LEAVES PEROXIDASE AND ESTERASE ISOZYMES IN SUNFLOWER CROPS EXPOSED TO SALINE ENVIRONMENT

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Water deficit stress is one of the main problems determining the yield of many crop plants. The study of the effects of salinity on plant growth and development and the search for biochemical stress markers are of great importance in the selection of resistant species. Accumulation and antioxidant activities in response to salinity at different sunflower varieties constitute markers for genetic selection and improvement of plants in the face of tolerance to salinity. Electrophoretic analysis of zymograms of different sunflower varieties grown in a saline environment for 40 days revealed zymograms of peroxidases and esterases specific to varieties tolerant to the saline environment. Peroxidases activity extracted from leaves is augmented in relation to their involvement in the physiological process related to salinity tolerance. In susceptible varieties, peroxidases activity is decreased compared to normal conditions. Variability of activity and polymorphism of isozyme peroxidases and esterases in response to salinity at different sunflower varieties constitute markers for genetic selection and plant improvement in the face of salinity tolerance.

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INTRODUCTION

Salinization is one of the most important dangers for agriculture on a global scale. 23 % of 63 million irrigated arable hectares are affected by salt. With salinization and repeated drought due to climate change in the world increases poverty and results in the loss of livelihoods for many farmers.

The increase in the productivity of salt-affected land can be achieved through the use of good hauling crops associated with water management conditions, the use of appropriate fertilizers, taking into account the climate and the region concerned. The concept of developing salt-tolerant plants, even to the degree that they can be grown with seawater, captures the imagination of both the scientific and the public sector.

Shannon has defined salinity tolerance as the ability of plants to increase and reach their life cycle on a substrate that contains high concentrations of soluble salts.[1] In this habitat, the plant must meet two requirements, osmotic adaptation and acquisition of the mineral elements it needs for the growth and functioning of metabolism.

Shannon and Grieve have described plant tolerance to salinity as an ability of plants to withstand influences from high salt levels in the parts of the organs, roots and leaves without adverse consequences.[2] The plant can adapt to saline stress in different ways, excluding selective transport can absorb useful nutrient ions and re-expel Na ions.[3] The effect of water stress results in the appearance of oxidative stress, the increase of reactive oxygen species that cause degradation to cellular organizations. These oxygen drift derivatives are the superoxide free radicals (O_2), the perhydroxide radical (HO_2), the hydroxyl radical (OH), the radical peroxide (RO_2) and the radical alkyl (RO), as well as non-radical forms such as hydrogen peroxide (H_2O_2). The development of oxygenated free radicals following the stress effect of water deficit plays a central role in the senescence of the leaves.[4]

The activities of biological molecules are affected by the development of reactive forms of oxygen and leading to cell degeneration.[5] Plants adopt antioxidant systems made up of enzymes and metabolites to counter the ROS.[4] Various authors have revealed the role of ROS in signalling mechanism responsible for the expression of many defence genes (chaperone proteins, heat shock proteins, antioxidant enzymes, ascorbate peroxidase (APX), gluthation-S-transferase).[6,7] The integrity of photosynthetic activity is maintained through the action of antioxidant molecules during the water stress processes.[5] During water or saline stress, the inhibition of photosynthesis, and more precisely the leakage of electrons due to the decrease in CO_2 fixation, results in a strong accumulation of ROS.[8,9] Numerous studies show that enzymes such as superoxides dismutases (SOD), peroxydases ascorbates (APX), catalases (CAT), glutathion-Stransferases (GST) and glutathion peroxydases (GPX) accumulate in response to water stress.[5] The expression of catalases is induced by H_2O_2, that of superoxides dismutases and glutathiones reduced by various stresses including ABA, ethylene and drought.[10]

Sunflowers are part of the genus Helianthus, rich in 50 species. In particular, the species *Helianthus annuus* is present in a wide range of environments and latitudes. It may have genes to adapt to contrasting climatic conditions.[11] The present work compares the zymograms of leaves peroxidases, esterases, and activity of peroxidases extracted from hybrid sunflower leaves and population in the anti oxidative defence during ageing and during saline stress of cultivated sunflower. This work provides the genetic resources needed for the breeder to create new varieties of saline-tolerant sunflower.

Table 1. List of sunflower varieties.

Variety	ALBENA	DK3790	RECORD	ORO9
Origin	France	France	INRA Morocco	INRA Morocco
Characteristic	Hybrid	Hybrid	Population	Population
Behaviour	Moderate	salinity tolerance	Sensitive	Sensitive

EXPERIMENTAL

Plant materiel

Four varieties of sunflower provided from France and local Moroccans are selected based on agronomic characteristics, and their behaviour in the face of a saline environment presented in Table 1.

