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Physiological responses of two halophytic grass species under drought stress environment

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Abstract – The physiological responses of two halophytic grass species, *Halopyrum mucronatum* (L.) Staph. and *Cenchrus ciliaris* (L.), under drought stress were evaluated. Biomass accumulation, relative water content, free proline, H_2O_2 content, stomatal conductance, photosynthetic performance and quantum yield (F_v/F_m ratio) were studied. Under drought conditions, these halophytic plants expressed differential responses to water deficit. Stomatal conductance and free proline content were higher in *H. mucronatum* than in *C. ciliaris*, while H_2O_2 content in *H. mucronatum* was substantially lower than in *C. ciliaris*. Performance index showed considerable sensitivity to a water deficit condition, more so in *C. ciliaris* than in *H. mucronatum*. Results were discussed in relation to comparative physiological performance and antioxidant enzymes activity of both halophytic grasses under drought stress.

Keywords: antioxidant enzymes activity, *Cenchrus ciliaris,* chlorophyll *a* fluorescence, drought stress, *Halopyrum mucronatum*, photosystem II

Introduction

Drought and salinity have long been known as the most prevalent abiotic stresses inhibiting the growth and productivity of many wild and domestic plant species across the world (Qadir 2008, Naz et al. 2010). In locations with limited water resources and an increasing human population, conventional crop production might not be able to meet food demands. In this distressing situation, effective measures are adopted not only to minimize crop losses but also to find alternate means of food production. The only economic solution considered in the present circumstance is the use of halophytic plants as an alternate source of food and therefore their growth performance should be tested in arid and saline habitats (Khan and Duke 2001, Nedjimi 2011). For that, focus on developing halophytes as cash crops in the future should be amplified (Breckle 2009).

In the sub-continent, the salt range and large coastal area enable a large number of halophytes or salt tolerant plant species to grow. Among them, *Halopyrum mucronatum* L. Staph. and *Cenchrus ciliaris* L. are important and widely occurring halophytic grasses. Most of these halophytic grasses have been investigated and physiological explanations regarding their salt tolerance have been provided (Khan and Ungar 1999, Saini et al. 2007, Siddiqui and Khan 2011). However, the effects of drought stress on these plants and their physiological mechanism have not been examined.

Generally, *C. ciliaris* L. is considered to be an important pasture grass and is being used for cattle and sheep production in arid and semiarid regions (Khan and Ungar 1999, Saini et al. 2007, Sidiqui and Khan 2011). *H. mucronatum* is an excellent salt-tolerant grass species, and is used as fodder (Siddiqui and Khan 2011). Furthermore, it was also reported that these two grasses might have the ability to tolerate long dry seasons under varying soil conditions indicating some degree of drought tolerance (Ayerza 1981, De Leon 2004).

It was reported that some of the metabolic reactions triggered by drought and salinity are similar. Among them, osmotic adjustment, changes in relative water content, maximum quantum yield and dry mass accumulations are well known (Munns et al. 2002, Kwon et al. 2009, Siddiqui et al. 2014). Early responses to drought or salt stress are generally the same, apart from acting as water stress either qualitatively (saline) or quantitatively (amount of water) and the specific ion effect. Therefore, it is hypothesized that those halophytic grasses that showed salt stress tolerance in saline habitats may also exhibit drought tolerance in dry environments. Hence, the physiological performance and

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antioxidant enzymes activity of two known salt tolerant grasses in drought stress environment have been examined.

Materials and methods

Plant materials

The two halophytic grass species: *H. mucronatum* (L.) Staph. and *C. ciliaris* L were used for experiments. Caryopses were collected at maturity from these plants growing on dunes of Hawks Bay beach, Karachi, Pakistan. They were collected on December 2013. Hulled seeds were cleaned and stored in a refrigerator prior to use.

Germination, growth and treatments

The seeds were surface sterilized in 0.52% sodium hypochlorite solution for one minute and rinsed thoroughly with sterilized distilled water. Seeds were pre-soaked in distilled water for 4 h. Ten seeds were placed in 90 mm sterilized Petri plate. Plants were allowed to grow in a growth chamber (Hotpack USA) at 25–28 ± 2 °C day / night temperature with 69–80% humidity. Light intensity varied between 2000 and 2300 µmol m^{-2} s^{-1}. After 20 days, equal-sized seedlings were then transferred to pots with a diameter of 30.48 cm. There were five plantlets in each pot with 6 replications for each treatment, i.e., one for control and other for the drought treatment. The plantlets were grown up to four leaf stage and then drought was induced up to 7 days when soil moisture content reached 15%. After 7 days, the stomatal conductance (g_s) and chlorophyll fluorescence was recorded on the youngest fully expanded leaf between 9:00 – 11:00 AM using a steady state diffusion porometer, Model SC-1 (Decagon devices) and a chlorophyll fluorescence meter (OS-30p+, Opti-Science, USA) respectively. Afterwards, plants were harvested and biomass production, relative water content, free proline quantification, H_2O_2 content and antioxidant enzymes activity were examined.

