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COMPARISON OF SALT TOLERANCE BETWEEN TWO POTENTIAL CULTIVARS OF *PHOENIX DACTYLIFERA* L. GROWING IN SAUDI ARABIA

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Abstract

The date palm is a woody plant growing in different regions of the world. Two potential cultivars of the date palm (Ajwa and Mabroom) from Saudi Arabia were studied for their tolerance to salinity stress (0, 50, 100 and 150 mM NaCl). Salinity stress affected morphometric traits as well as biomass yield in both cultivars. The percent germination under different concentrations of NaCl was greater in the Ajwa compared to the Mabroom. The Ajwa cultivar showed enhanced activities of antioxidant enzymes (Catalase: CAT; superoxide dismutase: SOD and glutathione reductase; GR) than the Mabroom. Thiobarbituric acid reactive substance (TBARS) content increased in both cultivars compared to the control. The osmolytes level (proline) was increased whereas soluble protein was decreased in both cultivars. The leaf relative water content (LRWC) was decreased as compared to the control and greater reduction was seen in the Mabroom cultivar. Abscisic acid content was found to be higher in the Ajwa cultivar than that in the control plant and Mabroom. In conclusion, the Ajwa cultivar showed greater salinity tolerance compared to Mabroom which possible owing to the presence of tolerance genes in the genome.

Key words: Salinity stress, Tolerant plant, Morphological marker, Abscisic acid.

Introduction

The date palm (*Phoenix dactylifera* L.) is a monocotyledon, dioecious, domesticated and evergreen tree. Initially, date palm was cultivated by humans in Southern Spain, North Africa, and Northern India and it plays an essential economic role in arid regions (El-Juhany, 2010). Date palm fruits have ample nutritive importance and various cultivars have different chemical constituents in their fruits (Hamad *et al*., 2015). Various cultivars of date palm are available in Saudi Arabia (Fayadh & Al-Showiman, 1990) and around 40 cultivars with economic importance are found to be in various Saudi provinces and growing well under prevailing environmental condition. The best dates production areas in Saudi Arabia are Eastern province, Al-Qassim, Riyadh and Medina.

The various cultivars of date palm vary in nutritional composition to each other (Hamad *et al*., 2015). The Ajwa and Mabroom cultivars have good nutritional as well as medicinal value. The fruits of Ajwa cultivar was used to cure various human diseases (Yasin *et al*., 2015; Eid *et al*., 2014; Khan *et al*., 2016) and the medicinal value of this cultivar could be correlated due to the occurrence of different secondary metabolites in its mature fruit. The water extracts of Ajwa fruit exhibited the occurrence of alkaloids phenols, carbohydrates, flavonoids and tannins which are good sources of antioxidants (Nor *et al*., 2018). The methanolic extract of Ajwa fruit showed antibacterial activity against some bacteria including *Bacillus cereus, Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus* (Samad *et al*., 2016). The methanolic extract of Ajwa and Mabroom fruits showed inhibitory activity against human gastric, lung, breast, prostate and colon tumor cell lines (Zhang *et al.,* 2017).

Salinity stress disturbs plant growth development and productivity. Excess salt in the soil causes toxicity in the plant cell due to osmotic or water-deficit stress. About 50% of the irrigated lands affected by salinity worldwide (Tuteja, 2007). More than 800 million hectares (ha) is affected by salt in the world, which constitutes 6% of the total land area. At present, 19.5% (45 million ha) of 230 million ha of irrigated land has already been destroyed by salinity stress (Anon., 2016).

Antioxidant enzymatic system including ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) in the plant play a significant role to protecting the cell from damage against reactive oxygen species (ROS). Under unfavorable conditions, the enzymes activities and biosynthesis of antioxidant molecules are altered (Mittler *et al*., 2004; Horling *et al*., 2003). An enhanced level of antioxidant enzymes activities in sensitive and salt tolerant genotypes has been correlated with salt tolerance in many studies (Mittova *et al*., 2003; Sumithra *et al*., 2006). Similarly, osmolytes can help in scavenging the free radical molecules and protect subcellular structure from salinity stress (Slama *et al*., 2015).

Accumulation of abscisic acid (ABA) in salinity stressed plants acts as a signaling molecule to cope with the adverse conditions of environment (Raghavendra *et al*., 2010). ABA is also called as stress hormone in plants (Mehrotra *et al*., 2014) and its level increases in plants under harsh environmental conditions. However, external application of ABA was also revealed to enhance drought tolerance in some plant species (Zeinali *et al*., 2014; Wei *et al*., 2009).

Date palm is a drought and salt tolerant plant (Zaid, 2000; Yaish & Kumar, 2015; Al Kharusi *et al*., 2017). In previous study, some cultivars of date palm showed more salinity tolerance at 12.8 dS m−1 NaCl without any visible effect on the phenotype of seedlings (Ramoliya & Pandey, 2003). In another study, some cultivars of date palm were found to tolerate up to 9 dS m−1 soil salinity (Alrasbi *et al*., 2010). Cultivars of date palm from Oman viz., Manoma and Umsila showed greater salinity tolerance compared to that in other cultivars (Al Kharusi *et al*., 2017). The Khalas cultivar showed higher salinity tolerance than the few cultivars of date palm (Aljuburi, 1992; Al-Mulla *et al*., 2013). However, the fruits of both cultivars have varied chemical compositions (Hamad *et al*., 2015).

