American Journal of Plant Physiology

ISSN 1557-4539

Academic Journals Inc.

www.academicjournals.com

American Journal of Plant Physiology 6 (3): 126-143, 2011
ISSN 1557-4539 / DOI: 10.3923/ajpp.2011.126.143

In vitro Selection and Photosynthetic Characterization of Date Palm Regenerated Seedling as Affected by Water Stress

[1]M.N.M. Helaly and [2]A.M. Hanan El-Hosieny
[1]Department of Agriculture Bot, Faculty of Agriculture, Mansoura University, Egypt
[2]Hort. Res. Inst. A.R.C. Egypt

Corresponding Author: M.N. Helaly, Department of Agriculture Bot, Faculty of Agriculture, Mansoura University, Egypt

ABSTRACT

The study was aimed to investigate the ability of two date palm genotypes (Shamia and Amri) to tolerate drought stress throughout their micropropagation period. Some morpho-physiological basis of drought responses in the regenerated seedlings were evaluated. This may help in selecting drought stress tolerance and detecting other quantitative traits. Direct organogenesis technique, using shoot tip explants and supplementation of mannitol to the MS basal media has been chosen to tackle this issue. The data indicated that emberyogenesis obtained in the two genotypes followed normal development and had ability to competent to regeneration and converted into plantlets under drought stress conditions. However, swelling, numbers of embryos and shoots/jar, plantlet height and root length were decreased. All these parameters recorded higher values in Shamia genotype than those of Amri. Photosynthetic rate, transpiration rate and stomatal conductance of the regenerated plants in both date palm genotypes, were decreased whereas water use efficiency was increased with an increase in water stress level. Shamia genotype obtained higher values than Amri genotype in this respect. Carbon isotope discrimination was higher (more negative values) in shoots than in the roots and decreased with decreasing water supply. The values of maximum (Fm) and variable (Fv) fluorescence of the chlorophyll showed a significant reducing trend in both genotypes under stressed condition. The quantum yield of photochemistry (Fv/Fm) was also decreased. The mean Fv values as well as Fv/Fm ratio of both date palm genotypes in water stressed plants were significantly lower than those in the control. It could be concluded that both date palm genotypes studied, with the superiority of Shamia, have an ability to tolerate water stress under *in vitro* system. Chlorophyll fluorescence can be used as a tool, with other examined useful physiological characters, to screening date palm genotypes for drought resistant.

Key words: *Phoenix dactylifera* (Shamia and Amri genotypes), photosynthetic rate, stomatal conductance, carbon isotope discrimination, chlorophyll fluorescence, photosynthetic activity radiation

INTRODUCTION

Date palm (*Phoenix dactylifera*, L., Arecaceae), is one of the most important and oldest fruit trees in the world and is mentioned in Quraan and Bible. In Egypt, it is used also as an ornamental plants as well as a source of fiber, fuel and furniture uses (Dowson, 1989; Khataab, 1985; El-Kosary, 2009). Dates produced about 680000 tons fruits from about 9463911 millions fruitful female plants. It decreased continually from the last century by about 12-15% from the total

number of dates (FAO, 2004). To avoid date palm from extinction and because of the limitation of the cultivated area in Egypt, it is important to select a suitable genotype for micropropagation from existing elite cultivars having an ability to grow under the newly reclaimed soils. Low water availability is one of most important limiting factor for date palm production in these regions (El-Kosary *et al*., 2009). El-Semea (2003) classified dates in Egypt arbitrary and conveniently according to their fleshiness and available heat units (calculated in relation to zero level or 18°C during the period from May up to the end of October) to soft (heat units 2100; Zaghloul, Barhee, Amri, Samani and Bent Aisha), semi dry (heat units 3600; Aglany, Sewi and Meghil) and dry dates (heat units 4700; Bartamuda, Sakkoty, Shamia Goundila, Karkoda, Digna, Khazali and Mekawi).

Low water availability impairing numerous biological roles causes yield reduction and affecting the majority of the arable land around the world. Due to the limitation of water resources for the agriculture uses, it is important to develop a suitable drought tolerant genotype (Bruce *et al*., 2002).

Increasing food production in stressful environments may be achieved through breeding of crops that are more tolerant to the stress. The classical breeding for abiotic stress tolerance is quite difficult, time-space and labour-consumming intensive because of the reduced genetic back ground, the incompatibility between the wild resistant species with the plant varieties and lines (El-Shihy and Monem, 1994; Costa Maria and Aloufa, 2006).

Generally, number of mechanics can contribute to an improved drought tolerance of the plants, including morphological characteristics like deep rooting or metabolic regulators mechanism like osmotic adjustment (Bloch and Hoffimann, 2005). Also, the use of physiological traits is very relevant for plant improvement in dry environments (Mohammadian *et al*., 2003; Joseph *et al*., 2010). It appears that *in vitro* selection for stress tolerance will continue to have its significant place in the strategy of establishing plant systems with optimal stress reaction and producing. However, no strictly confirmed reports and little information on the direct effects of mannitol as a stress factors on the physiological processes through the regeneration procedure of date palm are available (Bartels and Sunkar, 2005).

Biotechnologies such as somaclonal variation obtained by tissue culture techniques offer a rapid and reliable alternative in crop. Improvement purified mannitol and Polyethylene Glycol (PEG) have been found to have no injurious or toxic effects on the plant but inhibit growth by lowering the water potential of the media. Thus, cultured explants are unable to take up water (Ehlers and Goss, 2003). These compounds have been long used to stimulate drought stress in plants as non-penetrating osmotic agent lowering the water potential; in a way similar to soil drying. However, studies at a whole plant level revealed that the nature of correlation between high productivity and stress tolerance is currently not well established and understood (El-Kosary *et al*., 2009).

The mechanism investigation of drought tolerance-date palm propagated by tissue culture technique therefore, requires research focused on physiological processes such as photosynthesis, assimilation and degradation as well as translocation mechanism (Van der Beek and Houtman, 1993).

Bloch *et al*. (2006) reported that when plants encounter water deficit there is a decline in photosynthesis. This can be due to a reduction in light interception as leaf expansion is reduced or as leaf senescence is accelerated. But it can also be attributed to reduction in CO_2 fixation per unit leaf area as stomata close or as photo-oxidation damages the photosynthetic mechanism (Bruce *et al*., 2002).

Much of the reduction in CO_2 assimilation under water deficit is due to stomatal closure and/or direct inhibitory effects of water deficiency (Sharkey and Seemann, 1989; Seckin *et al*., 2010). In plants, higher stomatal conductance increases CO_2 diffusion into the leaf thereby favoring higher net photosynthetic rates (Lopez *et al*., 2008). Genotypic differences in stomatal conductance can be linked to variability for drought resistance (Ashraf *et al*., 2002).

The aim of the present investigation was to obtain tolerant date palm genotypes to water stress using *in vitro* selection procedure. Photosynthetic activity parameters and water use efficiency; WUE of the regenerated seedlings after acclimatization were evaluated for drought resistant.

MATERIALS AND METHODS

The present investigation was carried out at the plant tissue culture Dep. Genetic of Eng. and Biotech. Res. Inst; GEBRI, El-Sadat city. Menofia Univ., Egypt and the laboratories of the Middle Eastern Regional Radio-isotope Center for Arab countries Dokki, Cairo Egypt during the period elapsed from April 2009 to November 2010.

The selected genotypes of date palm (*Phoenix dactylifera* L.) were expected to represent a wide range of drought tolerance as comprised of the commercial arbitrary and conveniently according to their fleshiness and available heat unit (El-Semea, 2003). The 1st is the Egyptian Shamia genotype; dry genotype (heat units 4700), a genotype which has been successful over years in the Aswan governorate and is regarded as drought tolerant (Abdallah *et al*., 1993). The second is the Egyptian genotype Amri; soft genotype (heat unit 2100) which is putative drought susceptible due to reduction in the root system and cultivated at Sharkia governorate (Abd-El-Hamid *et al*., 2001).