Chlorophyll fluorescence

After half an hour of dark adaptation, the chlorophyll fluorescence parameters, minimal chlorophyll florescence (F_0) and maximal fluorescence (F_m), were measured in order to determine the maximum quantum yield (F_v/F_m ratio) (Maxwell and Johnson 2000) of ten fully expanded leaves using a portable fluorometer, model OS-30p+ (Opti-Science, USA).

Pigment analysis

Leaf samples (500 mg) were ground in 10 mL of 96% methanol and then centrifuged at 4000 rpm for 10 min. Total chlorophyll (Chl $_{a+b}$), chlorophyll *a* (C_a), chlorophyll *b* (C_b) and total carotenoid (C_{x+c}) contents were determined (Lichtenthaler 1987). The supernatant was separated and the absorbance was read at 666, 653 and 470 nm in UV – Vis Spectrophotometer (Shimadzu), respectively. Later the pigments were quantified according to the following formulas:

$$C_a = 15.65 \times A_{666} - 7.340 \times A_{653}$$

$$C_b = 27.05 \times A_{653} - 11.21 \times A_{666}$$

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

Free proline content

Free proline content was estimated according to Bates et al. (1973). Fresh leaf samples (500 mg) were homogenized in 10 mL of sulphosalicylic acid (3% w/v). Later, the extract was filtered through Whatman No. 2 filter paper. To 2 mL of the aliquot, 2 mL of acid ninhydrin and 2 mL of glacial acetic acid were added and the contents were boiled at 100 °C for an hour. The mixture was further extracted with 2 mL of toluene by mixing thoroughly with vigorous stirring for 15 to 20 s. The upper layer was separated from the aqueous phase and absorbance was read at 520 nm against toluene blank.

Hydrogen peroxide content

Hydrogen peroxide (H_2O_2) was estimated by the procedure of Sergiev et al. (1997). Fresh leaf samples (500 mg) were homogenized in 5 mL 0.1% (w/v) trichloroacetic acid (TCA) using ice bath. Afterwards, the homogenate was centrifuged at 12,000 g for 15 min. To 0.5 mL supernatant, 0.5 mL of 10 mM potassium phosphate buffer and 1 mL of 1 M potassium iodide (KI) were added. The absorbance was read at 390 nm. The H_2O_2 contents were estimated using a standard curve.

Relative water content

Four leaf strips of 4 cm^2 were excised randomly and fresh weights (FW) were determined. For the measurement of turgid weight (TW), leaves were left in distilled water for 24 h under low irradiance conditions. Samples were then oven-dried at 80 °C for 48 h and dry weight (DW) was determined. Relative water content (RWC) was calculated according to Barrs and Weatherley (1962) according the formula:

$$\text{Relative water content} = (FW - DW / TW - DW) \times 100$$

Enzyme assays

Leaf samples (500 mg) were randomly collected and crushed in liquid nitrogen at 4 °C and homogenized in 10 mL protein extraction buffer containing Tris-HCl pH 6.8, 50 mg polyvinylpyrrolidone, 0.05 mM ethylenediaminetetraacetic acid (EDTA). The contents were centrifuged at 12,000 rpm in a refrigerated micro centrifuge (Smart R-17, Hanil) for 10 min. Total protein was estimated by the method of Bradford (1976).

Catalase (CAT; EC 1.11.1.6) activity was estimated by the method of Patterson et al. (1984). The decomposition of H_2O_2 was measured at 240 nm taking Δε as 43.6 mM cm^{-1}. Reaction assay (3.0 mL) consisted of 10.5 mM H_2O_2 in 0.05 M potassium phosphate buffer (pH 7.0) and the reaction was initiated after the addition of 0.1 mL enzyme extract at 25 °C. The decrease in absorbance at 240 nm was used to calculate the activity. One unit of CAT activity is defined as the amount of enzyme that catalyzes the conversion of 1 mM of H_2O_2 min^{-1} at 25 °C.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was performed by the method of Nakano and Asada (1981). The reaction mixture (2.0 mL) contained 50 mM potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid and 0.25 mM H_2O_2. The reaction was started after the addition of 0.1 mL enzyme extract at 25 °C. The decrease in absorbance at 290 nm for one minute was recorded and the amount of ascorbate oxidized was calculated from the extinction coefficient 2.8 mM cm^{-1}. The unit of activity is expressed as micromole of ascorbic acid oxidized min^{-1} at 25 °C.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was performed by the method of Beyer and Fridovich (1987). The reaction mixture consisted of 27.0 mL of 0.05 M potassium phosphate buffer (pH 7.8), 1.5 mL of L-methionine (300 mg per 2.7 mL), 1.0 mL of nitroblue tetrazolium salt (14.4 mg per 10 mL), and 0.75 mL of Triton X-100. Aliquots (1.0 mL) of this mixture were delivered into small glass tubes, followed by the addition of 20 mL enzyme extract and 10 mL of riboflavin (4.4 mg per 100 mL). The cocktail was mixed and then illuminated for 15 minutes in an aluminium foil-lined box, containing 25 W fluorescent tubes. In a control tube the sample was substituted for by 20 mL of buffer and the absorbance was measured at 560 nm. The reaction was stopped by switching off the light and placing the tubes in the dark. Increase in absorbance due to the formation of formazan was measured at 560 nm. Under the described conditions, the increase in absorbance in the control was taken as 100% and the enzyme activity in the samples were calculated by determining the percentage inhibition per minute. One unit of SOD is the amount of enzyme that causes a 50% inhibition of the rate for reduction of nitroblue tetrazolium salt under the conditions of the assay.

