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Morpho-physiological and biochemical responses of muskmelon genotypes to different degree of water deficit

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Abstract

Morpho-physiological and biochemical analyses were carried out in eight diverse indigenous muskmelon (*Cucumis melo* L.) genotypes exposed to different degrees of water deficit (WD). The ability of genotypes MM-7, and especially MM-6, to counteract better the negative effect of WD was associated with maintaining higher relative water content (RWC), photosynthetic rate, efficiency of PSII, and photosynthetic pigments compare to other genotypes. Furthermore, MM-6 showed a better ability to maintain cellular homeostasis than the others. It was indicated by a stimulated antioxidative defense system, *i.e.*, higher activities of antioxidant enzymes, accumulation of nonenzymatic antioxidants together with lower concentration of reactive oxygen species and malondialdehyde. However, the genotypes MM-2 and MM-5 suffered greatly due to WD and showed reduced RWC, photosynthetic rates, pigment content, and exhibited higher oxidative stress observed as lower antioxidant enzyme activities.

Additional key words: antioxidant enzyme; muskmelon; photosynthesis; proline; reactive oxygen species.

Introduction

Muskmelon (*Cucumis melo* L.) is an important vegetable that is frequently cultivated in arid and semiarid regions, where water availability is a major limitation (Cabello *et al*. 2009, Ibrahim 2012). Despite being water a scarce resource in (semi)arid regions, increasing demands of industrial as well as domestic sectors, particularly in developing countries like India, are forcing a decline in water availability for agriculture. Further, the foreseen consequences of change in global climate may worsen the situation in future. This highlights the urgency for developing a new strategy to identify potential genetic resources with specific traits and technological developments that can improve productivity of vegetables under declining natural resources and increasing environmental stresses (Pandey *et al*. 2016). Drought or water stress represents the most significant environmental constraint, limiting growth and yield efficiency of plants worldwide (Chaves *et al*. 2002, Adibah and Ainuddin 2011). Plants show either susceptible or tolerant response to water stress that is ascertained by interactive effects of physiological, biochemical, and morphological determinants (Penella *et al*. 2014).

Plants exposed to WD conditions show reductions in shoot and root biomass, leaf chlorosis and necrosis, while under mild WD, these symptoms are less aparent, but various cellular processes may be altered (Dhillon *et al*. 2011, Kusvuran 2012). Water stress induces in plants WD and loss of cell turgor, which in turn results in stomatal closure, and ultimately retarded photosynthesis and finally also plant growth (Lawlor 2002). Besides, at the cellular level, WD results in enhanced generation of reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\bullet -}$),

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Abbreviations: APX – ascorbate peroxidase; Car – caroteinods; CAT – catalase; DM – dry mass; DMRT – *Duncan*'s multiple range tests; DWD – days of water deficit; EL – electrolyte leakage; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; GR – glutathione reductase; g_s – stomatal conductance; MDA – malondialdehyde; P_N – net photosynthetic rate; POD – guaiacol peroxidase; RWC – relative water content; SOD – superoxide dismutase; SWC – soil water content; WD – water deficit; WW – well watered. *Acknowledgements*: The authors acknowledge the generous support of the National Agricultural Innovation Project (NAIP), New Delhi. We thank Dr. A.B. Singh for guidance during the data collection. A Maulana Azad National Fellowship provided to WAA by the University Grant Commission (UGC), New Delhi, India, is fully acknowledged.

hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet-}$), which can directly affect membrane lipids and inactivate metabolic enzymes, as well as cause damage to nucleic acids, leading to cell death. The half-life of H_2O_2 is comparatively longer than that of other ROS, and the enhanced H_2O_2 in plant cells may lead to oxidative stress (Deeba *et al*. 2012). However, in order to cope with such disorders, plants employ various mechanisms. A complex antioxidant defense system exists against ROS to protect the plant cells (Gill and Tuteja 2010). It includes antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) ascorbate peroxidase (APX), and peroxidase (POD), as well as nonenzymatic antioxidants, such as reduced glutathione, ascorbic acid, α-tocopherol, and carotenoids (Kumar *et al*. 2015a). During the enzymatic detoxifying process, the first enzyme, SOD, dismutates $O_2^{\bullet-}$ to H_2O_2, then CAT and peroxidases scavenge the accumulated H_2O_2 to render it to nontoxic concentrations by conversion to H_2O and O_2 (Colla *et al*. 2013). In addition, the accumulation of compatible solutes (*e.g*., sugars, proline, glycinebetaine, or potassium) is an important strategy that plants adopt to protect against cellular dehydration by maintaining the osmotic strength of the cytoplasm and thus sustaining plant physiological processes and growth (Lee *et al*. 2009, Kravić *et al*. 2013, Penella *et al*. 2014). Plant responses to WD can be determined by comprehensive analyses of their physiological, biochemical, and molecular traits. Therefore, plant's capacity to withstand WD is of great significance in an ever-changing climate. Once a genotype with high water-use efficiency (WUE) and better yield has been identified, it can be utilized for future breeding programs to develop more efficient varieties (Pandey *et al*. 2013).

The reactions, as well as a potential to withstand drought environments, depend on the species and genotype, duration and level of water loss, age and stage of development, organ, cell type, and type of subcellular compartment (Jaleel *et al*. 2008). Hence, many reports demonstrate differential responses of species or genotypes to drought stress (Dhillon *et al*. 2011, Pandey *et al*. 2013). Although, a number of drought-tolerant genotypes have been identified in many crops (Fleury *et al*. 2010), limited information is available for muskmelon (Cabello *et al*. 2009, Kusvuran 2012). In fact, the Indian muskmelon varieties were developed under best agronomic practices, including irrigation, but they have been hardly tested for yield efficiency under drought conditions (Pandey *et al*. 2008). Like many other vegetables, muskmelon plant is sensitive to water stress probably due to its larger leaf surface which evokes more transpiration, as reported in pepper by Penella *et al*. (2014). In general, muskmelon growth and productivity are greatly affected by drought stress. For example, the total production of melon in Gansu province (China) declined by 50% as a result of severe drought (Feng and Wu 2007). Therefore, in the area with decreased water availability, an improvement in crop yield is the main scientific and economic challenge (Penella *et al*. 2014). The potential of indigenous genetic materials for Indian muskmelon has been revealed in earlier studies for a range of traits (Pandey *et al*. 2008, 2013, 2016; Ansari *et al*. 2017). However, detailed study of genotypes required based on various physiological and biochemical parameters to explore the intrinsic potential of selected materials against the specific environmental constraints. In view of this, the present study aimed to elucidate the differencial responses of diverse indigenous genotypes of muskmelon by assessing their intrinsic potential at different physiological and biochemical processes under varying degree of WD.

