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Response of differently adapted populations of blue panic grass (*Panicum antidotale* Retz.) to water deficit conditions

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Summary

To explore plant species that could tolerate harsh environment, six ecotypes of *Panicum antidotale* were collected from different habitats varying in water availability, salt content and agricultural practices within the Faisalabad city. All six ecotypes were grown under normal growth conditions for six months, after which time they were subjected to three drought levels (control (normal irrigation), 60% and 30% field capacity). Imposition of drought caused a marked reduction in shoot and root fresh and dry biomass, shoot length, chlorophyll *b*, *a/b* ratio, net CO_2 assimilation rate, transpiration rate, and water use efficiency in all six populations. While shoot P, root N, P and Ca^{2+} were remained unaffected due to application of drought stress conditions. Of the six ecotypes, population collected from sludge of disposal channel and that from the along the botanical garden produced higher quantity of plant biomass and net CO_2 assimilation rate as compared to the others.

Introduction

The genus *Panicum* comprises over 500 species distributed mostly in tropical and subtropical regions of the world. *Panicum antidotale*, Blue panic or Giant panic is a native of Southeast Asia. It is a robust and shortly rhizomatous perennial grass that grows up to 1.5 m with very deep root system (JACOBS and WALL, 1993). The flowers of *Panicum antidotale* are hermaphrodite (have both male and female organs) and are cross-pollinated through wind. Reproduction of this grass is either through seeds or by vegetative structures like rhizome (ENCYCLOWEEDIA, 2002). It is an excellent sand binder and prefers arid and semi-arid conditions (COPE, 1982). Blue panic has ability to withstand a variety of climatic conditions including severe environmental stresses like drought and salt stress (ASHRAF, 2004; AHMAD et al., 2010). It can tolerate salinity up to 15,000 mg L^{-1} and drought, using almost 50% less water than alfalfa (BOKHARI et al., 1988). Blue panic is an ideal fodder grass because of its high protein contents (15-18%) (BOKHARI et al., 1988).

Due to high tolerance against a multitude of stressful environments, blue panic is widely distributed in a variety of climatic conditions including the whole Indo-Pakistan region (AHMAD et al., 2010). It extensively occurs in arid and semi-arid region where plants usually experience drought stress (COPE, 1982).

Plants exhibit resistance to various stresses including drought by under-going morphological changes and alterations in genetic and biochemical attributes (LEVITT, 1972). Alteration in various mechanisms by plants to produce resistance against drought, tolerance at the cellular level is essential, because the cellular processes are most sensitive due to change in cell turgor under drought (TAIZ and ZEIGER, 2006). Reduced cell turgor may lead to impaired growth in most plant species (COLEMAN, 2008).

Drought-induced decrease in plant biomass has been extensively reported in the literature in many crops like wheat, maize, rice grasses etc. (MANABENDRA et al., 1998; AKRAM et al., 2008; KAMRAN

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et al., 2009). Drought stress also has a negative effect on net CO_2 assimilation rate, transpiration rate and stomatal conductance (ARES et al., 2000). Mineral composition of soil is also changed under drought stress which causes poor absorption of minerals (GARG et al., 2004; SAMARAH et al., 2004).

The responses of plants to drought depend on the severity of drought, i.e., intensity and duration of drought. It also depends on the plant species and developmental growth stage (CHAVES et al., 2003). Of morphological traits required to resist to early drought stress conditions, a deep and dense root system is probably the most important one (GREGORY, 1989; ROBERTSON et al., 1993). High root growth by diverting assimilates from shoots towards roots is also a strategy of plants to cope with the adverse effects of drought stress, and resulting in to high root/shoot ratio (O'TOOL and BLAND, 1987).

Ecotypes of *Panicum antidotale* are found cultivated on ecologically distinct habitats in the Faisalabad region (GELANI, 2000). Due to the distribution of this grass on a wide range of habitats, it is expected that all these ecotypes are potentially adapted to a variety of environmental stresses like drought, salinity, toxic nutrients etc. It is also hypothesized that ecotypes inhabiting a particular habitat since a long period must have genetically adapted on this environment. Thus, the main objective of the present study was to examine variation in drought tolerance of differently adapted blue panic grass ecotypes using some key physiological attributes.

