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Influence of drought stress on physiological traits of crossed okra varieties

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Abstract

Drought and heat stresses are major constraints to agriculture worldwide, reducing crop productivity and affecting global food security. Two okra cultivars (Japanese and Egyptian) with different physiological attributes were crossbred for producing F1 hybrids. All cultivars and their hybrids were evaluated for drought stress tolerance at two water regimes (12% and 2%). At limited water condition, photosynthesis and stomatal conductance tended to decrease in Egyptian parent and Japanese ×Egyptian hybrid while transpiration rate showed no significant changes in both parents and F1 hybrids. Maximum quantum yield of PSII (Fv/Fm) ratio was decreased with decreasing soil moisture content in Japanese cultivar, E×J and J×E hybrids with mean value of (0.14, 0.16 and 0.15, respectively). Chlorophyll content in both parents and their hybrids was decreased under severe drought stress. Significant high activity levels of the anti-oxidative enzymes, peroxidase (POX) and superoxide dismutase (SOD) was observed in water-stressed plants than in well-watered (12% water regime) plants. The highest activity of POX was recorded in E×J hybrid (234.9 U/g FW) and the highest activity of SOD was found in Japanese cultivar (18.69 U/g FW). Accumulation of proline content under drought severity stress in both hybrids (E×J and J×E) was recorded (16.7 and 10.4 mg/g DW, respectively). The performance of E×J hybrid was more prominent than parents because of the strong antioxidant defence system and accumulation of higher proteins, proline and chlorophyll content than other cultivars.

Keywords: *Abelmoschus esculentus*, metabolic changes, antioxidant enzymes, cross breeding

1. Introduction

Global water scarcity and change in climate are responsible for the occurrence and severity of drought, heat and salinity stresses in the environment. Combined drought and heat events are the most destructive abiotic stress to crop growth and productivity worldwide, which promote evapotranspiration and affects photosynthetic rate (Mir et al., 2012; Lamaoui et al., 2018). Drought and heat stresses are both important threat limitations impairing plants' photosynthetic rate and altering stomatal function (Silva et al., 2010). Anjum et al. (2017) concluded that exposure to environmental stresses increases reactive oxygen species (ROS) production and thus leads to harmful oxidative damage, impairing the normal cellular functions and causing damages to lipids, proteins and nucleic acids. Heat stress and water deficit also affect the electron transport rate (ETR) and damage the photosynthetic apparatus PSII (Guo et al., 2016). Chlorophyll fluorescence is a non-invasive measurement detecting the authenticity of photosystem II (PSII) (Harb and Lahham, 2013). Chl fluorescence parameters such as photosystem efficiency (Fv/Fm), minimal fluorescence (Fo) and maximal fluorescence (Fm) are useful tools for detection of drought stress severity, genetic variation and determine any damage to PSII (Rahbarian et al., 2011).

In order to cope and adapt stress conditions, plants respond by triggering molecular, physiological and biochemical processes such as altering gene expression, inducing osmolyte accumulation and activation of the antioxidant system whether enzymatic or non-enzymatic (Reddy et al., 2004; Hussien et al., 2019). Plants can regulate their rates of photosynthesis by modifying photosystem II, low electron transport rate and enhance stomatal closure (Khan et al., 2016).

Finding new strategies for maintaining crop productivity under adverse drought, salt and heat stress conditions is probably the major challenge being faced by recent agriculture (Lizana et al., 2006; Albdaiwi et al., 2019). Plant breeders exploit the genetic diversity to make significant improvement towards developing drought adapted crops.

Okra (*Abelmoschus spp*, (L) Moench) is an important cultivated vegetable crop in Egypt, and it is grown for its edible fruits. It is energy supplier to the human diet as well as carbohydrates, protein, fat, fibers, minerals and vitamins (Adejumo et al., 2018). Its usage is increasing in pharmaceutical industry due to high content of polysaccharides and bioactive compounds (Petropoulos et al., 2017). Based on Kusvuran et al. (2012) study, okra genotypes exhibited differences in physiological responses to drought stress; these differences are related to the valuable genetic variation between okra genotypes (Aaron

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et al., 2016). Genetic variation in okra is necessary for crop improvement (Sawadogo et al., 2006).