The agriculture is affected in arid and semi-arid regions due to poor irrigation as more soluble salts are accumulated in the soil which increases the salinity. There are some areas in Saudi Arabia which have high salinity including Skaka city of Al-Jouf (Al-Hassoun, 2007), Wadi Al-Dawasir (Elhag, 2016), Al-Hassa oasis (Hussain, 1982) etc. In such regions, the priority of date palm cultivation should be according to the salinity tolerance of cultivars as growth, reproduction and productivity could be affected in sensitive cultivars.

The Ajwa date is most expensive and popular cultivar which is cultivated in Madeena Al Munawara and its neighboring areas. The Mabroom date is less expensive than Ajwa and also cultivated in Madeena Al Munawara. However, cultivation of both cultivars is limited in other provinces of Saudi Arabia. Therefore, their cultivation should be spread in other provinces of Saudi Arabia for the enhancement of date production. The cultivation of date palm cultivars depends on many parameters which may be physical, physiological or geographical. As from the literature, the Ajwa and Mabroom, both are potential medicinal valued cultivars, however, salinity tolerance has not been assessed till date. Therefore, in the present study, Mabroom and Ajwa cultivars were studied for their salinity tolerance comparison using various markers, biomass yield, germination study and antioxidant system response.

Materials and Methods

One-year-old seeds of Ajwa and Mabroom were used for the experiment set up in a growth chamber at temperature 26-27°C, relative humidity of 72% and a 16-h photoperiod per day. The percent germination of seeds was counted under different concentrations of NaCl; 0 mM (control), 50 mM (T-50), 100 mM (T-100) and 150 mM (T-150). One seed per pot of each cultivar (Ajwa and Mabroom) was planted for the study of morphological markers, biochemical parameters and biomass yield under salinity stress. For accurate comparison, salinity treatment was given at same height of the plants for same duration. Two-months-old seedlings were treated with different concentrations of salt (50, 100 and 150 mM NaCl) along with the control (0mM NaCl) in triplicate to ensure the accuracy of the results. The plants were irrigated weakly with saline water at different concentrations as mentioned above and the control plants were irrigated with distilled water.

Percent seed germination: The germination rate was calculated using the equation as described by Shamsaddin-Saied *et al*., (2007). Percent germination was counted on the 8th day and continued till the 14th day for complete germination of both cultivars.

$$\text{Germination} = \sum_{i=1}^{n} \frac{si}{di}$$

where: Si: indicates the number of seeds germinated each day, Di: the number of days until nth count; n: total number of count.

Morphological traits and biomass yield: The shoot and root length along with biomass yield were measured after two-months of salinity treatment. All treatments were compared to the controls to determine salinity tolerance.

Total chlorophyll estimation: Total chlorophyll was estimated following the method developed by Arnon (1949). Chlorophyll was assessed in fresh leaves. The pigment contents (mg g^{-1} fw) were calculated using the following formula:

Chlorophyll a (mg g^{-1} fw) = (12.7 × OD_{663}) - (2.69 × OD_{645}) × 1/10

Chlorophyll b (mg g^{-1} fw) = (22.9 × OD_{645}) - (4.68 × OD_{663}) × 1/10

Total Chlorophyll (mg g^{-}1 fw) = chl a + chl b

Proline and total soluble protein estimation: Proline was measured using the protocol developed by Hanson *et al*., (1979). 0.3 g of fresh leaves were ground in 10 ml, 3% sulphosalicylic acid and centrifuged. Two ml of supernatant was mixed with equal volume of acid ninhydrin. The reaction mixture was placed in boiling water for 1h and further reaction was stopped by putting it on ice water bath. The equal volume of toluene was added to the above mixture and vortexed. The chormatophore containing toluene was separated and absorbance was taken at 520nm using Model UB-1800, Shimadzu, Japan).

The soluble protein content was estimated following the method developed by Bradford (1976). The fresh leaves (0.5 g) were homogenized in 1 ml phosphate buffer. The equal volume of supernatant and TCA were mixed, centrifuged and pellet was dissolved in 1 ml of 0.1N NaOH.

Thiobarbituric acid reactive substances (TBARS): TBARS content was measured following the method developed by Cakmak & Horst (1991). It was extracted from fresh leaves following the steps as mentioned in the method. The TBARS content was calculated using the following formula:

$$\text{TBARS (nmol g}^{-1}\text{ fw)} = \frac{(A_{532} - A_{600}) \times V \times 1000}{155 \text{ (extinction coeff.)} \times W}$$

(A532 = Absorbance at 532 nm, A600 = Absorbance at 600 nm, W = Fresh tissue weight, V= Extraction volume)

Catalase (CAT) (EC 1.11.1.6): Catalase activity in fresh leaves was determined using the method generated by Aebi (1984). The leaf samples (0.5g) were ground in phosphate buffer (0.5 M, pH 7.3) and centrifuged at 10,000×g at 4°C for 20 min. The reaction was completed in a reaction mixture 2 ml volume (0.1ml 3mM EDTA, 0.1ml of enzyme extract, and 0.1ml of 3mM H_2O_2) for 5 min. Enzyme activity was measured at 240 nm and expressed in EU mg^{-1} protein min^{-1}.

