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RESEARCH ARTICLE

Supplemental exogenous NPK application alters biochemical processes to improve yield and drought tolerance in wheat (*Triticum aestivum* L.)

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Abstract The recent food security issues, combined with the threats from climate change, demand future farming systems to be more precise and accurate to fulfill the ever increasing global food requirements. The role of nutrients such as nitrogen (N), phosphorous (P), and potassium (K) in stimulating plant growth and development is well established; however, little is known about their function, if applied in combination, in improving crop yields under environmental stresses like drought. The aim of this study was to evaluate the effects of combined foliar spray of supplemental NPK (NPK_c) on physiological and biochemical mechanisms that enhance the drought tolerance potential of wheat for improved yield. Foliar NPK_c markedly influenced the accumulation of osmoprotectants and activity of both nitrogen assimilation and antioxidant enzymes. It significantly improved the concentration of proline (66 %), total soluble sugars (37 %), and total soluble proteins (10 %) and enhanced the activity of nitrate reductase, nitrite reductase, catalase, and peroxidase by 47, 45, 19, and 8 %, respectively, with respect to no spray under water-deficit conditions which, in turn, improve the yield and yield components. The accumulation of osmolytes and activity of antioxidant machinery were more pronounced in drought tolerant (Bhakkar-02) than sensitive genotype (Shafaq-06).

Keywords NPK_c · Osmoprotectants · Antioxidants · Wheat · Drought

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Introduction

Drought stress is one of the most important abiotic factors constraining harvestable yield of crops across the globe (Nawaz et al. 2012). It negatively affects plant growth and development due to impaired germination and seedling growth (Ashraf et al. 2006), decreased water relations (Siddique et al. 2003), reduced photosynthetic activity (Wahid and Rasul 2005), and increased production of reactive oxygen species (ROS). The drastic effects of water stress have been well reported in many crops such as sunflower (Sajjan et al. 1999; El-Midaoui et al. 2001), sugar beet (Sadeghian and Yavari 2004), maize (Moussa and Abdel-Aziz 2008), sorghum (Gill et al. 2002), kochia (Masoumi et al. 2010), and wheat (Waraich et al. 2012).

The supplemental supply of plant nutrients such as silicon (Sacala 2009), selenium (Nawaz et al. 2013, 2014), salicylic acid (Waseem et al. 2006), and zinc (Weisany et al. 2012) has found to be effective in improving the drought tolerance potential of plants. The role of nitrogen (N), phosphorous (P), and potassium (K) as stress ameliorants is also well established (Kaya et al. 2001; Raza et al. 2012; Gevrek and Atasoy 2012). However, most of the literature indicated the evaluation of individual effects of each nutrient on plants' life cycle, and the reports regarding combined effects of these nutrients are scant.

The effectiveness and bioavailability of nutrients to plants are influenced by several factors such as soil moisture (Zhao et al. 2007), organic matter, increase in acidity, and high clay content of soil (Kabata-Pendias and Pendias 2001). The foliar application can be used as an alternative approach to supply nutrients to plants (Smrkolj et al. 2006) because it reduces the impact of soil chemistry and microbiology on uptake and accumulation of nutrients particularly N, P, and K. Moreover, it results in proper leaf nutrition as well as carbon balance and improves water relations and photosynthetic capacity of plants (Oosterhuis and Bondada 2001; dos Santos et al. 2004; Ihsan et al. 2013) due to direct transfer and accumulation in plants.

In our previous study, we successfully demonstrated that foliar spray of NPK, in combination, was more effective than individual effects of these nutrients in improving growth and physiological processes in wheat under drought stress (Shabbir et al. 2015). However, the report was a short-term study and was limited to the estimation of effects of combined foliar spray on biomass accumulation and water relations of wheat seedlings; therefore, the present study aimed at investigating the effect of NPK foliar spray, applied in combination (NPK_c), on biochemical mechanisms and yield in wheat under normal and water-deficit conditions and can be considered as a continuation of the early reports. We hypothesize that supplemental foliar NPK_c enhanced accumulation of osmoprotectants and increased activity of antioxidant machinery to improve growth and yield of wheat under water stress conditions.