Egyptian cultivar (African variety) and Japanese cultivar (Asian variety) of okra showed differences in their morpho-physiological characteristics (Ahmed and El-Sayed, 2019) and were expected to respond differently when subjected to a drought stress. High productivity Japanese variety was crossed with local nutritional low productivity variety and the produced hyprids were tested in this study for their tolerance to water deficiency. Ultimately, this study aims to investigate the effect of drought stress on physiological traits and metabolic variability, and to assess the performance of new okra hybrids under drought stress.

2. Materials and methods

2.1. Plant material and growth conditions

The experiments were performed on cultivars of okra (*Abelomoschus esculantus*) i) Egyptian cultivar and ii) Japanese cultivar and two intraspecific F1 hybrids derived from a cross between these two parents (Ahmed and El-Sayed, 2019). These cultivars and their F1 offspring were planted in summer season in nursery under normal climatic conditions at Research Unit for Studying plants of Arid Lands (RUSPAL). They were grown in pots containing a mix of sand and clay (1:1 w/w), and three month old plants were subjected to two moisture levels including 12% (100 % pot water holding capacity as well-watered (Control) and 2% of the maximum capacity for water retention that applied for stressed plants. Healthy expanded leaf samples were collected at time of the measurement of physiological responses, metabolic content and defensive antioxidant enzymes.

2.2. Gas exchange measurements and Chlorophyll fluorescence

Gas exchange (including Photosynthesis rate (pn), transpiration rate (E), leaf intercellular CO_2 concentration, stomatal conductance (Ci)) were measured using infrared gas analyzer (IRGA, CI 340) photosynthesis system (CID Bio-Science, Inc.) on one randomly selected fully expanded healthy leaf. Timing of data recording was between 11:00 AM to 1:00 PM and between 5:00 to 7:00 PM.

The fourth last fully expanded leaf from different plant samples were used fo measuring chlorophyll fluorescence including maximum fluorescence (Fm), initial fluorescence (Fo), variable fluorescence (Fv), and PSII maximum quantum efficiency of PSII (Fv/Fm) using IRGA (CI- 510 CF) chlorophyll fluorescence module.

2.3. Measurement of Enzymatic and Metabolic changes

2.3.1. Assays for PAL, SOD and POX enzymatic activities

Frozen plant tissues (200 mg) were homogenized in a medium composed of 2 ml of extraction buffer which consists of 0.2 M (pH 7.2) of phosphate buffer, 0.1 mM EDTA, 1 mM DTT, and 2 U protease inhibitor. The homogenates were centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was collected and used for the assays of enzymatic activities.

PAL activity was determined according to (Nagarathna et al., 1993).by measuring the L-phenylalanine formation. The supernatant was added to reaction mixture containing 100 mM Tris−HCl, 40 mM trans-cinnamic acid. The mixture was incubated at 40 ºC for 1 h and an ice bath was used to stop the reaction. The absorbance was measured at 290 nm in U g^{-1} FW using a spectrophotometer (Genesys 5, Thermo Spectronic, Rochester, NY, USA).

The method by (Giannopolitis et al., 1977) was followed to assay SOD activity. Three ml of reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 50 µM NBT, 10 µM riboflavin, 12 mM L-methionine and 100 µl of crude extract. To start the reaction, the tubes were placed under two 15W florescent lamps and last for 15 min. Absorbance was read through a spectrophotometer at 560 nm. The ability of SOD to inhibit NBT by 50% was considered as 1 unit for enzyme activities.

POX activity was estimated using method of Kim and Yoo, (1996). 0.2 ml of crude enzyme extract was added to reaction mixture containing 0.8 ml of 0.2 M phosphate buffer (pH 7.2), 1 ml of 15 mM guaiacol, 1 ml of 3 mM hydrogen peroxide. The absorbance was determined at 470 nm. POX activity was calculated as per extinction coefficient of its oxidation product: U/ml = [Change in absorbance min^{-1}× Reaction mixture volume (ml) × Dilution factor]/ [ε470× Enzyme extract volume (ml)]

2.3.2. Total saponins determination

Total saponins content was assayed by spectral reading at 473 nm using spectrophotometer (Ebrahimzadeh and Niknam, 1998). Standard curve of the Diosgenin compound was used to calculate the saponin contents.