Statistical analysis

All data from treated and control were subjected to statistical analysis using SPSS 17.0 (IBM, USA). The values were expressed as mean and standard errors. t-test ($p = 0.05$) was computed between drought treatments and corresponding controls for each species separately. Levels of significance were expressed on bar graph with different letters.

Results

Drought stress significantly reduced leaf fresh and dry mass in both halophytic grass species (Fig. 1). Fresh weight in both species was lower than in the control. However, decrease in turgid weights was non-significant within a species. On the other hand, dry weights in both the species were substantially lower under drought stress conditions than in the control. In comparison with their respective controls, a greater decrease was observed in *H. mucronatum* than in *C. ciliaris*. Subsequently, relative water content in leaves was slightly higher in *H. mucronatum* than in *C. ciliaris* in a drought stress environment.

Halophytic grasses subjected to drought showed a decrease in chlorophylls a and b and total chlorophyll that was significant compared to control (Fig. 2). However, *C. ciliaris* showed higher decrease than *H. mucronatum* under drought stress conditions. Similarly, total carotenoid content decreased in both species, although more so in *C. ciliaris*.

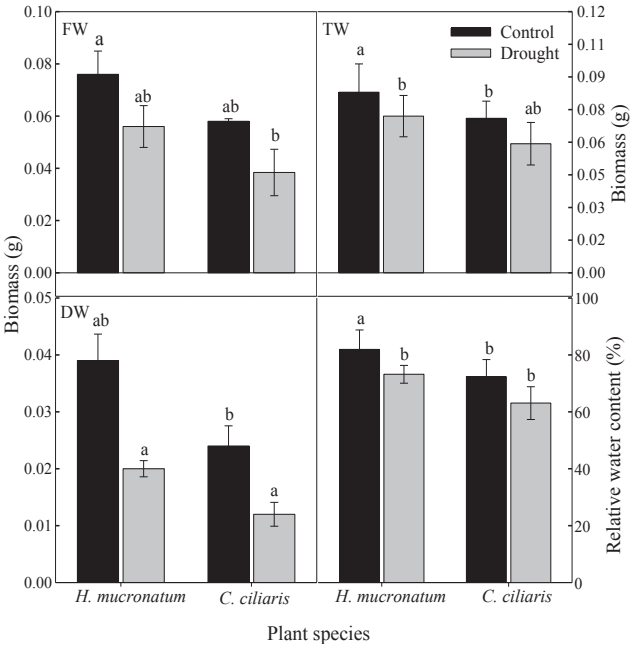

Fig. 1. Biomass and relative water content of *Halopyrum mucronatum* (L.) Staph. and *Cenchrus ciliaris* L. under drought stress. Bars followed by the same letter denote no significant difference between drought treatment and control, for each species separately, according to paired "t" test at $p < 0.05$. Vertical lines on bar graphs represent mean ± standard error. FW – fresh weight, TW – turgid weight, DW – dry weight.

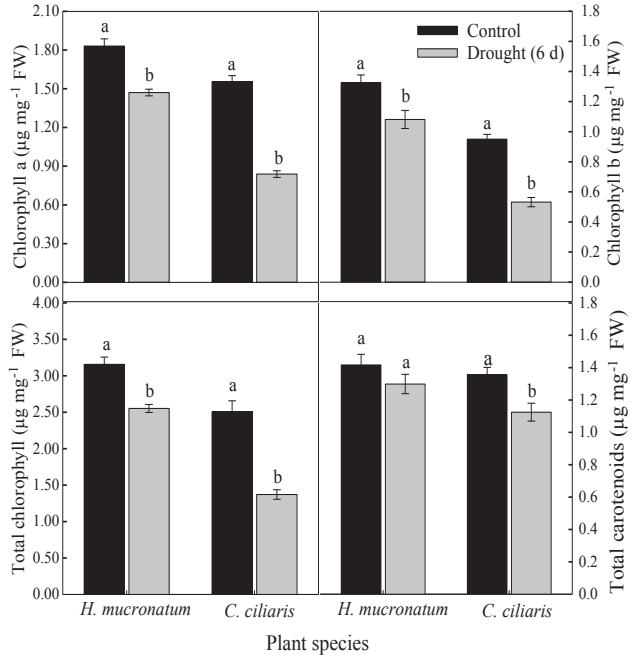

Fig. 2. Total chlorophyll and carotenoid content of *Halopyrum mucronatum* (L.) Staph. and *Cenchrus ciliaris* L. under drought stress. Bars followed by the same letter denote no significant difference between drought treatment and control, for each species separately, according to paired "t" test at $p < 0.05$. Vertical lines on bar graphs represent mean ± standard error.

