In Vitro Screening of Cucmis Melo L. Against Drought Mediated By PEG and Sorbitol

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Abstract

Water deficit in first growth stages of melon (*Cucumis melo* L.) in formation of first true leaves after germination can be a factor limiting production. The first step for resolve the problem is genotypes evaluation and identification of drought tolerant melons. An effective method to achieve the goal is use of osmotic solution in tissue culture. Responses of Iranian melon landraces to drought was evaluated using sorbitol at 0.1, 0.2 and 0.4 M or polyethyleneglycol (PEG 6000) at 0.009, 0.012 and 0.015 M concentrations, and MS medium without treatment as the control. Coleoptile length, fresh and dry weight of shoots and roots, photosynthetic pigments, protein, proline, malondialdehyde (MDA) and antioxidant enzymes superoxide dismutase, guaiacol peroxidase and ascorbate peroxidase were measured. The PEG or sorbitol decreased coleoptile length, fresh weight and photosynthetic pigments, and led to enhancement of proline and MDA. Contents of protein and antioxidant enzymes was completely dependent on genotype and type and concentration of osmotic material. The *in vitro* culture for screening and identification of tolerant and sensitive drought genotypes could be rapid, useful and effective, with sorbitol mimicing drought better than PEG. After *in vitro* evaluation, the genotype responses to induced water deficit need to be confirmed under field conditions.

Introduction

Shortage of water, which detrimentally reduces plant growth, is becoming more drastic as desertification increases worldwide[1]. Drought significantly affects vegetable production, disturbs plant water relationships, reduces leaf size, root growth and various physiological characteristics[2]. Melon (*Cucumis melo* L.) is an important vegetable cultivated in arid and semiarid areas of the world, and generally is moderately tolerant to water deficict[3].

Landraces, or local varieties, often have useful genes for adaptation and tolerance to biological and non-biological challenges and are a source for breeding[4,5]. Some Iranian landraces of melon are tolerant, and resistant to water-deficit. Evaluation and identification of tolerant genotypes, or landraces, is important in development of drought-tolerant melons. An effective method to evaluate plants is with *invitro* culture which is relatively easy, mimics environmental conditions[6], but needs to be confirmed under field conditions to appraise effects of water deficit on drought-tolerant genotypes. Polyethylene glycol (PEG) and sorbitol could be used for *in vitro* screening[7]. The PEG and sorbitol reduce water potential and simulate drought in the medium. The PEGs are macrogols, a polyether constructed of repeated ethylene glycol units [-(CH_2CH_2O) n] with grades from 100 to 700 molecular weight (MW) liquid at 21-24°C, those between 1000 and 2000 MW are soft solids, and at MW>2000 are hard crystalline solids with melting points around 63°C. The high polarity of PEG rises hydrophilicity and increases water solubility[8]. The PEG do not react with chemicals or biologicals compounds, are non-toxic, cannot be absorbed by plants and its concentration remains constant throughout the test period[9]. Sorbitol is a water-soluble polyhydric alcohol (polyols) having 0.6 sweetness from sucrose, made from corn syrup and synthesized from glucose, and naturally detected in some plants[10].

Studies on effects of drought in melons have been done under field and greenhouse conditions[2,3,11], and none study has been conducted using *in vitro* screening selection in cucurbitaceae family

and especially in *C. melo* by means of seed culture. Some studies were conducted in association with effects of osmotic materials by creation of *in vitro* and *in vivo* drought in other crops, but it has not been done on *C. melo* seedlings in tissue culture[6,7,9].

This study was undertaken to assess drought-tolerant melons using PEG or sorbitol *in vitro* with objectives of: 1) rapid and accurate identification of tolerant genotypes at 7 days after application of osmolytes; 2) use of one, or some, traits as indicators for identification of drought tolerant melons in tissue culture 3) to extend *invitro* screening for drought tolerance using landraces of melons with different water use efficiency; and 4) finally, comparison of sorbitol with PEG as drought simulators, while they are completely different structurally and chemically, and selection of better osmolyte.

Methods

Seed of the *C. melo* landraces: Ghobadloo (GHO), Girke (GIR) and Toghermezi (TOG) were collected from Ajbashir (37.4788° N, 45.8929° E), Boukan (36.5187° N, 46.2094° E) and Isfahan (32.6539° N, 51.6660° E), respectively. At GIR dry land-farming is the norm; at TOG and GHO irrigation is used. Seeds have been collected based on Maragheh University license and for Maryam Nekoee's master's thesis. All seed were pre-treated with 10% sodium hypochlorite (NaOCl) solution for 15 min and subsequently washed with distilled water and air dried. Seed were cultured in ¼ MS medium[20]. After germination, plantlets were subcultured to MS media containing sorbitol (Merck, Darmstadt, Germany) and PEG 6000 (Merck) and placed in a growth chamber. The growth chamber had a photoperiod of 16/8 h (light/dark), temperature of 25°C and air relative humidity of 70±5%. The sorbitol or PEG concentrations of 0.1, 0.2 and 0.4 mM (osmotic potentials were -0.88, -1.12 and -1.80 Mpa), respectively, estimated following Reid[21] and 0.009, 0.012 and 0.015 M (osmotic potentials were -0.55, -0.87 and -1.2 Mpa), estimated following Michel and Kaufmann[22] respectively, was added to the MS medium, containing 8 g•L^{-1} agar and 30 g•L^{-1}sucrose. The control was MS medium without sorbitol and PEG. After 7 days, plants were harvested and evaluated.

