ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/315799819

Enhanced Tolerance to Water Deficit By Overexpressing of The Enzyme Superoxide Dismutase (Sod) in Some Algerian Populations of Peanut (Arachis Hypogaea L.)

Article · January 2017

CITATIONS
0

READS
148

2 authors:

Sihem Tellah
Ecole Nationale Supèrieure Agronomique ENSA Algèrie
27 PUBLICATIONS **136** CITATIONS
SEE PROFILE

Sidi Mohamed Ounane
Ecole Nationale Supérieure Agronomique
105 PUBLICATIONS **413** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Résidus de pesticides et évaluation du risque View project

Amélioration des légumineuses alimentaires en Algérie View project

All content following this page was uploaded by Sidi Mohamed Ounane on 23 July 2017.

The user has requested enhancement of the downloaded file.

International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-7, Issue-4, 2016, pp1765-1772
http://www.bipublication.com

Research Article

Enhanced Tolerance to Water Deficit By Overexpressing of The Enzyme Superoxide Dismutase (Sod) in Some Algerian Populations of Peanut (*Arachis Hypogaea* L.)

Tellah Sihem[1 *], Latati Mourad[1], Benselama Amel[2], Nabi Fahima[1], Laribi Abdelkader[1], Kaci Ghiles[1], Mebdoua Samira[3], Ounane Ghania[1], and Ounane Sidi Mohamed[1]

[1]Ecole Nationale Supérieure Agronomique,
Département de « Productions végétales »,
Laboratoire "Amélioration Intégrative Des Productions Végétales" (*AIPV*),
Hassan Badi 16200 El Harrach, Alger, Algérie.
[2]Université d'Oran, Es-Senia, Faculté des Sciences de la Nature et de la Vie,
Département de Biotechnoogie, Laboratoire de biotechnologie Des Rhizobiums
Et Amélioration Des Plantes, BP 1524 ELM_Naouer 31000 oran, Algérie.
[3]Université Akli Mohand Oulhadj, Bouira, Département De Sciences Agronomiques.
Rue DRISSI Yahia - Bouira 10000 - Algérie.
*Corresponding Author: E-mail: sihemtellah@yahoo.fr, Tel: 00213554042153

ABSTRACT

Water stress is known to induce active oxygen species in plants. The accumulation of these harmful species must be prevented by plants as rapidly as possible to maintain growth and productivity. This study was carried out for understanding of antioxidant mechanisms of peanut under water stress conditions. For this aim, after six weeks of growth (47 DAS), four peanut (*Arachis hypogaea* L.) landraces: EL FRIN (FR), TONGA (TO), OUED SOUF (OS) and SEBSEB (SEB) were subjected to drought stress for 25 days. Plants were submitted to water stress by withholding water supply and the attention was given to the expression analysis of the superoxide dismutase (SOD) gene, in view of its central role for antioxidant defence in all aerobic organisms and of its involvement in antioxidant responses to water shortage. PCR conditions for SOD antioxidant enzyme were optimized. Then, total RNA was isolated from stressed and non-stressed plant shoots. The gene expression levels SOD were examined by semiquantitative reverse transcription polymerase chain reaction (RT-PCR) technique. Results show how water stress regulates the synthesis and the activity of superoxide dismutase and how these enzymes contribute to protect against the damageable effects of superoxide radicals in peanut. Relative expression levels of SOD increased after 25 days of drough treatment in shoots of Tonga (TO) landrace. On the other hand, expression levels of SOD decreased on SEBSEB (SEB) landrace after 25 days of water stress in shoot tissues. Although in the presence of an equal sample loading, still the results reveal ample "constitutive" differences in SOD gene expression among the four peanut landraces. Indeed, SOD transcripts appeared to be most abundant in TO and least abundant in SEB. Further work will reveal whether contrasting SOD expression maybe associated to varying responsiveness to water stress in the peanut landraces at study.

Keywords: Peanut, landraces, drought stress, antioxidant defence, SOD, gene expression.

