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ENZYMATIC ACTIVITY AND SOLUBLE PROTEIN CONTENT IN SEEDLINGS OF *CALENDULA OFFICINALIS* L. UNDER SALT STRESS

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Abstract: Enzymatic activity and soluble protein content in relation to salt stress tolerance were investigated in *Calendula officinalis* seedlings after 24 days of treatment with different salt treatments, including NaCl, $CaCl_2$, $MgCl_2$ and mixtures of them. The marigold seedlings were used in order to investigate the possible salt-inducible responses and the possible alleviative role of calcium and magnesium salts in respect with adverse salinity conditions. Activity of superoxide dismutase (SOD; EC 1.15.1.1) and peroxidase (POD; EC 1.11.1.7) generally slightly decreased under salt treatments, with minor variations from the value recorded for control series and within applied treatments. Catalase (CAT; EC 1.11.1.6) activity was stimulated by NaCl salinity and $MgCl_2$ addition; its activity was found to be lowest under calcium and magnesium chloride treatments solely.

Keywords: antioxidant system, calcium, flavonoids, magnesium, salinity, seedlings.

Introduction

Marigold (*Calendula officinalis* L.) is the common name for *Calendula* genus of *Asteraceae* family. *Calendula* is stated to possess antispasmodic, mild diaphoretic, anti-inflammatory styptic, antihemorrhagic, emmenagogue, vulnerary, and antiseptic properties [DUKE & al. 2002; BARNES & al. 2007; EBADI, 2007; YBERT & DE LAAGE, 2007]. Traditionally, it has been used to treat gastric and duodenal ulcers, amenorrhea, dysmenorrhea and epistaxis; crural ulcers, varicose veins, hemorrhoids, anal eczema, proctitis, lymphadenoma, inflamed cutaneous lesions (topically) and conjunctivitis (as an eye lotion) [EBADI, 2007; KHALID & al. 2012]. Phytochemical studies have reported four main groups of constituents, for *Calendula*, namely flavonoids, polysaccharides, volatile oil and triterpenes [AZZAZ & al. 2007; BARNES & al. 2007].

Animal studies have reported wound-healing and anti-inflammatory effects, supporting the traditional uses of calendula in various dermatological conditions. The anti-inflammatory effect is due to the triterpenoid constituents, although flavonoids may contribute to the activity. The reputed antispasmodic effect may be attributable to the volatile oil fraction. In addition, immunostimulant activity has been reported for high molecular weight polysaccharide components. Clinical research assessing the effects of calendula

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preparations is limited, and rigorous randomized controlled clinical trials are required [BARNES & al. 2007].

Salinity is one of the most severe environmental factors limiting the productivity of agricultural crops. Most crops are sensitive to salinity caused by high concentrations of salts in the soil [PITMAN & LAUCHLI, 2004]. The cost of salinity to agriculture is estimated conservatively to be about $US 12 billion a year, and is expected to increase as soils are further affected [GHASSEMI & al. 1995]. An alarming scenario has been described in this context, including a future dominated by salinity and aridity in agriculture, fresh water scarce and consequently food crisis [GRIGORE & al. 2014]. Hence, the necessity to elucidate the salt-mechanism tolerance in plants with medicinal properties is a condition of great interest.

There are several studies focused to salt stress in *C. officinalis*; they refer to influence on germination [GHARINEH & al. 2013; SEDGHI & al. 2010; TORBAGHAN, 2012], or impact of exogenous salicylic acid on growth parameters in marigold under salt stress [BAYAT & al. 2012]. Few studies refer on antioxidant activity under salt stress conditions [CHAPARZADEZ & al. 2004].

Therefore, the aim of this study is to investigate several biochemical responses of the medicinal plant *C. officinalis* subjected to different salt treatments; in addition, calcium and magnesium salts are discussed in relation to the possible role in alleviating NaCl effects.

