International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Evaluation of the Retarding Effect of Salt Stress on the Activity and Expression of Peroxidases in Nine Durum Wheat Cultivars (*Triticum durum* Desf.)

Mohammed Saadallah[a]*, Fatima Zohra Fyad-Lamèche[b]

[a,b]*Laboratoire de Biotechnologie des Rhizobiums et Amélioration des Plantes. Equipe de Génétique et d'Amélioration des Plantes. Département de Biologie, Faculté des sciences de la Nature et de la vie. Université Oran-1, Ahmed Benbella, BP 1524 ELM_Naouer 31000, Oran. Algerie.*

[a]*Email: saadallah.mohammed@univ-oran.dz*

Abstract

Research on stress tolerance is imperative to be able to stabilize and improve yields of a species such as durum wheat in Algeria. Several studies have shown that salt stress causes, in fact, a delay and a marked inhibition of growth and development. Furthermore, several studies have clearly shown the important role of peroxidases in plant tolerance to salt stress. In nine cultivars of wheat (*Triticum durum* Desf.), a daily peroxidase enzymatic profiles and activities from 1st to 7th day of germination in the absence of stress (0 mM NaCl), and only those of the treated groups (51,3 mM; 28,3 mM and 256,6 mM NaCl) aged of 7 days are followed, with the aim to demonstrate the delay caused by salt stress on activity and profiles of peroxidases, as well as search for possible differences between the cultivars studied. The results of this work have enabled to suggest the existence of a significant delay and a clear inhibition of the expression of peroxidase isoforms in durum wheat cultivars studied. The cultivars Belikh2 and Eider known for their tolerance to salt, present in zone with an average rate of migration, bands with significantly superior intensity, compared to other cultivars whose tolerance is not specified. Whereas, the comparison of the enzymatic activity during the application of a salt stress of a continuous way from setting seed germination, appears not to allow the assessment of delay caused.

Keywords: delay; durum wheat; PAGE; Peroxidase, salt stress

---

* Corresponding author.

E-mail address: saadallah.mohammed@univ-oran.dz.

1. Introduction

Salt stress is one of the main environmental factors limiting crop productivity. This affects the germination, growth and development of plants and the cellular metabolic processes. The effects of salinity are more important in arid and semi-arid regions, where rainfall is limited, associated with high evapotranspiration due to high temperatures and poor water and soil management, contributing to the problem of production agriculture in these regions [1]. Crop tolerance to salinity varies with species, genotypes and stages of development [2, 3].

It is now widely accepted that reactive oxygen species (ROS) induced by stress are responsible for various damage of the macromolecules and ultimately, cell structure [4, 5]. Therefore, the role of antioxidant enzymes suchas peroxidases, responsible for the scavenging ROS becomes very important [6, 7, 8].

Peroxidases are oxidoreductases involved in several physiological processes in plants whose growth, development and resistance to biotic and abiotic stresses [9]. By their specificity, peroxidases are divided into 16 classes. The role of most characterized physiologically five classes is taken into account: catalases (EC 1.11.1.6), peroxidase plants (EC 1.11.1.7), the glutathione-peroxidase (EC 1.11.1.9) or (EC 1.11.1.12) ascorbate-peroxidase (EC 1.11.1.11) and peroxiredoxins (EC 1.11.1.15) [10]. Plant peroxidases (PRX) (EC 1.11.1.7), also known as class III peroxidases, catalyze the oxidation of several substrates with phenols in the presence of hydrogen peroxide (H_2O_2), which are involved in a number of mechanisms, such as the formation connections between various cell wall polymer and the polymerization of lignin and suberin. These peroxidases play an important role in cell growth and differentiation and in plant defense against pathogens and other stress [11, 12]. PRXs are encoded by multigene families, consisting for example of 73 genes in *Arabidopsis thaliana* and 138 in rice (*Oryza sativa japonica*) [11, 12]. The work of Liu and collaborators have confirmed the existence of four genes coding for the synthesis of PRX isozymes and showed the existence of a new gene in the cultivar "*Chinese Spring*" wheat. The set of genes code for the synthesis of PRX isoenzymes are: Prx-1 gene is carried on the short arm of chromosome 1 homologue group, they have a high degree of conservation and are expressed in coleoptile tissues. The Prx-2 gene is carried on the short arm of chromosome 2 group shows some polymorphism and is expressed in root tissue. The Prx-3 gene is located on the long arm of chromosome 3 of the latter group is highly variable and is expressed in embryonic tissues. The Prx-4 gene is carried on the arm of chromosome 7AS, 4AL and 7DS, the additional variables, and is most active in the endosperm tissue. A single locus Prx-D5 is found on the arm of chromosome 2DS [13].