All chemical used for propagation were obtained from Sigma Comp (Merch, India). MS basal nutrient solution, sucrose as energy source (30 g L^{-1}), gelrite as a gelling agent at 1.9 g L^{-1} and activated charcoal; A.C (3 g L^{-1}) were used. For oxidative browning control, a combination of antioxidant citric and ascorbic acids both at 75 mg L^{-1} were added as recommended by Helaly *et al*. (2008). Polyvinylpyrrolidene, PVP (1500 mg L^{-1}) and Ca-pantothenates (2.5 mg L^{-1}) were also added to the basal media based on the data of El-Bellaj *et al*. (2000). The pH of the media was adjusted to 5.6±0.1 with 0.1 N KOH or 0.1 N HCl prior to addition of gelling agent.

Hardening and callus induction: Shoot tips explants from the two genotypes of female date palm; Shamia and Amri were selected for plantlets regeneration through direct organogenesis technique (Helaly *et al*., 2008). They were cultured on the MS basal media (Murashige and Skoog, 1962) supplemented with mannitol at the levels of 0.0, 0.2 and 0.3 ML^{-1} corresponding to about -1, -5 and -7 bar osmotic pressure. The culture media of each treatment was distributed in the culture jars (250 mL^{-1}), each one dispensed with 30-35 mL of the specific prepared media. Each treatment was replicated 6 times. The culture jars were immediately copped with polypropylene closures and autoclaved at 121°C lb $inch^2$ for 20 min. The sterilized shoot tip explants were cultured on the specific medium at the rate of one explant per jar and incubated at 25-27°C for 16/18 h day/night condition using white fluorescent tubes giving intensity of about 1500 Lux.

The incubation period was took place for 30 weeks. The survived explants were transferred and sub cultured on the same fresh specific media every six weeks. At each sub culturing date, the survived and established degrees of the initial explants in both date palm genotypes were calculated according to Pottino (1981).

Induction embryos to tolerant stress: To induce drought stress on the regenerated embryos the specific basal MS media were used. The incubation was took place for 6 months with 4-sub-culturing, 6 weeks intervals at the same conditions previously mentioned.

Plantlets formation: The obtained developed embryos clusters were induced to shoot formation using a specific fresh basal media previously mentioned (0, 0.2 and 0.3 M mannitol/L). Small clusters, each contained about 10-15 embryos were cultured under the same condition with 4 replicates. The mean numbers of produced shoots/jar, secondary embryos and shoot height were recorded after twelve weeks.

The regenerated shoots were induced to form roots in cultured glass tubes (25×150 mm; Borosil) containing 20 mL of specific modified MS medium supplemented with 3 g L^{-1} activated charcoal and 2 mg L^{-1} indol buteric acid; IBA as recommended previously Helaly *et al.* (2008). They were incubated in the growth room at the same conditions previously mentioned for 8 weeks. At the end of incubation, plantlet heights, as well as root length were recorded.

Acclimatization and drought stress treatments: Acclimatization was took place during the two growing seasons of 2009 and 2010. At 15th March in both seasons, the produced well-rooted plantlets were selected from each treatment from the two genotypes at the end of plantlets formation. They were removed carefully from the gelling media found in the cultured glass tubes, under tap water, to minimize dehydration effects and obtained gelling media free plantlets.

The plantlets were immersed in Rizolex 5% fungicide (2 g L^{-1}) containing 2 drops of Tween 20 for 20 min and planted individually in plastic pots; tyrpido (5.5×6.5 cm) containing optimum media for hardening (peatmoss: sand: perlite; 1:1:1 v/v) as recommended previously by Helaly *et al.* (2009). The pots containing plantlets were arranged, at random, in the acclimatization room under 25±1°C and 1500 Lux light intensity for 16/8 h, photoperiod and about 80% relative humidity. The pots were covered, enveloped and tightly closed with a transparent polyethylene sheets to provide high humidity atmosphere and light intensity around the plantlets. Acclimatization was achieved by gradual reduction of relative humidity around the plantlets by removing the plastic sheets along with four weeks. After two weeks, the bags were ruptured from two places for two weeks before its completely opened and removed.

Hardening was took place for 48 days from transplanting. Throughout the hardening period, Crystalone (20:20:20); commercial fertilizer of NPK, at the rate of 1 g L^{-1} was used with the irrigation of the pots every two weeks. The plantlets were transferred to plastic greenhouse and were left to grow for 4 weeks, and percentage of survived plantlets, was recorded.

Seventy five hardening plantlets from each treatment of the two genotypes were selected and transferred into plastic pots 25 cm inner diameter containing 9.0 kg fine sand at the rate of five plantlets/pot.

Seedlings of both genotypes were thinned to 2 seedlings after 3 weeks. The substrates were additionally covered with 1 kg of coarse sand to present evaporation. Seedling received optimal nutrient solution according to El-Bellaj *et al.* (2000). Water holding capacity; WHC of the media was determined gravimetrically (Piper, 1950) as the amount of water retained by representative samples of the substrate at pF 1.8 by subsequent drying of the samples at 105°C for 24 h. Water supply was varied in three levels to 75% (control), 50 and 25% of WHC and were implemented two weeks after transplanting. The pots were arranged completely randomized with 5 replications per each treatment. Adjustment of the intended water content was accomplished on a weight basis every two days. The vegetative period under the treatments was performed to 6 months in two growing seasons. At the end of experiment, certain physiological parameters were estimated. The plants were separated into roots and shoots. They were cleaned and the fresh weight was recorded. Samples were oven-dried at 105°C for 24 h to a constant weight and the dry matter content was weighted.

Photosynthetic characteristic: Net photosynthesis rate, stomatal conductance, internal CO_2 partial pressure and transpiration rate were determined at the upper leaf surface well exposed to sunlight of recently fully-expanded leaf (three leaves per treatment). CO_2/O_2 gas exchange and reradiating intensity were measured using the portable promoter CIRAS S/N 110 (Combined Infrared gas Analysis system, p.p system, GB). The reference air stream had a flow rate of 5 cm^3/s at 20°C and 1 bar air pressure. Its CO_2 concentration and water content were set to 350 ppm and 90% of the ambient air at the time of the measurement. The Photosynthetic Active Radiation (PAR) was determined by a sensor of the leaf cuvette. A chlorophyll meter model SPAD-502 was used for chlorophyll measurement and the fast phase of chlorophyll-a fluorescence variation was determined by the use of Plant Efficiency Analyzer (PEA) instrument. As dark adaptation causes reaction centers of PSÐ to rest i.e., not involved in any photosynthetic reactions (Papageorgiou, 1975) a portion of dark-adapted leaf was measured. Dark adaptation was induced by a clip having a sliding opening. Measurements were made from 11.0 till 14.00 O'clock after 30 min of dark adaptation. The plant efficiency analyzer was set on the light level of 4 and the measuring time of two seconds, before starting up. Initial; fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v) and the ratio F_v/F_m were recorded according to Schreiber and Bilger (1987). The used fluorescence parameter was $\Delta F/F'_m$ (effective quantum yield of photosynthesis), which is calculated by the following equation:

$$(F'_m - F_t)/F'_m$$

where, F'_m being the chlorophyll fluorescence signal at its intermediate maximum and F_t being the steady state signal.

^{13}C discrimination was determined for shoot and root system. Carbon isotopes were analyzed using an isotope mass spectrometer Finnigan MAT (Bermen, Germany), located at the Middle Eastern Regional Radio-isotope Center for Arab countries, Dokki, Cairo Egypt as described by Werner *et al.* (1999) and the effects of carbon isotope are calculated as:

$$\delta^{13}C = [R_{sample} - R_{standard})]/R_{standard}] \times 1000$$

where, $R_{sample} - R_{standard}$ being the $^{13}C/^{12}C$ ratio of the sample and the standard Pee Dee Belemnite, respectively. Carbon isotop discrimination was calculated as:

$$\Delta(\%) = (\delta_{air} - \delta_{plants})/(1 + \delta_{plants} Bc \times 1000)$$

where, $\delta^{13}C$ of air CO_2 is –8%

Water-use efficiency was calculated for total dry matter as well as for shoots and root system separately according to Bloch *et al.* (2006) by dividing accumulated dry matter accumulation by cumulative water use.

