Plant Archives Vol. 20 Supplement 1, 2020 pp. 1029-1034 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IN VITRO EVALUATION OF SOME GRAPEVINE ROOTSTOCKS GROWN UNDER DROUGHT STRESS

A.T. Mohsen, R.G. Stino, A.M. Abd Allatif and N.M. Zaid

Pomology Department, Faculty of Agriculture, University of Cairo, Giza, Egypt.

Abstract

The use of rootstocks is a key element to face the problem of water shortage. For this reason, the aim of the present investigation was to evaluate *in vitro* grown grapevine rootstocks for drought tolerance using polyethylene glycol as water stress stimulator. The evaluated rootstocks were grown in DWK media supplemented with different PEG concentrations (0, 1.5%, 3%, 4.5% and 6% PEG). Four rootstocks Paulson 1103 (1103P), Ruggeri 140 (140 RU), Ramsey, and Dog Ridge were used. Shoot length, node number, leaves number/shoot, shoot fresh weight, total chlorophyll and surviva l% were decreased, while total proline, defoliation % and DSI were found to be increased gradually in response to increasing PEG concentration. At the end of this investigation the studied rootstocks can be ordered from the drought tolerance perspective as follow; Dog ridge is the more sensitive rootstock where it began to break down after 20 days of stress, then 1103P and 140RU are considered a moderate tolerance where it began to break down after 30 days of stress, the best rootstock in this investigation is Ramsey where it began to break down after 60 days of stress.

***Key words*:** Grapevine rootstocks, Micropropagation, Water stress, Polyethylene glycol,

Introduction

Abiotic stresses including drought, salinity and heat are the major environmental factors which affect plant growth, productivity and commonly constitute serious threats to agriculture production (Shashidhar *et al.,* 2013). The impact of climate change on rainfall patterns, salinization of agricultural lands leads to increase the attention of the abiotic stress and its effects on plants. Drought and salinity are particularly widespread in the Mediterranean zone and by the year 2050 may cause serious salinization of more than 50% of all arable lands (Wang *et al.,* 2003). The drought stress can define as a decreasing of the soil available water, this decreasing makes it more difficult for the plant to uptake water (Shashidhar *et al.,* 2013). Drought stress leads to a series of morphological, physiological, biochemical and molecular changes that negatively affect plant growth and productivity (Jaleel *et al.,* 2009). The use of grapevine rootstocks were mainly for phylloxra and nematode resistance; however, several other characteristics are also required, such as rooting, grafting compatibility, its effect on vine vigour, yield and fruit quality and tolerance to drought and salinity are also considered (Granett *et al.,* 2001, Koundouras *et al.,* 2008). Grapes are commonly grown in semi-arid environments, where drought is a series problems (Cramer *et al.,* 2007). Because of differences in root system properties, drought tolerance of plants is significantly influenced by rootstocks (Pavlousek, 2011). The grapevine rootstocks response to drought stress tolerance depends on their genetic structure (Sommer, 2009). Hence, selection and evaluate of genotypes that will survive under drought conditions is a potential solution to overcome drought problem. The traditional evaluation method is time consuming, need wide area and affected by environmental condition. Tissue culture offers opportunities to study plant responses to drought stress and determine the tolerance level. PEG has been widely used to impose water stress on plants (Lawlor, 1970). The addition of PEG to the tissue culture media can be used successfully to decrease the water potential (Gopal and Iwama, 2007). PEG were successfully used to *in vitro* screening of drought tolerant in almond genotypes (Karimi *et al.,* 2012) and olive (Shibli and Al-Juboory, 2002). PEG is one of the reliable agent for drought screening than the often used solute mannitol because its dose not has a toxic effects on plant growth (Hohl and Schopfer, 1991, Verslues *et al.,* 1998). PEG has a high

molecular weight and does not enter the apoplast, therefore, PEG simulate the soil drying in a similar way compared to other stress agent (Nepomuceno *et al.,* 1998). In the light of above discussion, the present study was designed to investigate the relative drought tolerance of some grape rootstocks under *in vitro* condition using PEG reagent.