Materials and methods

An experiment was conducted in a wire-house in the Botanical Garden, University of Agriculture, Faisalabad (UAF), Pakistan (latitude 31°30 N, longitude 73°10 E and altitude 213 m). Six populations of blue panic (*Panicum antidotale*) grass were collected from different habitats from the suburbs of Faisalabad city. Brief description of the habitats is presented in Tab. 1. the habitats were selected on the basis of water availability, salt content, soil texture, physico-chemical characteristics and environmental conditions.

Soil analysis

The soil adhering to the roots was used to analyze various physico-chemical characteristics. The soil pH and ECe were determined by using pH and EC meter respectively. Analysis of soil was carried out following the specific methods described in Hand Book No.60 (US SALINITY LABORATORY STAFF, 1954).

Experimental detail:

A total of 90 to 100 plants of each population were collected from each habitat and all plants were established in normal soil under natural conditions for six months in the Botanical Garden of the University of Agriculture, Faisalabad-Pakistan. Young tillers of uniform size from each population were collected and then transplanted in plastic pots (30 cm diameter; at a rate of 6 tillers per

Tab. 1: Habitats details and physico-chemical characteristics of soils from where *Panicum antidotale* ecotypes were collected.

Characteristics Sites of collection	Population 1 Along a water channel, UAF	Population 2 On sludge of a disposal channel, UAF	Population 3 Dry shady condition, UAF	Population 4 Along roadside, UAF	Population 5 A derelict field, UAF	Population 6 Botanical garden, UAF
Habitat description	Roots are exposed to highly saline water	Soft textured soil	Very hard textured saline-sodic soil	Dry rain-fed loamy soil	Undisturbed land	Moist shady loamy soil
K$^+$ (mg kg^{-1} dry soil)	90	70	175	40	115	60
Ca^{2+} (mg kg^{-1} dry soil)	44	19	20	13	12	16
N (%)	4.70	7.90	0.52	1.86	1.24	0.86
P (%)	0.06	0.04	0.06	0.04	0.03	0.05
Saturation percentage	40	35	23	29	27	22.2
pH	6.6	7.35	6.8	8	7.65	6.70
ECe (dS m^{-1})	19.8	2.5	17.4	1.5	1.4	2.9

pot) each having 8 kg dry sandy soil. Soil with pH = 7.4, ECe = 2.8 dS m^{-1}, and saturation percentage = 27.7 was used for this study. When the plants grown in pots attained a height of about 60 cm, their shoots were clipped at the height of 30 cm and then subjected to three levels of drought (Control = normal irrigation, 60% field capacity, and 30% field capacity) in four replications. A total 72 pots were used in this study. After four weeks of plant growth under drought, all plants were harvested from each pot carefully, washed with distilled water and data for the following attributes were recorded:

1. Shoot and root fresh and dry weights (g): Four plants from each pot were used for appraising shoot and root fresh and dry biomass, while the remaining two used for quantifying chlorophyll contents and mineral nutrients. Both parts, i.e., shoots and roots were weighed separately and then oven-dried at 65 °C for one week.

2. Chlorophyll pigments: The chlorophyll contents were determined from green leaves following ARNON (1949). Fresh leaf material (0.2 g) was extracted in 80 % acetone and centrifuged at 10,000 x *g* for 5 minutes. Absorbance of the supernatants of all samples was measured at 663 and 645 nm using UV-Visible spectrophotometer (Hitachi-U2001, Tokyo, Japan).

3. Gas exchange parameters: Net CO_2 assimilation rate and other gas exchange characteristics were measured using an open system portable infrared gas analyzer (LCA-4; Analytical Development Company, Hoddesdon, England). These measurements were made on a fully expanded youngest leaf from each plant from 10:15 to 12:45 hours with the following adjustments of leaf chamber:ambient CO_2 concentration (C$_{ref}$) 353 µmol mol^{-1}, molar flow of air per unit leaf area (Us) 221.04 mol m^{-2} s^{-1}, temperature of leaf chamber varied from 31.4 to 37.9 °C, leaf surface area 11.35 cm^2, ambient pressure 99.3 kPa, water vapor pressure into the chamber ranged from 0.0006 to 0.00089 MPa, leaf chamber gas flow rate (V) 252 µmol s^{-1}, *PAR* (Q$_{leaf}$) at the leaf surface was maximum up to 1048 µmol m^{-2} s^{-1}.

