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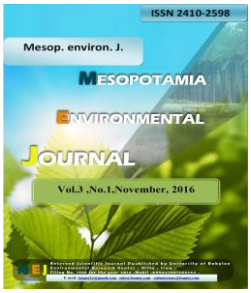

Biochemical responses of Romanian *Calendula officinalis* L. under salinity stress

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Abstract:

Total flavonoids and polyphenols content, and pigment amount under salt stress were analyzed in *Calendula officinal is* L. seedlings after 24 days of treatment with different salt combination, including NaCl, $CaCl_2$,and $MgCl_2$.The content of photosynthetic pigments increased according to increasing salinity concentration. Total polyphenols content generally decreased under salt stress, while flavonoids biosynthesis significantly increased almost under all salt treatments.

Keywords: seedlings, salinity, non enzymatic antioxidants, calcium, magnesium, flavonoids**.**

Introduction

Calendula officinalis (L.), generally known as marigold, belongs to *Asteraceae* family and should not be confused with *Tagetes* species; it is an annual herb native to the Mediterranean region [1]. It is originally from Europe, Asia and the U.S.A, and has been widely used in folk medicine [2; 3]. The therapeutic properties of *C. officinalis* are attributed to the existence of a large variety of biologically active substances such as terpenoids, flavonoids, carotenoids, volatile oils, quinones, and amino acids[4; 5].Several studies suggested that extracts of *C. officinalis* may have actions that include the following properties: anti-inflammatory [6; 5; 7], antibacterial [8], antifungal [9], anticancer [7], hepatoprotective [10], antioxidant [11], wound healing effects following skin burns [4] and potential capacity to prevent UV irradiation-induced oxidative stress in skin [1]. The interest in the study of salt tolerant plants and/or in plants with assumed salt tolerance is still argued by theoretical reasons, and especially by the current context of human condition, regarded as a well-defined part of surrounding environment [12; 13]. Salinity has affected agriculture from millennia, having a deeply negative impact in agriculture and most likely, being involved in the fall of some ancient flourishing civilizations [14].

The Earth's total surface area covers about 13.2 billion ha, but no more than 7 billion ha are arable and 1.5 billion are cultivated [15]. Of the cultivated lands, about 340 million ha (23%) are saline (salt-affected) and another 560 million ha

(37%) are sodic (sodium-affected) [16]. Here are many different projections, suggesting that human population will increase over 8 billion by the year 2020 that will worsen the current scenario about food insecurity [17]. There are often not sufficient reservoirs of freshwater available and most of the agronomical used irrigation systems are leading to a permanent increase in soil-salinity and slowly to growth conditions unacceptable for most of the common crops [18].A global study of land use over 45 years found that 6 % had become saline [19]. Soil salinity expands, and some studies suggest that this process is almost irreversible and difficult to control. According to a FAO Repport [20], despite unprecedented global economic growth, 1.1. bilion people continue to live in extreme poverty and more than 850 milion people suffer from chronic hunger while ecosystems are being threatened as never before. Not accidentally the first goal of *The Millennium Development Goals Report* [21] is to eradicate extreme poverty and hunger.

This data suggests that salt tolerant plants should be taken into consideration, since they could play an important role in bio saline agriculture [14].

Therefore, the aim of this study is to investigate the antioxidant 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.

Material and methods

Plant material, treatment and growth conditions

Seeds of marigold (*C. officinalis*) were obtained from Agricultural Research and Development Station, Secuieni Neamt, Romania. Only intact seeds were chosen. These seeds were then sterilized with sodium hypochlorite 10% for 30 seconds and were washed with sterile distilled water. Consequently, marigold seeds were sown and germinated in plastic pots. After 7 days, salt treatments started, by adding 100 mL of salt solutions (or distilled water for the control variants) to pots once per week. Eleven treatments were applied (Table 1).

Table 1. Concentrations of salt treatments

1	50mM NaCl
2	50mM NaCl +10mM $CaCl_2$
3	50mM NaCl+ 20mM $MgCl_2$
4	100mM NaCl
5	100mM NaCl + 10mM $CaCl_2$
6	100mM NaCl+ 20 $MgCl_2$
7	150mM NaCl
8	150mM NaCl+10mM $CaCl_2$
9	150mM NaCl+20mM $MgCl_2$
10	10mM $CaCl_2$
11	20mM $MgCl_2$

The biochemical analyses were conducted at 24-days old seedlings; five different individuals corresponding of each treatment were selected for analysed parameters.

