Plant Archives Vol. 19 No. 2, 2019 pp. 2158-2162 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF COPPER-SALINITY INTERACTION ON PROLINE AND SOLUBLE SUGARS CONTENTS IN RADISH (*RAPHANUS SATIVUS* L.)

Nour Elhouda Neggaz[1*] and Houcine Abdelhakim Reguieg Yssaad[2]

[1*]Department of Biology, Abdelhamid Ibn Badis University, Mostaganem 27000, Algeria.
[2]Laboratory of Biodiversity and Conservation of Waters and Soils, Mostaganem 27000, Algeria

Abstract

Soil degradation by salinity and heavy metal pollution is a serious environmental problem. This work consists to determine the action of Copper and NaCl on *Raphanus sativus* L.; a suitable plant for eco-toxicological investigations. Stress is applied for two weeks after 45 days of Radish culture. The parameters analyzed; proline and soluble sugars are measured by a UV-Visible Spectrophotometer JENWAY6505. The results obtained reveal an increase in proline and sugars as a function of NaCl and copper-NaCl interaction increase, particularly in the aerial system and a decrease in these as Copper increases. The highest levels of proline (0.143Mg/g DM to 80 Meq/L) and sugars (2.73 Mg/g DM in the control) are recorded at the arial part of Radish. From these results, it appears that salinity improves tolerance to metallic stress in the aerial part of Radish by increasing proline and sugars contents, which shows that salinity promotes phytoextraction and transport of copper to these aerial parts.

Key words: *Raphanus sativus*, Copper, NaCl, Proline, Sugars.

Introduction

The presence of heavy metals in saline environments is an ever more important problem due to the increasing pollution of saline soils by metallic elements. The highest salinity levels are found in arid and semi-arid regions. Agricultural land is polluted by heavy metals through the irrational use of fertilizers and other plant protection methods (Valentina *et al.,* 2010). Copper is a potentially phytotoxic trace element in high concentration. It's mainly manifested through rhizotoxicity and sometimes by Fe deficiency induction (Kabata-Pendias and Pendias, 1992; Marschner, 1995).

The majority of plant species accumulate some organic solutes (sugars, alcohol, proline) in response to stress. These organic solutes are called osmoprotectants because they don't interfere with enzymatic activities even at high concentrations (Chen *et al.,* 2007; Tavakkoli *et al.,* 2012). Proline plays multiple roles in plant tissues exposed to abiotic stresses such as nutritional reserve for growth, protein and membrane stabilization, osmoprotection and free radical scavenging (Zouari *et al.,* 2016). Soluble sugars are indicators of stress levels, because of its significant increase during severity; metabolic sugars allow resisting different stresses (Zerrad *et al.,* 2006). They also appear to play an important role in maintaining turgidity pressure which is the basis of the different processes controlling a plant's life (Hare *et al.,* 1998).

The plant material used in this study is Radish (*Raphanus sativus* L.) from the Brassicaceae family, the plant was chosen for its use in the laboratory as a model plant for eco-toxicology studies of different pollutants (Sun *et al.,* 2010), as well as for its better germination rate, rapid growth and high biomass (Bitour, 2012).

The objective of this work is to study the behaviour of Radish under various stresses; metallic (Copper), saline (NaCl) and the interaction between both stresses, by estimation of osmoregulatory contents; proline and soluble sugars and determination of the action of salinity on Radish response to copper stress.

Materials and Methods

Plant material and culture method

Radish culture was carried out in a controlled greenhouse at the Mostaganem University Experimental

---

****Author for correspondence :*** E-mail : nourelhouda.neg@gmail.com

Farm during winter 2017. The seeds of *Raphanus sativus* L. of the National variety are disinfected with 2% sodium hypochlorite for 10 min and then washed several times in distilled water. Then, they are germinated in alveoli containing compost for 15 days before being transplanted into cylinders of 30 cm high and 20 cm in diameter for 45 days, these cylinders contain a mixture of sand and compost with proportions of (2V/V). Watering was done 3 times a week, twice with distilled water and once with a nutrient solution of Hoagland (1938). After 60 days of culture in the alveoli and cylinders, Radish plants are harvested and transported to the laboratory to be cut into two aerial and root parts, then dried at 80°C for 48h (Neggaz and Reguieg Yssaad, 2018).