The coleoptile length was measured, shoot and root fresh weights were determined and tissues dried in a convection oven at 70° C for 24 h. Dry weights of shoots and roots were determined and percents calculated.

Chlorophylls (Chl *a* and *b*) and carotenoids (CARs) content were determined spectrophotometrically using equations described by Arnon[23]. Leaf samples (0.5 g) were ground in liquid nitrogen and suspended in 10 mL of 80% acetone, mixed and extracted in 2 mL of extract and poured into tubes. The concentration was determined by measuring the extinction of the extract at the major red absorption maxima of Chl *a* (664 nm) and b (647 nm) and CARs (470).

To determine proline fresh leaves (0.5 g) were ground with liquid nitrogen, then 10 mL of 3% sulphosalicylic acid was added and centrifuged at 1120 g for 20 min at 4°C, and finally 2 mL of ninhydrin acid and 2 mL of glacial acetic acid were added to the supernatant. The mix was placed in a boiling water bath for 60

min, removed and immediately cooled on ice for 5 min. Then 4 mL of toluene was added and the mix vortexed for 20 sec. Absorbance was recorded at 520 nm[24].

The amount of malondialdehyde (MDA) was estimated following Heath and Packer[25]. A 0.5 g of fresh leaves was ground in 1.5 mL trichloroacetic acid 1% w/v (TCA), and centrifuged at 1120 g for 10 min and 1 mL thiobarbituric acid 0.1% w/v (TBA) added to 0.5 mL of the supernatant. The mix was boiled at 95°C for 30 min and cooled on ice for 15 min. Absorbance was recorded at 532 and 600 nm. The extinction coefficient of MDA was calculated.

Total protein content was determined with fresh leaf samples (0.2 g) ground in liquid nitrogen and homogenized in 1.5 mL of 50 mM Na buffer phosphate (pH:7.8) including 1 mM EDTA and 2% (w/v) polyvinylpolypyrolidone. The homogenate was centrifuged at 1344 g for 15 min at 4°C. Supernatants were used for total soluble protein and extracts for enzyme activity. Total protein content was measured following Bradford[26], bovine serum albumin (BSA) was used as a standard with the serial solutions containing 0, 0.2, 0.4, 0.6, 0.8 or 1 mg•mL^{-1} to which 100 µL of Bradford solution was added to each standard. The Bradford reagent was prepared with 50 mg of coomassie brilliant blue G-250 dissolved in 50 mL of methanol and 100 mL of 85% (w/v) phosphoric acid added. The solution was added into 850 mL H_2O and gravity filtered through Whatman filter paper #1. Finally, 1000 µL of the Bradford reagent was added to 50 µL protein buffer phosphate samples and mixed, incubated for 5 min and absorbance read (A595 nm) at taken with a spectrophotometer (model 100, Cary, Richmond, VA).

Antioxidant enzymes were determined with ascorbate peroxidase specific activity (APX) assayed according to Nakano and Asada[27] with oxidation of ascorbate by APX. The reaction mix included 2550 µL 0.5 mM ascorbate, 450 µL 3% H_2O_2 and 50 µL extract and recorded at 290 nm. Guaiacol peroxidase specific activity (GPX) was measured by reduction of H_2O_2 with oxidation of guaiacol following Chance and Maehly[28]. The reaction mix was 1500 µL sodium buffer phosphate (100 mM) pH:7, 120 µL H_2O_2 (15 mM), 480 µL guaiacol (20 mM) and 50 µL of enzyme extract. Increase in absorbance was recorded at 470 nm. Superoxide dismutase specific activity (SOD) was determined according to Giannopolites and Rice[29]. The mix was contained 100 µL sodium carbonate (1.5 mM), 200 µL methionine (0.2 M), 100 µL EDTA (3 mM), 1500 µL sodium phosphate buffer (0.1 M), 900 µL distilled water, 100 µL nitro blue tetrazolium (2.25 mM), 50 µL extract enzyme. The reaction was started by addition of 100 µL riboflavin (60 µM). The mix was incubated under light for 15 min, then absorbance was recorded at 560 nm. The reaction mix without enzyme extract was the control.

The experiment was arranged as a factorial based on a completely randomized design with 4 replicates. The data were subjected to ANOVA in a general linear method (GLM) using SAS software (ver. 9, SAS Institute, Cary, NC), nevertheless correlation, principal component analysis and cluster analaysis were done by SPSS (ver. 23) and minitab (ver. 17) software.

Results

Sorbitol or PEG individually, and in the interaction with genotype affected coleoptile length, and shoot and root fresh and dry weight and the interaction was used to explain results (Table 1). In all three genotypes, coleoptile length was reduced under simulated drought compared to the control. The longest coleoptile was for TOG control and the least was for TOG at 0.2 M sorbitol, GHO at 0.2 M and 0.4 M sorbitol. The maximum reduction belonged to TOG (50%) and the minimum was for GIR (31%) (Fig. 1).

