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Effect of Drought on Water Relations, Growth and Solute Accumulation in Two Sesame Cultivars

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Abstract: The effects of drought on water potential, osmotic potential, relative water content of leaves and growth, content of proline, sugars and ion in leaves and roots of *Sesamum indicum* L. cvs Darab 14 and Yekta were determined. Four weeks after sowing, plants were grown under soil moisture corresponding to 100, 75, 50 and 25% field capacity for next four weeks. Water potential, osmotic potential, relative water content of leaves in both cultivars decreased, fresh and dry masses, proline content, reduced, soluble and total sugars and oligosaccharides increased and polysaccharides decreased with increasing of stress. Na⁺ content in leaves and roots of both cultivars increased but K⁺, Ca²⁺ and K⁺ to Na⁺ ratio decreased. The mechanisms of drought tolerance in the sesame may be obtained by production of proline, changes in sugars and ion accumulation for maintenance of suitable pressure potential.

Key words: Drought, water relation, solute accumulation, *Sesamum indicum*

INTRODUCTION

Plants are exposed to various types of environmental stress. Among these stresses, osmotic stress, in particular that due to drought and salinity is the most serious problem that limits plant growth and crop productivity in agriculture (Chen *et al.*, 2000).

Under water stress, growth is inhibited and a variety of molecular, biochemical and physiological modifications are produced (Sgherri and Navari-Izzo, 1995; Büssis and Heineke, 1998; Pattanagul and Madore, 1999; Bajji *et al.*, 2001; Hsu *et al.*, 2003; Ratnayaka *et al.*, 2003).

The production of low-molecular-weight metabolites seems to be a virtually universal response to stress. The most common hypothesis to explain the role of these molecules in stress tolerance is that they serve as osmolytes, helping cells to osmotically adjust when faced with low water potentials (Gilbert *et al.*, 1997). The solutes that accumulate during osmotic adjustment include ions such as K⁺, Na⁺ or organic solutes such as proline and other amino acids, polyamines and glycinebetaine (Good and Zaplachinski, 1994; Tamura *et al.*, 2003; Martinez *et al.*, 2004; Karimi *et al.*, 2005).

Sesame is considered a drought-resistant crop (Weiss, 2000) and its cultivation is extended beyond the tropic and subtropic zones to temperate and subtemperate zones of the world (Ali *et al.*, 2000).

The objective of the present investigation was to study the effect of drought stress on some aspects of physiological and biochemical changes in leaves of two sesame cultivars.

MATERIALS AND METHODS

Plant growth: Seeds of two sesame (*Sesamum indicum* L.) cultivars (Darab 14 and Yekta) were sown in plastic pots filled with 3 kg of sandy-loam soil maintained to field capacity (FC). Pots of each cultivar were divided into four groups (four replicates each) and then were watered for four week to 100, 75, 50 and 25% FC. The water supplies were maintained by weighing the pots.

Plant maintained in controlled condition: Sixteen hours photoperiod, irradiance of 250 $\mu\text{mol m}^{-2}\text{s}^{-1}$, 32/22°C day/night temperature and 40-50% air humidity. Third leaves of each plant were sampled and assayed in the experiments.

Water relations measurement: Leaf water potential (Ψ_w) was evaluated by using the pressure chamber (Eijkelkamp, Giesbeek, Netherland) on four randomly chosen leaves for each treatments (Richardson and Mckell, 1980).

Osmotic potential (Ψ_s) was measured on expressed sap of frozen/thawed leaves using a microosmometer

(model 5520, Wescor, Inc., Logan, UT, USA). The estimated osmotic contribution of mineral and organic solutes to leaf Ψ_s was obtained by the Van't Hoff equation, using total tissue water and ionic and sugar content (Morant-Manceau *et al.*, 2004).

Relative Water Content (RWC) of the leaves was determined as $(FW - DW) / (TW - DW) \times 100$, where FW is the fresh mass, DW is the dry weight at 80°C for 48 h and TW is the turgid weight of the leaves after equilibrium in distilled water for 20-24 h at 4°C in the dark (Bajji *et al.*, 2001).