Materials and methods

Experimental layout and plant material

A manually operated rainout shelter, equipped with movable sheet of transparent flexible plastic sheet, at Department of Crop Physiology, University of Agriculture, Faisalabad (Pakistan) was used as experimental site to conduct the experiment. The experiment was laid out in randomized complete block design (RCBD) with split plot arrangements and four replications. The pots (15 cm dia × 11 cm length) were wrapped with plastic bags filled with 2 kg sand (sun dried, grounded, sieved, and well mixed at the beginning of the experiment) used as experimental material. The characteristics of the growth medium and basal nutrients (using urea, diammonium phosphate, and potassium sulfate as sources of N, P, and K, respectively) applied in solution to each pot were according to Shabbir et al. (2015).

The seeds of two local wheat genotypes, i.e., Bhakkar-02 and Shafaq-06, identified as drought tolerant and sensitive ones in our early reports (Shabbir et al. 2015), were obtained from Ayyub Agricultural Research Institute, Faisalabad (Pakistan). Randomly selected 100 g seeds of each genotype were sterilized with sodium hypochlorite (5 %) solution for 5 min and later washed with distilled water and air-dried to their original moisture level before the start of the experiment. Initially, ten seeds of each genotype were sown in each pot, but only five plants were kept after thinning in each replication. The plants were grown till maturity during the normal spring wheat growth season of about 150 days and then harvested.

Drought stress and NPK concentration

The plants were exposed to water stress 8 days after sowing (completion of germination). The field capacity (FC) level (100 % FC) was measured for normal plants, i.e., 364 mL for each pot. Tap water was used for irrigating normal plants with this amount, whereas 218.4 mL (60 % FC) was applied to water-stressed plants. For the maintenance of FC levels, each treatment pots were weighed twice daily in the morning (9:00 am) and evening (05:00 pm), calculated the amount of water consumed in evapotranspiration, and re-irrigated until the pot weight reached the predetermined weight. Two foliar spray treatment, i.e., no spray and NPK_c (a mixture of N=1.5 %, P=2 %, and K=3 %) containing 33 % each nutrient and 0.1 % Tween-20 as a surfactant, were applied twice with a difference of 2 weeks in each application. The final foliar spray was carried out during anthesis stage. A compression sprayer of 1 L capacity was used for spraying, performed in the morning (between 08:00 and 10:00) on a sunny day, to ensure even distribution of NPK_c on all leaves. The leaf samples randomly collected 1 week after foliar treatment were used for the biochemical analyses.

Determination of osmoprotectants

For the estimation of total soluble sugars (TSSs), fresh leaf material (0.5 g) from each treatment was extracted in 80 % ethanol and incubated for 6 h at 60 °C. This extract was taken in 25-mL test tubes, and 6 mL anthrone reagent (always prepared fresh by dissolving 150 mg of anthrone in 72 % H_2SO_4 solution) was added to each tube, which was later heated in boiling water bath for 10 min. The tubes were then ice-cooled for 10 min and incubated for 20 min at room temperature (25 °C). Optical density was read at 625 nm on a spectrophotometer (Hitatchi, 2800, Japan). The concentration of TSS was calculated from the standard curve according to the method published by Riazi et al. (1985).