Statistical analysis: The experimental design was randomized complete blocks design with six replications. All data were subjected to statistical analysis according to Steel and Torrie (1980). Duncan's multiple range test for genotypes, water stress levels and their interactions were employed using SAS (2003).

RESULTS AND DISCUSSION

In vitro parameters

Establishment of the initial explant: Data in Table 1 show that the survived and establishment of the initial explant, during the embryo induction stage, was significantly increased with advancement in culturing period up to 24 weeks and thereafter decreased. It increased from 1.54 at 6 weeks to 2.33 at 24 weeks. At 30 weeks the established degree was decreased to 2.9 level compared with 1.45 at 6 weeks from the incubation period. These results are true overall drought treatments and genotypes examined. At the end of culturing period (30 weeks), the survived explants were subjected to the browning of the tissues and the adjacent media. These results are in agreement with the finding of date palm plant tissues (Helaly *et al*., 2008), which not only deplete the nutrients that are furnished in the medium but also release substances that can accumulate in the culture. These substances such as phenols may have profound physiological effects on the culture tissues. Browning of the tissues and the adjacent medium is assumed to be due to the oxidation of polyphenols and formation of quinines which are toxic to the tissue (El-Meskaoui and Tremblay (2001) and often making growth and developments impossible.

Table 1: Surviving and establishment degree of the initial explants as affected by mannitol levels(M), genotypes G), culturing period (W) and their interactions

	Mannitol treatments (M)			
Genotype (G)	MS alone (control)	MS+0.2 M	MS+0.3 M	Mean
6 weeks(W)				
Shamia	1.75^{a}	1.75^{a}	1.50^{b}	1.67^{A}
Amri	1.50^{b}	1.50^{b}	1.25^{c}	1.42^{B}
Mean	1.63^{A}	1.63^{A}	1.38^{B}	1.54^{E}
12 weeks(W)				
Shamia	2.00^{a}	2.00^{a}	1.75^{b}	1.92^{A}
Amri	1.75^{b}	1.50^{c}	1.25^{d}	1.50^{B}
Mean	1.88^{A}	1.75^{B}	1.50^{C}	1.71^{D}
18 weeks(W)				
Shamia	2.25^{a}	2.00^{b}	1.75^{c}	2.00^{A}
Amri	2.00^{b}	2.00^{b}	1.75^{c}	1.92^{B}
Mean	2.13^{A}	2.00^{B}	1.75^{C}	1.96^{C}
24 weeks(W)				
Shamia	2.75^{a}	2.50^{b}	2.25^{c}	2.50^{A}
Amri	2.25^{c}	2.25^{c}	2.00^{d}	2.17^{B}
Mean	2.50^{A}	2.38^{B}	2.13^{C}	2.33^{A}
30 weeks(W)				
Shamia	2.25	2.00	2.00	2.09^{NS}
Amri	2.25	2.00	2.00	2.09^{NS}
Mean	2.25^{NS}	2.00^{NS}	2.00^{NS}	2.09^{B}
Means				
Shamia	2.20^{a}	2.05^{b}	1.85^{d}	2.03^{A}
Amri	1.95^{c}	1.85^{d}	1.65^{e}	1.82^{B}
Mean	2.08^{A}	1.95^{B}	1.75^{C}	

Means with the same letter are not significantly different at 5% level. For each culturing period, the capital letters in the column are comparing the genotypes. The capital letters in the rows are comparing the effects of mannitol levels. The capital letters of the gross means are comparing the effects of culturing period. The small letters refer to the interaction treatments (M×G, M×W, G×W and M×G ×W). Degree of surviving and establishment of the initial explant were scored according to Pottino (1981) as follows: Negative result (-) = 1, below result (+) = 2, average result (++) = 3 and good result (+++) = 4

Therefore, the establishment period can be extended to about six months by continuously exposing the explant to high auxin levels as shown in the present investigation (Table 1). The appearance of browning at 30 weeks from the incubation period may be due to the perforation of embryogenic calli and consequently an increase in IAA oxidase activity. In this study, Seckin *et al.* (2010) reported that IAA oxidase activity in embryogenic calli was three fold that of non-embryogenic. Similarly, Roy and Banerjee (2003) added that, IAA oxidase activity in embryogenic calli was higher than that of ionic oxidases whereas no such differences were detected in non-embryogenic. Thus, IAA oxidase and endogenous IAA may have a crucial role in date palm somatic embryos.

Supplementation of mannitol to the basal media decreased significantly the values of swelled survived and establishment of the explants especially at the high level compared with the mannitol-free Ms Media (control) and the decreased has been a concentration dependent. The mean surviving values was decreased from 2.08 in the control to 1.75 under the highest level of mannitol (MS+0.3 M/L.). These results are true overall the date palm genotypes examined and regardless culturing period. Similar results were reported by Debnath (2008) who attributed this effect to the hormonal unbalances caused by stress condition. El-Bellaj *et al.* (2000) found that there is a positive correlation between the level of auxins and the level of methylation in DNA.

Regarding the effects of genotype, it was found that Shamia genotype, in general, recorded higher values regarding the survived and establishment of the initial explant compared with Amri in all culturing period. The gross mean values recorded 2.03 and 1.82 for Shamia and Amri genotypes, respectively regardless mannitol levels and culturing period. The interaction between genotypes and drought stress was found to be existed Van der Beek and Houtman (1993). In this context, Roy and Banerjee (2003) recorded that the swelled explants and induction its establishment is dependent on the genotype and the explants used. In addition, the point of arrest is more or less typical of each cell line. Thus, the establishment of the explants in date palm are dependent on the genotype and medium component especially plant growth substances used. This conclusion was supported by Moghaieb *et al.* (2006) who found a correlation between DNA fingerprinting using AFLP technique for five commercial canola cv (s) and the embrygenic callus formation.

Growth of the regenerated plantlets and Plantlet characters: The initial explants produced were developed to different types of morphogenesis after 30 weeks from incubation during embryogenesis induction period. Some established explants formed callus whereas others developed directly to somatic embryos. The different types of morphogenesis and their development were affected by the level of mannitol supplemented to the MS media and depended on the genotypes used.

Table 2 shows that Shamia genotype recorded higher numbers of embryos and secondary embryos/jar, number of formed shoots, plantlet height and root length rather than Amri, overall mannitol treatments. The mean values of these parameters in Shamia genotype recorded about 18.8 embryos/jar, 5.9 secondary embryos/jar, 6.5 shoots/jar, 4.9 plantlet height in cm and 4.8 root length in cm compared with 14.6, 4, 5.6, 3.9 and 4.3 in Amri in a descending order.