A noticeable reduction was observed in performance index (PI_{abs}) and photochemical quenching (qP) in both the species under drought stress environment (Fig. 3). However, results showed that a higher reduction in PI_{abs} and qP was recorded in *C. ciliaris* than in *H. mucronatum* under drought stress (Fig. 3). A marked reduction in stomatal conductance (g_s) was observed in two tested halophytic grass species due to drought stress. However, reduction in stomatal conductance was greater in *C. ciliaris* than in *H. mucronatum*.

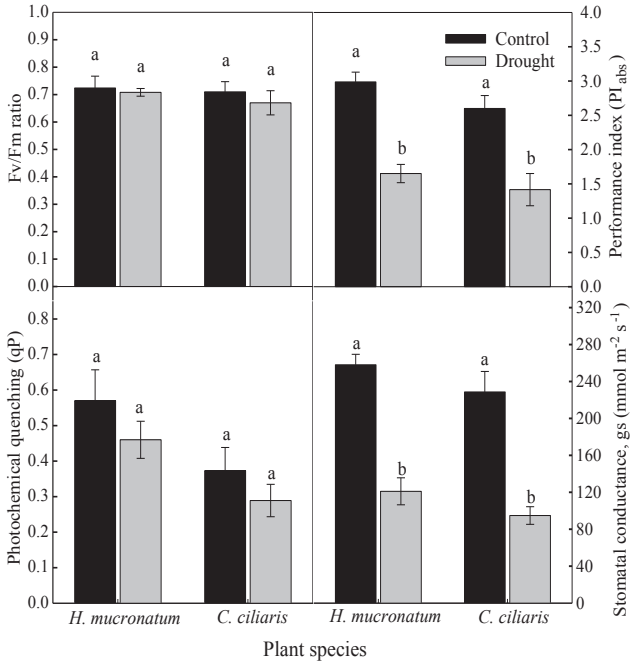

Fig. 3. Maximum quantum yield (F_v/F_m ratio), performance index (PI_{abs}) and photochemical quenching (qP), and stomatal conductance (g_s) of *Halopyrum mucronatum* (L.) Staph. and *Cenchrus ciliaris* L. under drought stress. Bars followed by the same letter denote no significant difference between drought treatment and control, for each species separately, according to paired "t" test at $p < 0.05$ level. Vertical lines on bar graphs represent mean ± SE.

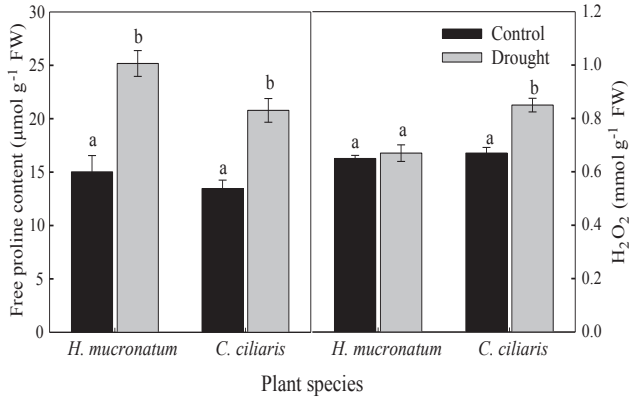

Fig. 4. Total free proline and H_2O_2 content of *Halopyrum mucronatum* (L.) Staph. and *Cenchrus ciliaris* L. under drought stress. Bars followed by the same letter denote no significant difference between drought treatment and control, for each species separately, according to paired "t" test at $p < 0.05$ level. Vertical lines on bar graphs represent mean ± SE.

The level of drought stress damage was examined in terms of free proline and hydrogen peroxide (H_2O_2) production (Fig. 4). Leaf proline concentration of both grasses increased due to drought stress. However, *H. mucronatum* accumulated more proline than *C. ciliaris* under drought stress condition. On the other hand, H_2O_2 content in *C. ciliaris* increased significantly as compared to *H. mucronatum* under stress.

Activity of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) was measured under drought stress against control and is illustrated in Fig. 5. Observations revealed that SOD and CAT activities of treated samples increased significantly in *H. mucronatum* as compared to *C. ciliaris*. However, antioxidant activities of all tested enzymes were higher in treated samples of both halophytes compared to control. Among the treated samples *H. mucronatum* showed an increase that was substantial in SOD and CAT as compared to *C. ciliaris*

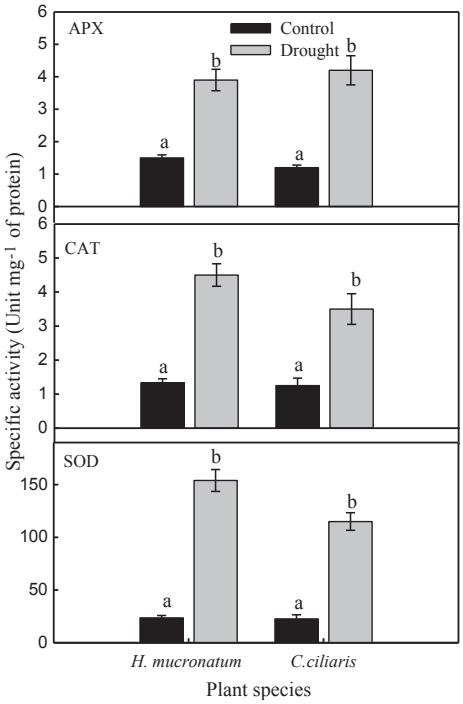

Fig. 5. Antioxidant enzyme activity of *Halopyrum mucronatum* (L.) Staph. and *Cenchrus ciliaris* L. under drought stress. Bars followed by the same letter denote no significant difference between drought treatment and control, for each species separately, according to paired "t" test at $p < 0.05$ level. Vertical lines on bar graphs represent mean ± SE.

