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Evaluation of Potassium Compared to Other Osmolytes in Relation to Osmotic Adjustment and Drought Tolerance of Chickpea Under Water Deficit Environments

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ABSTRACT

Line source sprinkling irrigation system was used to evaluate potassium (K) and other major osmolytes (sugars, proline, total amino nitrogen, and potassium) in respect with differential yielding capabilities of two groups of chickpea varieties (BR group with brown-red seed and PY group with pale yellow seed) under water deficit. Leaf water relation parameters, osmolytes, and total osmotic potential at full turgor ($\Psi_{\pi 100}$) were studied at early flowering (EF), late flowering (LF), and early fruiting (Eft) stages. Of the osmolytes, K accumulated in the highest amount (35 to 55% of the total $\Psi_{\pi 100}$) in chickpea leaves regardless of growth stages and varietal groups. Varietal groups showed substantial accumulation of SPN (sugars + proline + amino nitrogen) and K with the increase in soil moisture stress irrespective of growth stages, the BR group proving to be a better accumulator of the osmolytes than PY group in most cases. However, the contribution of SPN to total $\Psi_{\pi 100}$ increased, while that of K decreased generally with increasing soil moisture stress, particularly at Eft stage. The OA capacity of BR varieties increased, while that of PY varieties decreased significantly at EF and LF stages along the line source moisture gradient. However, potassium-contribution to OA decreased largely with the increase in water deficit and crop age. In fact, it contributed negligibly to OA at the Eft stage in case of both the varietal groups. Osmotic parameters, namely, total OA and $\Psi_{\pi 100}$ as well as $\Psi_{\pi 100}$ due to SPN and K, correlated linearly ($P \leq 0.05$ or $P \leq 0.01$) with seed yield, relative water content, and drought tolerance efficiency under water deficit, indicating their significant role in drought tolerance of the crop. Since BR varieties proved generally superior to PY varieties regarding all the osmotic parameters, the superior yielding capability of BR to PY varieties (26–30% yield benefit

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under water deficit), could be ascribed to the favorable effect of osmotic parameters on the sustenance of crop yield under water deficit. Thus, chickpea varieties could be selected for improved drought tolerance on the basis of total OA, $\Psi_{\pi 100}$ and $\Psi_{\pi 100}$, due to osmolytes. Moreover, the $\Psi_{\pi 100}$, due to K, could prove as a good selecting tool; however, further research is required to know if the chickpea varieties with high efficiency of K accumulation across the growth stages are able to withstand water deficit more efficiently.

Keywords: chickpea, osmolytes, osmotic adjustment, osmotic potential, potassium, RWC, water potential

INTRODUCTION

A favorable effect of potassium (K) application on plant water relations during water deficit has been reported for several crops (Pier and Berkovitz, 1987; Shahid Umar and Moinuddin, 2002). Increased application of K has also been shown to enhance photosynthetic rate, plant growth, yield, and drought resistance in different crops under water stress condition (Shahid Umar and Moinuddin, 2002; Egilla et al., 2001). Further, a favorable relationship between K content of leaves and plant water relations under water deficit has been observed (Arneke, 1981). Besides being used as osmoticum, K has been reported to stimulate the osmotically active solutes such as malate (Beringer, 1978) and proline (Sashidhar et al., 1981), which could be used as potential osmotica during osmotic adjustment (OA) under water deficit. In fact, K is a predominant low molecular weight inorganic ion accumulating during drought stress in case of various crops (Jones et al., 1980; Premachandra et al., 1991; Morgan, 1992; Renu Khanna-Chopra et al., 1994; Iannucci et al., 2002). It can accumulate in plants in concentrations ranging from 50 to 150 mM in the cytoplasm and vacuoles of the cells without imposing any harmful effect on plant metabolism (Leigh and Jones, 1984). Thus, it is desirable to evaluate the role of K in crop plants during water stress in relation to sustenance of their yield.

The present investigation was carried out on two groups of chickpea varieties differing in seed color (brown-red, BR and pale yellow, PY). The two groups also differed significantly in seed yield under drought stress. Since OA has been reported to be an important drought-adaptation mechanism in many crop plants under water deficit (Morgan, 1984; Subbarao et al., 2000), this investigation was carried out (1) to explore if the difference in seed yield of the two groups of chickpea varieties was due to the difference in OA and (2) to evaluate K and other major osmolytes (sugars, praline, and free amino nitrogen(N)) with respect to OA and quantify their contribution to OA and drought tolerance of the two chickpea groupsunder water deficit. Such knowledge of plant adaptive strategies to water stress and their physiological basis may serve to formulate plant breeding and management strategies adapted to semi-arid environmental conditions.

MATERIALS AND METHODS

Plant Material and Field Experimentation

The field experiment was conducted in the winter season of 1992 to 1993 at the Indian Agricultural Research Institute, New Delhi, India. Eight varieties of chickpea (*Cicer arietinum* L.) namely, BG-329, BG-365, BG-372, BG-380, BG-384, BG-1001, PUSA-256, and PUSA-267 were field grown with a line source sprinkler irrigation system. The experimental field comprised of 12 m long and 1.5 m wide beds laid out on either side of the line source. These beds were further divided length-wise into four moisture treatments, viz. T_1, T_2, T_3, and T_4, of 3 m $\times$ 1.5 m (4.5 m^2) each. The results reported in this paper are from the T_1, T_3, and T_4 moisture levels only, because in most cases there was no significant difference in plant water relations parameters at the T_1 and T_2 moisture levels which were nearest to the line source. In each treatment bed, seeds were sown at 80 kg ha^{-1} in six rows. Row to row distance was 0.25 m. There were four replications. The treatment plot nearest to the line source (T_1) received maximum water, and the water deficit increased progressively with the distance from the line source. Nitrogen, phosphorus (P_2O_5), and potassium (K_2O) were supplied as urea, single superphosphate, and muriate of potash, respectively, at the rate of 20: 60: 60 kg ha^{-1} at the time of sowing. The soil of the experimental field was sandy loam with a mean depth of 3 m and bulk density of 1.55 Mg m^{-3}. The available surface (0–180 cm soil depth) soil water content measured gravimetrically at the time of sowing was 233 mm. Subsequently, three irrigations were given at 30, 50, and 80 d after sowing (DAS) using the line source sprinkler irrigation system. Total rainfall during the crop season was 51.3 mm. The line source irrigation cumulatively supplied 92.7, 51.7, and 2.8 mm water, with the total water available to the crop being 377, 336, and 287 mm in the T_1, T_3, and T_4 moisture levels, respectively. The line source irrigation applications were always made when the wind speed was low (<1 m s^{-1}). All the chickpea cultivars flowered within 5 d and matured almost at the same time. At harvest, four 2 m rows of plants were hand harvested (total harvest area 2 m $\times$ 1 m = 2m^2) from each of the four plots in each moisture treatment. Seed yield of each treatment plot was recorded at harvest. The performance of the genotypes regarding all the parameters studied was assessed group-wise, viz. brown-red and pale yellow color of seed. Of the 8 chickpea genotypes tested, 6 (Pusa-256, BG-329, BG-365, BG-372, BG-384, and BG-386) belonged to brown-red seed color (BR group) and two (Pusa-267 and BG-1001), to pale yellow seed color (PY group).

