



NITROGEN METABOLISM, GROWTH AND YIELD RESPONSES OF WHEAT (*TRITICUM AESTIVUM* L.) TO RESTRICTED WATER SUPPLY AND VARYING POTASSIUM TREATMENTS

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The aim of study was to get insight into the role of potassium in water stress tolerance of wheat. Laboratory and field experiments were conducted to study the responses of two wheat varieties: MP 4010 (V_1) and Sujata (V_2) to restricted water supply and varying potassium doses viz., 20 (K_1), 40 (K_2) and 60 kg ha⁻¹ (K_3). Two types of experiments were set up i.e. laboratory and field. Water stress was imposed by withholding water at different stages i.e. I_0 (rainfed), I_2 (20 DAS) and I_3 (40 DAS), wherein I_0 served as normal irrigation. In laboratory experiments polyethylene glycol-6000 (15%) was used for imposing stress. Potassium treatments resulted in an increase in total chlorophylls, carotenoids and yield parameters in both the cultivars. Relative water content (RWC) improved with increasing potassium doses under all the irrigation schedules. Free amino acids and free sugar contents increased in plants grown with added potassium, particularly under rainfed condition in comparison to normal irrigation. Potassium treatments resulted in increased nitrate reductase, aspartate and alanine aminotransferase activity, indicating positive role of added potassium on nitrogen metabolism and ultimately on growth and yield parameters. Potassium supplementation compensated to some extent the reduction caused due to water stress/ sub optimal water supply.

Key words: Water stress, relative water content, aminotransferases, nitrate reductase, free amino acids, Na⁺/K⁺ ratio.

Potassium, an inorganic cation and important macronutrient in plants has been attributed a vital role in metabolism, growth and stress adaptation. In plant cells potassium acts as an osmolyte (Mengel and Arneke 1982). Potassium plays significant role in plant metabolism particularly in physiological processes like photosynthesis, enzyme activation and water use efficiency besides imparting resistance to drought, lodging, insect, pest and diseases (Umar and Moinuddin 2002, Agarwal *et al.* 2009).

Potassium maintains charge balance by counter balancing the negatively charged ions present in cytoplasm and controls phloem transport, adds to crop quality, overall growth, development and yield (Marschner 1995). Positive effects of potassium on growth and development have been reported by many workers e.g. Sangakara *et al.* (2000) and Yin and Vyn (2003). However, much needs to be done regarding the role of potassium under water stress conditions on different physiological, biochemical, growth and yield parameters in different crops. In the present study, an attempt has been made to examine the water stress induced changes at

different developmental stages of two wheat cultivars and to determine whether the water stress effects could be alleviated by potassium supplementation.

MATERIALS AND METHODS

Experimental set up

Laboratory and field experiments were conducted with two varieties of wheat (*Triticum aestivum* L.) viz, MP 4010 and Sujata procured from College of Agriculture, Gwalior.

Details of experiments are given below:

Laboratory experiments: For laboratory experiment PEG-6000 (15%) was used to impose water stress following Hegarty (1977), after initial screening using three concentrations of PEG-6000 i.e. 5, 10 and 15% for germination and seedling growth experiments (Jatav *et al.* 2011). Petriplates lined with Whatmann filter paper number 1 were used for germination and seedling growth experiments in which activities of nitrate reductase and aspartate and alanine aminotransferases were worked out. Treatments consisted of 0.01 and 0.001 M K₂O with and without 15% PEG-6000.

Field experiments: The field experiments were conducted on agricultural farm on the outskirts of Gwalior during 2007-08. The experiments were laid down in split-split plot design with four replications. Total field area was 821.5 m², which was divided in main plots (6×5 m²), sub plots (6×2.5 m²) and sub-sub plots (2×2.5 m²). Where, main plot to plot distance was 50 cm and space between two plant rows was maintained at 25 cm. Seeds were sown at 4 cm depth.

Nutrient application and water supply

Recommended doses of nitrogen (urea) and phosphorus (single super phosphate) were applied at the rate of 120 kg ha⁻¹ and 60 kg ha⁻¹ respectively and potassium (K₂O) was applied in three varying doses i.e. K₁: 20, K₂: 40 and K₃: 60 kg ha⁻¹. Four irrigation treatments were employed viz., I₀: completely rainfed, I₁: normal irrigation i.e. irrigated 20, 40, 60 and 80 days after sowing, whereas I₂ and I₃ were subjected to restricted irrigation i.e. I₂ and I₃ plots were not provided irrigation at 20 and 40 DAS, respectively.

Sampling, analysis and statistical analysis.

After 65 DAS, soluble sugars, free amino acids, nitrogen, potassium contents and Na/K ratio were determined along with other growth parameters i.e. plant height, number of tillers per plant, leaf area etc. However, yield data like thousand grain weight, grain yield and biomass yield were recorded at maturity i.e. 120 DAS.

Soluble sugar content was estimated using anthrone method (Fong et al. 1953, Jain and Guruprasad 1989). Starch was determined using anthrone method as outlined by Sadasivam and Manickam (2004). Nitrogen estimation was done following micro-Kjeldahl's method as suggested by Jackson (1973) and modified by Iswaran and Marwaha (1980).

The analysis of potassium and sodium was done flame photometrically (Singh *et al.* 2010). One gram dried powdered plant material was taken in a conical flask and digested in triacid mixture (H₂SO₄+HNO₃+HClO₄ in 9:3:1 ratio).

The colorless digested material was filtered through Whatman filter paper number 1 into 100 ml volumetric flask making up the total volume to 100 ml and was read directly on digital flame photometer, employing K and Na filters.