Application of stress

Radish plants are exposed to abiotic stresses twice for two weeks, the doses used in copper stress are (0, 400, 800 and 1000 PPM) and the doses used in NaCl stress are (0, 40, 80 Meq/L), as well as an interaction between these two stresses has been applied on Radish plants.

Parameters analyzed

The parameters analyzed in this work are proline and soluble sugars. The method used to determine the proline is that of (Troll and Lindsley, 1955) modified by (Dreier and Goring, 1974) and then by (Monneveux and Nemmar, 1986) and the determination of soluble sugars is made by the method of (Dubois, 1956). The instrument used to measure these parameters is the uv-visible spectrophotometer JENWAY 6505.

Statistical analyses

The software chosen to analyze the results obtained is the STATBOX software Version 6.4. The statistical analyses performed are the variance (ANOVA) and the NEWMAN-KEULS test with a significance level (P=5%).

Results and Discussion

Effect of copper-salinity interaction on proline content

The quantitative study of the proline content of Radish exposed to increasing concentrations of NaCl, reveals a significant increase in proline in both aerial and root parts of Radish, these levels are higher than those observed in the control. By contrast, the treatment with Cu causes a significant decrease in proline in the both parts of Radish (Table 2, Fig. 1). When Cu is combined with NaCl, root proline levels decrease proportionally with increasing metal and salt doses, but these levels remain higher in the Cu-80Meq/L interaction than Cu-40 Meq/L and Cu treatment. However, aerial proline concentrations decrease in the 800 PPM combined with 40 Meq/L. Beyond this dose, the proline content starts to increase gradually with the increase of Cu-NaCl doses (Table 2, Fig. 1).

Statistical analyses indicate that NaCl and Cu effect on the aerial and root proline levels of Radish is highly significant ($Pd \leq 0.005$) and the effect of the Cu-NaCl interaction is not significant on the root proline content ($P > 0.05$), but highly significant on its aerial content ($Pd \leq 0.005$).

The proline content of *Raphanus sativus* L. decreases gradually with increasing doses of copper. These results are in accordance with several researchers showing a decrease in proline content in *Raphanus sativus* L. stressed by zinc (Tihana *et al.,* 2008), *Brassica juncia* exposed to lead and cadmium stress (Jhon *et al.,* 2009). The accumulation of proline in plants exposed to heavy metal pollution has been examined in several species, like *Cicer arietinum* L. (Tantrey and Agnihotri, 2010), *Rosa hybrida* L. (Kumar *et al.,* 2010), *Pinus sylevstris* L. (Kandziora-Ciupa *et al.,* 2016). Overall, the sensitive species accumulate proline more quickly. However, tolerant species, present a relative stability or low proline accumulation (Lemziri *et al.,* 2007). Brinis and Belkhoudja (2015) justified the reduced proline levels in stressed plants by the stress level that does not appear to have triggered the proteolysis necessary to obtain a high quantity of proline, therefore there

Table 1: Proline content (Mg/g Dry Matter) in Radish (*Raphanus sativus* L.) under copper (PPM) and NaCl (Meq/L) interaction.

	Copper / NaCl	0	400	800	1000
Arial part	**0**	0.065	0.054	0.037	0.031
	40	0.14	0.049	0.028	0.037
	80	0.143	0.035	0.038	0.056
Root part	**0**	0.079	0.059	0.05	0.046
	40	0.086	0.066	0.04	0.042
	80	0.1	0.091	0.069	0.065

Table 2: The soluble sugars content (Mg/g Dry Matter) in Radish (*Raphanus sativus* L.) under copper (PPM) and NaCl (Meq/L) interaction.

	Copper / NaCl	0	400	800	1000
Arial part	**0**	2.73	2.479	1.484	1.378
	40	2.529	2.382	1.987	1.254
	80	2.703	2.108	1.532	2.19
Root part	**0**	1.877	1.758	1.408	1.359
	40	2.248	1.876	1.868	2.603
	80	2.381	1.396	1.325	1.804

is no response to the point of envisaging a possible blockage of the metabolic activity in progress. Thus, Kadri and Midoun (2015), justify the reduction in proline content in some species under stress by their ability to provide osmotic adjustment using other osmoregulators.