INTRODUCTION

Plants respond to water deficit and adapt to drought conditions by various physiological changes including transition in gene expression during water deficit. The mechanisms of drought response have been investigated most extensively in a model plant, Arabidopsis (18) and (19). The most feared and widespread plant stress agents are active oxygen species. These include redox intermediates in the reduction and oxidation between dioxygen and water; superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$), and the

electronically- excited oxygen species, singlet excited oxygen ($^1O_2$). These species are able to react with DNA, lipids, proteins, and almost any other constituent of plant or animal cells (5,9) These reduced oxygen species are not only generated as by-products of endogenous biological reactions, but also their formation increases during biotic and abiotic stresses. Superoxide dismutase (SOD, EC 1.15.1.1) is a key enzyme which constitutes the first line of defence against oxygen toxicity and catalyses the dismutation of the superoxide anions to dioxygen and hydrogen peroxide (10, 11). The three known types of this enzyme can be distinguished according to their metal cofactor made of manganese (MnSOD), iron (FeSOD) or copper and zinc (Cu/Zn SOD) (4)**,** and according to their behaviour towards specific inhibitors (7). The identification of the three types of isozymes is based on the differential inhibition of SOD activity on polyacrylamide gels preincubated with KCN or H_2O_2 (7). H_2O_2 generated from the activity of SOD is scavenged by catalase in peroxisomes and by ascorbate peroxidase in Halliwell- Asada cycle (10, 14). Identification of resistance mechanisms to water stress is often difficult because resistance to drought stress is a quantitative trait. Induction of antioxidative enzymes can be assumed to reflect a general strategy required to overcome increased oxidative stress induced by water stress. In this paper, we point out superoxide dismutase (SOD) in some peanut populations, and we study it activity during water stress induction in order to understand the level of implication of this enzyme in the physiology of resistance to water stress. We presented isolation, characterization and expression analysis of genes coding for superoxide dismutase in Algerian peanut (*Arachis hypogaea* L.) landraces under water stress conditions.

MATERIAL & METHODS

2.1. Plant material

Peanut (*Arachis hypogaea* L.) is a leguminous crop that is widely cultivated all over the world, especially in the semi-arid tropical regions. This species presents a good resistance to drought (20) but a large number of varieties and cultivars have been created that are adapted to varied local environmental conditions. In this study, experiments were performed on 04 peanut Algerian landraces obtained from a prospection conducted by the Ecole Nationale Supérieure Agronomique, Algiers, Algeria, during the years 2003-2005. Based on the locations in which they were collected, the peanut landraces were assigned the following names: Tonga (TO), Oum Tboul (OT), El Frin (FR) and SebSeb (SEB). Botanical and agronomic features of peanut landraces, as well as their geographical and climatic distribution within the Algerian territory, are reported in (2, 15). To avoid confusion with the acronyms used for plant parameters, the full names of the peanut landraces are used in the text thereafter, whereas their acronyms are used in figures.

2.2. Seeds inoculation

Two days before sowing, healthy seeds of uniform size were selected for each peanut landrace. Selected seeds were first soaked into an aqueous solution of 40% (w/v) arabic gum and then evenly coated with peat inoculated with the rhizobial strain BRE 1.2 which was previously isolated from nodules of the same peanut populations (6). A trapping test conducted before seeds inoculation allowed to conclude that the soil mix used for plant growth did not contain any non specific rhizobia able to infect the experimental plant material.

2.3. Plants' growth conditions

Four seeds for each landraces were sown in each of six 6 L-pots, each filled with 4 kg of a 50/20/30 (v/v/v) agricultural soil/peat/washed quartz sand mixture. The soil physico-chemical characteristics are reported in (12). Peat medium properties were (%, w/w dry weight): organic C 52%, organic N 0.8 %, organic matter 90%, pH $_{H2O}$ 7, 3. Tthe particle size of quartz sand (Progettinelblu.com) was 0.5 mm. After the emergence (5 days after sowing, DAS), two seedlings of each landraces per pot was used for the experiment. Planted pots were randomly distributed within a walk-in climatic chamber, where an air temperature of 26 ± 1°C, a relative air humidity of 70 ± 5 %, and a photoperiod of 12 h were set. Photosynthetic photons flux density at plants' height was 350 µmol m-2 s-1

(LI-190SA quantum sensor, Li-Cor, Lincoln, NE). Since both agricultural soil and peat medium were reputed to contain adequate levels of N, P and organic matter, no further fertilizer was supplied during the plants' growth. Planted pots were given 200 mL of tap water every four days because preliminary trials indicated that such amount and frequency of water supply was required to compensate water losses by evapotranspiration.