Superoxide dismutase (SOD) (EC 1.15.1.1): Superoxide dismutase activity was measured in fresh leaves at 560 nm wavelength spectrophotometrically using the protocol by Dhindsa *et al*., (1981). Fresh leaf samples (0.05 g) were homogenized in extraction buffer, centrifuged and supernatant was collected for the enzymatic assay. The reaction mixture consisted of 1.5ml of reaction buffer, 0.2ml of methionine, 1ml of DDW and 0.1ml each of (riboflavin 1M-NaCO3, 3mM-EDTA, 2.25mM-NBT

solution and enzyme extract) and was incubated under the light. The blank was kept in dark with all the components as taken for light reaction. The activity of enzyme was represented in EU mg^{-1} protein min^{-1}.

Glutathione reductase (EC 1.6.4.2): Glutathione reductase activity was measured in fresh leaves at 340 nm wavelength using the protocol by Rao (1992). The leaves (0.5g) were homogenized with extraction buffer, centrifuged, and supernatant was used for the enzymatic assay. The reaction was completed in reaction mixture (1ml) containing 0.1ml enzyme extract, and 0.05ml each of (0.2 mM NADPH and 0.5 mM GSSG). Enzyme activity was calculated using the molar absorptivity constant of NADPH (6.2 $mM^{-1}cm^{-1}$) and expressed as EU mg^{-1} protein min^{-1}.

Photochemical efficiency (Fv/Fm): Photochemical efficiency (Fv/Fm) was measured by Li-CoR equipment (USA).

Leaf relative water content (LRWC): Relative water content (RWC) was measured at different NaCl concentrations in both cultivars along with control.

Abscisic acid (ABA) extraction and estimation: The content of ABA in the leaves of the date palm was measured using the protocol developed by Cabot *et al*., (1986). Fresh leaves (2.5 g) from salinity stressed and control plants were collected. The leaves were freeze dried for 24 h and stored at -80°C. The leaf tissues were then ground in liquid N_2 and dissolved in 90% (v/v) methanol with 5 mg^{-l} (BHT) and were kept shaking at 4°C for 12 h. The mixture was filtered and evaporated at 35°C to the aqueous phase. The pH of the aqueous phase was set using 6N NaOH followed by extraction with ethyl acetate. The ethyl acetate containing chlorophyll was discarded and aqueous phase was taken for further extraction. The 6N HCL was used to adjust the aqueous phase to pH 2.51. The aqueous solution of above step and ethyl acetate were taken in equal volume for the extraction of ABA. The fraction of ethyl acetate was collected for ABA extraction and dried at 35°C under vacuum. The dried filtrate was dissolved in methylene chloride (2ml), eluted through Sep Pack cartridge and further washed with 5% acetone in methylene chloride, 5% methanol in methylene chloride and then 2ml, 10 % methanol in methylene chloride. The collected pooled fractions were dried and dissolved in 0.5ml of 60% hexane in ethyl acetate with 1% acetic acid. The extracted ABA was quantified with UHPLC (Agilent Technologies, USA). The gradient was established with the mobile phase, 0.6% acetic acid, and methanol (20:80) in Sep column. Sample injection was set up with 1µl for 10 min. ABA content was calculated from peak areas with the standard curve obtained using a chemical-grade ABA.

Statistical analysis

The data were statistically analyzed using SPSS. One-way analysis of variance (ANOVA) was used for data recorded from each parameter. Duncan's test was performed for comparison of data and four replicates were used to ensure the accuracy of the results. The data were considered statistically significant at $p<0.05$.

Results and Discussion

Salinity stress affects plant metabolic activity by inhibiting water uptake and eventually produces hindrance in seed germination, plant growth and plant biomass. Free radicals are produced in the plant cell under salinity stress which causes slow growth and development. Two nutritionally and medicinally important cultivars of date palm (Ajwa and Mabroom) were chosen for the evaluation of salt tolerance. The seeds of both cultivars were germinated in the presence of 0, 50, 100 and 150 mM NaCl. The percent seed germination varied between cultivars under salinity stress (Fig. 1). The germination was observed earlier in Ajwa by the 8^{th} day after incubation than in Mabroom under salinity stress. The seeds were germinated in control of both cultivars by the 7^{th} day after incubation. No effect was observed on the percent germination in both cultivars at 50mM NaCl and all seeds germinated similar to the control. Germination was decreased in Mabroom (98% and 97%) compared to that in Ajwa (100% and 100%) at 100 and 150 mM NaCl, respectively. On the 12^{th} day almost all seeds of Ajwa were germinated whereas seeds of Mabroom showed lower germination at 100 and 150 mM NaCl. It is also reported that the germination of seeds was decreased in various crops grown in salinity stress (Houle *et al*., 2001; Hadi *et al*., 2007). Soil salinity stress also affects germination by either ionic toxic effects or osmotic stress (Bewley & Black, 2012).