Leaf area measurement: The leaf area was determined with a Δ -T Image Analysis System (Δ -T, LTD, Cambridge, UK).

Growth analysis: Fresh and dry mass of leaves and roots were directly recorded. Dry mass was determined after drying the samples at 60°C for 48 h to constant mass.

Solute accumulation quantification: Free proline was determined by the method of Bates *et al.* (1973). Plant material (0.5 g) was homogenized for 5 min in 5 cm³ of 3% aqueous sulfosalicylic acid. Two cm³ of the extract reacted with 2 cm³ of glacial acetic acid and 2 cm³ of acid-ninhydrin reagent for 1 h at 100°C. The reaction mixture was extracted with 4 cm³ toluene and the absorbance was read at 520 nm.

For determination of sugar content, 0.5 g of powder was extracted using 10 cm³ of ethanol-distilled water (8:2, v:v) and supernatants were collected after twice centrifugation at 7000×g. The residue from ethanol extraction was subsequently used for polysaccharide extraction by boiling water (Seyyednejad *et al.*, 1998). Total sugars content were estimated by the method of DuBois *et al.* (1956). Reducing sugars were quantified according to Nelson (1944). Oligosaccharides content was obtained by difference between soluble and reducing sugars content.

Ions concentration measurement: After digestion of dry matter in nitric-perchloric acid mixture (Johnson and Ulrich, 1959) concentration of Na⁺ and K⁺ were assayed by flame photometer (model PFP7, Jenway, West Yorkshire, UK). Ca²⁺ concentration was obtained by atomic absorption spectrometer (model 3110, Perkin-elmer, Norwalk, USA).

Statistical analysis: The experiments were carried out in Randomized Complete Block Design (RCBD), with two cultivars and three levels of drought treatments per each

cultivar and four replicates. The significance of differences was determined according to Duncan's Multiple Range Test (DMRT). $p < 0.05$ are considered to be significant.

RESULTS

Water relations: Ψ_w in leaves of both cultivars at all levels of drought decreased significantly compared to control. The responses of two cultivars at same treatments were different. Ψ_s of leaves decreased significantly at 50 and 25% FC in both cultivars. The difference of Ψ_s at 25% FC between two cultivars was significant (Table 1).

RWC in leaves of both cultivars decreased. The RWC of both cultivars were different at same levels of drought, except at 50% FC (Table 1).

Leaf area: Leaf area decreased significantly in both cultivars under 50 and 25% FC. The decrease of these values in Darab 14 was same as those of Yekta (Table 1).

Growth: Fresh mass at 50 and 25% FC and dry mass at 25% FC decreased significantly in leaves of Darab 14. Fresh and dry mass in leaves of Yekta decreased significantly at 50 and 25% FC. Fresh mass of Darab 14 leaves at control and 75% FC were different significantly from Yekta (Table 1).

Fresh and dry mass decreased in roots of both cultivars at all levels of stress. Fresh mass in Darab 14 at 25% FC and dry mass in Yekta under 50 to 25% FC decreased significantly. Fresh mass of Darab 14 roots at control and 75% FC differ from those of Yekta, obviously (Table 2).

Solute accumulation: Free Proline content in leaves of Darab 14 increased significantly at 25% FC. The values in Yekta increased significantly under all of the treatments compared to the control. Both cultivars were different obviously at same levels of treatments, except in control (Table 1).

Reduced sugars in leaves of Darab 14 increased significantly at 50 and 25% FC. These sugars in leaves of Yekta increased under all levels of drought relative to control. Oligosaccharides and soluble sugars in leaves of both cultivars increased significantly under treatments. While polysacchrides in both cultivars decreased significantly. Total sugars in leaves of Darab 14 but not in Yekta increased significantly at 25% FC. Overall, the variation of polysaccharides, reduced sugars, soluble and total sugars was significantly different under all levels of stress (Table 1).