The proline content recorded in *Raphanus sativus* L. under saline stress rises gradually with increasing saline doses. These results are compatible with several studies on various species, namely *Brassica juncea* (Mittal *et al.,* 2012), *Portulaca aleraceae* (Rahdari *et al.,* 2012) and *Phaseolus vulgaris* (Tahri, 2018). The accumulation of proline in response to saline stress may be due to increased its biosynthesis, decreased their catabolism, or their combination (Trinchant *et al.,* 2004).

Effect of copper-salinity interaction on total soluble sugars content

The increase in Cu doses causes a progressive decrease in the soluble sugars content in the both parts of Radish compared to that recorded in the control. Unlike Cu, NaCl and the Cu-NaCl interaction cause an increase in soluble sugars levels in the aerial and root system of plant (Fig. 2). The highest soluble sugars content of the aerial parts is recorded in the control and that of the root parts is marked at 1000 PPM of Cu combined with 40 Meq/L of NaCl (Table 3). However, the lowest sugars content in both aerial and root parts are recorded at 1000 PPM combined with 40 Meq/L and 800 PPM combined with 80 Meq/L interactions respectively (Table 3).

Statistically, the variation in soluble sugars contents of Radish treated with Cu and Cu-NaCl is highly significant for the both parts of plant (Pd≤0.005). However, the effect of NaCl on soluble sugar levels is not significant in the aerial part (P>0.05), but highly significant in the root part of plant (Pd≤0.005).

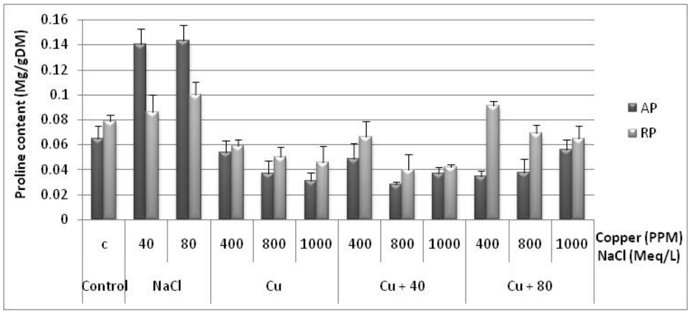

Fig. 1: Effect of Copper-NaCl interaction on proline content (Mg/g Dry Matter) in the aerial and root part of *Raphanus sativus* L.

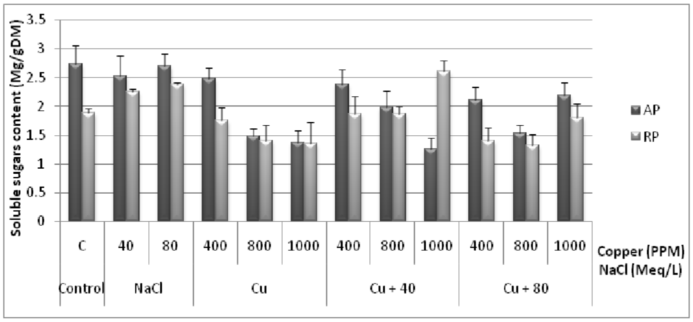

Fig. 2: Effect of Copper-NaCl interaction on soluble sugars content (Mg/g Dry Matter) in the aerial and root part of *Raphanus sativus* L.

Soluble sugars decrease in the aerial and root parts of Radish as copper doses increase. This response is similar to that reported by (Choudhary *et al.,* 2012 a,b), where the soluble sugars content of Radish was reduced by about 2.1 times in response to Cu stress and 2.80 times under Cr stress. Other studies also signal a reduction of soluble sugars levels in Radish under Zn effect (Vijayarengan, 2012) and under Co effect (Kaliyamoorthy *et al.,* 2007). Various studies have reported the decrease in soluble sugars content in other species, namely: *Cyamopsis tetragonoloba* L. Taub (Manivasagaperumal *et al.,* 2011) and the leaves of *Triticum aestivum* under Zn action (Kumar *et al.,* 2012), *Phaseolus vulgaris* under Cd and Pb effect (Bhardwaj *et al.,*2009). This decrease is caused by copper toxicity since it manifests mainly through rhizotoxicity (Kabata-Pendias and Pendias, 1992; Marschner, 1995), because they reduce the transport of sugars and carbohydrates to growing cells and inhibit amylases and phosphatases (Mihoub *et al.,* 2005). According to (Rosa *et al.,* 2009), salinity and low temperatures increase soluble sugars concentrations and heavy metals in general reduce it.

