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Changes in antioxidant enzyme activities and gene expression in two muskmelon genotypes under progressive water stress

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Abstract

Responses of two muskmelon (*Cucumis melo* L.) genotypes (drought tolerant SC-15 and drought susceptible EC-564755) were analyzed at 0, 7, 14, and 21 d of progressive water stress. Although water deficit caused a significant decline in relative water content, the magnitude of reduction was lower in SC-15. Electrolyte leakage, hydrogen peroxide, and malonydialdehyde generation were higher in EC-564755, whereas accumulation of proline was higher in SC-15. Higher activities of antioxidant enzymes, such as catalase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and glutathione reductase, and higher expression of the respective genes were recorded in SC-15 than in EC-564755. Expressions of *DREB2C* and *DREB3* in SC-15 revealed a fluctuating pattern with down-regulation on days 7 and 21 of water stress, whereas up-regulation was observed on day 14. Concurrently, both genes in EC-564755 showed continuous down-regulation on days 7, 14, and 21 of water stress. Expressions of *RD22* and *dehydrin* recorded on days 7, 14, and 21 were lower in SC-15. The cluster analysis showed that, these two genotypes had a clear distinction in physiological and biochemical properties and gene expressions under water stress and the genotype SC-15 had more efficient osmoprotectant mechanism than genotype EC-564755 under water deficit conditions.

Additional key words: ascorbate peroxidase, catalase, *Cucumis melo*, *DREBs*, glutathione reductase, guaiacol peroxidase, *RD22*, RWC, superoxide dismutase.

Introduction

Muskmelon (*Cucumis melo* L.), a member of the *Cucurbitaceae* family, belongs among most cultivated vegetable crops. Water stress, a major limitation of agricultural production (Chaves *et al*. 2003), also severely affected muskmelon yield. Plants exhibit a series of morpho-physiological, biochemical, and molecular responses to drought stress, which render drought tolerance – a complex multi-genic trait (Jimenez *et al*. 2013). Reactive oxygen species (ROS) are essential for regulating the vital processes in cells but their overproduction causes oxidative stress. Increased ROS production due to abiotic stresses causes changes in their homeostasis (Polle 2001), but also act as signal for activating stress responses. To overcome the possible oxidative stress, plants generate enzymatic [*e.g.*, ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), guiacol peroxidase (POD), and superoxide dismutase (SOD)] and non-enzymatic [*e.g.*, ascorbate, glutathione (GSH), carotenoids, tocopherol] antioxidants (Choudhary and Agrawal 2014). The enhanced transcriptions of many antioxidative enzyme genes reflect genetic regulations toward oxidative stress. The relatively high mRNA amount of *cyt-SOD*, *CAT*, *GR*, and those encoding ascorbate-glutathione cycle enzymes confirm their relevance for enzymatic ROS scavenging (Foyer and Noctor 2011).

Dehydration-responsive element-binding proteins (DREBs), also referred to as C-repeat binding factor, belonging to the ethylene responsive factor subfamily, considerably influence plant responses to abiotic stresses (Akhtar *et al*. 2012). *DREB2C* induces by many abiotic stress factors, binds C-repeat/dehydration response element *in vitro* and possesses transcriptional activity (Lee *et al*. 2010). Drought stress induced overexpression

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; DREB - dehydration-responsive element-binding; MDA - malondialdehyde; nding protein, DWD - days of water deficit; EL - electrolyte leakage; GR - glutathione reductase; LPO - lipid peroxidation; POD - guaiacol peroxidase; RWC - relative water content; SOD - superoxide dismutase.
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of *RD22* (a gene encoding dehydration-responsive protein) in an abscisic acid-dependent manner was also reported (Shinozaki and Yamaguchi-Shinozaki 2007). The correlation between tolerance to stresses and accumulation of *dehydrin* was established in barley (Zhu *et al*. 2000) and sunflower (Cellier *et al*. 1998).

Muskmelon is a warm-season crop, frequently cultivated in arid and semi-arid regions with scarce rainfall (Cabello *et al*. 2009). Although drought tolerant cultivars of other crops have been abundantly reported, limited information is available for such cultivars in muskmelon (Cabello *et al*. 2009). Therefore, the present experiment was conducted to examine the effect of water deficit on physiological, biochemical, and gene expression changes in muskmelon genotypes.

Materials and methods

Plants and treatments: The muskmelon (*Cucumis melo* L., 2n = 2x = 24) genotypes SC-15 (drought tolerant) and EC-564755 (drought susceptible) were selected for the present experiment (Ansari *et al*. 2013, Pandey *et al*. 2013). They were grown in pots (9 dm^3) at the experimental site of the Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India (four sets with 5 pots for each genotype). The pots were filled with a mixture of sand, loamy clay, and farmyard manure in the ratio of 1:2:1, the soil bulk density was 1.34 g cm^{-3} and pH 6.8. During the trial period, the mean temperature ranged from 21.4 °C to 38.2 °C and relative humidity ranged from 59 % to 89 %, 14-h photoperiod, and a daily maximum irradiance of 400 μmol m^{-2} s^{-1}. Each pot was irrigated with 2 dm^3 of water at 3 d interval until flowering initiation. Water deficit for, 7 d [soil water content (SWC), 27 %], 14 d (SWC, 13 %) and 21 d (SWC, 8.5 %) was imposed by withholding irrigation in three sets; concurrently the fourth set, kept as the control, was irrigated regularly and an average SWC of 45 % was maintained. Experiments were conducted in triplicates and the SWC was calculated using the method reported by Coombs *et al*. (1987).