2.4. Imposition of water stress

After six weeks of growth (47 DAS), plant material was divided into two halves, each consisting of 3 pots for each peanut landrace. One half pots continued to receive adequate water supply (see above) and acted as the well-watered control (W-plants), whereas irrigation was withheld on the other half of pots, thus imposing a progressive water stress to plants (S-plants). Keeping in mind the results of (2), who observed that nodulation, plant height and dry matter production of Algerian peanut landraces were affected after 15 days of irrigation withdrawal, water deprivation on the present S-plants lasted for 25 days. During water stress treatment, S- and W-plants were kept together in the same controlled environment where they were previously grown (see above).

2.5. Isolation, characterization and expression analysis of genes coding for superoxide dismutase in Algerian peanut (*Arachis hypogaea* L.) landraces under water stress conditions

Attention was given to the expression analysis of the superoxide dismutase (SOD) gene, in view of its central role for antioxidant defence in all aerobic organisms and of its involvement in antioxidant responses to water shortage (3,13) . The expected complexity of the SOD gene family in *Arachis* spp was further exacerbated by finding that scanty previous work was carried out so far on the SOD genomic organisation in such species, except in seeds, which are of course the economically valued part of the plant.

2.6. Searching databases of complete sequences or EST (*Expressed Sequence Tags*) coding for the enzyme SOD in Peanut (*Arachis hypogaea* L.) The first part of the work was focused on identification of sequences coding for SOD in databanks. Such database search included SOD-codifying sequences not only in peanut, but also in cowpea and in the model species *Arabidopsis*, in order to evaluate interspecific similarities and differences in the genomic organisation of the SOD gene. It was conducted by performing a research using the BLAST program (1) in different protein databases (GenBank, PDB, SwissProt, PIR and PRF), nucleotide (EMBL, Genbank, DDBJ and PDB) and EST (GenBank, EMBL and DDBJ) using EMB-BLAST (European Molecular Biology network - Swiss node -).

2.7. RNA extraction and cDNA synthesis

Leaves were rapidly cut off, immediately frozen in liquid nitrogen and stored at - 80°C until used. For RNA extraction, leaf samples were ground in liquid nitrogen with a pestle and mortar. From the W-25 leaves of the four peanut landraces selected for gene expression experiments, total RNA was extracted using the CTAB method (16, 8). The resulting RNA was treated with Rnase-free DNase I (Promega) according to the manufacturer's protocol. Following digestion, nucleotides were removed from RNA using a G50 sepharose buffer exchange column (Amersham). Absence of genomic DNA contamination in DNase I-treated samples was checked by PCR of 0.125 µg of RNA template using a primer pair (5'-GGTTCCCTGAGATCACAACT-3' and 5'-CCAAACGTTCCCATCTATGAGG- 3') designed to amplify an intron sequence of a gene encoding SOD. When a single DNase treatment did not completely remove interfering genomic DNA, a second DNase incubation was performed to eliminate any detectable DNA. RNA concentration and integrity were checked with a UV/VIS spectrophotometer Lambda 3B (Perkin Elmer) before and after DNase I digestion. Only RNA samples with 260/280 wavelength ratio between 1.9 and 2.1 and 260/230 wavelength ratio greater than 2.0 before and after DNase I digestion were used for cDNA synthesis. The quality of RNA samples was also assessed by electrophoresis on 1% formaldehyde agarose gels and stained with ethidium bromide. First-strand cDNA was synthesized from 3 µg of total RNA by Expand™ Reverse Transcriptase

(RT) (Roche) and RACE (Rapid Amplification of cDNA Ends) for obtaining cDNA clones spanning the entire coding sequence (full-length) of SOD and diluted 1:5 before use in PCR assays.

2.8. Amplification by PCR using specific primers designed on database sequences

A search in the BLAST program found 13 sequences of *peanut* encoding SOD displaying a high degree of homology among each other. The primers used for PCR analysis (forward: 5'-GGTTCCCTGAGATCACAACT-3' and reverse: 5'-CCAAACGTTCCCATCTATGAGG- 3') wich were designed in conserved regions of the 13 transcripts. First-strand cDNA was synthesized as described above and the PCR reactions were performed by the TripleMaster PCR system (Eppendorf) using 1 µl of the RT reaction. After initial denaturation at 94°C for 2 min, amplification conditions were 35 cycles each at 94°C for 30 s, 60°C for 1 min and 72°C for 3 min, followed by a final extension step at 72°C for 7 min. Samples of the amplification products (3 µl) were collected after 28, 32 and 35 PCR cycles and analysed by electrophoresis on 2% agarose gel. Each PCR experiment was independently repeated twice to test amplification reproducibility. The specificity of the amplicons was checked by sequencing of the PCR products in order to confirm that its sequence corresponded to the target gene.