Table 1: Water potential, osmotic potential, relative water content, leaf area, fresh and dry mass, free proline content, reduced sugars, polysaccharides, oligosaccharides, soluble sugars, total sugars, Na⁺, K⁺, Ca²⁺ concentrations, K⁺/Na⁺ in leaves of two sesame (*Sesamum indicum* L.) cultivars under drought stress. Values are mean±SE of four replications. The same letters do not differ statistically by Duncan's multiple range test (p≤0.05)

FC (%)				Cultivars	Parameters
25	50	75	100		
1.25 ±0.032a	0.97±0.021c	0.54±0.030e	0.48±0.021f	Darab 14	Water potential [-MPa]
1.02±0.034b	0.78±0.022d	0.45±0.021f	0.40±0.034g	Yekta	
1.67±0.245a	1.12±0.037bc	0.70±0.033de	0.57±0.021e	Darab 14	Osmotic potential [-MPa]
1.35±0.037b	0.95±0.028cd	0.60±0.035e	0.51±0.030e	Yekta	
59.57±0.330f	67.07±0.300e	75.36±1.110d	85.98±0.510c	Darab 14	RWC (%)
67.78±1.730e	68.62±0.700e	89.40±0.850b	98.05±0.830a	Yekta	
16.75±2.780c	30.00±3.030b	46.25±2.430a	51.75±3.540a	Drab 14	Leaf area [cm ²]
20.00±3.370c	32.75±2.810b	44.25±2.870a	48.00±3.370a	Yekta	
109.80±7.200c	152.50±14.930c	222.60±28.400b	235.15±12.600b	Darab 14	Fresh mass [mg]
117.50±14.930c	154.93±14.980c	340.58±34.910a	363.75 ± 30.170a	Yekta	
15.93±1.320d	21.27±3.740b-d	28.65±4.880a-c	29.88±2.110ab	Darab 14	Dry mass [mg]
15.50±3.140d	17.75±1.600cd	34.35±4.250a	35.58±6.710a	Yekta	
189.00±5.740d	124.60±10.250e	118.19±8.490e	98.77±7.260e	Darab 14	Free proline [µg g ⁻¹ (f.m.)]
406.10±16.230a	306.00±8.130b	253.00±7.390c	118.30±9.710e	Yekta	
1.28±0.060b	0.82±0.048c	0.65±0.029d	0.57±0.037d	Darab 14	Reduced sugars [% (d.m.)]
1.67±0.085a	1.27±0.062b	1.27±0.067b	0.83±0.045c	Yekta	
1.86±0.058e	2.66±0.076d	2.65±0.062d	2.98±0.073c	Darab 14	Polysaccharides [% (d.m.)]
2.56±0.074d	3.43±0.078b	3.42±0.086b	4.24±0.085a	Yekta	
1.86±0.028a	1.09±0.074cd	1.18±0.067bc	0.86±0.077e	Darab 14	Oligosaccharides [% (d.m.)]
1.97±0.095a	1.38±0.096b	1.33±0.065b	0.90±0.030de	Yekta	
3.14±0.086b	1.91±0.080d	1.82±0.039d	1.43±0.108e	Darab 14	Soluble sugars [% (d.m.)]
3.64±0.094a	2.65±0.100c	2.60±0.005c	1.73±0.055d	Yekta	
5.00±0.073b	4.57±0.108c	4.47±0.089c	4.41±0.170c	Darab 14	Total sugars [% (d.m.)]
6.20±0.131a	6.08±0.135a	6.02±0.904a	5.97±0.131a	Yekta	
3.40±0.149a	3.24±0.149ab	2.93±0.439bc	2.51±0.159d	Darab 14	Na ⁺ concentration [mg g ⁻¹ (d.m.)]
3.17±0.153ab	3.07±0.148ab	2.67±0.172cd	2.59±0.149d	Yekta	
43.53±1.090e	48.30±1.660de	54.60±1.260cd	51.20±2.400c-e	Darab 14	K ⁺ concentration [mg g ⁻¹ (d.m.)]
57.55±2.470c	72.63±2.850b	109.92±3.020a	114.95±6.400a	Yekta	
19.00±0.890d	18.40±1.270d	27.80±1.400c	33.80±1.510b	Darab 14	Ca ²⁺ concentration [mg g ⁻¹ (d.m.)]
12.20±0.890e	18.40±1.030d	21.80±1.550d	38.40±1.760a	Yekta	
12.94±0.890e	14.95±0.200de	20.73±3.130bc	20.54±0.700bc	Darab 14	K ⁺ /Na ⁺
18.16±0.270cd	23.76±1.200b	41.71±3.740a	44.56±1.290a	Yekta	