Material and Methods

Plant materials and growth conditions: This study was conducted in Tissue Culture Laboratory, Pomology department, Faculty of Agriculture, Cairo University, Giza, Egypt, during 2018-2019. Four grapevine rootstocks Paulson 1103 (1103P, *Vitisberlandieri X V. rupestris*) Ruggeri 140 (140 RU, *Vitisberlandieri X V. rupestris*), Ramsey (63Salt Creek, *Vitischampinii*) and Dog Ridge (*Vitischampinii*) were used. The newly sprouted, vegetative shoots of the rootstocks were collected, stripped of leaves, washed with tap water and divided into single node cutting. Node cuttings were then surface sterilized for 10 min in sodium hypochlorite (1% v/v), followed by mercuric chloride (0.1% w/v) for 8 min and finally rinsed three times with sterile distilled water. The single-node cuttings were cultured on solid DKW (Driver and Kuniyuki, 1984) supplemented with 0.7 mg l^{-1} 6-benzyladenine, 30 g l^{-1} sucrose and 6.5 g l^{-1} agar. Media pH was adjusted to 5.7-5.8 before agar adding. The media was disturbed on glass jars (40ml per jar) and media were autoclaved at 121°C for 15 min. All of the cultures were incubated at 25 ± 1°C under a 16 h photoperiod, with light supplied by white fluorescent tubes (40-60 µmol m^{-2} s^{-1}). After three weeks, the sprouted buds were segmented to single node and subculture on fresh media of the same composition for another three weeks to obtain homogeneous shoots with appropriate length and leaves number.

Polyethylene glycol treatment: Homogeneous shoots obtained from the 2nd subculture, were transferred to fresh DKW basal medium supplemented with 0.7 mg l^{-1} 6-benzyladenine, 30 g l^{-1} sucrose, 6.5 g l^{-1} agar and different PEG levels (0, 1.5, 3, 4.5 and 6 % PEG 6000). At the end of the stress exposed period the shoots were removed from the culture media and gently washed with tap water and the following measurements were recorded; survival percentage, defoliation percentage drought severity index**:** visible symptoms of drought injury in shoots were observed and the severity index was calculated according to the following formula (Booth, 1970),

$$\text{DSI} = \frac{(A\times 0) + (B\times 1) + (C\times 2) + (D\times 3) + (E\times 4)}{M}$$

where A is the number of healthy shoots, B is the number of shoots with chlorosis leaves, C is the number of shoots with necrosis leaves, D is the number of wilting shoots, E is the number of dead shoots and M is the total number of shoots (Cirulli *et al.,* 2008).

Growth analysis: Shoot length, node number, green leaves number per shoot and shoot fresh weight were measured at the end of stress period for each rootstock in different PEG concentrations.

Chemical analysis: Total chlorophyll was analysed following Lichtenthaler and Wellburn (1983) method using 0.25 g fresh leaves sample immersed in 20 mL of 80% acetone. Free proline was determined in 0.5 g of fresh leaves sample using the ninhydrin method (Bates, 1973). Proline concentration was expressed as µmole proline/g FW.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) using the SAS software (version 9.0, SAS Institute, Cary, NC). The mean and standard error (SE) were calculated from three replicates per treatment. Mean values per rootstocks were calculated and the corresponding SE was calculated. The significance of the differences among control and drought stress treatments for each rootstock was evaluated with Duncan range test (Duncan, 1955). One way ANOVA was performed using the rootstock means of both control and drought stress conditions in order to detect the effect of drought stress level within the rootstock, as well as between the rootstocks.

Results and Discussion

The end of the stress period was determined according to the drought severity index and survival % (Table 1). Ramsey, 1103P, 140RU and Dog Ridge were harvested after 60, 30, 30 and 20 days of water stress, respectively (Fig. 1).