Materials and methods

Plant materials, experimental conditions and water-stress treatments: Eight diverse indigenous muskmelon genotypes: Arka Jeet (MM-1), IIHR-663 (MM-2), Dharwad Selection 1 (MM-3), Hara Madhu (MM-4), IIHR-595 (MM-5), MJ-7 (MM-6), BS-25 (MM-7), and IIHR-659 (MM-8), were selected for the present study. Genotypes were identified according to preliminary field screening (Pandey *et al*. 2013). The experiment was conducted at the experimental field of ICAR – Indian Institute of Vegetable Research, Varanasi (25.10ºN, 82.52ºE and 76.1 m above mean sea level in the Eastern Indo-Gangetic Plain of India). Plants were grown in 10-L pots (22 cm in diameter and 23.8 cm in height) and the experiment consisted of four sets [*i.e*., 0 (for control well irrigated), 7, 14, and 21 d of water deficit, DWD]. Each set was replicated thrice and each genotype included five pots in each set. The soil in the pots was a mixture of sand, loamy clay, and farmyard manure (1:2:1, v/v) with a bulk density of 1.34 g cm^{-3} and pH 6.8. The soil had 0.39% (w/w) organic carbon content, 0.30% (w/w) total nitrogen content, and 0.51 mg(available phosphorus) g^{-1}, and 0.35 mg(potassium) g^{-1}. During the experiment, mean temperature and relative humidity ranged from 21.4 to 38.2°C and 59–89%, respectively. Water (2 L) was applied at 3-d intervals to each pot untill imposition of WD treatment (*i.e.,* 30 d after seed germination). WD was imposed by withholding water for 0, 7, 14, and 21 d (DWD). The arrangement of experimental pots were random and periodically rotated to minimize the effects of environmental heterogeneity. Plants of muskmelon genotypes were grown under greenhouse conditions and natural light/dark cycle.

Soil moisture was measured according to the formula: soil water content [%] = [(FM − DM)/DM] × 100, where FM is the fresh mass of the soil portion taken from the pot and DM is the dry mass of the soil portion after drying in a hot air oven at 85°C for 4 d (Cha-um *et al*. 2013). The fully expanded 6–7th leaf from the tip was harvested on

0, 7, 14, and 21 DWD, frozen immediately in liquid N_2 for the biochemical measurements, and kept at −80°C until used, while the other observation were carried out on fresh leaves. Three samples were taken from each genotype in each replication.

Root length, dry-to-fresh mass ratio of root, shoot and leaf: Plants were removed from the soil with intact roots to measure root length on 0, 7, 14, and 21 DWD. The root, shoot, and leaf parts were carefully separated and their FM was recorded. Respective plant parts were dried in an oven at 80°C for 48 h and DM was recorded. The ratio of dry-to-fresh mass (DM/FM) was calculated in percentage according to the formula given by Kausar *et al*. (2012) with minor modifications:

RDM/RFM [%] = (RDM/RFM) × 100
SDM/SFM [%] = (SDM/SFM) × 100
LDM/LFM [%] = (LDM/LFM) × 100

where RDM is root dry mass, RFM is root fresh mass, SDM is shoot dry mass, SFM is shoot fresh mass, LDM is leaf dry mass and LFM is leaf fresh mass.

Relative water content (RWC) and electrolyte leakage (EL): At 0, 7, 14, and 21 DWD, the RWC and EL of leaf tissues were measured as described by Khare *et al*. (2010). To determine the RWC, 12 leaf discs were weighed (FM). The same tissues were rehydrated in water for 6 h until fully turgid, surface-dried, and reweighed [turgid mass (TM)] followed by oven-drying at 80°C for 24 h, and reweighing (DM). The RWC was calculated by the following equation: RWC [%] = [(FM – DM)/(TM – DM)] × 100. EL was measured using a conductivity meter (*CM-180*, *Elico*, India): 10 leaf discs were placed in 25 ml of Milli Q water and their conductivity was measured after 4 h at room temperature (a) and then after autoclaving at 121°C for 30 min (b). The EL was calculated as: EL [%] = $a/b \times (100)$.

Net photosynthetic rate, stomatal conductance, and chlorophyll (Chl) fluorescence: Net photosynthetic rate (P_N) and stomatal conductance (g_s) were measured after 0, 7, 14, and 21 DWD in three fully expanded intermediate leaves in each replications and each genotype with a portable photosynthetic system (*LI 6400*, *LICOR*, Lincoln, NE, USA) from 11.00 to 13.00 h using a 250-cm^3 closed-circuit cuvette (Cha-um *et al*. 2007). These measurements were completed with the following specifications: ambient CO_2 concentration of 350 mmol mol^{-1}, cuvette air temperature set to 32°C before and after the respective treatments, and to 28°C during the relative treatment, relative humidity ranged between 65–70%. PAR at leaf surface was maximum up to 1,060 µmol(photon) m^{-2} s^{-1}. Photosynthetic efficiency was determined after 0, 7, 14, and 21 DWD in three fully expanded intermediate leaves using a portable *Handy Plant Efficiency Analyzer* (*Hansatech Instruments*, King's Lynn, Norfolk, UK). The leaves were dark-adapted for 30 min using leaf clips on the adaxial side. Red light was used to irradiate the leaf surface; the fluorescence signal was collected at excitation irradiance, set at 3,000 µmol(photon) m^{-2} s^{-1} from the same surface. Minimum (F_0) and maximum (F_m) Chl fluorescence of the dark-adapted leaf was recorded and maximum quantum efficiency of PSII was calculated according to: $F_v/F_m = (F_m - F_0)/F_m$ (Maxwell and Johnson 2000).

Photosynthetic pigments: For Chl and carotenoid (Car) content estimations, leaf samples (300 mg) were crushed in 80% chilled acetone using a mortar and pestle. Absorbance of supernatant was read (*UV-vis 1601 Shimadzu*, Japan) at 663, 645, and 470 nm and calculated according to Lichtenthaler and Buschmann (2001). Chl and Car contents [mg g^{-1}(DM)] were calculated using the formula: Chl a = [(12.7 × A_{663}) − (2.69 × A_{645})]; Chl b = [(22.9 × A_{645}) − (4.68 × A_{663})]; Car = [{(1,000 × A_{470}) − (3.27 × Chl a + Chl b)}/227].

Estimation of H_2O_2, lipid peroxidation and proline: The H_2O_2 contents were determined according to Jana and Choudhuri (1981). Leaf samples (200 mg) were crushed in 5 ml of 50 mM sodium phosphate buffer (pH 6.5). Supernatant (3 ml) was mixed with 1 ml of 0.1% (w/v) titanium sulfate in 20% (v/v) H_2SO_4 and centrifuged at 6,000 × g for 15 min, absorbance was recorded at 410 nm (*UV-vis 1601 Shimadzu*, Japan).