Sowing sunflower varieties

After rinsing with water with added sodium hypochlorite, sunflower seeds are germinated in separate trays containing soil and sand from the region of Rabat and maintained in a growth for a period of 40 days. Control seedlings are irrigated with tap water, and those conditioned in the saline environment are irrigated with 60 mM and 120 mM of NaCl solution. The bins are placed in places sheltered from the rain and exposed to the sun.

Extraction of crude protein fraction from sunflower leaves

0.1 g of leaves of the different control varieties and grown in a 60 mM and 120 mM NaCl environment are taken and rinsed with tap water with added NaOCl. After grunding with mortar in a buffer with pH 8.6, containing cys 0,1 mM, pvp 1% at 4°C, homogenates were filtered and centrifuged at 3500 pm for 10 min, cellular debris are eliminated and the crude fractions of total proteins contained in the supernatants are conserved at -20°C.

Analytical electrophoresis

Analytical electrophoresis was carried out in a fraction of total protein. The fraction was subjected to electrophoresis (12 % acrylamide) to separate proteins.[12] A zymogram of peroxidase activity were localised on the gels by incubating at 25 °C for 5 min in solution buffer containing acetate pH = 5 buffer and benzidine 0.05 g (acetone) made to 100 mL with distilled water. After incubation 1 % H_2O_2 (1 mL) was added for the appearance of tapes. Esterase's activity were localised on the gels by incubating them at 25 °C for 15 min in solution of buffer tris HCl pH = 7.2, containing α-naphtyl acetate 0.03g (acetone 50 %), β-naphthyl acetate and made up to 100 mL with distilled water. After rinsing with distilled water, the gels were treated for 20 min with fast blue salt at pH = 7.2 tris buffer solution salt and diluted with 100 mL of distilled water.

Assay of peroxidase activity

Peroxidase activity was determined by the described method by using guaicol as a substrate. The reaction was started by the addition of 50 µl of crude extract in 2 mL of the acetate 0.1M buffer containing 6 mM of Guaicol. The reaction was initiated by the addition of 1 mmoles solution of H_2O_2 and monitored as increase of absorbance at 470 nm.

RESULTS AND DISCUSSION

Analysis of zymogram of peroxidase

As seen in figure 1, electrophoretic analysis of peroxidase isozymes extracted from control sunflower leaves reveals peroxidase zymograms composed of 4 peroxidases pox1, pox2, pox3 and pox4 of varying color intensity.

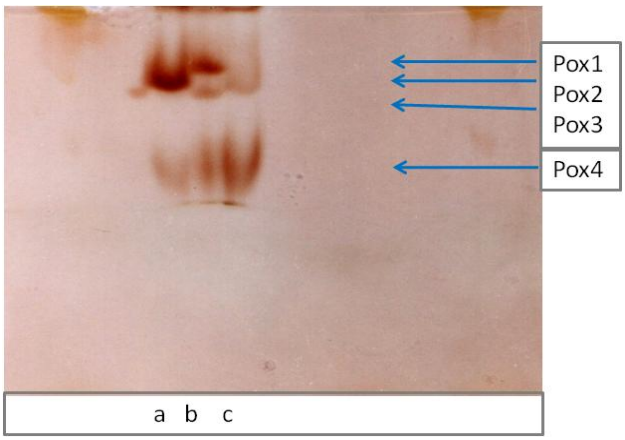

Figure 1. Zymogram of peroxydases extracted from sunflower leaves of controls varieties albena (a), DK 3790 (b) and Record (c).

The Albena variety has 2 high-intensity pox2 and pox4 isozymes, the isozymes pox1 and pox3 are absent. The variety DK 3790 presents 3 high-intensity pox1, pox3 and pox4 isozymes, isozyme pox2 is absent. The variety Record presents 2 low-intensity pox2 and pox4 isozymes, the isozymes pox1 and pox3 are absent. Isozyme pox4 is common to these three varieties. The isozymes pox1 and pox3 are specific to the DK 3790 variety. Isozymes pox2 and pox4 are common to both Albena and Record varieties. Electrophoretic analysis of peroxidase isozymes reveals a zymogram composed of Pox 1 bands under control conditions, with increasing color intensity under the conditions of NaCl 60 mM and 120 mM at the variety albena.