2.9. Cloning of the amplification products

The differential amplification products were inserted by ligase in the modified EcoRV restriction site of the plasmid vector pGEM-T (Promega) and sequenced. Plasmid purification was performed using the Quiagen "Mini Plasmid Prep Kit".

2.10. DNA sequencing

The nucleotide sequence was determined on one strand of denatured plasmid DNA using the dideoxy Chain-termination method (17) with the Oncor sequencing kit, using the electrophoretic system of Kodak; the other strand was sequenced by ESGS (Paris, France).

In addition, the F4 primer was designed within the SOD-coding region in **[Figure-2]**, in order to capture differences among the components of the SOD gene family and among the two genomes present in *Arachis*. Such differences are in fact expected to reside mostly in the 3' region, beyond the stop codon and before the polyA tail, which are transcribed but not translated. The F4 primer allowed to apply the RACE methodology to the 3' region, to obtain complete transcripts until the polyA tail in **[Figure-2]**.

RESULTS

As a result, 13 transcripts (complete or partial) coding for SOD were identified in peanut sequences databases, displaying a high degree of homology among each other in **[Figure-1]**. Aligning the sequences shown in **[Figure-1]** allowed to identify highly conserved regions, against which oligonucleotidic primer pairs (forward and reverse) were designed in [**Figure-2]**. In particular, the primers F1 and R1 allowed to obtain the entire SOD transcript, expected to encompass in length about 500 base pairs (bp).

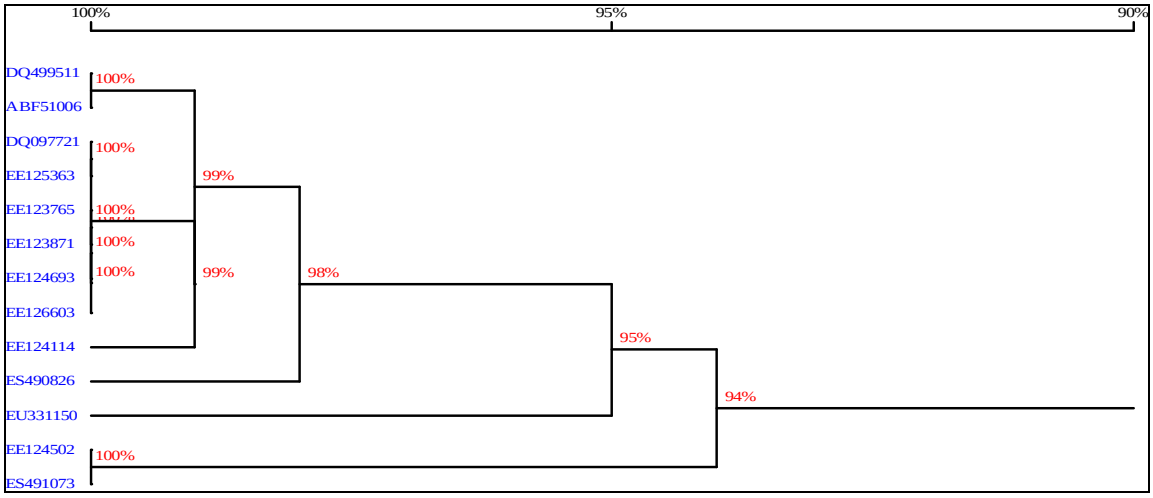

Fig: 1. Dendrogram of the 13 SOD-coding sequences identified in *Arachis* spp by data base search.

The lower panel in **[Figure-3]** shows quality control and equal loading of total RNA extracted from the W-25 leaves of the four peanut landraces selected for gene expression experiments. To isolate the SOD gene(s), an aliquot of such total RNA was reverse-transcribed and PCR-amplified by using the F1-R1 primer pairs shown in **[Figure-2]**.

