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# COMBINED EFFECTS OF DROUGHT STRESS AND NPK FOLIAR SPRAY ON GROWTH, PHYSIOLOGICAL PROCESSES AND NUTRIENT UPTAKE IN WHEAT

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## Abstract

The present study investigated the effects of supplemental foliar nitrogen (N), phosphorous (P) and potassium (K) spray, alone or in various combinations, on physiological processes and nutrients uptake in wheat under water deficit conditions. The study comprised of two phases; during the first phase, ten local wheat (*Triticum aestivum* L.) genotypes were evaluated for their response to PEG-6000 induced osmotic stress. One drought tolerant (Bhakkar-2002) and sensitive (Shafaq-2006) genotype selected from screening experiments were used in the second phase to determine the individual and combined effects of N, P and K foliar spray on physiological mechanisms in wheat under drought stress. The results revealed that limited water supply significantly reduced germination, growth and uptake of N, P and K. Supplemental foliar fertilisation of these macronutrients alone or in different combinations significantly improved the water relations, gas exchange characteristics and nutrient contents in both the genotypes. Bhakkar-2002 maintained higher turgor, net $CO_2$ assimilation rate ($P_n$), transpiration rate ($E$), stomatal conductance ($g_s$) and accumulated more N, P and K in shoot than Shafaq-2006. The foliar spray of NPK in combination was effective in improving wheat growth under both well-watered and water-deficit conditions.

**Key words:** Foliar spray, Macronutrients, Water relations, Gas exchange, Drought stress, Wheat.

## Introduction

Drought stress is one of the major abiotic stresses that drastically affects crop production around the globe (Shahbaz *et al*., 2011). Exposure to drought stress poses serious challenges for the survival of plants, because it results in impaired germination and seedling growth (Ashraf *et al*., 2006) and affects plant growth (Xu *et al*., 2007), and reduced harvestable yield of plants (Nawaz *et al.,* 2012).

Several approaches have been used by researchers to improve wheat growth and yield under water deficit conditions. Breeding for drought tolerance is one of the key approaches that significantly contributed to successful wheat production, especially in arid and semi-arid regions, for many years but is time consuming. The screening of available wheat germplasm to identify drought tolerant and sensitive genotypes is a viable, rapid and effective shotgun approach to ensure sustainable agricultural productivity in water deficit areas of the world (Ashraf *et al.,* 2006). The simulation of drought conditions in the laboratory by using osmotic agents is an important technique for efficient screening of genotypes. Among different osmotic agents, PEG-6000 is recommended due to its non-toxicity and non-penetration into the seeds (Willenborg *et al.,* 2005) and there exists a strong relation between this agent and wheat emergence percentage (Zhu *et al*., 1997).

Plants have adopted certain mechanisms to respond to various environmental stresses. Maintenance of turgor through accumulation of osmoprotectants, decrease in rate of transpiration and closure of stomata help to minimize the drastic effects of drought stress (Nawaz *et al.,* 2013). However, such limitations like closure of stomata also reduce the intercellular concentration of $CO_2$ that prevents the Calvin-cycle at moderate water stress (Shangguan *et al*., 1999) thus ultimately reducing the potential yield of the crop plants.

The limited water conditions decrease the uptake and translocation of nutrients therefore, the foliar application may be an alternate and effective approach to improve the nutrients availability to plants. Foliarly applied NPK-fertilizers significantly contribute towards improved yield through increase in biomass of the plants (Ling &Silberbush, 2002). The positive effect of foliar applied nitrogen (N), phosphorus (P), and potassium (K) to sustain proper leaf nutrition as well as carbon balance, and improving photosynthetic capacity is well established (Ihsan *et al*., 2013). However, most of the studies involved the assessment of effects of either N, P or K foliar fertilization on plants and combined effects of these nutrients are seldom investigated. The present study was therefore, planned to screen the available wheat genotypes for drought tolerance and to investigate the individual and combined effects of supplemental NPK on physiological mechanisms of wheat under water deficit conditions. We hypothesize that foliar NPK spray improves the drought tolerance potential of wheat through enhanced uptake of nutrients and maintenance of turgor and gas exchange characteristics.

## Materials and Methods

**Experimental site and conditions:** The experiments were carried out in collaboration with Crop Stress Management Group of Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan and Stress Physiology Laboratory, Department of Crop Physiology, University of Agriculture, Faisalabad (Pakistan) under Lab and glasshouse conditions. Two screening experiments were conducted under Lab conditions (25±3°C) using available ten wheat genotypes (Lasani-2008, Shafaq-2006, Ufaq-2006, Chakwal-86, Farid-2006, Miraj-2006, Manthar-2003, Bhakkar-2002, FSD-2008, and $V_0$-4178). Twenty seeds of each genotype were randomly selected and sterilized for 1-2 minutes with 5% sodium hypochlorite solution, washed with distilled water, and then air dried. The seeds were placed in covered sterilized petri-dishes (9 cm diameter) containing filter paper moistened with 10 ml of PEG-6000 solution in the first experiment. The data regarding germination parameters were recorded daily and the experiment was terminated after eight days. Seeds were considered to be germinated when gained approximately 2 mm of root length (Afzal*et al*., 2004). Complete germination of seeds was considered when no further germination occurred in two consecutive days.

The second experiment was conducted in plastic pots (15cm dia × 11cm length) containing thoroughly washed river sand. Randomly selected five healthy plants were maintained in each pot after completion of germination (eight days after sowing). The seedlings were harvested after four weeks to record the biomass data for the estimation of various physiological indices. Both the experiments were laid out in completely randomized design (CRD) with three replications.