Table 2: Fresh and dry mass, free proline content, reduced sugars, polysaccharides, oligosaccharides, soluble sugars, total sugars, Na⁺, K⁺, Ca²⁺ concentrations, K⁺/Na⁺ in roots of two sesame (*Sesamum indicum* L.) cultivars under drought stress. Values are mean±SE of four replications. The same letters do not differ statistically by Duncan's multiple range test (p≤0.05)

FC (%)				Cultivars	Parameters
25	50	75	100		
94.20±7.880c	107.95±12.810bc	137.75±13.120bc	149.40±10.270b	Darab 14	Fresh mass [mg]
128.93±15.210bc	147.10±19.820b	202.88±22.450a	233.23±13.940a	Yekta	
22.25±1.040cd	23.57±2.500b-d	29.48±3.220a-c	31.70±3.130a-c	Darab 14	Dry mass [mg]
21.50±3.290d	23.88±3.330b-d	32.25±3.170ab	35.42±2.040a	Yekta	
142.60±4.790a	120.80±3.510b	82.08±4.320cd	76.84±3.880d	Darab 14	Free proline [µg g ⁻¹ (f.m.)]
152.40±2.600a	123.40±2.100b	90.39±2.960c	79.00±3.930d	Yekta	
0.42±0.013b	0.32±0.018c	0.27±0.015d	0.17±0.013e	Darab 14	Reduced sugars [% (d.m.)]
0.50±0.033a	0.36±0.021c	0.35±0.017c	0.18±0.013e	Yekta	
1.47±0.088c	1.58±0.095c	1.57±0.099c	1.92±0.076b	Darab 14	Polysaccharides [% (d.m.)]
1.92±0.104b	2.09±0.099ab	2.20±0.114ab	2.22±0.045a	Yekta	
0.46±0.032a	0.32±0.056ab	0.33±0.023ab	0.20±0.012b	Darab 14	Oligosaccharides [% (d.m.)]
0.46±0.040a	0.42±0.057a	0.42±0.062a	0.21±0.031b	Yekta	
0.88±0.034a	0.64±0.047c	0.60±0.021c	0.37±0.023d	Darab 14	Soluble sugars [% (d.m.)]
0.96±0.043a	0.78±0.038b	0.77±0.051b	0.39±0.025d	Yekta	
2.35±0.059cd	2.22±0.097d	2.14±0.099d	2.29±0.086d	Darab 14	Total sugars [% (d.m.)]
2.87±0.101ab	2.87±0.129ab	2.97±0.159a	2.61±0.062bc	Yekta	
3.25±0.055a	3.09±0.070b	2.54±0.058d	2.05±0.049f	Darab 14	Na ⁺ concentration [mg g ⁻¹ (d.m.)]
2.83±0.012c	2.85±0.062c	2.47±0.073d	2.19±0.090e	Yekta	
31.61±1.210e	44.54±1.210d	53.34±1.480c	59.27±2.640c	Darab 14	K ⁺ concentration [mg g ⁻¹ (d.m.)]
45.26±1.310d	65.92±1.260b	66.01±3.390b	90.52±2.620a	Yekta	
9.80±0.480g	11.20±0.420f	11.20±0.570d	21.80±1.520a	Darab 14	Ca ²⁺ concentration [mg g ⁻¹ (d.m.)]
13.20±0.630e	14.60±0.550d	16.60±0.570c	20.00±0.910b	Yekta	
9.71±0.270e	14.45±0.710d	21.09±1.020c	28.88±0.700b	Darab 14	K ⁺ /Na ⁺
16.00±0.530d	23.13±0.110c	26.67±0.650b	41.74±2.930a	Yekta	

Proline content in roots of Darab 14 increased significantly at 50 and 25% FC. The values in Yekta increased significantly under all levels of drought (Table 2).