4. Determination of mineral elements in plant tissues
The dried ground plant material (shoot or root) (0.1 g) was digested with a mixture of sulphuric acid and hydrogen peroxide following WOLF (1982). The digested samples were analyzed for potassium and calcium using a flame photometer (PFP7; Gransmore Green, Dunmow UK). Phosphorus was determined spectrophotometrically

(Hitachi-U2001, Tokyo, Japan) following the method of JACKSON (1962) and N by titration method following ALLEN et al. (1998).

Statistical analysis
Data for different parameters were analyzed statistically by adopting two-way analysis of variance technique based on completely randomized design with four replications according to STEEL and TORRIE (1997). The least significance difference test (LSD) was used for appraising the significant difference between the mean values (SNEDECOR and COCHRAN, 1980).

Results
Drought stress significantly reduced shoot fresh and dry weights of all populations of *Panicum antidotale* (Tab. 2; Fig. 1A and B). Since the populations differed significantly in control values, so it is not legitimate to interpret the results on mean fresh and dry weights basis. Thus, the populations were compared on percent of control basis (In Fig. 1 values presented in parenthesis are percent of control). At 60 and 30 % field capacity, populations 2 and 6 were higher in percent shoot fresh and dry weights as compared to the other populations (Fig. 1), while the population 1 was the lowest of all six populations.

Root fresh and dry weights also decreased significantly under water deficit conditions. Of the six diverse populations collected from different habitats, populations 3 and 4 were better as compared to the others under well watered or water deficit conditions. Populations 2 and 6 were lower in root fresh and dry weights than those of the other populations of *P. antidotale* (Tab. 2; Fig. 1C and D).

Shoot length of all populations was significantly reduced under water deficit conditions (Tab. 2; Fig. 1E). Of different populations, populations 2 and 3 were higher and populations 5 and 6 lower in shoot length than the other populations at 60% and 30% field capacities, respectively.

Drought stress significantly affected chlorophyll *a* and *b* contents of all populations of *P. antidotale* (Tab. 2; Fig. 1F and G), while the populations did not differ significantly in both chlorophyll *a* and *b* pigments under non-stressed or drought stress conditions. In chlorophyll *a/b* ratio (Fig. 1H), population behavior was different. Populations 2 and 3 showed an increase in chlorophyll *a/b* ratio at

Tab. 2: Mean squares from analyses of variance of data for growth attributes and gas exchange characteristics of *Panicum antidotale* when plants were subjected to control or water deficit conditions for three weeks ($n = 4$)

Source of variation	Degrees of freedom	Shoot fresh weight	Shoot dry weight	Root fresh weight
Drought (D)	2	17367.7***	3933.6***	2837.1***
Populations (P)	5	518.21***	133.61***	259.37***
D x P	10	452.66***	114.70***	139.47***
Error	54	70.968	20.380	29.651
Source of variation	**Degrees of freedom**	**Root dry weight**	**Shoot length**	
Drought (D)	2	1030.2***	7278.9***	
Populations (P)	5	123.63**	230.72**	
D x P	10	69.783***	233.01***	
Error	54	21.064	51.279	
Source of variation	**Degrees of freedom**	**Chlorophyll *a***	**Chlorophyll *b***	**Chlorophyll *a/b* ratio**
Drought (D)	2	0.592***	3.314***	0.010***
Populations (P)	5	0.035ns	0.078ns	0.041***
D x P	10	0.058***	0.160**	0.013***
Error	54	0.015	0.047	0.00011
Source of variation	**Degrees of freedom**	***A***	***E***	***g_s***
Drought (D)	2	4259.5***	44.252ns	76834.1***
Populations (P)	5	320.72***	16.67ns	105407.2***
D x P	10	213.25***	32.694ns	13381.1***
Error	54	52.53	18.52	19.85
Source of variation	**Degrees of freedom**	***C_i***	**WUE**	***C_i/C_a***
Drought (D)	2	177589.6ns	420.86ns	0.012ns
Populations (P)	5	47355.7ns	705.34*	0.043ns
D x P	10	165651.3**	243.97ns	0.040**
Error	54	61140.6	207.12	0.015

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively.
　　　ns = non-significant

60% field capacity, while all other populations showed a decrease in this attribute. Maximum reduction in chlorophyll *a/b* ratio was observed in population 6 at 30 % field capacity.