Materials and methods

Plant material, treatment and growth conditions

In order to investigate the effect of salt solutions on several biochemical parameters in *C. officinalis* L. seedlings, an experiment was conducted in controlled laboratory conditions based on a completely randomized design. Marigold (*C. officinalis*) seeds were obtained from Agricultural Research and Development Station, Secuieni Neamt, Romania. Intact seeds, which were homogeneous and identical in size and colour, and free from wrinkles, were chosen. These seed were then sterilized with sodium hypochlorite 10% for 30 seconds and were washed with sterile distilled water. After that, 30 marigold seeds were sown and germinated in plastic pots. After 7 days (corresponding to a uniform seedlings emergence) salt treatments started and were carried out by adding 100 mL of salt solutions (or distilled water for the control treatments) to pots once per week. The follow treatments were applied: 50mM NaCl, 50mM NaCl + 10mM $CaCl_2$, 50mM NaCl + 20mM $MgCl_2$, 100mM NaCl, 100mM NaCl + 10mM $CaCl_2$, 100mM NaCl + 20 $MgCl_2$, 150mM NaCl, 150mM NaCl + 10mM $CaCl_2$, 150mM NaCl + 20mM $MgCl_2$, 10mM $CaCl_2$, 20mM $MgCl_2$. The NaCl concentrations were chosen as optimal after performing previous trial at different concentrations. Concentrations of calcium and magnesium chloride were preferred according to previous research experiments [GRIGORE & al. 2012]. The biochemical analyses were conducted at 24-days old seedlings; five different individuals from each treatment were selected to measure all the analyzed parameters.

Preparation of extracts and assay enzyme

Marigold seedling sample (0.3g) were homogenized with phosphate buffer (pH=7.5). After that the homogenates were centrifuged the supernatants were used for enzyme assays.

Superoxide dismutase (SOD) activity was estimated by recording the decrease in absorbance of superoxide-nitroblue tetrazolium complex by the enzyme [WINTERBOURN & al. 1975]. About 3 mL of reaction mixture, containing 0.1 mL of 1.5 mM nitroblue tetrazolium (NBT), 0.2 mL of 0.1 M EDTA, 2.55 mL of 0.067 M potassium phosphate buffer, and 0.01 mL of enzyme extraction, were taken in test tubes in duplicate from each enzyme sample. One tube without enzyme extract was taken as control. The reaction was started by adding 0.05 mL of 0.12 mM riboflavin and placing the tubes below a light source of 215 W florescent lamps for 5 min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal colour. A non-irradiated complete reaction mixture, which did not develop colour, served as blank. Absorbance was recorded at 560 nm and 1 unit of enzyme activity was taken as the quantity of enzyme that reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

Peroxidase (POD) activity was determined spectrophotometrically by measuring the oxidation of o-dianisidine at 540 nm [MOLLER & OTTOLENGHI, 1966] with slight modification. The reaction was started by adding 0.1 H_2O_2 0.05% on mixture reaction containing 0.2 mL of enzyme extraction, 0.8 mL distillated water and 1.5 mL 1% o-dianisidine. After 5 min. the reaction was stopped with 2.5 mL H_2SO_4 50%. One unit of POD activity was expressed as the amount of enzyme that produced a change of 1.0 absorbance per min.

Catalase (CAT) activity was measured according to the method described by Sinha, 1972. Briefly, the assay mixture consisted of 0.4 mL phosphate buffer (0.01 M, pH 7.0), 0.5 mL hydrogen peroxide (0.16 M) and 0.1 mL enzymatic extract in a final volume of 3.0 mL. About 2 mL dichromate acetic acid reagent was added in 1 mL of reaction mixture, boiled for 10 min, cooled. Changes in absorbance were records at 570 nm. CAT activity was expressed as the amount of enzyme needed to reduce 1 µmol of H_2O_2 per min. The activity of these enzymes (SOD, POD and CAT) was expressed as unit per mg proteins (U/mg protein).

