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Drought Stress Responses of *Prunus microcarpa* C. A. Mey. *subsp. tortusa* Rootstocks under *in Vitro* Conditions

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ABSTRACT

Background: *Prunus microcarpa* C.A. Mey. *subsp. Tortusa* (MK)**,** a wild deciduous plant species, is native to Caucasus and West Asia and grows in a dry temperate climate zone. The plant is mainly used to obtain a dark greyish green dye from its leaves and it has an economic value due to ornamental characteristics. Therefore, this study aimed to determine the appropriate Polyethylene Glycol 8000 (PEG) doses for early detection of drought resistance using MK, SL-64, Garnem, Pixy and Gis-6 rootstocks.

Methods: The microshoots of each rootstock were planted at the end of the fourth subculture in Magenta GA7 vessels containing NRM medium. The medium contained 1.0 mg L^{-1} BA + 0.01 mg L^{-1} IBA supplemented with 30 g L^{-1} sucrose, gelled with 5.5 g L^{-1} agar and 0, 2, 3 and 4% PEG doses (four treatments). The cultures were kept at 23±2 °C temperature under 16 h light (80 µmol m^{-2} s^{-1}) and 8 h dark photoperiod.

Result: The highest number of shoots was recorded in control treatment for MK rootstock (2.81 shoots $plant^{-1}$) and in 4% PEG treatment for Gis-6 rootstock (2.81 shoots $plant^{-1}$). Mean chlorophyll a (10.66 mg/f.w) and chlorophyll b (3.63 mg/f.w) contents in the control treatment were lower compared to the mean chlorophyll a (2.03 mg/f.w) and chlorophyll b (1.15 mg/f.w) contents of plants grown in PEG 4% dose. *Prunus microcarpa* genotypes of Turkey origin tested in this study could be used as potential rootstocks in arid and semi-arid region as safe as the other standard rootstocks tested.

Key words: Drought Stress, *In vitro*, MDA, PEG, Proline.

INTRODUCTION

The *Prunus* genus contains more than 200 species of deciduous evergreen trees and shrubs and are widely distributed in temperate zones (Kalinina *et al*., 2007). The *Prunus* includes many temperate stone fruit species; thus, it is an economically important plant due to its ornamental characteristics. *Prunus microcarpa* C. A. Mey. *subsp. tortusa* is usually found in the temperate climate zone, is one of the wild stone fruit species native to dry temperate climate zone of Caucasus and West Asia.

Genetic variations in germplasm are relatively low for *Prunus* species (Scorza *et al*., 1985; Kaur *et al.,* 2006). Many studies have been carried out on wild *Prunus* species, however studies on *P. microcarpa* subsp. *Tortusa* are missing in the literature (Nas *et al.,* 2011). Native germplasms are used to expand the gene pool and to achieve successful reproduction (Webster *et al*., 2000). Since wild species constitute an essential genetic resource, studies using different wild species are important to increase the number of breeding materials and diversify the gene pool. Rootstocks control tree size through a direct effect on growth and indirectly by increasing crop load (Sonkar *et al*., 2002; Sharma *et al*., 2004; Sharma *et al*., 2009). Drought is the most important environmental stress that severely impairs plant growth and development and limits plant production worldwide (Shinde *et al*., 2018). Conservation of *Prunus* species and development of new rootstock germplasms are important (Cheong and Pooler, 2004) for drought tolerance (Sivritepe *et al.,* 2008). Therefore, development and screening of drought-tolerant rootstocks are needed to improve production in modern fruit cultures and to alleviate water deficiency related production losses.

The information on genetic diversity and relationship with other *Prunus* species is important to assess the potential uses of *P. microcarpa* C.A. Mey. *subsp. tortusa* and develop effective conservation strategies (Nas *et al.,* 2011). Screening of Prunus and many other wild plant species against drought stress have been successfully carried out using different Polyethylene Gligol (PEG) doses (Joshi *et al.,* 2011; Sorkheh *et al.,* 2012). The purpose of this study was to investigate the responses of some *P. microcarpa* rootstocks on resistance to drought conditions by the application of different (PEG) doses *in vitro* conditions and measuring physiological and biochemical parameters.

