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ORIGINAL ARTICLE

The influence of drought stress on photosynthetic pigments and some metabolic contents of two *Kochia scoparia* ecotypes in saline condition

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ABSTRACT
Drought stress is considered as the main factor of yield limitations in arid and semi-arid areas, where drought and salinity stress are combined the negative effects of these stresses are more conventional on crop yield. Kochia species have recently attracted the attention of researchers as forage and fodder crop in marginal lands worldwide due to their drought and salt tolerant characters. This field experiment was performed at the Salinity Research Station (36°15´N, 59°28´E) of Ferdowsi University of Mashhad, Iran, in a split plot based on a randomized complete block design with three replications in 2008. Three levels of drought stress (control, no irrigation in vegetative stage (recovery treatment) and no irrigation at reproductive stage for one month (stress treatment)), and two Kochia ecotypes (Birjand and Borujerd) were allocated as main and sub plots, respectively. Plants were irrigated with ground water having electrical conductivity (EC) of 5.2ds.m^{-1}. Photosynthetic pigment, proline, soluble carbohydrate, Na and K concentrations were assayed at the beginning of anthesis. Results showed that stress treatment caused a significant increase in proline, soluble sugar and photosynthetic pigment compared with control and recovered conditions. It seems that these parameters may play a role in minimizing the damage caused by dehydration. Na and K concentration didn't differ between treatments. According to the results, there were no differences between recovered plants and control treatment, therefore, Kochia can recover quickly after removing stress. Birjand ecotype from the arid regions, revealed a better response to drought stress than Borujerd ecotype. This could be due to initial adaptation of Birjand ecotype especially in stress condition. Proline showed that the most differential between measured parameter, therefore proline can be one of the best stress indexes in this condition.
Keywords: *Drought stress, photosynthetic pigment, proline, soluble carbohydrate*

INTRODUCTION

Drought is commonly defined as a below-normal water availability, types of drought is the meteorological or climatological, agricultural, hydrological, and socioeconomic which is a major climatic issue that measured based on precipitation index.(10). Different level of soil water content beside such other essential element caused adverse effects on plant growth (18). Drought stress is one of the major constrains of crop production systems in many parts of the world including Iran. Water shortages and soil water losses due to environmental and land use changes are challenges to crop production. Kochia is a hardy, drought and salinity resistant plant widely used as emergency forage for livestock. Kochia can establish on saline soils, not only produce protective short-lived vegetation coverage, but also is being used as an alternative fodder crop, especially in regions faced with forage shortage. Several researchers showed that Kochia produces high biomass in saline-sodic soils.

plants vary greatly in their capability to tolerate stress conditions, hence some of them are unable to endure stress so wilt and die (sensitive plants), while others can tolerate stress by undergoing certain physiological changes in their tissues which thus maintain their cell water potential turgidity and at normal level, in spite of soil drought (tolerant plants) (22). One of the most common strategies of plants for avoiding water stress is the accumulation of the osmoprotectors or osmolytes (4). During osmotic stress, plant cells accumulate solutes to prevent water loss and reestablish cell turgour. The solutes that accumulate during osmotic adjustment include ions such as K^+ and Na^+ or organic solutes that include compounds that contain N, such as proline and other amino acids and polyamines (23). Proline accumulates in a great variety of plant species in response to stress such as drought, salinity, and extreme temperatures. Although its osmotolerant role in plants is not clear under stress conditions, proline can act as a mediator of osmotic adjustment, stabilizer of subcellular structures, eliminator of free radicals, and as a buffer of redox potential (13, 16). Moreover, proline has a protective action that prevents membrane damage and protein denaturation during severe drought stress (1). However, the improvement of stress tolerance due to proline accumulation is species-dependent (5). Despite this research, there is currently great controversy on the protective properties of proline accumulation. (12) concluded that proline accumulation is not an adaptive feature, but rather only a symptom of stress. In agreement with this contention, some findings show that drought tolerant wheat plants have higher relative water content ((RWC) related to a lower proline concentration (19).

Carbohydrate changes are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration. Among the soluble carbohydrates, sucrose and fructans have a potential role in adaptation to these stresses (24, 14). Soluble sugar content proved to be a better marker for selecting improvement of drought tolerance in durum wheat (*Triticum durum* Desf.) than was proline content (2).