The results obtained reveal a gradual increase in soluble sugars content as doses of NaCl rise. These results are consistent with those obtained in *Cucurbita pepo* (Ialelou *et al.,* 2013), *Medicago sativa* (Kadri and Midoun, 2015), for which an increase in soluble sugars is recorded as salt doses rise. According to (Ialelou *et al.,* 2013), the increase in sugars has been linked to the degradation of starch and (Subba *et al.,* 2014), justify this increase by the disruption of photosynthetic activity which could modify the metabolism of sugars.

Effect of salinity on the tolerance of *Raphanus sativus* L. to copper stress

The results obtained show that proline and soluble sugars levels in Radish treated with Cu-NaCl increase compared to those recorded under Cu effect alone. It appears that salinity promotes the tolerance of Radish against copper by increasing the synthesis of Proline and soluble sugars; in order to maintain its osmotic adjustment.

The Cu-NaCl interaction revealed a positive effect of salinity on the increase in proline and soluble sugars levels in the aerial part of Radish compared to the effect of copper alone on these levels. This indicates that salinity favours copper phytoextraction and their passage to the arial part of Radish. These results are similar to those obtained by (Azzouz, 2011), showing that *Vicia faba* accumulate soluble sugars and proline under the influence of lead-NaCl interaction in an excessive manner compared to their accumulation under stress of lead and NaCl separately. Thus, this accumulation remains important in the aerial part that the root part of *Vicia faba*. According to (Valentina *et al.,* 2010), it is not totally unexpected that salinity exercised a positive effect on the plants' tolerance to metal stress. By contrast, significant data indicating a reduction or neutralization of heavy metal toxicity and an improvement in plant condition are presented in a few reports only, (Volkov *et al.,* 2006; Ghnaya *et al.,* 2007).

Conclusion

The Radish reduces its proline and sugars levels under copper stress and increases these levels under NaCl and Copper-NaCl interaction stress, this means that salinity has a positive action on the tolerance of plants to copper and it can increase the level of these osmoregulators to maintain its osmotic adjustment. The content of proline and soluble sugars is higher in root part under Cooper stress. By contrast, the addition of NaCl to copper increases this content in the arial part, which shows that the copper level rises in this part. The results obtained show that moderate salinity can improve the tolerance of *Raphanus sativus* L. to copper quite effectively, which should be taken into account during the development of phytoremediation technologies.

References

Azouz, F. (2011). Effect of lead-salinity interaction on the physiological and biochemical responses of a halophyte (*Atriplex halimus* L.) and a glycophyte (*Vicia faba* L.). Memory of Magister, Oran University, Algeria, pp:62-68.

Bhardwaj, P., K.C. Ashish and P. Prasad (2009). Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris*. *Nature Science*, **8:** 63–75.

Biteur, N. (2012). Tests for the use of Radish h (*Raphanus sativus* L.) in phytoremediation (biodepollution) in soil contaminated by heavy metals (Lead): Study of oxidative stress and some enzymatic parameters. PhD thesis, Oran University, Algeria p:61.

Brinis, A. and M. Belkhodja (2015). Salinity effect on physiological and biochemical parameters of *Atriplex halimus* L. *Science and Technology*, Synthèse, **31:** 42 -51.

Chen, Z., T.A. Cuin, M. Zhou, A. Twomey, B.P. Naidu and S. Shabala (2007). Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. *Journal Of Experimental Botany*, **58:** 4245–4255.

Choudhary, S.P., M. Kanwar, R. Bhardwaj, J.Q. Yu and L.S.P. Tran, (2012b). Chromium Stress Mitigation by Polyamine-Brassinosteroid Application Involves Phytohormonal and Physiological Strategies in *Raphanus sativus* L. *Public Library of Science,* **7(3):** e33210.

Choudhary, S.P., O.H. Volkan, R. Bhardwaj, J.Q. Yu and L.S.P. Tran, (2012a). Interaction of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus sativus* L. *Journal of Experimental Botany*, **63(15):** 5659-5675 Published by: Oxford University Press

Dreier, W. and M. Göring (1974). Dereim slushohersolz kongentrasion en aiesverschideu physiologcshe parameter van-maiswrzelnwiss. *Z. Drh. Berlin Nath. Naturwiss R.*, **23:** 641-4.