Relative water content, electrolyte leakage, hydrogen peroxide, lipid peroxidation, and proline content: The relative water content (RWC) was calculated as RWC [%] = [(FM - DM)/WSM - DM)] × 100, where FM, DM, and WSM were fresh mass, dry mass, and water saturated mass, respectively. Electrolyte leakage (EL) of leaf tissues was measured according to Khare *et al*. (2010) using a conductivity meter (*CM-180*, *Elico*, India) and calculated as EL [%] = (EC1/EC2) × 100, where EC1 is conductivity at room temperature and EC2 conductivity after autoclaving. The H_2O_2 content was measured as described by Jana and Choudhury (1981). Leaf samples (0.2 g) were crushed in 5 cm^3 of 50 mM sodium phosphate buffer (pH 6.5). The supernatant (3 cm^3) was mixed with 1 cm^3 of 0.1 % (m/v) titanium sulphate in 20 % (m/v) H_2SO_4 and centrifuged at 6 000 *g* for 15 min. Absorbance was recorded at 410 nm on spectrophotometer *UV-vis 1601* (*Shimadzu*, Japan). Using the method reported by Heath and Packer (1968), lipid peroxidation (LPO) was estimated as malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction. Leaf samples (0.4 g) were crushed in 4 $cm^3$of 0.1 % (m/v) trichloroacetic acid (TCA) comprehended with 0.5 % (v/v) of butylated hydroxytoluene and 1 % (m/v) polyvinylpyrrolidone. After centrifugation at 12 000 *g*, the supernatant (2.5 cm^3) was mixed with 0.5 % (m/v) TBA and 20 % (m/v) TCA and boiled for 30 min. Absorbance of supernatant was measured at 532 nm; and unspecific turbidity was corrected by subtracting its absorbance at 600 nm. For proline estimation, leaf tissues (0.2 g) were crushed in 3 % (m/v) sulfosalicylic acid and centrifuged at 13 000 *g* for 10 min. Thereafter, 0.5 cm^3 of the supernatant with 0.5 cm^3 of glacial acetic acid and 0.5 cm^3 of freshly prepared ninhydrin reagent was incubated at 100 ºC for 60 min. Toluene (1 cm^3) was added to the mixture and absorbance was recorded at 520 nm (Bates *et al*. 1973).

Antioxidant enzyme activities: For extraction of enzymes, fresh leaf samples (0.2 g) were homogenized in 5 cm^3 of extraction buffer by using chilled mortar and pestle. The extraction buffers used were 50 mM Tris-NaOH buffer (pH 8.0), 100 mM potassium phosphate buffer (pH 7.5), 50 mM potassium phosphate buffer (pH 7.8), 0.1 M Tris-HCl buffer (pH 7.8), and 60 mM sodium phosphate buffer (pH 7.0), for CAT (EC 1.11.1.6), SOD (EC 1.15.1.1), APX (EC 1.11.1.11), GR (EC 1.6.4.2), and POD (EC 1.11.1.7), respectively. Activities of CAT, APX and GR were estimated according to McKersie *et al.* (1990), Nakano and Asada (1981) and Sanchez *et al*. (2010), respectively. While SOD and POD activities were measured using the method described by Shah *et al*. (2001). For CAT, the degradation of H_2O_2 was recorded at 240 nm, absorbance was recorded at 290 nm for APX and the decrease in absorbance was recorded at 340 nm in case of GR. SOD was measured by recording adrenochrome formation at 470 nm, while POD activity was measured as oxidation of guaiacol determined as the increase in absorbance at 470 nm.

Gene selection and expression analysis: Drought stress associated 10 genes were selected and their nucleotide sequences were downloaded (www.melonomics.net). Primers were designed using *Primer3* (*v. 0.9*) software (Rozen and Skaletsky 1998). Selected genes were *CAT*, *SOD*, *APX*, *GR*, *POD*, *DREB2C*, *DREB2D*, *DREB3*, *RD22*, and *dehydrin*.