Understanding the mechanisms of physiological and biochemical response to salt stress is important and useful to assess the degree of tolerance of cultivars and expand the use of tolerant varieties in regions facing this problem. Several studies have shown that the salt stress actually causes a delay and growth inhibition. Only delayed germination is caused by osmotic and salt stress moderates in seeds of three cultivars of wheat (*Triticum durum* Desf.) differing in resistance to salinity and drought; while high concentrations of NaCl and PEG reduces the final germination percentage [14]. The study of the responses in terms of germination and growth of a hybrid of *Carpodrotus* at the concentrations of 0, 10, 20, and 50 % of saline water showed that the germination is inhibited is the presence of salt, but that the inhibition is lifted after transfer of seeds in fresh water. Furthermore, growth of *Carpodrotus* is lightly increased at low concentrations of salt, but it is reduced by the

high concentrations [15]. Salt stress induces both a reduction in seed germination and a delay in the initial germination process in glycolphytes and to a lesser extent in halophytes [16]. The increase in salinity does not only decrease germination, but also delays the initial germination rate [17]. Inhibition or delay in germination under saline conditions is due to an osmotic effect [18], which limits the uptake of water during seed germination [19] by obstructing membrane, or cytosolic enzymes and hormones [20].

Several studies have demonstrated that salt-tolerant species increase their antioxidant enzyme activities and antioxidant contents in response to salt stress, while salt- sensitive species fail to do the same [21]. PRX has often served as a parameter of metabolism activity during growth alterations and environmental stress conditions [22]. Treatment with salt for four days in two varieties of lettuce, led to an increase in PRX activity in the Great Lakes susceptible variety, and a decrease in the tolerant variety Kagraner Sommer tolerant variety KS, suggesting that salinity induces increased production of O^{2-} in the susceptible variety which has been counterbalanced by the increased activity of PRX. This study showed that KS (tolerant variety) seeds possessed an important mechanism of delaying initiation of the germination process in order to adapt to the saline environment; as well as, the effects of NaCl on antioxidant PRX enzyme activities in the two lettuce varieties exhibit the important PRX activity in seedlings of Great Lakes (sensitive type) at 100 mM of NaCl. However, at this concentration, the increase represented 2,21 fold compared to the control. PRX activity decreased significantly with increasing NaCl concentrations in seedlings of Kagraner Sommer (tolerant variety). This decrease was 66,72 and 75% under 50, 100 and 150 mM NaCl, respectively. The increased PRX activity in sensitive variety might enable plants to protect themselves against salt stress [23].

Our study aims to highlight the delay caused by salt stress on activity and profiles of peroxidases, as well as search for possible differences between the cultivars studied.

2. Materials and Methods

The study is focused on nine cultivars of durum wheat (*Triticum durum* Desf.) with local and introduced originally, provided by ITGC (Institut Technique des Grandes Cultures) of Oued Smar, El-Harrach and Constantine of Algiera, multiplied in the laboratory of genetic and plants improvement at the University of Oran-1 (Table 1).

2.1. Plants culture and treatments

Seeds of each wheat variety are disinfected for 1 min in 75% alcohol, and then rinsed with distilled water. The seeds are germinated in food plastic dishes containing a doubled filter paper dampened in Knop solutions with added: 0 mM; 51,3 mM; 28,3 mM and 256,6 mM NaCl, corresponding to T0 (control), T1, T2 and T3. The seeds are germinated in a growth chamber at 23 °C temperature in the dark until the 4^{th} day, and then transferred in photoperiodic 10/14h day/night. The filter paper is changed every two days.

2.2. Enzyme extraction

The peroxidases extraction is performed daily from young seedlings from the first day until the seventh day in

the control (T0) and only for the 7th day of the treated groups T1, T2 and T3. Frozen seedling (5 invidious) were ground to a fine powder with liquid nitrogen and were extracted with ice-cold 10 mM Tris-KCl (pH 6.8) and 1 mM PMSF containing 10% (w/v) sucrose (1:1 buffer volume/FW). The homogenate was centrifuged at 14 000 g for 20 min, at 4°C, and the supernatant was used for enzyme activity and the separation of PRX isoenzymes.

Table 1: Durum wheat cultivars studied and their origins.