The experiment was completed in pots to evaluate salinity tolerance based on morphological markers, biomass yield and biochemical parameters. The seeds were sowed in pots with a soil and peat moss ratio of 3:2. Salinity treatment was performed at the same height of plants (two-months old). Some plants within a cultivar showed variation in height and were excluded from the experiment at the time of salinity treatment. In our experiment, morphological traits as well as biomass yield were affected in both cultivars under salinity stress. The shoot length, chlorophyll content, and root length were greater in Ajwa as compared to Mabroom cultivar under salinity stress. In the present study, shoot length was less affected than root length under salinity stress and result was lined according with our previously published work on the Khalas cultivar of date palm under same NaCl concentrations (Al-Qurainy *et al*., 2017). Similarly, the shoot length of Ajwa was less affected compared to Mabroom and the result was found to be non-significant within the Ajwa cultivar (Fig. 2). On contrary to shoot length, the root length reduction was observed more between both cultivars as well as within cultivar significantly (Fig. 3). The shoot mass under salinity stress was found non-significant between cultivars and also within Mabroom (Fig. 4). However, the observation on shoot mass were significant within the Ajwa cultivar when compared to control. The impact of salinity was observed to be greater on root mass and significant results were found between cultivars and also within Mabroom (Fig. 5). However, root mass was found to be non-significant within the Ajwa cultivar. Our result was in agreement with the results reported on different plant species grown in salinity stress where biomass yield and morphological traits were affected (Alam *et al*., 2015; Kotagiri *et al*., 2017).

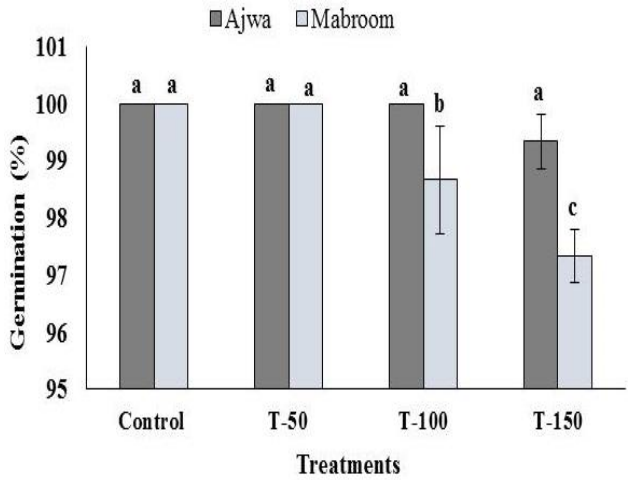

Fig. 1. Percent seed germination at different concentrations of NaCl; data are means of three replicates with standard deviation; various letters indicate the significant values according to Duncan's test (p <0.05).

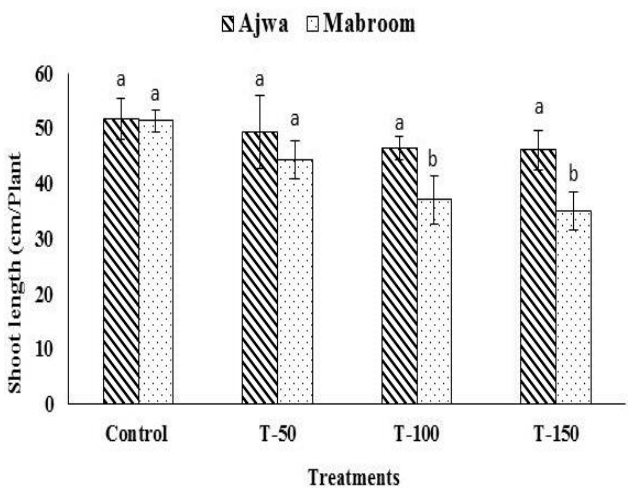

Fig. 2. Shoot length of both cultivars; data are means of three replicates with standard deviation; different letters represent the significant values according to Duncan's test (p <0.05).

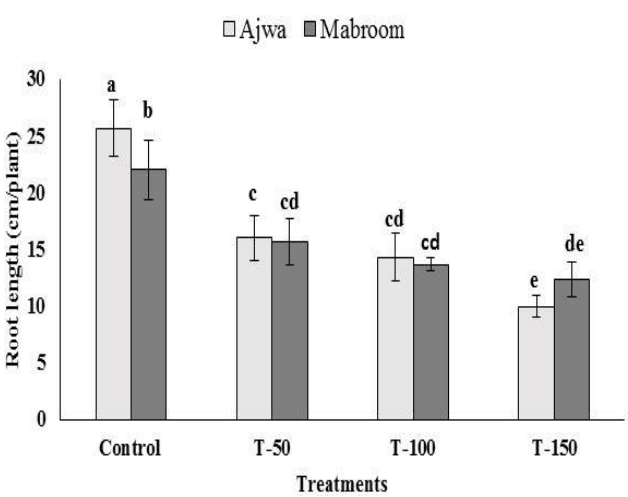

Fig. 3. Effect of salinity stress on root length; data are means of three replicates (means ± standard deviation); different letters indicate the significant values according to Duncan's test (p <0.05).

