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Comparison of biomass production-based drought tolerance indices of pistachio (*Pistacia vera* L.) seedlings in drought stress conditions

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Abstract

Pistachio (*Pistacia vera* L.)has a high tolerance to soil drought and salinity.Especially adult trees are well-known for drought resistance. We carried out a greenhouse experiment to evaluate the effects of two drought stress levels (Ψs= -0.75 MPa, Ψs= -1.5 MPa) and subsequent recovery on relative chlorophyll content and biomass production in three Iranian pistachio cultivars i.e. Akbari, Kaleghochi and Ohadi. Both drought stress levels lowered leaf relative chlorophyll content and total plant dry weight. Ohadi had significantly higher rates of relative chlorophyll and plant dry weights (biomass) under drought stress conditions compared to Akbari, whereas Kaleghochi showed intermediate results.Six drought tolerance indices including stress susceptibility index (SSI), stress intensity (SI), stress tolerance index (STI), stress tolerance (TOL), mean productivity (MP) and geometric mean productivity (GMP) were calculated from total plant dry weight (biomass) under severe drought and non-stressed (control) conditions.Our results show a significant relationship between both absolute plant biomass and plant biomass reduction (TOL) with STI, MP and GMPfor pistachio cultivars. Tolerance indices including STI identified cultivars which produce high plant biomass in both favorable and unfavorable moisture conditions.These results demonstrate that Ohadi may be more tolerant to drought in terms of biomass productivity as it performs better than other cultivars.

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Introduction

Pistachio belongs to the Anacardiaceae family even though only *Pistacia vera* L., i. e. cultivated pistachio, has an economic importance. Iran, as the region of origin of pistachio, has always had the largest cultivation area (450000 ha) in the world (Esmaeilpour *et al.*, 2010).About 90 percent of Iran is categorized as semi-arid and arid. These areas are characterized by low rainfall and deficiency of fresh water, high evapotranspiration rates, soil salinization, dust storms, extreme heat and desertification(Cheraghi, 2004). In Iran, pistachio is usually cultivated under dry and saline soil conditions(Sheibani, 1995)as the species has a high tolerance to drought and salinity of soil and water. Still, water deficiency and salinity can cause a reduction in growth, yield and nut quality. These harsh growing conditions already led to the loss of important local genetic resources of pistachio cultivars and rootstocks(Panahi *et al.*, 2002).

Drought stress adversely affects growth, dry mass and productivity in most of the plants(Anjum *et al.*, 2011; Zhao *et al.*, 2006). Drought tolerance of wild pistachio species could be related to a deep taproot, high water conservation ability by stomatal adjustment, stomatal features, leaf characteristics, and leaf shedding (Fardooei, 2001; Germana, 1996; Spiegel-Roy *et al.*, 1977). Drought stress was evaluated for *P. vera* Kerman grafted onto three different pistachio rootstocks. Grafting onto hybrid rootstock (UCB#1) and *P. terebinthus* resulted in a higher growth reduction compared with *P. atlantica* under drought stress (Gijón *et al.*, 2010). Bagheri *et al.* (2011)found that Qazvini was more tolerant to drought stress than Badami as it maintained a higher photosynthetic activity under drought. Net photosynthetic rates were more reduced for *P. mutica* than *P. khinjuk* under increasing osmotic drought stress, indicating a higher tolerance of *P. khinjuk* (Ranjbarfordoei *et al.*, 2000). Drought indices which predict drought tolerance based on yield loss under drought stress conditions as compared to optimal conditions, have been used for screening drought-tolerant genotypes in arable crops(Mitra, 2001). These indices are either based on drought tolerance or susceptibility(Fernandez, 1992).Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the difference in yield between drought-stressed (YS) and irrigated (YP) environments, and mean productivity (MP) as the average yield of YS and YP. Fischer and Maurer (1978)proposed a stress susceptibility index (SSI)of the cultivar.Fernandez (1992) defined astress tolerance index (STI), which can be used to identify genotypes that produce high yield under both stressed and well-watered conditions. Another yield-based estimate of drought tolerance is the geometric mean productivity (GMP). The geometric mean is often used by breeders interested in relative performance since drought stress can vary in severity in a field environment over years (Ramirez and Kelly, 1998).Golabadi *et al.* (2006) and Sio-Se Mardeh *et al.* (2006) suggested that selection for drought tolerance inwheat (*Triticum aestivum* L.) could be conducted through high MP, GMP and STI under rain fed and irrigatedfield conditions.Among the stress tolerance indicators, a larger value of TOL and SSI represents relatively more sensitivity to stress, thus a smaller value of TOL and SSI is favored. Selection based on these two criteria favors genotypes with low yield potential under non-stressed conditions and high yield under stressed conditions. On the other hand, selection based on STI and GMP will result in the selection of genotypes with higher drought tolerance and yield potential (Fernandez, 1992).In spring wheat (*Triticum aestivum* L.)cultivars, Guttieri *et al.* (2001)using SSI suggested that a value > 1 indicates above-average susceptibility whereas a value of less than 1 indicates below-average susceptibility to drought stress.