As for the effects of mannitol level, overall the genotypes, data in the same table show that increasing mannitol level decreased number of formed embryos, shoots and axillary buds with different types of morphogenesis. The lowest numbers (8.3 and 3 for emberyos and

Table 2: Certain growth characters of Shamia and Amri genotypes of date palm (*Phoenix dactylifera*, L.) regenerated plantlets as affected by water supply (W), genotype (G) and their interactions

	Mannitol treatments (M)			
Genotype (G)	MS alone (control)	MS+0.2 M	MS+0.3 M	Mean
No. of embryos /jar				
Shamia	25.50^{a}	21.25^{b}	9.68^{d}	18.81^{A}
Amri	21.42^{b}	15.48^{c}	6.86^{e}	14.59^{B}
Mean	23.46^{A}	18.37^{B}	8.33^{C}	
No. of secondary embryos/jar				
Shamia	8.62^{a}	5.88^{b}	3.25^{d}	5.92^{A}
Amri	5.22^{b}	3.85^{c}	2.80^{e}	3.96^{B}
Mean	6.92^{A}	4.87^{B}	3.03^{C}	
No. of shoots /jar				
Shamia	8.75^{a}	6.20^{c}	4.48^{e}	6.48^{A}
Amri	7.54^{b}	5.62^{d}	3.67^{f}	5.61^{B}
Mean	8.15^{A}	5.91^{B}	4.08^{C}	
Plantlet height (cm)				
Shamia	5.28^{a}	4.95^{b}	4.50^{c}	4.91^{A}
Amri	4.65^{b}	3.86^{d}	3.10^{f}	3.87^{B}
Mean	4.97^{A}	4.41^{B}	3.80^{C}	
Root length				
Shamia	5.85^{a}	4.68^{c}	3.89^{e}	4.84^{A}
Amri	5.25^{b}	4.10^{c}	3.62^{d}	4.32^{B}
Mean	5.55^{A}	4.39^{B}	3.76^{C}	

ans with the same letter are not significantly different at 5% level. For each trait the capital letters in the columns are comparing the two genotypes overall mannitol levels. The capital letters in the rows are comparing the effects of mannitol levels overall genotypes. The small letters refer to the interaction between G×M

secondary embryos) were shown at the highest level of mannitol (0.3 M) compared with 23.5 and 6.9 at the control. Plantlet growth expressed as plantlet height and root length were also decreased from about 5 and 5.5 cm in the control to 3.8 and 3.7 cm under the highest mannitol level, respectively.

The retarding effects of drought stress, caused by Mannitol supplementation on plantlet growth parameters may be related to its effects on the availability of water and certain nutrients as well as on cell division and elongation. Similar results were reported by Mathur *et al.* (2006) and Karimi *et al.* (2009) under salt and drought stress, respectively. Hatung (2004) reported that drought stress decreased the absorption of minerals nutrients and GA level within the plant tissues whereas increased both plant inhibitors and endogenous ethylene. Seckin *et al.* (2010) supported our results and attributed the reduction in growth under drought stress to the changes in plant hormones within the plant tissues which may exert an important influence on the physiological processes such as CO_2 fixation, assimilation, transport and others including the modification of anatomical and physiological changes. El-Meskaoui and Tremblay (2001) suggested that, the accumulated ethylene under stress condition could cause tissue browning as shown in the present investigation and activate the synthesis of oxidative enzymes or could inhibit the synthesis of protective enzymes.

The role of nutrients on plant growth and metabolism was reviewed previously (Hatung, 2004; Pak *et al.*, 2009). Nitrogen is an essential element for protein formation and its derivatives (amino and nucleic acids, enzymes as well as energy transfers molecules). Similarly, K

is an important element for plant growth. It is involved in very metabolic processes including, carbohydrate metabolism, protein biosynthesis, assimilation, translocation, conformation of enzymes and stomatal movement. Moreover, K has regulating and catalytic role on plant metabolism and involved in numerous function in the plant such as enzyme activities, cation-anion balance, phloem loading, turgor regulation (Krauss, 2001). The important role of K on energy conversion, carbohydrates assimilation and its translocation was also reported. The important of Ca and Mg was also reported (Hatung, 2004). These effects of nutrient reflected on vigorous vegetation growth such as number of shoots and secondary embryos.

As for the effects of genotype, it was found that all plantlet growth parameters in Shamia genotypes were higher than those of Amri (Table 2). Numbers of embryos, secondary embryos, shoots as well as plantlets height and root length registered 18.8, 5.9, 6.5, 4.9 and 4.8 for Shamia genotype compared with 14.6, 4, 5.6, 3.9 and 4.3 values for Amri genotype, respectively. These results indicated that the retardation of growth and development as well as the restriction to the unfavorable condition depending on plant genotype. Similar conclusion was reported by Bloch and Hoffimann (2005) on sugar beet cultivars.

The performance against drought stress gives some sort of resistance against wilting. Such mechanism is well known as osmotic adjustment which can be accomplished by creating more negative osmotic potentials through the accumulation of the organic osmolytes (sugars and others) within the root cell as an adaptable mechanism against either biotic or abiotic stresses (Seckin *et al*., 2010). Sugars as osmolytes enable plants to keep better water relation under stress condition by increasing the ability of their roots to extract more water (Gupta and Kaur, 2005). The relationships between the organic osmolytes and stress tolerance may be correlated with the gene action in the different plant species (Bloch and Hoffimann, 2005).

Physiological characters of the selected seedlings

Photosynthetic parameters: Data presented in Table 3 show that photosynthetic rate, stomatal conductance and transpiration rate of both date palm genotypes, were decreased with increasing water deficit continuously. These results are true in the two growing seasons. Internal CO_2 partial pressure was significantly decreased under 50% WHC and still nearly constant at 25% WHC in the first season. However it was not affected significantly under the condition of the second season. Instantaneous WUE (WUEi) and effective quantum yield of photosynthesis were decreased significantly only under severe drought, at 25% WHC in both growing seasons.

As for the effects of genotype, data in the same table show that Shamia genotype recorded higher values that Amri regarding photosynthetic rate, stomatal conductance, transpiration rate and effective quantum yield overall salinity levels. These results are true in the two growing seasons. The reverse trend was noticed for internal CO_2 partial pressure and WUEi; since Amri genotype showed higher values than Shamia in this respect. The interaction treatments showed different behaviors depending on drought level and genotype examined.

The noticed decrease in the various parameters of photosynthesis due to drought stress especially under the severe level (25% WHC) could be explained by reduction in stomatal conductance, which reduced CO_2 diffusion. The internal CO_2 partial pressure decreased significantly due to stress condition particularly under severe drought. Therefor, reduced stomatal conductance is not supposed to be the major cause of reduced photosynthesis as shown in the present investigation. In this context, it was reported that the effect of severe drought stress on

Table 3: Certain photosynthetic parameters of date palms as affected by water supply, genotypes and their interactions during the two growing season of 2009 and 2010

	Water supply % WHC (W)							
	Season 2009				Season 2010			
Genotype (G)	(control) 75%	50%	25%	Mean	(control) 75%	50%	25%	Mean
Photosynthetic rate (µmol CO_2/m^2/sec)								
Shamia	15.3^{a}	9.5^{d}	5.4^{e}	10.1^{A}	14.5^{a}	9.8^{c}	4.9^{d}	9.7^{A}
Amri	13.1^{b}	10.2^{c}	4.6^{f}	9.3^{B}	12.7^{b}	9.5^{c}	4.8^{d}	9.0^{B}
Mean	14.2^{A}	9.9^{B}	5.0^{C}		13.6^{A}	9.7^{B}	4.9^{C}	
Stomatal conductances mmol/m^2/sec								
Shamia	430^{a}	210^{c}	70^{d}	236.7^{A}	465^{a}	198^{c}	76^{d}	246^{A}
Amri	368^{b}	213^{c}	60^{e}	213.7^{B}	354^{b}	221^{c}	58^{e}	211^{B}
Mean	399^{A}	212^{B}	65^{C}		410^{A}	210^{B}	67^{C}	
Transpiration rate (mmol/m^2/sec)								
Shamia	6.2^{a}	3.1^{c}	2.4^{c}	3.9^{A}	6.4^{a}	3.3^{c}	2.1^{d}	3.9^{A}
Amri	5.4^{b}	2.7^{c}	1.3^{d}	3.1^{B}	5.7^{b}	3.1^{c}	1.5^{e}	3.4^{B}
Mean	5.8^{A}	2.9^{B}	1.9^{C}		6.4^{A}	3.2^{B}	1.4^{C}	
Int. CO_2 partial pressure (µmol mol^{-1})								
Shamia	230^{a}	210^{b}	200^{c}	231.3^{B}	224^{a}	202^{c}	198^{c}	208^{B}
Amri	210^{b}	213^{b}	225^{c}	216^{A}	200^{c}	226^{a}	214^{b}	213^{A}
Mean	220^{A}	212^{B}	213^{B}		212^{A}	214^{A}	211^{A}	
Effective quantum yield (Δ F/F'$_m$)								
Shamia	0.83^{a}	0.83^{a}	0.50^{c}	0.72^{A}	0.76^{a}	0.73^{b}	0.52^{d}	0.67^{A}
Amri	0.77^{b}	0.75^{b}	0.51^{c}	0.68^{B}	0.72^{b}	0.68^{c}	0.47^{d}	0.63^{B}
Mean	0.80^{A}	0.79^{A}	0.50^{B}		0.74^{A}	0.71^{A}	0.50^{B}	
WUEi (µmol CO_2 /mmol H_2O)								
Shamia	2.5^{b}	2.5^{b}	2.0^{d}	2.3^{B}	2.3^{c}	2.4^{b}	2.0^{d}	2.2^{B}
Amri	2.7^{b}	3.1^{a}	2.3^{c}	2.7^{A}	2.5^{b}	2.9^{a}	2.1^{d}	2.5^{A}
Mean	2.6^{A}	2.8^{A}	2.2^{B}		2.4^{A}	2.7^{A}	2.1^{B}	