Discussion

The physiological responses of two halophytic grass *H. mucronatum* (L.) Staph., and *C. ciliaris* L., were evaluated under drought conditions. It was observed that biomass production was reduced in the two tested species under drought stress. It is well known that drought stress conditions cause substantial reduction in biomass and growth in many plant species (Mahiwal and Sutaria 1992, Ashraf et

al. 1998, Karsten and MacAdam 2001, Tavakol and Pakniyat 2007, Siddiqui 2013). In the present study, RWC of both the halophytic species was significantly reduced under drought conditions compared to control (unstressed) plants. However, the reduction of RWC in *C. ciliaris* was higher than that in *H. mucronatum*. Hence, it can be suggested that *H. mucronatum* has a better drought tolerance through the maintenance of higher water content in leaf under drought. Furthermore, it was observed that RWC in crop plants and the tolerance of the plants to stress are directly related (Schonfeld et al. 1988, Merah 2001, Siddiqui et al. 2014). Therefore, it may be suggested that *H. mucronatum* may have a better ability to regulate intracellular water relations through biomass accumulation than *C. ciliaris* under drought stress conditions. It is well documented that decline in RWC is related to cell membrane properties and its adaptability to environmental changes such as drought (Katerji et al. 1997, El Hafid et al. 1998, De Pereira-Neto et al. 1999, Liu et al. 2002, Molnar et al. 2002, Blokhina et al. 2003). However, spatial differences among the species cannot be ruled out as water relation characteristics reflect the physiological differences among species and cultivars. Nevertheless, RWC is a good indicator of drought tolerance or adaptation in various plant species (Ashraf et al. 1994, Siddiqui et al. 2014).

Photosynthetic performances of the two halophytic grasses under drought conditions were examined in terms of their components, such as maximum quantum yield (F_v/F_m ratio), photochemical quenching (qP), performance index and photosynthetic pigments analysis. Drought stress caused a significant reduction in total chlorophyll and carotenoid content. However, a higher decrease was observed in *C. ciliaris* as compared to *H. mucronatum.* Photosynthetic pigments like chlorophylls and carotenoids are responsible for converting energy and/or trapping it in chemical forms for almost all green plants. It was observed that plant metabolism is clearly linked with photosynthetic pigment and adversely affected by abiotic stress like drought (Li et al. 2012, Siddiqui et al. 2013, Reza and Hassan 2014). The decrease in chlorophyll under water stress is primarily a result of injury in chloroplasts caused by reactive oxygen species, which are usually elevated as a consequence of drought (Smirnoff 1995, Siddiqui et al. 2014). In this study the performance index and quenching substantially were reduced from the maximum yield in both the species under drought stress. Observations showed that greater reductions in stomatal conductance, PI_{abs} and qP, were recorded in *C. ciliaris* than in *H. mucronatum* under drought. The F_v/F_m ratio, characterizes the maximal efficiency yield of excitation energy captured by "open" photosystem II reaction centres. This suggests that the photosynthetic activity of *C. ciliaris* might be decreased due to inhibition in chlorophyll synthesis and their quenching ability which may have an effect on the performance index (Lutts et al. 1996, Tijen and Ismail 2006, Siddiqui 2013, Siddiqui et al. 2014). Photosystem II (PSII) in photosynthetic response is related to chlorophyll and carotenoids concentration and it was varied against fluctuating environmental (Baker 1991). Therefore, changes in photosynthesis under water stress conditions are to be expected. Likewise, performance index (PI_{abs}) is an excellent indicator that showed plant fitness and provides useful quantitative information about photosynthetic apparatus (Strauss et al. 2003, Xia et al. 2004, Oukarroum et al. 2007, Mehta et al. 2010, Stefanov et al. 2011). Likewise, photosynthetic pigments and maximum quantum yield are important physiological parameters reflecting the photosynthetic ability of plants in stressful environments. (Colom and Vazzana 2002, Parida et al. 2003, Waseem et al. 2006, Siddiqui et al. 2008).

H. mucronatum accumulated more proline and less hydrogen peroxide content than *C. ciliaris*. A greater accumulation of proline in response to drought stress is well documented in many plants and maintains homeostasis in leaf (Abdel-Nasser and Abdel-Aal 2002, Parida et al. 2007, Slama et al. 2007, Mostajeran and Rahimi-Eichi 2009, Kumar et al. 2011). It was suggested that an amino acid like proline might play a highly valuable role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signalling molecule, which results in substantial reduction in ROS activity (Hayat et al. 2012).