Total RNA was extracted from the leaves of muskmelon plants using the *TRI* reagent (*Ambion*, USA) in combination with RNAase-free DNAase treatment

(*Qiagen*, Germany). First-strand cDNA synthesis was performed using 1 μg of total RNA in 20 mm^3 reaction volume, using first-strand cDNA synthesis kit, according to the manufacturer's instructions (*Bio-Rad*, Hercules, USA). Expression analysis was performed using *IQ SYBR Green Supermix* (*Bio-Rad*) according to the manufacturer's instructions on *iQ5* thermal cycler (*Bio-Rad*) with *iQ5* software. Gene specific primers for the target genes were used in combination with *actin* gene as an internal control (Table 1 Suppl.). Quantitative polymerase chain reaction (PCR) was performed in an optical 96-well plate. Each reaction mixture contained 5 mm^3 of cDNA, 1 mm^3 of the specific forward/reverse primer (200 nM), and 10 mm^3 of *SYBR Green qPCR Mix* (*Bio-Rad*). Real-time quantitative PCR was performed using the following temperature program: an initial incubation at 95 °C for 1 min, followed by 35 cycles at 95 °C for 30 s, at 55 - 60 °C (varied according to primer melting temperature) for 30 s, at 72 °C for 40 s, and finally one cycle at 72 °C for 5 min. The relative level of gene expression was detected using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). ΔΔCT values reflect the relative fold change expression (induction ratio) of the transcription of the target gene upon exposure to water deficit.

Statistical analyses: The means and standard errors were calculated. The data were analyzed by a one way analysis of variance (*ANOVA*) at ($P \leq 0.05$) using *SPSS v. 16.0* (*SPSS*, Chicago, USA). Duncan's multiple range test was performed to compare the mean values when *ANOVA* results were significant. In order to visualize the performance of two genotypes at different treatments, cluster analysis was conducted on the basis of the unweighted pair group method with arithmetic mean (UPGMA) clustering method using the software *PAST v. 2.15* (Hammer *et al.* 2001) on correlation matrix basis to normalize the quantitative data.

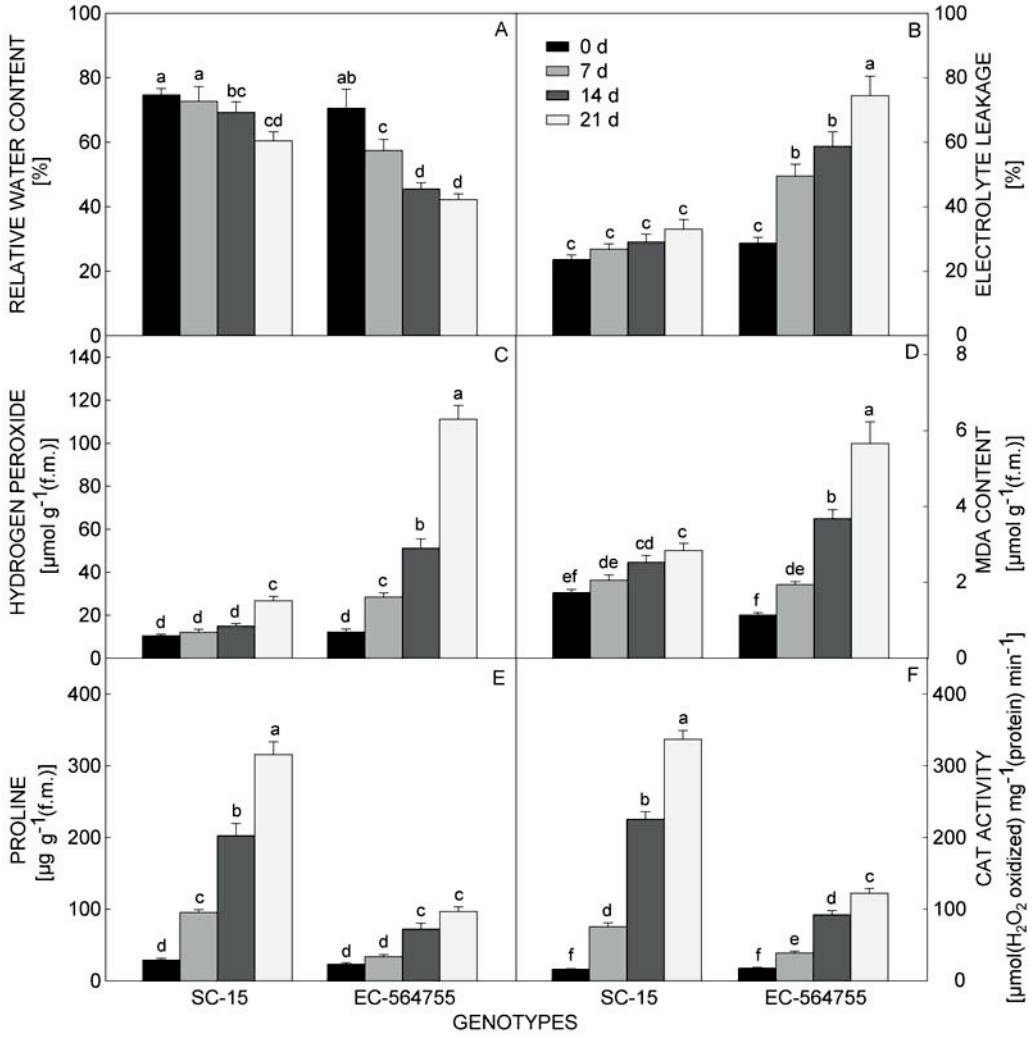

Fig. 1. Relative water content (*A*), electrolyte leakage (*B*), hydrogen peroxide content (*C*), lipid peroxidation (MDA content) (*D*), proline content (*E*), and catalase activity (*F*), in muskmelon leaves under 0 (well-watered), 7, 14, and 21 d of water deficit. Means of three replicates ± SEs, values followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