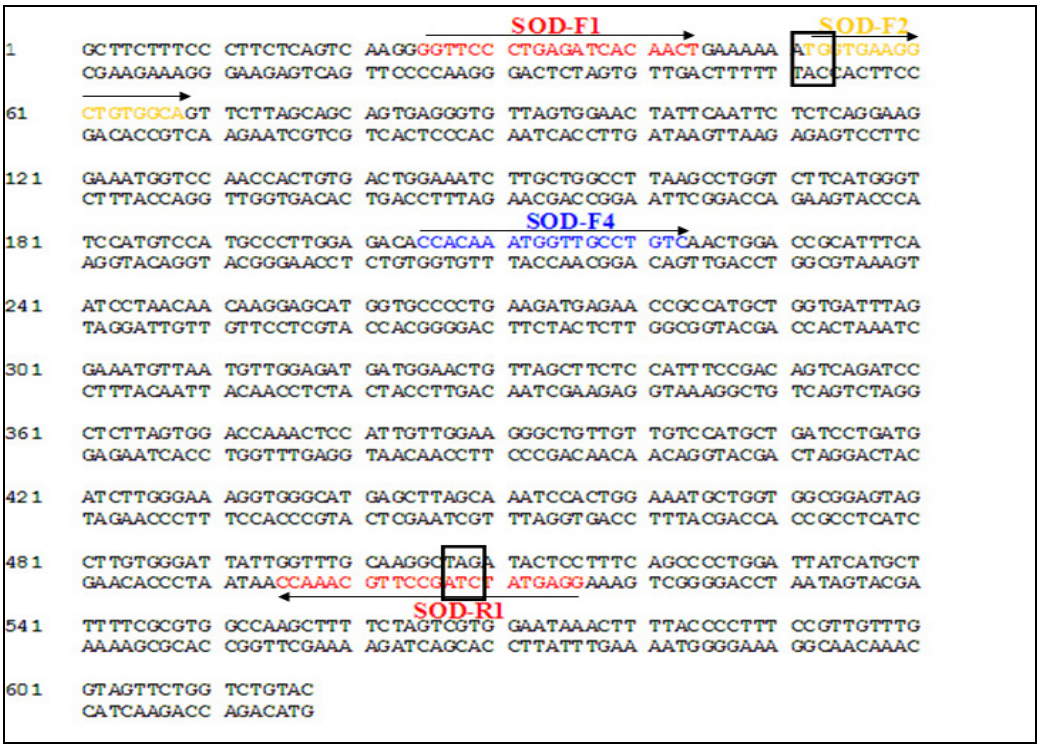

Fig: 2. Sequence alignment and oligonucleotidic primers designed for delimiting highly conserved regions within SOD-coding DNA sequences

Electrophoretic separation of amplification products gave the banding pattern shown in the upper panel in **[Figure-3]**. Although they are at the very beginning of the work leading to SOD gene characterisation and expression analysis in the peanut landraces at study, the results reported in **[Figure-3]** (upper panel) might deserve some comments.

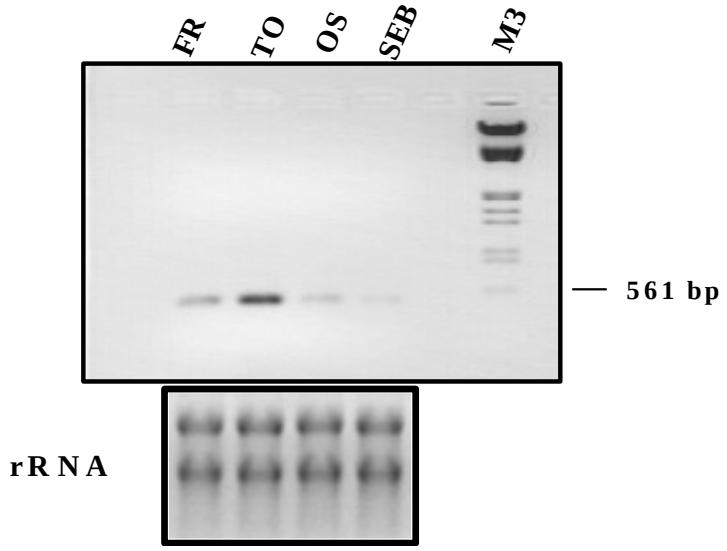

Fig: 3. Lower panel: gel electrophoregram showing quality control and equal loading of total RNA (3 µg for each lane) extracted from the leaves of W-25 plants belonging to four peanut landraces (for landraces acronyms, see the text). Upper panel: gel electrophoregram of cDNA fragments coding for superoxide dismutase obtained after the reverse-transcription of total RNAs (2 µL each) and their PCR-amplification (35 cycles) by using the F1-R1 primers pair shown in Fig. 5. M3, DNA marker.