Imposition of different water regimes caused a significant reduction in net CO_2 assimilation rate, transpiration rate and stomatal conductance (Tab. 2; Fig. 2A, B and C). This reduction was maximum at 30% field capacity. Populations 4, 5, and 6 were lower in photosynthetic rate than the others at 30 or 60% field capacity. However, populations 2 and 5 had significantly lower transpiration rate than the other populations under water deficit conditions. Stomatal conductance was lower in all populations except populations 3 and 4 at both 30 and 60% field capacities and population 1 had high g_s value at 60% field capacity (Fig. 2).

Populations 1, 2 and 5 were higher in water use efficiency (*A/E*) as compared to the other populations under normal or drought stress conditions (Tab. 2; Fig. 2E). Data for sub-stomatal CO_2 concentration and C_i/C_a showed that neither the drought caused a significant effect nor populations differed significantly for this attribute (Tab. 2; Fig. 2D and F).

All six populations of *P. antidotale* differed significantly for shoot N. Shoot N was high in populations 2, 3 and 5 at 30% field capacity while in others the difference in shoot N was not discernable (Tab. 3; Fig. 3A). However, maximum root N contents were observed in population 4 at 30% field capacity (Fig. 3B).

Imposition of water deficit conditions had no significant effect on shoot P (Tab. 3; Fig. 3C). Of various populations, an increase in shoot P was observed in population 4 and a decrease in populations 5 and 6 at 60% and 30% field capacities. Population 5 was the lowest in root P of all populations under drought stress conditions, while in the others the difference was not significant (Fig. 3D).

Water deficit conditions significantly increased shoot K^+ concentration of all populations, but for root K^+, the pattern of increase or decrease was not consistent (Tab. 3; Fig. 3E and F). Water deficit conditions had no significant effect on shoot Ca^{2+} or root Ca^{2+} concentrations. Populations also did not differ significantly in both these attributes (Tab. 3; Fig. 3G and H).

Discussion

Water scarcity is known to directly reduce the plant productivity (WASEEM et al., 2006). However, responses of plants to water deficit conditions are very complex. Of these, adaptive changes are of importance (CHAVES et al., 2002) like decrease in leaf area which further causes reduction in net CO_2 assimilation rate and growth (PEREIRA and CHAVES, 1993). Decrease in growth is common in all crop plants under drought stress. Plants tend to cope this adverse effect by adopting various strategies. Thus, there is a need to explore such mechanisms or criteria adopted by the plants which might differ on locality basis in even a single species.