Results

The genotype SC-15 exhibited greater potential of maintaining tissue water than EC-564755. Under control condition, RWC in both genotypes was similar, 74.7 and 70 %, respectively. As compared to well-watered plants, at 7, 14, and 21 d of water deficit (DWD), the reduction in RWC for SC-15 was 2.7, 7.31, and 19.1 %, respectively, and that for EC-564755 was 18.6, 35.5, and 40.1 %, respectively (Fig. 1*A*). With an increase in DWD, an increase in EL of SC-15 was lower than that of EC-564755. At 7, 14, and 21 DWD, the recorded EL elevation for SC-15 was 13.6, 22.9, and 40.1 %, respectively, and that for EC-564755 was 72.3, 104, and 159 %, respectively, compared to that for well-watered plants. The results indicated that SC-15 maintained a more favorable physiological status than EC-564755 under water deficit conditions (Fig. 1*B*).

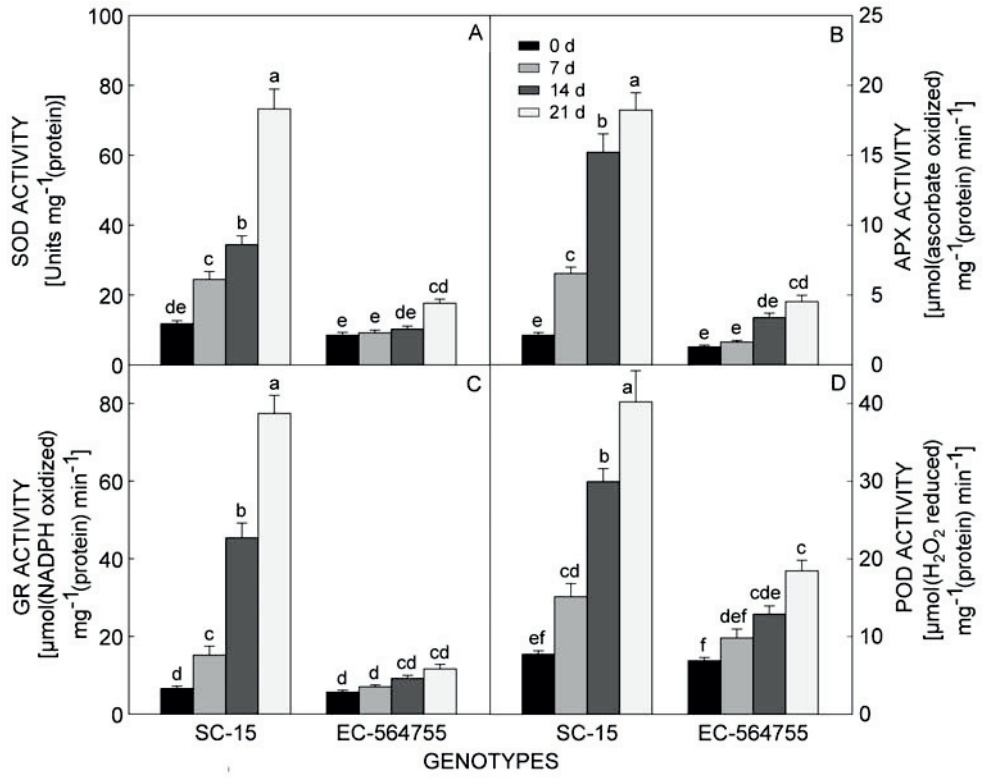

Fig. 2. Activities of key antioxidant enzymes in muskmelon leaves under 0 (well-watered), 7, 14, and 21 d of water deficit: superoxide dismutase (*A*), ascorbate peroxidase (*B*), glutathione reductase (*C*), guaiacol peroxidase (*D*). Means of three replicates ± SEs, values followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

An increase in DWD increased content of H_2O_2, LPO, and proline. At 7, 14, and 21 DWD, H_2O_2 content in SC-15 leaves increased 1.16-, 1.43-, and 2.56-times and in EC-564755 2.33-, 4.19-, and 9.09-times, respectively, compared to that in the well-watered plants (Fig. 1*C*). As the duration of water deficit progressed, MDA content increased at 7, 14, and 21 DWD of 19, 46, and 64 % in SC-15 and 70, 223, and 396 % in EC-56475, respectively, compared to well-watered plants (Fig. 1*D*). Proline content increased at all stages of water deficit in both genotypes. However at 7, 14, and 21 DWD, a higher increase in proline was recorded for SC-15, compared to EC-564755 (Fig. 1*E*).