The same amount of fresh leaf material (0.5 g), extracted in 0.2 M phosphate buffer (pH 7.0), was used for the determination of total soluble proteins (TSPs) and total free amino acids (TFAs) following the procedures reported by Lowry et al. (1951) and Hamilton and Van Slyke (1973), respectively. Copper reagents, for the estimation of TSP, were prepared as follows: Na_2CO_3 (2.0 g), NaOH (0.2 g), and sodium

potassium tartarate (1.0 g) were dissolved in distilled water, and the volume was made to 100 mL (solution A). $CuSO_4 \cdot 5H_2O$ solution was prepared by dissolving 0.5 g $CuSO_4 \cdot 5H_2O$ in 100 mL distilled water (solution B). Fifty milliliters of solution A and 1.0 mL of solution B were mixed to prepare alkaline solution (solution C). This solution was always prepared fresh. One milliliter of the leaf extract from each treatment was taken in a test tube. The blank contained 1 mL of phosphate buffer (pH 7.0). One milliliter of solution C was added to each test tube. The reagents in the test tube were thoroughly mixed and allowed to stand for 10 min at room temperature. Then, 0.5 mL of Folin-phenol reagent (1:1 diluted) was added, mixed well, and incubated for 30 min at room temperature. The optical density (OD) was read at 620 nm on a spectrophotometer (Hitachi 2800, Japan) for recording TSP.

Total free amino acids were determined by adding 1 mL of the leaf extract from each treatment in 1 mL of pyridine (10 %) and 1 mL of ninhydrin (2 %) solution in a test tube. Ninhydrin solution was prepared by dissolving 2 g ninhydrine in 100 mL distilled water. The test tubes with sample mixture were heated in boiling water bath for about 30 min. Volume of each test tube was made up to 50 mL with distilled water. The optical density of the colored solution was recorded at 570 nm using spectrophotometer (Hitachi 2800, Japan). TFA were estimated by a standard curve developed with leucine.

Proline contents were determined according to the method of Bates et al. (1973). Fresh leaf material of 0.5 g was ground and dissolved in 10 mL of 3 % sulfosalicylic acid. The sample material was filtered by using Whatman No. 2 filter paper. Two milliliters of the filterate was taken in a test tube and reacted with 2 mL acid ninhydrin solution. Acid ninhydrin solution was prepared by dissolving 1.25 g ninhydrine in 30 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid. Two milliliters of glacial acetic acid was added in the test tube and kept for 1 h at 100 °C. After terminating the reaction in an ice bath, the reaction mixture was extracted with 10 mL toluene. Continuous air stream was passed vigorously for 1–2 min in the reaction mixture. The chromophore containing toluene was aspirated from the aqueous phase and warmed at room temperature, and the absorbance was noted at 520 nm on spectrophotometer. Toluene was used as a blank. The proline concentration was calculated by using a standard curve and estimated on fresh weight basis using the formula reported by Nawaz et al. (2015):

$$\text{mmol proline } g^{-1}\,\text{FW} = \left[\left\{\left(\text{mg of proline mL}^{-1}\right) \times (\text{mL of toluene})\right\}/(\text{wt. of sample}/5)\right]/115$$

Estimation of nitrite reductase and nitrate reductase activity

The method published by Sym (1984) was used for the estimation of nitrate reductase (NR) activity in leaves using KNO_3 as a substrate. At first, 0.02 M KNO_3 solution was prepared in chilled phosphate buffer (pH 7.0). Fresh plant (leaves) material (0.5 g) from each treatment was extracted in 5 mL phosphate buffer (pH 7.0) containing 0.02 M KNO_3 and incubated in the dark at 32 °C for 1 h. This reaction medium (1 mL) was later mixed with 0.5 mL sulfanilamide prepared in 2 N HCl. After this, 0.5 mL of 1-naphthylethylenediamine dihydrogen chloride was added which resulted in the formation of pink diazo color complex due to NO_2. A set of standards developed with $NaNO_2$ were used to record absorbance at 542 nm on a spectrophotometer (Hitachi 2800).

The nitrite reductase (NiR) activity in leaves was determined following the procedure reported by Ramarao et al. (1983) using $NaNO_2$ solution (0.02 M) prepared in phosphate buffer of pH 5.0. Fresh leaf material (0.5 g) was extracted in 4.5 mL phosphate buffer (pH 7.0) and 0.5 mL of $NaNO_2$ (0.02 M) and was later incubated at 30 °C in water bath for 30 min. The reaction was terminated by transferring the sample in boiling water for 2 min and then cooled. One milliliter of cooled extract was taken in test tubes, and 1 % sulfanilamide was prepared in 2 N HCl, and 0.02 % of aqueous solution of *N*-1-naphthylethylenediamine dihydrochloride was added in each tube. The optical density was read at 542 nm on a spectrophotometer (Hitachi 2800) using $NaNO_2$ to develop standard curve. The NiR activity was expressed as NO_2 utilized g^{-1} FW h^{-1}.