Leaf Water Relations Parameters

Leaf water relation parameters comprised of water potential (ψ_w), osmotic potential (ψ_π), and relative water content (RWC) were measured during the crop reproductive phase at early flowering (104 DAS), late flowering (114 DAS), and early fruiting (130 DAS) stages using leaves having flowers in their exils. Leaf ψ_w was measured between 1000 and 1100 h on each sampling date in four replicates by the use of a pressure chamber (Soil Moisture Equipments Corp., Santa Barbara, CA). The same leaves were then frozen in sealed polyethylene vials in a freezer at -20°C. After thawing at room temperature ($\approx$15 min), cell sap was expressed using a hand press, and the ψ_π of the cell sap was measured with a vapor pressure osmometer (model 5500, Wescor, Inc., Logan, UT). The osmometer was calibrated with known concentrations (mmol kg^{-1}) of NaCl solutions. The values of ψ_π in mmol kg^{-1} were converted to pressure unit (MPa) according to the following equation: ψ_π (MPa) $= -R \times T \times$ mol kg^{-1}, where R is the gas constant (0.008314) and T is the temperature measured in the Kelvin scale (298 K). The ψ_π values were corrected ($\psi_\pi + 0.1\ \psi_\pi$) for the dilution of symplastic sap by apoplastic water, assuming 10% apoplastic water (Kramer, 1983). The osmotic potential at full turgor ($\psi_{\pi 100}$) was calculated, using the following equation: $\psi_{\pi 100} =$ (Corrected $\psi_\pi \times$ RWC)/100. Osmotic adjustment was expressed as the difference between leaf $\psi_{\pi 100}$ of irrigated (at T_1 moisture level) and stressed (at T_3 and T_4 moisture levels) plants. Turgor potential (ψ_ρ) was computed as the difference between ψ_w and ψ_π measured on the same leaves. Relative water content (RWC) was determined by the method of Barrs and Weatherly (1962) according to the following equation: RWC $= 100 \times (FW - DW)/(TW - DW)$, where FW is fresh weight, DW is dry weight, and TW is turgid weight of the leaf samples (4-mm diameter leaf discs). The turgid weight was determined after floating the leaf discs on distilled water for 24 h at room temperature (about 20°C) under dim light, whereas, dry weight was measured after oven-drying the samples at 80°C for 48 h.

Chemical Analyses of Osmolytes

The leaf sap was analyzed chemically to quantify the osmolytes, viz. sugars, proline, amino nitrogen, and K. The cell sap, squeezed from the frozen and thawed leaves, was stored in the freezer in plastic Eppendorff tubes with attached airtight lids. At the time of chemical analyses, the Eppendorff tubes were again thawed and analyses regarding the osmolytes were carried out. 50 μL of leaf sap were dispensed into separate test tubes for the estimation of total sugars, total amino acids, praline, and K. To minimize sucrose hydrolysis, hot 80% ethanol was immediately added to the sap samples for sugar analysis, bringing their volume to 1 mL. Total sugars were determined colorimetrically by the method of Dubois et al. (1956). Total free amino nitrogen was also assessed with the help of colorimeter using the method of Herridge (1984) for xylem exudates. The colorimetric method described by Bates et al. (1973) was employed for proline measurements. Potassium was determined using a flame photometer

(410 Corning). The contribution of measured osmolytes to total $\psi_{\pi 100}$ and OA was computed from their concentration in osmotic volume of tissue at 100% RWC. The contribution of each solute to total $\psi_{\pi 100,}$ calculated as mmol kg^{-1}, was expressed as percent of $\psi_{\pi 100}$ measured from the same sample. The osmotic potential (mmol kg^{-1}) of individual solutes were corrected and converted to pressure units (MPa) as expressed above for total $\psi_{\pi 100}$.

Statistical Analysis

Statistical analyses were carried out according to split block design, with irrigation levels arranged systematically in each replicate along the gradient of applied water and genotypes randomized in each replicate. All the parameters were subjected to analysis of variance (ANOVA). Fischer's least significant difference (LSD) was used to test for the significance of the differences between means of varietal groups and moisture levels at $P \leq 0.05$ according to Gomez and Gomez (1984). Regression analyses were determined between the parameters of interest.

RESULTS

Leaf Water Relations Parameters

In general, the values regarding leaf water relations parameters, namely, water potential (ψ_w), osmotic potential at full turgor ($\psi_{\pi 100}$), turgor potential (ψ_ρ), and relative water content (RWC), decreased progressively from T_1 to T_4 moisture level regardless of growth stages and varietal groups. The lowest values of all the water relations parameters were generally obtained at Eft stage irrespective of moisture levels. The $\psi_{\pi 100}$ generally increased (turned less negative) at LF stage, decreasing thereafter to the lowest extent at Eft stage. However, mostly ψ_w, ψ_ρ and RWC decreased at LF stage, regardless of varietal groups. In general, BR varieties showed significantly lowered values than PY varieties regarding ψ_w and $\psi_{\pi 100}$, whereas, they showed greater values than PY varieties regarding ψ_ρ and RWC (Table 1).