One drought tolerant and sensitive wheat genotypes selected from Lab experiments were used in a glass house experiment using plastic pots to evaluate the effect of various combinations of foliar NPK fertilization on physiological mechanisms of wheat under water deficit conditions. Two kg dry sand with the following characteristics was used in each pot: pH = 5.8; $NH_4$-N = 2.1 mg $kg^{-1}$, $NO_3$-N = 4.8 mg $kg^{-1}$; P = 8.4 mg $kg^{-1}$; K = 37 mg $kg^{-1}$; sulphur = 4.3 mg $kg^{-1}$. Basal nutrients in solution were applied to each pot at ½ strength (Hoagland &Arnon 1950).

**Drought stress and NPK foliar spray:** Polyethylene glycol (PEG-6000) was used as an osmotic agent to induce water stress in the first experiment. Drought treatments included a non-stress control (0 MPa) along with -0.2, -0.4, -0.6 and -0.8 MPa. Solutions for the required four concentrations were prepared by dissolving 6.65 g (-0.2 MPa), 13.30 g (-0.4 MPa), 20 g (-0.6 MPa), and 26.6 g (-0.8 MPa) of PEG-6000 in distilled water. Different concentrations of the solution (100 ml) were confirmed by Vapor Pressure Osmometer (Wescor 5520, USA) at 25°C according to the method of Michel & Kaufmann, (1973).

Drought stress was imposed in pot experiments by withholding water after the completion of germination (eight days after sowing). The normal plants were irrigated with tap water on demand, whereas no water was applied to water stressed plants after the onset of drought stress. Nine fertiliser levels i.e. no-spray , water spray, N (alone) 1.5% , P (alone) 2% , K (alone) 3% , N and P (50%-50%), N and K (50%-50%), P and K (50%-50%) and N, P, K (33%-33%-33%) were applied as foliar spray on14 days old plants. The seedlings were foliarly sprayed with N, P and K alone or in different combinations after three days of imposition of drought stress and were harvested after four weeks.

**Physiological indices:** The biomass data collected from these experiments were used to calculate the following physiological indices, like germination stress index (GI) was calculated by the formula given by Anonymous (1983), where as germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) were calculated by the formulae published by Ashraf *et al*., (2006).

**Determination of water relations and gas exchange characteristics:** Fully expanded flag leaf from each plant was used to determine the leaf water potential ($\psi_w$). The measurements were made from 8.00 to 10.00 a.m. with Scholander type pressure chamber. The same leaf, as used for $\psi_w$, was preserved at -20°C for one week and then osmotic potential ($\psi_s$) was determined. The frozen leaf material was thawed and cell sap was extracted with a glass rod. The sap was directly used for the determination of $\psi_s$ using an osmometer (Wescor 5520). Turgor potential ($\psi_p$) was calculated as the difference between $\psi_w$ and $\psi_s$.

A fully expanded youngest leaf of each plant was used to measure the photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*) by using Photosynthesis System CI-340 (Inc.USA) portable infrared gas analyser These measurements were recorded from 9.00 to 11.00 a.m. with the following adjustments: molar flow of air per unit leaf area 403.3 mmol $m^{-2}$ $s^{-1}$, atmospheric pressure 99.9 kPa, water vapour pressure into chamber ranged from 6.0 to 8.9 mbar, PAR at leaf surface was maximum up to 1711 mol $m^{-2}$ $s^{-1}$, temperature of leaf ranged from 28.4 to 32.4°C, ambient temperature ranged from 22.4 to 27.9°C and ambient $CO_2$ concentration was 352 μmol $mol^{-1}$.

**Nutrients analyses:** The harvested plant material was oven dried at 65°C for 72 hours. The dry samples of shoots were ground with grinding mill. About 0.5 g sample was digested with 5ml concentrated sulfuric acid for 30 min at about 300°C, cooled for 10 min, five drops of 30% $H_2O_2$ were added and the sample was boiled for 15 min. If the sample was not colorless, the last step was repeated. On cooling, it was diluted to 50 ml with distilled water and shaken thoroughly (Wolf 1982). The solutions were used for the determination of the concentration of the nutrient elements N, P and K. The measurement of N concentration was determined by using the Kjeldahl method (Bremner, 1965); P was determined by colorimetric method with spectrophotometer (Jackson, 1962). Potassium (K) was determined by flame photometer (Jenway PFP 7, UK). The nutrient elements concentration in wheat shoot was expressed as mg $g^{-1}$ DW (dry weight).

**Statistical analyses:** Significant differences among genotypes means were determined by analysis of variance according to Duncan's Multiple Range Test ($p \leq 0.05$) for experiment 1 and 2 and least significant difference test ($p \leq 0.05$) using statistical software STATISTICA.

## Results

**Screening experiments:** The data showed a highly significant ($P \leq 0.001$) interaction between genotypes and PEG-6000 induced stress levels for germination percentage (GP), germination index (GI), promptness index (PI), and germination stress tolerance index (GSI). In all tested ten genotypes, maximum GP, GI, PI, and GSI were observed under non-stress conditions which consistently decreased in response to increasing osmotic stress (Figs.1-4). Drought tolerant Bhakkar-2002 showed the maximum GP, GI, PI, and GSI under non-stress conditions. Drought sensitive genotype Shafaq-2006 showed the minimum GP, GI, PI, and GSI under water stress particularly when imposed at the rate -0.8 MPa. The GI of Bhakkar-2002 was statistically at par with Lasani-2008 under non-stress conditions. The Bhakkar-2002 and Lasani-2008 were also statistically at par in treatment where water stress was imposed at the rate -0.2 MPa. The maximum GSI of Bhakkar-2002 was statistically at par with Fareed-2006 and Lasani-2008 in the treatment where water stress was imposed at the rate -0.2 MPa (Fig. 4).

