley cultivars collected from Tunisian different climatic stage to salt stress using some physiological traits and peroxidases activity at germination level.

MATERIALS AND METHODS

Seed germination rate and shoot/root ratio: This study was carried out at the National Agronomic Research Institute of Tunisia (INRAT) in 2003. The cultivars studied were landrace barley (Tozeur, Kebelli, Jandouba, Kalaa and Kelibia). They are originated from two different geographic regions: Tozeur and Kebelli are from the South, which is characterized by high temperature and lower rainfall (≤150 mm), whereas Jandouba, Kalaa and Kelibia were originated from the North where the temperature is mild and the rainfall varied from 400 to 600 mm/year. The physiological parameters studied were germination rate, shoots/roots ratio and peroxidase activity.

From each barley cultivar, 25 seeds were germinated in Petri dishes filled with 10 mL of distilled water as control (T_0) and under three salt concentrations (3, 6 and 9 g L^{-1} of NaCl); T_1, T_2 and T_3, respectively. The experiment was carried at 25°C in dark per cultivar in a complete randomized block design with 4 replications.

After 7 days of germination, the physiological parameters and the peroxidases activity were measured.

Peroxidases activity

Peroxidases extraction: The peroxidases were extracted from barley roots according to Brian *et al.* (1999) method. Samples were taken from each treatment (0, 3, 6 and 9 g L^{-1} of NaCl).

For each sample, 100 mg of fresh matter have been ground with mortar and pestle in liquid nitrogen. The harvested powder was mixed with an extraction buffer (phosphate buffer 50 mM, pH 7, KCl 100 mM, NaCl 1 M; $CaCl_2$ 1 mM; triton X-100 0, 1% and PVP 1%). After homogenization, the mixture was centrifuged at 15000 rpm for 15 min at 4°C. The pellet was discarded and the supernatant was used.

Peroxidase kinetic study

Gaïacol test: This test was carried out according to Vallejos (1983) method. Dosage has been done at wavelength of 470 nm, every 30 sec for 12 min. The total peroxidase activity = (Δ DO/Δ t) X (1/δ) X (Vc/Vext).

Vc : Volume of the bowl (1 mL)
Vext : Volume of vegetal extract
δ : Coefficient of extinction
Δ DO/Δ t : The curve tangent of the optic density according to the time

$$\delta = 26.6 \text{ mM cm}^{-1}$$

The specific activity = total activity/protein content.

The protein content was determined according to Bradford (1976) method.

RESULTS

Germination rate: Results showed that the behavior of the different barley cultivars, at germination level, was variable according to salt stress intensity (Fig. 1). In fact, a depressive effect of salt on the germination rate was observed for all the studied cultivars especially under severe saline conditions.

At 3 and 6 g L^{-1} of NaCl levels, most of the cultivars showed a reduction in their germination rate compared to the control. However, Tozeur and Kebilli showed an improvement of their germination rate compared with their control.

When the stress intensity was higher (9 g L^{-1} NaCl), the rate of reduction varied from 20 to 36% for Tozeur and Kebilli cultivars, respectively and from 52 to 100% for Jendouba, Kelibia and Kalaa cultivars.

Shoot/root ratio: The shoot and root length, as well as, their ratio were measured. We remarked that salinity stress

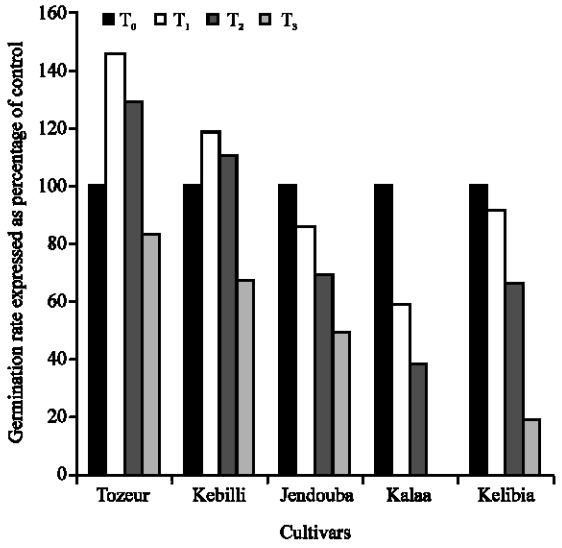

Fig. 1: Seed germination rate of some barley cultivars under salt stress

reduced the barley cultivars shoot/root ratio especially at 6 and 9 g L^{-1} NaCl (Fig. 2). The cultivars Kalaa and Kelibia showed the lowest shoot/root ratio. At 6 and 9 g L^{-1} of NaCl, Tozeur, Kebilli and Jendouba were able to keep a steady ratio varying from 0.80 to 0.90, whereas Kelibia showed only 0.27.

ANOVA followed by Newman-Keuls test at 5% level showed significant differences among cultivars (Table 1).