MATERIALS AND METHODS

Microshoots (1 to 4 cm) of *P. microcarpa C.A. Mey. Subsp* (6 to 8-year old stock plants) were used as the plant materials

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of the study. The material was obtained from the Department of Horticulture laboratory in Sirnak University, Turkey**.** Nas and Read Medium (NRM) containing 1.0 mg L^{-1} BA and 0.01 mg L^{-1} IBA supplemented with 30 g L^{-1} sucrose was used as beginning medium. Four different concentrations of PEG 8000 (0, 2, 3 and 4%) were added to the standard NRM medium. Sixty-four uniformly selected microshoots were equally divided and four microshoots were planted into each magenta containing four different drought concentrations. Each treatment was allowed to grow under 16 h light and 8 h dark photoperiod in the climate chamber at 23±2°C temperature.

Five grams of each sample were taken and stored at -80°C for proline, malondialdehyde (MDA) and chlorophyll analyses. The explants exposed to drought stress were evaluated by using the number of shoots and the shoot length criteria similar to the multiplication factor *in vitro*.

The leaf samples (0.5 g) were taken from the surviving plants and were homogenized in a mortar using 5 ml of 80% acetone solution. The concentrations of chlorophyll a and chlorophyll b pigments were measured after filtering the solution and the absorbance of solutions at 645 nM and 663 nM were read on the spectrophotometer. The chlorophyll contents were calculated using the equations proposed by Güneş *et al.* (2007);

Chlorophyll a (mg g^{-1}) = 11.75 ×A663 – 2.35 × A645
Chlorophyll b (mg g^{-1}) = 18.61 ×A645 – 3.96 × A663

The proline content of the leaf samples was determined spectrophotometrically using the method described by Bates *et al.* (1973). Lipid peroxidation of the leaf samples was determined as malondialdehyde (MDA) content of the shoots (Heath and Packer, 1968). The fresh leaf samples (0.5 g) in each treatment were homogenized using 6 ml of 10% trichloroacetic acid (TCA) centrifuged at 10000 rpm for 15 minutes. Then, 2 ml of supernatant was mixed with 2 ml of 0.6% thiobarbutric acid (TBA) containing 20% TCA. The mixture was heated at 100 p C for 20 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 10000 rpm for 10 min and absorbance of the supernatant was measured by a spectrophotometer at 450, 532 and 600 nm wavelengths, respectively. The MDA concentration was calculated following the method of Zhang *et al.* (2005).

Statistical analysis

The experimental lay out was a randomized plot with four replications (4 explants per replication). The number of shoots, shoot lengths and the number of plants survived were recorded at the end of the fourth week. Variance analysis was used to evaluate the data using JMP 13 statistical software (SAS Institute Inc. North Carolina USA). The mean values of the experimental parameters investigated were compared using Tukey's multiple comparison test at $p<0.05$ level of significance.

RESULTS AND DISCUSSION

Number and Length of Shoots

The results of variance analysis indicated a significant effect ($p\geq0.05$) of the drought stress on the number of shoots, shoot length and chlorophyll a, MDA and proline contents. In contrast, the effect of drought on chlorophyll b was not significant (Table 1). The highest mean number of shoots in the control treatment was obtained for MK rootstock as 12.81 shoots $plant^{-1}$ and the lowest mean number of shoots was recorded as 5.47 shoots $explant^{-1}$ for SL-64 rootstock. The mean number of shoots sharply decreased in 4.0% PEG treatment, it was 1.30 shoots $explant^{-1}$ for MK and 1.54 shoots $explant^{-1}$ for SL-64 rootstocks. The highest number of shoots in the highest PEG dose (4%) was recorded in Pixy (2.81 shoots $explant^{-1}$) rootstock (Table 2). The adverse effects of PEG applications on the number of shoots and shoot length in different rootstocks have also been reported by Deblonde and Ledent (2001). The number of shoots recorded in 2% PEG treatment for SL-64 and Pixy rootstocks indicated that these two rootstocks are more active under slight drought stress than the control conditions. In contrast, the highest number of shoots for MK (12.81 shoots $explant^{-1}$) and Pixy (10.28 shoots $explant^{-1}$) rootstocks were obtained in the highest PEG application dose (Table 2).