Lipid peroxidation was measured as malondialdehyde content (MDA) by thiobarbituric acid reaction according to the method of Heath and Packer (1968). Approximately 400 mg of leaf sample was crushed in 4 ml of 0.1% (v/v) trichloroacetic acid mixed with 0.5% (v/v) butylated hydroxytoluene and 1% (w/v) polyvinylpyrrolidone (PVP). The supernatant (2.5 ml) was mixed with 0.5% (v/v) and 20% thiobarbituric acid and trichloroacetic acid, respectively, and boiled for 30 min, absorbance of the supernatant at 532 nm was noted and correction for nonspecific turbidity was performed by subtracting the supernatant's absorbance at 600 nm (*UV-vis 1601 Shimadzu*, Japan).

To estimate the proline content, leaf tissues (200 mg) were homogenized in 3% (v/v) sulfosalicylic acid. After centrifugation (13,000 × g for 10 min), 0.5 ml of the supernatant was incubated at 100ºC for 60 min with 0.5 ml glacial acetic acid and 0.5 ml freshly prepared ninhydrin reagent. Toluene (1 ml) was added to the mixture and the absorbance was recorded at 520 nm (Bates *et al*. 1973) (*UV-vis 1601 Shimadzu*, Japan).

Determination of antioxidant enzyme activity and protein contents: Catalase (CAT; EC 1.11.1.6) activity was estimated according to Rai *et al*. (2012). Fresh leaf sample (200 mg) was homogenized in 5 ml of 50 mM Tris-NaOH buffer (pH 8.0) containing 0.5 mM EDTA, 2% PVP, and 0.5% (v/v) *Triton X-100*. The 1.5-ml assay mixture contained 1,000 µl of 100 mM KH_2PO_4 buffer (pH 7.0), 400 µl of 200 mM H_2O_2, and 100 µl of the

enzyme extract. The degradation of H_2O_2 was recorded at 240 nm, CAT activity was expressed as μmol(H_2O_2 oxidized) min^{-1} mg^{-1}(protein). The superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed according to the method of Shah *et al*. (2001). Fresh leaf sample (200 mg) was homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.5). Homogenate was centrifuged at 22,000 × *g* for 10 min at 4°C and SOD activity was assayed in the supernatant. The assay mixture contained 50 mM sodium carbonate-bicarbonate buffer (pH 9.8) containing 0.1 mM EDTA, 0.6 mM epinephrine, and enzyme extract in a final volume of 3 ml. Adrenochrome formation was recorded at 470 nm (*UV-vis 1601 Shimadzu*, Japan). One unit of SOD activity is equal to the amount of enzyme required to cause 50% inhibition of epinephrine oxidation and is expressed as U mg^{-1}(protein). Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to the method of Nakano and Asada (1981). Fresh leaf sample (200 mg) was homogenized in 5 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 1.0 mM EDTA, 1% PVP, 1.0 mM ascorbic acid, and 1.0 mM phenylmethylsulfonyl fluoride. The reaction mixture in a final volume of 3 ml contained 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 0.2 mM H_2O_2, and the extracted enzyme, and the absorbance was recorded at 290 nm (*UV-vis 1601 Shimadzu*, Japan). The specific activity of APX was expressed as μmol(ascorbate oxidized) min^{-1} mg^{-1}(protein). Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as per the mechanism described by Sánchez-Rodríguez *et al*. (2010). Fresh leaf sample (200 mg) was crushed in 5 ml of 0.1 M Tris-HCl buffer (pH 7.8). The reaction mixture in a final volume of 2 ml contained 100 mM Tris-HCl buffer (pH 7.8), 0.2 mM NADPH, 0.5 mM oxidized glutathione, 3 mM $MgCl_2$, and 200 μl of the enzyme extract. The assay was initiated by addition of NADPH at ambient temperature and the decrease in absorbance was recorded at 340 nm (*UV-vis 1601 Shimadzu*, Japan) and the specific activity of GR was expressed as μmol(NADPH oxidized) min^{-1} mg^{-1}(protein). Guaiacol peroxidase (POD; EC 1.11.1.7) activity was assayed according to Shah *et al*. (2001). Leaf sample (200 mg) was homogenized in 5 ml of 60 mM sodium phosphate buffer (pH 7.0), centrifuged, and the supernatant was used for enzyme assay. The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 6.5), 15 mM guaiacol, 0.05% (v/v) H_2O_2, and 60 μl enzyme extract. The reaction was initiated by adding H_2O_2 and the oxidation of guaiacol was determined by the increase in absorbance at 470 nm, the specific activity of POD was expressed μmol(H_2O_2 reduced) min^{-1} mg^{-1}(protein). All the absorbance were measured using spectrophotometer (*UV-vis 1601 Shimadzu*, Japan). Lowry's method was followed to measure the protein in each enzyme preparation, using bovine serum albumin (BSA) as the standard (Lowry *et al*. 1951).

Statistical analysis: All statistical analyses were performed using the statistical package *SPSS* (*SPSS Inc.*, *Version 16.0*). Three samples from each replication were taken and mean value of each replication was used for statistical analysis and significance difference was assessed by one-way analysis of variance (*ANOVA*), and further analyzed by *Duncan*'s multiple range tests. The significance level for post hoc test was set at 5% and $P<0.05$ was treated as significant.

Results

The morphological, physiological and biochemical analyses revealed that there were significant variations among the genotypes in all the parameters analyzed at each point of WD treatments even before the imposition of WD treatments or under well watered (WW) conditions, except for proline and Car contents at WW conditions (Tables 1–5, Fig. 1*A–E*). The soil water content (SWC) of the pot was 45, 27, 13, and 8.5%, respectively, at 0, 7, 14, and 21 DWD (the value presents average of all pots in each set).

Morphological parameters: As presented in Table 1, root length increased in all the genotypes as WD advanced. With exception of MM-2, statistically similar root length was observed in all the genotypes analyzed at WW conditions. In response to stage of WD, the maximum root length was recorded in MM-1 followed by MM-6 within each point of WD. The DM/FM ratio of shoots, roots, and leaves were significantly affected by DWD, with maximum at 21 DWD in MM-5 for shoots, in MM-5 and MM-8 for roots, and in MM-2, MM-4, MM-5 for leaves, respectively. Under similar conditions, the minimum value was observed in MM-6 for shoots, in MM-6 and MM-7 for roots, and in MM-1, MM-3, MM-6, MM-7, and MM-8 for leaves, respectively (Tables 1 and 2).

Physiological parameters: Significant variation among the genotypes was observed with respect to all the physiological parameters, except F_v/F_m at WW conditions. The RWC progressively decreased in all genotypes as the level of WD advanced (Table 2). Genotype MM-6 and MM-7 exhibited higher RWC at each WD level. At 7 DWD, except MM-6 and MM-7, all genotypes showed insignificant differences in their RWC. However, at 14 DWD, the highest RWC was found in MM-6 and MM-7, while MM-5 showed the lowest RWC. At 21 DWD, genotypes MM-2, MM-3, MM-8, and MM-4, MM-5 were grouped together with lower RWC (Table 2). Compared to the others, a significant and consistently the highest P_N was recorded for MM-6, and the lowest for MM-5 at each WD level. The F_v/F_m was the highest in MM-6 until 14 DWD, however, at 21 DWD, it was the highest in MM-1, MM-7, and MM-6. The lowest F_v/F_m was recorded in MM-5 at 0, 7,

14, and 21 DWD (Table 3). The g_s was reduced with respect to the increasing WD (from 7 to 21 DWD) in each genotype, but genotype MM-7 mainatined higher g_s at each WD level compare to other genotypes, and especially MM-5, which con-sistently showed the lowest g_s at each WD level (Table 3).

