IN VITRO EVALUATION OF SOME GRAPEVINE ROOTSTOCKS GROWN UNDER DROUGHT STRESS

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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 survival% 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 phylloxera 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, *Vitis berlandieri X V. rupestris*) Ruggeri 140 (140 RU, *Vitis berlandieri X V. rupestris*), Ramsey (63Salt Creek, *Vitis champinii*) and Dog Ridge (*Vitis champinii*) 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), 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).

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

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
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).

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.
and 140RU without any significant difference (0.045 and 0.044 µg . 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...
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 (Kliwer 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, Koecs 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**