Three possibilities can be predicted from the results: (1) *H. mucronatum* may have a certain chloroplast protein against oxidative damages, (2) high carotenoid concentrations and proline enhance the antioxidant ability of the *H. mucronatum* (3) the increased chlorophyll in *H. mucronatum* as compared to *C. cilliaris* provides a continuous and substantial energy supply to maintain quantum yield in drought stress. It has been suggested that the sensitivity, tolerance, and response timing of plants to drought vary among species. For example, slow-growing species have been found to be more sensitive than fast-growing species (Waseem et al. 2006, Munns 2002). This was seen in water stress; some drought-tolerant plants developed fitness by reducing leaf area and stomatal conductance to transpiration (Nativ et al. 1999, Ares et al. 2000). Thus, plants might adapt physiologically to drought conditions by reducing stomatal conductance to water vapour, increasing their water-use efficiency (WUE). Tolerant plants have been observed to adapt two different strategies during drought: long-living annuals and perennials decrease their leaf size and/or stomatal conductance (Geber and Dawson 1997, Querejeta et al. 2003), while shorter-living annuals maximize fitness by increasing stomatal conductance (decreasing WUE) to increase carbon gain and avoid drought stress. This strategy lets them grow rapidly, flower early, and increase yield before the start of substantial soil drying (Geber and Dawson 1997, Mckay et al. 2003). When drought is experienced at later developmental stages, selection should favour decreased stomatal conductance (high WUE) and smaller leaves, whereas when plants experience drought at early developmental stages, increased stomatal conductance (low WUE) should be selected for and leaf size may be of no adaptive value.

It was observed that antioxidant enzyme activity like SOD and CAT were higher in *H. mucronatum* than in *C. ciliaris* in a drought stress environment However, compared to control, both halophytes showed substantial increases in

antioxidant enzymes activities. It was reported that antioxidant enzymes like SOD, CAT, APX and GR played a significant role in combating drought stress and maintaining substantial growth rate under stress (Siddiqui 2013, Vujčić and Radić Brkanac 2014). It was observed that under stress, two different defensive mechanisms are provoked: a) an antioxidant non-enzymatic system such as a synthesis of osmolytes and phenols, and b) antioxidant enzyme systems such as synthesis and activity of enzymes like SOD, APX, CAT etc. (Siddiqui et al. 2014)

In conclusion, through higher photosynthetic performance, photo-quenching and lower stomatal conductance, maintaining substantial higher relative water content, *H. mucronatum* may be considered to have more drought tolerance than *C. ciliaris*. Furthermore, it was concluded that *H. mucronatum* maintains a substantially better performance index and lower H_2O_2 contents than *C. ciliaris,* which may be due to greater antioxidant enzyme activity, such as SOD and CAT.

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References

Abdel-Naseer, L. E., Abdel-Aal, E., 2002: Effects of elevated CO_2 and drought on proline metabolism and growth of safflower (*Carthamus mareoticus* L.) seedlings without improving water status. Pakistan Journal of Biological Sciences 5, 523–528.

Ares, A., Fownes, J. H., Sun, W., 2000: Genetic differentiation of intrinsic water-use efficiency in the Hawaiian native *Acacia koa*. International Journal of Plant Sciences 161, 909–915.

Ashraf, M. Y., Azmi, A. R., Khan, A. H., Naqvi. S. S. M., 1994: Water relation in different wheat (*Triticum aestivum* L.) genotypes under water deficit. Acta Physiologiae Plantarum 3, 231–240.

Ashraf, M. Y., Ala. S. A., Bhatti, A. S., 1998: Nutritional imbalance in wheat genotypes grown at soil water stress. Acta Physiologiae Plantarum 20, 307–10.

Ayerza, R., 1981: El Buffel grass: utilization and productivity of a promising grass. Hemisferio Sur, Buenos Aires Argentina (In Spanish).

Baker, N. R., 1991: Possible role of photosystem II in environmental perturbations of photosynthesis. Physiologia Plantarum 81, 563–570.

Barrs, H. D., Weatherley, P. E., 1962: A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences 15, 413–428.

Bates, L. S., Waldren, R. P., Teare, L. D., 1973: Rapid determination of free proline for water stress studies. Plant and Soil 39, 205–207.

Beyer, W. F., Fridovich, I., 1987: Assaying for superoxide dismutase activity: some large consequences of minor changes in condition. Annals of Biochemistry 161, 559–566.

Blokhina, O., Virolainen, E., Fagerstedt, K. V., 2003: Anti-oxidative damage and oxygen deprivation stress. Annals of Botany 91, 179–194.

Bradford, M. M., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annals of Biochemistry 72, 248–254

Breckle, S. W., 2009: Is sustainable agriculture with seawater irrigation realistic? In: Salinity and water stress. Tasks for Vegetation Sciences 44, 187–196.

Colom, M.R., Vazzana, C., 2002: Water stress effects on three cultivars of *Eragrostis curvula*. Italian Journal of Agronomy 6, 127–32.

De-Leon, M., 2004: Guidelines for the management of subtropical pastures. In: De Leon, M. Boetto, C. (eds.), Broadening the Cattle Frontier, Technical Note No. 1, INTA Cordoba, Argentina (In Spanish).