Nitrate reductase activity (in vivo) was determined in root and shoot tissues following Srivastava (1974). Aminotransferase activity was determined following the method given by Sadasivam and Manickam (2004). 100 mg fresh plant sample was homogenized using pestle and mortar in 1.5 ml chilled potassium phosphate buffer (0.05M; pH 7.4) at low temperature. After centrifugation for 5 minutes at 5000 g the pellet was discarded and supernatant was collected and used as enzyme source. Entire procedure was carried out at 4°C. 50 µl of supernatant was added to 0.1 ml buffer-substrate broth (100 mM phosphate buffer containing 2 mM 2-oxoglutarate and 100 mM L-aspartate or 200 mM DL-alanine), mixed and incubated for 60 minutes at 37°C in a water bath. Thereafter, 0.1 ml of DNPH (1 mM 2, 4-dinitrophenyl hydrazine in 1 M HCl) was added and resultant was allowed to stand for 20 minutes. Finally 1 ml sodium hydroxide (400 mM) was added and mixed. After five minutes absorbance was recorded at 540 nm. For control, enzyme source and for blank water was taken and mixed with 0.1 ml buffer substrate and 0.1 ml DNPH making up the volume as in the case of sample. For standards, enzyme source was substituted by standard solution. Free amino acids were estimated following the method given by Sadasivam and Manickam (2004).

Data presented is the mean of three replicates and was subjected to statistical analysis by adopting method of analysis of variance as described by Fisher (1959). Wherever variance ratio (calculated 'F' values) was found significant, critical difference (C.D. at 5%) values were computed between the treatments.

RESULTS AND DISCUSSION

Decline in nitrate reductase activity was observed under water stress conditions. Nitrate

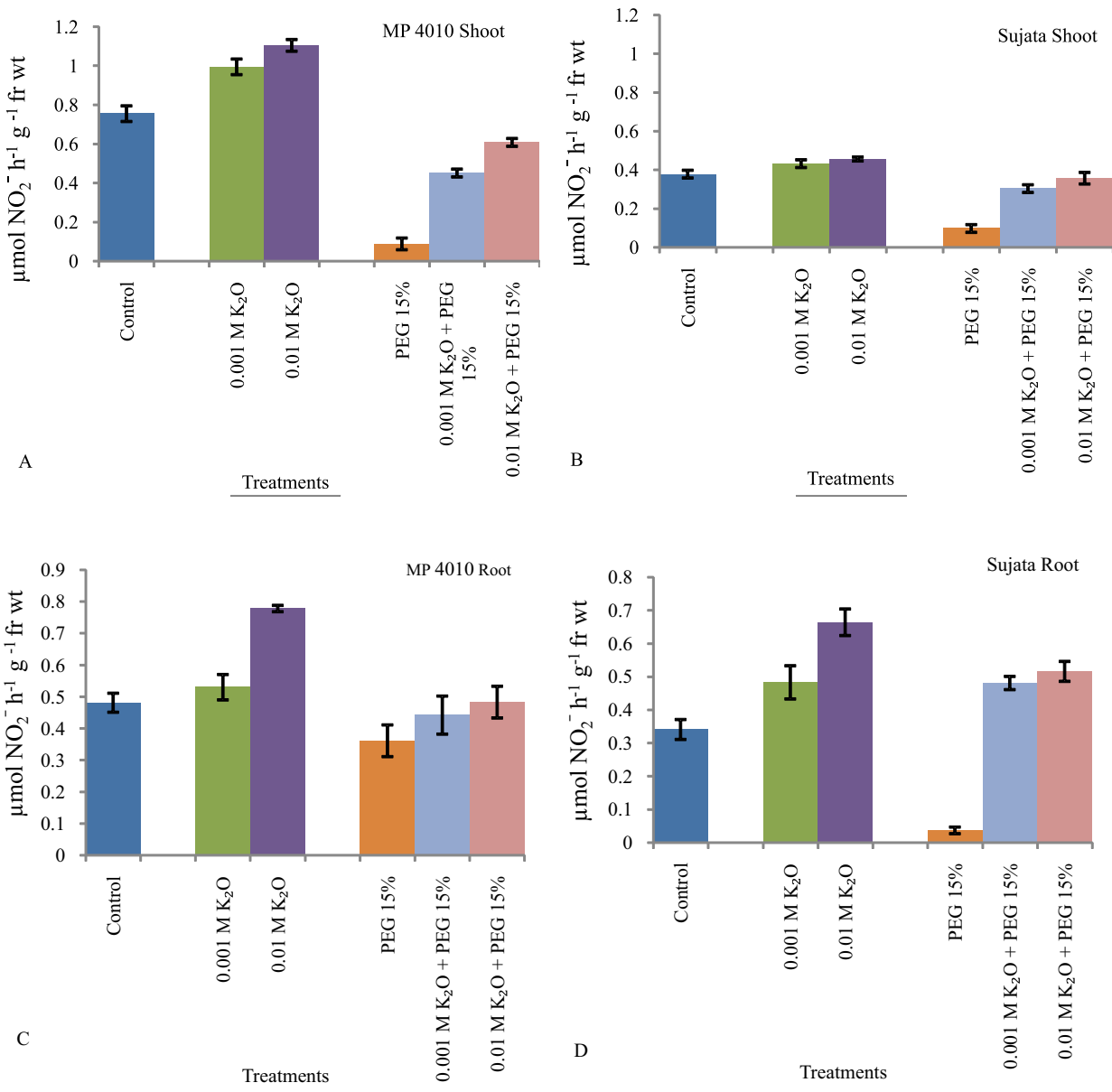


Figure 1. Nitrate reductase activity ($\mu\text{mol NO}_2^- \text{h}^{-1} \text{g}^{-1} \text{fr wt}$) in shoot and root of 7 days old wheat seedlings under PEG-induced water stress and potassium treatments

reductase activity increased in both shoot and root under potassium treatments, which was higher in MP 4010 (Fig. 1). The decrease in nitrate reductase activity due to water stress was to some extent compensated by added potassium (Fig. 1). Decreased nitrate reductase activity under water stress has been reported in castor seedlings (Thatikunta *et al.* 2003). Sharma and Agarwal (2002), and Krauss (2004) have also reported decrease in NR activity under potassium deficiency. Enzymes like

nitrate reductase are sensitive to water stress. Water stress induced imbalance in nutrients leads to inactivation of several enzymes.