2.3.3. Total carbohydrates determination

An anthrone-sulfuric acid method was used to determine the total contents of carbohydrates following Fales (1951). The developed blue-green color was read at 620 nm spectrophotometrically.

2.3.4. Total flavonoids determination

The total contents of flavonoids of the dried leaves extract were quantified by the aluminum chloride method; the reading absorbance wavelength was at 510 nm through spectrophotometer (Chang et al., 2002). The total flavonoids concentration was calculated from a standard curve, and the result was estimated as mg quercetin equivalent per g dry weight.

2.3.5. Total proteins assay

Total proteins were assayed according to the method of (Lowry et al., 1951).

2.3.6. Determination of total phenolics

Total phenolic compounds were examined using the Folin-Ciocalteu method (Ough et al., 1988).

2.3.7. Ascorbic acid determination

Ascorbic acid content was determined according to (Omaye et al., 1979).

2.3.8. Proline determination

Proline content was examined by modification of the method of (Bates et al., 1973). Dry powdered okra leaves (250 mg) were homogenized with 10 ml of 3 % sulfo-salicylic acid and centrifuged at 3000 × g for 10 min. Two ml of the supernatant were mixed with 2.0 ml of the

prepared reagent (2.5 g ninhydrin in 40 ml of 6 m orthophosphoric acid and 60 ml glacial acetic acid), and 2.0 ml of glacial acetic acid in a test tube. The test tubes were heated at 100 °C for 1 h and 4 ml of toluene were added to each test tube after cooling. The absorbance was read at 520 nm through spectrophotometer. The concentration of proline was quantified using calibration curve of proline standard and calculated on dry weight basis.

2.3.9. Chlorophyll content assay

To quantify chlorophyll content by spectrophotometer, the method described by (Ni et al., 2009) was used.

2.4. Statistical analysis

The obtained data were analyzed by a one-way analysis of variance (ANOVA) using Minitab. Student t-test was used to compare the control with stressed plants. Statistical significance was considered at the level ($P \leq 0.05$). Values shown in the figures were reported as means ± standard errors (SEs) of three independent replicates. The relationships among individual variables were determined using Principal Component Analysis (PCA).

3. Results

3.1. Gas exchange measurements and Chlorophyll fluorescence parameters

A significant reduction ($p<0.05$) in net photosynthesis and stomatal conductance was detected for Egyptian mothers and J×E hybrid (0.07 and 0.0 µmol $s^{-1}m^{-2}$, respectively) and (8.09 and 0.0 mmol $s^{-1}m^{-2}$, respectively) Fig.1. Declines in the intercellular CO_2 concentrations were observed following the reduction in stomatal conductance. In contrast, Japanese cultivar and E×J hybrid exhibited higher photosynthesis rate with high stomatal conductance as soil water content decreases in comparison with their unstressed plants and stressed Egyptian mothers and J×E. Transpiration rate of J×E hybrid showed significant decrease (0.06 mmol $m^{-2}s^{-1}$) at severe drought stress. Parents and E×J plants showed no significant change in transpiration rate (0.12 mmol $m^{-2}s^{-1}$) at the same drought condition.

Fig. 1 shows that the quantum efficiency (Fv/Fm) of PSII was affected significantly by the water shortage. This led to reduction in PSII efficiency. Both hybrids and Japanese cultivar showed decreasing in Fv/Fm under stress condition ranged between 0.14 and 0.15 for stressed plants ($P<0.05$), while Egyptian mothers showed no significant change compared to unstressed plants, the Fv/Fm remained higher than other cultivars under stress 0.38 . Drought stress was also affected by the efficiency of electron transfer (F0) which is necessary for regulating the primary photochemical activity.