Osmotic Potential Due to Osmolytes

Among the measured osmolytes, the lowest (most negative) osmotic potential was shown by K, followed by amino nitrogen, praline, and sugars at EF and LF stages, while at Eft stage, proline occupied the second position. This coincides with the sequence of accumulation in chickpea leaves. The cumulative values of sugars, proline, amino nitrogen, and K recorded at the highest stress level (T_4), is shown in Figure 1 in terms of $\psi_{\pi 100}$ and osmotic adjustment (OA) offered

Table 1
Effect of line source moisture levels on leaf water relation parameters of two groups of chickpea varieties differing in seed color

Leaf water relations parameters[a]	Line source moisture levels						LSD ($P < 0.05$)	
	T_1		T_3		T_4		Water stress	Varietal group[b]
	BR	PY	BR	PY	BR	PY		
Early flowering								
Ψ_w (-MPa)	0.780	0.700	1.110	1.000	1.430	1.250	0.090	0.110
$\Psi_{\pi 100}$ (-MPa)	0.900	0.810	1.072	0.975	1.182	1.000	0.011	0.086
Ψ_ρ (MPa)	0.330	0.325	0.305	0.279	0.247	0.253	0.011	NS
RWC (%)	81.09	78.99	75.77	c76.22	70.46	66.51	1.550	1.600
Late flowering								
Ψ_w (−MPa)	0.760	0.710	1.240	1.030	1.540	1.160	0.080	0.110
$\Psi_{\pi 100}$ (-MPa)	0.792	0.615	1.058	0.800	1.158	0.835	0.021	0.092
Ψ_ρ (MPa)	0.288	0.259	0.219	0.116	0.181	0.131	0.010	0.025
RWC (%)	75.56	73.27	72.49	69.83	67.28	64.68	1.250	1.930
Early fruiting								
Ψ_w (-MPa)	1.130	1.050	1.870	1.500	2.000	1.720	0.120	0.070
$\Psi_{\pi 100}$ (-MPa)	0.958	0.885	1.300	1.060	1.428	1.175	0.043	0.083
Ψ_ρ (MPa)	0.193	0.188	0.130	0.109	0.114	0.108	0.010	0.010
RWC (%)	72.38	71.46	68.41	65.86	67.54	64.27	0.660	1.160

[a] Ψ_w, Water potential; $\Psi_{\pi 100}$, Total osmotic potential at full turgor; Ψ_ρ, Turgor potential; RWC, Relative water content.
[b] Varietal groups: BR, Having brown-red seed; PY, Having pale-yellow seed.
NS, Nonsignificant.

by them. Since sugars, praline, and amino nitrogen were minor contributors to total $\psi_{\pi 100}$ compared to K, the osmolytes measured were divided into two groups, viz. SPN (sugars + proline + amino nitrogen) and K and described accordingly (Table 2; Figure 1).

The SPN-$\psi_{\pi 100}$ decreased progressively with the increase in moisture stress along the line source moisture gradient irrespective of growth stages and varietal groups. However, the decrease in SPN-$\psi_{\pi 100}$ was usually greater in BR than PY varieties. On the other hand, the pattern of changes in K-$\psi_{\pi 100}$ was variable with regard to growth stages and varietal groups. At EF stage, K-$\psi_{\pi 100}$ related to BR varieties decreased significantly with the decrease in soil moisture content, whereas, that related with PY varieties decreased up to T_3 level only and thereafter it leveled off at the T_4 level. At LF stage, K-$\psi_{\pi 100}$ related to BR varieties decreased up to the T_3 level and leveled off thereafter at the T_4 level, whereas that associated with PY varieties increased (turned less negative) significantly at T_4 level. The K-$\psi_{\pi 100}$ followed exactly similar trend at Eft stage in case of BR varieties, while the apparent progressive decrease

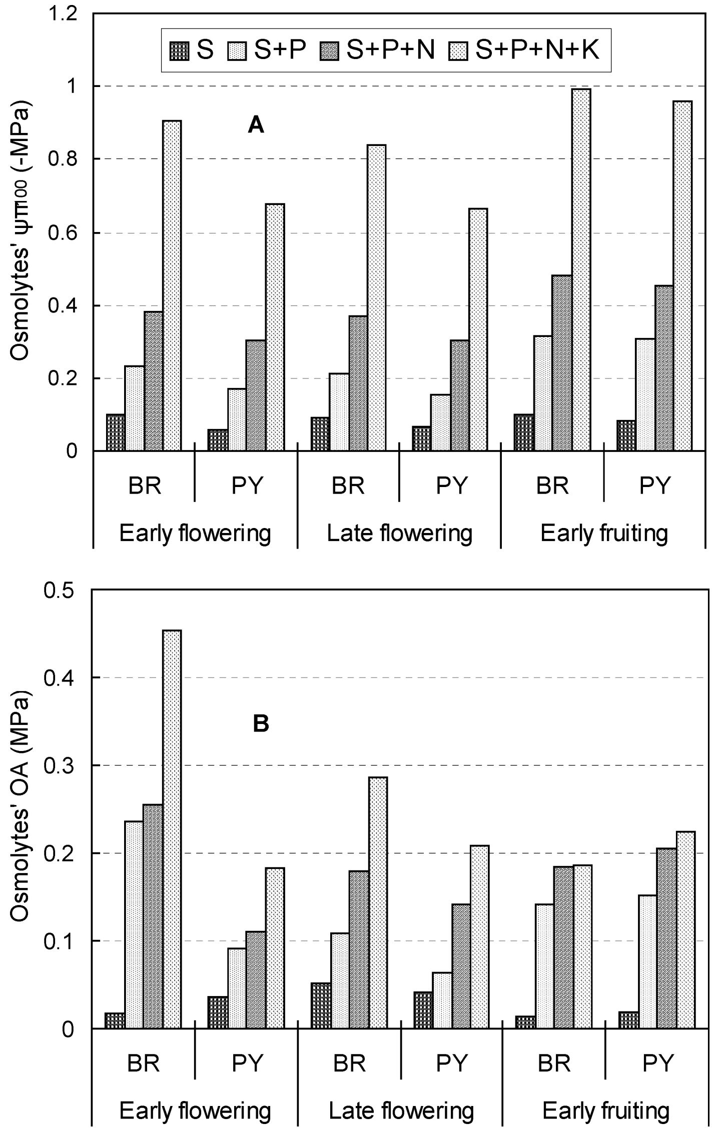

Figure 1. Cumulative values of sugars (S), proline (P), amino nitrogen (N) and potassium (K) in terms of the osmotic potential ($\Psi_{\pi 100}$) offered by them to (A) total osmotic potential and (B) osmotic adjustment (OA) estimated at full turgor.