The determination of **soluble protein content** was determined according to BRADFORD method (1976) with bovine serum albumin as standard. Thus, this assay is refers to the binding of Coomassie Brilliant Blue G-250 at aromatic amino acid radicals and measuring the colour at 595nm.

Statistical analysis. The statistical analysis was performed using Student *t*-test. Values with $p<0.005$ were considered as statistically significant.

Results and discussion

Enzymatic activity

There is no uniform response of *Calendula* seedlings with respect to salinity stress, regarding the investigated enzymatic activity: SOD, CAT, and POD (Fig. 1, Fig. 2 and Fig. 3).

Superoxide dismutase activity was registered to be visibly higher only in plants subjected to elevated concentration of 150 mM NaCl (14.27%), comparatively with un-stressed plants and other salt treatments. However, the other values remain only slightly lower than in control samples. In all cases, the addition of calcium and magnesium chloride to NaCl corresponds to lower values than those recorded for NaCl treatments solely. SOD

activity showed a significant decrease ($p<0.05$) in case of singular treatment with $CaCl_2$ and, respectively, $MgCl_2$.

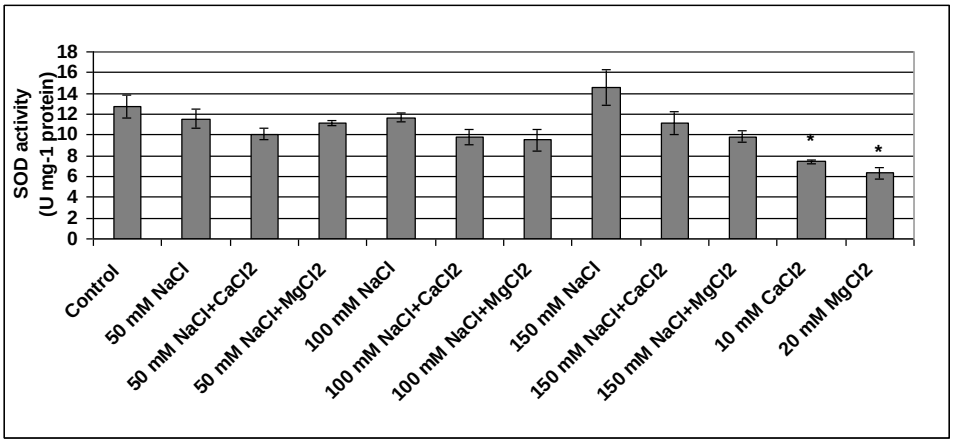

Fig. 1. Effect of NaCl, $CaCl_2$, $MgCl_2$ and their combination on SOD activity of *C. officinalis* (Values are means ± SE of 5 replicates); *significant at $p< 0.05$

SOD is an important antioxidant enzyme and is the first line of defence against oxidative stress in plants [ALSCHER & al. 2002; JITESH & al. 2006]. The production of toxic superoxide free radicals appears to be a universal problem in aerobic cells exposed to different type of stress. The elimination of superoxide by superoxide dismutase (SOD) produces H_2O_2 which in turn is removed by various peroxidase or catalase.

Usually, salt stress in cultivars differing in salt tolerance evidenced an increased SOD activity in salt-tolerant genotypes of pea, cotton, tomato and wheat; therefore, induction of SOD activity was suggested as a reason for improved tolerance to salinity in these species [HERNANDEZ & al. 1993, 1995, 1999; GOSSETT & al. 1994; MITTOVA & al. 2003; SAIRAM & al. 2005] and in general [SHARMA & al. 2011]. However, there are exceptions from this general statement; SOD activity may decrease in several species under salt conditions or it may vary in the same species in different investigated organs. For instance, in tobacco leaves, SOD activity increased, while in the roots of the same plant, SOD activity decreased [MYTINOVA & al. 2010]. In *Plantago major* exposed to NaCl stress, the roots were characterized, unlike leaves, by high constitutive activity of SOD [RADYUKINA & al. 2009]. In forage sorghum seedlings, salinity of 50 and 100 mM NaCl induced significant increase in SOD activity, in tolerant genotypes compared to sensitive group [HEFNY & ABDEL-KADER, 2009]. Other data report that under salinity conditions (50 and 100 mM NaCl), CAT (and POD) activity decreased in *Calendula officinalis* [CHAPARZADEH & al. 2004].