De-Pereira-Netto, A. B., De-Maganhaes, C. A. N., Pinto, H. S., 1999: Effect of soil water depletion on the water relation in tropical Kudzu. Pesquisa Agropecuaria Brasileira 7, 1151–1157.

El Hafid, R., Smith, D., Karrou, M., Samir, K., 1998: Physiological responses of spring durum wheat cultivars to early seasons drought in a Mediterranean environment. Annals of Botany 81:363–370.

Geber, M. A., Dawson, T. E., 1997: Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. Oecologia 109, 535–546.

Hayat S., Hayat Q., Alyemeni M. N., Wani A. S., Pichtel J., Ahmad A., 2012: Role of proline under changing environments. Plant Signal and Behavior 7, 1456–1466.

Karsten, H. D., MacAdam, J. M., 2001: Effect of drought on growth, carbohydrates and soil water use by perennial ryegrass, tall fescue and white clover. Crop Science 41, 156–66.

Katerji, N., van-Hoorn, Hamdy, J. W., Mastrorilli, A., Mou-Karzel, M. E., 1997: Osmotic adjustment of sugar beets in response to soil salinity and its influence on stomatal conductance, growth and yield, growth and yield. Agricultural Water Management 34, 57–69.

Khan, M. A., Ungar, I. A., 1999: Effect of salinity on the seed germination of *Triglochin maritima* under various temperature regimes. The Great Basin Naturalist 59, 144–150.

Kumar, R. R., Karajol, K., Naik, GR., 2011: Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeon pea (*Cajanus cajan* L. Millsp.). Recent Research in Science and Technology 3, 148–152.

Kwon, T. R., Siddiqui, Z. S., Harris P. J. C., 2009: Effect of supplemental Ca^{++} on ion accumulation, transport and plant growth of salt sensitive *Brassica rapa* landraces. Journal of Plant Nutrition 32, 644–667.

Li, X., Zhang, L., Li, Y., Ma, L., Bu, N., Ma, C., 2012: Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd. Plant Soil 352, 377–387.

Lichtenthaler, H. K., 1987: Chlorophylls and carotenoids: pigments of photosynthetic membranes. Methods in Enzymology 148, 350–382.

Liu, Y., Fiskum, G., Schubert, D., 2002: Generation of reactive oxygen species by mitochondrial electron transport chain. Journal of Neurochemistry 80, 780–787.

Lutts, S., Kinnet, J. M., Bouharmont, J., 1996: NaCl induced senescence in leaves of rice cultivars differing in salinity resistance. Annals of Botany 78, 389–398.

Mahiwal, G. L., Sutaria, P. M., 1992: Salt tolerance in wheat. Indian Journal of Plant Physiology 35, 258–61.

Maxwell, K., Johnson, G. N., 2000: Chlorophyll fluorescence: a practical guide. Journal of Experimental Botany 5, 1659–668.

Mckay, J. K., Richards, J. H., Mitchell-Olds, T., 2003: Genetics of drought adaptation in *Arabidopsis thaliana*. I. Pleiotropy contributes to genetic correlations among ecological traits. Molecular Ecology 12, 1137–1151.

Mehta, P., Jajoo, A., Mathur, S., Bharti, S., 2010: Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. Plant Physiology and Biochemistry 48, 16–20.

Merah, O., 2001: Potential importance of water status traits for durum wheat improvement under Mediterranean conditions. Journal of Agricultural Science 137, 139–145.

Molnar, I., Gaspar, L., Stehli, L., Dulai, E., Sarvari, E., kirali, I., Galiba, G., Molnar-Lang, M., 2002: The effect of drought stress on the photosynthetic processes of wheat and of *Aegilops biuncialis* genotype originating from various habitats. Acta Biologica Szegediensis 4, 115–116.

Mostajeran, A., Rahimi-Eichi, V., 2009: Effects of drought stress on growth and yield of rice (*Oryza sativa* L.) cultivars and accumulation of proline and soluble sugars in sheath and blades of their different ages leaves. American-Eurasian Journal of Agricultural and Environmental Sciences 5, 264–272.

Munns, R., 2002: Comparative physiology of salt and water stress. Plant Cell Environment 25, 239–250.

Munns, R., Hussain, S., Rivelli, A. R., James, R. A., Condon, A. G., Lindsay, M. P., Lagudah, E. S., Schachtman, D. P., Hare, R. A., 2002: Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant and Soil 247, 93–105.

Nakano, Y., Asada, K., 1981: Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiology 22, 867–880.

Nativ, R., Ephrath, J. E., Berliner, P. R., Saranga, Y., 1999: Drought resistance and water-use efficiency in *Acacia saligna*. Australian Journal of Botany 47, 577–586.

Naz, N., Hameed, M., Ashraf, M., Arshad, M., Ahmad, M. S. A., 2010: Impact of salinity on species association and phytosociology of halophytic plant communities in the Cholistan Desert, Pakistan. Pakistan Journal of Botany 42, 2359–2367.

Nedjimi, B., 2011: Is salinity tolerance related to osmolytes accumulation in *Lygeum spartum* L. seedlings? Journal of Saudi Society of Agricultural Sciences 10, 80–87.