Availability of water is an effective selective pressure which is an important factor in driving the evolution of natural plant populations
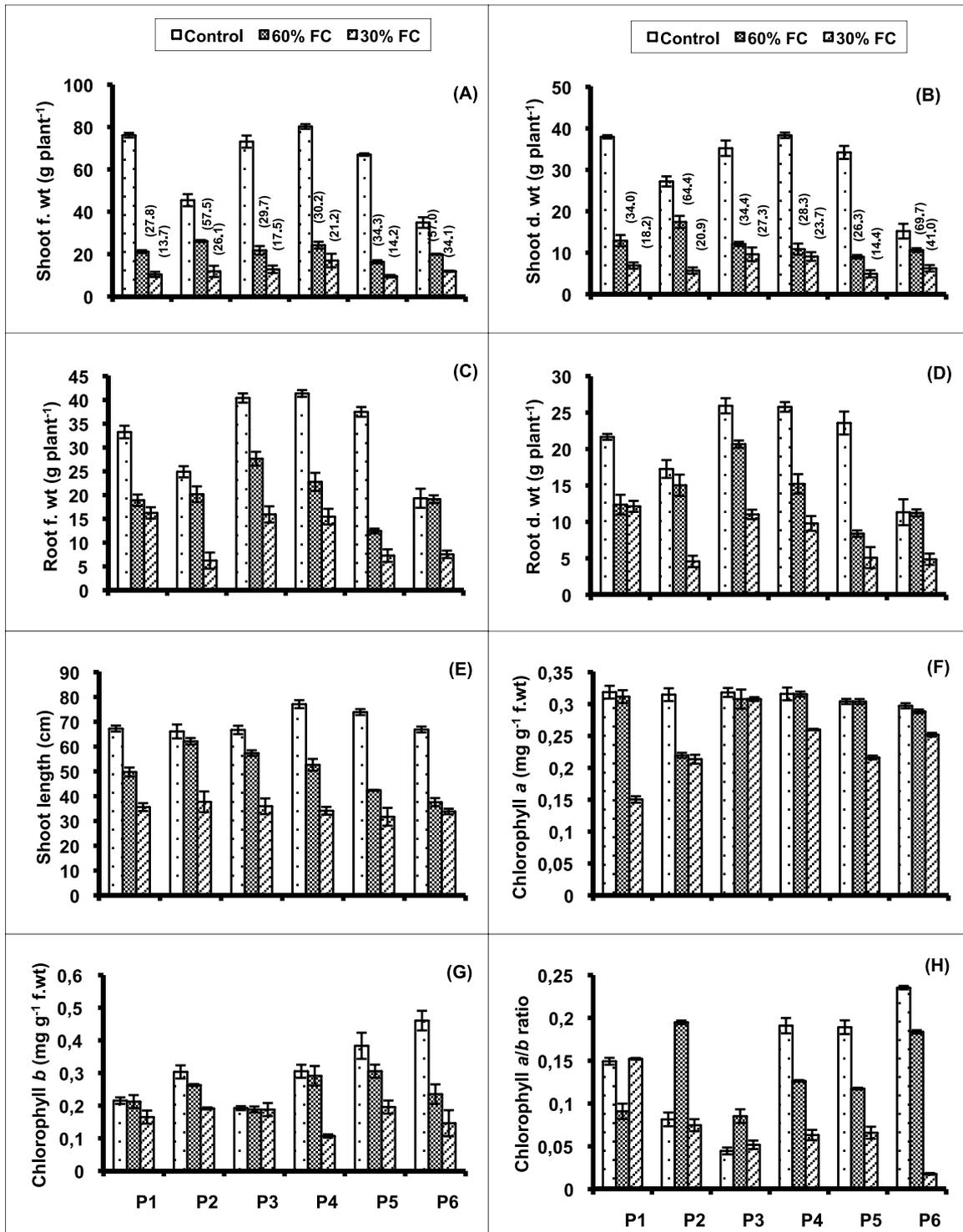
Fig. 1: Growth attributes and chlorophyll contents of *Panicum antidotale* Retz when plants were subjected to various water deficit conditions for three weeks (Figs. in parenthesis are percent of control) (P1 to P6 = Populations 1 to 6), FC = Field capacity

for drought tolerance (BENNINGTON and MCGRAW, 1995; DUDLEY, 1996a, b; HESCHEL et al., 2002). However, natural populations evolve a multitude of morpho-anatomical and physiological characteristics that enable the plants to thrive well under harsh environmental conditions including drought stress.

In present study, water deficit conditions reduced plant biomass (both fresh and dry biomass) of all six ecotypes of blue panic grass. Reduction in biomass under water deficit conditions has already