Abbreviations	Name	Origin
MBB	Mohamed Ben Bachir	Local (selection 1931)
BLK2	Belikh2	Selection ICARDA
HDB3	Hedba3	Local (selection1921)
BD1.94	Blé dur 1.94	Origin CIMMYT, selection ITGC
OFN	Ofanto (Ouarsenis)	Italy, selection ITGC
ARD	Ardente	France, selection ITGC
POLO	Polonicum (Chougrane)	Origin INRA FR. selection ITGC 1973
HIE	Eider	Origin CIMMYT, Selection ITGC
DUL	Duilio	Italy, selection ITGC

2.3. Gel electrophoresis

For the separation of PRX isoenzymes, non-denaturating PAGE was performed on 6-9% gradient acrylamide gels at 4°C on 18 x 16 cm vertical slab gel (Hoffer, USA). Equal amounts of sample (15 µL) were loaded on to each lane. The gels were then equilibrated with 100 ml 0,2 M acetate buffer pH 4,6 and the bands visualized in the same buffer containing 1% O-dianizidine (Sigma), dissolved in 3 ml ethanol, and 200 µL of H_2O_2 at 30%. Scanned Isozymes profiles gels were analyzed by Software GELANALYZER (Istvan Lazar Hungary Copyright, 2010).

2.4. Enzyme activity

The activity of PRX is carried by the method of Shannon and his colleagues [24] cited by Kumar and his colleagues [25] modified, the PRX activity (EC.1.11.1.7) is measured by monitoring the change in absorbance at 460 nm through to oxidation of O-dianizidine in the presence of H_2O_2 and enzymatic extracts using a spectrophotometer (UK- JENWAY 7305). The reaction mixture 2,8 ml of O-dianizidine buffer consists of 16 ml of 0,5% O-dianizidine, 48 ml of sodium acetate pH 4,6 and 10 µl of the enzyme extract. The reaction is initiated by adding 0,1 ml of H_2O_2. The amount of enzyme is calculated in the basis of the molar extinction coefficient used for the O-dianizidine at 460 nm which is 30 mM^{-1}. cm^{-1}.

2.5. Statistical analysis

Each experiment was repeated at least three times. For the antioxidant enzymes activities, Statistical analysis was performed using one way analysis of variance (ANOVA) with the software STATISTICA Version 10.0

(STATISTICA Inc., France). $P \leq 0.05$ was considered as significant. Data were subjected to Duncan's multiple range tests Error bars are in figures, mean ± SD.

3. Results and Interpretation

Profiles PRX isoenzymes are followed during the first seven days in seedlings germinated in the absence of stress (T0), and only the seventh day in seedlings in the presence of the three treatments (T1, T2 and T3).

The observations of different profiles of PRX reveal the existence of twelve (12) bands in total for all cultivars. All cultivars studied show a similar pattern of both PRX isoenzyme in the presence and absence of stress; only small differences are observed between the profiles of the cultivars studied, particularly after applying stress. To facilitate the study, gels were divided into three zones of different mobility: a first zone (a) contains the low mobility bands, a second zone (b) contains the bands having an average rate of migration, and a third zone (c) contains the fastest bands (Figure 1).

In the zone (a), three bands (a1, a2 and a3) were observed with low migration speed. Bands a1 (Rf =0,08) and a2 (Rf =0,11) appear with a very low intensity from the 1st and 2nd day of germination in the absence of stress, and continue with mild increase in intensity up the 7th day. The band a3 (Rf =0,20) appears only from the 3rd day. The intensity of the three bands in this zone gradually increases from the third day to the 6th day, whereas a slight decrease in intensity is observed at the 7th day in the absence of stress.

The three bands in this zone (a) were observed in the treated groups, but with a significant decrease in intensity especially in the T2 and T3 which are similar to those of the early days of the control. After seven days of germination, the profile of the extract of seedlings treated with T1, presents the band with a similar observed in the 6-day-old control activity. As for the profile of the extract of seedlings treated with T2 present similar to that observed in the 4-day-old control profile. Seedlings treated with T3 have a greater intensity of these bands relative to that seen in elderly control two days.

In the Zone (b), five bands (b4, b5, b6, b7 and b8) were observed, with an average rate of migration. The band b4 (Rf=0,48) appears with low intensity only in aged controls from 2 and 3 days of germination. The band b5 (Rf=0,55) appears in aged controls of 2, 3 and 4 days of germination with low intensity. The bands b6 and b8 (Rf=0.63 and Rf=0.69) respectively appear constitutively at the 1st day of germination, while the band b7 (Rf=0,67) appears also constitutively from the 2nd day.

The zone (b) seems to be the one that presents the most significant difference between the control and treated groups. No qualitative difference on all the profiles of the treatments groups is recorded in this zone (b), while a significant quantitative increase in activity is observed through the high intensity of the bands in this zone. The cultivars BLK2 and HIE known for their tolerance to salt, present in this zone, bands with significantly superior intensity, compared to other cultivars whose tolerance is not specified.