The data showed highly significant difference ($P \leq 0.001$) among genotypes and water stress treatments for root length stress tolerance index (RLSI), plant height stress tolerance index (PHSI) and dry matter stress tolerance index (DMSI) (Figs. 5-7).

Among all tested genotypes, maximum PHSI and RLSI were recorded in Bhakkar-2002, where PHSI in Bhakkar-2002 was statistically at par with Lasani-2008 under water stress conditions (Fig. 5). The maximum RLSI was recorded in Bhakkar-2002 (146.05%) under water stress conditions. Minimum PHSI was calculated in Shafaq-2006 (75.32%) and RLSI in Faisalabad (99.27%), (Fig. 6). Maximum DMSI was recorded in Chakwal-86 which was statistically at par with Bhakkar-2002. Minimum DMSI was recorded in Shafaq-2006 under water stress condition (Fig. 7). Maximum relative root: shoot was calculated in Ufaq-2002 under water stress conditions and minimum relative root: shoot was recorded in Lasani-08 under non-stress conditions. Relative root: shoot of Ufaq-2002 was statistically at par with Bhakkar-2002 under drought stress conditions (Fig. 8).

**Effects of NPK foliar spray:** The data showed that genotypes, various fertilizer doses and the interaction between (GXF) were markedly ($P \leq 0.01$) different for PHSI, and RLSI. Foliar application of nutrients (N, P, K alone or in various combinations) significantly increased these attributes under various water regimes (Figs. 9-10)

Plant height stress tolerance index (PHSI) significantly decreased under water stress conditions. Foliar application of N, P, and K alone or in combinations was highly significant for PHSI. The highest value for PHSI was recorded in both wheat genotypes, i.e., Bhakkar-2002 and Shafaq-2006 with foliar application of NPK under water stress conditions, while minimum value of PHSI was recorded in Shafaq-2006 at water spray treatment under water limited conditions (Fig. 9).

Data for root length stress tolerance index revealed highly significant ($P \leq 0.001$) interaction between GXF. Both wheat genotypes Bhakkar-2002 and Shafaq-2006 showed the highest values for RLSI with foliar applied NPK in combination under drought stress conditions (Fig. 10). Dry matter stress tolerance index (DMSI) significantly decreased under water deficit conditions. Application of NPK in combination, significantly increased DMSI under water stress conditions. Maximum value for DMSI was recorded in NPK treatment while minimum value was obtained in water spray treatment (Fig. 11).

Wheat plants exhibited significantly lower ($P \leq 0.001$) *Pn* under water stress as compared to well-watered conditions (Table 1). The exposure to drought stress decreased *Pn* rate by 50% as compared to well-watered conditions. Analysis of variance for the data regarding *Pn rate* showed highly significant ($P \leq 0.001$) difference between genotypes. The plants of Bhakkar-2002 maintained significantly higher *Pn* rate than Shafaq-2006. The foliar spray of NPK significantly ($P \leq 0.001$) increased *Pn* rate in wheat plants and gave maximum value for this variable, which is statistically at par with NK treatment whereas no NPK spray resulted in minimum *Pn* rate. The data regarding *gs* showed that water stress had highly significant ($P \leq 0.001$) on *gs*. The exposure to drought stress decreased *gs* (30%) as compared to normally grown wheat plants (Table 1). The interaction between WXF was significant. All other interactions were non-significant (Table 1).

The data regarding *E* showed that water stress had highly significantly ($P < 0.001$) effect on *E* (Table 1). A significant decrease of 44% was recorded in water stressed plants than well-watered ones. It was observed that Bhakkar-2002 genotype maintained significantly higher (7%) *E* than Shafaq-2006 genotype (Table 1). Highly significant ($P \leq 0.001$) effect of NPK application was observed on *E* of wheat plants. The plants foliarly sprayed with NPK gave maximum value. The interaction between WXF was significant. All other interactions were non-significant (Table 1).

Analysis of variance for the data of *gs* showed significant ($P \leq 0.05$) difference for genotypes (Table 1). Drought tolerant genotype (Bhakkar-2002) gave higher value for *gs* as compared to drought sensitive genotype (Shafaq-2006). Foliar application of NPK significantly ($P \leq 0.001$) increased *gs* in wheat plants which was statistically at par with NK, PK, K and N foliar sprays while minimum was recorded in no spray treatment (Table 1). The interactions between WXF and GXW were significant. All other interactions were non-significant (Table 1).

The plants exposed to water stress showed a significant ($P \leq 0.05$) decrease in $\Psi_w$(Table 1). Drought stress reduced $\Psi_w$ by 39% with respect to well-watered conditions. The decrease in $\Psi_w$ was more pronounced in Shafaq-2006 than Bhakkar-2002 genotype. A highly significant difference ($P \leq 0.001$) was observed between foliar spray treatments. The application of NPK as foliar spray increased $\Psi_w$ of plants and gave significantly higher value as compared to water spray (Table 1). The interaction between WXF was significant. All other interactions were non-significant (Table 1).