Potassium induced changes in alanine aminotransferase activity in the shoot and root of wheat seedlings were observed in both the varieties (Fig. 2). Application of potassium also mitigated to some extent the reduction in the alanine aminotransferase activity due to PEG-induced stress (Fig. 2). Applied potassium also increased aspartate aminotransferase activity in

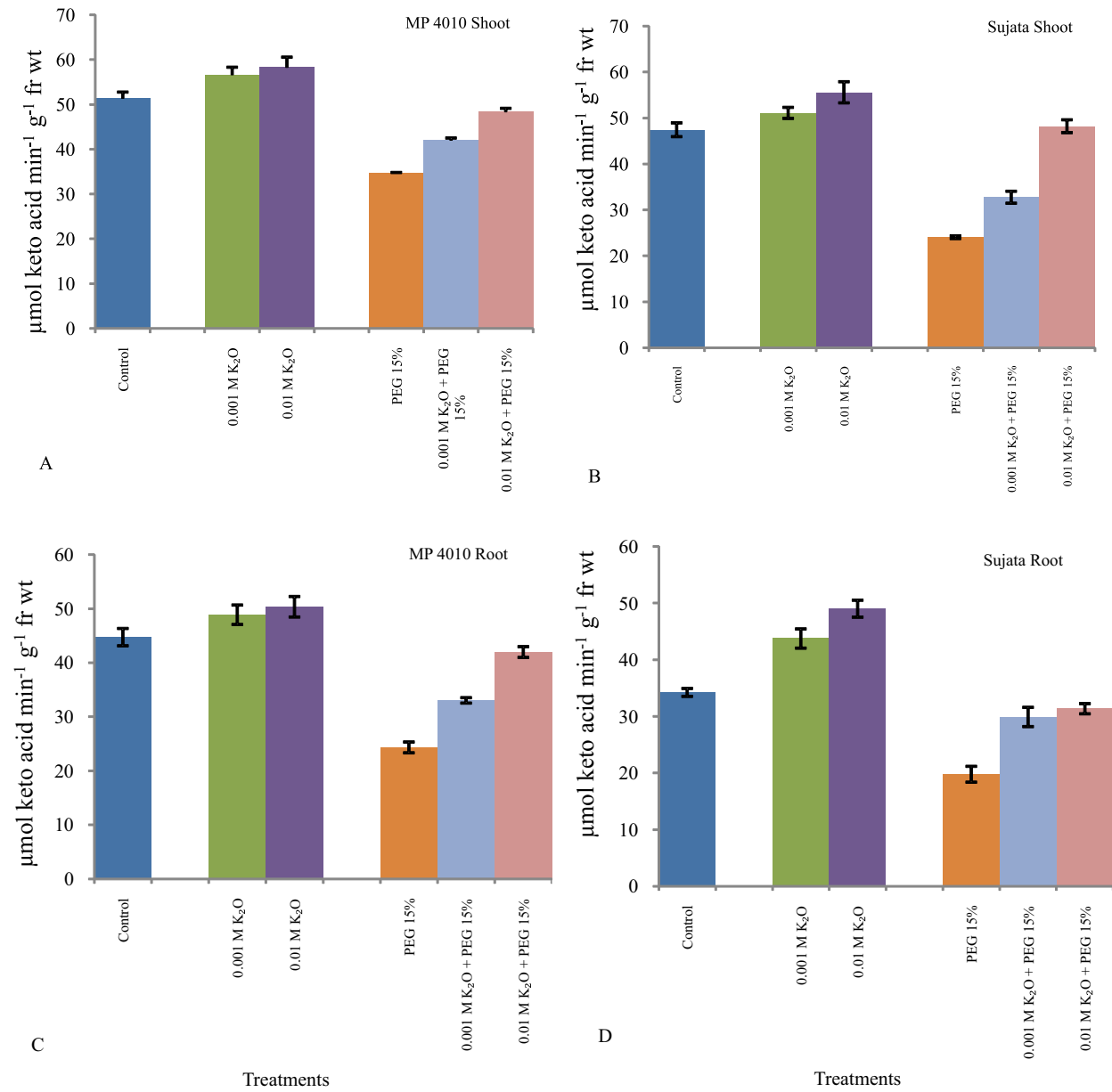


Figure 2: Alanine aminotransferase activity ($\mu\text{mol keto acid min}^{-1} \text{g}^{-1} \text{fr wt}$) in 7 days old wheat seedlings under PEG-induced water stress and potassium treatments

wheat seedlings (Fig. 3). Water stress imposed using polyethylene glycol-6000 caused reduction in aspartate aminotransferase activity, which however, was overcome substantially by the application of potassium (Fig. 3).

Osmotic stress induced decrease in aspartate and alanine aminotransferase activity along with increase in free proline content and altered amino acid contents have been reported by Pandey *et al.* (2004). Applied potassium alters

aspartate aminotransferase activity resulting into greater synthesis of aspartic acid (Sharma *et al.* 2006). Aminotransferases serve as a link between carbohydrate and amino acid metabolism. Potassium-induced increase in aspartate and alanine aminotransferase activity could be the reason for altered amino acid contents.

Increase in free amino acids in flag leaf, stem and root was higher in potassium treatments and highest under rainfed condition, indicating