The four bands b5, b6, b7 and b8 are observed in the treated groups T1 and T2 with a significant increase in their intensity, their profiles is qualitatively similar to the 3rd and 4th day controls, but with a much higher band

intensity. The group treated by T3 presents profiles rather similar to the 2nd control day, but with a greater intensity also particularly for b6 band.

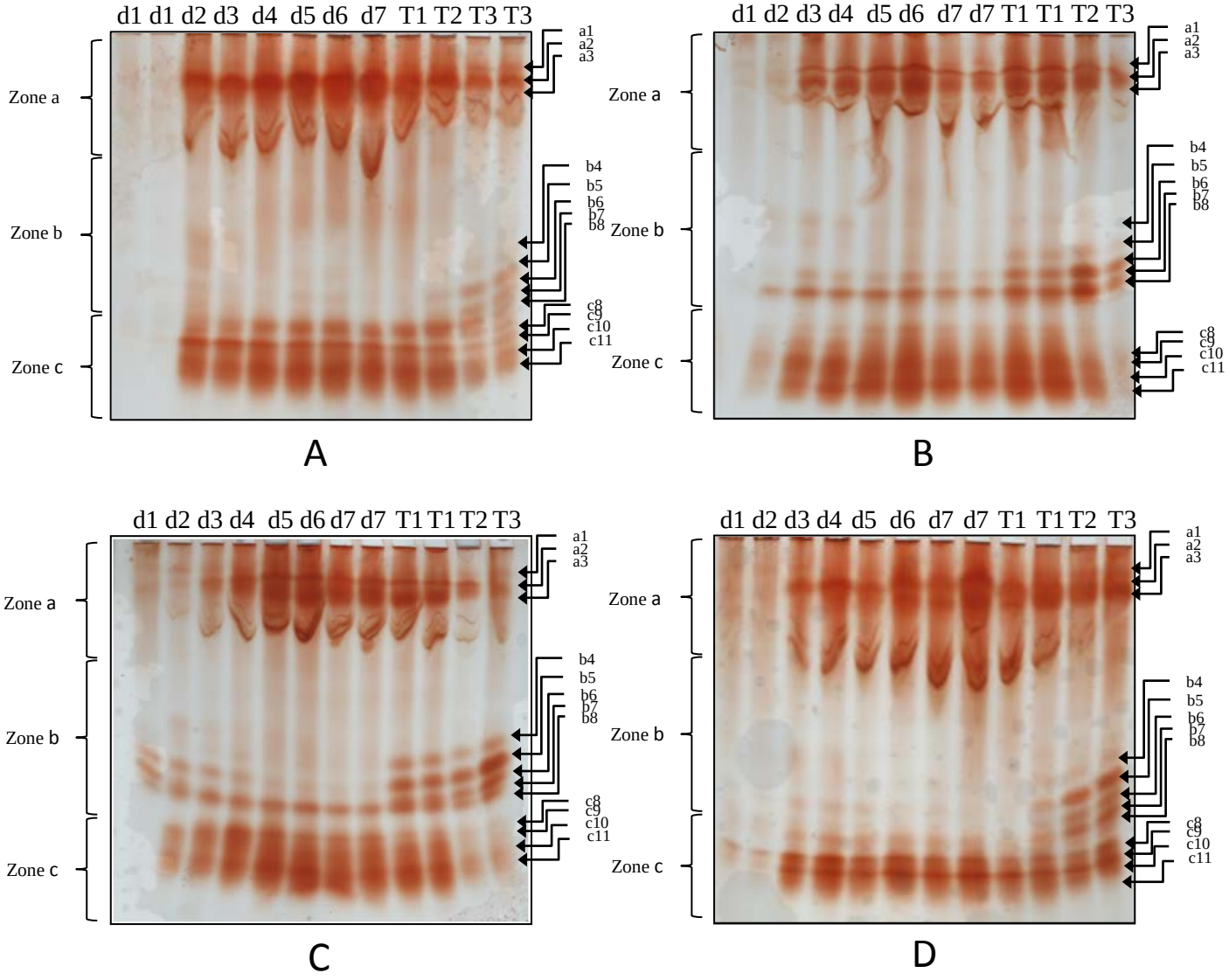

Figure 1: Profiles of peroxidase isozymes of controls and treated seedlings in the cultivars: A: BLK2, B: HIE, C: HDB3, D: MBB. The extracts d1, d2, d3, d4, d5, d6 and d7 corresponding to older control seedlings of 1, 2, 3, 4, 5, 6 and 7 days of germination; The extracts T1, T2 and T3 corresponding of seedlings treated by T1, T2 and T3, respectively.