At 7, 14, and 21 DWD, a rapid increase in CAT activity was observed in SC-15, whereas EC-564755 reported a relatively slow increase. The maximum CAT activity was after 21 DWD when CAT activity was ~20-times higher in SC-15 and ~6-times in EC-564755 than in controls (Fig. 1*F*). SOD activities in SC-15 and EC-564755 were comparable under well-watered conditions (Fig. 2*A*). Compared to well-watered plants in genotype SC-15, the increase in SOD activity was 107, 192, and 522 % at 7, 14 and 21 DWD, respectively, whereas in EC-564755 the activity increased only about twice. A higher increase in APX activity was observed in SC-15 compared to that in EC-564755. Increase in APX activity at 7, 14, and 21 DWD was 3.08-, 7.18-, and 8.6-fold for SC-15 and 1.26-, 2.6-2, and 3.51-fold for EC-564755, respectively, compared to well-watered plants (Fig. 2*B*). Similarly, significantly higher increase in GR and POD activities was recorded for SC-15 than in EC-564755 (Fig. 2*C*,*D*). At 7 DWD, a slight increase in GR and POD activities was observed. At 14 and 21 DWD, 590 and 1076 % as well as 62.9 and 105 %

increase in GR activity was recorded for SC-15 and EC-564755, respectively, compared to well-watered plants. In case of POD, 287 and 420 % as well as 87.2 and 168 % increase was recorded for SC-15 and EC-564755, respectively, compared to well-watered plants under similar condition.

Water deficit caused slight over-expression of *CAT* at 7 DWD in SC-15 (1.58 fold) and EC-564755 (1.45 fold), compared to well-watered plants (Fig. 3*A*). At 14 and 21 DWD, the *CAT* expression was higher in SC-15 (4.93 and 14.4 fold, respectively), than in EC-564755 (3.23 and 3.71 fold, respectively. Differences in expression of *SOD*, *APX*, *GR,* and *POD* were low at 7 DWD in both genotypes. However, the expression peaked at 21 DWD and expressions of *SOD*, *APX*, *GR*, and *POD* were 17-, 10.3-, 8.63-, and 8.57-fold in SC-15, whereas 7.46-, 5.13-, 3.18- and 2.62-fold in EC-564755, compared to well-watered plants (Fig. 3*B-E*).

The expression of *DREB2C* did not follow a fixed pattern. In SC-15, compared to well-watered plants, expression was reduced at 7 DWD, increased at 14 DWD, and again decreased at 21 DWD. The expression was down-regulated in EC-564755 under similar conditions (Fig. 4*A*). At 7 DWD, an increase in *DREB2D* expression was higher in EC-564755 (3.31-fold) than in SC-15 (3.03-fold). At 14 DWD, the expression declined in both genotypes and it was similar. However, at 21 DWD, *DREB2D* expressions peaked in SC-15, whereas it was down-regulated in EC-564755 (Fig. 4*B*). Water deficit of 7, 14 and 21 d, resulted in repression of *DREB3* expression in both genotypes, except at 21 DWD in EC-564755, where the expression was enhanced. The respective changes in *DREB3* expression were 0.95-, 0.92-, and 0.81-fold in SC-15 and 0.67-, 0.45-, and 0.52-fold in EC-564755, at 7, 14, and 21 DWD, respectively (Fig. 4*C*).