CAT activity has been found to be higher in NaCl stressed plants (50 and 100 mM NaCl), while under 150 mM NaCl, the activity was almost imperceptibly lower than the control (Fig. 2). Interestingly, the smallest value of CAT in plants under 150 mM NaCl salinity treatments (-1,6%) is inversely correlated to SOD activity under the same conditions (14.27%). The sodium chloride mixed to $CaCl_2$ and $MgCl_2$ induces different responses. The addition of magnesium chloride to all NaCl treatments implies higher values of CAT activity

than the control plants; addition of calcium chloride show lower and higher values comparatively with the control. However, only under elevated 150 mM NaCl salinity, the added calcium and magnesium chloride seem to increase the CAT activity compared to NaCl treatment alone. By application of the singular treatment with $CaCl_2$ the activity of CAT showed a significant decrease ($p < 0.01$).

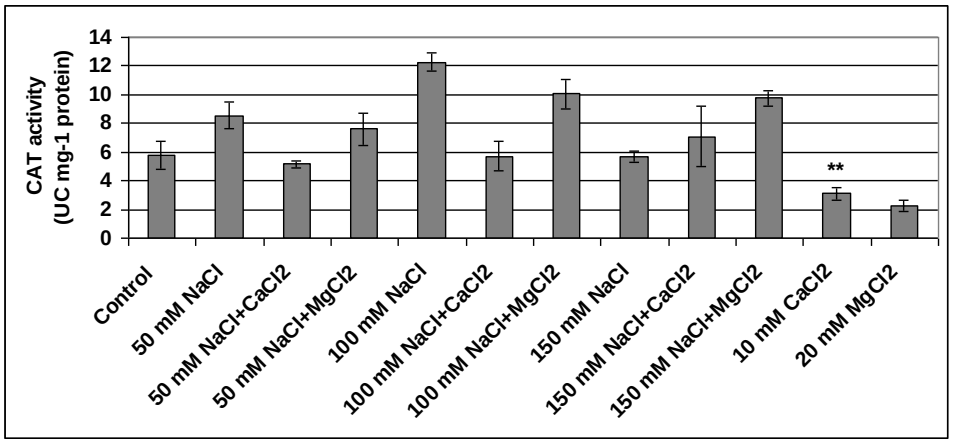

Fig. 2. Effect of NaCl, $CaCl_2$, $MgCl_2$ and their combination on CAT activity of *C. officinalis* (Values are means ± SE of 5 replicates); **significant at $p < 0.01$

Catalases are haem-containing tetrameric enzymes involved in the removal of H_2O_2 and during salt stress and other abiotic stress conditions [WILLEKENS & al. 1997]. Abiotic stresses cause either enhancement or depletion of CAT activity [EL-SHINTINAWY & al. 2004; SHARMA & DUBEY, 2005; NOREEN & ASHRAF, 2009]. For instance, in strawberry (*Fragaria* x *ananassa* Duch., cv. *Selva*) leaves under NaCl stress, there was an inhibition in CAT activity [TANOU & al. 2009]. In *Plantago major* roots subjected to NaCl stress a lower CAT activity has been reported [RADYUKINA & al. 2009]. In nine genetically diverse pea (*Pisum sativum*) cultivars exposed to salt stress, a decreased CAT activity has been found, while SOD activity was enhanced by salinity conditions [NOREEN & ASHRAF, 2009]. In tobacco leaves, CAT activity declined under salinity stress [MYTINOVA & al. 2010].

POD activity in NaCl stressed plants show fairly lower values when compared with control plants (Fig. 3). The mixed salts (NaCl with calcium and magnesium chloride) induced different values, as compared with control or series stressed with NaCl solely. For instance, $MgCl_2$ added to 50 and 150 mM NaCl imply slightly higher values than those recorded in plants subjected only to corresponding NaCl treatments.