In the zone (c), four bands (c9, c10, c11 and c12) with a rapid rate of migration were observed. The bands c9 and c10 (Rf = 0.77 and Rf = 0.80, respectively) appears in all extracts controls diced the 3rd day of germination with medium intensity. The c11 and c12 bands (Rf=0.84 and Rf = 0.88, respectively) appear on the 2nd day of germination with medium intensity and increases diced the 3rd day.

The four bands of this zone c9, c10, c11 and c12 appears in treated groups T1 and T2. The band c10 seems to

disappear in the treated group T3, while the three other bands seem reduced their intensities. The profile of the extracts from plants treated with T1 present after 7 days of germination the four bands of this zone with an activity similar to that observed in the control aged 6 days of germination. The profile of the treated group by T2 has a similar intensity to that of the old seedlings of 3 days, while the profile of the extract of the seedling treated by T3 has an activity similar to that observed in the control aged 2 days of germination.

The results of the enzymatic activities of daily PRX in control groups from 1st to7thday of germination, and those of the treated groups aged of 7 days of germination are summarized for all varieties studied in (Table2) and (Figure 2). The PRX activity was followed during the first seven days of germination of control plants, in nine durum wheat cultivars studied. This study shows that in a general way, this activity involves three main phases with some differences between cultivars: an initial phase of low enzyme activity, generally exponentially, goes from 2 to 4 days depending on the cultivar. A second phase, with a stationary trend, variant 4th to 6th day; then a third phase of increase in PRX activity starting in the 6th and7th day.

Table 2: Results of one way analysis of variance on the activity of PRX

during the seven days of Control and in treated seedlings.

Cultivars	F	P
ARD	1,543899	0,199912 ^{ns}
BD1.94	**2,419371**	**0,047929***
BLK2	**6,969721**	**0,000157***
DUL	**2,487946**	**0,042990***
HDB3	2,335371	0,054808 ^{ns}
HIE	**2,515153**	**0,041182***
MBB	0,766165	0,648047 ^{ns}
OFN	2,072058	0,083913 ^{ns}
POL	1,835194	0,123756 ^{ns}

Number represents F-valus at 5% level. ^{ns} Non-significant, * significant at P<0,05.

The cultivars BLK2 and DUL have the weakest PRX activities in the first days during Phase I. whereas the cultivars MBB and HDB3 show the most important PRX activities during this phase. The second stationary phase is clearly observed in all varieties, as well as the third phase of increased PRX activity, generally from the 6th day.

The results of the analysis of variance did not revealed significant differences than among four cultivars on the whole (BD1.94, BLK2, DUL and HIE). Variable responses were observed between the cultivars studied in the presence of low stress intensity (T1). The cultivars ARD, DUL, HDB3 and POL have higher PRX activities than those within seven days of the control of each cultivar respectively. This increase in PRX activity appears to be an exuberant response to this low stress intensity. The cultivars BD1.94 and BLK2 have an intermediate levels PRX activity between the 6th and 7th days of the control, marking delayed by one day; while the cultivars HIE,

MBB and OFN show a levels PRX activity between those of the 5th and 6thdaysof control, thus a marking delay of about two days.

