

Biochemical Changes in Grapevines Roots in Responses to Osmotic Stress

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Abstract- We studied osmotic stress-induced biochemical changes in hydroponically grown plants of grapevine, *Vitis vinifera* (var. doukkali). Our focus was on the effect of peg (applied at 0,2, 0,4 or 0,8 MPa) on roots hydrogen peroxide, polyphenols, and proline

Osmotic stress induced an accumulation of proline and hydrogen peroxide in a dose dependent manner. The total amount of the phenolic increased after high level of stress (0,8MPa) and decrease under 0,2 and 0,4 MPa and start to increase after 24h. These findings could offer a partial explanation for the physiological role of the varying endogenous levels of proline that could govern the mechanism of osmotic stress tolerance in drought grapevine cv. Doukkali.

Index Terms- *Vitis vinifera*- hydrogen peroxide- osmotic stress- proline- polyphenols

Abbreviations: DW: dry weight- FW: fresh weight- PEG: polyethylene-glycol- Pro, proline; WC, water content.

I. INTRODUCTION

Grapevine an important economical crop is widely distributed throughout the arid and semiarid regions of the world. Grapevines grown in the Mediterranean zone are usually subject to water stress conditions caused by high evaporative demand and low soil water availability (Rodrigues *et al.*, 2008). It is also well known that grapevine varieties are characterized by high heterogeneity concerning their adaptation ability to such adverse environmental conditions (Schultz, 2003; Duan *et al.*, 2007). Doukkali cv. is a local variety cultivated in the region of doukkala (morocco) Characterised as semi arid region and wich know increasing salinity.

Drought and salt stress are the main abiotic stresses subjected to plants during their whole growth and development and are also one of the major causes of osmotic stress to plants under natural conditions (Xiong *et al.*, 2002). Plants under these conditions initiate some defense mechanism to reduce stress injury such as accumulation of organic compounds like polyamines and proline.

Proline is most common osmolyte and osmoprotectant in plants under stress conditions (Hasegawa *et al.*, 2000), and is often considered to be involved in stress resistance mechanisms and osmotic adjustment in stressed tissues of plant (Ashraf and Foolad, 2007). When exposed to drought or a high salt content in soil, many plants have been observed to accumulate high amounts of proline, (Mansour 2000). Trotel *et al.*, 1996 suggest

that proline is a nitrogen source available for recovery from stress and for restoration of growth. Additionally, it was shown that proline possesses antioxidant capacity, either as a potent ROS scavenger (Matysik *et al.* 2002) or by protecting and stabilizing ROS scavenging enzymes and activating alternative detoxification pathways (Szabados and Savouré 2010).

Therefore, this paper was aimed to study the changes occurring in proline, hydrogen peroxide and phenolic compounds in the roots of grapevine plants growing hydroponically under PEG osmotic stress.

II. MATERIAL AND METHODS

II.1. Plant material and osmotic treatments

The study was carried out with hydroponically grown grapevine cuttings (*Vitis vinifera* L.) var. Doukkali, a high-quality table grape variety in Morocco. Hardwood cuttings of grapevines were collected at the dormant bud stage from the vineyard of Doukkala (El jadida, Morocco) and planted in sand for rooting in growth chamber at 28°C, 70% humidity, and 16h/8h photoperiod. After 4-months, the well-developed healthy plants were transferred to hydroponic culture containing the Hoagland nutrient solution (Hoagland, ref). The nutrient solution was renewed every 4 days. Plants were grown in the same conditions as described above. For osmotic treatments, PEG-20000 was added to the nutritive solution in order to obtain a final osmotic potential of -0.2 MPa, -0.4 MPa and -0.8 MPa. Samples were taken after 0, 6, 12, 24 and 48h and stored at -20°C until use or immediately used for PAs extraction.

II.2. Determination of water content (WC)

In addition to fresh weight (FW), dry weight (DW) was determined after desiccation at 70°C for 48 h. Leaf water content was estimated using the equation: $WC = (FW - DW/FW) * 100$.

II.3. Hydrogen peroxide content

Hydrogen peroxide was measured by using Sergiev *et al.*, (1997) method. Samples (300 mg) were extracted with 3 ml of 0.1 % trichloroacetic acid (TCA). After centrifugation at 4500 rpm during 20 min an aliquot (0,5ml) of the supernatant was added to 1 ml of 1 mM potassium iodure and 10 mM phosphate buffer, pH7 (1:2; v/v). H₂O₂ concentration of the supernatant was evaluated by comparing its absorbance at 390 nm to a standard calibration curve.