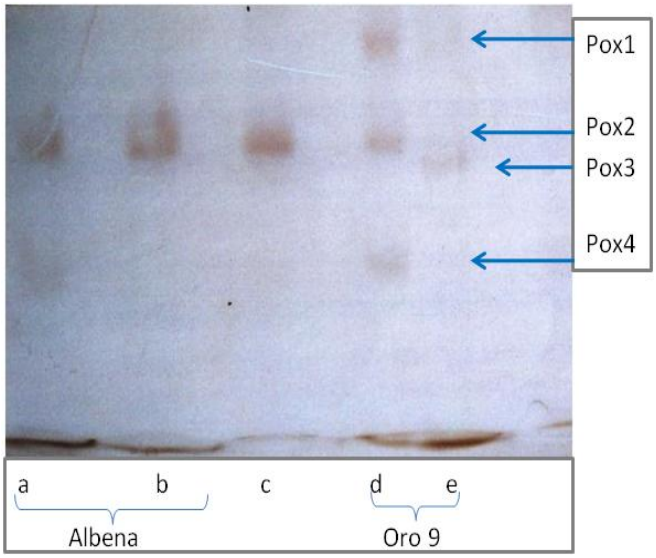

Figure 2. Zymogram of peroxidases extracted from sunflower leaves of varieties albena (sowing period November, january), Oro9 (sowing period december january) cultivated under environment of NaCl 60 mM and 120 mM.

In the variety Oro 9, 3 peroxidases Pox 1, Pox 2 and pox 4 are revealed under control conditions. Under NaCl 60 mM conditions a Pox3 peroxidase is revealed of different electrophoretic migration to that of control conditions Pox 1, pox 2 and Pox 4 are absent.

The variability of the peroxidases zymogram reveals various expressions of specific isozymes of tolerant varieties and varieties sensitive to salinity. Isozymes pox1 and pox3 may be related to the response to salinity tolerance in the DK3790 variety. This hybrid variety has a higher salinity tolerance than the Record population variety.[13] In saline environment conditions, the expression of isozymes is revealed in sunflower varieties in relation to their involvement in the physiological process related to salinity tolerance. Peroxidases have been detected in all organs of the plant and at different stages of development. [14,15] In the cultivars varieties of safflower, four guaiacol peroxidases were identified in the leaves of tolerant varieties and two in saline stress sensitive varieties.[16]

Peroxidases may be involved in changes in the plant wall by incorporating other compounds such as flavonoids, hydroxycoumarines, according to a physiological process or in response to stress.[17,18] Ascorbate peroxidases has a higher affinity for H_2O_2 and reduces it to H_2O in chloroplasts, cytosol, mitochondria and peroxisomes as well as in the aplastic space.[19] Peroxidases are involved in the polymerization of the precursors of lignin. Lignin is a polymer responsible for rendering the plant stronger and more rigid and making the cell walls hydrophobic. [20]

Analysis of activity of peroxidase

Activity of Peroxidases extracted from the sunflower leaves are 15.2 and 0.96 EU Kg^{-1} of fresh weight respectively in the controlled sunflower leaves Record and DK 3790 varieties (Table 2). Under the 60 mM environmental conditions peroxidase activity is 2.2 EU g^{-1} fresh weight of the variety DK 3790. Peroxidases activity is 12.4 and 3.2 respectively in the Record and DK 3790 varieties in the NaCl 120 mM environment. Peroxidase activity is increased a factor (F) of 3.33 in the DK 3790 variety grown in the NaCl 120 mM environment compared to control conditions. In the Record variety, activity is reduced by a factor (F) of 1.22 compared to the normal environment. The DK3790 variety reveals a higher anti-oxidative action compared to the Record variety caused by the effect of the saline environment. Salt stress has an effect on plant metabolism depending on the genotype and the intensity of the salt stress. The activity of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX) in the corn variety 'Lluteño' was significantly greater than in 'Jubilee' under the effect of saline stresses.[21] The work of Hiraga S et al[22] has revealed the variability of amino acids sequences of plant isozymes associated with their variable expression in various physiological processes. The change in the structure of the cell wall in transgenic potato seeds grown under saline stress conditions is affected by the increase in peroxidase activity.[23]

Salicylic acid treatment enhances peroxidase activity in the leaves of the cassava variety tolerant to this environment.[24]

Analysis of zymogram of esterases

Electrophoretic analysis of esterases isozymes (Figure 3) reveals the zymogram composed of the bands est1 of high intensity, est2 and est3 of low intensity in the albena variety, 4 isozymes est1 est2 of high intensity and 2 isozymes of low intensity est3 and est5 in the variety DK 3790, 3 isozymes est1 est2 of high intensity, est4 of high intensity in the variety Record. Isozyme est1 is common to albena, Dk3790 and record varieties. Isozyme est2 is of higher intensity in the Record variety compared to the albena and Dk3790 varieties. Isozyme est3 is specific to the varieties albena and Dk 3790. Isozyme is4 is specific to the record variety. Isozyme is5 is specific to the DK3790 variety.