As mentioned above, there are several reports on the resistance and sensitivity of pistachio cultivars to drought stress in different growth stages. According to drought tolerance indices, there were no reports on drought stress and biomass production (dry weight) relation in pistachio plants.There is a wide variationin edible pistachio (*P. vera*) cultivars in Iran(Esmaeilpour and Khezri, 2006; Sheibani, 1995)whereby theyare grown in different environmental conditions. Akbari, Kaleghochi and Ohadi are the most common cultivars in the country. We hypothesize, however, that there is (are) cultivar (s) among pistachio cultivars that are more tolerant to drought stress.The objectives of the present study were to evaluate: (1) the effects of osmotic drought stress on relative chlorophyll as a fast indicator of reduced growth potential and on plant dry weight (biomass) production; and (2) six drought indices and their potential use for drought resistance evaluation in pistachio, and to provide a reference for the selection of drought-tolerant genotypes. These investigations should lead to appropriate recommendations for development of new pistachio orchards.

Materials and methods

Plant material and experimental set-up

This study was carried out in a greenhouse at the Faculty of Bioscience Engineering, Ghent University, Belgium (51°3' N, 3°42' E). Certified seeds of three pistachio cultivars, *Pistacia vera* L. Akbari,Kaleghochi and Ohadiwere obtained from the Iranian Pistachio Research Institute, Rafsanjan, Iran (30° 39 ' N, 55° 94 ' E). Seeds of these pistachio cultivars were first soaked in water for 12 hours and then pre-treated for 20 minutes with 0.01 % captan, a broad-spectrum fungicide(Panahi *et al.*, 2002). All seeds were sown in 4-L polyethylene pots containing sand and organic material (10% washed sand and 90 % sphagnum peat with a diameter less than 0.5 mm) in June 2011. Plants cropping requirements, including soil preparation, planting, irrigation, thinning, staking, pruning and pest and disease control werefollowing good agricultural practices starting fromthe first growing season. In March 2012, seedlings (twenty-seven plants for each cultivar) were transplanted to 5-L polyethylene pots filled with vermiculite. Transplanted 1-year-old seedlings were grown in a controlled glasshouse environment in a hydroponic system using Hoagland's solution (Picchioni *et al.*, 1991) for fertigation. Temperature and relative humidity in the glasshouse ranged between 21.7-27.1°C and 49.4-71% RH,respectively. Polyethylene glycol (PEG) solutions with molecular mass of 6000 and above are often used to create an osmotic stress (Nepomuceno *et al.*, 1998). In May 2012, drought treatments were applied using PEG 6000, they consisted of a control (osmotic potential of the nutrition solution (Ψ_s) = -0.10 MPa), and two drought stress levels ($\Psi_{s=}$ -0.75 MPa, $\Psi_{s=}$ -1.5 MPa). Drought stress levels were maintained for two weeks; then all solutions were replaced by the control treatment (-0.10 MPa), and this level was maintained for two recovery weeks.

Measurements

Relative chlorophyll content (SPAD)

Measurements were done on the fifth fully expanded leaf counting from the top of the pistachio seedlings using a chlorophyll content meter (CCM-200 plus chlorophyll content meter, ADC, UK). For each leaf, three readings were performed and averaged. Measurements were done after four weeks afterwarddroughts stress, respectively,usingthree replicates.

Plant growth parameters

After four weeks when plants subjected to drought stress treatments, seedlings were harvested. Dry weightsof leaves, shoots and roots were measured with a precision of ± 0.1 mg (Mettler Toledo PB602-L, Greifensee, Switzerland). Dry weight of the plant fractions was determined after drying at 85°C for 72 hours (L031, Jouan laboratory oven, UK).

Evaluation of drought indices

Drought tolerance/susceptibility indices werecalculated to describe drought tolerance and resistance in pistachio cultivars. These indices were calculated as follows for each cultivar:

Stress tolerance (TOL) and mean productivity (MP) (Rosielle and Hamblin, 1981);

MP= (Yp + Ys) / 2, and

TOL= (Yp - Ys).