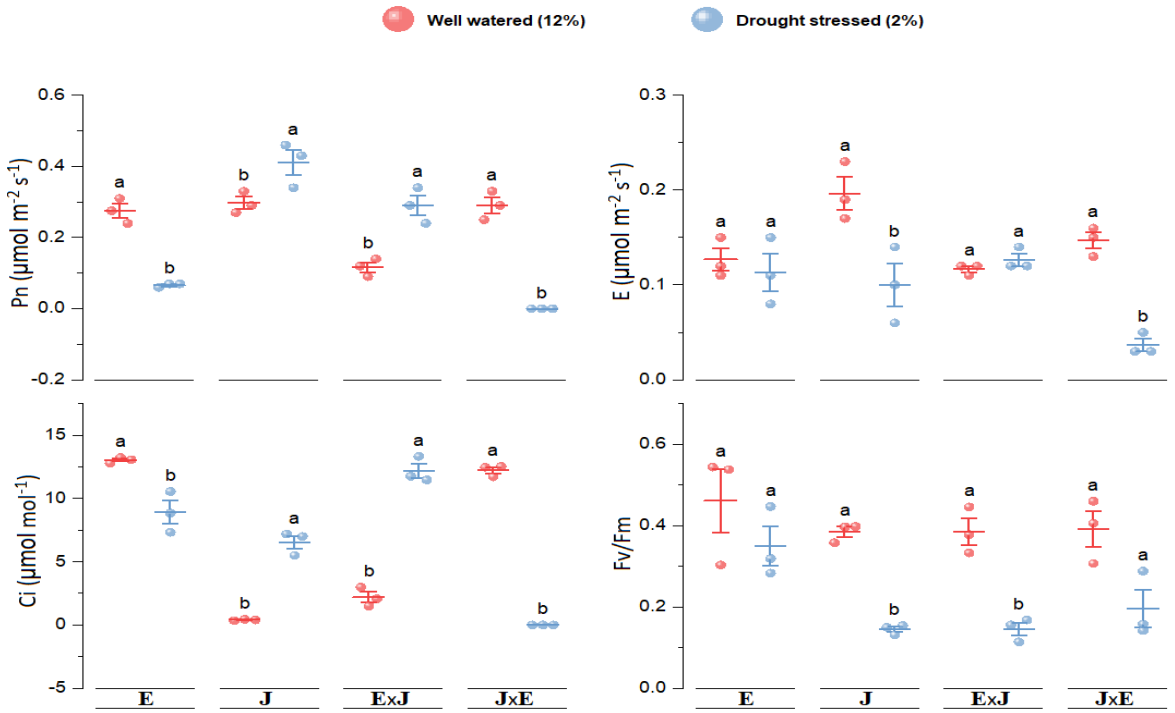

Figure 1. Diagram showing the effect of drought stress on photosynthesis rate (Pn), Transpiration rate (E), stomatal conductance (Ci) and Fv/Fm of Japanese cultivar, Egyptian cultivar, E×J and J×E at two water regimes (12%and 2%), a and b letters indicate significant changes ($P<0.05$) between well watered and stressed plants according to Student's t-test.

3.2. Activities of Enzymatic Antioxidants under Drought Stress

Fig. 2 shows the varied significant influence of osmotic stress ($P < 0.05$) on the antioxidant enzymes (SOD), (POD) and (PAL). The activities of SOD in Egyptian mothers, Japanese mothers, E×J and J×E were significantly increased with the severity of drought stress in comparison to the well-watered control plants. The maximum activity of SOD was observed in Japanese

cultivar at 2% water regime level (18.69 U g^{-1} FW) followed by Egyptian mothers, J×E and E×J (17.961, 14.078 and 13.835 U g-1 FW, respectively). The obtained results showed that highly significant increase in POX for E×J reaching the maximum activity value of (234 U g^{-1} FW), followed by Japanese mothers and J×E with value of (167.5 and 101 U g^{-1} FW, respectively). In Egyptian mothers, there is no significant change in POX activity in well watered (62.6 U g^{-1} FW) compared with stressed plants (60.3 U g^{-1} FW). Activities of PAL were significantly increased in the Egyptian mothers and E×J under severe drought stress conditions while in Japanese mothers and J×E the PAL activities were decreased significantly.