Shoot FW in all genotypes declined in media with sorbitol and PEG. The genotype TOG had the maximum and highest reduction (73%) of shoot FW in 0.015 M PEG; the lowest reduction was in the GIR genotype (50%). The control GIR had the minimum shoot FW compared to GHO and TOG controls. Shoot FW was reduced more in all genotypes under drought simulated by sorbitol than PEG (Fig. 2a). Root FW was reduced in media with PEG and sorbitol. The TOG produced greater root FW than controls of GHO and GIR. The most, and least, reduction of root FW was in TOG (84%) and GIR (65%), respectively. Sorbitol decreased root FW more than PEG (Fig. 2b).

Shoot DW was affected by increasing PEG and sorbitol concentration; genotypes in all media increased compared to the control. The increase was more pronounced in GHO with 0.0.4M (7.27%), TGO with 0.4 and 0.2 M (7.38%, 6.33%) of sorbitol, respectively; and GIR had the lowest shoot DW (1.36%). Shoot DW of GHO was 3.15 times greater than the control, and 1.18 greater than for GIR. Sorbitol increased shoot DW more compared with PEG (Fig. 2c).

In genotypes root and shoot dry weights increased under simulated drought created with sorbitol and PEG. Root DW of GIR (11.44%) in 0.4 M sorbitol was highest; the lowest was for TOG (1.52%) controls. The greatest root DW was in GIR for 0.4 M sorbitol, and 2.8× more than in TOG (0.4 M sorbitol) more than the GIR. Generally, sorbitol caused a greater increase of root DW than did PEG (Fig. 2d).

The interaction of sorbitol, or PEG, with genotype affected photosynthetic pigments and was used to clarify results (Table 2). There was a decrease in Chl *a* content when osmolytes were used. The highest Chl *a* content was in control GHO, TOG and GIR genotypes with 30.30, 31.72 and 30.58 mg•kg^{-1} FW, respectively, and in the MS media with 0.015 M PEG, leaves of GIR genotype had lower Chl *a* than all of genotypes under simulated drought. Reduction of Chl *a* content was more in GIR (78%) than TOG (68%) and GHO (54%), as well as Chl *a* content in GIR, decreased more quickly than other genotypes in MS media with 0.2 M sorbitol and 0.009 M PEG (Fig. 3a). Chl *b* decreased in all the genotypes exposed to osmotlytes. In the MS media with sorbitol and PEG, Chl *b* was decreased more in GIR than TOG and GHO. Chl *b* in GIR was decreased 83% in MS media with 0.015M PEG compared to the control, while the reduction was 70% and 52% for TOG and GHO, respectively. The GIR had more Chl *b* in controls than other genotypes (Fig. 3b). Chlorophylls *a* and *b* declined with sorbitol and PEG in the genotypes, and total chlorophyll was reduced more in GIR with PEG and sorbitol treatment than other genotypes (Fig. 3c). The CARs content was decreased by simulated drought induced with PEG and sorbitol in all genotypes. The most, and least, CARs was in GHO without osmotmolytes and GIR with 0.015 M PEG, respectively. The CARs content of GIR was reduced more than GHO and TGO (Fig. 3d).

The interaction of sorbitol, or PEG, with genotype affected proline content (Table 2). Use of osmotytes leads to enhanced proline content at 0.1, 0.2 M of sorbitol and 0.009 and 0.012 M PEG, and at higher PEG and sorbitol concentrations decreased, although genotypes under simulated drought produced more proline than controls. The most, and least, proline content was in GIR with 0.2 M sorbitol and MS medium without PEG and sorbitol, respectively. In GIR the most proline content occurred (8.45-fold) at 0.2 M sorbitol (Fig. 4a).

The interaction of sorbitol or PEG with genotype affected MDA content (Table 3). The MDA in all genotypes increased with increased PEG and sorbitol concentration in MS medium. The MDA was higher in TGO and GHO at 0.4 M sorbitol, and these genotypes had the most increasing 6.96 and 4.75 times compared to controls, respectively, and the GIR was least. The MDA in plants treated with sorbitol was higher compared to plants treated with PEG (Fig. 4b).

The interaction of sorbitol or PEG with genotype affected total protein contant (Table 3). Total protein was decreased in MS media containing sorbitol and PEG compared to controls, but there was no difference at 0.009 M PEG in GHO. The highest total protein was in GHO controls and under 0.009 M PEG, and in the TOG control, GHO had the most reduction, up to 63.55%, and TOG had the least reduction, up to 59.70%, compared with control in 0.4 M of sorbitol. Sorbitol decreased total protein more than PEG (Fig. 4c).

Sorbitol or PEG individually, and in the interaction with genotype affected antioxidant enzymes activity and the interaction was used to explain the results (Table 3). Both sorbitol and PEG stimulated APX special activity; activity induced by sorbitol was greater than for PEG. The highest APX special activity was in TOG under 0.2 M sorbitol and GHO under 0.2 and 0.4 M sorbitol; the most APX activity was in GIR (13.6 times) (Fig. 5a).

The GPX special activity increased in MS medium with PEG and sorbitol in all concentrations and all genotypes. The highest GPX special activity was in GHO in MS medium with 0.4 M sorbitol, the highest increase was in GIR with 0.4 M sorbitol, nearly 3-times than GHO and TOG (Fig. 5b).

The SOD special activity was increased by adding PEG and sorbitol to MS medium in all genotypes; differences compared to control at higher concentration were not different. The most SOD special activity was in GIR in MS medium with 0.2 M sorbitol, the highest, compared to the control, was in GHO with 0.1 M sorbitol. Sorbitol induced activity of SOD better than did PEG (Fig. 5c).