The harvest of the shoots was done when the DSI reached or exceeded 4. For 140RU, the harvest was a little bit late because the survival % was still high compared to 1103P. A noticeable decrease in survival % and increasing of defoliation % and DSI was shown in water stressed-plants for all rootstocks. The response was gradually in response to water stress which has been stimulated by increasing PEG %. The highest defoliation % values were seen on 6% PEG level to be 57.48, 39.17, 37.10 and 33.41% for Dog ridge, 1103P, Ramsey and 140RU respectively. Concerning Survival %, the lowest values were recorded in 6% PEG treatment values to be 61.21, 62.5, 75 and 83.27% for 1103P, 140RU, Dog ridge and Ramsey respectively. Regarding DSI, the highest values were noted in 6% PEG treatment values to be

Table 1: The effect of the rootstock and PEG on survival % and drought symptoms.

Rootstock	PEG	Severity index	Survival %	Defoliation%
	Control	0.00 ± 0.00 e	100 ± 0.00 a	0.00 ± 0.00 c
	1.5%	1.92 ± 0.19 d	100 ±0.00 a	16.20 ± 1.09 b
Ramsey	3%	2.58 ± 0.52 c	100 ± 0.00 a	32.01 ± 1.56 a
	4.5%	3.17 ± 0.19 b	100 ±0.00 a	34.50 ± 1.23 a
	6%	4.00 ± 0.21 a	83.27 ±4.39 b	37.10 ± 2.37 a
Mean		2.34 b	96.654 a	23.962 b
	Control	0.00 ± 0.00 d	100± 0.00 a	0.00 ± 0.00 e
	1.5%	0.28 ± 0.19 d	100± 0.00 a	19.89 ± 1.55 d
Dog Ridge	3%	1.46 ± 0.50 c	100± 0.00 a	26.61 ± 0.24 c
	4.5%	2.57 ± 0.60 b	93.3± 10.64 b	32.82 ± 2.26 b
	6%	4.05 ± 0.14 a	75± 0.00 c	57.48 ± 3.66 a
Mean		1.67 c	93.67 b	27.361 a
	Control	0.00 ± 0.00 c	100± 0.00 a	0.00 ± 0.00 d
	1.5%	2.82 ± 0.24 b	90.3± 0.525 b	3.20 ± 0.045 c
1103P	3%	3.41 ± 0.17 b	83.08± 2.67 c	14.25 ± 3.62 b
	4.5%	4.44 ± 0.32 a	68.79± 4.66 d	18.27 ± 1.04 b
	6%	4.70 ± 0.61 a	61.21± 2.102 e	39.17 ± 3.004 a
Mean		3.07 a	80.677 d	14.978 c
	Control	0.00 ± 0.00 e	100± 0.00 a	0.00 ± 0.00 d
	1.5%	2.42 ± 0.07 d	100± 0.00 a	4.77 ± 0.914 c
140RU	3%	3.34 ± 0.36 c	87.50± 0.00 b	5.00 ± 0.00 c
	4.5%	4.04 ± 0.26 b	75± 0.00 c	10.30 ± 2.35 b
	6%	5.71 ± 0.59 a	62.5± 0.00 d	33.41 ± 5.32 a
Mean		3.10 a	85 c	10.697 d

Values followed by different letters within the root stock are significantly different (P < 0_05). Mean values of different rootstocks followed by bold different letters are significantly different (P < 0_05)

5.71, 4.70, 4.05 and 4% for 140RU, 1103P, Dog ridge and Ramsey respectively. The obtained results showed that osmotic stress affected the whole plant growth, which may be due to the reduction of cell division and expansion under water deficit (Taiz and Zeiger, 2002). Moreover, drought disrupts plant physiological parameters and changes leaf water status (Boyer, 1982, Chartzoulakis *et al.,* 1999).