The Newman-Keuls test showed three different classes when the cultivars were grown under favorable conditions or under 6 g L^{-1} of NaCl, two classes when they were subjected to 3 g L^{-1} of NaCl and four classes when they were subjected to 9 g L^{-1} of NaCl (Table 2).

Generally, Tozeur and Kebilli cultivars occupy the first or the second class (A or B) whereas Kelibia and Kalaa occupy the latest ones.

Peroxidase activity: The specific peroxidase activity, at the germination stage, is shown in Table 3.

Results showed a similar behavior of peroxidase activity for most studied cultivars under control with the exception of Jendouba (Table 3 and 4). Under moderate stress conditions (3 and 6 g L^{-1} NaCl) high increase of peroxidase activity was recorded for all studied cultivars. However Jendouba cultivar showed only a moderate increase. The most important activity increase was observed at Kelibia (222.74%), Tozeur (161.71%) and Kalaa (132.89%), explaining an ability to prevent oxidative stress under 6 g L^{-1} of NaCl.

At the highest salt stress (9 g L^{-1} NaCl), the response was different. Southern cultivars reacted by keeping a

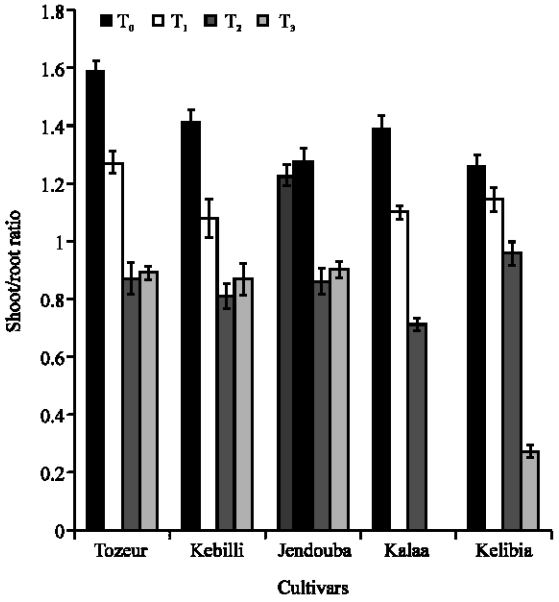

Fig. 2: Variation of shoot/root ratio of some barley cultivars under salt stress conditions

Table 1: Summary of ANOVA analysis for shoot/root ratio (PA/PS)

Variables	SCE	DDL	CM	F-test	Proba
Variable total	10.87	79	0.14	-	
Variable 1: cultivars	1.32	4	0.33	198.82	0.00
Variable 2: treatment	6.87	3	2.29	1378.59	0.00
Variable 3: interaction 1×2	2.58	12	0.22	129.71	-
Residual	0.10	60	0.00	-	-

Table 2: Barley cultivars classification (Newman-Keuls method, 5%) based on shoot/root ratio under different salt concentrations

Control		3 g L^{-1} NaCl		6 g L^{-1} NaCl		9 g L^{-1} NaCl	
Cultivars	H. class	Cultivars	H. class	Cultivars	H. class	Cultivars	H. class
Tozeur (A1)	A	Tazeur (A1)	A	Kelibia (A5)	A	Kebilli (A2)	A
Kebilli (A2)	B	Jendouba (A3)	A	Tozeur (A1)	B	Tozeur (A1)	B
Jendouba (A3)	B	Kebilli (A2)	B	Kebilli (A2)	B	Jendouba (A3)	B
Kalaa (A4)	C	Kalaa (A4)	B	Jendouba (A3)	B	Kelibia (A5)	C
Kelibia (A5)	C	Kelibia (A5)	B	Kalaa (A4)	C	Kalaa (A4)	D

H. class = Homogenous class

Table 3: POD activity of barley cultivars under different salt stress concentrations

POD activity	NaCl (g L^{-1})							
(µmoles min^{-1} mg^{-1})	0 (control)	3	%*	6	%*	9	%*	%**
Tozeur	25.25±0.025	46.10±0.030	82.570	66.08±0.036	161.71	77.11±0.030	205.39	16.69
Kebilli	20.14±0.035	29.85±0.040	48.210	39.94±0.100	98.31	54.17±0.065	168.97	35.63
Jendouba	58.44±0.035	69.39±0.055	18.737	94.33±0.051	61.41	39.26±0.096	-32.82	-58.38
Kalaa	27.12±0.110	46.18±0.020	70.280	63.16±0.049	132.89	39.78±0.026	46.68	-37.02
Kelibia	22.21±0.075	29.6±0.0350	33.270	71.68±0.075	222.74	25.65±0.015	15.49	-64.22

The measurements were performed after 7 days of germination; Means and confidence intervals (p = 0.05); *% of control; **% of 9 g/6 g L^{-1} of NaCl; The values are the means of 4 replications

Table 4: Classification of barley cultivars (Newman-Keuls method, 5%) based on peroxidase activity under different salt stress intensity