Means with the same letter are not significantly different at 5% level. For each trait the capital letters in the column are comparing the two genotypes regardless WHC levels. The capital letters in the rows are comparing the effects of WHC levels overall genotypes. The small letters refer to the interaction effects (Water supply; WH ×Genotype)

photosynthesis may be attributed to non-stomatal effects (Bruce *et al*., 2002). Generally, the severity of the stress appears to be important in determining whether non-stomatal factors affect photosynthesis, as inhibition of mesophyll activity (non-stomatal inhibition of photosynthesis) in addition to stomatal closure occurs only during severe or prolonged stress (Bloch *et al*., 2006). In accordance with that, effective quantum yield of photosynthesis was reduced only under severe drought (Table 3). Damage of the photosynthetic pathway under water deficit was previously reported (Mohammadian *et al*., 2003). In contrast, Clover *et al*. (1999) did not observe any effect of drought on chlorophyll fluorescence in sugar beet leaves and attributed findings of damage of the photosynthetic pathway to a premature senescence of drought stressed leaves. The contradicting effects on the photosynthetic parameters may be due to the gene action and the level of drought stress as indicated by the interaction treatments noticed in the present investigation.

Water use efficiency (WUE): Data in Table 4 show that water use efficiency calculated as integrated values of the entire experimented period of the acclimatization was increased with an increase in drought level based on total plant D.Wt especially under sever drought stress level.

Table 4: Water use efficiency (WUE) of date palm as affected by genotypes (G), water supply (W) and their interactions during the two growing seasons of 2009 and 2010

	Water supply % WHC (W)							
	Season 2009				Season 2010			
Genotype (G)	75% (control)	50%	25%	Mean	75% (control)	50%	25%	Mean
Root WUE (g root D.Wt/kg H_2O)								
Shamia	2.4^{e}	3.3^{b}	4.0^{a}	3.23^{A}	2.6^{e}	4.1^{c}	5.2^{a}	3.97^{A}
Amri	2.0^{d}	3.2^{b}	4.0^{a}	3.07^{B}	2.4^{f}	3.8^{d}	4.5^{b}	3.57^{B}
Mean	2.2^{C}	3.3^{B}	4.0^{A}		2.5^{C}	3.9^{B}	4.8^{A}	
Shoot WUE (g shoot D.Wt/kg H_2O)								
Shamia	4.1^{b}	5.3^{a}	4.0^{b}	4.47^{A}	4.6^{c}	5.4^{a}	5.6^{a}	5.20^{A}
Amri	3.5^{c}	4.2^{a}	3.0^{d}	3.57^{B}	3.8^{d}	4.9^{b}	4.3^{c}	4.33^{B}
Mean	$3.8\|^{B}$	4.7^{A}	3.5^{B}		4.2^{B}	5.2^{A}	5.0^{A}	
Root and Shoot WUE (g total plant D.Wt/kg H_2O)								
Shamia	6.5^{e}	8.6^{a}	8.0^{b}	7.00^{A}	7.2^{c}	9.5^{b}	10.8^{a}	9.17^{A}
Amri	5.5^{f}	7.4^{c}	7.0^{d}	6.63^{B}	6.2^{d}	8.7^{b}	8.7^{b}	7.90^{B}
Mean	6.0^{B}	8.0^{A}	7.5^{A}		6.7^{C}	9.1^{A}	9.7^{A}	

Means with the same letter are not significantly different at 5% level. For each trait, the capital letters in the column are comparing the two genotypes overall WHC levels. The capital letters in the rows are comparing the WHC levels regardless genotypes. The small letters refer to one interaction effects (WHC×Genotype)

These results are true during the two growing seasons for root and/or shoot system. However, shoot-based WUE was higher for stressed plants as well; root system-based WUE was not distinctly affected by water supply. Roots recorded highest values (4 and 4.8 in the first and second season respectively) at 25% WUE compared with the control which showed minimum values(2.2 and 2.5) in this respect. The maximum values in the shoots was detected at 50% WHC (4.7 in the first season and 5.2 in the second). Therefore, root and shoot system at the control (75% WHC) registered minimum WUE values (6 and 6.7 in both seasons, respectively) compared with root and shoot stressed systems. In tendency Shamia genotype obtained higher significant values of WUE than Amri genotype in all treatments. In Shamia genotype it recorded about 4, 5.2 and 9.2 for root, shoot and root+shoot WUE compared with 3.6, 4.3 and 7.9 in Amri genotype in a descending order.

The data also indicated clearly that dry matter accumulation and water use efficiency, in general, are closely related. This relation is to a large extent independent on the level of water supply and water use. Similar results were reported by Ehlers and Goss (2003). However, Bloch *et al*. (2006) found that the relationship between water use and yield of sugar beet was modified by water supply. The higher efficiency of water use under stress is due to the fact that drought-stressed plant wilt far more than unstressed plants and wilting invariably occurs in times when the saturation deficit of the atmosphere is large. Therefore, the plant assimilates only in times when the saturation deficit is small and hence loses less water for every carbon molecule fixed Clover *et al*. (2001).

Shamia genotype obtained higher dry weight in all treatments under the present investigation and hence used the water more efficiency than Amri genotype. However, the lack of interaction between genotype and water supply for WUE suggests as reported by Bloch and Hoffimann (2005) that genotypes which are most productive under well-watered conditions will be superior in dry environments.

Data in the same table show also that, WUE; values obtained were not clearly affected by water supply. In this context, Ashraf *et al*. (2002) reported that often the relationship between

short-term gas exchange efficiency and the actual water-use efficiency for the whole growing seasons is poor, because there are a number of factors affecting dry matter accumulation but not gas exchange. Bloch *et al.* (2006) stated that, the biomass production of a plant is not only determined by photosynthesis but also by respiratory losses at night. It is altered by the molecular composition of the dry matter.

^{13}C discrimination: Carbon isotope discrimination was higher (more negative values) in shoot than in roots and decreased with decreasing water supply (Table 5). Genotypic differences for carbon discrimination were not significant. Differences in carbon discrimination between plant organs have been supported for other plant species by Bloch *et al.* (2006) but reason for these findings have not been clearly established. In this context, Zhao *et al.* (2004) found a contradiction results in two cultivars of upland rice during different developmental stages under three water regimes. However, they reported that products of secondary metabolism, usually have lower carbon discrimination values than primary photosynthetic product. Bloch *et al.* (2006) added that, the formation of carbon skeletons for some amino acids involves phosphoenolpyruvate carboxylation, which discriminates in favor of ^{13}C.

The reduction values of carbon isotope discrimination which detected in the stressed date palm plantlets compared with the control (Table 5) have been reported for many other plant species (Tsialtas and Karadimos, 2003). Bloch *et al.* (2006) found a relationship between carbon discrimination and water availability for both leaves and tap roots in sugar beet.

Chlorophyll fluorescence: The trend of chlorophyll fluorescence variation, as measured by Fo, Fm, Fv and the ratio Fv/Fm as well as the mean chlorophyll concentration (SPAD) as affected by water supply in both date palm genotypes during the two growing seasons of 2009 and 2010 are presented in Table 6.