Dubois, M., K. Gilles, J. Hamilton, P. Rebers and F. Smith (1956). Colorimetric Method for Determination of sugar and related substances. *Analytical Chemistry*, **28:** 350-356.

Ghnaya, T., I. Slama, D. Messedi, C. Grignon, M.H. Ghorbel and C. Abdelly (2007). Cd-induced growth reduction in the halophyte *Sesuvium portulacastrum* is significantly improved by NaCl. *Journal of Plant Research*, **120:** 309–316.

Hare, P.D., W.A. Cress and J. Van Staden (1998). Dissecting the rôles of osmolyte accumulation during stress. Plant, Cell and Environment, Natal University Research Unit for Plant Growth and Development, University of Natal Pietermaritzburg, Private Bag X01, Scottsville, 3209, South Africa, P:542.

Hoagland, D.R. and D.I. Arnon (1938). The water-culture method for growing plants without soil. Berkeley, California: College of Agriculture, University of California. Circular, **347**:1-39.

Ialelou, F.S., J. Shafagh-Kolvanagh and M. Fateh (2013). Effect of various concentrations of Zn on chlorophyll, starch, soluble sugars and proline in naked pumpkin (*Cucurbita pepo*). *International Journal of Farming and Allied Sciences*, **2(24):** 1198–1202.

John, R., P. Ahmad, K. Gadgil and S. Sharma (2009). Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *International Journal of Plant Production*, **3:** 65-75.

Kabata-Pendias, A. and H. Pendias (1992). Trace Elements in Soils and Plants. 2nd Edition, CRC Press, Boca Raton.

Kadri, A. and N. Midoun (2015). Effect of salt stress on some biochemical parameters of cultivated alfalfa (*Medicago sativa* L.). Kasdi Merbah University, Ouargla, Algeria, pp:31-40.

Kaliyamoorthy, J., A. Cheruth and V. Packirisamy (2007). Changes in Growth, Biochemical Constituents and Antioxidant Potentials in Radish (*Raphanus sativus* L.) under Cobalt Stress. *Turkish Journal of Biology*, **31:** 127-

136.

Kandziora-Ciupa, M., R. Ciepa³, A. Nadgórska-Socha and G. Barczyk (2016). Accumulation of heavy metals and antioxidant responses in *Pinus sylvestris* L. needles in polluted and non-polluted sites. *Ecotoxicology*, **25:** 970–981.

Kumar, N., M. Pal, A. Singh, R. Kumar SaiRam and G.H. Srivastava (2010). Exogenous proline alleviates oxidative stress vase life in rose (*Rosa hybrid*a L. 'Grand Gala') *Scientia horticulturae*, **127:** 79–85.

Kumar, V., G. Awasthi and P.K. Chauhan (2012). Cu and Zn tolerance and responses of the Biochemical and Physiochemical system of Wheat. *Journal of Stress Physiology & Biochemistry*, **8(3):** 203-213.

Lemzeri, H. (2007). Ecophysiological responses of three forest species of the genus Acacia, Eucalyptus and Schinus (*A. cyanophylla, E. gomphocephala* and *S. mölle*) under saline stress. Memory of Magister, Constantine University, Algeria 180p.

Manivasagaperumal, R., S. Balamurugan, G. Thiyagarajan and J. Sekar (2011). Effect of Zn on Germination, Seedling Growth and Biochemical Content of Cluster Bean (*Cyamopsis tetragonoloba* L. Taub). *Current Botany*, **(2):** 11-15.

Marschner, H. (1995). Mineral nutrition of higher plants. 2nd Edition. *Academic Press*, San Diego. 889pp.

Mihoub, A., A. Chaoui and E.E. Ferjani (2005). Biochemical changes induced by cadmium and copper during pea seed germination (*Pisum sativum* L.). *Comptes rendus Biologies*, **328:** 33-41.

Mittal, S., N. Kumari and V. Sharma (2012). Differential response of salt stress on *Brassica juncea*: photosynthetic performance, pigment, proline, D1 and antioxidant enzymes. *Plant Physiology and Biochemistry*, **54:** 17–26.

Monneveux, P.H. and M. Nemmar (1986). Contribution to the study of drought resistance in soft wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.): study of proline accumulation during the development cycle. Agronomy, *Édition Diffusion Presse Sciences*, **6(6):** 583-590.