Correlation, principal component, and clustering analyses

Bivariate pearson coleration analysis explained significant positive and negative correlation among the evaluvated traits at 5% and 1% of P value. Significant positive correlation of coleoptile length with shoot FW, root FW, photosynthetic pigments and negative with shoot DW, root DW, proline, MDA, total protein, APX and GPX special activity was represented. In this term, shoot FW was positively correlated with root FW, photosynthetic pigments and protein, and other hand were negetaivly correlated with root DW, proline, MDA and GPX special activity. Similarly, root FW positively was correlated with photosynthetic pigments and total protein, while negatively was correlated with root DW, total protein MDA and SOD special activity. In the same way, root DW and photosynthetic pigments, protein and root DW and MDA, APX, GPX and SOD special activity, photosynthetic pigments together, MDA and GPX special activity, protein and SOD special activity, APX and GPX special activity, SOD and GPX special activity represented sifnificant positive or negative correlation at 1% and 5% P Value (Table 4).

The principal component analysis (PCA) were drawn out to reveal pattern of variation among the assessed morphological and physiological traits and to list a more effective interpretation of the weight of each attribute in the total construction of variation. In the present experiment, among 15 PC, foure PC elucidated 81.03% of the total variance (Table 5). The PC1 was the principal and most efficient component, responsible for nearly 41.35% of the exploited total variance (eigenvalue 6.20). In the PC1, Chl *a*, Chl *b*, total Chl and carotenoids were prominent and most effective vriables or trait which played the major role in the observed variation. Nevertheless, PC2, PC3 and PC4 accounted for 17.22% (eigenvalue 2.58), 12.509% (eigenvalue 1.87) and 9.94% (eigenvalue 1.49) of total variation, respectively.

Loading plot of the studied variables included in the PCA revealed that correlated variables were placed on the plot with close distances (Fig. 7). , as well as the correlations were showed in the pearson correlation. For instance, photosynthetic pigments had highest correlation and they had closet distance in loading plot.

In agreement with bivariate correlation, dedrograme of traits that represented using cluster analaysis were classified in four groups. The evaluvated attributes that correlated to eac other were placed in the same group suh as coleoptile length, shoot FW and photosynthetic pigments (Fig. 6).

Discussion

Coleoptile length, and shoot, and root FW were adversely affected by addition of PEG and sorbitol to the MS medium, but shoot and root DW were positively affected. Sorbitol, or PEG, decrease water potential and simulate drought which has adverse effects on cell division, cytoplasm volume, cell turgor pressure and nutritional balance. Increase drought effects reduce cytokines and increase ABA that affect cell division, reduce fresh weight and restrict plant growth[12,13] Reduction of fresh weight was reported in cucumber[13], melon[3] under drought. Dry weight of drought tolerant plants increase under water deficit[17] as does dry matter partitioning and biomass disposition which is strongly related to plant efficiency under water shortage Enhancement of dry mass is probably due to dehydration and new substance synthesis required for protection of higher osmotic level to continue water uptake[11].

In general, photosynthetic pigments were reduced in genotypes under water-deficit. One reason for pigment reduction during drought can br increasing abscisic acid and ethylene, chlorophyllase and peroxidase enzymes[14]. A decline of chlorophyll content was reported in melon[3,2,11], and cucumber[15]. The GIR had the greatest decrease in photosynthetic pigments in most treatments, which probably is related to leaves being pale green in color during the dry season.

Proline, an amino acid, plays a role in maintenance of plant osmotic potential and in cells osmotic adjustment[16]; it has a role in plant tolerance under biotic and abiotic challenge via antioxidant activity and ROS scavenging[3]. Proline content increased with higher PEG, or sorbitol, in the genotypes, accumulation of

proline depends on genotype and osmotic substances interaction, the more proline in GIR indicated greater tolerance than others under simulated drought.

Increasing MDA due to membrane lipid peroxidation is an indicator to show damage caused by oxidative challenge[3]. Drought causes peroxidation of chloroplast thylakoid glycolipids followed by production of diacylglycerol, triacylglycerol, and free fatty acids which may result in increased MDA in plant tissue[17]. Plants with higher drought tolerance produce less MDA because of their ability to maintain membrane integrity [3,18] and GIR is likely more tolerant than the others due to less MDA under drought.

Increased simulated drought resulted in reduction in total protein content of plants[11]. Total protein content of plants under PEG and sorbitol treatments decreased compared to control genotypes. Total protein content decreased in genotypes under MS medium with sorbitol more than PEG, probably due to greater penetration of sorbitol at high concentration, and these

The antioxidant enzymes APX, GPX, and SOD special activity increased with water-deficit. Sorbitol enhanced enzyme special activities more than PEG and genotype responses were different compared to control' GIR had the most APX, GPX, and SOD special activity. Increasing APX, GPX and SOD special activity under simulated drought in response to ROS is probably due to increased expression of antioxidant enzyme genes and activity of enzymes by activating transcriptional signaling pathways, and because of toxicity of hydrogen peroxide that produced more than plants under oxidative challenge, the antioxidant enzymes may play an important role in neutralizing the toxic effect[19].

Growth and biochemical characters were been affected by drought. Coleoptile length, root and shoot fresh weight, root dry weight, proline, MDA content and GPX activity, can be used as indicators for *in vitro* screening of *C. melo* because there were genotype responses. Protein content, photosynthesis pigment and SOD activity are not reliable for *in vitro* selection because the characteristics rely on genotypes.