Photosynthetic pigment content assay

Chlorophyll *a*, chlorophyll *b* and carotenoids content assays were performed according to Lichtenthaler method [22]. Thus fresh leaves of samples were homogenized in 80% acetone and then were centrifuged at 4°C for 15 min (3000 rpm). Finally the volume was made to 5 mL and used then for the analysis. The absorbance was measured at 645, 663 and 470 nm respectively and pigment contents were evaluated and expressed in mg/g fresh weight (FW) using the following equations:

Chlorophyll *a* = (11.24×A662 - 2.04×A645)

Chlorophyll *b* = (20.13×A645 - 4.19×A662)

Carotenoids = [(1000×A470 - 1.9×Chl *a* - 63.14×Chl *b*)/214]

Total polyphenols and flavonoids assay

The total polyphenols content was determined through a slightly modified Folin - Ciocalteau method [23]. Folin-Ciocalteau reagent was added to appropriately diluted samples and mixed thoroughly. After four minutes, 15% Na_2CO_3 was added. After two hours, the absorbance of resulted coloured solution was determined at 765 nm, against the blank (distilled water). The amount of the total polyphenols content was expressed as mg gallic acid equivalents per g fresh weight (mg GAE g-1 FW) (R^2=0.99). The flavonoids content was measured following a spectrophotometric method [24]. Initially, 5% $NaNO_2$ solution was added to each test tube; after five minutes, 10% $AlCl_3$ solution was added followed by 1.0 M NaOH. Absorbance of resulting coloured solutions was read at 510 nm against the blank (distilled water). Flavonoids content was expressed as mg catechin equivalent per g fresh weight (mg CE g-1 FW) (R^2 = 0.98).

All experiments were carried out with three independent repetitions and the results were expressed as the mean values ± standard error (ES). The statistic significance of the differences between treated samples and control ones was assessed by means of the Student *t* test.

Results and discussions
Chlorophyll content

Obtained results show that chlorophyll content displays a variant and heterogeneous picture in respect to salt treatments. Surprisingly, increasing NaCl concentrations increase the content of chlorophylls *a* and *b* (Table 2). The combination of NaCl salt with calcium or magnesium chloride did not affect the pigments content, in terms of alleviating the effects of NaCl treatment. Only the combination of 50 mM NaCl with $CaCl_2$ induces a slightly increased content of chlorophyll *a* and *b*, comparatively with NaCl solely. The unique treatment with 10 mM $CaCl_2$ induced however a decreased content in chlorophyll *a* and *b* in plants treated both with NaCl solely and in combination with calcium chloride. Only at higher salinity (150 mM NaCl), but in combination with $CaCl_2$, the treatment with 10 mM $CaCl_2$ induced a slightly increase in chlorophylls content. Generally, the same is true for 20 mM Mg Cl_2, corresponding to a higher value of chlorophyll content, only comparatively with 50 mMNaCl treatment; nevertheless, in all cases when NaCl was combined with magnesium chloride, the chlorophyll content was lower than in plants subjected solely to 20 mM $MgCl_2$. This finding may be explained by the fact that in green leaves a major function of magnesium is its role as the central atom of the chlorophyll molecule [25]. However, at higher concentration of NaCl (150 mM), combined with magnesium chloride, the values remain lower, as compared with mixtures with 50 and 100 mM NaCl. In this case, perhaps the concentration of magnesium chloride was not efficient in order to counteract the effects of salt stress induced by NaCl.

Table 2. Variation in pigment content in seedlings of *C. officinalis* aftersalt treatments

	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
Martor	0.247±0.0238	0.075±0.013	0.0041±0.0005
50 mMNaCl	0.215±0.0366	0.068±0.014	0.0036±0.0008
50 mM+$CaCl_2$	0.266±0.0192	0.083±0.009	0.0046±0.000394
50 mM+$MgCl_2$	0.196±0.0143	0.063±0.0101	0.0034±0.00005
100 mMNaCl	0.269±0.0443	0.080±0.012	0.0048±0.00075
100 mM+$CaCl_2$	0.191±0.0075	0.067±0.006	0.0034±0.00039
100 mM+$MgCl_2$	0.195±0.031	0.061±0.012	0.0033±0.000635
150 mMNaCl	0.291±0.0488	0.095±0.019	0.0050±0.000553
150 mM+$CaCl_2$	0.158±0.027	0.052±0.005	0.0027±0.000712
150 mM+$MgCl_2$	0.185±0.038	0.061±0.012	0.0033±0.000941
10 mM $CaCl_2$	0.171±0.007	0.050±0.003	0.0031±0.00021
20 mM $MgCl_2$	0.234±0.028	0.068±0.011	0.0042±0.000488