in K-$\psi_{\pi 100}$ of PY varieties with decreasing soil moisture content was non-significant ($P \leq 0.05$). Generally, BR varieties gave lower K-$\psi_{\pi 100}$ than PY varieties, indicating their respective accumulation in the two varietal groups (Table 2).

Table 2
Effect of line source moisture levels on leaf-osmolytes and their contribution to total osmotic potential in two groups of chickpea varieties differing in seed color

	Line source moisture levels						LSD ($P < 0.05$)	
	T_1		T_3		T_4		Water	Varietal
parameters[a]	BR	PY	BR	PY	BR	PY	stress	group[b]
Osmotic potential[a] of osmolytes (-MPa)								
Early flowering								
SPN	0.303	0.194	0.343	0.260	0.384	0.305	0.021	0.025
Potassium	0.325	0.300	0.435	0.391	0.522	0.371	0.036	0.039
Late flowering								
SPN	0.190	0.164	0.253	0.222	0.370	0.305	0.013	0.052
Potassium	0.362	0.293	0.453	0.397	0.469	0.361	0.025	0.043
Early fruiting								
SPN	0.297	0.246	0.441	0.400	0.481	0.451	0.013	0.014
Potassium	0.508	0.490	0.533	0.492	0.511	0.510	0.021	0.023
Percent contribution of osmolytes to total osmotic potential[a]								
Early flowering								
SPN	33.67	23.95	32.00	26.67	32.48	30.50	1.062	1.160
Potassium	36.11	37.04	40.58	40.10	44.16	37.10	1.585	1.654
Late flowering								
SPN	23.98	26.66	23.91	27.75	31.95	36.52	1.169	0.940
Potassium	45.71	47.64	42.82	49.62	40.50	43.23	2.078	1.150
Early fruiting								
SPN	31.00	27.80	33.93	37.74	33.68	38.37	0.942	1.321
Potassium	53.03	55.36	41.00	46.41	35.78	43.40	1.521	1.203

[a]Estimated at full turgor.
[b]Varietal groups: BR, Having brown-red seed; PY, Having pale-yellow seed.
SPN: Sugars + proline + amino nitrogen.

Contribution of Osmolytes to Total Osmotic Potential

Percent contribution of all measured osmolytes to total $\Psi_{\pi 100}$ was computed at all moisture levels and growth stages as: 100 × ($\Psi_{\pi 100}$ due to particular osmolyte/total $\Psi_{\pi 100}$). Soil moisture levels generally increased the contribution of SPN to total $\Psi_{\pi 100}$, regardless of growth stages and varietal groups. However, the increase in SPN-contribution of PY varieties with increasing soil moisture stress was greater than that of BR varieties, particularly at LF and Eft stages (Table 2).

Potassium contributed more than SPN to total $\Psi_{\pi 100}$ invariably. Line source moisture levels significantly affected the contribution of K to $\Psi_{\pi 100}$ regardless of growth stages and varietal groups. However, PY varieties showed a greater

K contribution than the BR varieties, particularly at LF and Eft stages. BR varieties showed an increase in K contribution with the increase in water stress at EF stage; however, later they showed significant ($P \leq 0.05$) decrease in K contribution with the increase in water stress, particularly at Eft stage. Whereas, PY varieties showed an increase in K contribution up to T_3 level at EF stage, decreasing significantly thereafter at T_4 level. At LF stage, PY varieties showed at par values at T_1 and T_3 levels, decreasing significantly at T_4 level. At Eft stage, the K contribution decreased significantly with the increase in water stress, irrespective of varietal groups, the decrease being more in BR than PY varieties (Table 2).

Osmotic Adjustment

Total osmotic adjustment (OA) attained at T_3 (mild stress) and T_4 (severe stress) moisture levels was computed by subtracting the total $\Psi_{\pi 100}$ attained at T_1 level (assuming T_1 as well-watered control) from that attained at T_3 and T_4 moisture levels, respectively. Total OA shown by BR and PY groups ranged from 0.172 to 0.282 and 0.165 to 0.190 MPa at EF stage, from 0.266 to 0.366 and 0.185 to 0.220 MPa at LF stage and from 0.342 to 0.470 and 0.175 to 0.290 MPa at Eft stage, respectively. In fact, the OA values reported here are underestimated because T_1 was regarded as well-watered control owing to its being nearest to the line source. However, T_1 level was also under mild water stress as evident by the corresponding Ψ_w and RWC values (Table 1). Water stress affected the OA capacity of both the varietal groups significantly regardless of growth stages. The varietal groups showed parity in values at EF and LF stages under T_3 level, while at T_4 level, BR group showed significantly higher OA than PY group. At Eft stage, OA capacity of both the varietal groups increased significantly with the increase in soil moisture stress, with BR varieties giving 95 and 62% higher value than PY varieties at T_3 and T_4 stress levels, respectively (Table 3).

The OA due to SPN increased significantly with the increase in moisture stress regardless of growth stages and varietal groups. However, PY varieties generally showed greater SPN-OA than BR varieties. The OA due to K was generally lower than that due to SPN, particularly at T_4 level. It decreased drastically with increasing crop age. Hence, both the varietal groups showed negligible K-OA at Eft stage with no significant ($P \leq 0.05$) effect of soil moisture levels (Table 3).