been observed in many crops, e.g., grasses (ASHRAF and YASMIN, 1995), wheat (PESCHKE et al., 1997; ASHRAF et al., 1998), maize (ABRECHIT and CARBERRY, 1993) and rice (MANABENDRA et al., 1998). However, reduction in plant biomass differed among different ecotypes of *P. antidotale*. Populations collected from sludge of disposal channel and that from the botanical garden were superior in shoot biomass production as compared to the other populations. One of important factor involved in plant growth is photosynthesis.
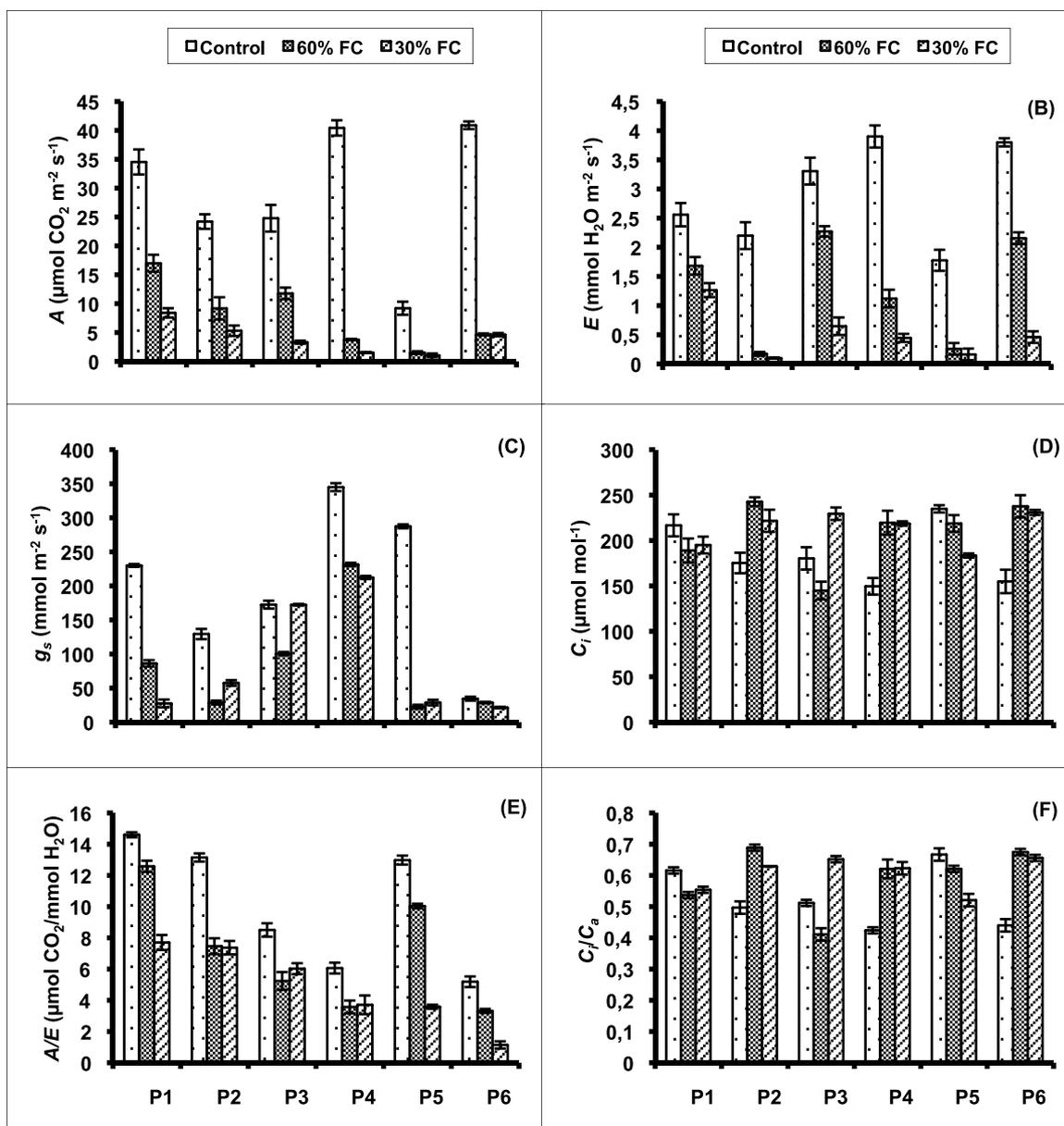
Fig. 2: Gas exchange of *Panicum antidotale* Retz when plants were subjected to various water deficit conditions for three weeks (P1 to 6 = Populations 1 to 6), FC = Field capacity

High biomass in above mentioned populations might due to high photosynthetic rate. Inter-cultivar difference has already been observed in barley (BLUM, 1996; MARTIN et al., 1991), blackgram (ASHRAF and KARIM, 1991), lentil and Indian mustard (WRIGHT et al., 2001), and *Brassica carinata* (ASHRAF and SHARIF, 1998).