The photosynthetic pigments, chlorophyll a, chlorophyll b and carotenoids are the main factors in photosynthesis and growth. Scientist reported many variations of pigments at stress situation. (3) and (21) have investigated that water stressed leaves contain less amounts of chlorophyll a, b and total pigments as compared to untreated control ones. One the other hand, (20) and (17) have shown that the carotene contents of leaves subjected to drought were much increased. Carotenoids are well known for their antioxidant activity within the chloroplasts, scavenging singlet oxygen and lipid peroxy radicals, as well as preventing lipid peroxidation.

The objective of this work was to examine the effects of drought stress on photosynthetic pigments, proline, soluble sugar, Na and K concentration in the leaves of Kochia under prolonged drought stress and natural field conditions irrigated with saline water.

MATERIAL AND METHODS

Field studies were conducted during the summer of 2008 at the Salinity Research Station (36°15´N, 59°28´E) of Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. This experiment was performed in a split plot based on randomized complete block design with three replications. Water treatment, including three levels control, no irrigation in vegetative stage (30th Jun. to 27th Jul.) then irrigated up to ripening and non irrigated in reproductive stage (21st Jul. to 17th Aug.) then irrigated up to ripening, by using saline irrigation water with 5.2 dS m^{-1} electric conductivity, because kochia is a halophyte plant, saline water is used until real situation is provided and two kochia ecotypes (Birjand and Borujerd) were allocated as main and sub plots, respectively. RWC measured every two weeks for monitoring drought stress. Two Kochia ecotypes were selected from different parts of Iran. Borujerd city include cold weather, without salinity in the soil and water and Birjand has arid climate and salty soil. This experiment designed to study stress and recovery treatment, therefore, relative Photosynthetic pigment, proline, soluble carbohydrate, Na and K concentration were assayed on the end of reproductive stress (15th August).

The main chemical properties of the soil and irrigation water are presented in Table 1. The amount of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}) sulfate ($\mathbf{SO_4}$), carbonate (CO_3) and bicarbonate (HCO_3) in a soil sample was measured before the experiment began. The concentration of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, $\mathbf{SO_4}$, CO_3 and HCO_3 was determined from the Manual on Soil Sampling and Methods of Analysis (McKeague, 1976). A counter was installed at the beginning of irrigation system and volume of water for irrigating was controlled separately at each plot.

Proline concentration

Samples of fresh leaves were weighed (0.1 g) and homogenized in liquid nitrogen into microtube by electrical homogenizer, after that one ml of 3 % sulphossalicilic acid as solvent was added to sample. Than the extract was centrifuged at 3500 g for 10 min and the supernatant was preserved 4°C for the proline determination. An aliquot of this supernatant was taken and after adding reactive ninhydrin acid reagent (ninhydrin, phosphoric acid 6 M, glacial acetic acid) and glacial acetic acid at 99%, was placed in a water bath at 100 °C for 30 min. Soon after removal from the water bath, the microtubes were cooled in ice bath. The absorbance was read in a spectrophotometer at 520 nm, as indicated. Proline content in fresh tissue was calculated by comparing the sample absorbencies with the standard proline curve in a concentration range of 0 to 25 mg/L. The result of proline concentration was expressed as μg /gfw (Fresh weight).

Soluble sugars

Soluble sugars were determined based on the modified method of phenolsulforic (8). Samples of fresh leaves (0.1 g) homogenized in liquid nitrogen into microtube by electrical homogenizer, after that 500 µl of ethanol as solvent was added to sample. The extraction was centrifuged at 3500 g for ten min and the supernatant was preserved 4°C. Than 500 µl of %70 ethanol was added to primary sample. The extraction was centrifuged at 3500 g for ten min. after 4h. and this supernatant added to primary supernatant. Finally 400µl of supernatant, 400µl chloroform and 200µl distilled water mixed together. 200µl of light phase, phenol and sulfuric acid mixed and after 20 min. in boiling water, their absorbance are read in 480nm. Soluble sugar concentration was calculated using glucose solution as a standard curve.

Chlorophyll pigment

Chlorophyll pigments were determined based on the modified method of (7). 0.1 g of fresh leaves homogenized in liquid nitrogen into microtube by electrical homogenizer, after that one ml of methanol as solvent was added to sample. This sample preserve in dark and cold situation for 24h. Than sample in microtubes were mixed by vortex for 5 minutes. Than the extract was centrifuged at 2500 g for ten min and the supernatant was preserved 4°C for the chlorophyll determination. Supernatant diluted 10 times with methanol. The absorbance was read in a spectrophotometer at 470, 653 and 666 nm. Finally chlorophyll a, b and total carotenoid were calculated from this equation.