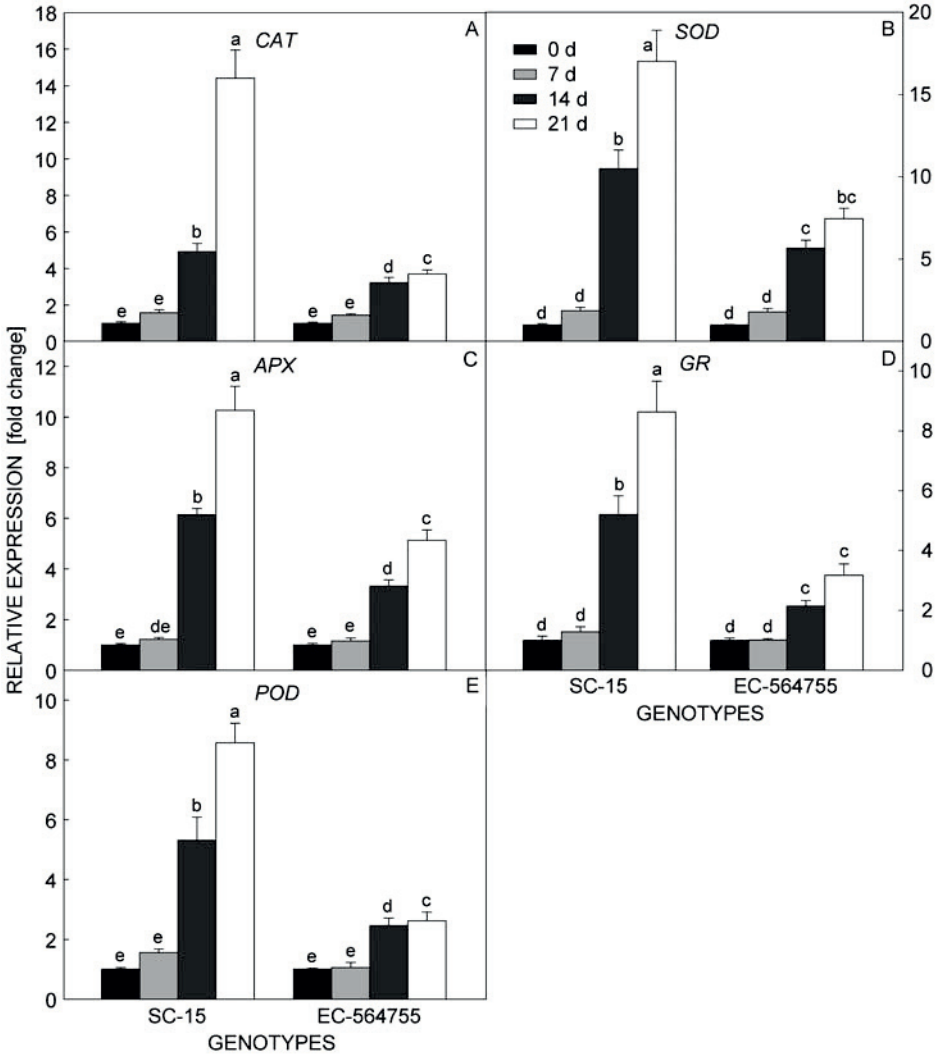

Fig. 3. Relative expressions of antioxidative enzyme genes in leaves of muskmelon genotypes under 7, 14 and 21 d of water deficit: *A* - *CAT* (MELO3C017024), *B* - *SOD* (MELO3C014007), *C* - *APX* (MELO3C013363), *D* - *GR* (MELO3C006322), and *E* - *POD* (*MELO3C014652*). Means of three replicates ± SEs, values followed by the same letter are not significantly different ($P > 0.05$).

The expression of *RD22* was higher in SC-15 (2.38-, 5.94- and 12.8-fold, respectively), at 7, 14, and 21 DWD. In the leaf of EC-564755, the expression of *RD22* increased until 14 DWD with their respective values of 1.95- and 5.78-folds, and thereafter decreased (3.1- fold) at 21 DWD (Fig. 4*D*). In addition, up-regulation of *dehydrin* expression was higher in SC-15 than in EC-564755. At 7, 14, and 21 DWD it was 1.12-, 1.44-, and 3.1- fold in SC-15 and 1.06-, 1.2-, and 2.33-fold in EC-564755 (Fig. 4*E*). Under control condition, both genotypes were grouped in cluster I. Cluster II constituted by performance of the drought tolerant genotype (SC-15) at three sampling dates of, 7, 14, and 21 DWD, whereas under similar sampling dates, cluster III constituted the performance of drought susceptible genotype EC-564755 (Fig. 5).