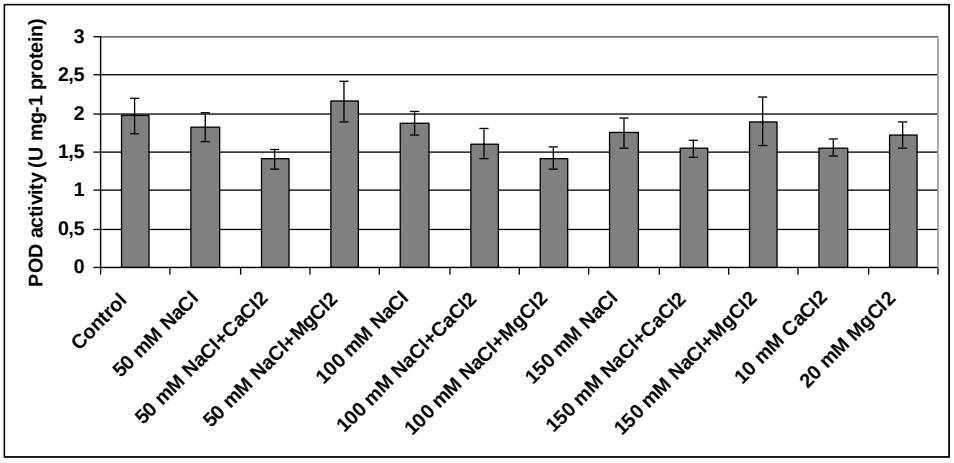

Fig. 3. Effect of NaCl, $CaCl_2$, $MgCl_2$ and their combination on POD activity of *C. officinalis* (Values are means ± SE of 5 replicates)

Plants contain abundant amounts of peroxidases that are involved in H_2O_2 scavenging; they are associated with the cell wall where they generate phenoxy compounds from cinnamic acids [JITESH & al. 2006]. Activity of POD has been markedly enhanced by salt stress in several genetically diverse pea cultivars [NOREEN & ASHRAF, 2009].

Soluble protein content

Under salinity conditions, the registered values are generally higher than in control (Fig. 4). The NaCl treatment induces values very closed to those registered for control; however, the mixture of NaCl with calcium and magnesium chloride increased the soluble protein content as compared with control series. Plants treated only with $CaCl_2$ and $MgCl_2$ have the highest values of soluble protein content. As regards the protein amount in stress condition, the data reported from literature is abundant numerous and greatly variable. Therefore, some authors [AGASTIAN & al. 2000] published that soluble protein content increases at low salinity and decreases at high salinity in *Morus* sp. Depending on NaCl concentrations, in some wheat varieties (Faur, Iasi, Fundulea) the protein content was reduced in 4 days old seedlings, but it tends to increase after 168 and 240h of salt treatment, as compared to control [OPRICĂ, 2011]. On the other hand soluble protein amounts of leaves significantly decreased in response to salinity at some species like *Paulownia imperialis* and *P. fortunei* [AYALA-ASTORGA & ALCAREZ-MELENDEZ, 2010; PARVAIZ & SATYAVATI, 2008; PARIDA & DAS, 2005]. However, in other study [FAYEZ & BAZAID, 2014], the soluble protein of barley leaves was slightly changed in response to salt and water deficit stresses as well as with the combined treatments (salicylic acid and KNO_3).