In both genotypes, Fo was reduced insignificantly, due to an increase in water deficit regardless the genotypes examined. These results are true during the two growing season. The least insignificant values of Fo (about 0.5 in the two growing seasons) were recorded under the severe stress condition (25% WHC) compared with maximum values under the control. The values

Table 5: ^{13}C discrimination in shoots and root system, of date palm as affected by Genotype (G), Water supply (W) and their interactions during the two growing seasons of 2009 and 2010

	Water supply % WHC (W)							
	Season 2009				Season 2010			
Genotype (G)	75% (control)	50%	25%	Mean	75% (control)	50%	25%	Mean
Roots								
Shamia	-27.1	-26.3	-25.6	-26.3^{NS}	-28.6	-27.5	-26.9	-27.7^{NS}
Amri	-28.2	-26.1	-24.8	-26.4^{NS}	-29.7	-26.4	-25.5	-27.2^{NS}
Mean	-27.7^{A}	-26.2^{B}	-25.2^{C}		-29.1^{A}	-27.0^{B}	-26.2^{C}	
Shoots								
Shamia	-29.1	-26.8	-26.5	-27.5^{NS}	-30.2	-27.1	-26.9	-28.1^{NS}
Amri	-29.7	-28.5	-26.0	-28.1^{NS}	-31.1	-28.5	-26.4	-28.7^{NS}
Mean	-29.4^{A}	-27.7^{B}	-26.3^{C}		-30.7^{A}	-27.8^{B}	-26.7^{C}	

Means with the same letter are not significantly different at 5% level. For each plant organ, the capital letters in the columns are comparing the two genotypes overall WHC levels. The capital letter in the rows are compared the WHC levels regardless genotypes. The small letters refer to the interaction effects (WHC x Genotype), NS: Not significant

Table 6: Chlorophyll fluorescence variations and chlorophyll concentration (SPAD) of date palm as affected by water supply (W), genotypes (G) and their interactions during the two growing seasons of 2009 and 2010

	Water supply % WHC (W)							
	Season 2009				Season 2010			
Genotype (G)	75% (control)	50%	25%	Mean	75% (control)	50%	25%	Mean
Fo								
Shamia	0.56^{NS}	0.53^{NS}	0.49^{NS}	0.52^{NS}	0.58^{NS}	0.57^{NS}	0.53^{NS}	0.56^{NS}
Amri	0.50^{NS}	0.49^{NS}	0.49^{NS}	0.50^{NS}	0.59^{NS}	0.55^{NS}	0.54^{NS}	0.56^{NS}
Mean	0.53^{NS}	0.51^{NS}	0.49^{NS}		0.59^{NS}	0.56^{NS}	0.54^{NS}	
Fm								
Shamia	2.0^{a}	1.7^{b}	1.6^{b}	1.8^{NS}	2.5^{a}	2.20^{b}	1.6^{c}	2.1^{NS}
Amri	1.9^{a}	1.7^{b}	1.52^{c}	1.7^{NS}	2.3^{c}	2.00^{c}	1.4^{d}	1.9^{NS}
Mean	1.95^{A}	1.7^{B}	1.55^{C}		2.4^{A}	2.00^{B}	1.5^{C}	
Fv								
Shamia	1.2^{a}	0.9^{b}	0.8^{b}	0.97^{A}	1.6^{NS}	1.4^{b}	0.9^{d}	1.3^{A}
Amri	1.1^{a}	0.9^{b}	0.6^{c}	0.87^{B}	1.4^{b}	1.2^{c}	0.60^{e}	1.00^{B}
Mean	1.15^{A}	0.9^{B}	0.7^{C}		1.5^{A}	1.1^{B}	0.65^{C}	
Fv/Fm								
Shamia	0.60^{a}	0.50^{b}	0.50^{b}	0.50^{NS}	0.64^{a}	0.64^{a}	0.56^{b}	0.44^{NS}
Amri	0.58^{a}	0.53^{b}	0.39^{c}	0.50^{NS}	0.61^{a}	0.60^{a}	0.43^{c}	0.47^{NS}
Mean	0.59^{A}	0.51^{B}	0.44^{C}		0.63^{A}	0.62^{B}	0.33^{C}	
Chlorophyll (SPAD)								
Shamia	48.22^{c}	51.83^{b}	54.74^{a}	51.60^{A}	42.41^{c}	46.57^{b}	49.16^{a}	46.05^{A}
Amri	43.42^{e}	46.29^{d}	48.62^{c}	46.11^{B}	40.11^{d}	42.12^{c}	43.28^{c}	41.84^{B}
Mean	45.82^{C}	49.06^{B}	51.68^{A}		41.26^{C}	44.35^{B}	46.22^{A}	

Means with the same letter are not significantly different at 5% level. For each trail, the capital letters in the columns are comparing the two genotypes overall WHC levels. The capital letters in the rows are comparing the effects of WHC levels regardless the genotypes. The small letters refer to the interactions effect (W×G), NS: Not significant

of Fm and Fv showed, in general, a significant reduction trend in both genotypes under stressed condition. These significant values were decreased from 1.95 and 1.15 under the control to 1.55 and 0.7 at 25%WHC in the first season. In the second season, Fm and Fv were decreased significantly from 2.4 and 1.5 under the control to 1.5 and 0.65 at 25% WHC. Therefore, quantum yield of photochemistry (Fv/Fm) were decreased significantly under the stress condition. Similar results were reported by Lopez *et al.* (2008). It registered 0.44 and 0.33 in the first and second season respectively under the severe condition (25% WHC) compared with 0.59 and 0.63 under the control. In this study Selmani and Wassom (1991) reported that chlorophyll fluorescence measurement at the laboratory or during night could not show the permanent damage to the photosynthetic apparatus caused by drought, because under these conditions, recovery night happens if the damage of chlorophyll is not severs enough. On the other hand, Cerovic *et al.* (1996) on maize, sugar beet and Kalanchoe (*Bryophyllum sp.*) found linear relationships between life time and yield for chlorophyll fluorescence *in vivo* studies under both optimal and non-optimal water condition.

The insignificant difference noticed with Fo in the present investigation between the control and stress condition was expected. Since Fo is resulted from the fluorescence of the antenna chlorophyll associated with PSI and PSII (Wilson and Greaves, 1993). Thus it could be suggested

that, drought stress in date palm does not have any damaging effects on Fo. This can be the result of increasing chlorophyll concentration under the stress condition compared to the non-stress conduction.

Chlorophyll assessment in both date palm genotypes under control condition was lower than the stressed condition (Table 6). It increased from 45.8 and 41.3 SPAD under the control in the first and the second seasons, respectively to 51.68 and 46.22 at highest stress level (25% WHC). Moreover, both genotypes were differing significantly in this respect due to gene action (Gupta and Kaur, 2005) and/or differential strategies between them to tolerate stress (Joseph *et al*., 2010; Sharma *et al*., 2011). The increase in chlorophyll concentration recorded in the present investigation under stress condition was similar to that found by several investigators. Mohammadian *et al*. (2003) found that water stress increased total chlorophyll and carotenoids concentrations in sugar beat leaves. Since there is a relationship between the amount of leaf chlorophyll and the level of leaf N (Hatung, 2004). Ehlers and Goss (2003) found a negative relationship between plant nitrogen concentration and water consumption. The high concentration of N under the drought stress condition may be effective in expending the recovery from drought stress conduction (Mohammadian *et al*., 2003).

The genotype x water supply interaction (GxW) was not significant for Fo. Moreover the two genotypes examined were almost equally affected by the stress treatments. It was reported that temperature damaging effect on photasystem II can be determined by a drastic increase in Fo while photoinhibtion lead to a slight increase in Fo and freezing damage does not affect Fo (Anonymous, 1993).