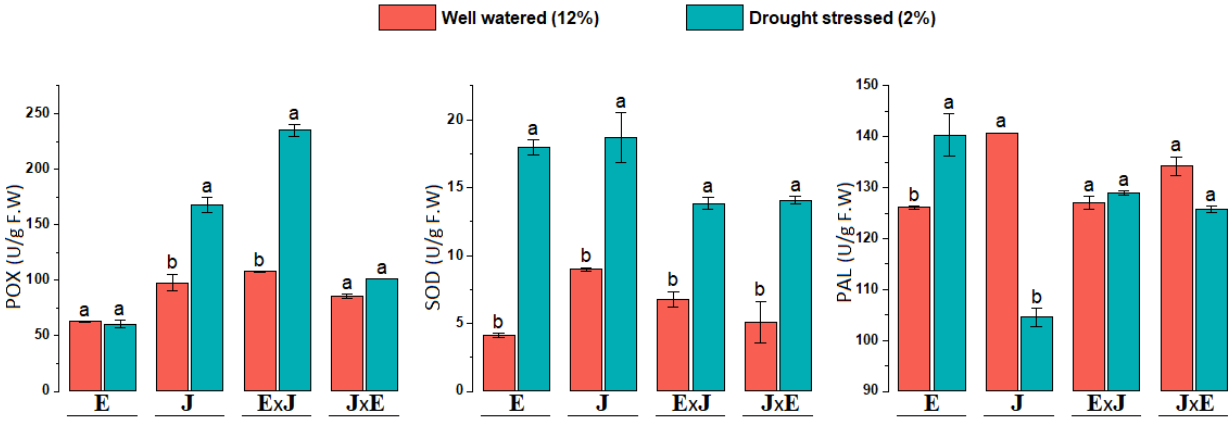

Figure 2. Antioxidant enzyme activities of peroxidase, POX; superoxide dismutase, SOD and Phenylalanine ammonia lyase, PAL representing well watered Japanese cultivar, stressed Japanese cultivar, well watered Egyptian cultivar, stressed Egyptian cultivar, well watered Japanese cultivar× Egyptian cultivar (J×E), stressed Japanese cultivar× Egyptian cultivar (J×E), well watered Egyptian cultivar × Japanese cultivar (E× J) and stressed Egyptian cultivar × Japanese cultivar (E× J). Values are means ± standard errors (SEs) of three independent replicates (n = 3), a and b letters indicate significant changes (P<0.05) between well watered and stressed plants as expressed by a Student's t-test.

3.3. Total chlorophyll content and Metabolic change

Under stress condition, the leaf chlorophyll concentration of Japanese and Egyptian parents and their J×E hybrid was decreased as compared with control from (1.84, 1.88 and 1.66 mg/g FW, respectively) to (1.66, 1.6 and 1.4 mg/g FW, respectively), while the total chl content in J×E hybrid remained constant under low water regime (1.9 mg/g FW). Changes in metabolite contents were induced by drought stress and led to different responses of the two Japanese and Egyptian mothers and their hybrids to water deficit conditions (Fig.3). Drought condition (2% water regime level) resulted in significantly higher protein content in Japanese cultivar and E×J (202.38 and 191.9 mg/g DW respectively) and in contrast, the content of protein in Egyptian and J×E decreased compared to their control plants (178.5 and 164.29 mg/g DW, respectively). Our results showed decrease in total carbohydrates content in Japanese, Egyptian and E×J while J×E hybrid showed significant increase (P < 0.05) in total carbohydrates under water deficit conditions.

In general, ascorbic acid content was unaffected by drought stress in both okra hybrids and Egyptian mothers except for Japanese mothers, where ascorbic acid contents were significantly increased (P<0.01) under 2% water regime with mean value of (30.6 mg/g DW). Proline content was increased due to drought stress in both F1 hybrids while their parents showed decreasing in proline content. Under low water regime (2%), there is no significant change in total phenolic for all okra cultivars and their F1 hybrids. Total flavonoids and saponins contents were declined in parental cultivars and both F1 hybrids under drought stress as compared to well-watered control plants. Principle component analysis PCA indicated that physiological traits are remarkable for distinguishing parental okra cultivars and their F1 offspring at both 12% and 2% water regimes (Fig.4). The PCA analysis was used to reduce the dimension of the data among all physiological and metabolic traits. PCA 1 was the most descriptive component which explained 31.9% of the variation. PCA 1 correlates positively to parameters related to peroxidase POX, super-oxide dismutase SOD and ascorbic in which characterize the plant under low water regime (2%), while physiological parameters such as chlorophyll fluorescence, transpiration (E) and stomatal conductance (Ci) has large negative loadings on PCA components. Furthermore, smaller variation was observed on PCA 2 (21.7%), corresponding mainly to proteins and photosynthesis (pn).