Values are means of three replicates ± SE

It is well known that photosynthesis is the source of organic carbon and energy used by plants for their growth, biomass production and yield [26]. Photosynthetic rates are usually lower in plants exposed to salinity and especially to NaCl. For instance, increasing salinity in the growth medium decreases content of chlorophyll and the net photosynthetic rate, which is expressed more obviously in salt-sensitive species, such as alfalfa [27], canola [28], pea [29] and wheat [30], while it increased in tomato [31].

Non enzymatic antioxidants

Total polyphenols. The highest value was recorded for control plants (Table 3); increasing NaCl salinity decreases the content of polyphenols, with the smallest value in the case of 100 mM NaCl treatment (with the biggest inhibition rate by -39.08%). Actually, this is the single situation where both added calcium and magnesium chloride slightly increased the content of total phenolics, as compared with 100 mM NaCl solely. In other situations, under 50 and 150 mM NaCl, the added calcium and magnesium chloride generally do not act as alleviative factors. There is some evidence of the induction of phenolic metabolism in plants as a response to multiple stresses [32].

Table 3. Effect of NaCl, $CaCl_2$, $MgCl_2$ and their combination on total polyphenols content of *C. officinalis* (Values are means ± Standard error of 3 replicates); significance level: $P \leq 0.001$(*)

	Means values ± ES
Control	68,42 ± 0.07
50 mM NaCl	57,81 ± 0.43
50 mM NaCl+CaCl2	41,92 ± 0.057*
50 mM NaCl+MgCl2	47,20 ± 0.18
100 mM NaCl	41,68 ± 0.09
100 mM NaCl+CaCl2	45,97 ± 0.13
100 mM NaCl+MgCl2	48,97 ± 0.07
150 mM NaCl	52,94 ± 0.44
150 mM NaCl+CaCl2	53,89 ± 0.09
150 mM NaCl+MgCl2	46,87 ± 0.17
10 mM CaCl2	53,49 ± 0.29
20 mM MgCl2	40,45 ± 0.11

Total flavonoids. The content of total flavonoids is generally higher in plants subjected to salt stress than in control (Table 4). At 50 mM NaCl, the content of flavonoids is increased when calcium and magnesium chloride were added. The same was found for the mixture of 150 mM NaCl mixed with $CaCl_2$ when compared to 150 mM NaCl solely. Only under moderate salinity conditions (100 mM NaCl), the flavonoids content was found to be greater than in plants exposed to the mixture of sodium chloride and calcium or magnesium chloride. When treated with $CaCl_2$ and $MgCl_2$, the flavonoids content was higher than in control and sodium chloride treated plants (at 50 and 150 mM concentrations). However, this could suggest that flavonoids can be clearly involved in the salt stress responses in *Calendula* seedlings.

Table 4. Effect of NaCl, $CaCl_2$, $MgCl_2$ and their combination on flavonoids content of *C. officinalis* (Values are means ± Standard error of 3 replicates); significance level: $P \leq 0.001$(*),$P \leq 0.01$(**)

	Means values ± ES
Control	50,35648 ±0.,08
50 mM NaCl	56,17834 ± 0.04*
50 mM NaCl+CaCl2	62,55472 ± 0.01**
50 mM NaCl+MgCl2	70,15582 ± 0.14
100 mM NaCl	73,88137 ± 0.006**
100 mM NaCl+CaCl2	67,00352 ± 0.009**
100 mM NaCl+MgCl2	47,99161 ± 0.74
150 mM NaCl	57,38672 ± 0.44
150 mM NaCl+CaCl2	82,95483 ± 0.01**
150 mM NaCl+MgCl2	55,7546 ± 0.09
10 mM CaCl2	66,48131 ± 0.09
20 mM MgCl2	70,61536 ± 0.06

Since different stresses have in common the generation of reactive oxygen species [33], it has been postulated that flavonoids are synthesized to effectively counter the stress-induced oxidative damage [34]. Flavonoids may act as antioxidant factors by both preventing the generation of ROS (through their ability to chelate transition metal ions such as Fe and Cu [35; 36] and scavenging ROS when formed [37].