Drought stress also decreased Fm in both date palm genotypes examined under the present investigation (Table 6). However, the GxW interaction was significant for Fm and the largest decrease of Fm was observed in the Amri genotype compared with Shamia genotype. Similar results were reported under high temperature condition (Anonymous, 1993) and under water stress (Seckin *et al*., 2010) on other plant species.

The mean Fv values of both date palm genotypes in water stressed plants were significantly lower than those in the control (Table 6). The GxW interaction treatments were also significant for Fv. Moreover, the decrease in Fv was different among the two genotypes examined as stress increased. Shamia genotype showed high significant value compared with Amri .Generally, Fv is sensitive to changes in the ultrastructure of the membrane and rates of electron transfer (Mohammadian *et al*., 2003). Usually, environmental stress decreased the Fv values, as the photo oxidizing side of PS II is inhibited (Wilson and Greaves, 1993; Lopez *et al*., 2008).

The largest decrease in Fv observed under stress condition especially with Amri genotype indicates that electron flow is blocked beyond acceptor Q and the electron flow is blocked on the water side of photosystem II as reported by Ranalli *et al*. (1997). Thus, it may be stated that in genotype Amri, water stress blocked electron flow on the water side of photosystem II more than genotype shamia. Ranalli *et al*. (1997) examined chlorophyll fluorescence of potato genotypes under drought stress and non-stressed conditions and stated that drought stress may block electron flow either on the water side of photosystem II or beyond acceptor Q.

The significant decrease of Fv /Fm ratio noticed under drought stress (Table 6) indicates the capacity of photosystem II electron transfer (Bjorkman and Demmig, 1987) which was a high relationship with quantum yield of photochemistry (Bolhar-Nordenkampf and Oquist, 1993).

In spite of the insignificant differences between the two genotypes of date palm for Fv/Fm, the GxW interaction showed significantly decreases on the ratio of Fv/Fm and the least values were

recorded with Amri genotype more than Shamia. Therefore, it seems that in Amri genotype the photosynthetic apparatus was affected more than genotype shamia by drought stress. Moreover the data in the present investigation indicated also the electron flow was restricted on the water-side of photosystem II in spite of the increase in chlorophyll concentration in date palm leaves and the both genotypes differed in this respect.

CONCLUSION

Data in the present investigation concluded that emberyogeneses obtaind in the two genotypes of date palm followed normal development and were competent to regeneration and converted into plantlets under drought stress media. They have an ability to tolerate water stress with a wide range of drought tolerance. The method adopted was successful in spite of decreased growth traits studied of both genotypes. However, the genotypes have different emberyogenic potential and growth vigor regardless drought regime levels. The values recorded with Shamia were found to be more than that of Amri genotype. The physiological characters showed distinct response to drought stress can be of importance for genotypic differences in the performance under stress. Drought stress decreased rates of photosynthesis and transpiration, stomatal conductance in the shoots as well as carbon isotope discrimination in both roots and shoot systems. The effective quantum yield of photosynthesis and WUEi were also reduced but only under severity level (25% WHC). In contrast, internal CO_2 partial pressure remained relatively stable and WUE in the roots and/or shoots was increased under drought stress. Moreover, no destructive effects of drought stress on the initial fluorescence, presumably due to increase in chlorophyll concentration of stressed date palm. Under stress condition, F_0 was not affected significantly whereas; Fv, Fm and Fv/Fm were restricted in spite of increases chlorophyll concentration in both date palm genotypes shoots. However, the two genotypes were varied in their reduction rate and showed a distinct response to drought stress. Amri genotype showed higher reduction than Shamia for all these parameters under drought condition, which suggests the highest sensitive to drought stress. The obtained results in the present investigation are repeatable. Therefore, it could be considered that measurements of chlorophyll fluorescence could be used by plant breeders to quantify rapidly and detect the response of different date palm genotypes to drought stress and may have relevance to screening for drought resistant. The other useful physiological characters studied including WUE can also used as a strategy to improve plant performance under water-limited condition.

ACKNOWLEDGMENT

We would like to thank Prof. Fahima, Helali National Res Center dokki Egypt for her technical help in carrying out carbon isotope determination.

REFERENCES

Abd-El-Hamid, A.M., M.H.Abo-Bakr, I.A. Ibrahim and M.A. Abd-El-Baky, 2001. Some aspects of *in vitro* micro propagation of date palm (*Phoenix dactylifera* L.). J. Agric. Sci. Mansoura Univ., 26: 5449-5466.

Abdallah, K.M., F. Hussein and A.S. Khalifa, 1993. Evaluation of productive and fruit characteristics of some Egyption date palm cultivers grown at Aswan. Proceedings of 3rd Symbosiom on Date Palm, Jan. 17-20, King Faisal University, Saudi Arabia, pp: 573-584.

Anonymous, 1993. An Introduction to Fluorescence Measurements with the Plant Efficiency Analyzer (PEA). Hansatech Instruments Ltd., England.

Ashraf, M., M. Arfan, M. Shahbaz, A. Ahmed and A. Jamil, 2002. Gas exchange characteristics and water relations in some elite okra cultivars under water deficit. Photosynthetica, 40: 615-620.

Bartels, D. and R. Sunkar, 2005. Drought and salt tolerance in plants. Crit. Rev. Plant. Sci., 24: 23-58.

Bjorkman, O. and B. Demmig, 1987. Photon yield of O_2 evolution and chlorophyll fluorescence at 77k among vascular plants of diverse origins. Planta, 170: 489-504.

Bloch, D. and C. Hoffimann, 2005. Seasonal development of genotypic differences in sugar beet (*Beta vulgaris* L.) and their interaction with water supply. J. Agron. Crop Sci., 191: 263-272.

Bloch, D., C.M. Hoffmann and B. Marlander, 2006. Impact of water supply on photosynthesis, water use and carbon isotope discrimination of sugar beet genotypes. Eur. J. Agron., 24: 218-225.

Bolhar-Nordenkampf, H.R. and G. Oquist, 1993. Chlorophyll Fluorescence as a Tool in Photosynthesis Research. In: Photosynthesis and Production in a Changing Environment: A field and Laboratory Manual, Hall, D.O., J.M.O. Scurlock, H.R. Balhar-Nordenkampf, R.C. Leegood and S.P. Long (Eds.). Chapman and Hall, London, pp: 193-206.

Bruce, W.B., G.O. Edmeades and T.C. Barker, 2002. Molecular and physiological approaches to maize improvement for drought tolerance. J. Exp. Bot., 53: 13-25.

Cerovic, Z.G., Y. Goulas, M. Gorbunov, J.M. Briantais, L. Camenen and I. Moya, 1996. Fluorosensing of water stress in plants: Diurnal changes of the mean lifetime and yield of chlorophyll fluorescence, measured simultaneously and at distance with at tau-LIDAR and a modified PAM-fluorimeter, in maize, sugar beet and kalanchoe. Remote Sens. Environ., 58: 311-321.

Clover, G.R.G., H.G. Smith, S.N. Azam-Ali and K.W. Jaggard, 1999. The effects of drought on sugar beet growth in isolation and in combination with beet yellows virus infection. J. Agric. Sci. Cambridge, 133: 251-261.

Clover, G.R.G., K.W. Jeggard, H.G. Smith and S.N. Azam-Ali, 2001. The use of radiation interception and transpiration to predict the yield of healthy, droughted and virus-infected sugar beet. J. Agric. Sci. Camb., 136: 169-178.

Costa Maria, D.S.M.N. and P.M. Aloufa, 2006. Direct organogenesis in *Phoenix dactylefera* through cotiledonar petiole. Trop. Agric. Res., 36: 195-198.

Debnath, M., 2008. Responses of *Bacopa monnieri* to salinity and drought stress *in vitro*. J. Med. Plants Res., 2: 347-351.

Dowson, V.H.W., 1989. Date production and protection with special reference to North Africa and the near East. FAO-UN, Plant Production and Protection Paper 35, FAO Rme, pp: 294.

Ehlers, W. and M. Goss, 2003. Water Dynamics in Plant Production. CABI Publishing, Cambridge, USA.