The PCA, correlation and cluster analysis showed the photosynthetic pigments had most effect on variation, and they can plays a most important role in selection of drought tolerant melins genotypes under *in vitro* culture. Based on the correlation among photosynthetic pigments and morphological traits such as coleoptile length and shoot and root FW can used for selection of genotypes under dought. And in the next steps, proline, protein and MDA can applied for selection.

Sorbitol mimicked drought better than PEG. Although sorbitol gradually penetrates into cells and may cause toxicity in higher concentration, the penetration can be minimized by reduced exposure. The PEG may affect root development because its viscosity is very sticky and hard[7]. The *in vitro* screening can be done for accurate and rapid selection of numerous melon genotyepes in the laboratory but the results need to be supported by field testing.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval All the authors mentioned in the manuscript have agreed for authorship, read and approved the manuscript, and given consent for submission and subsequent publication of the manuscript.

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Author contribution

Maryam NM carried out culture of the experiment and laboratory analysis and writing as her MS c. dissertation. Farzad R contributed with the writing of this manuscript, data processing and creation of figures and tables. Mousa TG had the idea, set the trial programming. Seyaed Morteza Z contributed by analyses of enzyme activity and photosynthesis pigments. Mohammad AA contributed by making their equipment and material available for culture.

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Tables

Table 1 ANOVA analysis of effects of PEG and sorbitol on morphological traits in melon genotypes.

Mean Square						
Source	df	Coleoptile length	Shoot FW	Shoot DW	Root FW	Root DW
Genotype (G)	2	42.21**	0.901**	17.612**	0.022**	12.827**
Treatment (T)	6	199.411**	0.310**	17.694**	0.046**	38.264**
G × T	12	38.582**	0.095**	6.582**	0.11**	8.639**
Error	42	5.664	0.004	1.107	0.001	1.108
C. V. (%)			7.64 14.56	29.91	19.19	15.57

** significant at p≤0.01.

Table 2 The ANOVA analysis of the effects of PEG and sorbitol on photosynthetic pigments in melon genotypes.						
Mean Square						
Source	df	Chl. a	Chl. b	Total Chl.	CARs	Proline
Genotype (G)	2	126.906**	7.460**	195.443**	5.202**	193.397**
Treatment (T)	6	624.092**	125.034**	1305.071**	31.152**	227.600**
G × T	12	23.462**	4.756**	44.513**	1.588**	126.294**
Error	42	1.983	0.658	3.885	0.251	2.174
C. V. (%)		8.5	11.51	8.35	12.46	8.52
** significant at p≤0.01.						

Table 3 The ANOVA analysis of the effects of PEG and sorbitol on biochemical and enzymes activity in melon genotypes.						
Mean Square						
Source	df	MDA	Total protein	APX special activity	GPX special activity	SOD special activity
Genotype (G)	2	65.835**	2.212**	0.802**	4133.235**	2.256**
Treatment (T)	6	27.153**	1.387**	0.627**	341.848**	0.584**
G × T	12	9.013**	0.146**	0.265**	62.531**	0.141**
Error	42	0.291	0.034	0.066	4.619	0.017
C. V. (%)		10.72	13.47	61.09	7.29	15.07
** significant at p≤0.01						

Table 4 Pearson correlation among studied traits of cucumis melo genotypes under drought that simulated by PEG and sorbitol

	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14
Y1	1													
Y2	0.497**	1												
Y3	-0.234*	-0.034	1											
Y4	0.585**	0.820**	-0.097	1										
Y5	-0.423**	-0.601**	0.162	-0.731**	1									
Y6	0.527**	0.665**	-0.403**	0.706**	-0.593**	1								
Y7	0.557**	0.595**	-0.321**	0.658**	-0.533**	0.962**	1							
Y8	0.541**	0.649**	-0.381**	0.697**	-0.579**	0.996**	0.982**	1						
Y9	0.495**	0.604**	-0.377**	0.567**	-0.451**	0.948**	0.929**	0.950**	1					
Y10	-0.457**	-0.199	-0.003	-0.263*	0.165	0.009	-0.010	0.003	0.044	1				
Y11	-0.315**	-0.045	0.230*	-0.293**	0.325**	-0.164	-0.151	-0.161	-0.043	0.382**	1			
Y12	-0.077	0.364**	-0.066	0.310**	-0.513**	0.139	0.023	0.104	0.019	0.257*	-0.163	1		
Y13	-0.120	-0.021	0.311**	-0.188	0.252*	-0.219*	-0.214*	-0.219*	-0.180	0.186	0.669**	-0.090	1	
Y14	-0.238*	0.120	0.249*	-0.130	0.266*	-0.161	-0.184	-0.169	-0.095	0.121	0.783**	-0.145	0.622**	1
Y15	0.112	-0.329**	-0.018	-0.278*	0.295**	-0.116	-0.051	-0.097	-0.014	0.100	-0.146	-.518**	-0.186	-0.340**

**, * are significant at 1% and 5% of P value, respectively. Y1: coleoptile length, Y2: shoot fresh weight Y3: shoot dry weight Y4: root fresh weight, Y5: root dry weight Y6: Chlorophyll *a*, Y7: Chlorophyll *b*, Y8: total Chlorophyll, Y9: carotenoids, Y10: proline, Y11: MDA, Y12: protein, Y13: APX special activity, Y14: GPX special activity, Y15: SOD special activity.