Assay of antioxidant enzyme activity

Catalase (CAT) activity was determined by measuring the conversion rate of hydrogen peroxide (H_2O_2) to water and oxygen molecules, following the method described by Chance and Maehly (1955). The activity was assayed in 3 mL reaction solution comprising 50 mM phosphate buffer with 7.0 pH, 5.9 mM of H_2O_2, and 0.1 mL enzyme extract. The catalase activity was determined by decline in absorbance at 240 nm after every 20 s due to consumption of H_2O_2. Absorbance change of 0.01 U min^{-1} was defined as one unit catalase activity.

The activity of peroxidase (POD) was determined by measuring peroxidation of H_2O_2 with guaiacol as an electron donor (Chance and Maehly 1955). The reaction solution for POD consists of 50 mM phosphate buffer with pH 5, 20 mM of guaiacol, 40 mM of H_2O_2, and 0.1 mL enzyme extract. The increase in the absorbance due to the formation of tetraguaiacol at 470 nm was assayed after every 20 s. One unit of the enzyme was considered as the amount of the enzyme that was responsible for the increase in OD value of 0.01 in 1 min. The enzyme activity was determined and expressed as U min^{-1} g^{-1} fresh weight basis.

Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance of ascorbic acid at

290 nm (extinction coefficient 2.8 mM cm^{-1}) in a 1 mL reaction mixture containing 50 mM phosphate buffer (pH 7.6), 0.1 mM Na-EDTA, 12 mM H_2O_2, 0.25 mM ascorbic acid, and the sample extract as described by Cakmak (1994).

Analyses of nutrients

The leaves were washed with deionized water and later oven dried at 72 °C before digestion and analyses. Dried ground material (0.5 g) was taken in digestion tubes, and 5 mL of concentrated H_2SO_4 was added to each tube (Wolf 1982). All the tubes were incubated overnight at room temperature. Then, 0.5 mL of H_2O_2 (35 %) was poured down the sides of the digestion tube, ported the tubes in a digestion block, and heated at 350 °C until fumes were produced. They were continued to heat for another 30 min. The digestion tubes were removed from the digestion block and cooled, and 0.5 mL of H_2O_2 was slowly added in each tube, and the tubes were placed back into the digestion block. The above step was repeated till the cooled digested material was colorless. The volume of the extract was maintained up to 50 mL in volumetric flasks. The extract was filtered using Whatman No. 40 filter paper and used for determining N, P, and K contents in the leaves of wheat.

Estimation of N

Nitrogen was estimated by micro-Kjeldhal method (Bremner 1965). The following reagents were used for N determination: 3 % boric acid solution, 0.01 N sulphuric acid standard (0.01 N), and mixed indicator of bromocresol green and methyl red. The digested material (5 mL) was taken in Kjeldhal tubes. The tubes were placed on the Kjeldhal ammonia distillation unit, and 5 mL of 40 % NaOH was added to each tube. Boric acid solution (5 mL) was taken in a conical flask with few drops of mixed indicator. When the distillate was approximately 40 mL, the distillation was stopped. The distillate was cooled for few minutes and titrated it against 0.01 N standard H_2SO_4 till the solution turned pink. A blank was run for the complete procedure.

N was estimated by the following formula:

$$\%\mathrm{N} = \frac{(V_2 - V_1) \times N \times 0.014 \times 100}{W}$$

where V_2 represents the volume of standard H_2SO_4 required to titrate the sample solution, V_1 indicates the volume of standard H_2SO_4 required to titrate the blank solution, N is the normality of H_2SO_4, and W stands for the weight of the sample.