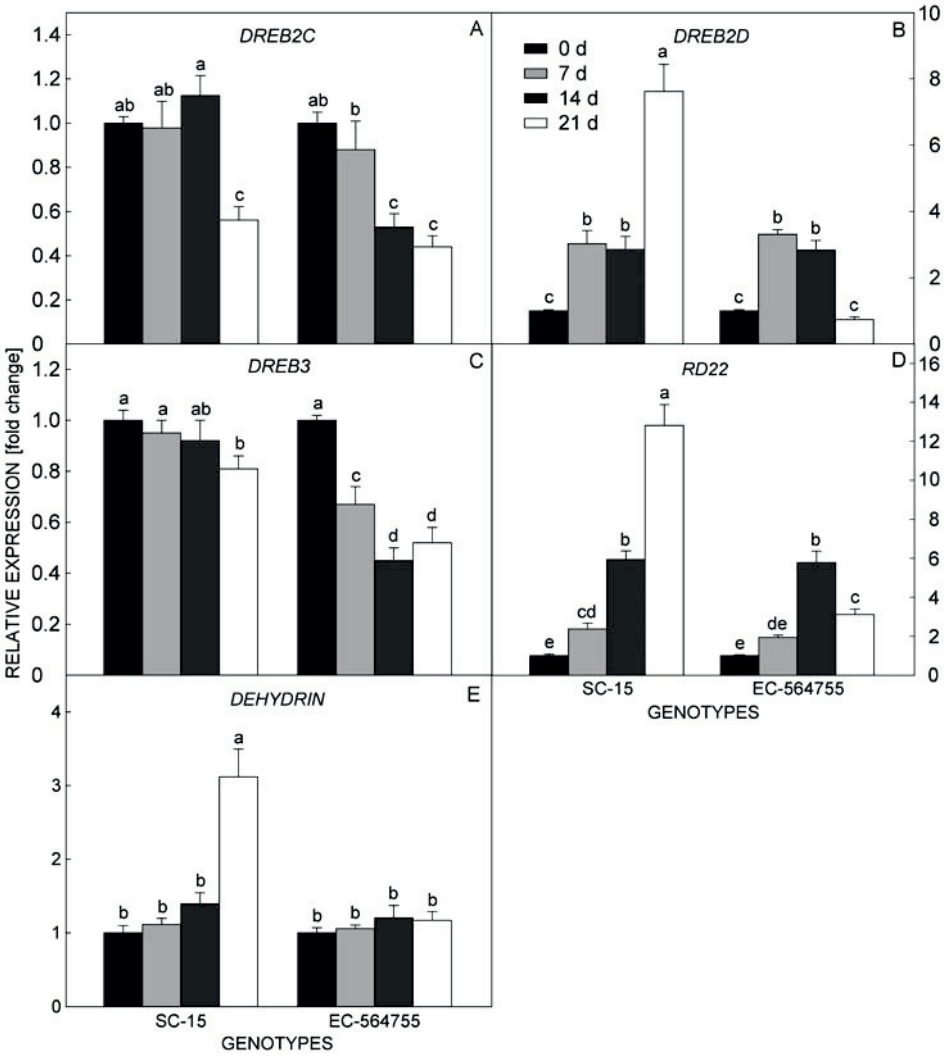

Fig. 4. Relative expression of the genes encoding dehydration responsible proteins in leaves of muskmelon genotypes under 7, 14, and 21 d of water deficit: *A* - *DREB2C* (MELO3C008318), *B* - *DREB2D* (MELO3C003785), *C* - *DREB3* (MELO3C003463), *D* - *RD22* (MELO3C003592), and *E* - *Dehydrin* (MELO3C016402). The means of three replicates ± SEs, values followed by the same letter are not significantly different ($P > 0.05$).

Discussion

Drought stress causes severe damages in plants at both cellular and molecular level, which influence the proper plant functioning. Under elevated water deficit, both genotypes exhibited lower RWC and higher EL compared to well-watered plants. RWC in given point of time is determined by the water uptake and transpiration by the plant. Relatively higher transpiration as compared to water uptake by the roots may lead to low RWC value also in control plants. The genotype SC-15 reported comparatively lower reduction in RWC and a minimal increase in EL, suggesting better water main-taining capacity and membrane integrity. A genotype is defined

as tolerant against drought stress if it maintains high RWC and low accumulation of EL under water deficit (Valentovic *et al*. 2006, Sanchez *et al*. 2010).

A complex regulatory network is involved in response to increased content of H_2O_2, which includes increased activities of antioxidant enzymes (Deeba *et al*. 2012). With an increase in DWD, H_2O_2 accumulation was higher in EC-564755 than in SC-15. Lower increase of H_2O_2 in SC-15 indicated a better balanced ROS scavenging. Lipid peroxidation was measured in terms of MDA and the increase of MDA was reported in both genotypes. Its accumulation was lower in SC-15 than in EC-564755. This indicates that SC-15 possessed an improved protective mechanism. Previous studies specified that the reduced up-regulation of MDA is associated with water deficit tolerance in tomato and muskmelon (Sebnem 2012, Rai *et al*. 2013). Enhanced accumulation of proline with an increase in DWD was crucial as it acts not only as compatible solute but also protects protein and membrane structure and scavenges ROS to regulate the cellular redox status (Yamada *et al*. 2005).

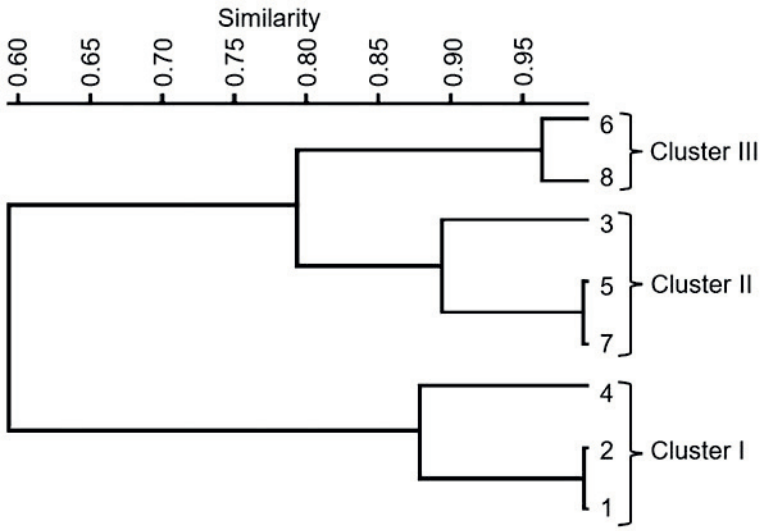

Fig. 5. Cluster analysis showing relationship between two genotypes at different sampling dates using data on 20 quantitative traits:. 1 = SC-15 at 0 DWD; 2 = EC-564755 at 0 DWD; 3 = SC-15 at 7 DWD; 4 = EC-564755 at 7 DWD; 5 = SC-15 at 14 DWD; 6 = EC-564755 at 14 DWD; 7 = SC-15 at 21 DWD, and 8 = EC-564755 at 21 DWD.