The contribution of SPN to total OA increased with water stress at EF and LF stages in case of both varietal groups. However, it decreased with water stress at Eft stage. In case of both the varietal groups, K contribution to total OA was the highest at EF stage, decreasing substantially with increasing crop age. Thus, K contributed very little to total OA at Eft stage regardless of varietal groups. At EF stage the K contribution to OA of BR varieties was greater than PY varieties, while at LF stage the reverse was true. At Eft stage, K contribution to OA of BR varieties decreased, while that of PY varieties increased significantly.

Table 3
Effect of line source moisture levels on total osmotic adjustment (OA), OA due to leaf-osmolytes measured and the percent contribution of the osmolytes to total OA in two groups of chickpea varieties differing in seed color.

	Line source moisture levels				LSD ($P < 0.05$)	
	T_3		T_4			
Osmolytes paramters	BR	PY	BR	PY	Water stress	Varietal group[b]
Osmotic potential[a] of osmolytes (-MPa)						
Early flowering						
Total OA[a]	0.172	0.165	0.282	0.190	0.0200	0.0860
SPN	0.040	0.066	0.081	0.111	0.030	0.019
Potassium	0.110	0.091	0.197	0.071	0.0012	0.0029
Late flowering						
Total OA	0.266	0.185	0.366	0.220	0.0210	0.0920
SPN	0.063	0.058	0.180	0.141	0.011	NS
Potassium	0.091	0.104	0.107	0.068	0.0033	0.0011
Early fruiting						
Total OA	0.342	0.175	0.470	0.290	0.0430	0.1080
SPN	0.144	0.154	0.184	0.205	0.018	0.015
Potassium	0.025	0.002	0.003	0.020	NS	0.0022
Percent contribution of osmolytes to total OA						
Early flowering						
SPN	22.69	40.00	28.73	58.43	3.6073	3.823
Potassium	63.95	55.15	69.86	37.37	2.706	2.068
Late flowering						
SPN	23.68	31.35	49.18	64.09	3.452	2.251
Potassium	34.21	56.22	29.23	30.91	5.270	1.051
Early fruiting						
SPN	42.11	87.99	39.14	70.68	3.312	4.065
Potassium	7.31	1.14	0.01	6.90	1.937	0.857

[a]Estimated at full turgor.
[b]Varietal groups: BR, Having brown-red seed; PY, Having pale-yellow seed.
SPN: Sugars + proline + amino nitrogen.
NS, Nonsignificant.

Seed Yield and Drought Tolerance Efficiency

Seed yield decreased significantly along the moisture gradient from T_1 to T_4 level regardless of varietal groups. Compared to T_1 level, seed yield at T_3 and T_4 stress levels decreased by 15 and 30% in BR varieties and by 35 and 56% in PY varieties, respectively. The grain yield attained by both the varietal groups was at par at T_1 level. However, BR group outyielded PY group by 26 and 30% at mild

(T_3) and severe (T_4) stress levels, respectively. The drought tolerance efficiency (DTE) computed as: 100× (seed yield under stress/ seed yield under no stress), was significantly affected by soil moisture levels, regardless of varietal groups. BR varieties showed significantly higher DTE than PY varieties at mild (T_3) as well as at severe (T_4) stress level (Figure 2).

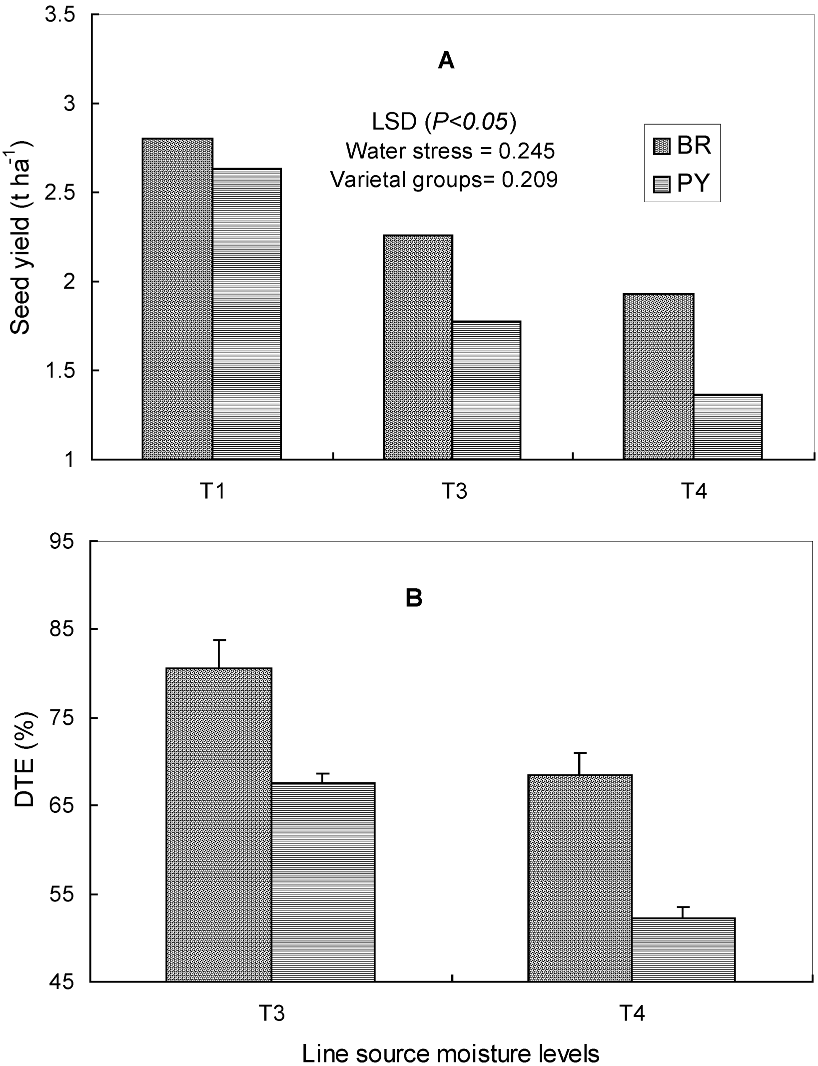

Figure 2. Effect of line source moisture levels (T1, T3 and T4) on (A) grain yield and (B) drought tolerance efficiency (DTE) of two groups chickpea varieties differing in seed color, namely, brown-red (BR) and pale-yellow (PY).