Growth and photosynthesis are two important aspects interlinked with each other. Ratio of photosynthetic rate and transpiration rate is known as water use efficiency (WUE). Under drought stress plants adapt themselves by improving water use efficiency and decreasing stomatal conductance (DUDLEY, 1996a; NATIV et al., 1999; ARES et al., 2000). Improvement in water use efficiency might be due to reduced in leaf size under drought stress as a result of which plants reduce water loss from leaves (GIVNISH, 1979) and stomatal conductance also decreases under water shortage (ZANGERL and BAZZAZ, 1984). In the present study, a marked reduction in stomatal conductance has been observed in all ecotypes collected from various habitats. The reduced stomatal conductance has positively

enhanced the water use efficiency in all populations but on the other hand, reduction in stomatal conductance resulted in decreased net CO_2 assimilation rate and it may happen even at moderate level of drought stress (ATHAR and ASHRAF, 2005). In most of the species reduction in growth is correlated well with reduced photosynthetic activity but this is not true for all species (ATHAR and ASHRAF, 2005). In the present study, plant growth was positively correlated with net CO_2 assimilation rate ($r = 0.719***$) and thus the drought-induced reduction in biomass production could have been due to reduced photosynthetic rate under drought conditions.

Plants require mineral nutrients for their proper growth and development, but under drought stress the soil mineral composition is also impaired (MARSCHNER, 1995). Decrease in water availability under drought generally results in reduced total nutrient uptake and frequently reduces the concentrations of mineral nutrients in crop plants (MARSCHNER, 1995; BALIGAR et al., 2001). Generally, plants showing small reduction in absorption of nutrients are considered

Tab. 3: Mean squares from analyses of variance of data for shoot and root mineral nutrients of *Panicum antidotale* when plants were subjected to control or water deficit conditions for three weeks ($n = 4$)

Source of variation	Degrees of freedom	Shoot N	Shoot P	Shoot K$^+$
Drought (D)	2	0.0004**	0.0023ns	423.04***
Populations (P)	5	0.0002**	0.0310**	73.86***
D x P	10	0.0004***	0.0242**	107.75***
Error	54	0.00006	0.0073	5.211
Source of variation	**Degrees of freedom**	**Root N**	**Root P**	**Root K$^+$**
Drought (D)	2	0.0003ns	0.0164ns	35.170***
Populations (P)	5	0.0005ns	0.0031ns	32.695***
D x P	10	0.0010*	0.0033ns	21.299***
Error	54	0.0005	0.0055	5.550
Source of variation	**Degrees of freedom**	**Shoot Ca^{2+}**	**Root Ca^{2+}**	
Drought (D)	2	9.753**	1.941ns	
Populations (P)	5	0.964ns	1.647ns	
D x P	10	2.128ns	6.541***	
Error	54	1.294	0.838	

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively.
         ns = non-significant

as drought resistant (GUNES et al., 2006) but plant species and even genotypes within species may vary in their response to mineral uptake under water stress (GARG, 2003). In the present study, all six *P. antidotale* ecotypes collected from different habitats showed a significant difference in shoot N, P, K$^+$ and root K$^+$. Likewise, a large amount of variation in N, P and K$^+$ uptake has already been reported in a set of 20 chickpea genotypes (GALLANI et al., 2003). Furthermore, shoot K$^+$ and Ca^{2+} concentrations were higher in all ecotypes under drought stress as compared to those in plants experienced normal watering. High Ca^{2+} concentration under drought stress has already been reported in pearl millet (ASHRAF et al., 2001). Both K$^+$ and Ca^{2+} are very important for plant growth in terms of stomatal regulation and membrane stability, respectively (MENGEL and KIRKBY, 2001). Efficient plants are active in uptake of N, P, and K$^+$ as compared to drought sensitive plants under drought stress (RENGEL, 2001). Similarly, drought tolerant cultivars accumulated more N, P, K$^+$, and Ca^{2+} as compared to sensitive as observed in chickpea (GUNES et al., 2006), soybean (SAMARAH et al., 2004), and grasses (AKRAM et al., 2008). In shoot K$^+$, populations collected from sludge of disposal channel and from dry shady conditions showed higher concentration of shoot K$^+$ as compared to the other ecotypes, but the difference in populations with respect to shoot or root Ca^{2+} was not prominent.

In conclusion, the ecotypes of *P. antidotale* collected from different habitats showed a significant reduction in plant fresh and dry biomass, chlorophyll pigments, photosynthetic and transpiration rates, while an increase in shoot N, P, K$^+$ and root K$^+$ was observed under water deficit conditions. Of all six ecotypes, one collected from disposable channel sludge and the botanical garden showed higher plant biomass in comparison with the others. Increase in plant biomass was found to be associated with higher photosynthetic rate in all six ecotypes. In other physiological attributes like net CO$_2$ assimilation rate, water use efficiency and shoot N the populations collected from habitats containing with high amount of sludge and that from dry shady conditions performed better performance as compared to the other populations.