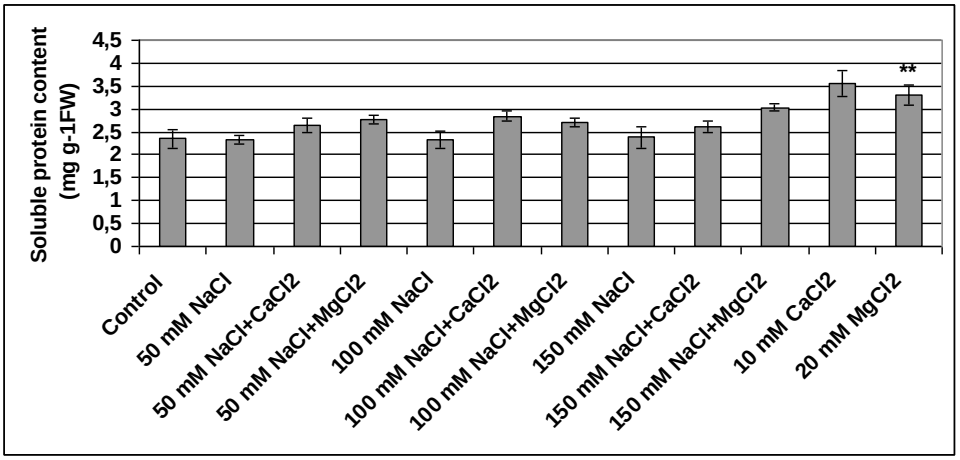

Fig. 4. Effect of NaCl, $CaCl_2$, $MgCl_2$ and their combination on soluble protein content of *C. officinalis* (Values are means ± SE of 5 replicates); **significant at $p < 0.01$

Conclusions

There is no uniform response of *Calendula* seedlings with respect to salinity stress, regarding the investigated enzymatic activity: SOD, CAT, and POD. SOD activity, usually considered as a first line of defense against oxidative stress, shows no important increase as a response of salt stress. The soluble protein content registered values generally higher than in control plants, with highest values recorded in the case of plant series subjected to calcium and magnesium chloride.

References

AGASTIAN P., KINGSLEY S. J. & VIVEKANANDAN M. 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes, *Photosynthetica*. **38**: 287-290.

ALSCHER R. A., ERTURK N. & HEATH L. S. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **53**: 1331-1341.

APEL K. & HIRT H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol*. **55**: 373-399.

AYALA-ASTORGA GLORIA IRMA & ALCARAZ-MELÉNDEZ L. 2010. Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* and *Paulownia fortunei* grown in vitro. *Electron. J. Biotechnol*. **13**(5): 13-14.

AZZAZ N. A., HASSAN E. A. & EL-EMAREY F. A. 2007. Physiological, anatomical, and biochemical studies on pot marigold (*Calendula officinalis* L.) plants. *African Crop Science Conference Proceedings*. **8**: 1727-1738.

BARNES J., ANDERSON L. A. & PHILLIPSON J. D. 2007. *Herbal medicines (third ed.).* Pharmaceutical Press, London, Chicago: 710 pp.

BAYAT H., ALIREZAIE M. & NEAMATI H. 2012. Impact of exogenous salicylic acid on growth and ornamental characteristics of calendula under salinity stress. *J. Stress Physiol. Biochem.* **8**(1): 258-267.

BRADFORD M. 1976. A rapid and sensitive method for microgram quantities of protein utilizing the principle of protein-dye binding. *An. Biochem*. **7**(72): 248-254.

CHAPARZADEH N., D'AMICO M. L., KHAVARI-NEJAD R. A., IZZO R. & NAVARI-IZZO F. 2004. Antioxidative responses of *Calendula officinalis* under salinity conditions. *Plant Physiol. Biochem*. **42**(9): 695-701.

DUKE J. A., BOGENSCHUTZ-GODWIN M. J., DU CELLIER J. & DUKE P-A. K. 2002. *Handbook of medicinal herbs (second ed.)*. CRC Press, Boca Raton, London, New Tork, Washington, D.C.: 870 pp.

EBADI M. 2007. *Pharmacodynamic basis of herbal medicine (second edition)*, Taylor & Francis Group Eds.: 699 pp.

EL-SHINTINAWY F., EBRAHIM M. K. H., SEWELAM N. & EL-SHOURBAGY M. N. 2004. Activity of photosystem 2, lipid peroxidation, and the enzymatic antioxidant protective system in heat shocked barley seedlings. *Photosynthetica*. **42**: 15-21.