Reduced and soluble sugars in roots of both cultivars increased obviously relative to control. Evaluation of differences between two cultivars showed that their reduced sugars at 75 and 25% FC and soluble sugars at 75 and 50% FC were significantly different from each other. In roots of Darab 14 polysaccharides content decreased drastically at all levels of stress and in Yekta only at 25% FC. There were significant differences between Darab 14 and Yekta. Oligosaccharides of Darab 14 roots at 25% FC and in Yekta at all levels increased significantly relative to control.

Total sugars in the roots of Yekta at 75% FC increased significantly compared to control. There were significant different at all treatments between Darab 14 and Yekta (Table 2).

Ions concentration: The Na⁺ concentration of leaves increased significantly at all levels of stress in Darab 14 and at 50 and 25% FC in Yekta. The concentration of K⁺ in Darab 14 decreased under stress, but a significant decline was observed in Yekta at 50 and 25% FC. The measurement of these ions between two cultivars showed that they were different from control to 25% FC. The Ca²⁺ concentration in leaves of both cultivars decreased drastically at all levels of drought. The decline of Ca²⁺ concentration in two cultivars at control, 75 and 25% FC was significant. K⁺ to Na⁺ ratio in root of cvs Darab 14 and Yekta at 50 and 25% FC decreased significantly (Table 1).

Changes in Na⁺ concentration in roots of both cultivars were obvious. There were significant differences between two sesames at 100, 50 and 25% FC. K⁺ concentration decreased in roots of Darab 14 at 50 and 25% FC and from 75 to 25% FC in Yekta. Two cultivars were different at all levels of stress. Decreases of Ca²⁺ concentration in roots of both cultivars were significant under stress. There were obvious differences between two cultivars at all levels of drought. K⁺ to Na⁺ ratio decreased significantly in roots of both cultivars at all levels of drought. Two sesames were different at all treatments from each other (Table 2).

DISCUSSION

The parameters characterizing plant water status, i.e., Ψ_w and RWC of leaves decrease with the increase of water deficit. Moreover, Ψ_s were lower in the high stress. These observations are similar to results obtained by Sgherri and Navari-Izzo (1995) about seedling of sunflower under

water deficit stress. Also, Hsu *et al.* (2003) reported that drought stress decreased RWC. Our results showed that the changes of Ψ_w and RWC were different between leaves of cv. Darab 14 and cv. Yekta. While, Ψ_s of leaves in both cultivars only at high level of stress were different. These results are in agreement with the results of Morant-Manceau *et al.* (2004) on a genotype of *Triticum* and its parental species under salt stress. Besides, Sairam *et al.* (2005) showed salinity sensitive wheat genotype had high decline in RWC.

Leaf area in both cultivars decreased drastically at moderate and high levels of drought. Researchers suggested that inhibition of leaf growth by water stress can be considered to be an adaptive response. Thus, it limits leaf area production and eventually, rates of transpiration per plant. Reduced transpiration may be then prolong plant survival by extending the period of availability of essential soil-water reserves in the root zone (Lu and Neumann, 1998).

It has been reported that the restriction of water supply from the soil in relation to genotype reduced plant biomass (Ogbonnaya *et al.*, 2003). Our results showed that drought decreased fresh and dry mass in leaves and roots of sesame cultivars. This is in accordance with the result of Ratnayaka *et al.* (2003). Free proline increased in leaves and roots of Darab 14 at high levels of stress and in Yekta at all levels of treatments. These results are similar to the results obtained by Singh *et al.* (1972, 1973), Stewart and Lee (1979), Navari-Izzo *et al.* (1990) and Hsu *et al.* (2003).