Table 1. Effect of increasing water deficit on root length, root and shoot dry-to-fresh mass ratio in muskmelon. Data are means ± SE of three replication. Means followed by *the same letter* within a column are not significantly different (P>0.05) according to *Duncan*'s multiple range tests; # − well-watered plants. 7, 14, and 21 d represents water-deficit treatment for respective day.

Genotype	Root length [cm]				Root dry-to-fresh mass ratio [%]				Shoot dry-to-fresh mass ratio [%]			
	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d
MM-1	29.7^{a}	33.1^{ab}	43.3^{a}	52.2^{a}	16.5^{bc}	22.1^{cd}	30.8^{c}	37.8^{c}	14.8^{a}	18.7^{b}	25.5^{bc}	34.2^{c}
MM-2	16.5^{b}	17.1^{c}	18.3^{d}	20.4^{d}	15.1^{c}	24.0^{bc}	37.3^{bc}	47.6^{bc}	10.0^{b}	20.1^{b}	32.0^{b}	48.0^{b}
MM-3	25.4^{a}	27.9^{ab}	33.3^{bc}	39.7^{bc}	15.1^{c}	21.6^{cd}	32.1^{c}	41.8^{bc}	11.2^{ab}	18.2^{b}	26.2^{bc}	34.3^{c}
MM-4	32.0^{a}	34.7^{a}	40.3^{ab}	43.2^{b}	17.8^{ab}	25.0^{bc}	35.3^{bc}	47.3^{bc}	10.6^{ab}	18.3^{b}	25.6^{bc}	35.6^{c}
MM-5	27.3^{a}	28.2^{ab}	29.9^{c}	31.0^{c}	18.8^{a}	31.4^{a}	51.4^{a}	63.6^{a}	13.2^{ab}	26.3^{a}	39.5^{a}	58.3^{a}
MM-6	25.7^{a}	29.2^{ab}	38.8^{abc}	52.5^{a}	15.0^{c}	17.7^{d}	21.6^{d}	24.6^{d}	9.6^{b}	11.6^{c}	16.6^{d}	23.1^{d}
MM-7	24.6^{a}	27.1^{b}	35.4^{ab}	41.1^{b}	17.4^{ab}	23.2^{bc}	33.8^{bc}	44.9^{bc}	12.5^{ab}	16.3^{b}	21.0^{cd}	29.4^{cd}
MM-8	28.8^{a}	30.2^{ab}	32.6^{bc}	36.2^{bc}	18.7^{a}	27.7^{ab}	41.5^{b}	53.9^{ab}	8.8^{b}	18.7^{b}	28.9^{b}	43.8^{b}

Table 2. Effect of increasing water deficit on leaf dry-to-fresh mass ratio and relative water content in muskmelon. Data are means ± SE of three replication. Means followed by *the same letter* within a column are not significantly different (P>0.05) according to *Duncan*'s multiple range tests; # − well-watered plants. 7, 14, and 21 d represents water-deficit treatment for respective day.

Genotype	Leaf dry-to-fresh mass ratio [%]				Relative water content [%]			
	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d
MM-1	27.3^{ab}	33.2^{a}	36.2^{a}	41.4^{bcd}	64.7^{c}	56.4^{c}	46.1^{b}	41.2^{b}
MM-2	26.8^{ab}	34.2^{a}	38.3^{a}	44.4^{ab}	76.7^{a}	59.0^{bc}	43.2^{b}	36.9^{bc}
MM-3	28.8^{ab}	35.8^{a}	37.6^{a}	41.5^{bcd}	64.5^{c}	54.5^{c}	42.7^{b}	35.3^{bc}
MM-4	18.8^{d}	34.2^{a}	38.8^{a}	43.4^{abc}	66.2^{bc}	57.2^{c}	46.0^{b}	34.8^{c}
MM-5	31.8^{a}	36.3^{a}	39.2^{a}	48.3^{a}	74.0^{ab}	55.9^{c}	38.5^{c}	32.0^{c}
MM-6	23.3^{cd}	24.8^{b}	31.4^{a}	37.2^{d}	74.1^{ab}	69.9^{a}	59.7^{a}	58.0^{a}
MM-7	24.1^{bc}	32.9^{a}	36.7^{a}	39.3^{cd}	74.8^{a}	66.7^{ab}	57.9^{a}	54.7^{a}
MM-8	31.7^{a}	35.8^{a}	38.2^{a}	42.2^{bcd}	72.0^{abc}	58.1^{c}	43.3^{b}	36.7^{bc}

Table 3. Effect of increasing water deficit on net photosynthetic rate, stomatal conductance, and F_v/F_m in muskmelon. Data are means ± SE of three replication. Means followed by *the same letter* within a column are not significantly different (P>0.05) according to *Duncan*'s multiple range tests; # − well-watered plants; F_v/F_m − maximal quantum yield of PSII photochemistry. 7, 14, and 21 d represents water-deficit treatment for respective day.

Genotype	Net photosynthetic rate [μmol m^{-2} s^{-1}]				Stomatal conductance [mmol(H_2O) m^{-2} s^{-1}]				F_v/F_m			
	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d
MM-1	13.2^{b}	10.6^{b}	7.3^{b}	5.1^{b}	1.18^{c}	1.02^{c}	0.81^{b}	0.68^{b}	0.76^{a}	0.70^{b}	0.57^{b}	0.48^{ab}
MM-2	7.2^{c}	4.7^{d}	3.3^{c}	2.3^{c}	1.08^{d}	0.82^{f}	0.60^{c}	0.46^{c}	0.74^{a}	0.66^{c}	0.50^{de}	0.39^{de}
MM-3	5.0^{d}	3.7^{de}	2.4^{de}	1.7^{d}	1.25^{b}	1.08^{b}	0.73^{b}	0.66^{b}	0.76^{a}	0.70^{b}	0.56^{cd}	0.46^{bc}
MM-4	13.3^{b}	9.44^{c}	7.0^{b}	4.8^{b}	1.09^{d}	0.91^{d}	0.61^{c}	0.52^{b}	0.76^{a}	0.69^{b}	0.55^{bc}	0.42^{cd}
MM-5	3.2^{e}	1.85^{f}	1.2^{f}	0.78^{f}	0.85^{e}	0.60^{g}	0.46^{d}	0.35^{d}	0.70^{a}	0.60^{d}	0.45^{f}	0.31^{f}
MM-6	18.4^{a}	14.9^{a}	10.8^{a}	8.9^{a}	1.09^{d}	0.99^{c}	0.76^{b}	0.70^{b}	0.77^{a}	0.74^{a}	0.61^{a}	0.51^{a}
MM-7	5.0^{d}	4.0^{de}	2.8^{cd}	2.2^{c}	1.55^{a}	1.34^{a}	0.98^{a}	0.85^{a}	0.75^{a}	0.70^{b}	0.57^{b}	0.49^{ab}
MM-8	4.3^{de}	3.0^{e}	1.9^{e}	1.3^{e}	1.15^{c}	0.87^{e}	0.64^{c}	0.49^{c}	0.72^{a}	0.64^{c}	0.47^{ef}	0.36^{e}