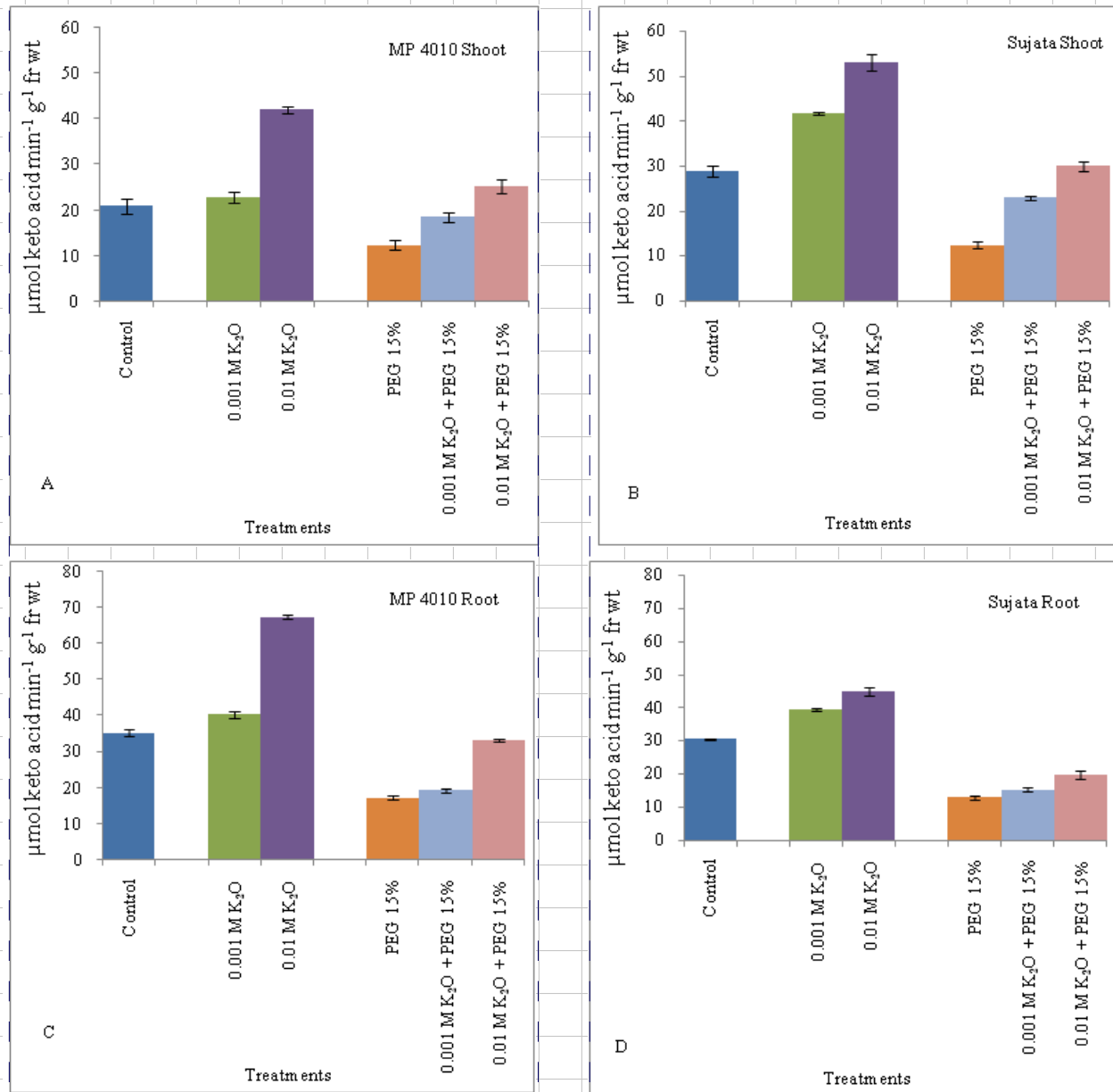


Figure 3: Aspartate aminotransferase activity ($\mu\text{mol keto acid min}^{-1} \text{g}^{-1} \text{fr wt}$) in 7 days old wheat seedlings under PEG-induced water stress and potassium treatments.

role of free amino acids in osmotic adjustment under water stress conditions. Increase in free amino acids was particularly greater in flag leaf of cultivar Sujata as compared to MP 4010 (Fig. 4a, 4b and 4c). Plants accumulate osmolytes such as soluble sugars, proline and free amino acids under stress conditions, thereby increasing osmotic potential and maintaining RWC (Turner and Beg 1981, Gupta *et al.* 2000). Drought induced increase in proline and free amino acid content and hydrolysis of

polysaccharides into simpler mono and disaccharides may lead to accumulation of osmotica. Uprety and Das (2006) found decrease in RWC, water potential and increase in the levels of free amino acid under water stress conditions, which possibly contributed to osmotic adjustment.

Higher total soluble sugars were recorded in the plants maintained under rainfed conditions. An increase was observed in all the plant parts under all the irrigation regimes as a result of

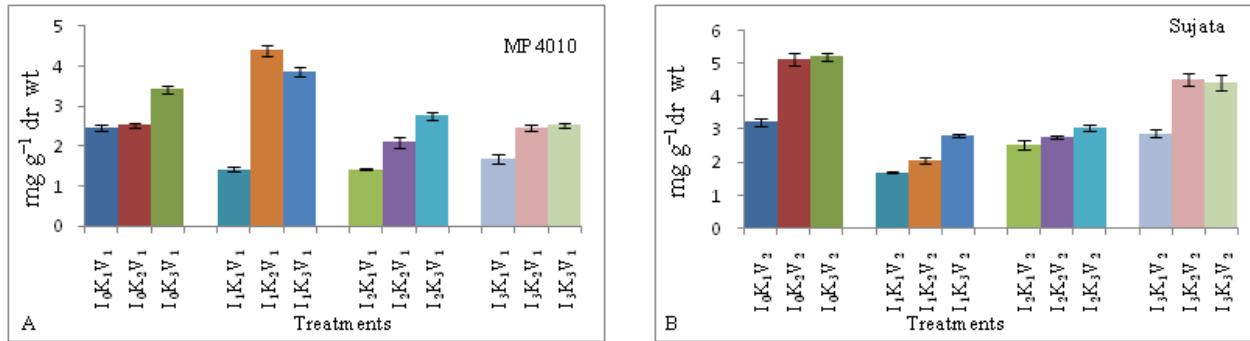


Figure 4a: Free amino acids content in flag leaf of different wheat varieties recorded at 65 DAS under different levels of irrigation and potassium treatments

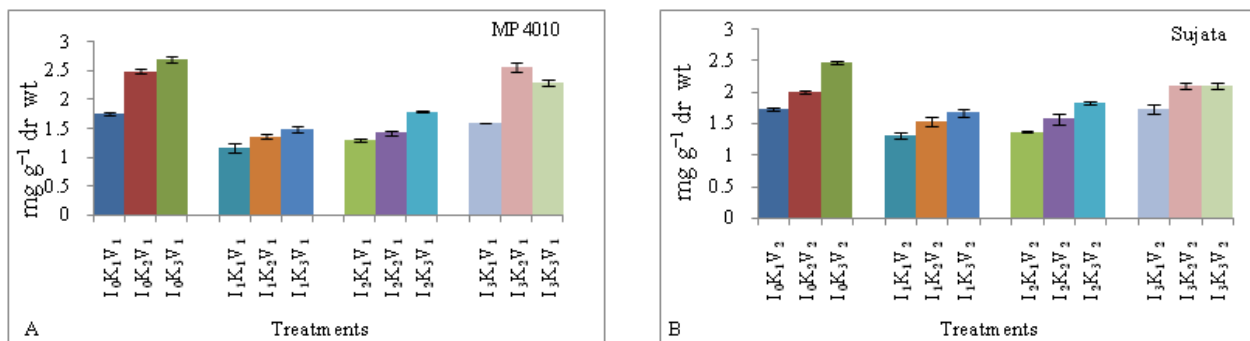


Figure 4b: Free amino acids content in stem of different wheat varieties recorded at 65 DAS under different levels of irrigation and potassium treatments