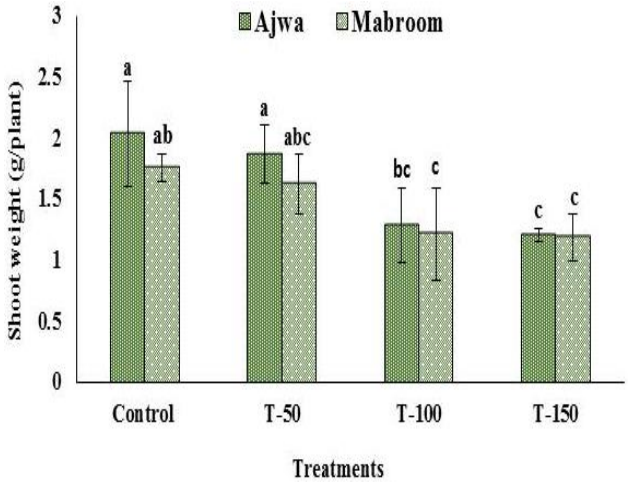

Fig. 4. Fresh shoot weight of both cultivars; data are means of three replicates (means ± standard deviation); different letters indicate the significant values according to Duncan's test (p<0.05).

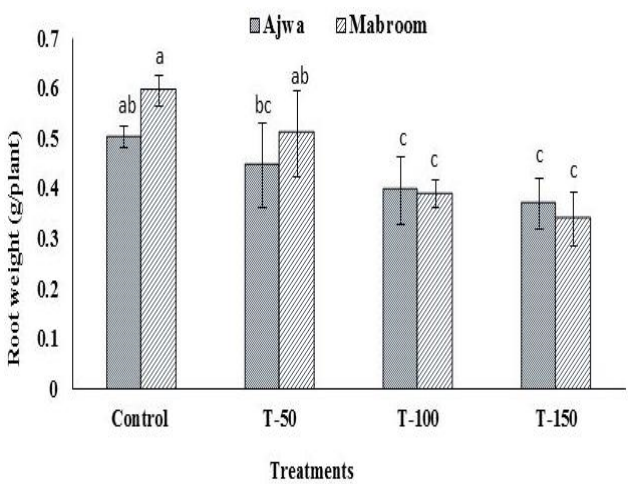

Fig. 5. Fresh root weight of cultivars; bar indicates means of three replicates with standard deviation; various letters indicate the significant values according to Duncan's test (p <0.05).

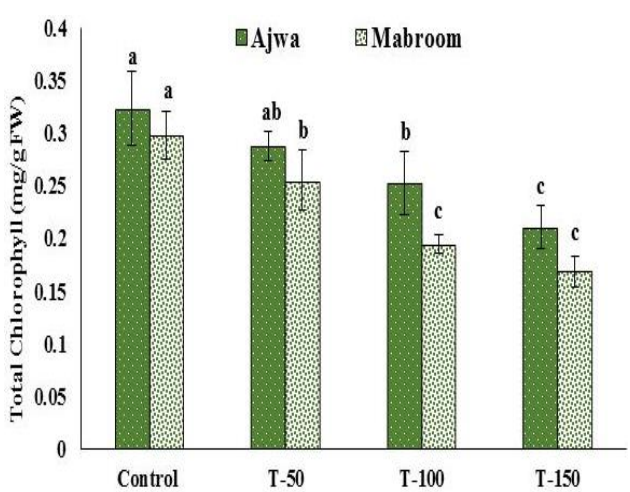

Fig. 6. Total chlorophyll content in fresh leaves; data are means of three replicates (means ± standard deviation); different letters.indicate the significant values according to Duncan's test (p <0.05).

The chlorophyll content was decreased with the salinity increase and significant results were observed within and between cultivars (Fig. 6). A greater reduction in chlorophyll content was observed in the Mabroom cultivar than that in the Ajwa. The total soluble protein content was increased in both cultivars compared to the controls and more accumulation was observed in the Ajwa cultivar (Fig. 7). Significant differences were observed between the cultivars, whereas, no-significant difference was seen within the Ajwa cultivar. Our result in Ajwa cultivar was in agreement with the report by Zhang *et al*., (2013) who performed an experiment on *Broussonetia papyrifera* grown in salinity stress where soluble protein level was same like as that of the control at 50 mM NaCl and its level was increased at 100 mM NaCl. Liu *et al*., (2016) reported increased accumulation of soluble protein in *Nitraria tangutorum* under salinity stress.

Proline is an amino acid that is produced in plant under different stresses and helps in plant development. In our study, the significant differences were observed within and between cultivars as the proline and TBARS levels were increased compared to those in the controls (Figs. 8 & 9). Mabroom showed greater accumulation of TBARS compared to that in the Ajwa cultivar. Our result was in agreement with previous results on date palm as both proline and TBARS were increased compared to those in the control (Al-Qurainy *et al*., 2017). Similarly, increased accumulation of TBARS was found in *Gypsophila aucheri* Boiss (Qureshi *et al*., 2005) and *Glycyrrhiza uralensis* Fisch (Abdallah *et al*., 2016) under salinity stress. Proline content was also enhanced in the leaves and shoots of *Phaseolus vulgaris* when grown with 150 mM NaCl (Jiménez-Bremont *et al*., 2006).

Photochemical efficiency of PSII (Fv/Fm) can be used for the evaluation of plant performance under stresses (Oukarroum *et al*., 2015; Husen *et al*., 2017). Photochemical efficiency was measured after 60 days of salinity treatment in both cultivars. At 50 mM NaCl, both cultivars (Ajwa and Mabroom) showed no variation in the Fv/Fm ratio relative to the control, which represented the plant without stress. The photochemical efficiency was decreased significantly within and between cultivars at 100 and 150 mM NaCl relative to the control (Fig. 10). Similarly, photochemical efficiency was decreased in *Zea mays* as the salinity increased (Husen *et al*., 2017).