FAYEZ K. A.&BAZAID S. A. 2014. Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. *J. Saudi Soc. Agri. Sci.* **13**(1): 45-55.

GHARINEH M. H., KHODDAMI H. R. & RAFIEIAN-KOPAEI M. 2013. The influence of diffrent levels of salt stress on germination of marigold (*Calendula officinalis* L.). *Int. J. Agr. Crop Sci*. **5**(14): 1581-1584.

GHASSEMI F., JAKEMAN A. J. & NIX H. A. 1995. *Salinisation of Land and Water Resources*. University of New South Wales Press Ltd, Canberra, Australia: 526 pp.

GOSSETT D. R., MILLHOLLON E. & LUCAS M. C. 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Sci*. **34**: 706-714.

GRIGORE M. N., BOSCAIU M., LINARES J. & VICENTE O. 2012. Mitigation of salt stressed-induced Inhibition of *Plantago crassifolia* reproductive development by supplemental calcium or magnesium. *Not. Bot. Horti Agrobot*. **40**(2): 58-66.

GRIGORE M. N., IVĂNESCU L. & TOMA C. 2014. *Halophytes. An integrative anatomical study*. Springer, Cham, Heidelberg, New York, Dordrecht, London: 548 pp.

HATIER J. H. B. & GOULD K. S. 2008. Foliar anthocyanins as modulators of stress signals. *J. Theor. Biol*. **253**: 625-627.

HEFNY M. ABDEL-KADER D. Z. 2009. *Antioxidant-enzyme system as selection criteria for salt tolerance in forage sorghum genotypes* (*Sorghum bicolor* L. Moench). In: ASHRAF M., OZTURK M., ATHAR H. R. (eds.). *Salinity and Water Stress*, Amsterdam, Netherlands, Springer: 25-36.

HERNANDEZ J. A., CORPAS F. J., GOMEZ M., DEL RIO L. A. & SEVILLA F. 1993. Salt-induced oxidative stress mediated by activated oxygenspecies in pea leaf mitochondria. *Physiol. Plant*. **89**: 103-110.

HERNANDEZ J. A., OLMOS E., CORPAS F. J., SEVILLA F. & DEL RIO L. A. 1995. Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Sci.* **105**: 151-167.

JITESH M. N., PRASHANTH S. R., SIVAPRAKASH K. R. & PARIDA A. K. 2006. Antioxidative response mechanisms in halophytes: their role in stress defence. *J. Genet.* **85**(3): 237-254 .

KHALID A. K. & TEIXEIRA DA SILVA J. A. 2016. Biology of *Calendula officinalis* Linn: Focus on Pharmacology, Biological activities and Agronomic Practices. *Medicinal Aromatic Plant Sci. Biotech.* **6**(1): 12-27.

MITTOVA V., TAL M., VOLOKITA M. & GUY M. 2003. Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennelli*. *Plant Cell Environ*. **26**: 845-856.

MÖLLER K. M. & OTTOLENGHI P. 1966. The oxidation of o-dianisidine by H2O2 and peroxidase at neutral pH. *Compt. Rend. Trav. Lab. Carlsberg*. **35**: 369-389.

MÝTINOVÁ Z., HAISEL D., MOTYKA V., GAUDINOVÁ A. & WILHELMOVÁ N. 2010. Effect of abiotic stresses on antioxidative enzymes and contents of phytohormones in wild type and AtCKX transgenic tobacco plants. *Biol. Plant*. **54**: 461-470.

NOREEN Z. & ASHRAF M. 2009. Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. *J. Plant. Physiol*. **166**(16): 1764-1774.

OPRICA L. 2011. Early effect of NaCl treatment on the protein content in the seedling of three wheat cultivars. *An. St. Univ. Al. I Cuza Iasi, Sect. Genetica si Biol. Molec*. **12**(4): 87-93.