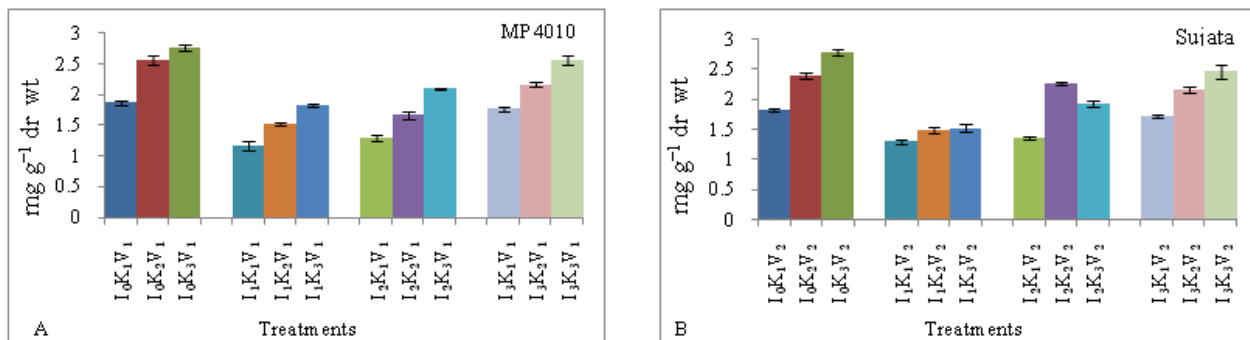


Figure 4c: Free amino acids content in root of different wheat varieties recorded at 65 DAS under different levels of irrigation and potassium treatments

potassium treatments. Increase in soluble sugar under rainfed and restricted water supply conditions indicate the contribution of soluble sugars in osmotic adjustment under water stress condition.

Soluble sugars were higher in Sujata under rainfed conditions in all the potassium treatments as compared to cultivar MP 4010 (Fig. 5a, 5b and 5c). Accumulation of solutes contributing towards maintenance of turgor and osmotic adjustment have also been reported earlier (Handa *et al.* 1983, Morgan 1984).

Pandey *et al.* (2004) reported that increase in total free sugars and α -amylase activity to be associated with a decrease in starch content under water stress conditions in rice.

Hydrolysis of starch to soluble sugars can contribute to osmotic adjustment and maintenance of turgor pressure under stress conditions (Wang *et al.* 1995). Considerable enhancement in free proline and total sugars during PEG-induced water stress indicates their contribution to osmotic adjustment (Jain *et al.* 1996). Applied potassium also enhanced

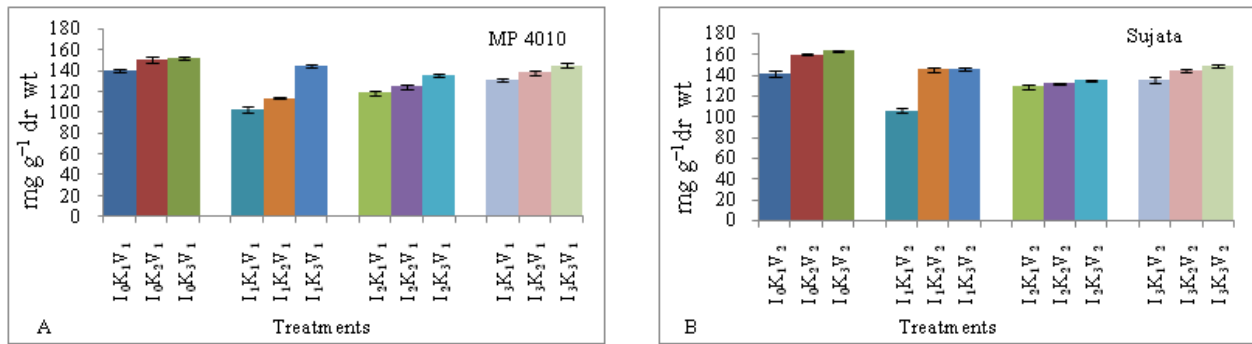


Figure 5a: Soluble sugar content in flag leaf of different wheat varieties recorded at 65 DAS under different levels of irrigation and potassium treatments

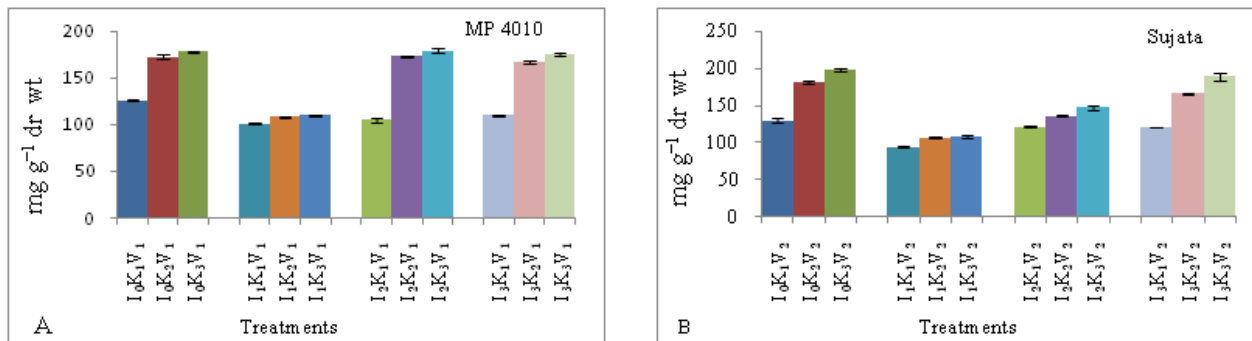


Figure 5b: Soluble sugar content in stem of different wheat varieties recorded at 65 DAS under different levels of irrigation and potassium treatments