Reduction in RWC was observed to be greater in the Mabroom cultivar than that in the Ajwa at 150 mM NaCl (Fig. 11). However, very low reduction in RWC and a non-significant result was found at 50 mM NaCl in the Ajwa cultivar. The RWC was affected in *Brassica juncea* grown under salinity stress, which causes injury to the root system and results in decreased water uptake (Liu *et al*., 2011). Increased production of toxic ions such as Na^+ and Cl^- might be the cause of lowered RWC (Hasegawa *et al*., 2000). Similarly, RWC was declined in cultivars of wheat grown under salinity stress and its reduction was more noticeable in Giza 168 (El-Bassiouny *et al*., 2005). As salinity increased, RWC was decreased in *Trigonella foenum-graecum* L. variety RM-1 (Kapoor and Pande, 2015).

Abscisic acid (ABA) plays significant role in plant under drought and salinity stress (Zhang *et al*., 2006). The content of ABA in treated and untreated samples was estimated using the standard chromatogram obtained with UHPLC (Fig. 12). In our study, ABA accumulation was found to be greater in Ajwa compared to that in Mabroom and the result was found to be significant at all NaCl concentrations used in the experiment compared to the control (Fig. 13). The highest accumulation of ABA, 475 and 445.66 µg/g fw was observed at 150 mM NaCl in Ajwa and Mabroom, respectively. The result was non-significant between cultivars at 100 and 150 mM NaCl, respectively**.** In some reports, it was found that the tolerant cultivars of the same species showed increased ABA accumulation relative to sensitive cultivar (Chen *et al*., 2002; Moons *et al*., 1995) and in barley cultivars without acclimatization (Bravo *et al*., 1998) [60]. ABA concentration was decreased in the salt-tolerant *Brassica napus* compared to salt-sensitive *B. carinata* (He and Cramer, 1996). Talanova and Titov (1994) studied the ABA level under low and high temperature and salinity where they found enhanced levels of this compound was found. ABA content was increased in sensitive variety of rice and was greater than tolerant variety grown under salinity stress (Saeedipour, 2011).

Antioxidant enzyme assay: Soil salinity results in generation of oxidative stress through an increase in ROS. Antioxidant enzymes play an important role in protecting the cell by scavenging ROS. In the present study, CAT enzyme activity was significantly higher compared to the control between both cultivars (Fig. 14) and result was also significant within the Mabroom cultivars as compared to the control. However, no significant difference was found within the Ajwa cultivar even though all the applied conditions were the same as those used for Mabroom. Contrary to CAT enzyme, SOD activity was non-significant between cultivars and also within Mabroom (Fig. 15). However, SOD activity was found to be highest in Ajwa at 150 mM NaCl (8.869 U/mg protein) compared to the control plants. At 100 mM NaCl, glutathione reductase (GR) activity was 0.103 and 0.076 U/mg protein in Ajwa and Mabroom relative to the controls (0.052 and 0.04 U/mg protein) (Fig. 16). However, a significant result was found in GR activity in Mabroom at all concentrations of NaCl used in the present study. Our result was supported by previously reported work on other plant species where the activity of GR and SOD was found to be high under salinity stress (Kachout *et al*., 2013; Kumar *et al*., 2009; Rossatto *et al*., 2017). The activities of antioxidant enzymes including SOD, peroxidase and CAT were enhanced under salinity stress in salt tolerant cultivar of wheat (Feki *et al*., 2017). Our result confirmed previously reported result on Khalas cultivars of date palm under salinity stress where the activities of antioxidant enzymes, CAT and SOD were increased (Al-Qurainy *et al*., 2017).

The result obtained from morphological markers, biomass yield and biochemical parameters indicated that, the Ajwa cultivar showed more salinity tolerance compared to Mabroom. However, the salt tolerance of both cultivars may be associated with salt tolerance genes which regulate the physiological and biochemical mechanism of the cell to cope with salinity stress. Further, salinity tolerance of these cultivars could be studied using the transcriptomic and genomic approaches. Thus, based on salinity tolerance, both cultivars could be cultivated in other regions of Saudi Arabia where soil has salinity problem.

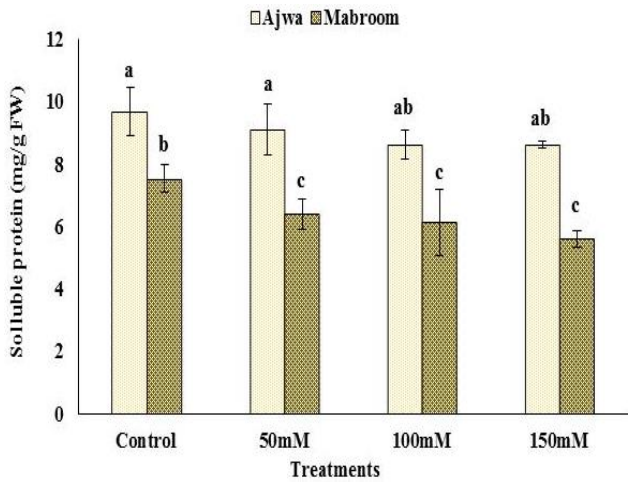

Fig. 7. Soluble protein content in treated (NaCl) and untreated plants (control); data are means of three replicates with standard deviation; different letters represent the significant values according to Duncan's test (p <0.05).