PARIDA A. K. & DAS A. B. 2005. Salt tolerance and salinity effect on plants: a review. *Ecotoxicol. Environ. Saf*. **60**: 324-349.

PARVAIZ A. & SATYAVATI S. 2008. Salt stress and phyto-biochemical responses of plants- a review. *Plant Soil Environ*. **54**: 89-99.

PITMAN M. G. & LÄUCHLI A. 2004. *Global impact of salinity and agricultural ecosystems*. In: LAUCHLI A. & LUTTGE U. (eds.). *Salinity: Environment-Plants-Molecules* Kluwer Academic Publishers, New York: 3-20.

RADYUKINA N. L., MAPELLI S., IVANOV Y. V., KARTASHOV A. V., BRAMBILLA I. & KUZNETSOV V. V. 2009. Homeostasis of polyamines and antioxidant systems in roots and leaves of *Plantago major* under salt stress. *Russ. J. Plant. Physiol*. **56**: 323-331.

REMORINI D., MELGAR J. C,. GUIDI L., DEGL'INNOCENTI E., CASTELLI S., TRAVERSI M. L., MASSAI R. & TATTINI M. 2009. Interaction effects of root-zone salinity and solar irradiance on the physiology and biochemistry of *Olea europaea*. *Environ. Exp. Bot.* **65**: 210-219.

ROMERO ARANDA R., SORIA T. & CUARTERO J. 2001. Tomato plant-water uptake and plant-water relationships under saline growth conditions. *Plant Sci*. **160**: 265-272.

SAIRAM R. K., SRIVASTAVA G. C., AGARWAL S. & MEENA R. C. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biol. Plant*. **49**: 85-91.

SEDGHI M., EMATI A. & ESMAIELPOUR B. 2010. Effect of seed priming on germination and seedling growth of two medicinal plants under salinity. *Emir. J. Food Agric.* **22**(2): 130-139.

SINHA K. A. 1972. Colorimetric assay of catalase. *An. Biochem*. **47**: 389-394.

SHARMA P., JHA A.B. & DUBEY R. S. 2011. *Oxidative stress and antioxidative defense systems in plants growing under abiotic stresses*. In: PESSARAKLI M. (ed.). *Handbook of plant and crop stress, third edition*, CRC Press, Taylor & Francis Group, Boca Raton, London, New York: 89-138.

SHARMA P. & DUBEY R. S. 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regul*. **46**: 209-221.

TANOU G., MOLASSIOTIS A. & DIAMANTIDIS G. 2009. Induction of reactive oxygen species and necrotic deathlike destruction in strawberry leaves by salinity. *Environ. Exp. Bot.* **65**: 270-281.

TORBAGHAN M. E. 2012. Effect of salt stress on germination and some growth parameters of marigold (*Calendula officinalis* L.). *Plant Sci. J*. **1**(1): 7-19.

WALIA H., WILSON C., CONDAMINE P., LIU X., ISMAIL A. M., ZENG L., WAMAKER S. I., MANDAL J., XU J., CUI X. & CLOSE T. M. 2005. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiol*. **139**: 822-835.

WILLEKENS H., CHAMNONGPOL S., DAVEY M., SCHRAUDNER M., LANGEBARTELS C. & VAN MONTAGU M. 1997. Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. EMBO J. **16**: 4806-4816.

XU C., SULLIVAN J. H., GARRETT W. M., CAPERNA T. J. & NATARAJAN S. 2008. Impact of solar Ultraviolet-B on the proteome in soybean lines differing in flavonoid contents. *Phytochemistry.* **69**: 38-48.

YBERT E. & DE LAAGE DE MEUX A. 2007. *Larousse Encyclopédie des Plantes Médicinales. Identification, preparations, soins*. Larousse, 21 Rue du Montparnasse 75283, Paris: 332 pp.

WINTERBOURN C., HAWKINS R., BRIAN M. & CARRELL R. 1975. The estimation of red cell superoxide dismutase activity. *J. Lab. Clin. Med*. **85**: 337.

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