Control		3 g L^{-1} NaCl		6 g L^{-1} NaCl		9 g L^{-1} NaCl	
Cultivars	H. class	Cultivars	H. class	Cultivars	H. class	Cultivars	H. class
Jendouba (A3)	A	Jendouba (A3)	A	Tozeur (A1)	A	Tozeur (A1)	A
Tozeur (A1)	B	Kalaa (A4)	B	Kebilli (A2)	A	Kebilli (A2)	B
Kebilli (A2)	B	Tozeur (A1)	B	Jendouba (A3)	A	Jendouba (A3)	C
Kalaa (A4)	B	Kebilli (A2)	C	Kelibia (A5)	B	Kalaa (A4)	C
Kelibia (A5)	B	Kelibia (A5)	C	Kalaa (A4)	B	Kelibia (A5)	D

H. class = Homogenous class

relative increase of their peroxidase activities (Kebilli 35.63% and Tozeur 16.69%) in relation to the previous rate (6 g L^{-1} NaCl). Thus, they were classified in first and second position (Table 4). However, the Northern cultivars (Jendouba, Kalaa and Kelibia) decreased their activity and were classified in the third or fourth class. In this case, these cultivars were so stressed that their peroxidases were inactivated.

DISCUSSION

At the germination level, we noticed a germination rate reduction under salt stress for all cultivars when salt concentration was relatively high (6 and 9 g L^{-1} NaCl). When salt concentration was moderate, some cultivars showed a decrease of their germination rate (Jendouba, Kalaa and Kelibia) whereas others (Kebilli and Tozeur) showed a germination rate increase. This behavior has been pointed out by Allagui (1994) on tomato varieties and by Rachidai *et al.* (1994), Mallek-Maalej *et al.* (1998) and Ben Naceur *et al.* (2001) on wheat varieties.

Statistical analysis of data followed by Newman-Keuls test at 5% level showed no difference between T_0, T_1 and T_2 However, a significant difference between those treatments and T_3 was found. Present result confirms the great tolerance of barley cultivars to salt stress as they had the same behavior under salt conditions (3 to 6 g L^{-1} NaCl). Theses results are widely described by several authors working on cereal varieties. At 9 g L^{-1} NaCl concentration, we have recorded three different classes where Tozeur and Kebilli occupy the first class and Kelibia and Kalaa the last one.

The germination rate improvement recorded at 3 and 6 g L^{-1} levels in Kebilli and Tozeur cultivars would be owed to a need of Na-ions for the balance between the cellular compartments and suggests that those cultivars are the most tolerant to salt stress. Similar results were also found by Ben Naceur *et al.* (2004) on tomato and by Heller *et al.* (2004) on many varieties. Those authors considered the germination improvement as an indicator to salt stress tolerance.

Reduction in shoot/root ratio was related to salt sensitivity. Most tolerant cultivars kept a steady or constant shoot/root ratio varying between 0.80 and 0.90 similar to what was demonstrated by Cheikh-M'hamed (2004). The statistical analysis, for the highest salt concentration (9 g L^{-1} NaCl), classified Kebilli cultivar in the first class, Tozeur and Jendouba in the second class, Kelibia in the third class and Kalaa in the last one. This classification means that Kebilli is the most tolerant, Tozeur and Jendouba are moderately tolerant and Kalaa and Kelibia are the most sensitive but with superiority of Kelibia to Kalaa.

The accumulation of ROS as singlet oxygen, super oxide anion radicals, hydroxyl radicals and hydrogen peroxide (Bartels, 2001; Apel and Hirt, 2004) under salt stress conditions extensively oxidizes proteins, lipids and requires a higher increase of peroxidase activity to prevent those toxic substances. In our case, we have demonstrated the limit of tolerance of Jendouba, Kalaa and Kelibia cultivars under high salinity stress by comparison to Kebilli and Tozeur. In fact, under high salt concentration Notehrn cultivars became seriously affected and were classed in last classes (Table 4).

CONCLUSIONS

The South of Tunisia is characterized by harsh environmental conditions: drought, high temperature and saline irrigation water and soil. However, the north benefits of a sufficient rainfall (≥450 mm), mild temperature and non-saline soil.

According to our results, the most tolerant cultivars to salt stress are those which had high germination rate, were able to maintain an important shoot/root ratio and showed a relatively high peroxidases activity. The southern cultivars (Kebilli and Tozeur) met the requirement. However, the Northern ones (Kelibia and Kalaa) were the most sensitive. Though it was from the North, the Jendouba cultivar behaved differently from both the northern and southern cultivars. Therefore, the geographic origin could not be considered as an indicator

for salt tolerance. Despite these amounts of information, we are far from understanding the stress reaction mechanism. It's urgently to refine our understanding by characterization of individual genes and assessing their contribution to stress tolerance reaction. As it is known, stress tolerance is governed by multiple genes that are expressed and inducible under salt stress, so their identification would be very useful to future program breeding.

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