II.4. Proline content

The free proline was measured by using Bates et al., (1973) method. Fresh plant material (300 mg) was homogenized in 40% methanol. After centrifugation at 4500 rpm during 20 min the supernatant was reacted (at 100°C) with ninhydrin and glacial acetic acid for 1h, and then kept in an ice bath. The chromophore was extracted with toluene and warmed to room temperature and then absorbance was recorded at 528 nm using L-proline as a standard.

II.5. Determination of total phenolic contents

The total phenolic compounds were extracted with a solution of ethanol/water/ chloroform (1v/1v/2v) using an Ultra Turrax homogenizer following the protocol described by Darné et al. (1979). The water-ethanol phase containing phenolic compounds was separated from the organic phase containing lipids, chlorophylls and other pigments. The ethanol was removed from the aqueous-phase using a rotavapor at low pressure at 35°C. The

aqueous solution containing the phenolic compounds was adjusted to a desired volume. Phenolic compounds were determined using Folin–Ciocalteu reagent method by reading the absorbance at 765 nm according to the method of Ainsworth and Gillespie (2007). Gallic acid was used as a standard and the results were expressed as milligrams of gallic acid equivalent (GAE)/ g of fresh weight.

III. RESULTATS

III.1. Induced changes in WC

WC in the leaves decreased significantly with the increase of PEG concentration when compared with the control ($P < 0.05$) (Figure 1). This effect was more severe with -0.8 MPa and after 48h of treatment.

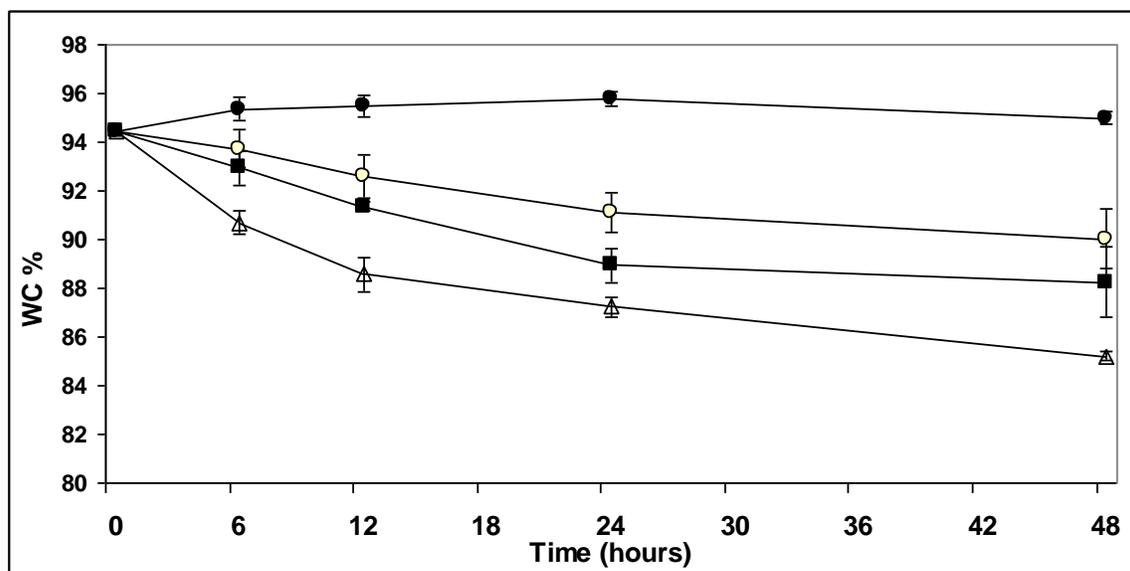


Figure 1. Time course change of water content in grapevine roots subjected to different levels of PEG-induced osmotic stress. PEG was added to the nutritive solution of hydroponic cultures in order to obtain a final osmotic potential of -0.2 MPa (○), -0.4 MPa, (■) and -0.8MPa (△). For control (●) no PEG was added. Values represent the means (\pm SE) of three replicates.

III.2. Induced changes in Proline content

Time course change of free Pro accumulation in response to different concentrations of PEG is shown in Figure 2. In response to PEG, Pro content started to increase after 6h of treatment. Pro accumulation increased with increasing PEG

concentration. It reached a maximum after 24h of treatment and its level started to decrease thereafter. This increase was highly noticeable with the highest osmotic pressure used (-0.8 MPa).