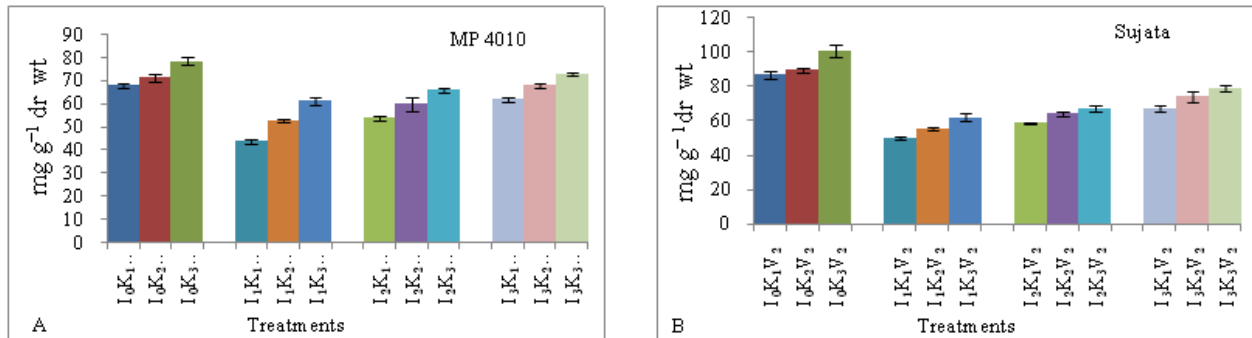


Figure 5c: Soluble sugar content in root of different wheat varieties recorded at 65 DAS under different levels of irrigation and potassium treatments

soluble sugar content, indicating its role in osmotic adjustment.

Total nitrogen contents in both the cultivars of wheat registered an increase with added potassium under all the irrigation schedules (Fig 6a, 6b and 6c). Flag leaves of plants grown under rainfed and restricted irrigation conditions exhibited relatively less nitrogen contents. Potassium has been reported to improve efficiency of nitrogen metabolism (Steineck and Header 1978, Bansal *et al.* 1993). Dibb and Thompson (1985) have reported

interaction of potassium with nitrogen and phosphorus. Uptake of nutrients like nitrogen, phosphorus and potassium and growth in terms of dry weight, shoot length, root length, number of spikes and grain yield are adversely affected by water stress. Increased potassium level in soil leads to enhancement in uptake of potassium and other nutrients under irrigated as well as under water stress conditions, improving the productivity and quality of wheat (Baque *et al.* 2006). Earlier also potassium fertilization has been reported to

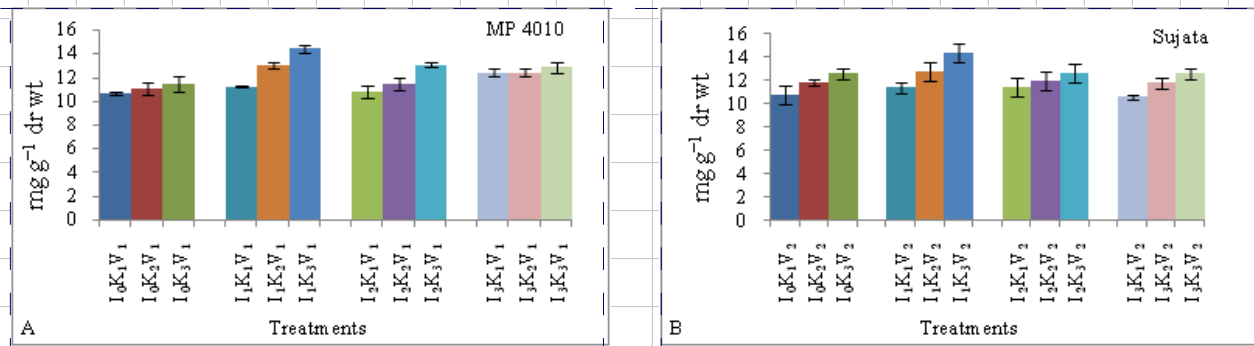


Figure 6a: Total nitrogen contents in flag leaf of different wheat varieties at 65 DAS under different levels of irrigation and potassium treatments

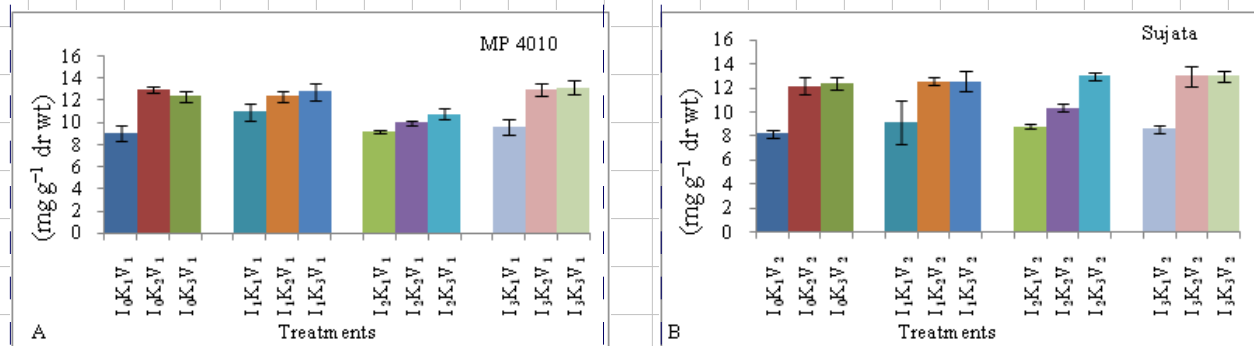


Figure 6b: Total nitrogen contents in stem of different wheat varieties at 65 DAS under different levels of irrigation and potassium treatments