Wang *et al*. (2010) reported that CAT, SOD, APX, GR, and POD protect plants from oxidative damage under environmental stresses. SOD is one of the ubiquitous enzymes in plants and substantially contributes to defense mechanism against the generated ROS. Enhanced SOD activity in white clover (Wang and Li 2008) and cabbage (Singh *et al*. 2010) are reported under drought stress. Increased SOD activity in SC-15 also correlates with improved protection against oxidative damage. CAT activity increased markedly in both genotypes, but it was higher in SC-15, suggesting the more effective H_2O_2 scavenging compared to EC-564755, similarly as previously observed by Rai *et al*. (2013). Enhanced activities of CAT under water deficit were reported by Qian *et al*. (2012) in cucumber and by Sebnem (2012) in muskmelon. In the present experiment, enhanced APX and GR activities in tolerant genotype corroborate with the results of Rai *et al*. (2013) and Sebnem (2012). However, APX and GR activities were higher in genotype SC-15 than in EC-564755. In addition improved adaptation to drought stress in SC-15 could be due to an increased POD activity. Increased POD activity in tolerant genotype under drought stress has been reported in sunflower (Zhang and Kirkham 1996).

In the present experiment, expressions of *CAT*, *SOD*, *APX*, *GR*, and *POD* genes increased with progression in DWD; and up-regulation of these genes was higher in SC-15 than in EC-564755. The insertion of *Cu/Zn-SOD*, *Mn-SOD*, and *APX* genes increased drought tolerance in transgenic tobacco (Li *et al*. 2009). The present experiment reported a similar observation; the expressions of *SOD* and *APX* enhanced with an increase in DWD and up-regulation was higher in SC-15 than in EC-564755, suggesting the relevance of these genes in water deficit tolerance of the muskmelon plant. Transcription of *GR* gene in muskmelon leaf was provoked with an increase in the severity of drought stress and it was higher in SC-15. Transgenic lines overexpressing *Capsicum annuum* ascorbate peroxidase-like 1 gene (*CAPOA-1)* showed 2-fold increase in total peroxidase activity which strengthen reactive oxygen scavenging system, leading to oxidative stress tolerance (Sarowar *et al.* 2005). It is well known that osmotic stresses induce oxidative damage, which can be reduced by the activation of antioxidant enzymes and the biosynthesis of osmolytes acting as ROS scavengers. Similar to POD activity, the expression of *POD* was up-regulated under water deficit conditions. The higher expression observed in the drought tolerant genotype SC-15 could be the reason of improved tolerance against

drought stress.

In the present experiment, the variable expression pattern of *DREB2C* did not confirm its role for drought stress tolerance in muskmelon plants. Hence, the discontinuous expression of *DREB2C* corresponded with the results of the previous study, which indicated the role of *DREB2C* in heat stress tolerance (Lim *et al*. 2006); however, *DREB2C* performance was also reported under drought and high salinity (Nakashima *et al*. 2000). The expression of *DREB2D* was significantly induced at 7 DWD in both genotypes, and then declined continuously until 21 DWD in EC-564755, whereas in SC-15, it declined until 14 DWD and again up-regulated at 21 DWD. This indicates that a high up-regulation of *DREB2D* provides improved tolerance in SC-15 under severe water deficit condition. Water deficit treatment induced the expression of *DREB3* gene until 21 DWD in the leaf of SC-15, whereas it was induced only until 14 DWD in EC-564755 followed by repressed expression. Improved tolerance to drought stress in transgenic barley by over-expressing *DREB3* was reported by Hackenberg *et al*. (2012).

AtMYC2 and *AtMYB2* bind to *cis*-elements in the *RD22* promoter and cooperatively activate *RD22* (Shinozaki and Yamaguchi-Shinozaki 2007). At 7, 14, and 21 DWD, *RD22* showed over-expression in both genotypes. However, the fold increase was comparatively higher in SC-15, which suggests that an enhanced expression of *RD22* in SC-15 could provide improved tolerance to water deficit. Previously, induced expression of *RD22* in transgenic cotton under drought stress was reported by Yue *et al*. (2012). Most of the *dehydrins* are up-regulated by abiotic stresses, such as drought, salinity, or low temperature (Close 1997). With an increase in DWD, the expression of *dehydrin* increased in both genotypes and it was higher in SC-15. This observation indicates that *dehydrin* has a functional role in the drought stress tolerance of muskmelon plant. Under well-watered condition, performance of genotypes was almost similar and both genotypes grouped in cluster I, because there was no change in the performance of both drought tolerant and susceptible genotypes. However, giving water deficit treatment, there was clear grouping of drought tolerant and drought susceptible genotypes over different sampling dates. It showed that drought tolerance mechanisms trigger at a very early stage in drought tolerant genotype SC-15. Similarly distinct clustering was reported in wheat genotypes (Nouri *et al*. 2011).

In conclusion, in the drought tolerant muskmelon genotype SC-15, a consistently high RWC, proline accumulation, low EL, LPO, and H_2O_2 accumulation under increased water deficit were reported. An adaptive strategy of SC-15 to balance a ROS production under water deficit was connected with enhanced antioxidative enzyme activities that positively correlated with enhanced expression of their genes. Further, enhanced expressions of the *DREBs*, *RD22*, and *dehydrin* genes in water deficit tolerant genotype SC-15 was found.

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