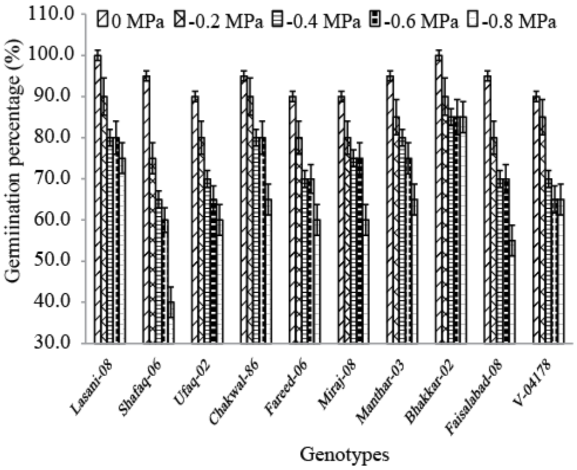

Fig. 1. Germination percentage of ten wheat (*Triticum aestivum* L.) genotypes under PEG induced water stress regimes (mean ± S.E).

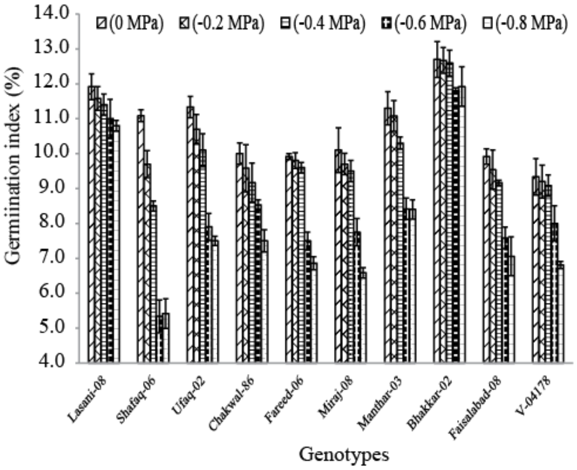

Fig. 2. Germination index of ten wheat (*Triticum aestivum* L.) genotypes under PEG induced water stress regimes of ten wheat genotypes (mean ± S.E).

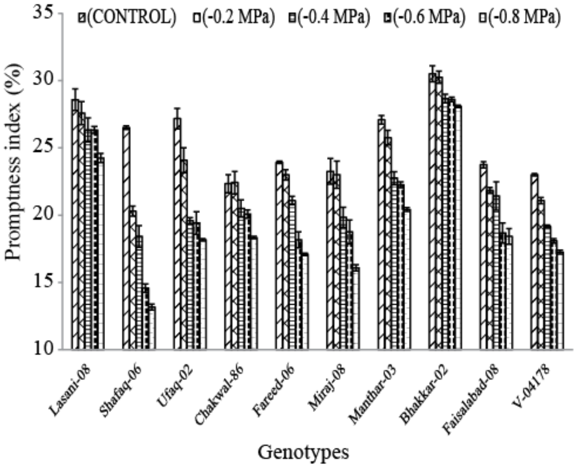

Fig. 3. Promptness index (PI) of ten wheat (*Triticum aestivum* L.) genotypes under PEG induced water stress regimes (mean ± S.E).

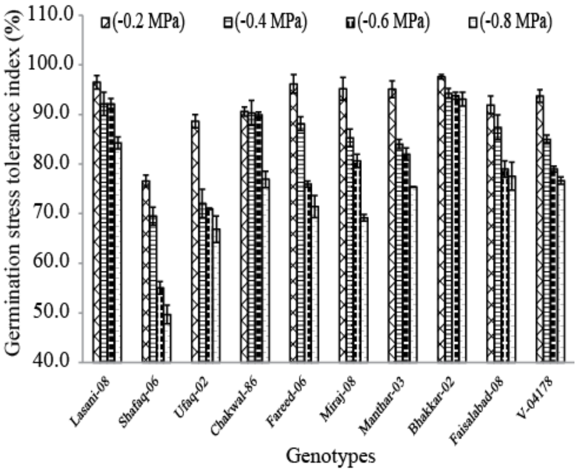

Fig. 4. Germination stress tolerance index of ten wheat (*Triticum aestivum* L.) genotypes under PEG induced water stress regimes (mean ± S.E).

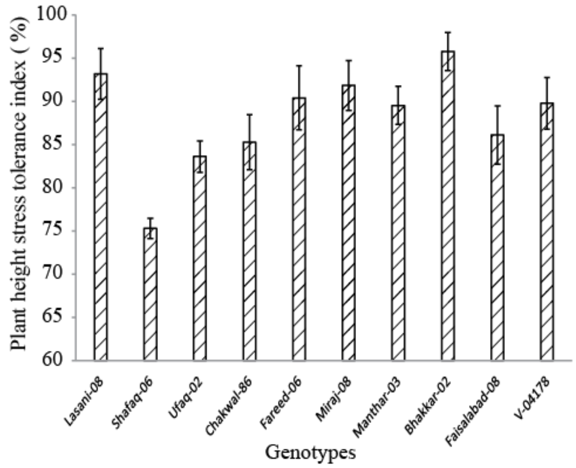

Fig. 5. Plant height stress tolerance index (PHSI) of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean ± S.E).