Estimation of elemental P

Leaf P contents were determined by measuring phosphomolybdenum complex colorimetrically at 460 nm using a spectrophotometer (Malavolta et al. 1997). The extracted material (2 mL) was dissolved in 2 mL of Barton's reagent, and total volume was made 50 mL. These samples were kept for half an hour before analyzing P. The values of P for each treatment were calculated by using standard curve.

Estimation of cations (K^+)

The extracted material obtained after digestion was used for the estimation of potassium (K^+) using flame photometer (Wolf 1982).

Determination of yield and yield components

The wheat plants from each treatment were manually harvested for the determination of yield components (spike length, spikelets $spike^{-1}$, and grains $spike^{-1}$). The seeds were cleaned, and their weight was recorded with the help of an electric balance to get 1000-grain weight in grams. The grain yield per pot was calculated, weighed, and expressed in g pot^{-1}.

Statistical analysis

The results were statistically analyzed using analysis of variance (ANOVA) technique, and significant means between treatments were compared by least significant difference test (LSD) at 0.05 significance level of probability using STATISTIX (version 8.1).

Results

Osmoprotectants

The exposure to drought stress significantly increased the concentration of TSS and TFA (24 and 19 %, respectively), whereas TSP decreased (50 %) in the leaves of water-stressed plants with respect to normal ones (Figs. 1–3). The accumulation of TSS was much higher (37 %) in plants foliarly sprayed with NPK_c under drought stress than normal conditions (13 %). Similarly, the supply of NPK_c increased TSP by 10 % as compared to no spray, while it did not significantly influence TFA (Fig. 2). Wheat genotypes also exhibited marked differences for the accumulation of osmoprotectants (Table 1) as Bhakkar-02 accumulated 13 % more TSS and TSP than Shafaq-06; however, both genotypes were statistically similar for TFA (Figs. 1–3).

Proline contents were found to be significantly higher (29 %) in water-stressed than normal plants (Fig. 4). The foliar

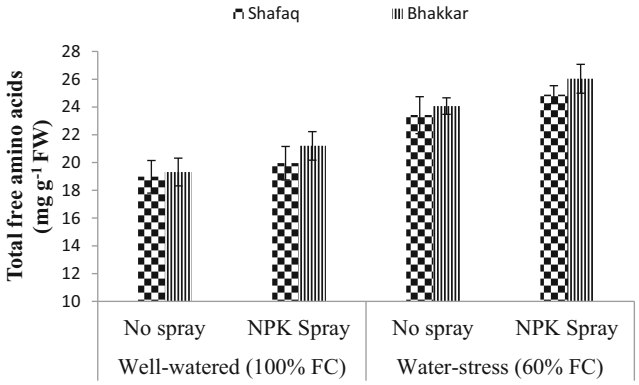

Fig. 1 Effect of supplemental foliar NPK application on total free amino acids (mg g^{-1} FW) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

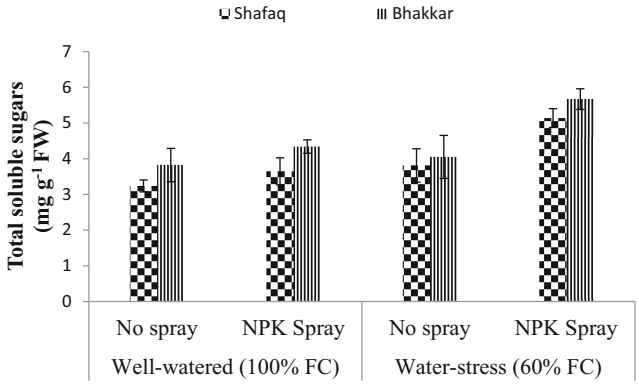

Fig. 2 Effect of supplemental foliar NPK application on total soluble sugars (mg g^{-1} FW) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

application of NPK_c markedly enhanced the proline concentration by 66 % than no spray under water-deficit conditions (Fig. 4), whereas non-significant effect of NPK_c on proline contents was recorded under normal conditions. Likewise, genotypes exhibited non-significant difference for this variable (Table 1).