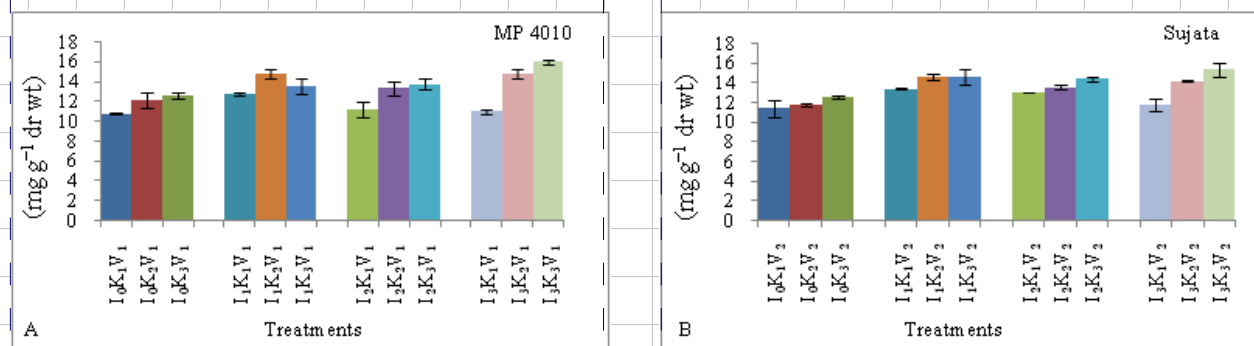


Figure 6c: Total nitrogen contents in root of different wheat varieties at 65 DAS under different levels of irrigation and potassium treatments

significantly improve the uptake of phosphorus and nitrogen in wheat plants accompanied by increase in seed index, grain and straw ratio and yield of grain (Ebtisam *et al.* 2010).

Potassium supplementation resulted in increase in leaf area under all irrigation schedules (Table 2). Potassium induced enhancement in leaf area has been reported by Sinha (1978), Behboudian and Anderson (1990) and Shivay *et al.* (2002). Increasing potassium doses have been reported to increase the number of productive tillers per plant, grain and straw yield (Mehdi *et al.* 2001).

Potassium has been reported to overcome the impact of water stress in mustard. Different levels of irrigation significantly affected grain yield, proline contents of seed and weight of individual seed (Ahmadi and Bahrani 2009).

Grain yield per plot in both the varieties was higher under potassium and normal irrigation (Table 1). Grain yield per plot was more in MP 4010 than in Sujata. Reduction in grain yield per plot was greater under rainfed conditions and irrigation restricted at a later stage (I3) than water supply withheld at early vegetative stage

Table 1: Growth and yield parameters of wheat under different levels of irrigation and potassium treatments (65 DAS)

Treatments	MP 4010					SUJATA				
	Plant height (cm)	Tillers per plant	*Thousand grain weight (g)	* Grain yield (kg plot ⁻¹)	* Biomass yield (kg plot ⁻¹)	Plant height (cm)	Tillers per plant	*Thousand grain weight (g)	* Grain yield (kg plot ⁻¹)	* Biomass yield (kg plot ⁻¹)
I ₀ K ₁	77.37±0.88	4.70±0.15	42.7±0.33	1.15±0.05	2.80±0.12	91.17±0.83	4.70±0.15	44.3±0.83	0.81±0.02	3.10±0.05
I ₀ K ₂	78.43±0.50	5.43±0.12	48.7±1.17	1.22±0.06	3.23±0.15	92.17±0.67	5.17±0.18	51.7±0.83	0.94±0.01	3.17±0.03
I ₀ K ₃	79.53±2.79	5.63±0.08	51.7±0.33	1.32±0.03	3.20±0.06	96.80±0.75	5.63±0.08	51.9±0.93	0.95±0.01	3.25±0.02
I ₁ K ₁	81.99±0.20	5.00±0.20	52.7±0.67	1.62±0.04	3.93±0.18	93.97±2.38	4.80±0.10	50.3±0.33	0.80±0.03	3.17±0.17
I ₁ K ₂	84.72±2.07	5.47±0.17	57.3±0.67	1.86±0.01	4.07±0.08	100.80±1.90	5.07±0.17	51.3±0.83	0.97±0.01	3.57±0.22
I ₁ K ₃	86.28±2.26	5.63±0.08	54.7±0.67	1.72±0.07	4.43±0.12	100.00±0.65	5.63±0.22	52.7±0.67	1.41±0.02	4.03±0.22
I ₂ K ₁	79.64±0.42	4.87±0.07	43.2±0.58	1.47±0.03	3.67±0.17	81.60±2.40	4.67±0.13	46.7±0.67	0.88±0.05	3.33±0.17
I ₂ K ₂	84.39±0.26	5.10±0.05	49.3±2.33	1.68±0.06	4.73±0.15	92.63±2.08	5.07±0.27	52.0±1.00	1.02±0.07	3.67±0.23
I ₂ K ₃	84.78±0.12	5.57±0.12	54.3±1.17	1.69±0.05	4.27±0.18	94.83±2.82	5.57±0.03	52.3±0.83	1.04±0.03	3.43±0.12
I ₃ K ₁	82.45±1.78	4.53±0.23	42.7±0.67	1.33±0.04	3.53±0.03	96.43±0.97	4.53±0.03	46.5±1.00	0.87±0.05	3.73±0.23
I ₃ K ₂	83.33±0.56	5.13±0.07	52.7±2.33	1.66±0.02	4.03±0.03	98.83±0.22	5.17±0.18	51.0±0.50	0.95±0.02	3.27±0.12
I ₃ K ₃	83.92±0.91	5.57±0.03	52.0±1.00	1.62±0.00	4.13±0.03	98.83±0.38	5.60±0.10	51.7±0.33	1.09±0.06	3.50±0.15

Table 1a: Growth and yield parameters of wheat (*Triticum aestivum* L.) under different levels of irrigation and potassium treatments (65 DAS)

Treatments	Irrigation C.D. (5%)	Potassium C.D. (5%)	Variety C.D. (5%)
Height	2.13	1.21	1.03
Tiller/plant	0.24	0.11	NS
* Thousand grain wt.	NS	0.958	NS
* Grain yield	0.06	0.05	0.06
* Biomass yield	0.20	0.20	0.13

* The data was recorded at the time of harvest i.e. After 120 days.

(I2) (Table 1). Thousand grains weight increased with increasing potassium doses. Restricted water supply resulted in a decline in thousand grains weight, which was overcome to some extent by potassium treatments, however, very little varietal differences were observed (Table 1).

Water stress has been reported to decrease water potential, relative water contents, photosynthetic rate, translocation, number of grains per spike, 1000 grains weight and grain yield and hastening maturity and senescence (Tiwari *et al.* 1996, Tyagi *et al.* 2003).