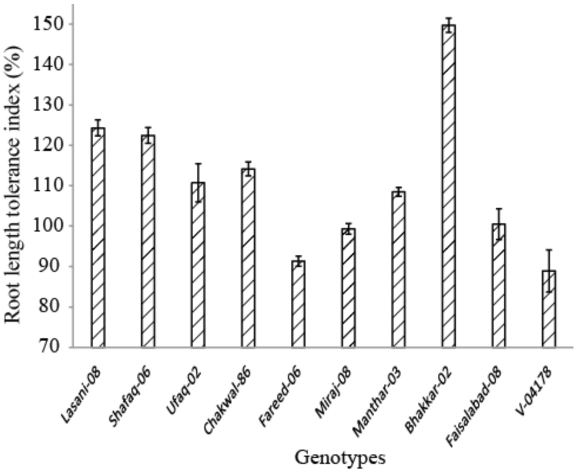

Fig. 6. Root length stress tolerance index (PHSI) of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean ± S.E).

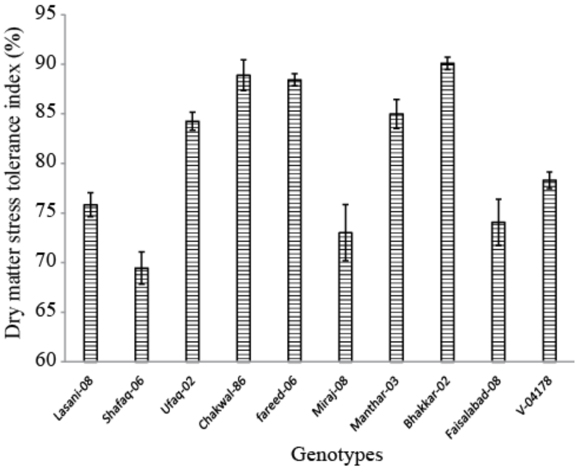

Fig. 7. Dry matter stress tolerance index (PHSI) of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean ± S.E).

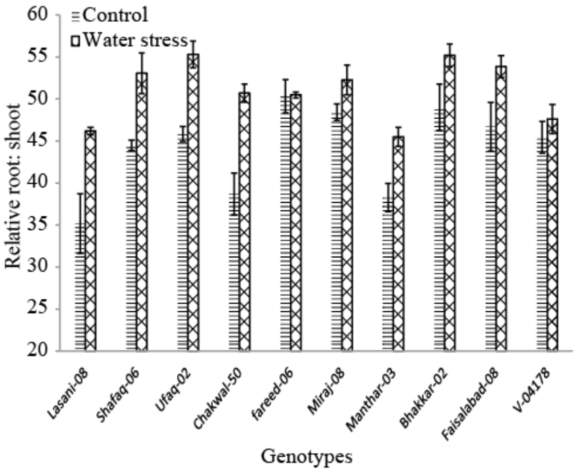

Fig. 8. Relative root:shoot of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean ± S.E).

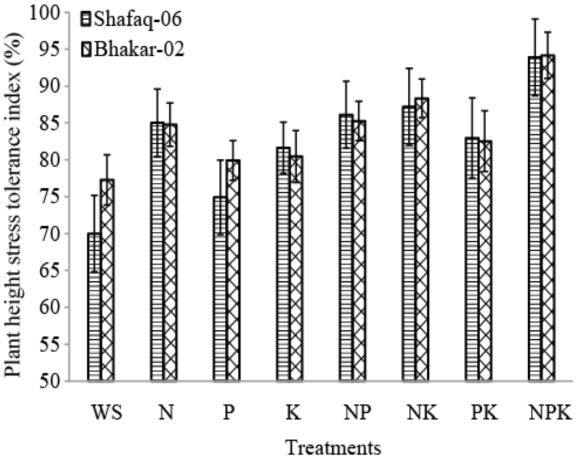

Fig. 9. Plant height stress tolerance index of wheat (*Triticum aestivum* L.) when plants were foliarly applied with various NPK levels under drought stress conditions (mean ± S.E).

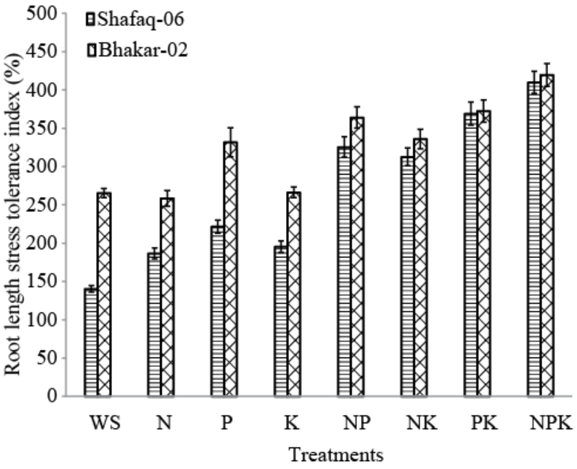

Fig. 10. Root length stress tolerance index (RLSI) of wheat (*Triticum aestivum* L.) when plants were foliarly applied with various NPK levels under drought stress conditions (mean ± S.E).

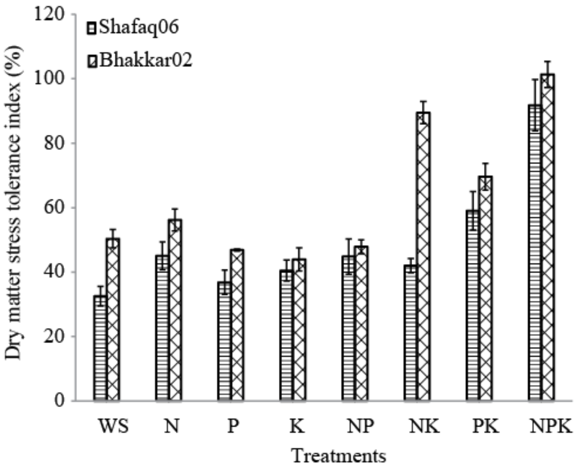

Fig. 11. Dry matter stress tolerance index of wheat (*Triticum aestivum* L.) when plants were foliarly applied with various NPK levels under drought stress conditions (mean ± S.E).