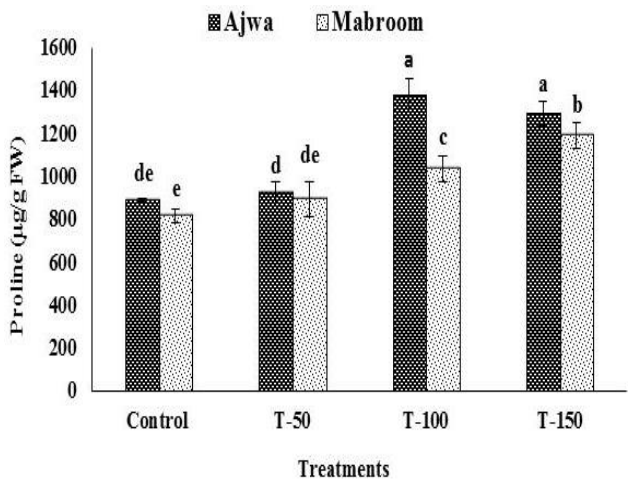

Fig. 8. Proline content in the fresh leaves of Ajwa and Mabroom; data are means of three replicates with standard deviation; various letters indicate the significant values according to Duncan's test (p <0.05).

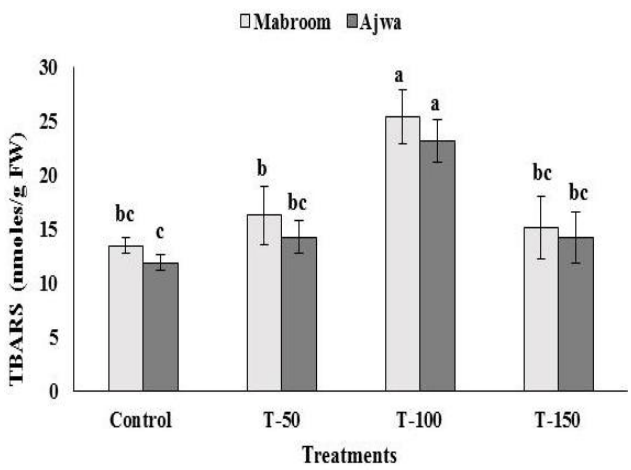

Fig. 9. TBARS content in the fresh leaves of Ajwa and Mabroom cv; data are means of three replicates (means ± standard deviation); different letters indicate the significant values according to Duncan's test (p <0.05).

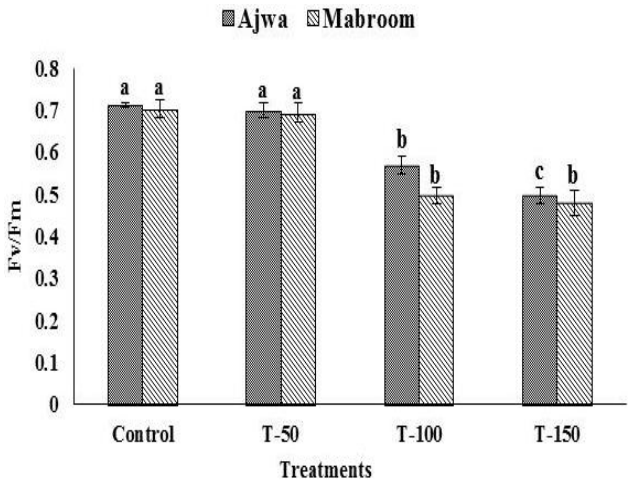

Fig. 10. Photochemical efficiency in treated and untreated plants; bar indicates means of three replicates with standard deviation; different letters indicate the significant values according to Duncan's test (p <0.05).

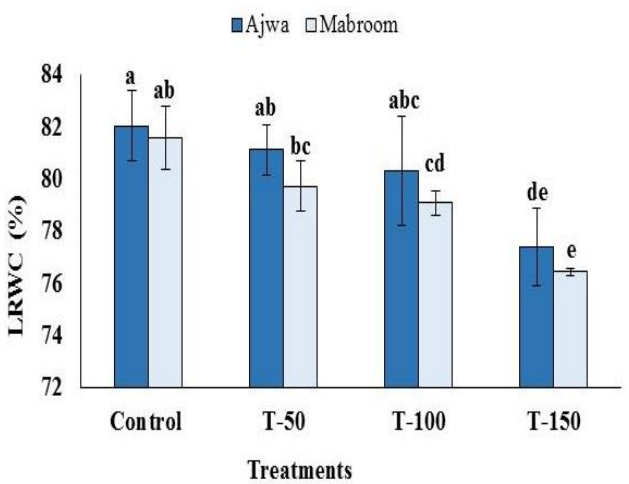

Fig. 11. Effect of salinity stress on leaf relative water content; data are means of three replicates (means ± standard deviation); different letters indicate the significant values according to Duncan's test (p <0.05).