Oukarroum, A., El Madidi, S., Schansker, G., Strasser, R. J., 2007: Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll fluorescence OLKJIP under drought stress and rewatering. Environmental and Experimental Botany 60, 438–446.

Parida, A. K., Dagaonkar, V. S., Phalak, M. S., Umalkar, G.V., Aurangabadkar, L. P., 2007: Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant Biotechnology Report 1, 37–48.

Parida, A. K., Das, A. B., Mittra, B., 2003: Effects of NaCl stress on the structure, pigment complex composition and photosynthetic activity of mangrove *Bruguiera parviflora* chloroplasts. Photosynthetica 41,191–200.

Patterson, B. D., Macrae, E. A., Ferguson, I. B., 1984: Estimation of hydrogen peroxide in plant extracts using titanium (IV). Annals of Biochemistry 139, 487–492.

Qadir, M., Qureshi, A. S., Cheraghi, S. A. M., 2008: Extent and characterization of salt-affected soils in Iran and strategies for their amelioration and management. Land Degradation and Development 19: 214–227.

Querejeta, J. I., Barea, J. M., Allen, M. F., Caravaca, F., Roldan, A., 2003: Differential response of delta C-13 and water-use efficiency to arbuscular mycorrhizal infection in two arid land woody plant species. Oecologia 135, 510–515.

Reza, M., Hassan, S., 2014: Physiological and biochemical changes of common Bermuda grass (*Cynodon dactylon* [L.] Pers.) under combined salinity and deficit irrigation stresses. South African Journal of Botany 92, 83–88.

Saini, M. L., Jain, P., Joshi, U. N., 2007: Morphological characteristics and nutritive value of some grass species in an arid ecosystem. Grass and Forage Science 62, 104–108.

Schonfeld, M. A., Johnson, R. C., Carver, B. F., Mornhiveg, D. W., 1988: Water relations in winter wheat as drought resistance Indicator. Crop Science 28, 529–531.

Sergiev, I., Alexieva, V., Karanov, E., 1997: Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. Proceedings of the Bulgarian Academy of Sciences 51, 121–124.

Siddiqui, Z. S., 2013: Effects of double stress on antioxidant enzyme activity in *Vigna radiata* (L.) Wilczek. Acta Botanica Croatica 72, 145–156.

Siddiqui, Z. S., Cho, J. L., Park, S-H., Kwon, T-R., Ahn B-Ok., Lee, K-S., Jeong, Mi-J., Kim, K-W., Lee S-K., Park S-C., 2014: Physiological mechanism of drought tolerance in transgenic rice plants expressing *Capsicum annuum* methionine sulfoxide reductase B2 (*CaMsrB2)* gene. Acta Physiologiae Plantarum 36, 1143–1153.

Siddiqui, Z. S., Khan, M. A., 2011: The role of enzyme amylase in two germinating seed morphs of *Halopyrum mucronatum* (L.) Stapf. in saline and non-saline environment. Acta Physiologiae Plantarum 33, 1185–1197

Siddiqui, Z. S., Khan, M. A., Kim, B. G., Huang, J. S., Kwon, T. R., 2008: Physiological response of *Brassica napus* genotypes in combined stress. Plant Stress 2, 78–83.

Slama, I., Ghnaya, T., Hessini, K., Messedi, D., Savoure, A., Abdelly, C., 2007: Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in *Sesuvium portulacastrum*. Environmental and Experimental Botany 61, 10–17.

Smirnoff, N., 1995: Antioxidant systems and plant response to the environment. In: Smirnoff N, (ed.), Environment and plant metabolism: flexibility and acclimation. Oxford: Bios Scientific 217–243.

Stefanov, D., Petkova, V., Denev, I. D., 2011: Screening for heat tolerance in common bean (*Phaseolus vulgaris* L.) lines and cultivars using JIP-test. Scientia Horticulturae 128, 1–6.

Strauss, A. J., Kruger, G. H. J., Strasser, R. J., Van Heerden, P. D. R., 2003: Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I-P. Environmental and Experimental Botany 56, 147–157.

Tavakol, E., Pakniyat, H., 2007: Evaluation of some drought resistance criteria at seedling stage in wheat (*Triticum aeativum* L.) cultivars. Pakistan Journal of Biological Sciences 10, 1113–1117.

Tijen, D., Ismail, T., 2006: Exogenous glycine betaine affects growth and proline accumulation and retards senescence in

two rice cultivars under NaCl stress. Environmental and Experimental Botany 56, 72–79.

Vujčić, V., Radić Brkanac, S., 2014: Physiological and biochemical responses of *Fibigia triquetra* (DC.) Boiss. to osmotic stress. Acta Botanica Croatica 73, 347–358.

Waseem, M., Athar, H. U. R., Ashrafi, M., 2006: Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. Pakistan Journal of Botany 38, 1127–1136.

Xia, J., Li, Y., Zou, D., 2004: Effects of salinity stress on PSII in *Ulva lactuca* as probed by chlorophyll fluorescence measurements. Aquatic Botany 80, 129–137.