Analysis of variance for the data showed highly significant ($P \leq 0.001$) reduction in $\Psi_s$ of water stressed plants (Table 1). The water deficit conditions decreased $\Psi_s$ by 8% and gave significantly lower value for this variable than normal conditions. A much higher reduction (14%) was recorded with foliar N spray than no spray treatment (Table 1). The genotypes did not differ significantly for $\Psi_s$ (Table 1). All interactions were non-significant.

The data regarding $\Psi_p$ revealed highly significant effect of drought stress on this variable. The limited water supply reduced $\Psi_p$ by 47% as compared to well-watered conditions. The plants maintained significantly higher $\Psi_p$ with foliar NPK spray, which was statistically at par with NP, N, K, and NK spray while lower $\Psi_p$ was recorded in no spray treatment (Table 1). The genotypes did not differ significantly for $\Psi_s$. The interaction between WXF was significant. All other interactions were non-significant (Table 1).

Table 1. Mean parameter values of gas exchange, water relations, and nutrient content for main effects of genotype, foliar sprays, water levels and their interactions.

| Parameter[a] | Genotype[b] | | Foliar spray[c] | | | | | | | | | Water level[d] | | Interactions | | | | CV[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | S | NS | WS | N | P | K | NP | NK | PK | NPK | WW | WS | GXW | GXF | WXF | GXWXF | |
| *Pn* | 7.18a | 6.79b | 5.43 e | 5.62 e | 7.06 c | 6.1 d | 7.46 b | 7.7 b | 7.8 ab | 7.46 b | 8.1 a | 9.29 a | 4.65 b | NS | NS | *** | NS | 6.83 |
| *E* | 4.09a | 3.8 b | 3.2 g | 3.39fg | 3.73def | 3.62 ef | 3.94cde | 4.07 cd | 4.48 b | 4.19 bc | 4.88 a | 5.06 a | 2.82 b | ** | NS | *** | NS | 11 |
| *gs* | 2.78a | 2.63b | 2.26 d | 2.46cd | 2.8 ab | 2.54 c | 2.85 ab | 2.63 bc | 2.94 a | 2.87 a | 2.99 a | 3.18 a | 2.23 b | NS | NS | *** | NS | 9.62 |
| $\Psi_w$ | -1.0a | -1.1b | -1.04c | -1.15d | -1.06 c | -1.07cd | -1.03bc | -1.03bc | -0.95 b | -1.02bc | -0.87 a | -0.78a | -1.28b | NS | NS | *** | NS | 7.18 |
| $\Psi_s$ | -1.6a | -1.64a | -1.47 a | -1.7cd | -1.71 d | -1.68 d | -1.677cd | -1.69 d | -1.6bc | -1.5ab | -1.58bc | -1.55a | -1.69b | NS | NS | NS | NS | 23.6 |
| $\Psi_p$ | 0.6 a | 0.59 a | 0.43 d | 0.51cd | 0.65 a | 0.61abc | 0.64 a | 0.65 a | 0.63ab | 0.52bcd | 0.72 a | 0.78 a | 0.41 b | NS | NS | * | NS | 10.8 |
| N | 38.36a | 36.04b | 34.34c | 35.68c | 38.36 b | 34.68c | 35.40 c | 39.68ab | 40.93a | 34.51 c | 41.22a | 39.28a | 35.11b | * | NS | *** | NS | 6.18 |
| P | 3.17 a | 3.08 a | 2.97cd | 2.98cd | 3.08bcd | 3.18bc | 2.93 d | 3.09bcd | 3.1bcd | 3.3 ab | 3.47 a | 3.42 a | 2.83 b | NS | NS | * | NS | 8.96 |
| K | 40.14a | 36.62b | 34.66f | 35.28ef | 36.59 e | 34.64 f | 38.59 d | 39.46cd | 40.63bc | 41.75 b | 43.8 a | 40.44a | 36.31b | * | ** | NS | NS | 5.62 |

[a] *Pn*=photosynthetic rate (µmol $m^{-2}s^{-1}$), *E*= transpiration (mmol $m^{-2}s^{-1}$), *gs*= stomatal conductance (mmol$m^{-2}s^{-1}$), $\Psi_w$= water potential (-MPa), $\Psi_s$= osmotic potential (-MPa), $\Psi_p$= turgor potential (MPa), [b] mean values across two genotypes and two water levels; B= Bhakkar-2002, S= Shafaq-2006; [c] mean values across nine foliar spray treatments; NS= No spray, WS= Water spray, N= 1.5% urea, P= 2% $KH_2PO_4$, K= 3% $K_2SO_4$, NP= 50% N 50% P, NK= 50% N 50% K, PK= 50% P 50% K, NPK= 33% N 33% P 33% K; [d] mean values across two water levels; WW= Well-watered, WS= Water stress; [e] coefficient of variation; Statistically significant (insignificant) differences for each main factor are indicated by different lower case letters; non-significant, *,**,*** significant at p≤ at 0.05, p≤ at 0.01, p≤ at 0.001respectively

Wheat plants exhibited significantly lower ($P \leq 0.001$) leaf N content under water stress as compared to well-watered conditions (Table 1). The exposure to drought stress decreased leaf nitrogen content by 11% as compared to normal supply of water. Analysis of variance for the data regarding leaf N content showed highly significant ($P \leq 0.001$) difference between genotypes. The plants of Bhakkar-2002 maintained significantly higher leaf N content than Shafaq-2006. The foliar spray of NPK significantly ($P \leq 0.001$) increased leaf N content in wheat plants and gave maximum value for this variable which was statistically at par with NK and NP spray whereas no NPK supply resulted in minimum leaf N content (Table 1). The interactions between WXF and GXW were significant. All other interactions were non-significant (Table 1).