C_a=15.65 A_{666} - 7.340 A_{653}

C_b=27.05 A_{653} - 11.21 A_{666}

C_{x+c} = 1000 A_{470} - 2.860 C_a - 129.2 C_b/245

C_a = Chlorophyll a, C_b = Chlorophyll b, C_{x+c} = Total carotene, A_{666} = Absorbance in 666nm, A_{653} = Absorbance in 653nm and A_{470} = Absorbance in 470nm

Mineral content analysis

The plant tissues were oven-dried (70°C for 2 days to constant weight), ground to pass a 0.5mm sieve. Na^+ and K^+ in the plant samples were extracted for 12 h. with HNO3 at 90°C in a water bath following the method of Gulati and (11). Sodium and Potassium contents were determined by flame photometers.

The data compiled were submitted to an analysis of variance (ANOVA) and means were compared by LSD test (P ≤ 0 05).

Result and Discussion

Proline concentration

Result showed that there were no significant differences in proline concentration between control and recovery treatment. Significant difference ($P < 0.01$) in proline concentration was observed between control and stress treatments. Proline concentration at stress treatment compared with control, increased by 300% (Fig. 1). Birjand ecotype had more proline concentration (28%) compared with Borujerd. Difference between ecotypes could return back to their original habitats. Birjand area has harsh, drought climate rather than Borujerd. Therefore, kochia in Birjand area could adapt with hard situation and in this experiment also showed a better performance than Borujerd. Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. For example, while in salt-tolerant alfalfa plants proline concentration in the root rapidly doubled under salt stress, in salt-sensitive plants the response was slow. In rice plants subjected to water deficit, the concentration of proline was increased in the leaves. In wheat, an assessment of the effects of drought stress on proline accumulation in a drought-tolerant and a drought-sensitive cultivar revealed that the rate of proline accumulation and utilization was significantly higher in the drought-tolerant cultivar. Engineered tobacco plants over-producing proline significantly reduced the level of free radicals and improved tolerance to 200mM NaCl.

In addition to proline role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions. It may also function as a protein compatible hydrotrope, alleviating cytoplasmic acidosis, and maintaining appropriate NADP+/NADPH ratios compatible with metabolism. Also, rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages. In response to drought or salinity stress in plants, proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment. High concentration of proline in kochia plants on stress situation coincided with this result especially amount of proline in recovered treatment proved role of proline in stress situation, Because after removing stress, proline reduced in plant.

Total soluble sugar

Result showed that there were no significant differences in total soluble carbohydrate between control and recovery treatment. However, significant differences ($P<0.05$) in total soluble carbohydrate of leaves were observed between control and stress treatments. Total soluble carbohydrate of the leaves increased by 25%, Compared with the control (fig. 2). There were no significant differences between ecotypes. (9) found that soluble sugars increased in sorghum more than in atriplex, as an effect of salinity. According to those authors, soluble sugars increased between 30 % to 144 % in stressed plants of sorghum, as compared to control plants. *Phaseolus vulgaris* grown under stress of 100 $mol.m^{-3}$ NaCl increased the content of soluble sugars to 51 % as compared to control plants (6). reported that relationship between carbohydrate accumulation and degree of salt and drought tolerance; indicate that total soluble carbohydrate content might be a useful trait to select drought or salt-tolerant wheat genotypes and the initial response to drought stress appears to be an increase in monosaccharides, while the more delayed response was an increase in fructan. On the other hand, fructan content could be a useful indicator of degree of salt tolerance.

Chlorophyll pigments

Result showed that there were significant difference between control and stress treatment but all of pigments including chlorophyll a, chlorophyll b and carotenoids had the most amounts in stress situation (fig. 3). Many of scientist reported that the stress situation increase carotenoids but decrease chlorophyll a, chlorophyll b. pointed that in a drought, plants usually increase endogenous α-tocopherol and carotenoid levels to cope with oxidative stress. Carotenoids also play a critical role in the assembly of the light-harvesting complex and in the radiationless dissipation of excess energy associated with the conversion of violaxanthin to zeaxanthin.

K and Na concentration

It is apparent from figure (4) that the sodium and potassium levels in both ecotypes didn't change significantly in different treatment. Probably in kochia plant other components are important in osmotic adjustment or this plant maybe could prevent from absorbing Na in control and stress situation, this subject can point to good resistance against Na absorbance. Potassium didn't change in stress and control situation in kochia plant in spite of most scientist reported reduction k in saline and drought situation in plants. For example, reported that the sodium amount in both shoots and roots increased progressively with the increase of period of water withdrawal from 1 to 6 days and they showed that when the two varieties of *Triticum* was subjected to stress treatments, their amounts of each of potassium, calcium magnesium and phosphorus in both shoots and roots were, in general, decreased so as to reach their minimum values in plants irrigated every 7 days as being compared to those of unstressed plants.