DISCUSSION

Firstly, it is noteworthy that, i) although not intended to put into evidence differences among SOD transcripts abundance, because of the high number of PCR amplification cycles, ii) although carried out on well watered plants, thus not taking into account any effect of water stress, and iii) although in the presence of an equal sample loading, still the results in **[Figure-3]** reveal ample "constitutive" differences in SOD gene expression among the four peanut landraces. Interestingly enough, SOD expression in the four landraces seemed to be roughly in accordance with the ranking of their overall tolerance to water stress, put into evidence by PCA. Indeed, SOD transcripts appeared to be most abundant in TO and least abundant in SEB in **[Figure-3]**. Further work will reveal whether contrasting SOD expression maybe associated to varying responsiveness to water stress in the peanut landraces at study. Secondly, it should be emphasised that each of the cDNA amplification products shown in **[Figure-3]** represents the global expression of all the SOD genes present in the peanut genome, with no possible discrimination at the genomic, transcriptional and translational levels (see above). Cloning of individual SOD-coding sequences isolated by means of the RACE approach will be helpful in deciphering the genomic architecture of SOD in Arachis, in revealing which components of its gene family are possibly involved in the response to water stress and in evaluating whether differences exist in SOD expression levels among the peanut landraces. In such context, and as an example of the work to be carried out, in **[Figure-4]**, upper panel, shows the alignment among a SOD-coding sequence obtained from data banks in **[Figure-1]** and the sequences obtained from two of the cloned SOD fragments from the landrace TO. As expected, a high overall homology between the three sequences was found, although differences emerged at the level of single bases. As single bases substitutions may lead to mere cases of triplet synonymy, or instead may give raise to translational changes into the aminoacidic sequence, the three nucleotidic sequences in **[Figure-4]** were

Fig: 4. Upper panel: aligning two cloned SOD-coding sequences isolated from the peanut landrace Tonga with a database peanut sequence; boxes denote start and stop translation codons. Arrows and red letters denote single base substitutions. Lower panel: alignment of the in silico translation products of the nucleotidic sequences above. Symbols as above.

translated in silico and then aligned again in **[Figure-4]** lower panel). This revealed that indeed single bases substitutions had consequences in terms of protein secondary structure, causing substitutions in aminoacids. It remains to be seen whether such changes may have in turn consequences in terms of tertiary structure and of catalytic properties of the SOD enzyme.

CONCLUSION

The results pertaining to the effect of drought stress on SOD indicated how water stress regulates the synthesis and the activity of superoxide dismutase and how these enzymes contribute to protect against the damageable effects of superoxide radicals in peanut. Relative expression levels of SOD increased after 25 days of drough treatment in shoots of Tonga (TO) landrace. On the other hand, expression levels of SOD decreased on SEBSEB (SEB) landrace after 25 days of water stress in shoot tissues. Although in the presence of an equal sample loading, still the results reveal ample "constitutive" differences in SOD gene expression among the four peanut landraces. Indeed, SOD transcripts appeared to be most abundant in TO and least abundant in SEB. Further work will reveal whether contrasting SOD expression maybe associated to varying responsiveness to water stress in the peanut landraces at study. In conlusion, learning from biodiversity with an equal loading of total RNA and saturating amplification conditions (35 PCR cycles) but still strong differences in SOD transcripts abundance among WELL-WATERED peanut landraces.So that interesting lessons expected from constitutive and stress-inducible antioxidant status in food legume landraces. Although it is nearly impossible to understand the whole antioxidant mechanism of plants under environmental stresses, this study was a step to learn about molecular background of some antioxidant enzymes. Gene expression profiles of CAT, chloroplast/mitochondrial GR and chloroplast/stromal APX will be the next step of this study. By this way, the comparison of gene expression profiles of different antioxidant enzymes with each other and also with enzyme activities will improve our knowledge of molecular protection mechanisms in peanut against drought stress.