Stress susceptibility index (SSI) was computed according toFischer and Maurer (1978):

SSI= 1 - [(Ys) / (Yp)] / SI,

Stress intensity (SI) = 1 - [($\bar{Y}$s) / ($\bar{Y}$p)].

Fernandez (1992) introduced the stress tolerance index (STI) to identify performance in both drought and stress conditions and the geometric mean productivity (GMP):

STI = [(Yp) × (Ys)] / (Ȳp) 2,

GMP = [(Yp) × (Ys)] 0.5.

In the above formulas, Ys is plant biomass of the cultivar under stress, Yp is plant biomass of the cultivar under non-stressed condition;Ȳs and Ȳp represent the means of plant biomass of all cultivars in stressed and non-stressed conditions, respectively.

Statistical analysis

Analysis of variance for each variable was applied to data analysis.Treatments and cultivars were consigned to a Randomized Complete Block Design (RCBD) with three replicates. A two-way analysis of variance was used to test for drought treatment differences and cultivar effects. Means were compared by a Tukey's test. Correlations between parameters were calculated using Pearson's correlation method.A principal component analysis (PCA) approach was used to analyze the tolerance indices using data from the control, and the most severe drought stress treatment ($\Psi_{s=}$ -1.5 MPa). The biplot of the PCAanalysis was generated to identify tolerant cultivar and high total plant biomass.All analyses were performed in SPSS 20 (IBM Corporation, USA) and JMP 10(statistical discovery software, SASInstitute,USA)which was used to draw the biplot display.

Results

Chlorophyll contents

SPADvalues significantly decreased for Akbari and Ohadi cultivarsas a result of the stress level imposed (Fig. 1A). Under osmotic drought stress, relative chlorophyll rates varied significantly between cultivars (P<0.01). Effects of cultivar and treatment at$P$< 0.01, and cultivar and treatment interactions were significant at P< 0.05during the drought stage. During recovery, treatments differed significantly at$P$< 0.01(data not shown).

Table 1. Drought tolerance indices of three pistachio cultivars, i.e. Akbari (AK), Kaleghochi (KA) and Ohadi (OH) under control, and severe drought-stressed conditions.

	YP	YS	TOL	MP	GMP	SI	SSI	STI
AK	2.79	2.33	0.46	2.56	2.54	0.17	1.08	0.95
KA	3.61	3.11	0.51	3.36	3.34	0.14	0.39	1.32
OH	5.62	3.71	1.91	4.66	4.51	0.34	0.70	0.83

Biomass characterization

Plant dry weight was significantly lowered for stressed plants (Fig. 1B). Leaf dry weight (LDW) significantly decreased while shoot (SDW) and root dry weight (RDW) did not differ significantly. Furthermore, cultivars significantly differed in drought stage. Drought stress treatments significantly decreased PDW and LDW for Akbari; LDW for Ohadi compared to control, whereas, there were no significant differences between moderate and severe drought stress levels.

Principal components analysis

Drought tolerant indices were calculated on the basis of plant dry weight (biomass) of cultivars (Table 1). As shown in table 1, plant biomass under stress conditions (YS) have decreased compared to plant biomass under non-stress conditions (YP). In the current experiment, plant biomass production figures for severe drought stress conditions were 16.49%, 13.85% and 33.99% lower than plant biomass under control conditions in Akbari, Kaleghochi and Ohadi cultivars, respectively. To determine the most-desirable drought tolerance standards, the correlation coefficients between YP, YS and other quantitative indices of drought tolerance were calculated (Table 2). Results showed that TOL, MP, GMP and STI were positively and significantly correlated to Ys, whereas SI and SSI did not correlate significantly with Ys. Significant correlation between Ys with TOL, MP, GMP and STI shows that these indices are good predictors to predict drought resistance while, the lack of correlation for Ys with SI and SSI indicates that these indices are not useful predictors for drought tolerance in pistachio.

Reduction in plant biomass (TOL) was utilized as a basis to detect drought tolerance. Our results show a significant positive correlation between TOL with all drought indices, i.e. MP, GMP, SSI, SI and STI (Table 2). Drought indices were significantly correlated with Yp except SI (Table 2).

Table 2. Correlation coefficients between plant biomass of cultivar under stress (YP) and plant biomass of cultivar under control (YS) conditions, and stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress index (SI) andstress susceptibility index (SSI) and stress tolerance index (STI) imposed on three Iranian pistachio cultivars.