Total biomass

Result showed significant difference ($P < 0.05$) in total biomass between treatments. The lowest total biomass was obtained in recovery treatment that was under stress during vegetative stage (fig 5). The results showed that although physiological parameters returned to normal in the recovery treatment but water deficits decrease growth, by slowing rates of cell division and expansion due to loss of turgor and increased synthesis of abscisic acid (15). Study the effect of total biomass between ecotypes showed that Birjand had more total biomass compare with Borujerd especially in stress condition (fig 5). Differences between ecotype probably return back to environmental conditions of Birjand area. Thus, kochia in Birjand area could adapt with hard situation and this experiment also showed better performance than Borujerd.

Generally environmental changes induce the activation of physiological processes in plants, allowing them to adapt to a new physiological status. In this experiment kochia also changed amount of proline, soluble sugar and carotenoids for adaptation in stress situation but there wasn't difference between control and recovery treatment and this subject emphasize resistance and good recovery in *kochia scoparia*.

Table 1. Main chemical properties of the waters and soil at the study site.

	Na	Ca	Mg	K	SO_4	CO_3	HCO_3	Cl	EC
					($meq.l^{-1}$)				$dS.m^{-1}$
Water	32.50	8.60	9.20	0.23	15.00	0.40	2.40	34.40	5.20
Soil	31.1	10.6	10.2	0.75	31.3	0	1.8	26.8	5.8

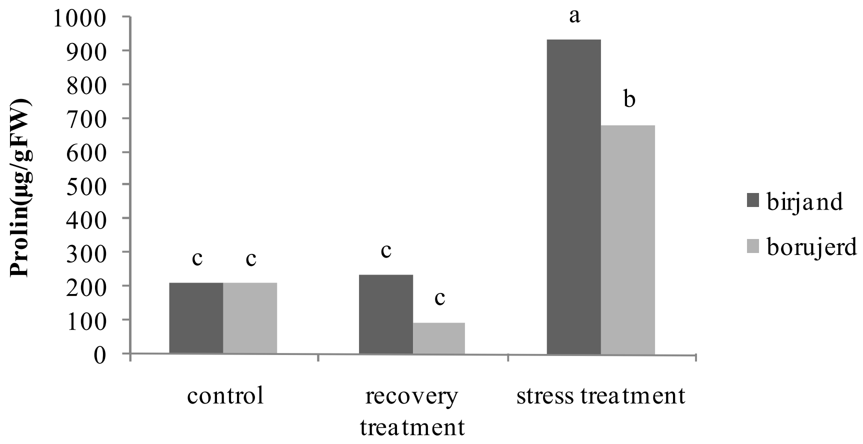


Fig 1. Difference of proline between ecotypes in control, drought stressed plants and after recovery.

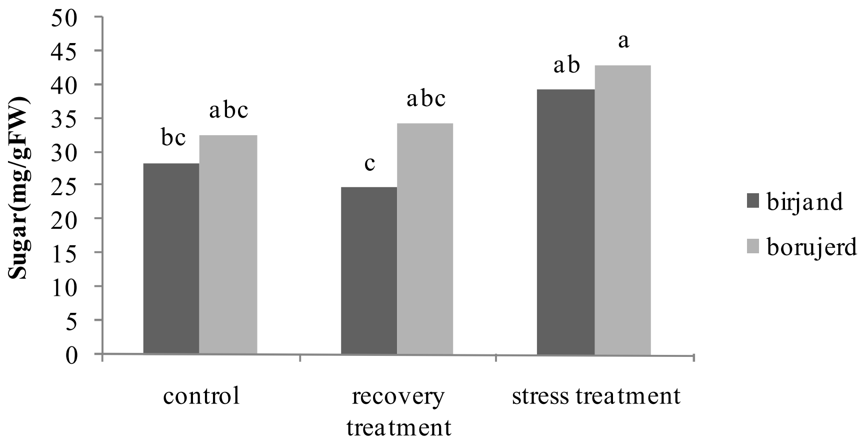
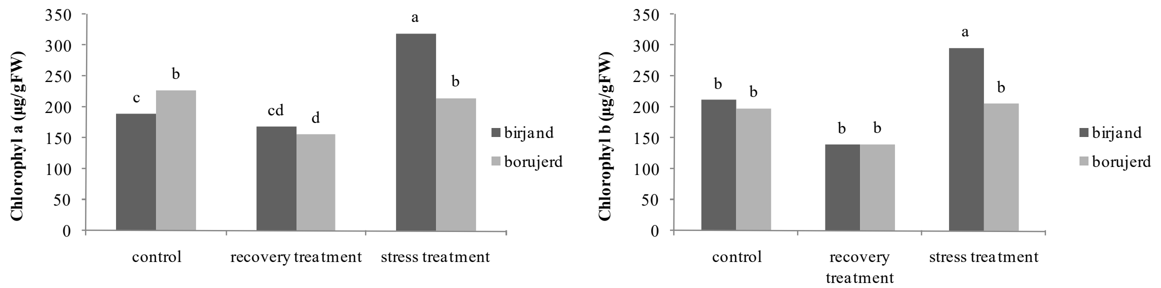