The growth of shoots as well as the number of node per shoot, the number of green leaves per shoot and shoot fresh weight from water stressed plants was significantly reduced in PEG treatments comparing to control (Table 2). This reduction was gradually in response to severity of water stress. According to the results Dog Ridge rootstock was highly sensitive for drought stress. In contrast, Ramsey rootstock recorded higher values in all parameters except the shoot length, it is probably due to the growth pattern of Ramsey which tends to branch. 1103P and 140RU seems to be very nearly from drought stress tolerance perspective, but 1103P was superior to 140RU on the number of green leaves per shoot, shoot fresh weight parameters. Reduction of growth parameters for grapevine exposed to drought stress has also been reported earlier (Bertamini *et al.,* 2006, Cramer *et al.,* 2007, Lebon *et al.,* 2006, Palliotti *et al.,* 2008, Pavlousek, 2011, Pellegrino *et al.,* 2005, Wani *et al.,* 2013). The length of plant shoots represents a sensitive indicator of water regime in grapevine plants (Lebon *et al.,* 2006, Pellegrino *et al.,* 2005). In addition, the reduction in leaf and shoot growth is one of grapevine water deficit signs (Stevens *et al.,* 1995).

Under drought stress condition, water deficiency can inhibit the cell elongation by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998). Also, the water stress reduced mitosis, cell elongation and expansion and cause growth reduction (Hussain *et al.,* 2008). Drought-induced reduction in the number of leaves per plant and cause a reduction in photosynthesis (Hussain *et al.,* 2008). Therefore the fresh and dry biomass production for the water-stressed plants is reduced (Zhao *et al.,* 2006).

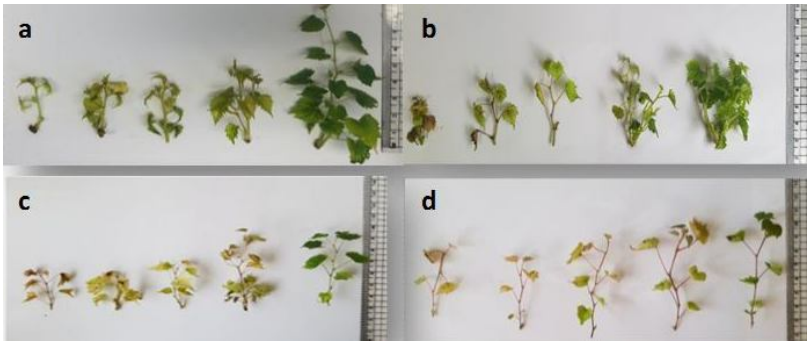

Fig. 1: Rootstocks growth and drought symptoms with different PEG % treatment.

Control, 1.5%, 3%, 4.5% and 6% respectively from right to left. (a) Ramsey after 60days of drought stress (b) Dog Ridge after 20days of drought stress (c) 1103P after 30days of drought stress (d) 140RU after 30days of drought stress.

The marked reduction of total chlorophyll in water-stressed plants was shown in (Fig. 2). The reduction was gradually in response to increasing drought stress. The lowest total chlorophyll value was in dog Ridge rootstock (0.027µg .g^{-1}), followed by Ramsey (0.036µg .g^{-1}) and the highest values were in 1103P

Table 2: The effect of the rootstock and PEG % on the growth and plant morphology.