Biochemical parameters: In general, an increasing trend in H_2O_2 concentration in all genotypes was observed with respect to DWD. The highest H_2O_2 concentration was measured in genotype MM-5, while it was the lowest in MM-6, especially at 21 DWD. At WW conditions, the lowest MDA content was found in MM-5, which was at par with MM-2, whereas the MDA content was the highest in MM-5 along with MM-8 under severe WD conditions (at 21 DWD). At this WD level, the lowest MDA content was in MM-7 (Table 4). The EL of genotype MM-3,

MM-7, and MM-6 were minimum and similar under WW conditions, while the maximum EL was observed in MM-2 under WW conditions. The EL was consistently recorded as maximal in MM-5 at each WD level (from 7 to 21 DWD), whereas, it was recorded minimal in MM-6 and MM-7 at 14 and 21 DWD, respectively (Table 4).

The concentration of total Chl and Car were found to be reduced in all genotypes with the increase of WD from 0 to 7, 14, or 21 DWD. At prolonged WD (21 DWD), the highest total Chl contents was in the genotype MM-6 and Car in MM-6 and MM-7, whereas the total Chl was the lowest in MM-2, MM-8, and MM-4, while genotype MM-5 showed the lowest Car content under similar conditions (Table 5). Though, similar amount of proline was found in all genotypes at WW conditions, its accumulation increased as the WD stress increased. However, the proline accumulation varied within the genotypes and the content was significantly higher in MM-6 at each WD level in comparison to other genotypes, while the lowest proline content was consistently observed in genotypes MM-2 and MM-5 at each WD level. Statistically, the values of MM-2 and MM-5 were similar to that of MM-7 under 7 DWD, and were also similar to that of MM-8 at 21 DWD (Table 5).

Table 4. Effect of increasing water deficit on hydrogen peroxide (H_2O_2), MDA, and electrolyte leakage in muskmelon. Data are means ± SE of three replication. Means followed by *the same letter* within a column are not significantly different ($P>0.05$) according to *Duncan*'s multiple range tests; # – well-watered plants. 7, 14, and 21 d represents water-deficit treatment for respective day.

Genotype	H_2O_2 [μmol g^{-1}(FM)]				MDA content [μmol g^{-1}(FM)]				Electrolyte leakage [%]			
	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d
MM-1	15.7^{ab}	21.3^{bc}	26.6^{cd}	52.1^{d}	1.25^{bcd}	1.62^{d}	2.14^{e}	3.35^{c}	23.7^{d}	27.5^{cd}	38.9^{c}	38.3^{e}
MM-2	8.28^{d}	13.5^{d}	19.1^{ef}	49.2^{d}	0.80^{f}	1.43^{e}	2.59^{d}	3.24^{c}	31.5^{a}	46.8^{a}	46.1^{b}	63.0^{b}
MM-3	12.6^{bcd}	17.4^{cd}	22.0^{def}	55.0^{d}	1.28^{bc}	2.02^{b}	3.28^{b}	4.25^{b}	22.0^{e}	29.4^{c}	35.3^{d}	38.7^{e}
MM-4	20.6^{a}	28.4^{a}	36.7^{b}	86.8^{b}	1.15^{cd}	1.77^{cd}	2.42^{d}	3.24^{c}	29.6^{b}	39.0^{b}	45.2^{b}	50.2^{d}
MM-5	16.0^{abc}	26.9^{a}	47.5^{a}	108.5^{a}	0.87^{ef}	1.87^{bc}	3.41^{b}	4.87^{a}	29.4^{b}	44.7^{a}	54.0^{a}	70.1^{a}
MM-6	10.8^{cd}	12.4^{d}	16.1^{f}	29.7^{e}	1.90^{a}	2.26^{a}	2.92^{c}	3.28^{c}	22.6^{de}	25.1^{d}	30.7^{e}	33.4^{f}
MM-7	17.9^{ab}	24.0^{ab}	30.4^{c}	67.8^{c}	1.05^{de}	1.38^{e}	2.01^{e}	2.72^{d}	21.7^{e}	26.8^{cd}	31.4^{e}	35.7^{ef}
MM-8	12.0^{cd}	17.4^{cd}	22.8^{de}	61.7^{c}	1.41^{b}	2.37^{a}	3.95^{a}	4.71^{a}	27.6^{c}	37.3^{b}	46.4^{b}	54.1^{c}

Table 5. Effect of increasing water deficit on total chlorophyll, carotenoid, and proline content in muskmelon. Data are means ± SE of three replication. Means followed by *the same letter* within a column are not significantly different ($P>0.05$) according to *Duncan*'s multiple range tests; # – well-watered plants. 7, 14, and 21 d represents water-deficit treatment for respective day.

Genotype	Total chlorophyll [mg g^{-1} (DM)]				Carotenoid [mg g^{-1} (DM)]				Proline [μg proline g^{-1} (FM)]			
	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d	0 d#	7 d	14 d	21 d
MM-1	49.2^{b}	40.3^{bc}	32.2^{b}	23.8^{b}	1.80^{a}	1.60^{b}	1.29^{a}	1.24^{bcd}	26.7^{a}	79.4^{b}	163.7^{b}	228.0^{b}
MM-2	56.1^{ab}	36.0^{cd}	27.6^{bc}	14.2^{cd}	2.01^{a}	1.54^{bc}	1.38^{a}	1.19^{cd}	24.1^{a}	39.7^{fe}	77.0^{f}	105.4^{f}
MM-3	63.3^{a}	46.9^{ab}	32.9^{b}	20.0^{b}	1.83^{a}	1.48^{bc}	1.28^{a}	1.18^{cd}	25.9^{a}	49.5^{de}	124.7^{d}	147.6^{d}
MM-4	55.5^{ab}	42.1^{bc}	31.6^{b}	18.5^{bc}	1.92^{a}	1.53^{bc}	1.36^{a}	1.29^{abc}	21.9^{a}	56.0^{cd}	103.5^{e}	132.3^{e}
MM-5	47.8^{b}	29.4^{d}	19.3^{d}	8.7^{d}	1.93^{a}	1.41^{c}	1.19^{a}	1.02^{e}	26.7^{a}	36.9^{f}	77.6^{f}	101.5^{f}
MM-6	62.3^{a}	54.0^{a}	44.9^{a}	34.9^{a}	1.98^{a}	1.78^{a}	1.49^{a}	1.39^{a}	28.0^{a}	90.3^{a}	189.2^{a}	288.2^{a}
MM-7	51.2^{b}	42.1^{bc}	31.0^{b}	23.9^{b}	1.96^{a}	1.65^{ab}	1.45^{a}	1.33^{ab}	24.4^{a}	37.6^{f}	144.7^{c}	173.4^{c}
MM-8	49.2^{b}	34.2^{cd}	22.6^{cd}	13.3^{cd}	1.91^{a}	1.42^{c}	1.24^{a}	1.15^{d}	22.2^{a}	63.9^{c}	92.8^{e}	102.8^{f}