Fig 2. Difference of total soluble sugar between ecotypes in control, drought stressed plants and after recovery.



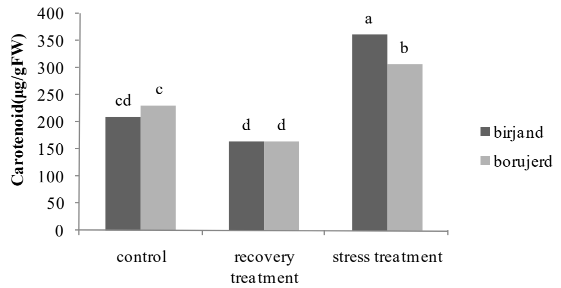


Fig.3. Difference of chlorophyll a, chlorophyll b and carotenoids between ecotypes in control, drought stressed plants and after recovery.

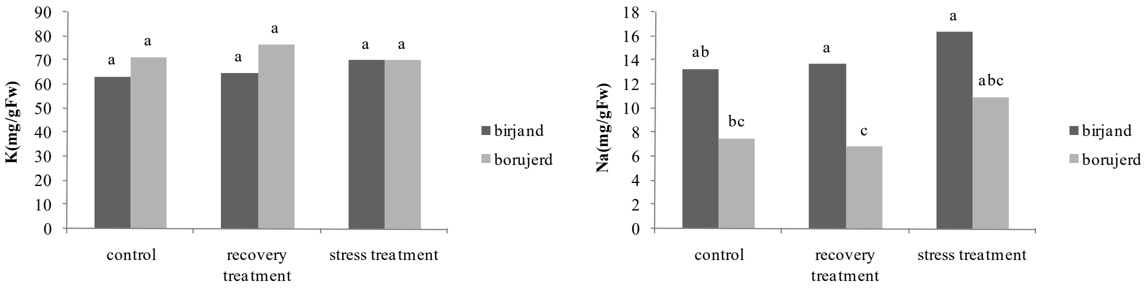


Fig 4. Difference of K and Na concentrations between ecotypes in control, drought stressed plants and after recovery.

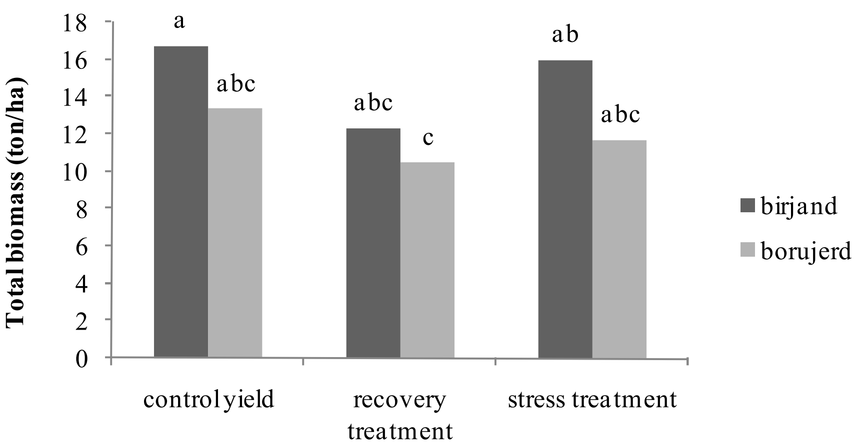


Fig.5. Difference of total biomass between ecotypes in control, drought stressed plants and after recovery.

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