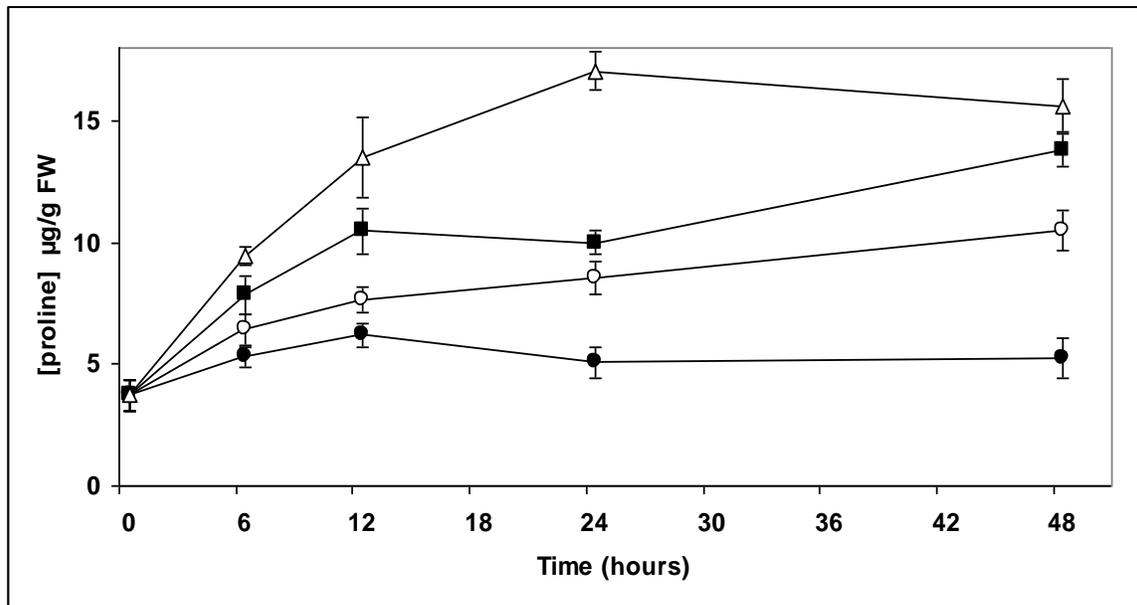


Figure 2. Time course change of free proline accumulation in grapevine roots subjected to different levels of PEG-induced osmotic stress. PEG 20000 was added to the nutritive solution of hydroponic culture in order to obtain a final osmotic potential of -0.2 MPa (○), -0.4 MPa (■) and -0.8 MPa (△). For control (●) no PEG was added. Values represent the means ($\pm\text{SE}$) of three replicates.

III.3. Induced changes in Hydrogen peroxide content

As shown in figure 6, PEG induced an increase in H_2O_2 contents in roots, in a dose-dependent manner. Under 0.8 MPa H_2O_2 accumulation was enhanced after 6h of stress and reached a maximum after 48 h and continue to increase (Figure 3).