Rootstock	PEG	Plant length	Node no.	Green leaf no.	Shoot FW
	Control	6.17 ± 0.38 a	17.33 ± 3.33 a	22.42 ± 2.63 a	1.10 ± 0.10 a
	1.5%	3.70 ± 0.42 b	14.58 ± 1.88 a	14.92 ± 2.38 b	0.81 ± 0.05 b
Ramsey	3%	3.53 ± 0.22 bc	14.83 ± 2.89 a	11.42 ± 2.38 bc	0.78 ± 0.02 b
	4.5%	2.92 ± 0.14 cd	12.25 ± 2.00 ab	9.42 ± 0.80 c	0.41 ± 0.06 c
	6%	2.53 ± 0.56 d	9.17 ± 2.13 b	7.25 ± 1.80 c	0.29 ± 0.07 c
Mean		3.77 b	13.63 a	13.08 a	0.68 a
	Control	4.47 ± 0.44 a	9.67 ± 0.38 a	12.67 ± 2.13 a	0.24 ± 0.03 a
	1.5%	3.63 ± 0.26 b	6.83 ± 0.52 b	7.67 ± 0.58 b	0.16 ± 0.02 b
Dog Ridge	3%	3.16 ± 0.04 bc	6.58 ± 0.88 bc	6.42 ± 0.38 bc	0.15 ± 0.05 bc
	4.5%	2.79 ± 0.01 cd	5.50 ± 0.66 cd	5.75 ± 1.39 bc	0.12 ± 0.02 bc
	6%	2.55 ± 0.18 d	5.25 ± 1.00 d	3.67 ± 1.23 c	0.10 ± 0.02 c
Mean	3.32 c	6.77 b	7.23 d	0.15 d	
	Control	8.79 ± 0.40 a	10.25 ± 0.50 a	15.75 ± 1.39 a	0.34 ± 0.00 a
	1.5%	6.29 ± 0.75 b	7.50 ± 0.66 b	15.00 ± 0.25 a	0.33 ± 0.00 a
1103P	3%	5.17 ± 0.31 c	7.67 ± 0.76 b	10.17 ± 1.42 b	0.27 ± 0.02 b
	4.5%	4.25 ± 0.50 cd	6.00 ± 0.00 c	7.75 ± 1.75 bc	0.20 ± 0.03 c
	6%	3.84 ± 0.40 d	5.42 ± 0.38 c	5.67 ± 1.13 c	0.14 ± 0.04 d
Mean	5.67 a	7.37 b	10.87 b	0.26 b	
	Control	7.67 ± 0.36 a	9.75 ± 3.90 a	14.00 ± 1.75 a	0.30 ± 0.06 a
	1.5%	6.46 ± 0.29 b	6.42 ± 0.38 ab	12.00 ± 0.75 b	0.23 ± 0.01 b
140 RU	3%	5.88 ± 0.82 b	5.92 ± 0.72 b	9.75 ± 0.43 c	0.20 ± 0.00 b
	4.5%	4.50 ± 0.33 c	5.00 ± 0.43 b	6.08 ± 0.95 d	0.20 ± 0.01 b
	6%	4.75 ± 0.10 c	5.67 ± 1.01 b	4.17 ± 0.14 e	0.13 ± 0.02 c
Mean		5.85 a	6.55 b	9.20 c	0.21 c

Values followed by different letters within the root stock are significantly different (P < 0_05). Mean values of different rootstocks followed by bold different letters are significantly different (P < 0_05).

and 140RU without any significant difference (0.045 and 0.044µg .g^{-1}, respectively).

Kaiser *et al*., (1981) found that the reduction in chlorophyll content under drought condition is due to damage of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets. The increasing drought stress also increased the necrosis on the leaf area; therefore changes in chlorophyll content in leaf tissue were occurred (Fig. 1). There are numerous reports of decreased levels of chlorophylls under water stress (Bertamini *et al.,* 2006, Haider *et al.,* 2017, Maroco *et al.,* 2002, Palliotti *et al.,* 2008, Pavlousek, 2011). This reduction may be related to the activity of proteolytic enzymes which causes chlorophyll degradation (Tuna *et al.,* 2008). The evaluation of chlorophyll content is very important; the reduction in chlorophyll content causes a reduction in