Nitrate reductase and nitrite reductase

Drought stress significantly reduced the enzymatic activity of NR and NiR by 27 % (Fig. 5) and 19 % (Fig. 6), respectively. The foliar spray of NPK_c was found to be effective in improving the activity of these enzymes under both normal and water stress conditions. It enhanced NR activity by 18 % in normal plants; however, water-stressed plants, foliarly sprayed with NPK_c, exhibited much higher activity (4.26 μmol $NO_2 g^{-1}$ FW h^{-1}) than those applied with no spray (2.89 μmol NO_2 g^{-1} FW h^{-1}) under limited water conditions (Fig. 5). Foliar application of NPK_c also enhanced NiR activity (45 %) of plants exposed to water stress with respect to no spray. The normal plants of Bhakkar-02 showed the highest NiR activity (5.93 μmol $NO_2 g^{-1}$ FW h^{-1}) by NPK_c foliar spray, whereas no spray resulted in the lowest NiR activity (2.88 μmol NO_2 g^{-1} FW h^{-1}) in Shafaq-06 under drought stress (Fig. 6).

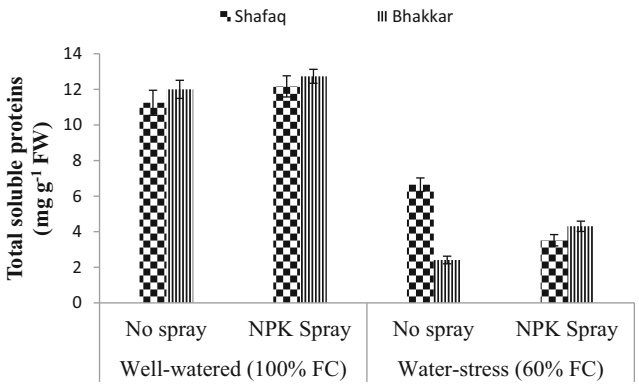

Fig. 3 Effect of supplemental foliar NPK application on total soluble proteins (mg g^{-1} FW) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

Antioxidants

A significant increase in the activity of CAT (14 %), POX (5 %), and APX (55 %) was recorded under water-deficit conditions (Figs. 7–9). The foliar NPK_c application resulted in the highest CAT (375 U min^{-1} g^{-1} FW h^{-1}) activity in water-stressed plants of Bhakkar-02, whereas no spray resulted in the lowest CAT activity (216 U min^{-1} g^{-1} FW h^{-1}) in Shafaq-06 under normal conditions. Wheat plants foliarly sprayed with NPK_c showed 19 % higher CAT activity than no spray under drought stress conditions (Fig. 7). Similarly, marked effect of NPK_c was recorded on the POX activity with an overall increase of 8 % as compared to no spray under water-deficit conditions (Fig. 8); however, it did not significantly affect the APX activity (Fig. 9).

Nutrient concentration

The foliar spray of NPK_c significantly increased concentration of nutrients like N, P, and K in wheat plants under water stress conditions. The supplemental foliar application of NPK_c improved nutrient accumulation in wheat leaves, but it was only significantly higher in individual factors sprayed with NPK_c, whereas NPK_c foliar spray did not affect the interactive effect of treatments with genotypes and water levels (Table 2). The water-stressed plants exhibited the lowest N contents (15.93 mg g^{-1} DW), P (3.58 mg g^{-1} DW) and K (31.35 mg g^{-1} DW) contents in the leaves of wheat. The application of NPK_c as foliar spray also improved N contents (17.76 mg g^{-1} DW), P (4.47 mg g^{-1} DW), and K (38.59 mg g^{-1} DW). The drought-tolerant genotype Bhakkar-02 showed higher N contents (18.93 mg g^{-1} DW), P (4.62 mg g^{-1} DW), and K (37.82 mg g^{-1} DW) than Shafaq-06. The only interaction between genotype and water levels was found to be significant for nitrogen contents. The highest