Unlike polyphenols, the role of flavonoids as antioxidants in plants under abiotic stress is well supported. Stress-responsive flavonoids have the greatest antioxidant potential, and the ratio of "effective antioxidant" to "poor antioxidant" flavonoids has been conclusively shown to increase gradually in response to many abiotic stresses [38; 39; 40].

The antioxidant enzymes are traditionally regarded as a first line of defense against stress-induced enhancement in ROS concentration. However, they have been reported to be ineffective to protect cells from oxidative damage during severe stress conditions [41]. Consequently, Hatier and Gould [41] have suggested that the conditions that lead to the accumulation of flavonoids are those that may inactivate key antioxidant enzymes. Salt stress has been reported to induce biosynthesis and accumulation of flavonoids in several species, such as: *Ligustrumvulgare*[42], *Oleaeuropaea*[43], *Oryza sativa* (salt-sensitive genotype) [44]. Nevertheless, a major question is if there is a relation between antioxidant enzymes and antioxidant flavonoids. There are several data suggesting that the biosynthesis of flavonoids is mostly upregulated under severe stress conditions, when the activities of antioxidant enzymes decline; therefore, flavonoids may complement

the action of other ROS – scavenging systems [34]. For instance, Xu et al. [45] have reported a higher accumulation of antioxidant enzyme proteins in soybean line with reduced flavonoid content. In *Ulvafasciata*, exposed to high UV-B doses that lead to the maximal flavonoid accumulation, the activity of antioxidant enzymes, particularly CAT and ascorbate peroxidase, declined significantly [46].

Conclusions

Salt treatments induced the increase in chlorophyll content, especially on moderate and severe stress intensity (100 and 150 mM NaCl). Under salt stress, significant differences occur in terms of polyphenols and flavonoids content.

References

[1] Fonseca, Y. M.; Catini, C. D.; Vicentini, F. T.M.C.; Nomizo, A.; Gerlach, R. F.; Fonseca M. J.V. Protective effect of Calendula officinalis extract against UVB-induced oxidative stress in skin: Evaluation of reduced glutathione levels and matrix metalloproteinase secretion. Journal of Ethnopharmacology, Vol.127, pp. 596-601. 2010.

[2] Aro, A.A.; Perez, M.O.; Vieira, C.P.;Esquisatto, M.A.M.; Rodrigues, R.A.F.; Gomes, L.; Pimentel, E.R. Effect of *Calendula officinalis*cream on Achilles tendon healing. The Anatomical Record, Vol. 298, pp. 428-435. 2015.

[**3] Borza, A.** Dicționaretnobotanic. Ed. Acad. Române, București, pp. 315. 1968.

[4] Muley, B.P.; Khadabadi, S.S.; Banarase, N.B. Phytochemical constituents and pharmacological activities of *Calendula officinalis*Linn (Asteraceae): a review. Tropical Journal of Pharmaceutical Research, Vol.8, No. 5, pp. 455–465. 2009.

[5] Barnes, J.; Anderson, L.A.; Phillipson, J.D. Herbal medicines (third ed.). Pharmaceutical Press, London, Chicago, pp. 710. 2007.

[6] Duke, J.A.; Bogenschutz-Godwin, M. J.; du Cellier, J.; Duke, P.A.K. Handbook of medicinal herbs (second Ed.), CRC Press, Boca Raton, London, New Tork, Washington, D.C., 870 pp. 2002.

[7] Ukiya, M.; Akihisa, T.; Yasukava, K.; Tokuda, H.; Suzuki, T.; Kimura, Y. Anti-inflammatory, anti-tumor-promoting and cytotoxic activities of constituents of marigold (*Calendula officinalis*) flowers. Journal of Natural Products, Vol.69, No. 12, pp. 1692–1696. 2006.

[8] Bissa, S.; Bohra, A. Antibacterial potential of pot marigold. Journal of Microbiology and Antimicrobials, Vol. 3, No. 3, pp. 51-54. 2011.