Table 5 Eigenvalue, Percent variation, and cumulative variance of the evaluvated traits (a) and four factors derived from principal component (PC) analysis (b).

a

Component	Total Variance Explained		
	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	6.204	41.359	41.359
2	2.584	17.227	58.586
3	1.876	12.509	71.095
4	1.491	9.942	81.037
5	0.853	5.690	86.727
6	0.583	3.885	90.612
7	0.397	2.649	93/261
8	0.314	2.097	95.357
9	0/230	1.530	96.887
10	0.198	1.319	98.207
11	0.134	0.895	99.101
12	0.071	0.472	99.574
13	0.046	0.305	99. 878
14	0.018	0.122	100. 000
15	0.000	0.000	100/000

Extraction Method: Principal Component Analysis.

b

	Rotated Component Matrix[a]			
	PC1	PC2	PC3	PC4
Y1	0.560	-0.161	-0.066	-0.622
Y2	0.636	0.152	0.508	-0.361
Y3	-0.411	0.395	0.095	-0.242
Y4	0.641	-0.101	0.484	-0.437
Y5	-0.492	0.275	-0.618	0.228
Y6	0.971	-0.115	0.148	-0.024
Y7	0.960	-0.103	0.039	-0.063
Y8	0.975	-0.113	0.116	-0.036
Y9	0.967	-0.034	-0.007	0.026
Y10	0.065	0.150	0.038	0.895
Y11	-0.020	0.874	-0.099	0.317
Y12	0.006	-0.195	0.885	0.260
Y13	-0.126	0.821	-0.001	0.036
Y14	-0.064	0.906	0.051	0.038
Y15	-0.005	-0.297	-0.763	0.040

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization

a. Rotation converged in 5 iterations.

Y1: coleoptile length, Y2: shoot fresh weight Y3: shoot dry weight Y4: root fresh weight, Y5: root dry weight Y6: Chlorophyll *a*, Y7: Chlorophyll *b*, Y8: total Chlorophyll, Y9: carotenoids, Y10: proline, Y11: MDA, Y12: protein, Y13: APX special activity, Y14: GPX special activity, Y15: SOD special activity.

Figures

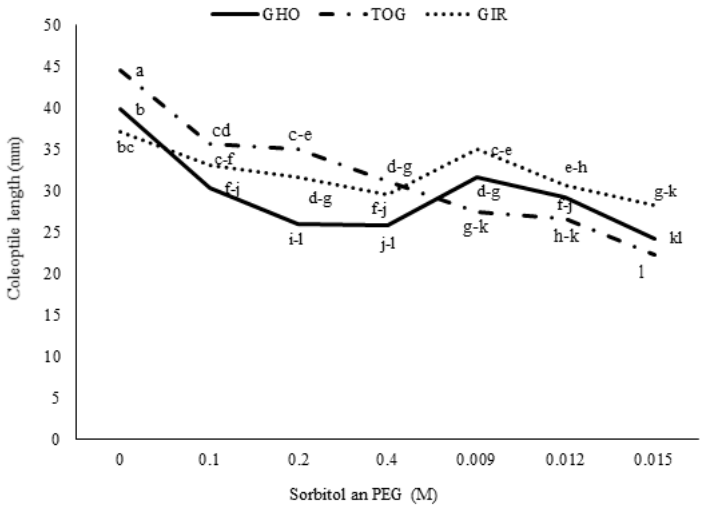

Figure 1

Effects of PEG and sorbitol on coleoptile length (mm) in melon genotypes. Different letters are significantly different based on Duncan's multiple range test ($p \leq 0.05$). GHO = Ghobadloo, TOG = Toghermezi, and GIR = Girke landraces.

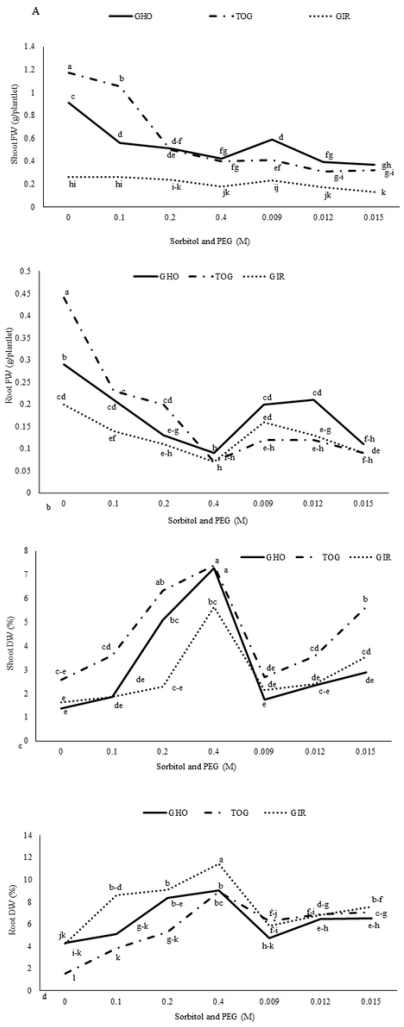

Figure 2

Effects of PEG and sorbitol on Shoot FW (a), Root FW (b), Shoot DW (c) and Root DW (d) in melon genotypes. Different letters are significantly different based on Duncan's multiple range test ($p \leq 0.05$). GHO, TOG and GIR are the Ghobadloo, Girke and Toghermezi landraces, respectively.