Relationship of Osmotic Parameters with Seed Yield, RWC, and Drought Tolerance Efficiency

To work out linear correlation of osmotic parameters (OA, total $\Psi_{\pi 100}$, potassium-$\Psi_{\pi 100}$ and SPN-$\Psi_{\pi 100}$) with seed yield, RWC and DTE, the mean values related to all the BR and PY varieties across the growth stages were used (n = 8). All osmotic parameters showed significant ($P \leq 0.05$) linear relationship with seed yield and RWC at mild (T_3) as well as severe (T_4) water stress level (Table 4). At T_4 level, there were also observed significant ($P \leq 0.05$) linear correlations of osmotic parameters with DTE.

The linear correlation of osmotic parameters with seed yield was mostly significant ($P \leq 0.05$ or $P \leq 0.01$). Total OA and $\Psi_{\pi 100}$was highly correlated ($P \leq 0.01$) with seed yield at both the stress levels (T_3 and T_4). The SPN- $\Psi_{\pi 100}$ was also positively correlated ($P \leq 0.01$) with seed yield at T_4 level. There was also

Table 4
Relationship of osmotic parameters with grain yield, relative water content and drought tolerance efficiency of chickpea under mild (T_3) and severe (T_4) moisture stress levels of line source (n = 8)

	Line source moisture levels			
	T_3		T_4	
Osmotic parameters[a]	Regression equation	Correlation coefficient (r)	Regression equation	Correlation coefficient (r)
Grain yield (t ha^{-1})				
OA	Y = 1.75 + 1.77×	0.8627**	Y = 1.17 + 2.04×	0.8969**
Total $\Psi_{\pi 100}$	Y = 1.12 + 0.92×	0.8376**	Y = 0.31 + 1.26×	0.9193**
Potassium-$\Psi_{\pi 100}$	Y = 0.63 + 3.22×	0.7904*	Y = -0.51 + 4.79×	0.7904*
SPN-$\Psi_{\pi 100}$	Y = 1.19 + 2.88×	0.6700†	Y = 0.25 + 3.99×	0.8977**
Relative water content (%)				
OA	Y = 69.13 + 12.17×	0.7489*	Y = 64.73 + 91.51×	0.7641*
Total $\Psi_{\pi 100}$	Y = 64.38 + 6.80×	0.7753*	Y = 60.73 + 5.85×	0.7812*
Potassium-$\Psi_{\pi 100}$	Y = 59.44 + 26.44×	0.7691*	Y = 54.97 + 26.29×	0.7924*
SPN-$\Psi_{\pi 100}$	Y = 62.51 + 28.33×	0.8304*	—	NS
Drought tolerance efficiency (%)				
OA	—	NS	Y = 51.06 + 42.47×	0.8800**
Total $\Psi_{\pi 100}$	—	NS	Y = 37.17 + 21.86×	0.7858*
Potassium-$\Psi_{\pi 100}$	—	NS	Y = 14.62 + 103.55×	0.7488*
SPN-$\Psi_{\pi 100}$	—	NS	Y = 30.76 + 86.63×	0.8624**

[a]OA. Osmotic adjustment; $\Psi_{\pi 100}$, Osmotic potential at full turgor; SPN-$\Psi_{\pi 100}$, $\Psi_{\pi 100}$ due to sugars + proline + amino nitrogen; Potassium-$\Psi_{\pi 100}$, $\Psi_{\pi 100}$ due to potassium.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; NS, Nonsignificant.

Note: There were used mean values of osmotic parameters and RWC across the growth stages in regression analysis.

a significant ($P \leq 0.05$) linear relationship between potassium-$\Psi_{\pi 100}$ and seed yield at both the stress levels. The correlation between SPN-$\Psi_{\pi 100}$ and seed yield was weak ($P \leq 0.10$) at T_3 level, but highly significant ($P \leq 0.01$) at T_4 level. With the exception of SPN at T_4 level, all osmotic parameters had significant ($P \leq 0.05$) linear correlation with RWC at both the stress levels. However, no significant ($P \leq 0.05$) correlation was recorded between osmotic parameters and drought tolerance efficiency at T_3 level, while all the correlations between osmotic parameters and drought tolerance efficiency were significant ($P \leq 0.05$ or $P \leq 0.01$) at T_4 level (Table 4).

DISCUSSION

Leaf Water Relations Parameters

Both Ψ_w and $\Psi_{\pi 100}$ decreased with the increase in water stress along the soil moisture gradient irrespective of the growth stages and varietal groups. The two potentials also decreased across the growth stages (Eft > LF > EF). Thus, both the varietal groups showed the lowest values of Ψ_w and $\Psi_{\pi 100}$ at Eft stage, indicating the marked effect of crop age on the depression of water status of plants. However, an increase (less negative values) in Ψ_w and $\Psi_{\pi 100}$ at LF stage was due to a rainfall of 9 mm, which occurred just a week before the sampling date at LF stage. Generally, BR varieties showed lower values of Ψ_w and $\Psi_{\pi 100}$ than PY varieties (Table 1). Additionally, they showed lower increase in $\Psi_{\pi 100}$ than PY varieties at LF stage. This proves that compared to PY group, BR group is not only a potential accumulator of solutes under water deficit, but also it is a weak dissipater of solutes after the drought stress is partly relaxed. Such a characteristic of BR varieties might turn them drought hardened in order to let them perform better under rainfed conditions during which plants experience occasional showers.