Table 1 Summary of the ANOVA for total free amino acid (TFA), total soluble sugars (TSSs), total soluble proteins (TSPs) (mg g^{-1} FW), free proline (mmol proline g^{-1} FW), nitrite reductase (NIR), nitrate reductase (NRA) (μmol NO_2 g^{-1} FW h^{-1}) catalase (CAT), peroxidase (POX) (U min^{-1} g^{-1} FW), and ascorbate peroxidase activity (APX) (ABA digested g^{-1} FW h^{-1}) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions with foliar applied nutrient NPK_c

SOV	TFA	TSS	Free proline	TSP	NIR	NR	CAT	POX	APX
Genotypes (G)	NS	**	NS	***	***	*	***	***	*
Water levels (W)	*	***	***	***	***	***	***	***	NS
Treatments (T)	NS	***	***	***	***	***	***	***	NS
GXT	NS	NS	NS	NS	*	NS	*	NS	NS
GXW	NS	NS	NS	**	NS	**	*	*	NS
TXW	NS	**	**	NS	***	*	***	**	NS
GXTXW	NS	NS	NS	NS	NS	**	**	*	NS
CV[b]	8.28	8.86	19.07	3.95	8.70	10.93	4.58	0.99	18.08

SOV source of variation, *CV* coefficient of variation

*$P \leq 0.05$, ** 0.01, *** 0.001

N contents were obtained in water stress plants of Bhakkar-02 which is statistically at par with N contents of Bhakkar-02 under normal water supply. The drought-sensitive Shafaq-06 exhibited minimum N contents under limited water supply (Fig. 10).

Yield and yield components

Water stress significantly decreased all the yield and yield components. The foliar spray of NPK_c was effective in obtaining the highest increased in yield components (spike length, spikelets $spike^{-1}$, grains $spike^{-1}$, and thousand grain weight) in drought-tolerant Bhakkar-02 (Table 2). The plants maintained the maximum grain yield by foliar spray (4.8 g $plant^{-1}$) under normal water supply with an overall increase of 12 %, respectively than those of the control. Similarly, foliar spray of NPK_c enhanced the grain yield by 7 % (3.73 g $plant^{-1}$) as compared to no NPK_c spray under water-deficit conditions. The interactive effect of genotypes, treatment, and water levels was found to be non-significant for yield and yield components.

Discussion

Our previous study showed that NPK_c was more effective than individual effects of these nutrients in maintaining the water relations and gas exchange characteristics which markedly improved the uptake of macronutrients (N, P, and K) under water-deficit conditions (Shabbir et al. 2015). The regulation of physiological processes by osmotic adjustment is the characteristic feature of plants exposed to limited water conditions (Nawaz et al. 2012). Such changes also result in alterations in biochemical mechanisms to enhance plant defense against reactive oxygen species (ROS) like singlet oxygen, hydrogen peroxide, superoxides, and hydroxyl radicals (Jung 2004) through increased activity of antioxidant enzymes (Salekjalali et al. 2012). The results of the present study confirmed our hypothesis that supplemental NPK_c foliar spray significantly improves the drought tolerance potential in wheat through accumulation of osmoprotectants and production of antioxidant enzymes. Moreover, the application of NPK_c at anthesis stage helped to evaluate the drastic effects

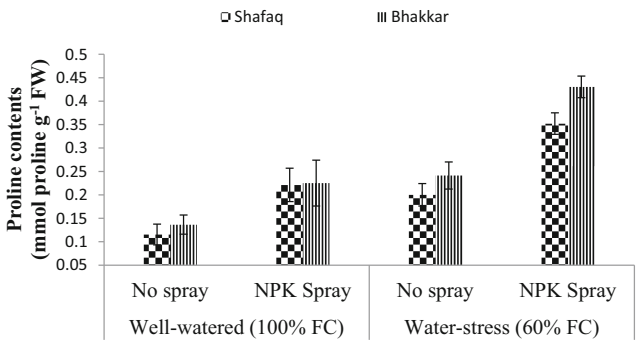

Fig. 4 Effect of supplemental foliar NPK application on proline contents (mmol proline g^{-1} FW) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