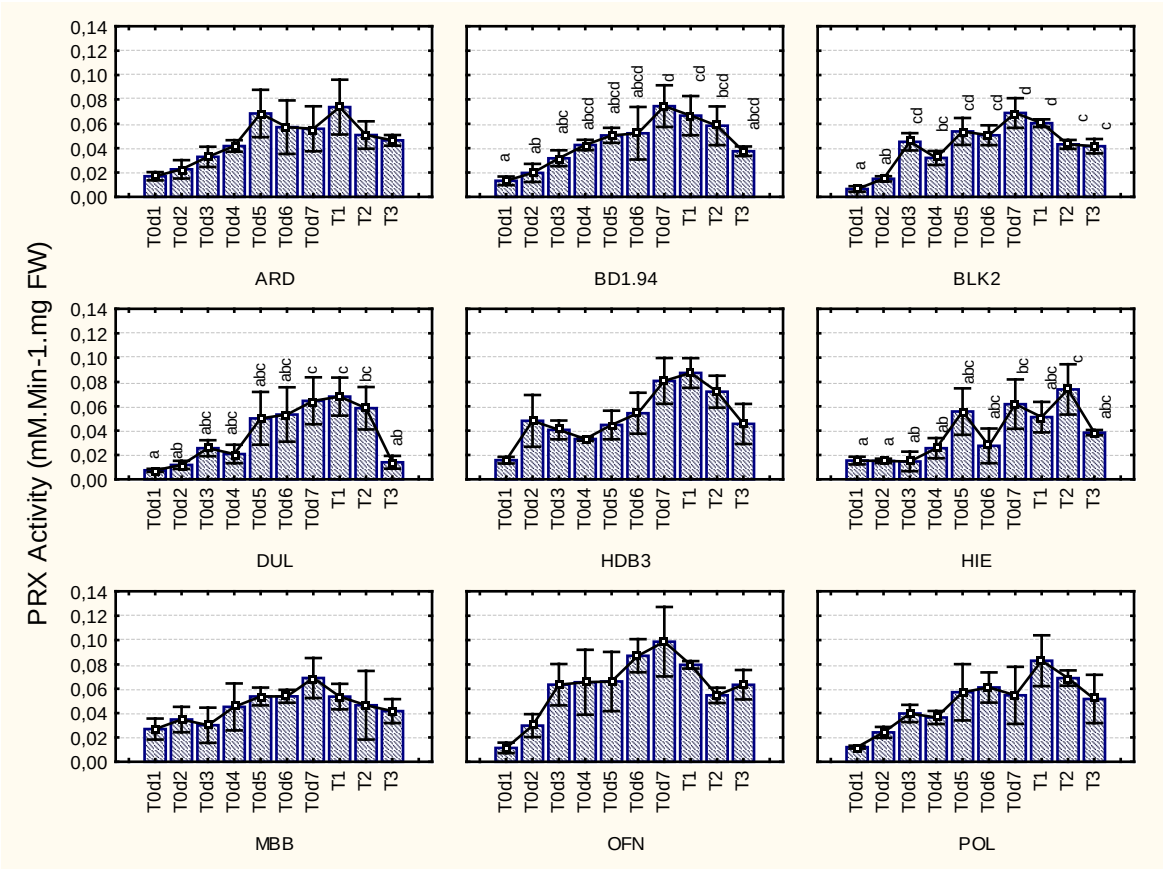

Figure 2: Effects of salt stress on PRX activity in seedlings of wheat cultivars: ARD, BD1.94, HDB3, HIE, MBB, OFN and POL. The extracts T0d1, T0d2, T0d3, T0d4, T0d5, T0d6 and T0d7 corresponding to older control seedlings of 1, 2, 3, 4, 5, 6 and 7 days of germination; The extracts T1, T2 and T3 corresponding of seedlings treated by T1, T2 and T3, respectively. Each value represents the mean of three replications and vertical bars indicate ±SE. The vertical bars of the different letters are significantly different from each other at $p \leq 0.05$ according to Duncan's Multiple Range test.

Variations of the PRX activity, between the cultivars studied, were also observed after the application of a stress at medium intensity (T2). With the exception for the cultivar HIE which exhibits activity superior to all PRX controls days, all the other cultivars showed a decrease of PRX activity compared to maximum activities recorded in controls of each cultivar studied respectively (mostly the 7th day control); consequently, HIE cultivar seems to tolerate this medium intensity of salt stress (T2) because it does not manifest delayed synthesis PRX activity.

The five cultivars BD1.94, DUL, HDB3, MBB and POL present an intermediate PRX activity between the 6th and 7th dayof control for each cultivar respectively, either a delay of one day. The cultivars ARD and BLK2 present an activity between that of the 4th and 5th day of control, indicating a more important delay of 2 to 3 days. The cultivar OFN has a low enzyme activity during this stress (T2) comprised between that of the 2nd and 3rd day of control, marking thus a more important delay of 4 to 5 days. A greater reduction of peroxidase activity is detected after the application of high stress intensity T3 (mM NaCl) in all studied cultivars of durum wheat. The cultivars ARD BLK2, HIE and POL show, after applying this T3 stress, PRX activity between those observed through the 4th and the 5th day of the respective controls, either a delay of 2 to 3 days. The cultivar BD1.94 present PRX activity between that of the 3rd and 4th day of control thus marking a delay of about 4 days; While cultivars HDB3, MBB and OFN show activities intermediary between the 2nd and 3rd day of their controls, so a delay of 5 days.

4. Discussion

Salinity is one of the most common abiotic stresses in plants; it causes major damage in agricultural productivity. A common response to salinity is the inhibition of germination and growth of plants; this inhibition is one of the most important agricultural indices of salt stress tolerance shown by various studies [26]. The PRX widely distributed in higher plants where they are involved in various processes, including lignification, metabolism auxin, salt tolerance and stress of heavy metals [27], often served as a parameter the metabolic activity in the growth alterations and environmental stress conditions.

In most of the work aimed to study the activities and isoenzyme profiles only consider the comparison between seedlings with the same age in two conditions of different salinity. But it received, that the application of salt stress causes, clearly on the morphological level, inhibition and delayed growth.