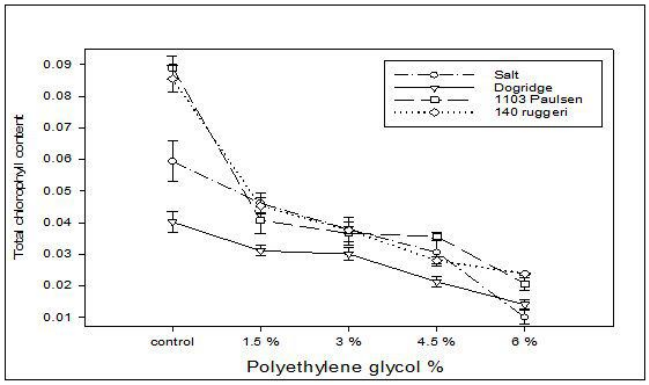

Fig. 2: The effect of the rootstock and PEG% on total chlorophyll

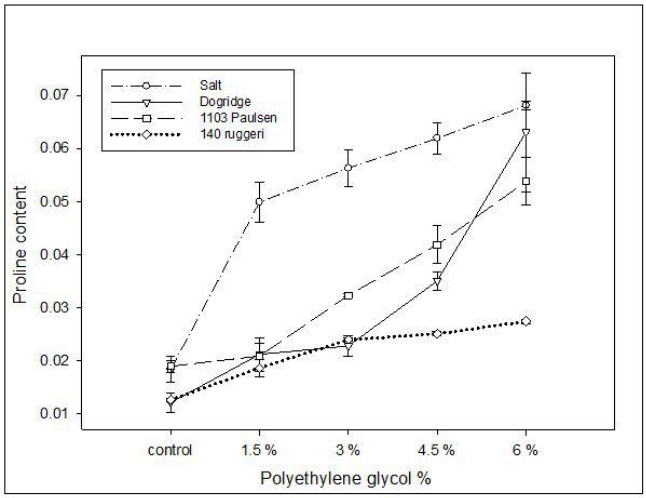

Fig. 3: The effect of the rootstock and PEG% on proline (µmole /g FW)

photosynthetic levels of the plant. Whereas and Schultz (1995) found that the responses of grapevine photosynthesis to water stress included many physiological processes as parts of stress tolerance strategies that varies within genotypes. Also, Gómez-del-Campo *et al.,* (2002) mentioned that the drought stress caused a reduction in the photosynthetic activity.

Proline content in leaves of all rootstocks was shown to increase gradually in response to increasing PEG % (Fig. 3). Low level of PEG cause slight increase in proline content; however, significant increase in proline content was seen on higher concentration. The increase was the highest in Ramsey rootstock (0.05 µmole $.g^{-1}$), followed by 1103P (0.034µmole $.g^{-1}$) and Dog Ridge (0.03µmole $.g^{-1}$) respectively and the lowest was in 140RU (0.02 µmole $.g^{-1}$). The use of biochemical markers, such as proline analysis can be used for evaluating crop resistance to osmotic stress. Accumulation of proline in cell exposed to drought stress has also been reported earlier (Bertamini *et al.,* 2006, Cramer *et al.,* 2007, Doupis *et al.,* 2011, Haider *et al.,* 2017). Proline is an important compatible organic solute that accumulate in many drought-stressed plant species including grapevine as a common responses of plant to dehydration (Cramer *et al.,* 2007, Delauney and Verma, 1993) and it is the most abundant free amino acid in grapevine leaves (Kliewer and Nassar, 1966). Proline plays a role in dehydration avoidance by increasing the cellular solute content and thus maintaining higher water content (Yancey *et al.,* 1982). At the same time, proline functions as an osmo-protectant which plays a role in dehydration tolerance by protecting protein and membrane structure, regulating redox status or acting as a scavenger of Reactive oxygen species (ROS) (Hare *et al.,* 1998, Kocsy *et al.,* 2005, Smirnoff and Cumbes, 1989).

Conclusion

The response of grapevine rootstocks to water stress was successfully investigated under *in vitro* conditions using different PEG concentrations. Marked reduction on growth was observed during the stress period. This reduction was appeared in decrease in shoots length, number of green leaves per shoot, shoot fresh weight and chlorophyll content in PEG treatments comparing with the control. Proline content increased in response to increasing the drought stress level. Finally, based on our findings, the drought tolerance of grape rootstocks can be ranked as Ramsey >1103P and 140RU >Doddridge.

References

Bates, L.S., R.P. Waldren and I. Teare (1973). Rapid determination of free proline for water-stress studies. *Plant and soil.*, **39(1):** 205-207.

Bertamini, M., L. Zulini, K. Muthuchelian and N. Nedunchezhian (2006). Effect of water deficit on photosynthetic and other physiological responses in grapevine (*Vitis vinifera* L. *cv. Riesling*) *plants. Photosynthetica.*, **44(1):** 151-154.

Booth, J. (1970). Verticillium albo-atrum. Crop loss assessment methods: FAO manual on the evaluation and prevention of losses by pests, diseases and weeds. Common Wealth Agriculture Bureaux., Farnham Royal, UK, 50-51.