Turgor potential (Ψ_ρ) and RWC decreased with the increase in water stress along the line source moisture gradient (Table 1). The decrease in Ψ_w and RWC was correlated ($P \leq 0.001$) linearly with the decrease in Ψ_ρ, when all the data points associated with both the varietal groups recorded at various growth stages ($n = 18$) were used in the regression analysis (figure not shown). However, generally BR varieties were superior to PY varieties in maintaining turgor as well as RWC all through the range of Ψ_w because the decrease in total $\Psi_{\pi 100}$ (solutes accumulation) shown by BR varieties was more than that exhibited by PY varieties in respect with Ψ_w, particularly at severe water stress level (Table 1).

Osmotic Potential Due to Osmolytes

Similar to total $\Psi_{\pi 100}$, osmotic potential due to SPN (SPN-$\Psi_{\pi 100}$) decreased progressively along the line source moisture gradient and the increasing crop age. Thus, in general, the lowest values were obtained at Eft stage under T_4 level. The decrease in SPN-$\Psi_{\pi 100}$ was invariably more in BR than PY varieties, indicating that BR varieties are better accumulator of sugars, praline, and amino nitrogen (SPN) than PY varieties at all the growth stages. Such a decrease in SPN-$\Psi_{\pi 100}$ (or an increase in SPN accumulation) due to water stress has been reported earlier (Morgan, 1992; Premachandra et al., 1995; Iannucci et al., 2002). However, this investigation reveals the effect of line source moisture levels on the accumulation of these osmolytes at different growth stages. At LF stage, the SPN-$\Psi_{\pi 100}$ generally increased (turned less negative) due to relaxation in water stress similar to total $\Psi_{\pi 100}$ as a result of a 9 mm rainfall a week before the sampling at LF stage. .

Potassium accumulated in chickpea leaves in the highest amounts regardless of growth stages and varietal groups. This is in line with the results obtained in case of several crops (Morgan, 1992; Jones et al., 1980; Iannucci et al., 2002; Premachandra et al., 1995). However, in this investigation, K accumulation was studied with regard to different growth stages and varietal groups. The decrease in K-$\Psi_{\pi 100}$ coincided with the increase in soil moisture stress at EF stage, indicating accumulation of K with increasing water stress. BR varieties showed marked progress in this regard compared to PY varieties. At LF stage, the K accumulation by BR varieties reached a plateau at T_3 and T_4 levels, while at Eft stage, they showed a significant increase in K-$\Psi_{\pi 100}$ (i.e. a decrease in K accumulation) at T_4 level. Thus, increasing water stress at late reproductive stage (Eft) did not support progressive K accumulation in case of BR varieties. Rather, potassium accumulation decreased at the highest plant water deficit (Eft stage, T_4 level). Similar results were obtained regarding PY varieties. However, PY varieties showed a plateau earlier than that shown by BR varieties. In fact, they reached a plateau even at EF stage under T_3 and T_4 levels. At LF stage, PY varieties showed significant decrease in K accumulation at T_4 level, while they showed no significant accumulation of K at Eft stage with increasing water stress. Such a behavior of K accumulation in chickpea leaves might, in fact, resemble with its uptake in respect with increasing moisture stress and crop age (Beringer et al., 1986). In fact, tolerant plants try to accumulate K in plant parts before the initiation of the stress to the extent of "luxury consumption," which is the insurance strategy of plants to withstand the forthcoming stress (Surya Kant and Kafkafi, 2001). However, it needs to be further explored if chickpea varieties having capacity to absorb more and more K from the soil with increasing soil moisture stress and crop age could prove drought tolerant under water deficit owing to their higher OA capacity across the growth stages. This investigation indicates towards this point, because BR varieties, with high OA and K accumulation capability, showed more drought tolerance efficiency and seed yield under water deficit than PY varieties. They proved to be not only good accumulator of K with increasing water stress, but also continued to accumulate it up to Eft stage in larger amounts compared to PY varieties

(Table 2), which showed low level of drought tolerance and seed yield under water deficit.

Contribution of Osmolytes to Total Osmotic Potential

At EF stage, BR varieties showed a greater contribution of SPN (sugars + proline + amino nitrogen) to total $\Psi_{\pi 100}$ than PY varieties, while at LF and Eft stages, PY varieties showed significantly higher SPN-contribution than BR varieties, particularly at T_3 and T_4 levels. This could be ascribed to lesser decrease in total $\Psi_{\pi 100}$ of PY varieties compared to that of BR varieties in respect with the decrease in SPN-$\Psi_{\pi 100}$ along with soil moisture gradient and increasing crop age (Tables 1 and 2), as while computing percent osmolyte contribution, the total $\Psi_{\pi 100}$ was used as denominator [percent SPN contribution = 100 × (SPN-$\Psi_{\pi 100}$/total $\Psi_{\pi 100}$)]. Thus, PY varieties showing much less decrease in total $\Psi_{\pi 100}$ than BR varieties with increasing soil moisture stress and crop age (Table 1), exhibited greater SPN-contribution than BR varieties under T_3 and T_4 levels at later growth stages (LF and Eft) (Table 2).

Potassium contribution to total $\Psi_{\pi 100}$ was highest invariably as per its accumulation in chickpea leaves (Table 2; Figure 1). At EF stage, BR varieties showed increased K-contribution with increasing water stress, while at LF and Eft stages, their K-contribution decreased with increasing water stress. On the other hand, PY varieties showed a decrease in K contribution with increasing water stress at all the growth stages, specifically under T_4 level. Thus, at early reproductive stage (EF), only BR varieties were able to contribute to total $\Psi_{\pi 100}$ progressively with the increase in water stress, presumably, due to the increased uptake of K, while the contribution of K decreased with increasing water stress regardless of varietal groups, presumably, because of its low uptake with increasing water stress at late reproductive stage (LF and Eft) (Beringer et al., 1986). However, BR varieties showed greater decrease in K contribution than PY varieties, particularly at Eft stage, due to higher decrease in their total $\Psi_{\pi 100}$ compared to PY varieties along the soil moisture gradient and crop age [percent K-contribution = 100 × (potassium-$\Psi_{\pi 100}$ / total $\Psi_{\pi 100}$)] (Tables 1 and 2).