The data regarding leaf P contents revealed highly significant ($P \leq 0.001$) differences of drought stress on this variable. The limited water supply reduced leaf P contents (17%) as compared to well-watered conditions. The foliar spray of NPK was highly effective in the uptake of higher leaf P. The plants foliarly applied with NPK gave significantly higher value that was statistically at par with PK spray and minimum was recorded in no spray and K spray treatment (Table 1).The genotypes did not differ significantly for leaf P contents. The interaction between WXF was significant. All other interactions were non-significant (Table 1).

Wheat plants exhibited significantly higher ($P \leq 0.001$) leaf K content under water stress than normal conditions (Table 1). The exposure to drought stress increased leaf K content by 10% as compared to normal supply of water. The increase in leaf K content was more pronounced (9%) in Bhakkar-2002 than Shafaq-2006 genotype. The plants maintained significantly higher (21%) leaf K content with foliar NPK spray as compared to no spray and P spray (Table 1). The interaction between GXW and GXF were significant. All other interactions were non-significant (Table 1).

## Discussion

Drought stress adversely affects plant growth and development (Shahbaz *et al*., 2011). Screening of drought tolerant genotypes is an effective approach to enhance the productivity in drought prone areas (Dhanda *et al*., 2004). In current study, PEG-induced various drought stress levels significantly reduced the seed germination ability of 10 wheat genotypes. All the genotypes showed a variable drought tolerant level under water deficit conditions. A great variation in wheat genotypes has already been observed by Ashraf *et al*. (1996) in response to PEG-induced water stress. In current experiment, PEG-induced water stress increased the time to germination and decreased the final germination percentage. The increase in PEG concentrations caused a decrease in the uptake of water by seeds, which caused a decline in germination percentage (Kaydan & Yagmur, 2008). The germination stress tolerance index (GSI) can be used as an effective criterion for genotype screening against various drought stress regimes (Fernandez, 1992). Our results showed that GSI decreased due to PEG-induced water stress irrespective of the genotype. Among tested genotypes, Bhakkar-2002 showed the maximum values of GSI at all levels of water stress. High values of GSI indicated the potential for drought tolerance (Zahra & Farshadfar, 2011) and in our study Bhakkar-2002 showed the maximum values of GSI under drought stress conditions as compared to other genotypes.

In current study, an increase in seedling root length was observed in all wheat genotypes under water stress conditions. This extension in root length happened at the cost of reduction in shoot length, thus shifting the relative root: shoot (R:S) equilibrium in favour of the roots which has also been described by various plant scientists (Ashraf & Sarwar, 2002; Guoxiong *et al*., 2002). The increase in R:S length under water limitations may be attributed to reduction in supply of water and nutrients to the shoot. Reduction in shoot length under water-limited conditions might be due to decrease in cell expansion, which ultimately reduced the plant height (Okçu *et al*., 2005; Shahbaz *et al*., 2011). Root length and seedling dry weight can be used as major selection criteria for screening genotypes against drought stress (Dhanda *et al*., 2004; Qayyum *et al*., 2012). Among tested genotypes, the drought-tolerant Bhakkar-2002 was high in root length, root length stress tolerance index(RLSI), and total seedling dry weight whereas drought-sensitive Shafaq-2006 was low in root length, RLSI, and total seedling dry weight. Deep roots and ability to accumulate dry biomass are considered typical characteristics of drought tolerant genotypes (Zhao *et al*., 2004). Germination rate and final GP correlate with root length and how much biomass is accumulated per unit area (Okçu *et al*., 2005).

Two selected wheat genotypes i.e. Bhakkar-2002 and Shafaq-2006 were used for further study. Our findings showed that supplemental foliar application of NPK in combination increased the plant height, root length, dry matter, photosynthetic rate (*Pn*), stomatal conductance ($g_s$), transpiration rate (*E*), water relations in both wheat genotypes i.e., Bhakkar-2002 and Shafaq-2006 under both well-watered and water-stress conditions. Fertilizers application increased nutrient contents under water stress conditions, which might be helpful for plants to cope with the adverse effects of water stress on morphological and physiological characteristics. For example, NPK application increased plant height and RLSI which might be due to the increased uptake of nutrients. Foliar application of NPK in combination was more effective as compared to alone application of N, P, K, or in combinations of two nutrients under both non-stress and water stress conditions. Under water limited conditions, P increased the early root growth (Noack *et al*., 2010) and K root weight (Baque *et al*., 2006) and root length (Ashraf *et al*., 2008). High root density plays crucial role in obtaining water from the deeper soil layers, whereas deeper roots decrease the moisture loss in soil. The promotion of root growth might increase water and nutrient uptake by plants (Ashraf *et al*., 2005). In addition to the role of P and K in promoting root growth, the use efficiency of N might also be indirectly affected. Foliar application of NPK in combination also helped in maintaining the dry matter stress tolerance index (DMSI) as compared to other treatments possibly by supplementing the plant's requirement for macronutrients- N, P, and K. Increase in plant dry weight (root and shoot) with foliar spray of

$K_2SO_4$ was reported in mung bean (Ihsan *et al*., 2013) and wheat (Imanparast *et al*., 2013) and with foliar spray of $KH_2PO_4$ in tomato by Kaya *et al.* (2001).