Boyer, J.S. (1982). Plant productivity and environment. *Science.*, **218(4571):** 443-448.

Chartzoulakis, K., A. Patakas and A. Bosabalidis (1999). Changes in water relations, photosynthesis and leaf anatomy induced by intermittent drought in two olive cultivars. *Environmental and Experimental Botany.*, **42(2):** 113-120.

Cirulli, M., C. Colella, M. D'Amico, M. Amenduni and G. Bubici (2008). Comparison of screening methods for the evaluation of olive resistance to Verticillium dahliae Kleb. *Journal of Plant Pathology.*, 7-14.

Cramer, G.R., A. Ergül, J. Grimplet, R.L. Tillett, E.A. Tattersall, M.C. Bohlman and C. Osborne (2007). Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Functional & Integrative Genomics.,* **7(2):** 111-134.

Delauney, A.J. and D.P.S. Verma (1993). Proline biosynthesis and osmoregulation in plants. *The Plant Journal.*, **4(2):** 215-223.

Doupis, G., K. Chartzoulakis, A. Beis and A. Patakas (2011). Allometric and biochemical responses of grapevines subjected to drought and enhanced ultraviolet B radiation. *Australian Journal of Grape and Wine Research.*, **17(1):** 36-42.

Driver, J.A. and A.H. Kuniyuki (1984). In vitro propagation of Paradox walnut rootstock (*Juglans hindsii X Juglans regia, tissue culture*). *Hort. Science.,* **19:** 507-509.

Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*., **11(1):** 1-42.

Gómez-del-Campo, M., C. Ruiz and J.R. Lissarrague (2002). Effect of water stress on leaf area development, photosynthesis and productivity in Chardonnay and Airén grapevines. *American Journal of Enology and Viticulture.*, **53(2):** 138-143.

Granett, J., M.A. Walker, L. Kocsis and A.D. Omer (2001). Biology and management of grape phylloxera. *Annual review of entomology.*, **46(1):** 387-412.

Haider, M.S., C. Zhang, M.M. Kurjogi, T. Pervaiz, T. Zheng, C. Zhang and J. Fang (2017). Insights into grapevine defense response against drought as revealed by biochemical, *physiological and RNA-Seq Analysis. Scientific Reports.*, **7(1):** 13134.

Hare, P.D., W.A. Cress and J. Van Staden (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell & Environment.*, **21(6):** 535-553.

Hohl, M. and P. Schopfer (1991). Water relations of growing maize coleoptiles: comparison between mannitol and polyethylene glycol 6000 as external osmotica for adjusting

turgor pressure. *Plant Physiology.*, **95(3):** 716-722.

Hussain, M., M. Malik, M. Farooq, M. Ashraf and M. Cheema (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *Journal of Agronomy and Crop. Science.*, **194(3):** 193-199.

Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneerselvam (2009). Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.*, **11(1):** 100-105.

Kaiser, W.M., G. Kaiser, S. Schöner and S. Neimanis (1981). Photosynthesis under osmotic stress: differential recovery of photosynthetic activities of stroma enzymes, intact chloroplasts, protoplasts and leaf slices after exposure to high solute concentrations. *Planta.*, 430-435.

Karimi, S., A. Yadollahi, R. Nazari-Moghadam, A. Imani and K. Arzani (2012). *In vitro* screening of almond (Prunus dulcis (Mill.)) genotypes for drought tolerance. *J. Biol. Environ. Sci.*, **6(18):** 263-270.

Kliewer, W. and A.R. Nassar (1966). Changes in concentration of organic acids, sugars and amino acids in grape leaves. *American Journal of Enology and Viticulture.*, **17(1):** 48-57.

Kocsy, G., R. Laurie, G. Szalai, V. Szilágyi, L. Simon Sarkadi, G. Galiba and J.A. De Ronde (2005). Genetic manipulation of proline levels affects antioxidants in soybean subjected to simultaneous drought and heat stresses. *Physiologia Plantarum.*, **124(2):** 227-235.