El-Bellaj, M., S. El-Jaafari and I. El-Hadrami, 2000. IAA- oxidase: Regulator and potential marker of somatic embryogenesis in date palm (*Phoenix dactylifera* L). through direct organogenesis. Indian J. Plant Physiol., 4: 323-326.

El-Kosary, S., 2009. Optimizing date palm aerial offshoots avail through vegetative propagation. J. Agric. Sci. Mansoura Univ. Egypt, 34: 3469-3481.

El-Kosary, S., E.I. Bakr, I.A. Hussein and A.S. Abdelhameed, 2009. Effect of soil moisture depletion and mulching of cv. Zaghloul date palm (*Phoenix dactylifera* L.) fruiting under desert conditions. J. Agric. Sci. Mansoura Univ., 34: 3437-3452.

El-Meskaoui, A. and F.M. Tremblay, 2001. Involvement of ethylene in the maturation of black spruce embryogenic cell lines with different maturation capacities. J. Exp. Bot., 52: 761-769.

El-Semea, M., 2003. Propagation of dry varieties of date palm. Ph.D. Thesis, Faculty of Agric. Cairo Univ. Egypt.

El-Shihy, O.M. and A.S Monem, 1994. Production of salt tolerant line of *Vicia faba* induced by protoplast irradiation with fast neutrons and gamma rays. Egypt. J. Applied Sci., 9: 393-418.

FAO., 2004. Food and Agriculture Organization Year Book. Vol. 40. Food and Agriculture Organization of The United Nations, Rome.

Gupta, A.K. and N. Kaur, 2005. Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. J. Biosci., 30: 761-776.

Hatung, W., 2004. Plant response to stress: Abscisic acid fluxes. Marcel Dekker Inc., New York, pp: 540-640.

Helaly, M.N., M.M. Darwish, Z.A. Mohamed, A.A. Hegazy and H.A.M.R. El-Hosieny, 2008. Botanical studies on the micropropagation of two dried from date palm (*Phoenix dactylifera*, L.) using tissue culture technique: ²- Effects of antioxidants; AOS on browning control and related compounds. J. Agric. Sci Mansoura Univ., 33: 7919-7929.

Helaly, M.N., A. Hegazy and A.H. El-Hoseiny, 2009. Botanical studies on the micropropagation of two dried genotypes from date palm (*Phoenix dactylifera*, L.) using tissue culture technique: II Carbohydrate contents and acclimization. J. Agric. Sci. Mansoura Univ., 34: 2041-2056.

Joseph, B., D. Jini and S. Sujatha, 2010. Insight into the role of exogenous salicylic acid on plants grown under salt environment. Asian J. Crop Sci., 2: 226-235.

Karimi, A., A. Abdolzadeh and H.R. Sadeghipour, 2009. Increasing salt tolerance in olive, *Olea europaea*,L. Plants by supplemental potassium nutrition involves changes in ion accumulation and anatomical attributes. Int. J. Plant Prod., 3: 49-60.

Khataab, H.A., 1985. Date palm in old Egypt. Agric. J., 40: 33-35.

Krauss, A., 2001. Potassium and abiotic stress. Physiol. Plantaram, 133: 682-691.

Lopez, M., N.A. Tejera and C. Lluch, 2008. Differential strategies of the model legumes *Lotus japonicus* and *Medicago truncatula* in the adaptation to salt stress: Photosynthetic and nutritional responses. Am. J. Plant Physiol., 3: 121-130.

Mathur, N., J. Singh, S. Bohra, A. Bohra and A. Vyas, 2006. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. Am. J. Plant Physiol., 1: 210-213.

Moghaieb, R.A.A., M.A. El-Awady, R.G. El-Mergawy, S.S. Youssef and A.M. El Sharkawy, 2006. A reproducible protocol for regeneration and transformation in canola (*Brassica napus* L.). Afr. J. Biotechnol., 5: 143-148.

Mohammadian, R., H. Rahimian, M. Moghaddam and S.Y. Sadeghian, 2003. The effect of early season drought on chlorophyll a luorescence in sugar beet (*Beta vulgaris* L.). Pak. J. Biol. Sci., 6: 1763-1769.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Pak, V.A., M. Nabipour and M. Meskarbashee, 2009. Effect of salt stress on chlorophyll content, fluorescence, Na^+ and K^+ ions content in rape plants (*Brassica napus* L.). Asian J. Agric. Res., 3: 28-37.

Papageorgiou, G., 1975. Chlorophyll Fluorescence: An Intrinsic Probe of Photosynthesis. In: Bioenergetics of Photosyntheis, Govindie, P. (Ed.). Academic Press, New York, pp: 319-371.

Piper, C.S., 1950. Soil and Plant Analysis. Interscience Publisher, New York.

Pottino, B.G., 1981. Methods in Plant Tissue Culture. Dep. of Hort. Agric. College Agric. College Mereland Univ., Coll. Perk. Mereland, USA., pp: 8-29.

Ranalli, P., M. Di Candilo and M. Bagatta, 1997. Drought tolerance screening for potato improvement. Plant Breed., 116: 290-292.

Roy, J. and N. Banerjee, 2003. Induction of callus and plant regeneration from shoot tip explants of *Dentrobium fimbriatum lindl.* Var. Oculantum HK.F. Sci. Hortic., 97: 333-340.

SAS, 2003. SAS/STARTR User's Guide: Statistics. Ver. 9.1, SAS Institute Inc., Cary, NC, USA.

Schreiber, U. and W. Bilger, 1987. Rabid Assessment of Stress Effects on Plant Leaves by Cholorophyll Fluorescence Measurments. In: Plant Response to Stress NATO ASI Series, Tenhunen, J.D., O.L. Lange and C. Oechel (Eds.). Vol. 15., Springer, Berlin/Heidelberg, pp: 27-53.

Seckin, B., I. Turkan, A.H. Sekmen and C. Ozfidan, 2010. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barleygrass) and *Hordeum vulgare* L. (cultivated barley). Environ. Exp. Bot., 69: 76-85.

Selmani, A. and C.E. Wassom, 1991. Leaf dark-adapter for measuring chlorophyll fluorescence *in situ* during daytime. Agron. J., 83: 652-654.

Sharkey, T.D. and J.R. Seemann, 1989. Mild water stress effects on carbonreduction-cycle intermediates, ribulose biphosphate cabohxylase activity and spatial homogeneity of photosynthesis in intact leaves. Plant Physiol., 89: 1060-1065.

Sharma, N., K.P. Sharma, R.K. Gaur and V.K. Gupta, 2011. Role of chitinase in plant defense. Asian J. Biochem., 6: 29-37.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill, New York, USA., ISBN-13: 978-0070609259.

Tsialtas, J.T. and D.A. Karadimos, 2003. Leaf carbon isotope discrimination and its relation with qualitative root traits and harvest index in sugar beet (*Beta vulgaris* L.). J. Agron. Crop Sci., 189: 286-290.

Van der Beek, M.A. and H.J. Houtman, 1993. Does interaction between varieties and drought stress exis. Proceedings of the 56th IIRB Congress, Feb. 10-11, Brussels, pp: 151-169.

Werner, R.A., B.A. Bruch and W.A. Brand, 1999. Conflo III-An interface for high precision d C13 and d N15 analysis with an extended dynamic range. Rapid Commun. Mass Spectrom., 13: 1237-1241.

Wilson, L.M. and J.A. Greaves, 1993. Development of Fluorescence-Based Screening Programs for Temperature and Water Stress in Crop Plants. In: Adaptation of Food Crops to Temperature and Water Stress, Kuo, C.G. (Ed.). AVRDC, Shanhua, Taitwan, pp: 389-398.

Zhao, B., M. Kondo, M. Maeda, Y. Ozaki and J. Zhang, 2004. Water-use efficiency and carbon isotope discrimination in two cultivars of upland rice during different developmental stages under three water regimes. Plant Soil, 261: 61-75.