[9] Gazim, Z.C.; Rezende, C.M.; Fraga, S.R.; Svidzinski, T.E.; Cortez, D.G. Antifungal activity of the essential oil from *Calendula officinalis*L. (Asteraceae) growing in Brazil. Brazilian Journal of Microbiology, Vol.39, pp. 61–63. 2008.

[10] Rusu, M.A.; Tamas, M.; Puica, C.; Roman, I.; Sabadas, M. The hepatoprotective action of ten herbal extracts in CCl4 intoxicated liver. Phytotherapy Research, Vol.19, No. 9, pp.744–749. 2005.

[11] Popovic, M.; Kaurinovic, B.; Mimica-Dukic, N.; Vojinovic-Miloradov, M.; Cupic, V. Combined effects of plant extracts and xenobiotics on liposomal lipid peroxidation. Part 1. Marigold extract-ciprofloxacin/pyralene. Oxidation Communications, Vol.22, pp. 487–494. 1999.

[12] Grigore, M.- N. Romanian Salt Tolerant Plants. Taxonomy and Ecology. Edit. Tehnopress, Iasi, pp. 455. 2012

[13] Grigore, M.- N.; Oprică, L. Halophytes as possible source of antioxidant compounds, in a scenario based on threatened agriculture and food crisis. Iranian Journal of Public Health, Vol. 44, No. 8, pp. 1153-1155, 2015.

[14] Grigore, M.- N.; Toma, C. Halophytes, between the fall of civilizations and biosaline agriculture. Ecological disturbances over time. MuzeulOlteniei Craiova. StudiişiComunicări. ŞtiinţeleNaturii, Vol. 26, No. 2, pp. 199-204.2010.

[15] Massoud, F. I. Salt affected soils at a global scale and concepts for control. FAO Land and Water Develop. Div., Tech. Paper, Rome, pp. 21, 1981.

[16] Tanji, K. K. Salinity in the soil environment. In: Läuchli, A.; Lüttge, U. [Eds.] Salinity: Environment-Plants-Molecules, Kluwer Academic Publishers, New York, Boston, Dordrecht, London, Moscow, pp. 21-52. 2002.

[17] Athar, H.R.; Ashraf, M. Strategies for crop improvement against salinity and drought stress: an overview. In: Ashraf M., Ozturk M., Athar H. R. [Eds.] Salinity and water stress. Improving crop efficiency. Springer Science + Business Media B. V., pp. 1-18, 2009.

[18] Koyro, H. – W.; Geissler, N.; Hussin, S. Survival at extreme locations: life strategies of halophytes. In: Ashraf, M.; Ozturk, M.; Athar, H. R. [Eds.]Salinity and water stress. Improving crop efficiency, Springer Science + Business Media B. V. pp. 167-177. 2009.

[19] Ghassemi, F.; Jakeman, A.J.; Nix, H.A. Salinisation of Land and Water Resources. University of New South Wales Press Ltd, Canberra, Australia, 520 pp. 1995.

[20] FAO. The state of food and agriculture. Food and Agriculture Organization of the United Nations, Rome, Electronic Publishing Policy and Support Branch, Communication Divison, pp. 222. 2007.

[21] United Nations. The Millennium Development Goals Report. New York, Published by the United Nations Department of Economic and Social Affairs (DESA), pp. 52. 2008.

[**22] Lichtenthaler, H.K.** Chlorophylls and carotenoids: pigments of photosynthetic membranes. Methods in Enzymology, Vol. 148, pp. 350- 382.1987.

[23] Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, Vol. 299, pp. 152-178.1999.

[24] Dewanto, V.; Wu, X.; Adom, K.K.; Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry, Vol. 50, pp. 3010-3014. 2002.

[25] Marschner, H. Mineral nutrition of higher plants (second edition), Academic Press Harcourt Brace&Company, Publishers, London, San Diego, New York, Boston, Sydney, Tokyo, Toronto, pp. 889. 1995.

[26] Heuer, B. Photosynthetic carbon metabolism of crops under salt stress. In Pessarakli, M. [Ed.] Handbook of Photosynthesis (second edition), Taylor&Francis Group, Boca Raton, London, New York, Singapore, pp. 776-789, 2005.

[27] Khavarinejad, R.A.; Chaparzadeh, N. The effects of NaCl and $CaCl_2$ on photosynthesis and growth of alfalfa plants. Photosynthetica, Vol. 35, pp/ 461-466.1998.