In our experiments, there were significant differences between two sesame cultivars in proline content. Other researchers such as Türkan *et al.* (2005) found a higher proline accumulation in drought-tolerant *Phaseolus acutifolius* than in drought-sensitive *P. vulgaris*. They suggested a directed consequence of higher proline concentration in tolerant species is the relatively higher water retaining capacity as reflected by RWC. These results were obtained by Al-Khayri and Al-Bahrany (2004) in palm, Vajrabhaya *et al.* (2001) in drought-tolerant lines of rice and Karimi *et al.* (2005) in *Kochia prostrata*.

Reduced, soluble and total sugars and oligosaccharides in leaves and roots of both cultivars increased, but polysaccharides decreased. Increases of sugars in response to water stress have been reported by Bajji *et al.* (2001), Karimi *et al.* (2005). Martinez *et al.* (2004) reported that the leaf soluble sugar concentration increased at a rate closely corresponding to the decrease in leaf RWC in *Atriplex halimus*. Moradshahi *et al.* (2004) explained that total soluble sugar tends to increase in cultivars of *Brassica napus*, which is probably due to

mobilization of reserved polysaccharides, by decreasing water potential. The decrease in starch content could result from a decrease in synthesis, since CO₂ assimilation was reduced in response to water stress as shown by Martinez *et al.* (2004). However, this reduction could also be caused by stimulation of starch degradation (Geiger *et al.*, 2000). According to Pattanagul and Madore (1999) starch levels were reduced in *Coleus blumei* in response to drought stress. They suggested that the increase in sucrose and hexose amounts to be due to the increase in starch hydrolysis. Besides, decrease of starch levels when water availability is limited, has been observed in many different species according to Geigenberger *et al.* (1997) and Geiger *et al.* (2000).

The production of low-molecular-weight metabolites seems to be a virtually response to stress (Bohnert *et al.*, 1995; Bohnert and Jensen, 1996). The most common hypothesis to explain the role of these molecules in stress tolerance is that they serve as osmolytes, helping cells to osmotically adjust when faced with low water potentials. However, it is becoming clear that these compounds frequently do not accumulate at high enough levels to be acting as true osmolytes and it is now envisioned that some may be serving alternate roles in stress protection, such as radical scavenging, a protection from photoinhibition, or detoxification (Rare, 1990).

Our results showed that Na⁺ concentration in leaves and roots of both cultivars increased. The increases of sodium ions in roots were different between two sesames in moderate and high levels. K⁺ and Ca²⁺ concentration of organs decreased. K⁺ to Na⁺ ratio in leaves and roots of both cultivars decreased. There were differences between two cultivars.

These results are similar in part to results obtained by Tozlu *et al.* (2000) and Karimi *et al.* (2005) under salt stress.

K⁺ and Ca²⁺ have important role under drought stress. K⁺ is vital for cell expansion, osmoregulation and cellular and whole-plant homeostasis (Maathuis and Sauters, 1996). The role of K⁺ in response to salt stress is also well documented, when Na⁺ and K⁺ may exchange during salt uptake (Maathuis *et al.*, 1996; Fox and Guerinot, 1998). Calcium has important role in preserving membrane integrity (Rengel, 1992), in osmoregulation (Mansfield *et al.*, 1990) and influencing K⁺/Na⁺ selectivity (Cramer *et al.*, 1985, 1987).

In conclusion, exposure of sesame cultivars to drought resulted in decreases in Ψ_w , Ψ_s , RWC and leaf area of leaves, decreases in fresh and dry mass of leaf and roots, changes in content of proline, reduced sugars, soluble and total sugars, oligosaccharides, polysaccharides, increases of Na⁺, decreases of K⁺, Ca²⁺

and K⁺/Na⁺ ratio in leaves and roots in both cultivars. However, in view of considerable variations in the molecular biochemical and physiological mechanisms of protection against drought in different plant cultivars, further work required to establish the general validity of this phenomenon.

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