The examination of the patterns of peroxidases, obtained by their follow in control seedlings for 7 days post-germination growth in nine cultivars of durum wheat can show clearly that there is no qualitative change in the appearance or disappearance of peroxidase isoforms, compared to plants treated with salt. The only visible change is that of a clear delay in the expression of peroxidase isoenzymes in stressed seedlings. This according what is reported by [27], that the PRX isoforms are known to be expressed in various plant tissues and their expression patterns varied depending on the condition and nature thereof, more that are regulated by the stage of development and the influence of environmental factors.

Furthermore, this work has shown that it is not possible to demonstrate a direct relationship between daily enzymatic activities of control and treated groups with the corresponding electrophoretic profiles, as these clearly show that isoenzymes increase their intensities in the zone (b) after the application of stress, particularly the stress T3, while the intensity of the isoenzymes in the zone (a) and (c) decrease. Therefore, the comparison of the enzymatic activity of peroxidases between the treatment and control groups did not evaluate the degree of the delay and tolerance to salt stress under conditions where stress is applied continuously from the setting seed

germination.

Plants exposed to high stress are known to up regulate their overall peroxidase activity. This reaction also occurs with various biotic and abiotic stresses. The Comparison of the situations described by [27] shows that the regulation of peroxidases is usually transient and peroxidases are often strongly induced at the start of an event and then slowly decreases with time. This type of reaction has been indeed shown in chemical stress, where the expression of peroxidase was significantly increased only in high stress [28]. This work has also shown that there is no difference in the profiles of the control plants and treated among the nine cultivars durum wheat studied because durum wheat is known to be genetically poor.

5. Conclusion

The results reported in this study suggest that the expression of peroxidases is strongly affected by the action of NaCl in durum wheat. Furthermore, a significant delay and a clear inhibition of the expression of isoforms peroxidases is observed in nine wheat cultivars studied. Therefore, this study suggest that a proper evaluation of the delay caused by salt could set the degree of tolerance to stress, and a good understanding of physiological, biochemical and genetics mechanisms of this tolerance. As well as expand the use of tolerant varieties in regions facing this problem. Whereas the comparison of the enzymatic activity at the application of stress in a continuous way from setting seed germination, appears not to allow nor the evaluation of delay, neither the degree of tolerance, since the level of expression of isoforms peroxidases varied within the same plant. And consequently does not reflect the global level in the same plant.

In the future, it is necessary search to identify and evaluate the delay peroxidases expression at the molecular level by quantifying mRNA either by real-time PCR or microarrays.

Acknowledgements

The authors wish to acknowledge Dr. A. BENBELKACEM Director INRAA Algeria, Biotechnology and Plant Breeding Division, for providing plant material; and Dr. O. KHAROUBI, Senior lecturer of University of Oran1 AHMED Benbella for such aid.

References

[1] A.D. Azevedo Neto, J.T. Prico, J. Eneas-Filho, C.E. Braga de Abreu and E. Gomes-Filho. “Effect of salt stres on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes”. *Environ. Exp. Bot*., 56: 87–94. 2006.

[2] F.E. Prado, C. Boero, M. Gallardo, J.A. Gonzalez. “Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* willd seeds.” *Bot. Bull. Acad. Sin*. 41:27-34. 2000.

[3] D.S. Pujari, S.V. Chanda. “Effect of salinity stress on growth, peroxidase and IAA oxidase activities in vigna seedlings.” *Acta Physiol. Plant*. 24(4): 435-439. 2002.

[4] L. Fridovich. “Biological effects of superoxide radical.” *Arch. Biochem. Biophys*. 247 (1): 1–11. 1986.

[5] J.A. Imlay. S. Linn, “DNA damage and oxygen radical toxicity.” *Science*, 240 : 1302–1309. 1988.

[6] C. Bowler, M. Van Montagu, D. Inze.. “Superoxide dismutasc and stress tolerance.” *Ann. Rev. Plant Physiol. Plant Mol. Biol*., 43: 83–116. 1992

[7] M. Menconi, C.L.M. Sgherri, C. Pinzino, F. Navari-Izzo. “Activated oxygen production and detoxification in wheat plants subjected to a water deficit programme.” *J. Exp. Bot*., 46: 1123–1130. 1995.

[8] J. Essemine, S. Ammar, S. Bouzid, “Impact of Heat Stress on Germination and Growth in Higher Plants: Physiological, Biochemical and Molecular Repercussions and Mechanisms of Defence.” *Journal of Biological Sciences*, 10 (6): 565-572. 2010.

[9] A. Khales, M. Baaziz. “Peroxydases d’Opuntia ficus indica L. Activités catalytiques et propriétés biochimique. ” presented at Congrès International de Biochimie, Marrakech, Maroc. 3-6 Mai 2004.