The leaf water relations of wheat genotypes significantly decreased under water deficit conditions however, foliar spray treatment of NPK in combination was effective in maintaining high water content. Foliar spray of NPK in combination also helped maintaining the water status of plants possibly through osmotic adjustment (Shabala & Lana, 2011). Increase in leaf K concentration decreased the water potential of plants through maintaining the turgor pressure in sunflower (Bajehbaj *et al*., 2009). In contrast, Ratnayaka & Kincaid, (2005) observed non-significant change in leaf water potential of senna plants with foliar-applied N under non-stress and drought stress conditions. A positive turgor is necessary for cell expansion and growth under water stress conditions (Zonia *et al*., 2006).

Photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*) increased by supplemental foliar application of combined NPK fertiliser in both wheat genotypes Bhakkar-2002 and Shafaq-2006 under water deficit conditions. Supplemental foliar fertilisation was also effective in improving the plant growth of drought-sensitive genotype. The decline in *Pn* under water stress may be associated with restriction of $CO_2$ diffusion into the leaf, and also inhibition of biochemical processes such as ATP synthase and Rubisco activity due to lower NPK accessibility for investment into photosynthetic apparatus. Nitrogen and K are involved in the regulation of *Pn* in plants (Baker, 1996). Nitrogen uptake decreased under water-limited conditions which ultimately lowers the chlorophyll contents resulting in decreased *Pn* (Toth *et al*., 2002). Foliar applied N increased the *Pn* under water stress conditions in various plants like maize (Zhang *et al*., 2012), senna (Ratnayaka & Kincaid, 2005), wheat (Shangguan *et al*., 2000). It is well recognised that N enhances the cell number and cell size and increases the efficiency of *Pn* in leaves (Lawlor *et al*., 1988). There was 51% increase in the *Pn* in the safflower plants supplied with soil applied N as compared to the control plants (Dordas & Sioulas, 2008). Decrease in *Pn* might be due to stomatal closure which restricts the carbon uptake by the leaves (Cornic & Massacci, 1996). Potassium has been shown to play a significant role in the opening and closing of leaf stomates which control the movement of $CO_2$ into the plant and water into the air, and would therefore, have an effect on $g_s$ (Bednarz *et al*., 1998). Potassium application improved the *Pn, E*, and intercellular $CO_2$ concentration in sweet potato-under water stress conditions (Zhu *et al*., 2012). Phosphorous under mild water deficit improved water use efficiency in P treated wheat plants (dos-Santos *et al*., 2004). The encouraging effects of P on plant growth under water stress have been ascribed as to enhancing the efficiency of P, stomatal conductance ($g_s$), and water use efficiency (Ackerson, 1985).

The concentration of N and P decreased while K increased in both wheat genotypes under water deficit conditions. Our findings correspond to the findings observed in linseed (Chourasia *et al*., 1992), maize (Foyer *et al*., 1998), rice (Beyrouty *et al*., 1994) and mungbean (Satyanarayanamma *et al*., 1996). Foliar application of urea increased the N, P, and K contents (Afifi *et al*., 2011) in maize (Murillo-Amador *et al*., 2006) in cowpea.

The diffusion coefficient of P in soil is very low, hence the root zone P is depleted and plants cannot absorb P (Clarkson, 1981). Therefore, the utilization of P as a foliar application becomes increasingly important. The mechanistic processes by which foliar applied nutrients are taken up are through leaf stomata (Eichert & Burkhardt, 1999) and hydrophilic pores within the leaf cuticle (Tyree *et al*., 1990). The decline in nutrient concentration of water-stressed plants can be described by the fact that under water-limited conditions, diffusion rate and mass flow of nutrient from rhizo-sphere to the root surface becomes slow due to the replacement of water by air in the soil pores (Chapin, 1991), ensuing in less availability of these nutrients to the plants. Hence, the transport of P from root to the leaf reduced. That is why low accumulation of phosphorous in water stressed plants of wheat genotypes was observed in our study, which is in line with the finding*s* of Ashraf, (1998) in wheat. In current study, it is clear that, under water stress conditions, foliar application of combined NPK fertilizer was more beneficial in enhancing the leaf P and K concentration than other combinations. Earlier reports suggested that increase in leaf P with foliar N sprays in maize (Afifi *et al*., 2011) and pearl millet (Ashraf *et al*., 2001). It is also reported that the increase in leaf $K^+$ concentration results in a parallel increase in the stomatal conductance and decrease in leaf osmotic potential (Patakas *et al*., 2002).

## Conclusion

Screening of drought tolerant genotypes at germination and seedling stages can be useful in decreasing the threat of poor stand establishment under water-limited conditions. On the basis of germination parameters and stress tolerance indices, we found that Bhakkar-2002 was the drought-tolerant and Shafaq-2006 drought-sensitive among tested genotypes. Furthermore, supplemental foliar application of NPK in combination improved gas exchange and water-relations, and nutrients concentration under both well-watered and water-stress conditions, which helped both tolerant and sensitive genotype to improve plant biomass at seedling stage but Drought tolerant Bhakkar-2002 showed more improvement than Shafaq-2006. This shows the foliar spray of NPK in combination is the most appropriate strategy for supplemental fertilisation as compared to their application as alone or in combination of two minerals.

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