Koundouras, S., I.T. Tsialtas, E. Zioziou and N. Nikolaou (2008). Rootstock effects on the adaptive strategies of grapevine (*Vitis vinifera* L. *cv*. Cabernet–Sauvignon) under contrasting water status: leaf physiological and structural responses. *Agriculture, ecosystems & environment.*, **128(1-2):** 86-96.

Lawlor, D. (1970). Absorption of polyethylene glycols by plants and their effects on plant growth. *New phytologist.*, **69(2):** 501-513.

Lebon, E., A. Pellegrino, G. Louarn and J. Lecoeur (2006). Branch development controls leaf area dynamics in grapevine (Vitis vinifera) growing in drying soil. *Annals of Botany.*, **98(1):** 175-185.

Lichtenthaler, H.K. and A.R. Wellburn (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions.*, **11(5):** 591-592.

Maroco, J.P., M.L. Rodrigues, C. Lopes and M.M. Chaves (2002). Limitations to leaf photosynthesis in field-grown grapevine under drought—metabolic and modelling approaches. *Functional Plant Biology.*, **29(4):** 451-459.

Nepomuceno, A., D. Oosterhuis and J. Stewart (1998). Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. *Environmental and Experimental Botany.*, **40(1):** 29-41.

Nonami, H. (1998). Plant water relations and control of cell elongation at low water potentials. *Journal of Plant Research.*, **111(3):** 373-382.

Pavlousek, P. (2011). Evaluation of drought tolerance of new grapevine rootstock hybrids. *Journal of environmental biology.*, **32(5):** 543.

Pellegrino, A., E. Lebon, T. Simonneau and J. Wery (2005). Towards a simple indicator of water stress in grapevine (*Vitis vinifera* L.) based on the differential sensitivities of vegetative growth components. *Australian Journal of Grape and Wine Research.*, **11(3):** 306-315.

Schultz, H. (1995). Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. *Strategies to Optimize Wine Grape Quality.,* **427:** 251-266.

Shashidhar, H., A. Kanbar, M. Toorchi, G. Raveendra, P. Kundur, H. Vimarsha and P. Bhavani (2013). Breeding for drought resistance using whole plant architecture—conventional and molecular approach. Plant breeding from laboratories to Fields. Vienna, Austria.

Smirnoff, N. and Q.J. Cumbes (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry.*, **28(4):** 1057-1060.

Sommer, K. (2009). Spring recovery-resilience of Sultana to drought. *The Vine.*, **5(3):** 32-34.

Stevens, R., G. Harvey and D. Aspinall (1995). Grapevine growth of shoots and fruit linearly correlate with water stress indices based on root weighted soil matric potential. *Australian Journal of Grape and Wine Research.*, **1(2):** 58-66.

Taiz, L. and E. Zeiger (2002). Plant physiology., 3rd edn. (Sinauer Associates: Sunderland, MA, USA).

Tuna, A.L., C. Kaya, M. Dikilitas and D. Higgs (2008). The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany.*, **62(1):** 1-9.

Verslues, P.E., E.S. Ober and R.E. Sharp (1998). Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. *Plant Physiology.*, **116(4):** 1403-1412.

Wang, W., B. Vinocur and A. Altman (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta.*, **218(1):** 1-14.

Wani, R.A., S. Sheema, N.A. Dar, S. Angchuk and G. Parray (2013). Irrigation regimes effecting drought tolerance of grape rootstocks under cold arid conditions. *Int. J. Sci. Technol. Res.*, **2:** 113-117.

Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero (1982). Living with water stress: *evolution of osmolyte systems. Science.*, **217(4566):** 1214-1222.

Zhao, T.J., S. Sun, Y. Liu, J.M. Liu, Q. Liu, Y.B. Yan and H.M. Zhou (2006). Regulating the drought-responsive element (DRE)-mediated signaling pathway by synergic functions of trans-active and trans-inactive DRE binding factors in Brassica napus. *Journal of Biological Chemistry.*, **281(16):** 10752-10759.