Antioxidant activities: Under WW condtions, the genotype MM-3 showed the maximum SOD activity, while the minimum was found in MM-1 and MM-2. At 7, 14, and 21 DWD, the maximum SOD activity was recorded for MM-6, whereas minimum was recorded in MM-5, MM-2, and MM-5 (Fig. 1*A*). At WW conditions, the maximum CAT activity was found in MM-8, though it was at par with MM-5. At 7 DWD, CAT activity was maximal in MM-1, MM-6, and MM-7. However, with the increase in severity of WD (from 14 to 21 DWD), the maximum CAT activity was observed in MM-6. The lowest CAT activity was found in MM-2 at 14 DWD and in MM-2 and MM-5 at 21 DWD (Fig. 1*B*). The maximum APX activity was recorded in MM-6 at 7, 14, and 21 DWD, while minimum was found in MM-5 at 7 DWD, in MM-2, MM-4, MM-5, MM-8 at 14 DWD, and in MM-5 and MM-8 at 21 DWD (Fig. 1*C*). Under WW conditions, the GR activity was maximum for MM-3, MM-7, and minimum for MM-2, MM-5, and MM-8, with insignificant difference. At 7 DWD, the GR activity reached maximum in MM-6 and MM-7, while at 14 and 21 DWD, the GR activity was maximal in MM-6. Minimum GR activity was recorded in case of MM-2, MM-5, and MM-8 at 7, 14, and 21 DWD, respectively (Fig. 1*D*). The POD activity in

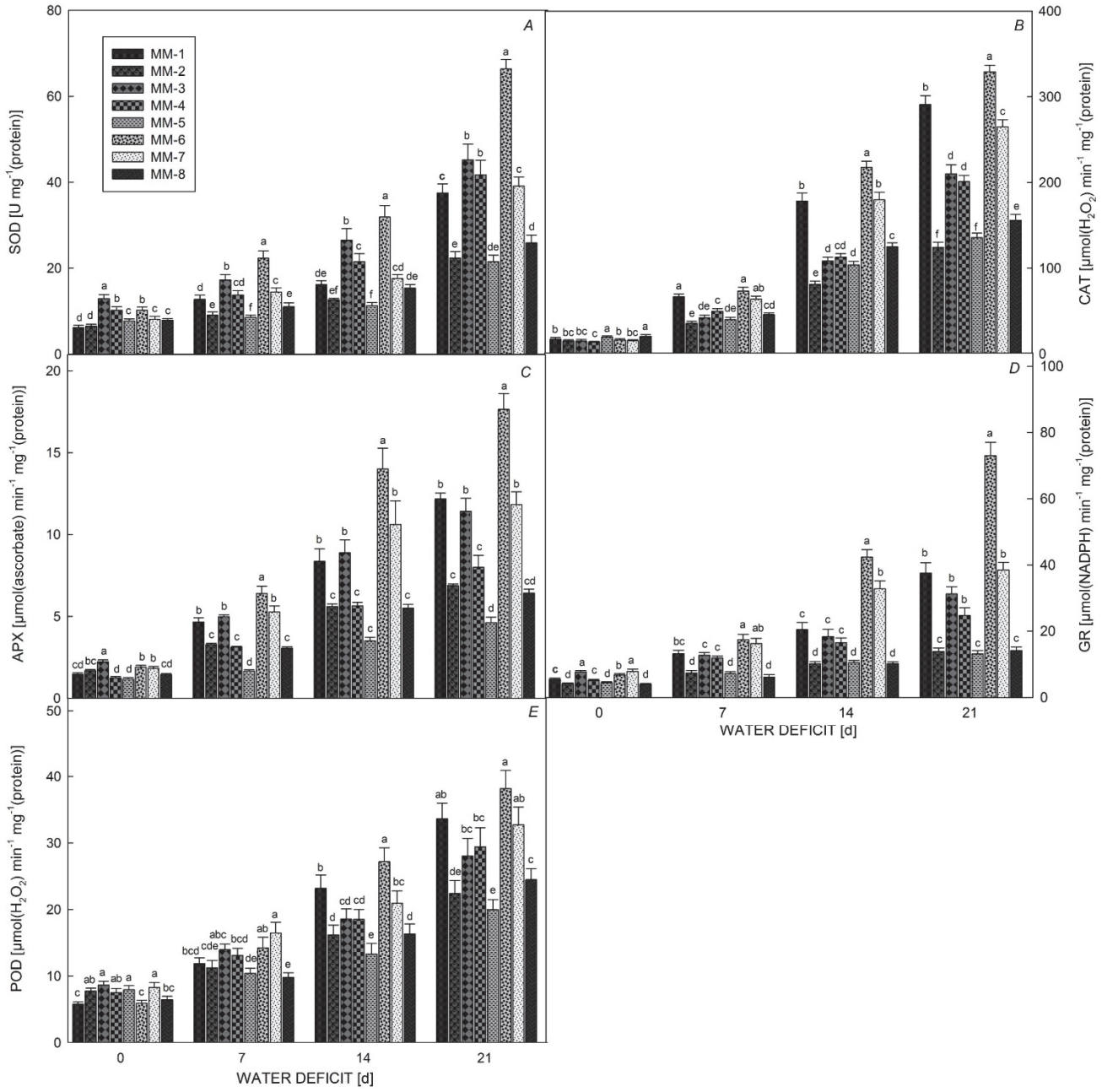

Fig. 1*A*-*E*. Effect of increasing water deficit on activities of key antioxidants in muskmelon leaves. *A* – superoxide dismutase (SOD); *B* – catalase (CAT); *C* – ascorbate peroxidase (APX); *D* – glutathione reductase (GR), *E* – guaiacol peroxidase (POD). The data are mean of three replicates ± SE. Those followed by *different letters* within similar water-stress treatment are significantly different according to *Duncan*'s multiple-range test at $P>0.05$.