	YP	YS	TOL	MP	GMP	SI	SSI	STI
YP	1							
YS	0.896**	1						
TOL	0.858**	0.541**	1					
MP	0.985**	0.959**	0.757**	1				
GMP	0.974**	0.973**	0.721**	0.998**	1			
SI	0.372 ns	0.238 ns	0.429*	0.33 ns	0.314 ns	1		
SSI	0.448*	0.244 ns	0.566**	0.38 ns	0.366 ns	0.02 ns	1	
STI	0.749**	0.867**	0.416*	0.814**	0.830**	-0.138 ns	0.346 ns	1

Within each column, means superscript with **, * are significantly different at $P< 0.01$ and$P< 0.05$, respectively and none significant with ns superscript.

Selection based on a combination of drought tolerance indices may provide a useful approach to identify drought tolerance. Principal component analysis (PCA) was used for treatment combinations (cultivars and drought treatments). The first two axes of the biplot explained 100% of total variation. The first axis explains the largest amount of variation (PCA 1=74.7%), and the second axis (PCA 2) 25.3 % (Fig. 2).

The biplot predicts that although Ohadi had a good plant biomass productivity (under drought stress, Ys), biomass was strongly affected by drought (high TOL and MP) compared to the other cultivars. On the other hand, the effect of drought stress on biomass production of Kaleghochi is limited, as reflected by its high STI (Fig. 2). Although total plant biomass of Akbari was lower compared to that of Kalegochi and Ohadi, its stress tolerance to drought has a middle value as shown by their high SSI (1.08).

Discussion

Biomass production was significantly lowered by drought stress compared to control plants for all cultivars. Under severe drought stress, cell elongation of higher plants will be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami, 1998).Drought stress inhibits the dry matter production largely through its inhibitory effects on leaf expansion, leaf development and consequently reduced light interception (Anjum *et al.*, 2011). Overall observed lowering in biomass production with increasing drought stress can be attributed to a decrease in leaf biomass but not to shoot or root biomass. The decrease in total plant dry weight of the tested pistachio cultivars with increasing drought is in line with results obtained by others with pistachio (Abbaspour *et al.*, 2012; Ranjbarfordoei *et al.*, 2000) and other species (Zhao *et al.*, 2006).

Ohadi had significantly higher leaf and total plant dry weights than both other cultivars in control treatment. Also, Ohadi had higher root dry weight than both other cultivars. Ohadi showed, however, the largest decrease for all plant growth parameters in reaction to drought stress. In contrast, small changes were observed in plant dry weight in response to drought stress for Kaleghochi (Tables 1). For plants that mainly grow in semi-arid areas, such as pistachio, a well-developed root system will allow to exploit deep soil water(Ferguson *et al.*, 2005; Panahi *et al.*, 2002). Ohadi had a higher root mass in both control and drought stress treatments indicating a better ability to cope with drought stress.

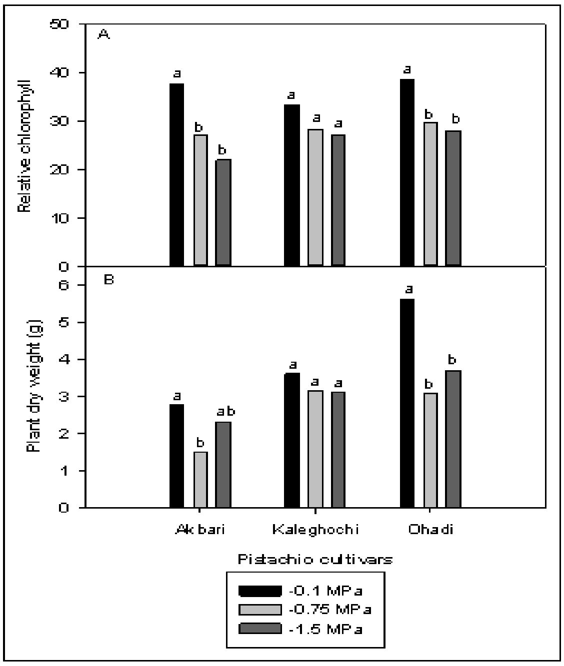

Fig. 1. Changes in relative chlorophyll content (A) and plant dry weight (B) in three pistachio cultivars (i.e. Akbari, Kaleghochi and Ohadi) at control (-0.1 MPa), and different drought stress levels (-0.75 and -1.5 MPa) induced by PEG (n = 9). Within each cultivar, means superscript with unlike letters are significantly different (P< 0.05).