Shoot length of rootstocks significantly decreased with the increased PEG doses (Table 3). The lowest shoot length in 4% PEG treatment was recorded in Garnem rootstock (0.50 cm). The shoot length of MK rootstock in PEG 2% dose was lower than the control. Similar to the number of shoots, the mean shoot length of all rootstocks significantly decreased with the increasing PEG doses. The mean shoot length in general was 1.38, 1.21, 0.92 and 0.66 cm for 0, 2, 3 and 4% PEG treatments, respectively. The lowest shoot length in control treatment was recorded in Pixy (1.00 cm) and the highest shoot length was in SL-64 rootstock (1.78 cm). The mean shoot length of Pixy rootstock in 2% dose PEG and control treatments (1.00 cm) was the same. Higher mean shoot length of Gis-6 and MK rootstocks in 2% PEG dose compared to the control indicated higher drought

Table 1: Effect of PEG treatments on physiological properties of *P. microcarpa* under *in vitro* conditions.

Treatments	Number of Shoots	Shoot length	Chlorophyll a	Chlorophyll b	MDA	Proline
Rootstock	**	**	**	*	**	**
PEG Doses	**	**	**	ns	**	**
Rootstock * Dose	**	**	**	ns	**	**
CV (%)	0.155	0.09	0.08	0.17	0.12	0.22

ns = not significant, * and **; Mean difference is significant at P<0.05 and P<0.01level of significance.

resistance of these two rootstocks compared to other three rootstocks. Therefore, prolongation of side shoots in arid regions can be attributed to the disruption of shoot apical meristem and growth inhibition of necrosis. Similar findings for the effects of drought stress on plant growth *in vitro* conditions have been reported by Dami and Harrison, (1995) and Al-Khayri and Al-Bahrany (2004).

Effects of drought stress on chlorophyll, proline, lipid peroxidation content

The chlorophyll a and chlorophyll b contents of all rootstocks investigated significantly decreased with the increasing PEG doses (Table 4 and 5). The lowest chlorophyll a (1.27 mg/f.w) and chlorophyll b (0.51mg/f.w) contents were recorded in 4% PEG treatment for Gis-6 rootstock. The chlorophyll a content in 0, 2, 3 and 4% PEG treatments were 10.66, 8.01, 3.68 and 2.03. mg/f.w, respectively. Similarly, the chlorophyll b content of rootstocks in 0, 2, 3 and 4% PEG doses were 3.63, 3.13, 1.55 and 1.15 mg/f.w, respectively. The chlorophyll contents gradually decreased compared to the control treatments in different almond rootstocks under drought stress (İpek, 2015). In addition, Cerci (2012) stated that the decrease in chlorophyll contents caused leaf color bleaching of six different rootstocks under different drought stress conditions. Yarsi *et al.* (2017) reported that abiotic stress reduced the chlorophyll a and b contents of leaves, which significantly reduced the plant growth and altered the morphology.

Malondialdehyde (MDA) contents of all rootstocks increased under drought stress. The highest MDA content in PEG treatment was obtained in Garnem rootstock (1.31µmolg^{-1}), while the lowest value was noted in MK (0.25 µmolg^{-1}) rootstock (Table 6). The mean value of MDA content for all rootstocks increased with the increasing PEG doses up to 3.0% then significantly decreased in 4.0% PEG dose (Table 6). The increasing MDA contents of rootstocks in 0, 2 and 3% PEG doses were ranked as 0.57, 0.80 and 1.02, respectively. The difference in MDA content of rootstocks was attributed to the relationship between MDA content and membrane coalescence; confirming that, the higher the MDA content, more damage occurs in the cell membranes (Liu and Zhao, 2005). Lipid peroxidation indicated by MDA

Table 2: Mean numbers of shoots (shoot/explant) for different rootstocks in PEG treatments.