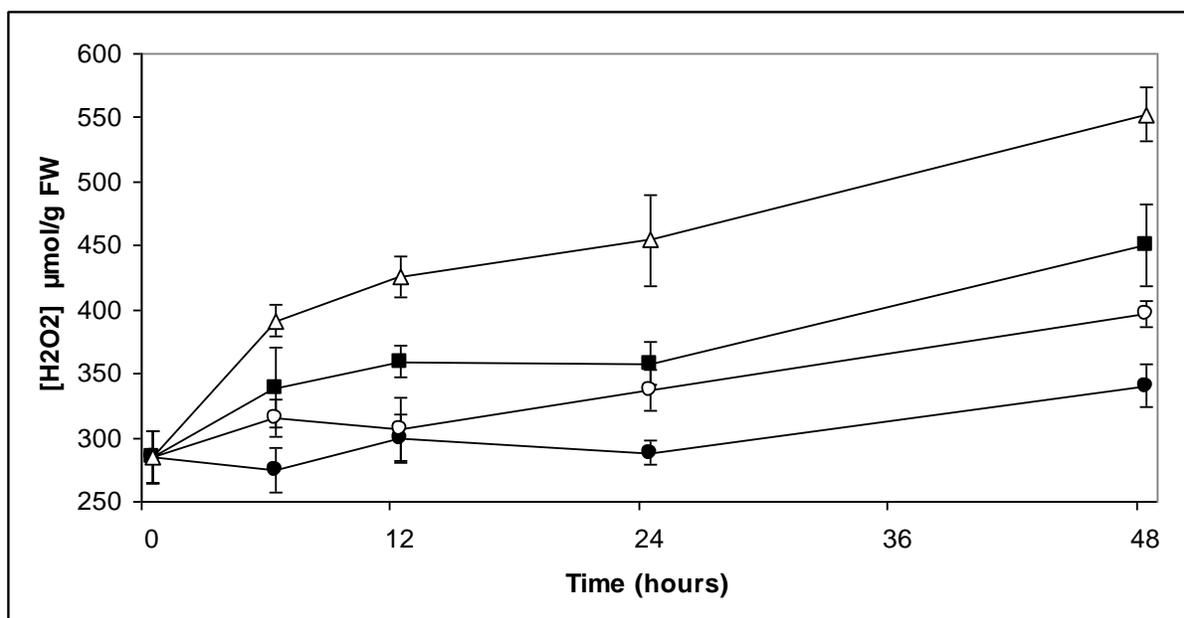


Figure 3. Time course change of hydrogen peroxide accumulation in grapevine roots subjected to different levels of PEG-induced osmotic stress. PEG was added to the nutritive solution of hydroponic cultures in order to obtain a final osmotic potential of -0.2 MPa (○), -0.4 MPa (■) and -0.8 MPa (△). For control (●) no PEG was added. Values represent the means ($\pm\text{SE}$) of three replicates.

III.4. Induced changes in total polyphenols content

The effect of osmotic stress on total phenols is shown in figure 4, at 0,8 MPa gave the highest content of total phenol after

24h of treatment. Under 0,2 and 0,4 MPa a slight decrease in TPC was observed in the beginning of the experiment and start to increase after 24h (Figure 4).

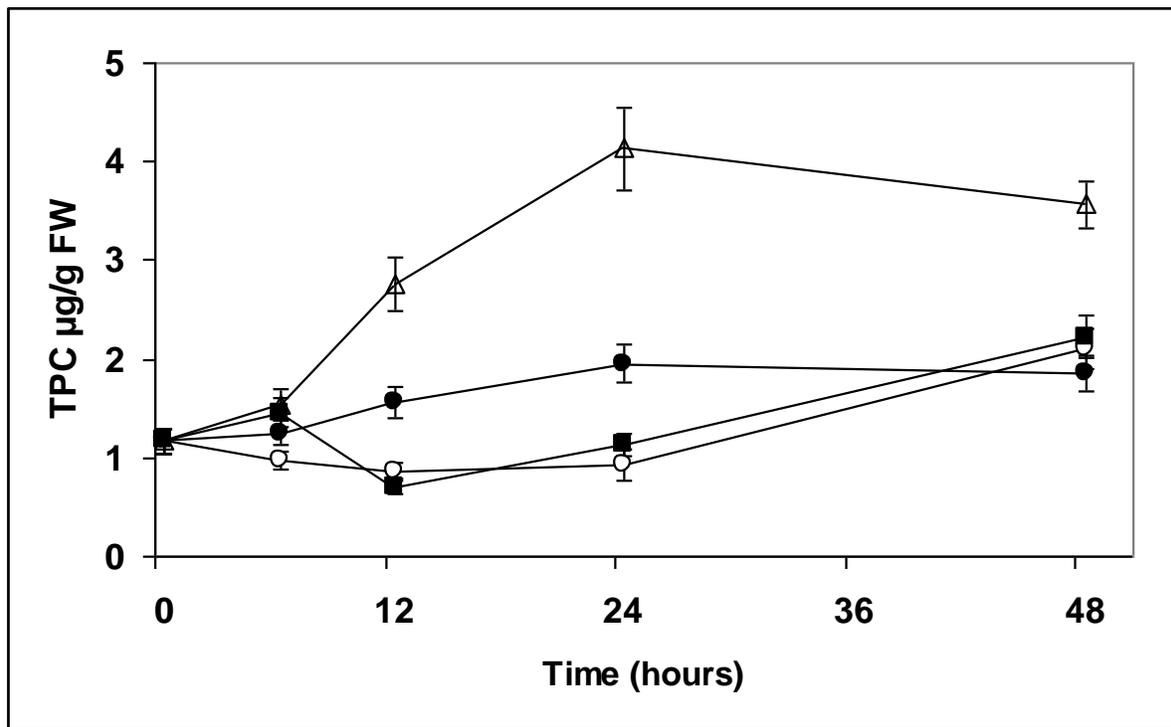


Figure 4. Time course change of polyphenols accumulation in grapevine roots subjected to different levels of PEG-induced osmotic stress. PEG was added to the nutritive solution of hydroponic cultures in order to obtain a final osmotic potential of -0.2 MPa (○), -0.4 MPa (■) and -0.8 MPa (△). For control (●) no PEG was added. Values represent the means (\pm SE) of three replicates.