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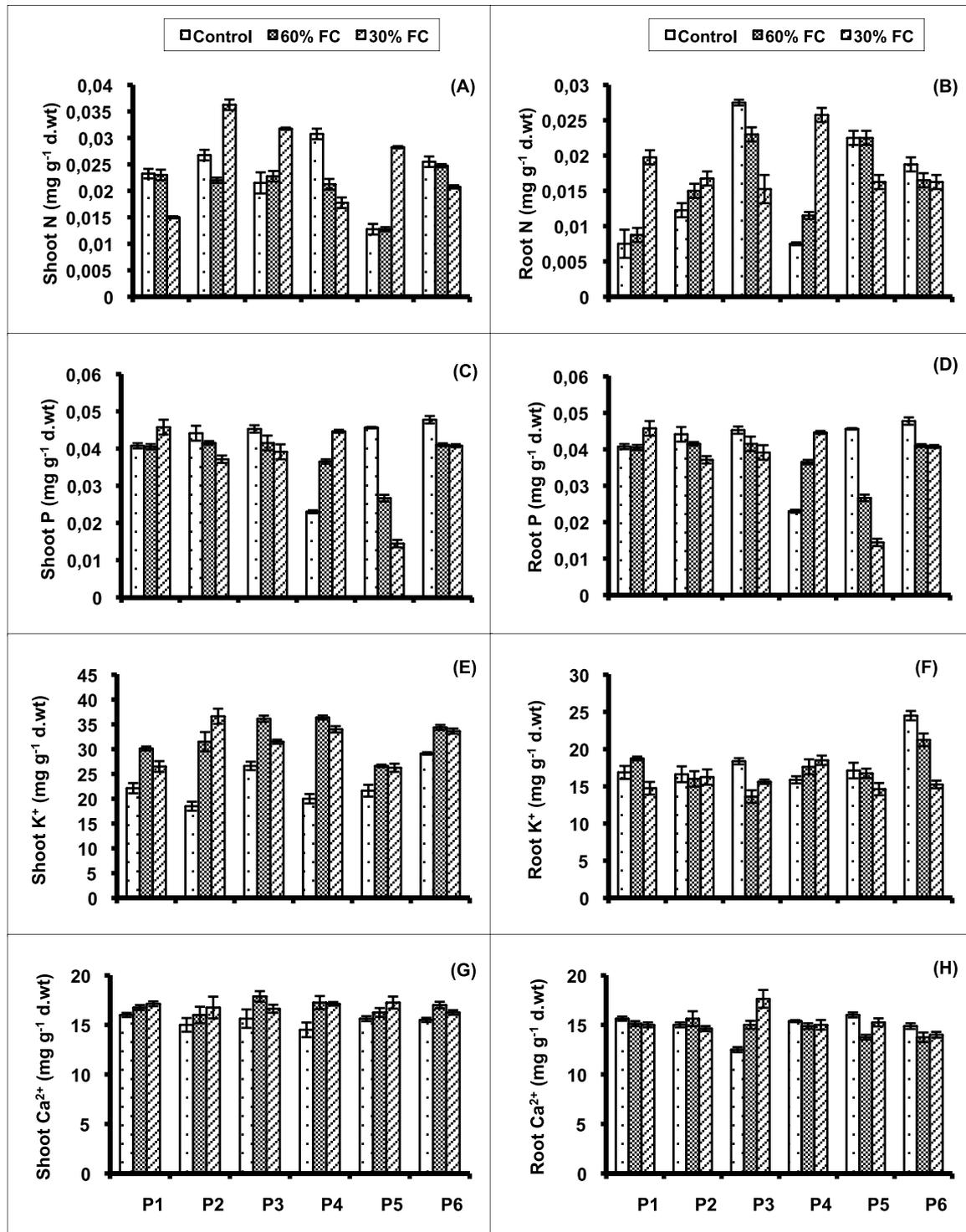
Fig. 3: Mineral nutrients in different populations of *Panicum antidotale* Retz when plants were subjected to various water deficit conditions for three weeks (P1 to 6 = Populations 1 to 6), FC = Field capacity

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