Application of K₂O (3%) before water stress imposition has been reported to improve the growth and yield parameters like number of spikes per m², number of grains per spike, weight of thousand grains and straw yield of wheat by many workers (El-Sabbagh *et al.* 2002, El-Ashrey *et al.* 2005).

Potassium contents in different plant parts were estimated and found to increase in all plant parts with increasing potassium doses under all the irrigation schedules substantiating the effectivity of potassium treatments, (Table 3). Higher potassium content was recorded in flag

Table 2: Leaf area (cm²) of flag leaf of wheat (*Triticum aestivum* L.) under different levels of irrigation and potassium treatments (65 DAS).

Treatments	MP 4010	SUJATA
I ₀ K ₁	7.20± 0.05	7.50± 0.03
I ₀ K ₂	8.20± 0.04	8.70± 0.02
I ₀ K ₃	8.90± 0.04	8.70± 0.02
I ₁ K ₁	7.40± 0.02	6.80± 0.04
I ₁ K ₂	7.70± 0.05	8.20± 0.04
I ₁ K ₃	8.80± 0.03	8.40± 0.03
I ₂ K ₁	7.90± 0.03	7.30± 0.02
I ₂ K ₂	8.30± 0.02	9.20± 0.02
I ₂ K ₃	8.70± 0.04	8.70± 0.06
I ₃ K ₁	8.00± 0.02	7.80± 0.01
I ₃ K ₂	9.00± 0.05	9.20± 0.07
I ₃ K ₃	10.40± 0.01	8.40± 0.03

Irrigation		Potassium		Variety	
S.E.(m) ±	C.D. (5%)	S.E.(m) ±	C.D. (5%)	S.E.(m) ±	C.D. (5%)
0.015	0.05	0.020	0.06	0.011	NS

Table 3: Potassium (mg g⁻¹ dr wt) in wheat (*Triticum aestivum* L.) under different levels of irrigation and potassium treatments (65 DAS).

Treatments	MP 4010			SUJATA		
	Flag leaf	Stem	Root	Flag leaf	Stem	Root
I ₀ K ₁	38.9± 0.05	19.2± 0.04	26.8± 0.05	46.9± 0.17	34.4± 0.15	23.1± 0.05
I ₀ K ₂	54.1± 0.19	35.5± 0.11	34.1± 0.13	54.2± 0.23	44.1± 0.11	34.7± 0.04
I ₀ K ₃	61.3± 0.07	47.3± 0.16	40.1± 0.14	57.3± 0.22	45.9± 0.11	38.1± 0.08
I ₁ K ₁	38.2± 0.02	17.3± 0.13	29.0± 0.16	43.8± 0.15	14.5± 0.05	22.6± 0.14
I ₁ K ₂	52.7± 0.21	34.8± 0.02	31.7± 0.10	57.5± 0.18	45.4± 0.21	36.9± 0.13
I ₁ K ₃	54.5± 0.21	56.1± 0.09	27.0± 0.14	56.1± 0.21	45.3± 0.11	38.0± 0.06
I ₂ K ₁	37.1± 0.12	36.8± 0.14	27.5± 0.05	48.5± 0.15	43.3± 0.15	25.3± 0.14
I ₂ K ₂	51.1± 0.13	43.2± 0.19	36.7± 0.08	52.4± 0.20	47.7± 0.17	34.5± 0.15
I ₂ K ₃	54.5± 0.25	43.4± 0.04	33.4± 0.05	57.5± 0.16	48.1± 0.16	34.7± 0.17
I ₃ K ₁	47.0± 0.11	44.2± 0.17	23.0± 0.11	54.2± 0.15	30.1± 0.05	29.7± 0.06
I ₃ K ₂	52.0± 0.12	41.1± 0.18	35.0± 0.12	57.9± 0.13	39.9± 0.14	37.3± 0.08
I ₃ K ₃	60.9± 0.20	50.7± 0.06	35.0± 0.10	64.7± 0.16	47.1± 0.02	37.1± 0.10

Plant sample	Irrigation		Potassium		Variety	
	S.E.(m) ±	C.D. (5%)	S.E.(m) ±	C.D. (5%)	S.E.(m) ±	C.D. (5%)
Flag leaf	0.135	0.47	0.143	0.43	0.075	0.22
Stem	0.092	0.32	0.066	0.20	0.062	NS
Root	0.080	NS	0.044	0.13	0.050	NS

leaf, indicating its efficient uptake. In both the varieties of wheat Na⁺/K⁺ ratio also decreased with increasing potassium doses (Table 4). Shiraji *et al.* (2005) have reported wheat genotypes maintaining higher K⁺/Na⁺ ratio to

perform better under salinity stress.

CONCLUSION

Results clearly indicate that potassium supplementation has a positive influence on the

Table 4: Na⁺/K⁺ ratio in wheat (*Triticum aestivum* L.) under different irrigation and potassium treatments (65 DAS).

Treatments	MP 4010			SUJATA		
	Flag leaf	Stem	Root	Flag leaf	Stem	Root
I ₀ K ₁	0.047	0.129	0.088	0.047	0.068	0.104
I ₀ K ₂	0.030	0.068	0.063	0.037	0.052	0.061
I ₀ K ₃	0.025	0.047	0.047	0.028	0.047	0.052
I ₁ K ₁	0.045	0.136	0.078	0.044	0.161	0.100
I ₁ K ₂	0.030	0.066	0.064	0.029	0.049	0.051
I ₁ K ₃	0.028	0.035	0.066	0.031	0.048	0.046
I ₂ K ₁	0.067	0.054	0.085	0.042	0.054	0.092
I ₂ K ₂	0.041	0.052	0.058	0.036	0.047	0.057
I ₂ K ₃	0.033	0.058	0.061	0.031	0.046	0.054
I ₃ K ₁	0.039	0.054	0.103	0.032	0.078	0.077
I ₃ K ₂	0.028	0.056	0.060	0.026	0.055	0.054
I ₃ K ₃	0.031	0.043	0.056	0.029	0.043	0.044

activity of nitrate reductase, aminotransferases and contents of free amino acids, proline, soluble sugars and potassium under normal and restricted water supply. Added potassium also has a positive influence on nitrogen uptake and the impact is ultimately reflected on the different growth and yield parameters of wheat cultivars.

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