[28] Qasim, M.; Ashraf, M.; Jamil, M.A.; Ashraf, M.Y.; Shafiq-Ur-Rehman; Rha, E.S. Water relations and leaf gas exchange properties in some elite canola (*Brassica napus*) lines under salt stress. Annals of Applied Biology, Vol. 142: 307-316. 2003.

[29] Hernandez, J.A; Campillo, A.; Jimenez, A.; Alarcon, J.J., Sevilla, F. Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytologist, Vol. 141, pp. 241–251.

[30] Zhu, X.G.; Wang, Q.; Zhang, Q.D.; Lu, C.M.; Kuang, T.Y. Response of photosynthetic functions of winter wheat to salt stress. Journal of Plant Nutrition and Fertilizer, Vol. 8, No. 2, pp. 177– 180. 2002.

[31] Romero-Aranda, R.; Soria, T.; Cuartero, J. Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Science, Vol. 160, No. 2, pp. 265–272. 2001.

[32] Michalak, A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies, Vol. 15, pp. 523–530.2006.

[33] Mittler, R. Abiotic stress, the field environment and stress combination. Trends in Plant Science, Vol. 11, pp. 15–19.2006.

[34] Di Ferdinando, M.; Brunetti, C.; Fini, A.; Tattini, M. In:Ahmad P, Prasad M.N.V. [Eds.] Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. Springer, New York, NY, USA, pp. 159–179. 2012.

[35] Brown, J.E.; Khodr, H.; Hider, R.C.; Rice-Evans, C.A. Structural dependence of flavonoid interactions with Cu^{2+} ions: implication for their antioxidant properties. Biochemistry Journal, Vol. 330, pp.1173–1178.1998.

[36] Agati, G.; Tattini, M. Multiple functional roles of flavonoids in photoprotecion. New Phytologist, Vol. 186, pp. 786–793.2010.

[**37] Ryan, K.G.; Swinny, E.E.; Markham, K.R.; Winefield, C.** Flavonoid gene expression and UV photoprotection in transgenic and mutant *Petunia* leaves. Phytochemistry, Vol. 59, pp. 23–32.2002.

[38] Kolb, C.A.; Käser, M.A. Kopecky, J.; Zotz, G.; Riederer, M.; Pfündel, E.E. Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant Physiology, Vol. 127, pp. 863–875. 2001.

[39] Lillo, C.; Lea, U.S.; Ruoff, P. Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. Plant, Cell& Environment, Vol. 31, pp. 587–601.2008.

[40] Kotilainen, T.; Tegelberg, R.; Julkunen-Tiitto, R.; Lindfors, A.; Aphalo, P.J. Metabolic specific effects of solar UV-A and UV-B on alder and birch leaf phenolics. Global Change Biology, Vol. 14, pp. 1294–1304.2008.

[41] Hatier, J.H.B.; Gould, K.S. Foliar anthocyanins as modulators of stress signals. Journal of Theoretical Biology, Vol. 253, pp. 625-627.2008.

[42] Agati, G.; Biricolti, S.; Guidi, L.; Ferrini, F.; Fini, A.; Tattini, M. The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in L. vulgare leaves. Journal of Plant Physiology, Vol. 168, pp. 204-212.2011.

[43] Remorini, D.; Melgar, J.C.; Guidi, L.; Degl'Innocenti, E.; Castelli, S.; Traversi, M.L.; Massai, R.; Tattini, M. Interaction effects of root-zone salinity and solar irradiance on the physiology and biochemistry of *Oleaeuropaea*. Environmental and Experimental Botany, Vol. 65, No. 2-3, pp. 210–219.2009.

[44] 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. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. Plant Physiology, Vol. 139, pp. 822–835.2005.

[45] Xu, C.; Sullivan, J.H.; Garrett, W.M.; Caperna, T.J.; Natarajan, S. Impact of solar Ultraviolet-B on the proteome in soybean lines differing in flavonoid contents. Phytochemistry, Vol. 69, pp. 38–48.2008.

[46] Shiu, C.T.; Lee, T.M. Ultraviolet-B-induced oxidative stress and responses of the ascorbate–glutathione cycle in a marine macroalgaUlvafasciata. Journal of Experimental Botany, Vol. 56, No. 421, pp. 2851–2865.2005.