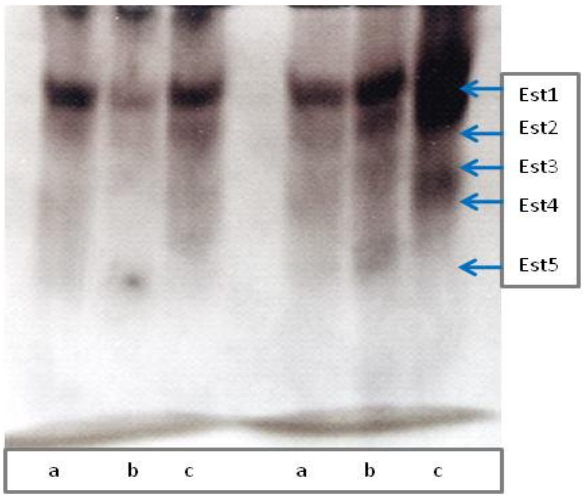

Figure 3. Zymogram of esterases extracted from controlled sunflower leaves of varieties albena (a), DK 3790 (b) and Record (c).

Table 2. Variation in activity (EU g^{-1} of fresh weight) of peroxidases extracted from the leaves of the sunflower varieties DK3790 and Record grown under normal conditions and those of the salty environment NaCl 60 Mm and 120 Mm.

Variety	Sowing period	Stress duration	Growth duration	Witness	60 Mm	120 mM
Record	June-July	33 days	50 days	15.2	-	12.4
DK3790	March-April	35 days	50 days	0.96	2.2	3.2

Electrophoretic analysis of esterases isozymes extracted from the control sheets of the DK 3790 variety reveals the zymogram composed of 2 esterases est1, est3. In the environmental conditions 60 mM, 3 isozymes est1, est 2 and est3 are expressed. The intensity of revelation est2 is increased. Under stress conditions 120 mM, 3 isozymes est1, est2 and est3 are expressed; the est2 is of increased intensity of revelation. The variability of esterous polymorphism reveals a correlation between isozyme expression and increased salinity in the environment. In the DK 3790 variety tolerant to the soil, in the saline environment conditions 60 mM and 120 mM, 3 esterases are of higher intensity of revelation compared to control conditions. The expression of est2 esterase is increased and is related to the response to salinity in the variety DK3790. Other work by Dasgupta et al.[25] has revealed the expression of specific esterases of mangroves conditioned to the saline environment. Nine different esterous isoenzymes were detected in seed embryos sprouted in 105 mM NaCl, while only five of them were detected in untreated seed embryos.[26] Amouri et al. showed quantitative and qualitative variations in esterase isoenzymes between two varieties, tolerant and sensitive of medicago during the germ process and during saline stress.[27]

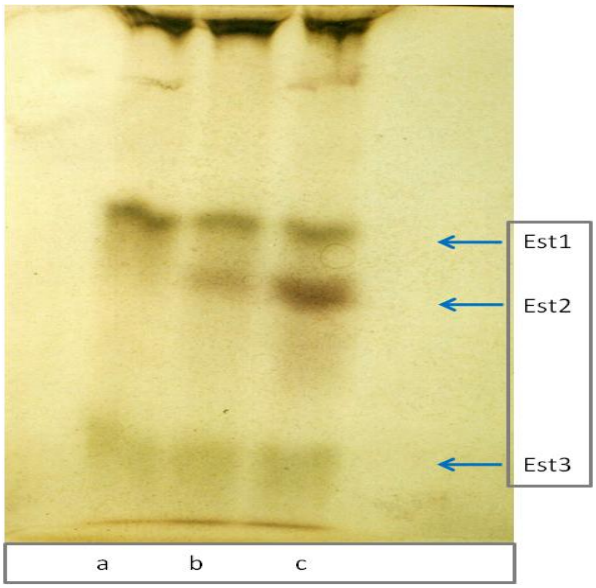

Figure 4. Zymogram of esterase extracted from sunflower leaves of varieties DK 3790 cultivated under normal and salin stress. a – absence of NaCl b –NaCl 60 mM c-NaCl 120 mM.

CONCLUSION

This work reveals the expression of specific peroxidase and esterase isozymes in the leaves of sunflower varieties tolerant to the saline environment. The activity of peroxidases extracted from the leaves is increased in the salinity-tolerant variety. The number of esterous isozymes is higher in sunflower varieties that are tolerant to the saline environment compared to susceptible varieties. The enzymatic activity of peroxidases could be biochemical markers of the adaptation of sunflowers in the conditions of the saline environment. Further work on the study of purification and isolated peroxidases will identify tissue and cell location and correlation with precise structure and function.

Further research is needed to better identify the factors of the conditions culture factors, regional variation and seasonal temperatures enzyme purification.

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