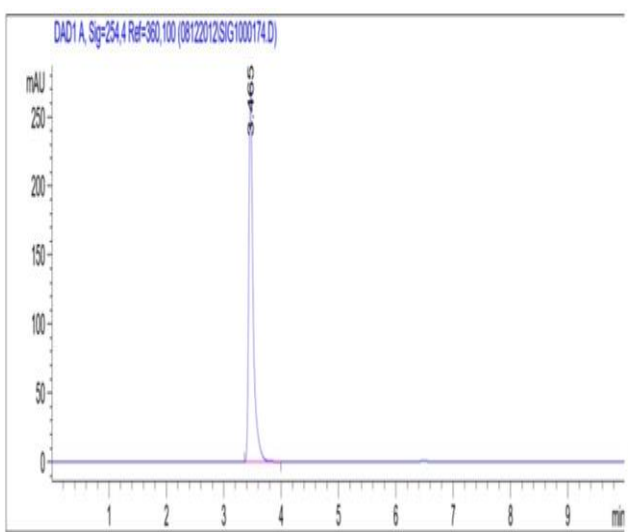

Fig. 12. Chromatogram of standard abscisic acid generated with UHPLC.

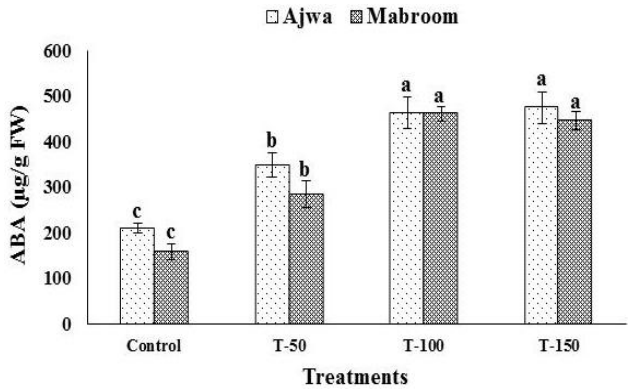

Fig. 13. Accumulation of abscisic acid content under salinity stress and compared from untreated plants (control); (data from means of three replicates ± standard deviation); different letters indicate the significant values according to Duncan's test (p <0.05).

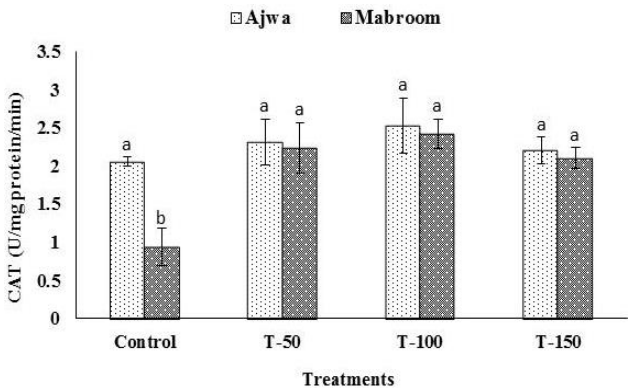

Fig. 14. Catalase activity in treated (NaCl) and untreated (control) plants of Ajwa and Mabroom cv.; bar indicates means of three replicates with standard deviation; different letters indicate the significant values according to Duncan'stest (p <0.05).

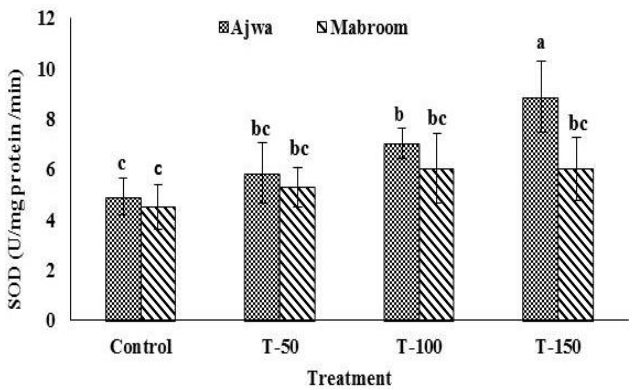

Fig. 15. Superoxide dismutase activity in treated (NaCl) and untreated plants of Ajwa and Mabroom cv.; bar represents means of three replicates with standard deviation; different letters indicate the significant values according to Duncan's test (p <0.05).

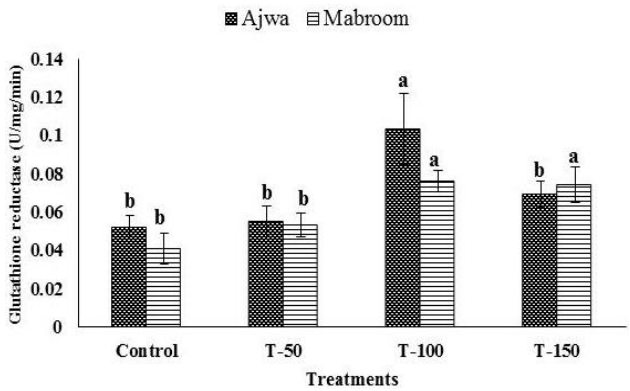

Fig. 16. Glutathione reductase activity in treated (NaCl) and untreated plants (control); data are means of three replicates (means ± standard deviation; different letters indicate the significant values according to Duncan's test (p <0.05).

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