IV. DISCUSSION

When grapevine was exposed to 0.2, 0.4 and 0.8 PEG for 2 days, the proline and hydrogen peroxide content in roots increased with PEG concentration (Fig. 2, 3), while the WC decreased with the increase of PEG concentration. Those results showed that the degree of plant injury of grapevine under water deficit increased with the PEG concentration and stress duration.

In this study, we show, using the whole plant system of grapevine that a conspicuous effect of salt stress on proline titers may already be observed after 6 h of stress. Plants accumulate compatible solutes, such as proline, in response to stresses to facilitate water uptake (Ashraf and Foolad, 2007). Proline accumulation was correlated to a variety of stress conditions and is now regarded as a major non-enzymatic antioxidant (Szabados and Saviouré, 2010) The accumulation of compatible solutes may help to maintain the relatively high water content necessary for plant growth and cellular function (Kishor *et al.*, 1997) And was associated to salt stress tolerance (Ramanjulu, and Sudhakar 2001; Madan *et al.* 1995. Giridara Kumar *et al.*, 2003) and water stress tolerance (Toumi *et al.*, 2007). In the present study, proline content increased with increase in PEG concentration (Figure 2). This increase could be explained by the activation of biosynthetic enzyme such as P5CS, which is the key regulatory and rate

limiting stress-inducible enzyme in proline biosynthetic pathway (Vaseva *et al.*, 2012), Activation of P5CS resulted in free proline accumulation in drought-stressed plants, in accordance with previous reports (Yamada *et al.*, 2005), or possible involvement and activation of other enzymes involved in proline biosynthesis, such as P5CR (Szabados and Saviouré, 2010), or the possible down-regulation of enzymes involved in proline catabolism. this increase of proline content with increase in severity and duration of stress helped the plants to maintain tissue water status (Figure1) and avoid the drought induced damages (Jiang and Huang, 2002). These results support a direct correlation between the degree of osmotic stress and proline accumulation, which is in agreement with previously studies revealing that the level of proline increased in parallel with the severity of environmental stress in different plant species (Chen and Kao, 1995; Sofu *et al.*, 2004; Claussen 2005) and that proline concentration could be used as a biochemical marker for drought stress level (Sofu *et al.*, 2004).

Phenol accumulation could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress (Mohamed and Aly, 2008). Several studies have reported that total phenol production is stimulated by salt and drought stress (Hanan *et al.*, 2008; Muthukumarasamy *et al.*, 2000).

V. CONCLUSION

The result of this experiment indicates that osmotic stress caused a number of biochemical changes in grapevine plant, including decreased water content, and increased proline content and total phenolic compounds. The increased synthesis of proline, total phenolic content and earlier in stressed plants exhibited a protective mechanism against the cellular structures from oxidative damage.