Fernandez (1992) reportedon different indices which are useful to score the performance of a genotype under both control and stress conditions. Although these stress indices are mainly used to screen herbaceous species, we evaluated pistachio cultivars (Akbari, Kaleghochi and Ohadi) for their biomass performance using these indices. Our results show a linkage between both plant biomass and plant biomass reduction (TOL) with STI, MP and GMP, suggesting that selection-based TOL is useful to distinguish between group C (cultivars with low Yp but high Ys) and group A (cultivars with high Yp and Ys) for pistachio cultivars. MP could be selected cultivars with high Yp but low Ys (group B). Correlations of STI to plant biomass reduction and Yp show its (STI) ability to separate group A from other groups. Furthermore, on average STI has higher ability than GMP to distinguish group A. On the other hand, there was no significant correlation between GMP and SSI (0.366^{ns}), suggesting that both indices are a potential indicator with different biological responses to drought. Ramirez-Vallejo and Kelly (1998) found no significant correlation between GMP and SI show that the high GMP with a low SI is biologically available in common bean. Tolerance index including STI identified cultivars with high plant biomass production in both favourable and unfavorable moisture conditions.

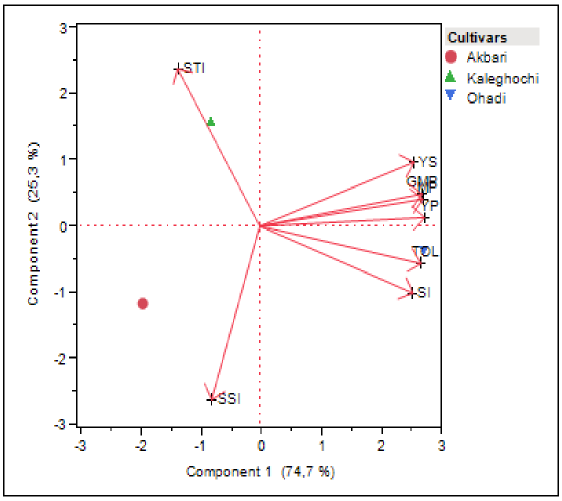

Fig. 2. Biplot of principal component analysis of drought tolerance indices in three Iranian pistachio (Akbari, Kaleghochi and Ohadi) cultivars according to sixdroughtstolerant indices to control (-0.1 MPa) and severe drought stress level (-1.5 MPa) induced by PEG.

Plant material (biomass) is often determined by breeders to select and explore a way for developing cultivars for drought stress environments (Sabaghnia *et al.*, 2011). The results of selection based STI was appropriate to distinguish group A. Among the mentioned cultivars; Kaleghochi fell in this group showing. Therefore, Kaleghochi had well performance of plant biomass productionunder both favorable and unfavorable conditions. Semi-arid areas characterized with large variability of conditions (Annicchiarico and Pecetti, 2003), our results showed Kaleghochi (with falling into group A) may be able to obtain well performance in a wider range of environment than other cultivars; it shows a low percentage of plant biomass changes in both stress and non-stress conditions (As also seen (Ramirez-Vallejo and Kelly, 1998). Moreover, our results revealedOhadi cultivar may have been had more drought-resistant due to drought stress condition, which has been emphasized the low potential of plant biomass production for non-water stress conditions. The researcher prefers cultivars that have well performance when water is not limited with a minimum loss in biomass during drought seasons in a plant breeding program (Uddin *et al.*, 1992).

Finding of this experiment showed that Ohadi is relatively more suited for drought and dry conditions. Although, performance reduction rate of Ohadi was higher and this value for Kaleghochi was lower compared to other cultivars in drought stress conditions.It needs to be taken into account that normally,the calculation of the drought indices is based on the obtained yield. However, since in the period of this study the measuring of yield was not possible, we used biomass (dry weight) to estimate the drought indices with assumption that cultivars with higher biomass can tolerant drought which needs to test by further studied.

On the other hand, evaluation of drought indices that based on plant biomass reduction in drought and control conditions, showed that Ohadi cultivar may have been had more drought-resistant due to drought stress condition. Therefore, it is relatively more suited for drought and dry conditions.

Among pistachio cultivars, Ohadi had the higher rates of relative chlorophyll, plant dry weights (biomass) and root dry weight in drought condition comparedto the othercultivars and significant with Akbari and non-significant to Kaleghochi. It is advisable to use Ohadi cultivar in regions more prone to soil drought as they are more drought tolerant and perform better than the other two evaluated cultivars.Kaleghochi cultivar may produce superior biomass in non-drought stages. Thus, productivity of this cultivar can be more stable than two others cultivars in irrigated conditions. Cultivars those changes between different environments would not have stability. However, further research is needed to support this idea in long-term studies, more severe drought treatments and on adult trees.

In conclusion, measured traits varied significantly with drought stress and seedlings use these traits to cope drought conditions. Cultivars responded to drought stress differently. Therefore, we concluded thatKaleghochimay be more tolerant in terms of productivity. However, Ohadi may be more tolerant to drought when survival is concerned because it was in group C.

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