MM-3 was maximal, but statistically similar to MM-7, MM-5, and MM-4 at WW conditions. Whereas under WD treatments, and especially at 14 and 21 DWD, the maximum POD activity was recorded in MM-6 followed by MM-3 and MM-7, which were statistically similar to each other (Fig. 1*E*).

Discussion

The response of genotypes to WD may vary with their specific ability to counteract the negative effects of WD. In the present study, WD invariably caused alteration in some of the plants physiological and biochemical characterstics, such as loss of cell turgor, changes in membrane fluidity and composition, changes in

compatible organic solute concentrations, and increased activities of antioxidant enzymes. In addition, WD resulted in increased root length. However, these responces varied among the genotypes. The overall responses of plants to stress can be categorized as (*1*) maintenance of ion homeostasis and osmotic adjustment, and (*2*) detoxification of harmful elements and metabolism.

A detailed study is required to understand the adaptive mechanisms and responses to WD stress that allow survival of plants under WD conditions. Root system is the main organ responsible for plant water uptake and essential for crop productivity, especially under water stress (Ober and Sharp 2007). Root length represents the morphological trait which better describes the capacity of the root to explore the deeper layer of soil (Ryser 1998). An increase in root length was observed at the progressive WD stages, with the highest root length found in MM-6 and MM-1, especially, under prolonged WD (14 and 21 DWD); this suggests that enhanced root length in these genotypes assist plants to retain maximum water under stress conditions. Matsui and Singh (2003) reported a similar trend of root distribution in various cowpea genotypes grown under WD. They observed a downward shift in root distribution in cowpea plants under WD conditions. Indeed, a deep and prolific root system was found to be associated with enhanced avoidance of drought in chickpea (Serraj *et al*. 2004) and muskmelon (Huang *et al*. 2011).

Plants exhibiting better tolerance to WD conditions showed superior water-withholding capacity. Any plant part, which retains more water, exhibits a low ratio of dry-to-fresh mass compared to that retaining less water. According to Kravić *et al*. (2013), a relative increase in the dry-to-fresh mass ratio of roots, shoots, and leaves due to WD could be an indicator of osmotic stress. In the present study, the dry-to-fresh mass ratio of roots, shoots, and leaves increased with duration of WD in all genotypes. The value of dry-to-fresh mass ratio of roots and shoots reached its maximum in MM-6 followed by MM-7, especially at 14 and 21 DWD. Similarly, in muskmelon, the increase in dry mass was observed (Ahmadi-Mirabad *et al*. 2013). RWC, which measures the maximum amount of water that a tissue can hold, is the most appropriate measure of plant water status in terms of the physiological consequences of cellular WD (Boyer 1968). RWC in all the muskmelon genotypes declined as the DWD progressed. However, the RWC was apparantly higher in MM-6 and MM-7, at 7, 14, and 21 DWD, and hence we propose, as reported by Hessini *et al*. (2009), the genotypes with higher RWCs (MM-6 and MM-7) can be defined as drought-tolerant, moderate tolerant with moderate RWCs, while the others having lower RWCs (MM-5, MM-4, MM-3, MM-8, and MM-2) as relatively drought-sensitive genotypes. Similar to our findings Ahmadi-Mirabad *et al*. (2014) also observed a reduction in RWCs in *Cucumis melo* under deficit irrigation. In tomato, Sánchez-Rodríguez *et al*. (2010) identified genotypes that were tolerant and sensitive to WD on the basis of RWC.

Drought stress can cause a serious damage to plants at cellular and molecular levels (Gill and Tuteja 2010). Plants facing drought stress show extensive ROS generation that damages their photosynthetic machinery, resulting in a significant decrease in P_N. This could be due to abscisic acid-mediated stomatal closure and lesser availability of CO_2, resulting in reduced photosynthetic activity (Tahi *et al*. 2008). In present study, photosynthetic rate declined in all genotypes as the DWD prolonged. The reduction in P_N corresponds with damage to photosynthetic machinery, and with the decline in g_s. However, among the genotypes, the maximum P_N was recorded in MM-6 at different WD level (*i.e*., 7, 14, and 21 DWD). This suggests that the damages caused to photosynthetic machinery due to WD were minimal in MM-6. Under WD, g_s decreased in all genotypes, although the extent of the reduction may vary among genotypes (Cha-um *et al*. 2010). A reduction in g_s suggests a relationship between WD and stomatal closure. The significantly higher g_s was recorded in MM-7 followed by MM-6 under different WD conditions. Together with P_N, the higher g_s in these genotypes suggests their better ability to tolerate WD, since it is widely accepted that increased g_s facilitates CO_2 influx into the leaves and the photosynthesis. The efficiency and stability of PSII can be described by the F_v/F_m ratio (Ranjbarfordoei *et al*. 2006). Chl fluorescence gives insights into the degree of stress injury to the photosynthetic apparatus. Under WD, restrictions of net CO_2 assimilation may promote an imbalance between photochemical activity at PSII, leading to an overexitation and subsequent photoinhibitory damage of PSII reaction centre (Petridis *et al*. 2012). In response to WD, the photoinhibition was much pronounced in MM-5, especially, under severe WD, while MM-6, MM-1, and MM-7 were able to maintain higher photosynthetic efficiency of PSII, which showed reduced photoinhibition with increasing WD in these genotypes. Petridis *et al*. (2012) also reported reduction in F_v/F_m in Greek olive. Reduction in PSII efficiency was also reported in watermelon plants when treated for different days by drought stress (Mo *et al*. 2016).

The level of each WD resulted in a decline of the Chl content for each muskmelon genotype. This might be due to damage to the chloroplast structure and a decrease in Chl biosynthesis (Wang *et al*. 2010). The total Chl content in all the genotypes decreased as the WD progressed. However, the genotype MM-6 could maintain relatively higher Chl content, especially at 14 and 21 DWD, which showed a better ability of MM-6 to tolerate WD, compared to others. Wang *et al*. (2010) reported reduction in total Chl content under WD conditions. Such a reduction in the Chl content might be attributed to a decrease in Chl biosynthesis or an increase in Chl degradation. Similarly, reduction also occurred in the Car content due to WD. Such a reduction might be due to Car degradation or a decrease in Car biosynthesis. However, the Car concentration was apparently higher in genotypes MM-6 and

MM-7 under WD conditions. Role of Car in conferreing tolerance to WD in sesame has been reported. The major role of Car in direct quenching of triplet Chl prevents the generation of singlet oxygen and protects from oxidative damage (Deng *et al*. 2003). Wang *et al*. (2010) reported that the content of Car in the leaves of winter wheat increased under drought stress.