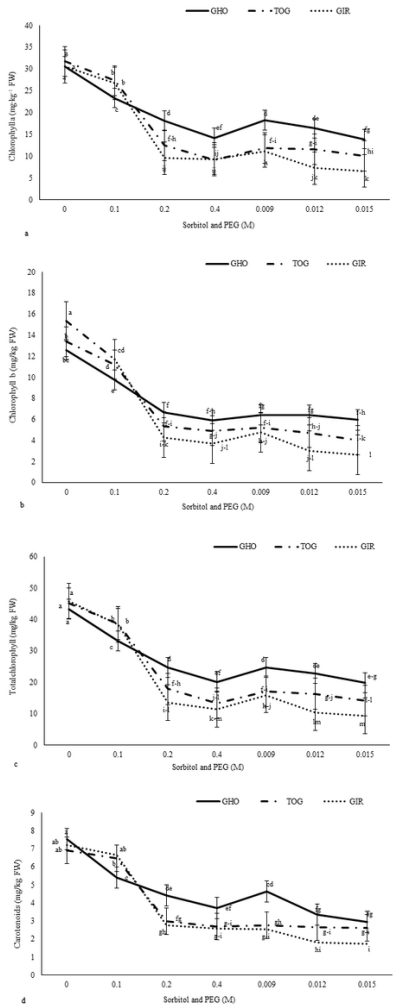

Figure 3

Effects of PEG and sorbitol on Chl a (a), Chl b (b), total Chl (c) and CARs in melon genotypes. Different letters are significantly different based on Duncan's multiple range test ($p \leq 0.05$). GHO, TOG and GIR are the Ghobadloo, Girke and Toghermezi landraces, respectively.

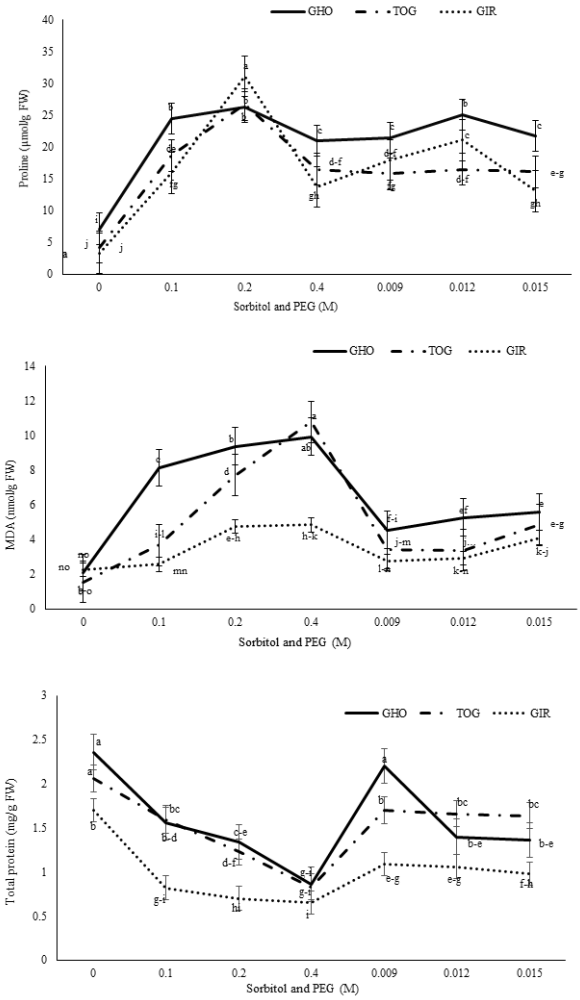

Figure 4

Effects of PEG and sorbitol on proline (a), MDA (b) and Total protein (c) in melon genotypes. Different letters are significantly different based on Duncan's multiple range test ($p \leq 0.05$). GHO, TOG and GIR are the Ghobadloo, Girke and Toghermezi landraces, respectively.