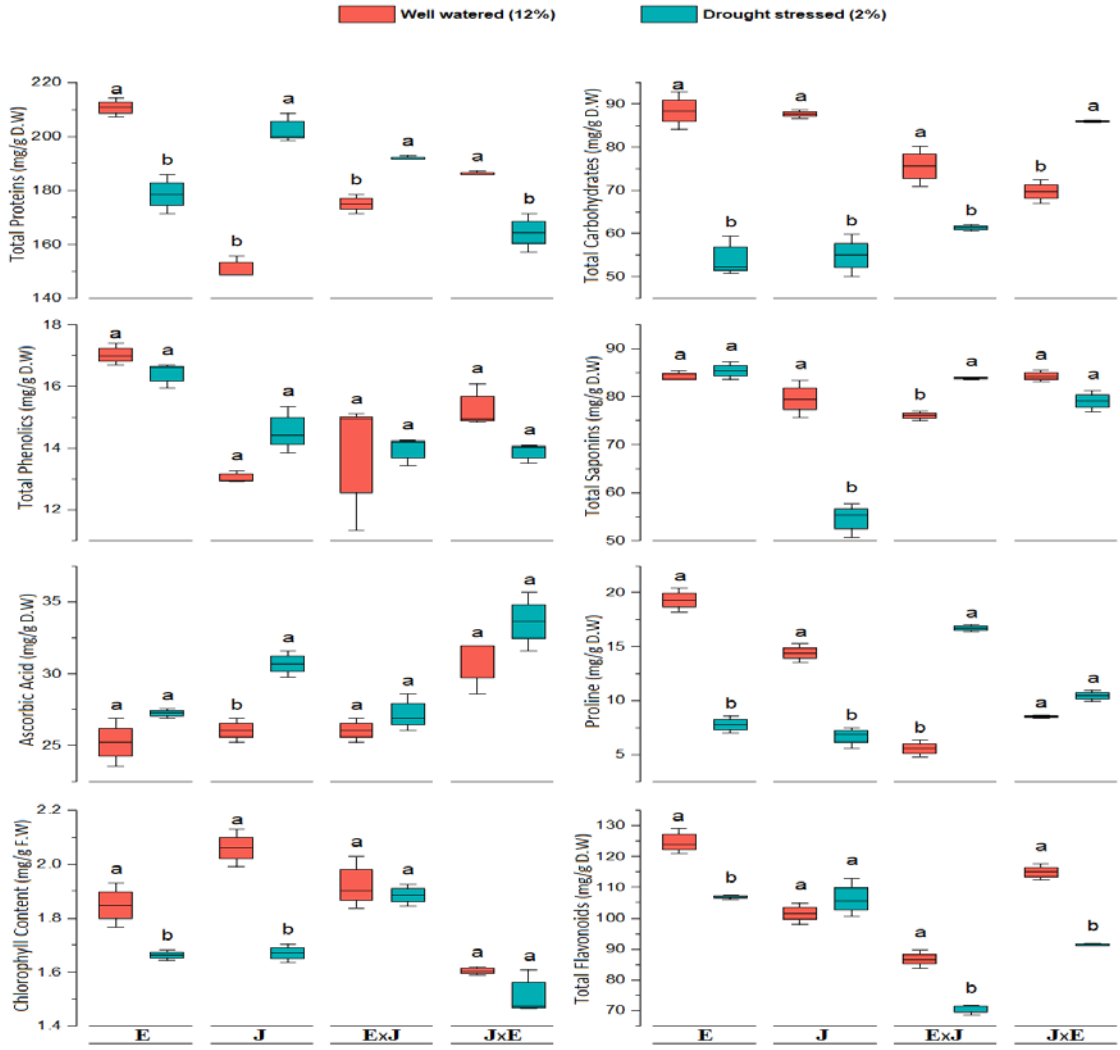

Figure 3. Differences in total protein contents, total carbohydrate contents, ascorbic acid and proline, total phenolics, flavonoids and saponins (mg/g DW) between well-watered Japanese cultivar, stressed Japanese cultivar, well watered Egyptian cultivar, stressed Egyptian cultivar, well-watered Japanese cultivar× Egyptian cultivar (J×E), stressed Japanese cultivar× Egyptian cultivar (J×E), well-watered Egyptian cultivar × Japanese cultivar (E×J) and stressed Egyptian cultivar× Japanese cultivar (E×J) Values are means ± standard errors (SEs) of three independent replicates (n = 3), a and b letters indicate significant changes (P<0.05) between well-watered and stressed plants as expressed by a Student's t-test.

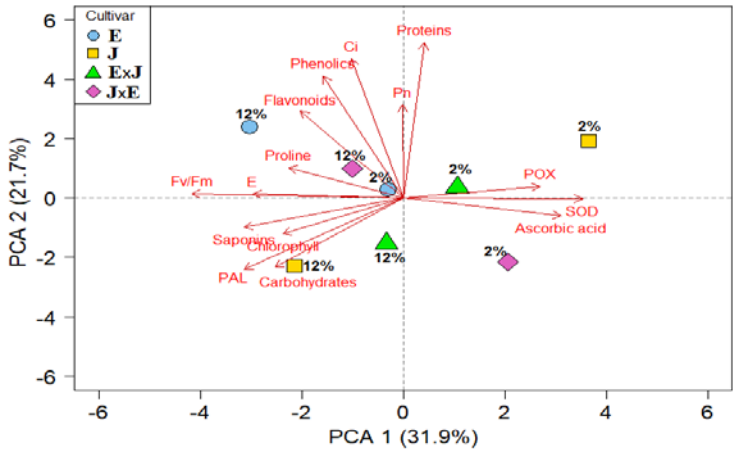

Figure 4. Principal component analysis (PCA) for identification of metabolic change and physiological attributes in two okra varieties (Egyptian and Japanese) and their hybrids (Egyptian cultivar ×Japanese cultivar and Japanese cultivar ×Egyptian cultivar) grown under two water regime 12% (well watered plants) and 2% (stressed plants). The factor loading values for variables are indicated by red arrows radiating from the center showing the direction (angle) and magnitude (length), allowing to separate well-watered and stressed plants. Arrows represent physiological traits with various length based on the impact of each trait on the separation of cultivars showing how each metabolite and physiological parameter contributes to the individual correlations represented by PCA1 (31.9%) and PCA2 (21.7%).

4. Discussion

Drought tolerance improvement and crops productivity are considered the most difficult challenges for plant breeders. Germplasm lines can be selected on the basis of their performance under conditions of water limit. Drought stress influence plant performance, by altering physiological processes such as disruptions of photosynthetic pigments, reduction of the gas exchange and regulation of stomatal function (Keyvan, 2010). Stomatal closure causes reductions in net photosynthesis rates in water stressed Egyptian okra cultivar and J×E hybrid. The stomatal clousure lead to reduction in CO_2 assimilation and minimized the rate of water loss transpiration. Similar findings were addressed for medicinal plants under shortage of water (Al-Gabbiesh et al., 2015). This response enables the plant to tolerate water stress (Souza et al., 2004). Fv/Fm is considered as one of the most commonly used features to estimate plant stress. The Fv/Fm ratio decreased in all cultivars and their hybrids under drought stress. Several researchers reported that Fv/Fm decreased under severe water stress (Miyashita et al., 2005; Banks, 2018). Thus, reduction in Fv/Fm ratio indicates the protective mechanism of light absorption in response to water deficiency (Paknejad et al., 2007).