Neggaz, N.E. and H.A. Regueig Yssaad (2018). Effect of lead stress on polyphenols, flavonoids and proline contents in Radish (*Raphanus sativus* L.). *International Journal of Biosciences*, **12(5):** 135-144.

Rahdari, P., S. Tavakoli and S.M. Hosseini (2012). Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in Purslane (*Portulaca oleracea* L.) Leaves. *Journal of Stress Physiology & Biochemistry*, **8(1):** 182–193.

Rosa, M., C. Prado and G. Podazza (2009). Soluble sugars—Metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant Signaling & Behavior*, **4(5):** 388-393.

Subba, P., M. Mukhopadhyay, S.K. Mahato, K.D. Bhutia, T.K. Mondal and S.K. Ghosh (2014). Zinc stress induces physiological, ultra-structural and biochemical changes in mandarin orange (*Citrus reticulata* Blanco) seedlings. *Physiology and Molecular Biology of Plants*, **20(4):** 461–473.

Sun, B.Y., S.H. Kan, Y.Z. Zhang, S.H. Deng, J. Wu, H. Yuan, H. Qi, G. Yang, L. Li, X.H. Zhang, H. Xiao, Y.J. Wang, H. Peng and Y.W. Li (2010). Certain antioxydant enzymes and lipid peroxydation of Radish (*Raphanus sativus* L.) as early warning biomarkers of soil copper exposure. *Journal of Hazardous Materials*, **183:** 833-838.

Tahri, M. (2018). Research of parameters related to salt tolerance in beans (*Phaseolus vulgaris* L.) PhD thesis from Mostaganem University of Algeria, pp:70-78

Tantrey, M.S. and R.K. Agnihotri (2010). Chlorophyll and proline content of gram (*Cicer arietinum* L.) under cadmium and mercury treatment. *Research Journal of Agricultural Sciences*, **1(2):** 119-122.

Tavakkoli, E., F. Fatehi, P. Rengasamy and G.K. McDonald (2012). A comparison of hydroponic and soilbased screening methods to identify salt tolerance in the û e ld in barley. *Journal of Experimental Botany*, **63(10):** 3853-67.

Tihana, T., T.H. John, E. Meri, P. Nada, C. Vera, L. Hrvoje, Š. Ivna and B. Drago (2008). Antioxidative responses in Radish h (*Raphanus sativus* L.) Plants stressed by copper and lead in nutrient solution and soil. *Acta biologica cracoviensia,* **50(2):** 79–86.

Trinchant, J.C., A. Boscari, G. Spennato, G. Van de Sype and D. Le Rudulier (2004). Proline betaine accumulation and metabolism in alfalfa plants under sodium chloride stress. Exploring its compartmentalization in nodules. *Plant Physiology*, **135(3):** 1583-94.

Troll, W. and J. Lindsley (1955). A photometric method for the determination of proline. *Journal of Biological Chemistry*, **215(2)**: 655-660.

Valentina, K., V. Kirill and K. Vladimir (2010). Plants Under Heavy Metal Stress in Saline Environments. I. Sherameti and A. Varma (eds.), *Soil Heavy Metals, Soil Biology*, Vol19.

Vijayarengan, P. (2012). Growth and biochemical variations in Radish under zinc applications *International Journal of Research in Plant Science*, **2(3):** 43-49.

Volkov, K.S., V.P. Kholodova and V.l.V. Kuznetsov (2006). Plant adaptation to salinity reduces copper toxicity. *Doklady Biological Sciences*, **411:** 479–481.

Zerrad, W., S. Hillali, B.S. Mataoui, E. Elantri and A. Elhmyene (2006). Study of the biochemical and molecular mechanisms of water stress resistance of two varieties of durum wheat. International Congress of Biochemistry, Agadir, Morocco.

Zouari, M., Ch. Ben Ahmed, W. Zorrig, N. Elloumi, M. Rabhi, D. Delmail, B. Ben Rouina, P. Labrousse and F. Ben Abdallah (2016). Exogenous proline mediates alleviation of cadmium stress by promoting photosynthetic activity, water status and antioxidative enzymes activities of young date palm (*Phoenix dactylifera* L.) *Ecotoxicology and Environmental Safety*, **128:** 100–108.