[10] I .V. Maksimov, E.A. Cherepanova, G.F. Burkhanova, A.V. Sorokan and O.I. Kuzmina. “Structural-Functional Feature of Plant Isoperoxidases.” *Biochemistry (Moscow)*. 76(6): 609-621. 2011.

[11] M. Tognolli, C. Penel, H. Greppin, P. Smon. “Analysis and expression of the class III peroxidase large gene family in *Arabidopsis thaliana*.” *Gene*, 288 : 129-138. 2001.

[12] C. Penel, L. Valerio, F. Passardi, J. Dobias, M. Perez, C. Dunand. “Aspects moléculaires, Biochimiques et physiologiques des peroxydases des plantes.” presented at Congrès International de Biochimie, Marrakech, Maroc. 3-6 Mai 2004.

[13] C.J. Liu, S. Chao, M.D. Gale, “The genetical control of tissue-specific peroxidases, Per-1, Per-2, Per-3, Per-4, and Per-5 in wheat.” *Theor Appl Genet*. 79: 305-313. 1990.

[14] M. Almansouri, J. M. Kinet, S. Lutts. “Effet of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.)”. *Plant and Soil*, 231: 243-254. 2001.

[15] E. Weber, C. M. D’Antonio, “Germination and growth responses of hybridizing *carpobrotus* species (Aizoaceae) from coastal California to soil salinity.” *American Journal of Botany*, 86(9): 1257–1263. 1999.

[16] A. El-Keblawy. “Salinity effects on seed germination of the common desert range grass, *Panicum turgidum*.” *Seed Sci Technol*. 32:943-948. 2004.

[17] A.S. Hajar, M. A. Zidan, H. A. Sahruni, “Effect of NaCl stress on germination, growth activities of black cumim (*Nigella sativa* L.).” *Arab Gulf. J. Scient. Res*. 14: 445-454. 1996.

[18] D.R. Gossett, E. P. Milhollon, M. C. Lucas,. “Antioxidant response to NaCl stress in salt-tolerant and salt-

sensitive cultivar of cotton." *Crop Sci*. 34: 706-714. 1994

[19] T.J. Flowers, S. A. Flower, S. A. Greenway. "Salt Effect of Sodium chloride on Tobacco plants." *Plant Cell. Environ*. 32: 645-51. 1986.

[20] W.L. Huang, F.L. Liu. "Carbohydtrate metabolism in rice during callus induction and shoot regeneration induced by osmotic stress." *Bot. Bull Acad. Sin*. 43: 107-113. 2002.

[21] A. Shalata, M. Tal. "The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*." *Physiologea Plantarum*, 104: 169-174. 1998.

[22] S. Gao, R. Yan, M. Cao, W. Yang, S. Wang, F. Chen. "Effects of copper on growth, antioxidant enzymes and phenylalanine ammonialyase activities in *Jatropha curcas* L. seedling." *Plant Soil Environ*. 54: 117-122. 2008.

[23] H. Mahmoudi, N. Nasri, I. Tarchoune, H. Zargouni I. Ben salah, MR. Ben, O. Baatour, NAM. Ben, A. Hannoufa, L. Mokhtar, O. Zeineb. "Salt stress induced changes in germination, lipid peroxidation and antioxidant activities in lettuce seedlings." *Afr. J. Biotechnol*., 10(65): 14498-14506. 2011.

[24] L.M. Shannon, E. Key, J. Lew. "Peroxidase isoenzymes rom horseradish roots. 1. Isolation and physical properties." *J. Biol. Chem*. 241: 2166–2175. 1966.

[25] N. Kumar, D.V.N. Sudheer Pamidimarri, K. Meenakshi, B. Girish, P. Muppala. Reddy. "Effects of NaCl on growth, ion accumulation, protein, proline contents and antioxidant enzymes activity in callus cultures of *Jatropha curcas*." *Biologia*. 63(3), 378-382. 2008.

[26] A.K. Parida, A.B Das. "Salt tolerance and salinity effect on plants: a review." *Ecotoxicol. Environ. Saf.* 60: 324–349. 2005.

[27] F. Passardi,·C. Cosio,·C. Penel,·C. Dunand." Peroxidases have more functions than a Swiss army knife" *Plant Cell Rep*., 24: 255–265. 2005.

[28] G. Klumpp,C.M. Furlan, M. Domingos, A. Klumpp. "Response of stress indicators and growth parameters of *Tibouchina pulchra* Cogn. exposed to air and soil pollution near the industrial complex of Cubatao," *Brazil. Sci Total Environ.* 246:79–91. 2000.