Water deficit conditions cause production of ROS, encouraging plant tissues under drought stress to produce various antioxidant enzymes to alleviate oxidative damage caused by ROS (Mattos et al., 2015). Previous researches have shown that antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and phenyl ammonia lyase (PAL) act as key defendant enzymes against ROS, and the higher enzymatic activity depends on genotypes genetic potential (Park et al., 2013; Martinez et al., 2018). Peroxidase is the most important enzyme providing for plant anti-oxidant defence (Kholodova et al., 2007). In our experiments, peroxidase was highly activated in Japanese cultivar, J×E and E×J plans under stress conditions as POX is lowering the concentration of H_2O_2 and limiting the oxidative damage. SOD activity was increased in parent cultivars and their both hybrids under severe drought condition to impair the ROS production while PAL activity increased only in Egyptian mothers. The protective effect of antioxidant enzymes against water stress has been demonstrated in several plant species (Sayfzadeh and Rashidi 2011; Ahmed et al., 2002; Ahmad et al., 2015).

The photosynthetic pigment contents (chlorophylls and carotenoids) are considered influential physiological crop indicators for stress tolerance, including drought stress (Pour-Aboughadareh et al., 2020). In this study, total chlorophyll value was decreased by drought stress in all parent cultivars and their F1 hybrids (E×J and J×E). This reduction in chlorophyll pigment is believed to be a result of inhibition of chlorophyll synthesis pathway (Anjum et al., 2011). Stress proteins are expressed intensively, and their accumulation in plants is a common feature of the response to drought stress. The total proteins increased in Japanese cultivar and E×J under low water regime while, in contrast, Egyptian mothers and J×E hybrid showed decrease in total proteins content. Increasing in protein content under drought stress is in agreement with a previous study carried out by (Qaseem et al., 2019). Total carbohydrates was higher in stressed J×E in comparison to the control while the other cultivars showed decrease in total carbohydrates, indicating that there might be a genetic variation in the accumulation of these compounds. Carbohydrates seem to play a key role in the regulation of carbon metabolism to be part of a wider mechanism for plant surviving during drought stress (Praxedes et al., 2005).

Free proline accumulation by plant tissue was dependent on the severity of osmotic stress conditions. Proline acts as osmolyte which regulates the osmotic pressure in the cytoplasm (Caballero et al., 2005). Adejumo et al. (2018) reported that accumulation of proline in okra leaves may serve as physiological tool to develop drought tolerance in okra plants. Our result revealed that proline content of well watered hybrid plants (E×J and J×E) was lower than those plants exposed to limited water condition. In contrast, the stressed parents (Japanese and Egyptian cultivars) showed lower proline content than well watered control plants. It seems that the hybrids have this drought-response character, and this result is similar to the observation by Castillo et al. (2017). PCA is the most frequently used multivariate statistical method (Liu et al., 2010). The output results of the PCA successfully identified variables which contribute most to response against drought stress amongst okra cultivars and hybrids. PCA analysis is useful tool for distinguishing between okra varieties and their hybrids based on the changes in their physiological traits and antioxidant enzymes activity (POX, PAL and SOD). Findings suggested that strong positive contributions were observed for POX and SOD on stressed hybrids, while they were in negative association with chlorophyll and carbohydrates according to PCA; also results showed that drought stress has a negative effect on photosynthesis, transpiration and proline content of parental cultivars.

Stress plants showed higher enzymatic activity and proteins content, indicating the most cultivar adapted to drought stress. Our findings in this study are in agreement with Liu et al. (2015) investigation.

5. Conclusion

In this investigation, okra cultivars and their F1 hybrids have various strategies to reduce the harmful effects of water deficit stress. It was concluded that photosynthesis and stomatal conductance tended to decrease under low water regime in parental cultivars and their hybrids. Transpiration rate was decreased in Japanese cultivar and E×J hybrid. Plants also responded against adverse conditions by up-regulation of antioxidant enzymes and accumulation of compatible solutes. The total chlorophyll, carbohydrates, proteins, proline contents were decreased across both parental cultivars under stressful conditions. In contrast, E×J hybrid was more tolerant to drought and performed better by enhancement of proline, proteins and total chlorophyll contents.

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