Rootstocks	PEG 0%	PEG 2%	PEG 3%	PEG 4%
MK	12.81±2.72 a*	7.80 ± 0.00 b	3.95 ±0.63 ab	1.30±0.07 bc
SL-64	5.47± 0.38 d	4.27± 0.10 c	1.80± 0.28 c	1.59 ±0.43 b
Garnem	7.40 ±0.39 c	3.71± 0.75 c	1.74 ±0.09 c	0.56± 0.00 c
Pixy	10.28± 0.16 b	11.59± 1.10 a	5.09± 0.39 a	2.81± 0.70 a
Gis-6	7.37±0.62 c	7.40 ±0.31 b	3.30 ±0.28 b	1.28 ±0.21 bc
Mean	8.67	6.95	3.18	1.51
$LSD_{0.05}$	1.38	2.10	1.31	1.00

*: Different letters in a column indicate significant differences (p<0.05) among rootstocks.

Table 3: The effects of PEG treatments on shoot lengths (cm) of different rootstocks under *in vitro* conditions.

Rootstocks	PEG 0%	PEG 2%	PEG 3%	PEG 4%
MK	1.31± 0.02 bc*	1.37± 0.07 ab	1.09 ±0.07 a	0.65± 0.00 b
SL-64	1.78± 0.12 a	0.89 ±0.33 c	0.73± 0.00 b	0.56± 0.04 c
Garnem	1.37 ±0.12 b	1.30 ± 0.13 a-c	1.18 ±0.09 a	0.50 ±0.00 d
Pixy	1.00 ±0.00 c	1.00± 0.00 bc	0.53 ±0.04 c	0.59 ±0.00 c
Gis-6	1.43 ±0.00 b	1.50± 0.00 a	1.07± 0.06 a	1.01 ±0.01 a
Mean	1.38	1.21	0.92	0.66
$LSD_{0.05}$	0.31	0.51	0.20	0.20

*: Different letters in a column indicate significant differences (p<0.05) among rootstocks.

Table 4: The effects of different PEG treatments on chlorophyll a (mg/F.W) content of different rootstocks under in *vitro* conditions.

Rootstocks	PEG 0%	PEG 2%	PEG 3%	PEG 4%
MK	15.75±0.65 a*	10.80 ± 0.28 a	2.89 ±0.40 b	2.33 ± 0.12 b
SL-64	7.25± 0.77 e	8.07± 0.24 b	5.10 ± 0.07 a	3.05± 0.14 a
Garnem	7.67± 0.82 d	7.00 ± 0.18 cd	2.62± 0.86 b	1.33 ± 0.44 c
Pixy	10.34± 0.84 c	8.44± 0.49 bc	4.50 ± 0.01 a	2.17 ± 0.14 b
Gis-6	12.31 ± 0.63 b	5.75± 1.05 d	3.27± 0.14 b	1.27± 0.09 c
Mean	10.66	8.01	3.68	2.03
$LSD_{0.05}$	0.30	1.88	1.31	0.80

*: Different letters in a column indicate significant differences (p<0.05) among rootstocks.

Table 5: The effects of different PEG treatments on chlorophyll b (mg/F.W) contents of different rootstocks under *in vitro* conditions**.**

Rootstocks	PEG 0%	PEG 2%	PEG 3%	PEG 4%
MK	5.21 ±0.60	4.86± 0.33 a*	2.60 ± 0.14 a	1.54 ±0.19 a
SL-64	3.02± 0.59	3.64 ± 0.28 b	1.00 ± 0.00 c	1.37 ± 0.07 a
Garnem	2.42± 0.53	2.07± 0.21 c	1.28± 0.05 bc	1.39 ± 0.44 a
Pixy	3.27 ± 0.38	1.71 ± 0.08 c	1.50 ± 0.41 b	0.95 ± 0.14 ab
Gis-6	4.25 ± 0.94	3.39± 0.65 b	1.38 ±0.19 bc	0.51 ± 0.55 b
Mean	3.63	3.13	1.55	1.15
$LSD_{0.05}$	ns	1.27	0.49	0.97

ns: not significant; *: Different letters in a column indicate significant differences (p<0.05) among rootstocks.