Osmotic Adjustment

Total osmotic adjustment (OA) increased with the increase in water stress and crop age. Thus, at severe stress level (T_4), OA induction was greater compared to mild stress level (T_3) regardless of growth stages and varietal groups. Further, since crop water stress also increased with increasing crop age (Table 1), the OA also increased with increasing crop age, the highest OA capacity being recorded at Eft stage irrespective of varietal groups. In fact, the induction of OA takes place only in stressed conditions, because the gene for OA induction, already recognized in wheat and rice, is expressed in stressed condition only. The OA differences are conditioned by alternative alleles of this gene at a single locus, with high response being recessive (Morgan and Tan, 1996). A greater OA capacity of BR varieties compared to that of PY varieties could be ascribed to the higher accumulation of the osmolytes in BR compared to PY varieties as evident by the decrease in osmotic potential associated with them (Table 2).

Similar to total OA, the SPN-OA decreased with the increase in moisture stress and crop age, however, PY varieties showed higher values than BR varieties, particularly at EF and Eft stages. This could be ascribed to the low value of SPN-$\Psi_{\pi 100}$ of PY than BR varieties at T_1 level ($\Psi_{\pi 100}$ at T_3 and T_4 levels—$\Psi_{\pi 100}$ at T_1 level = OA at T_3 and T_4 levels, respectively) (Table 2). Soil moisture levels significantly affected K-OA only at EF and LF stages. The BR varieties showed an increase, while PY varieties showed a decrease in K-OA with increasing water stress. There was the highest K-OA at EF stage, decreasing drastically at later growth stages, the decrease in K-OA being significantly higher in PY than BR varieties. Thus, K contributed maximally to OA at early reproductive stage, and BR varieties were superior to PY varieties in this respect. At late reproductive stage (Eft), the K-contribution to total OA was negligible irrespective of varietal groups. This could be ascribed to the K uptake pattern by the crop along with increasing soil moisture stress and crop age (Beringer et al., 1986).

Seed Yield and its Relationship with Osmotic Parameters

Seed yield decreased with the increase in water stress regardless of varietal groups (Figure 2). Varietal groups showed no difference in seed yield in control (T_1 level). However, BR group outyielded the PY group by 26 and 30% at mild (T_3) and severe (T_4) stress levels, respectively. That is, the two varietal groups were differentiated in terms of seed yield only under stress conditions. This coincided with drought tolerance efficiency (DTE) of the two varietal groups accordingly (Figure 2). In fact, there was a significant ($P \leq 0.001$, $P \leq 0.05$) correlation of DTE with seed yield and RWC, when all the data points associated with both the varietal groups recorded at various growth stages ($n = 18$) were used in the regression analysis (figure not shown). This depicts that under water deficit environment drought tolerance in chickpea varieties is associated with maintenance of high water content (RWC) in plants. Thus, higher values of RWC of BR varieties compared to PY varieties at all the growth stages, in general and at Eft stage in particular (Table 1), could interpret the difference in seed yield between the two varietal groups grown under water deficit. Since osmotic adjustment (OA) could enable plants to have greater RWC and Ψ_ρ under water deficit (Morgan, 1984), such results are expected, as in the present investigation BR varieties showed higher OA capacity than PY varieties both at mild (T_3) and severe (T_4) water stress levels at all the growth stages

(Table 3). Moreover, they showed maintenance of a higher RWC and turgor (Ψ_ρ) all through the range of Ψ_w during the crop span compared to PY varieties (Table 1). Owing to the greater OA at T_4 than at T_3 level, the yield benefit of BR varieties over PY varieties was also larger at T_4 compared to T_3 level. Moreover, significant linear correlations of osmotic parameters (total $\Psi_{\pi 100}$, SPN-$\Psi_{\pi 100}$ and potassium-$\Psi\pi_{100}$) with seed yield, RWC and DTE, particularly at T_4 level could be expected because these osmotic parameters contribute to total OA ultimately (Table 4). These results corroborate the findings of Blum et al. (1999) regarding the positive correlation of OA with grain yield of wheat. Further, significant ($P \leq 0.05$) linear relationship of potassium-$\Psi_{\pi 100}$ with seed yield, RWC, and DTE coincides the major contribution of K to total $\Psi_{\pi 100}$ in the present investigation, indicating that K is helpful in plants' survival under water deficit by having favorable effect on OA and, thereby, on plant water relations (Premachandra et al., 1991; Pier and Berkovitz, 1987). This is further confirmed by significant ($P \leq 0.05$) correlation between potassium-$\Psi_{\pi 100}$ and DTE at T_4 level (Table 4).

CONCLUSIONS

Potassium accumulated in the chickpea leaves more than sugars, praline, and amino nitrogen (SPN) both under mild and severe water deficit conditions. It maximally accounted for total $\Psi_{\pi 100}$ and OA regardless of growth stages. In general, The $\Psi_{\pi 100}$ associated with SPN and K decreased progressively along the line source soil moisture gradient regardless of growth stages and varietal groups, the decrease being usually greater in BR than PY varieties. Regardless of varietal groups, the contribution of SPN to total $\Psi_{\pi 100}$ generally increased, while that of K decreased with the increase in water stress, particularly at early fruiting stage. Osmotic adjustment capacity of chickpea varieties increased progressively along the line source moisture gradient and across the growth stages. It was invariably greater in BR than PY varieties. OA capacity of the two varietal groups coincided well with the seed yield under stress. It positively contributed to sustenance of seed yield and drought tolerance of chickpea, maintaining high levels of plant water content (RWC) and turgor (Ψ_ρ) in plants under water deficit. The BR group proved superior to the PY group under water deficit, in terms of seed yield, due to a greater accumulation of osmolytes assayed (particularly potassium) and a comparatively higher value of total $\Psi_{\pi 100}$ and OA. The study revealed that the chickpea varieties could be selected for drought environments on the basis of OA and total $\Psi_{\pi 100}$. Potassium-$\Psi_{\pi 100}$ could also prove as a tool to select improved chickpea genotypes. However, further research is required to know whether the chickpea varieties with efficiency of accumulating high amounts of K across the growth stages are able to withstand water stress more efficiently.

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