As a result of WD, generation of ROS is enhanced. The presence of H_2O_2 is considered indicative of ROS as the half-life of H_2O_2 is comparatively longer than that of other ROS, and excess accumulation of H_2O_2 in plant cells may lead to oxidative stress (Deeba *et al*. 2012). Increased H_2O_2 production in response to drought stress was reported. In this experiment, the severity of WD induced H_2O_2 production in leaves of all genotypes. H_2O_2 accumulation in MM-6 was the lowest one, when exposed to WD for longer period, whereas in MM-5, H_2O_2 accumulation was the highest one, indicating balanced ROS production in the tolerant as compared to susceptible genotypes (Esfandiari *et al*. 2008). Enhancement of H_2O_2 concentration was earlier reported in watermelon plants under varied drought stress conditions (Mo *et al*. 2016). MDA is the product of lipid peroxidation and is usually used as a criterion for stress-induced oxidative damage to the membrane. Here, relative to WW plants, stressed plants of all the genotypes showed a gradual increase in MDA concentration as drought intensified, which indicates that MDA concentration was directly related to drought stress. However, the increase in MDA was considerably lower in genotype MM-7 and especially MM-6, which indicates their better tolerance response to WD. Similarly, the increase in MDA concentration under drought conditons was found lower in wild drought-tolerant watermelon plants (*Citrullus lanatus* var. Citrode) than the cultivated sensitive one (*Citrullus lanatus* var. *lanatus*) (Mo *et al*. 2016). The reduced accumulation of MDA in tomato and muskmelon was also associated with WD tolerance (Kusvuran 2012, Rai *et al*. 2013). Simova-Stoilova *et al*. (2010) found more pronounced weakening of membrane integrity and oxidative damage to lipids in the sensitive varieties of wheat plants under WD. Cell membrane stability, assessed by EL method, is widey used to differentiate susceptible and tolerance of a genotype to abiotic stresses; the lower EL is indicative of higher membrane stability and better stress tolerance (Kumar *et al*. 2015b). The exposure of plants to WD for a longer period proved severe membrane damage, resulting in higher EL and loss of ions, vital factors for proper cell functioning. The present study showed that ion leakage was lower in genotype MM-6 and MM-7, indicating these genotypes as having better membrane stability at WD stress, thus considered as drought-tolerant genotypes. Previously, Valentovič *et al*. (2006) reported a susceptible maize cultivar that showed a more pronounced increase in EL than that of a tolerant one.

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. Stress-induced variation of antioxidants is dependent on the severity and duration of the treatment, and also the species and age of the plant (Shao *et al*. 2007). SOD is an ubiquitous enzyme in plants that plays a major role in the defense mechanism against generated ROS through catalyzing the disproportionation of superoxide radicals to molecular oxygen and H_2O_2. In fact, SOD reduces the risk of hydroxyl radical formation from superoxide *via* the metal-catalyzed Haber–Weiss-type reaction by removing the superoxide (Arora *et al*. 2002). Enhanced SOD activity has been reported in white clover (Wang and Li 2008) and cabbage (Singh *et al*. 2010) under drought stress. In present experiment, WD also led to relatively higher activity of SOD in all genotypes, where the highest SOD activity was observed in genotypes showing a drought-tolerant response (*e.g.*, MM-6) relative to drought-sensitive genotypes (*e.g.*, MM-2 and MM-5). H_2O_2 produced as a result of SOD activity and other reactions is destroyed by CAT in a further reaction (Foyer *et al*. 1994). CAT is tetrameric heme containing enzyme that is abundant in the glyoxysomes of lipid storing tissues. In this study, enhanced CAT activity observed at each WD in all genotypes, but the highest CAT activity was recorded in MM-6 compared to other genotypes, suggesting better H_2O_2-scavenging ability of tolerant genotypes. Rai *et al*. (2013) also observed that better performance of tomato under drought stress is accompanied by an increase in CAT activity. APX plays a key role in decreasing H_2O_2 accumulation and eliminating MDA. Higher APX activity was observed in drought-exposed plants, with MM-6 showing the highest APX activity analyzed at different DWD. Increased APX activity has been reported in a drought-stressed soybean genotype (Zhang *et al*. 2006). It is assumed that the increased activities of the enzymes associated with the ascorbate/glutathione pathway, especially APX, confer general resistance to environmental stresses (Carvalho 2008). High GR activity maintains the pool of glutathione in the reduced state. Increased GR activity as WD progresses ensures availability of $NADP^+$, which accepts electrons from the photosynthetic electron-transport chain (Sofo *et al*. 2005). Ratnayaka *et al*. (2003) reported enhanced GR activity in cotton during drought stress. In the present study, muskmelon genotypes exposed to WD conditions showed significantly higher GR activity, and the highest GR activity was observed in MM-6. Under WD conditions, induction of POD activity compensates for CAT and APX activities. PODs degrade indole-3-acetic acid and participate in the biosynthesis of lignin; POD also consumes H_2O_2. Higher POD activity has been reported in drought-tolerant genotypes of common bean, sunflower, and sorghum (McKersie *et al*. 1999). Here, POD activity increased with duration of the WD and genotype MM-6 and MM-7 showed significantly higher POD activity. Whereas, the minimum POD activity was obvious in susceptible genotype MM-5 at 21 DWD.

A common response of plants to WD is accumulation of free proline. Proline acts as a mediator for osmotic adjustment and eliminates free radicals, and it is also an important component of cell-wall proteins. Proline accumulation is well recognized in drought-tolerant genotypes as compared to susceptible ones under WD conditions (Ashraf and Foolad 2007). Similarly, we observed enhanced accumulation of proline under elevated WD, with the highest concentration found in the genotype MM-6, at each WD level. This indicates that free proline also contributes to better protection against oxidative damage in this muskmelon genotype (Ansari *et al*. 2016).

Conclusion: The general response of muskmelon plants to WD included the increase in root length and decrease in RWC, photosynthetic rate, stomatal conductance, maximum quantum-use efficiency of PSII (F_v/F_m), and photosynthetic pigments. However, the extent of these changes differed between the genotypes with respect to degree of WD. The negative effects of WD were less pronounced in genotypes MM-6, MM-7, and MM-1, which showed a drought-tolerance response, whereas the other genotypes were susceptible. An indicator of membrane damage (EL) increased under WD conditions, but tolerant genotypes exhibited a lesser enhancement of this parameter. The level of oxidative stress, as evidenced by increased EL and lipid peroxidation, associated with higher H_2O_2 production, was lower in genotypes MM-6 and MM-7 compared to the other genotypes, especially MM-5 and MM-2. Similarly, relatively higher activities of antioxidant enzymes (SOD, CAT, APX, GPX, and GR) and concentrations of metabolites, such as proline, indicated in MM-6, MM-7 and MM-1 a tolerant response that can more efficiently restrain oxidative stress.

Thus, it was apparent from the present investigation that drought tolerance in muskmelon is genotype-specific and can determined by the combined effects of various physiological, biochemical, and growth-related parameters. These factors are indicators at the cellular level and may serve as *in vitro* selection criteria for drought tolerance in muskmelon. Overall, MM-6 can be considered the most drought-tolerant genotype, followed by MM-7, and these lines may be useful in future breeding programs as potential donors of drought-tolerance genes.

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