Table 6: The effects of different PEG treatments on MDA (µmolg^{-1}) contents of different rootstocks under *in vitro* conditions.

Rootstocks	PEG 0%	PEG 2%	PEG 3%	PEG 4%
MK	0.25± 0.07 bc*	0.64 ±0.12 bc	1.08 ± 0.02 bc	0.90± 0.12 b
SL-64	0.26± 0.05 c	0.45± 0.07 c	1.43± 0.05 a	1.30± 0.12 a
Garnem	1.31 ± 0.05 a	1.45 ± 0.07 a	1.19 ± 0.08 b	0.56± 0.15 c
Pixy	0.66± 0.07 b	0.84 ± 0.05 b	1.02 ± 0.07 c	0.33 ± 0.14 d
Gis-6	0.36 ± 0.14 bc	0.63 ± 0.18 bc	0.40 ± 0.02 d	0.17± 0.01 e
Mean	0.57	0.80	1.02	0.65
$LSD_{0.05}$	0.33	0.38	0.14	0.17

*: Different letters in a column indicate significant differences (p<0.05) among rootstocks.

Table 7: The effects of different PEG treatments on proline (µmolg^{-1}) contents of different rootstocks under *in vitro* conditions.

Treatments	PEG 0%	PEG 2%	PEG 3%	PEG 4%
MK	0.24 ± 0.02 a*	0.99 ± 0.10 a	1.30± 0.07 b	1.09± 0.04 b
SL-64	0.44 ± 0.49 a	1.05± 0.01 a	1.07± 0.01 b	1.33 ± 0.18 a
Garnem	0.17 ± 0.05 a	0.63± 0.16 b	2.38± 0.09 a	0.93 ± 0.10 b
Pixy	0.22± 0.17 a	0.27± 0.20 c	0.44± 0.27 c	0.30± 0.03 d
Gis-6	0.22 ± 0.04 a	0.32 ± 0.08 c	0.61 ± 0.13 c	0.58 ± 0.03 c
Mean	0.26	0.65	1.16	0.85
$LSD_{0.05}$	ns	0.22	0.42	0.20

ns: not significant; *: Different letters in a column indicate significant differences (p<0.05) among rootstocks.

content increased markedly in all rootstocks up to 3.0% PEG dose. The results obtained in this study are consistent with the findings of Asghar *et al.,* (2016). The highest impact of drought stress on MDA content of rootstocks was recorded in 3%PEG treatment. The results indicated that all rootstocks have higher adaptability to 4% PEG dose compared to 3% PEG dose (Table 6).

The proline content of rootstocks under drought stress significantly differed from the control treatment. Similar to the MDA values, the mean proline contents of all rootstocks in PEG treatments increased up to 3% PEG and significantly decreased by the application of 4.0% PEG dose. The mean proline content of all rootstocks in 0, 2, 3 and 4% PEG treatments were 0.26, 0.65, 1.16 and 0.85 µmolg^{-1}, respectively (Table 7). The highest proline content in 4.0% PEG treatment was recorded in SL-64 (1.33 µmolg^{-1}) and MK (1.09 µmolg^{-1}) rootstocks, which can be considered more tolerant under high drought stress conditions compared to the other rootstocks (Table 7). Proline controls the production of free radicals like different antioxidants and proline is the only component that protects plant cells from oxidative damage (Baxter *et al.,* 2014). The differences in proline accumulation in various rootstocks under abiotic stress have also been mentioned by Sorkheh *et al.* (2012).

CONCLUSION

This study was carried out to compare the drought tolerance of four stone fruit rootstocks and *P. microcarpa* under *in vitro* conditions. The number of shoots and shoot lengths in high PEG doses indicated that SL-64 and P. *microcarpa* rootstocks are drought tolerant. The proline and MDA contents of SL-64 and MK rootstocks also supported the drought resistance of these two rootstocks. To our knowledge, this is the first report on drought stress of *P. microcarpa* with high potential to use as rootstock of stone fruits. *Prunus microcarpa* could be used as potential rootstocks in arid and semi-arid at least as safe as the other standard rootstocks tested.

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