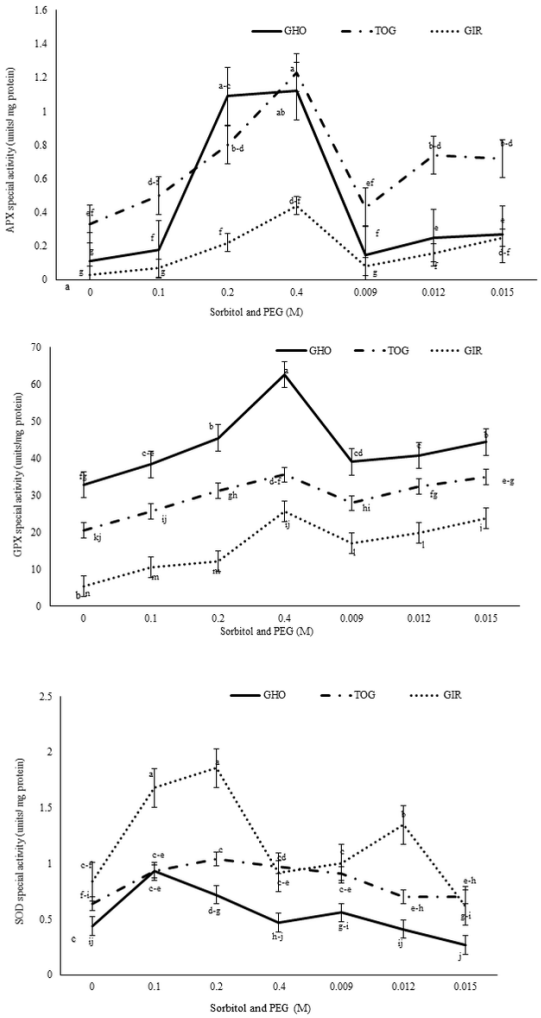

Figure 5

Effects of PEG and sorbitol on APX special activity (a), GPX special activity (b) and SOD special activity (c) in cucurbits genotypes. Different letters are significantly different based on Duncan's multiple range test ($p \leq 0.05$). GHO, TOG and GIR are the Ghobadloo, Girke and Toghermezi landraces, respectively.

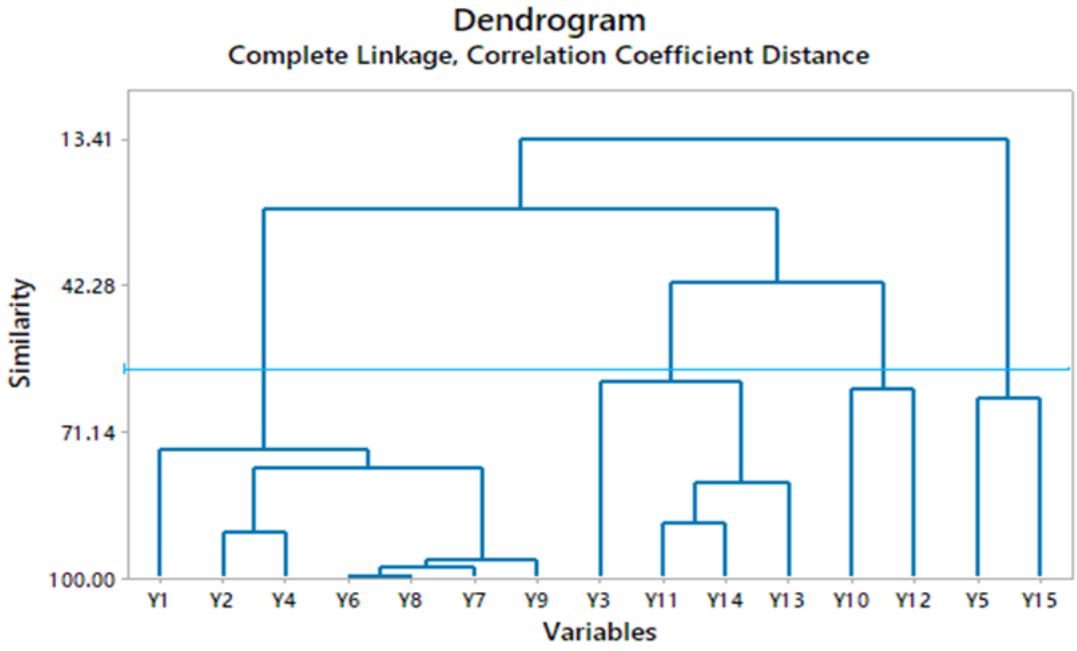

Figure 6

The cluster dendrogram based on the Ward method for evaluvated traits under under drought that simulated by PEG and sorbitol. Y1: coleoptile length, Y2: shoot fresh weight Y3: shoot dry weight Y4: root fresh weight, Y5: root dry weight Y6: Chlorophyll a, Y7: Chlorophyll b, Y8: total Chlorophyll, Y9: carotenoids, Y10: proline, Y11: MDA, Y12: protein, Y13: APX special activity, Y14: GPX special activity, Y15: SOD special activity.

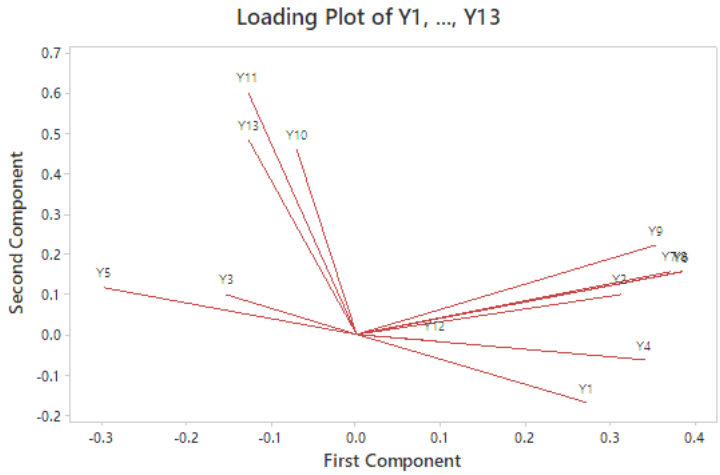

Figure 7

The loading plot of evluvated variables included in the PC analysis. The vriables were Y1: coleoptile length, Y2: shoot fresh weight Y3: shoot dry weight Y4: root fresh weight, Y5: root dry weight Y6: Chlorophyll a, Y7: Chlorophyll b, Y8: total Chlorophyll, Y9: carotenoids, Y10: proline, Y11: MDA, Y12: protein, Y13: APX special activity, Y14: GPX special activity, Y15: SOD special activity.