REFERENCES

- [1] Ashraf M., Foolad M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp.Bot.*, 59, 206–216.
- [2] Ainsworth E. A., and Gillespie K. M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols.*, 2, 875–877.
- [3] Bates L.S., Waldren R.D., Teare I.D., 1973. Rapid determination of free proline for water stress studies. *Plant and Soil.*, 39, 205–207.
- [4] Chen S.L., Kao C.H., 1995. Cd induced changes in proline level and peroxidase activity in roots of rice seedlings. *Plant Growth Regul.*, 17, 67–71.
- [5] Claussen W., 2005. Proline as a measure of stress in tomato plants. *Plant Sci.*, 168, 241–248.
- [6] Duan B., Yang Y., Lu Y., Korpelainen H., Berninger F., Li C., 2007. Interactions between water deficit, ABA, and provenances in *Picea asperata*. *J. Exp.Bot.*, 58, 3025–3036.
- [7] Giridara Kumar S., Matta Reddy A., Chinta Sudhakar., 2003. NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. *Plant Sci.*, 165, 1245–1251.
- [8] Hasegawa P.M., Bressan R.A., Zhu J.K., Bohnert H.J., 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Mol. Biol.*, 51, 463–499.
- [9] Hanen F., Ksouri R., Megdiche W., Trabelsi N., Boulaaba M., Abdelly C., 2008. Effect of salinity on growth, leaf phenolic content and antioxidant scavenging activity in *Cynara cardunculus* L. In: *biosaline agriculture and high salinity tolerance*. Abdelli C, Ozturk M, Ashraf M, Grignon YC, (Eds.). Birkhauser Verlag, Switzerland, pp: 335-343.
- [10] Jiang Y. and Huang B., 2002. Protein alterations in tall fescue in response to drought stress and abscisic acid., *Crop Sci.* 42, 202- 207.
- [11] Kishor P.B.K., Hong Z., Miao G.H., Hu C.A.A., Verma D.P.S., 1995. Overexpression of pyrroline-5-carboxylate synthetase increase pro-line production and confers osmotolerance in transgenic plants. *Plant Physiol.*, 108, 1387–1394.
- [12] Madan S., Nainawate H.S., Jain R.K., Chowdhury J.B., 1995. Proline and proline metabolising enzymes in in vitro selected NaCl-tolerant *Brassica juncea* L. under salt stress. *Ann. Bot.*, 76, 51–55.
- [13] Madan S., Nainawate H.S., Jain R.K., Chowdhury J.B., 1995. Proline and proline metabolising enzymes in in vitro selected NaCl-tolerant *Brassica juncea* L. under salt stress. *Ann. Bot.*, 76, 51–55.
- [14] Mansour MMF., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol Plant.*, 43, 491–500.
- [15] Matysik J., Alia A., Bhalu B., Mohanty P., 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr Sci.*, 82, 525–532.
- [16] Mohamed AA., Aly AA., 2008. Alternations of some secondary metabolites and enzymes activity by using exogenous antioxidant compound in onion plants grown under seawater salt stress. *American-Eurasian Journal of Scientific Research.*, 3, 139-146.
- [17] Muthukumarasamy M., Gupta S.D., Pannerselvam R., 2000. Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L. *Biol. Plant.*, 43, 317-320.
- [18] Ramanjulu S., Sudhakar C., 2001. Alleviation of NaCl salinity stress by calcium is partly related to the increased proline accumulation in mulberry (*Morus alba* L.) callus. *J. Plant Biol.*, 28, 203–206.
- [19] Rodrigues M.L., Santos T.P., Rodrigues A.P., Souza C.R., Lopes C.M., Maroco J.P., Pereira J.S., Chaves M.M., 2008. Hydraulic and chemical signaling in the regulation of stomatal conductance and plant water use in field grapevines growing under deficit irrigation. *Funct. Plant Biol.*, 35, 565–579.
- [20] Schultz H.R., 2003. Differences in hydraulic architecture account for near isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant Cell Environ.*, 26, 1393–1405.
- [21] Sergiev L., Alexieva E., Karanov E., 1997. Effect of spermine, atrazine and combination between them on some endogenous protective systems and markers in plants. *Compt. Rend. Acad. Bulg.Sci.*, 51, 121-124.
- [22] Sofo A., Dichio B., Xiloyannisa C., Masia A., 2004. Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. *Physiol Plant.*, 12, 158–65.
- [23] Szabados, L., Savouré, A., 2010. Proline: a multifunctional amino acid. *Trends in Plant Sci.*, 15, 89–97.
- [24] Toumi I., M'Sehli W., Bourgo S., Jallouli N., Bensalem-Fnayou A., Ghorbel A., and Mliki A. 2007. Response of ungrafted and grafted grapevine cultivars and rootstocks (*Vitis* sp.) to water stress. *Int J of Vine and Wine Sci.*, 41(2), 85-93.
- [25] Trodel P., Bouchereau A., Niogret M.F., Larher F., 1996. The fate of osmo-accumulated proline in leaf discs of rape (*Brassica napus* L.) incubated in a medium of low osmolarity. *Plant Sci.*, 118, 31-45.
- [26] Vaseva I., Akiscan Y., Simova-Stoilova L., Kostadinova A., Nenkova R., Anders I., Feller U., Demirevska K., 2012. Antioxidant response to drought in red and white clover. *Acta Physiol Plant.*, 34, 1689–1699.
- [27] Veal E.A, Day AM, Morgan BA. 2007. Hydrogen peroxide sensing and signaling. *Mol Cell.*, 26, 1-14
- [28] Yamada M., Morishita H., Urano K., Shiozaki N., Yamaguchi-Shinozaki K., Shinozaki K., Yoshida Y., 2005. Effects of free proline accumulation in petunias under drought stress. *J. Exp Bot.*, 56, 1975–1981.

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