FINANCIAL DISCLOSURE

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Altschul S.F., Madden T.L., Schaffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucl. Acids Res.*, 25, 3389-3402.
2. Amri-Tilioune W.(2008). Etude de la symbiose à rhizobium chez l'arachide (*Arachis hypogaea* L.): aspects microbiologiques, physiologiques et biochimiques et agronomiques. *Magister Thesis*, Institut Nationale Agronomique-El-Harrach, Algeria.
3. Apel K., Hirt H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, 55, 373–399.
4. Bannister J.V., Bannister W.H., Rotilio G. (1987). Aspects of the structure, function and applications of superoxide disinutase. *CRC Crit Rev Biochem* 22 111-180.
5. Beauchamp C., Fridovich I. (1971). Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Analytical Biochemistry.*, 44, 276-287.
6. Benmohamed A., Ounane S.M., Ghalmi N., Djebara-Lehamel M., Benamara A. (2004). Etude de la diversité génétique de l'arachide local (*Arachis hypogea* L*). The 2nd Mediterranean Conferene on Rhizobiology* (Oran, Algeria, 23-25 May 2004).
7. Bowler C., Van Montagu M., Inze´ D. (1992). Superoxide dismutase and stress tolerance. *Annu Rev Plant Physiol Mol Biol* 43: 83–116.
8. Ciaffi M., Paolacci A.R., Dominici L., Tanzarella O.A., Porceddu E. (2001). Molecular characterization of gene sequences coding for protein disulfide isomerase (PDI) in durum wheat (*Triticum turgidum* ssp *durum*). *Gene*, 265:147-156.

9. Deming B., Bjorkman O. (1987). Photon yield of O_2 evolution and chlorophyll fluorescence at 77k among vascular plants of diverse origins. *Planta* 170: 489-504.
10. Elstner E.F. (1982). Oxygen Activation and Oxygen Toxicity. *Annual Review of Plant Physiology*. Vol. 33: 73-96.
11. Foyer C.H., Noctor G., Lelandais M., Lescure J.C., Valadier M.H., Boutin J.P., Horton P. (1994). Short-term effects of nitrate, nitrite and ammonium assimilation on photosynthesis, carbon partitioning and protein phosphorylation in maize. *Planta* 192, 211–220.
12. Gelsomino A., Panuccio M.R., Sorgonà A., Abenavoli M.R., Badiani M. (2012). Effects of Carbon Sequestration Methods on Soil Respiration and Root Systems in Microcosm Experiments and In Vitro Studies. In: *Carbon sequestration in agricultural soils. A multidisciplinary approach to innovative methods (Piccolo A., ed.). Springer-Verlag*, Berlin, Heidelberg, Germany, pp. 261-290.
13. Gill S.S., Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.*, 48, 909-930.
14. Halliwell B., Gutteridge J.M.C. (1989). Free Radicals in Biology and Medicine, Ed 2. *Clarendon Press, Oxford.*
15. Lazali M. (2009). Etude de la symbiose à rhizobium chez l'arachide (*Arachis hypogaea* L.) cultivée sous contrainte hydrique: aspects morpho-physiologiques et agronomiques. *Magister Thesis*, Institut Nationale Agronomique-El-Harrach, Algeria.
16. Rogers S.O., Bendich A.J. (1991). Extraction of DNA from plant tissues.In: Gelvin S.A., Schilperoort R.A (eds) *Plant Molecular Biology Manual*, pp. A6/1–A6/10. Kluwer Academic Publishers, Dordrecht, Netherlands.
17. Sanger F.S., Nicklen S., Coulson A.R. (1977). DNA sequencing with chain termination inhibitors. *Proc Natl Acad Sci USA* 74: 5463–5467.
18. Shinozaki K., Yamaguchi-Shinozaki K. (1997). Gene expression and signal transduction in water-stress response. *Plant Physiol.*, 115: 327- 334.
19. Shinozaki K., Yamaguchi-Shinozaki K. (1999). Molecular responses to drought stress. In Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants (Shinozaki K., Yamaguchi-Shinozaki K., eds). *Austin, TX: RG Landes*, pp. 11±28.
20. Singh S.R., Rachie K.O. (1985). Cowpea Research, Production and Utilization. *John Wiley and Sons Ltd. UK.*

View publication stats