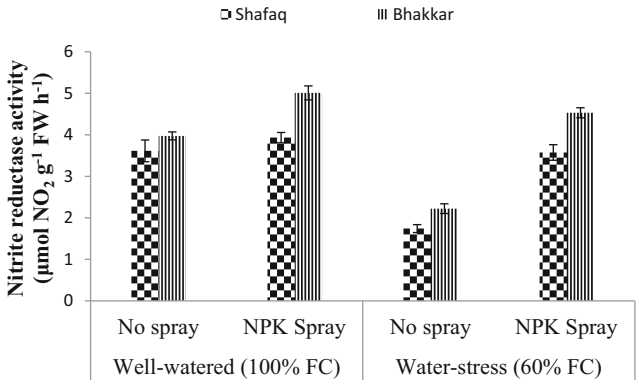

Fig. 5 Effect of supplemental foliar NPK application on nitrite reductase activity (μmol NO_2 g^{-1} FW h^{-1}) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

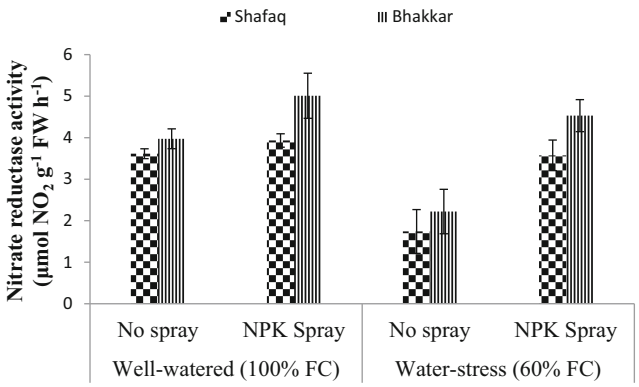

Fig. 6 Effect of supplemental foliar NPK application on nitrate reductase activity (µmol $NO_2 g^{-1}$ FW h^{-1}) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

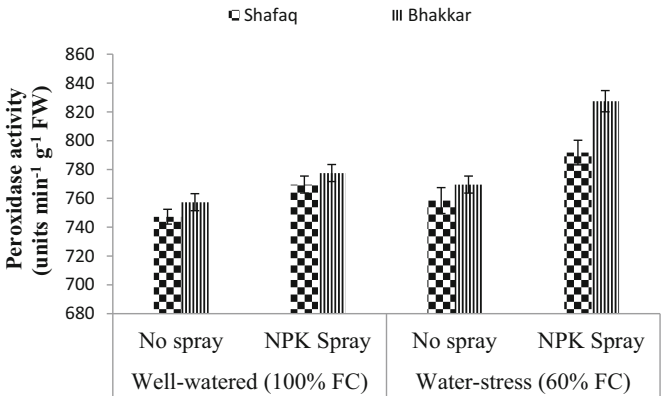

Fig. 8 Effect of supplemental foliar NPK application on peroxidase activity (U min^{-1} g^{-1} FW) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

of water stress at later stages of growth which may provide a better insight to the regulation of defense mechanisms for maintenance of growth and ultimately yield under environmental stresses like drought. Proline accumulation is a typical mechanism of biochemical adaptation in plants under environmental stresses (Ozturk and Demir 2002; Hsu et al. 2003; Kavi Kishore et al. 2005). It was observed that the plants foliarly treated with NPK_c under drought stress accumulated more proline than normal and water-stressed plants applied with no spray. The high proline concentration might be due to reduced protein biosynthesis under water-deficit conditions (Cechin et al. 2008) and suggests a significant influence of NPK_c on protein metabolism. An increase in proline contents by N or K has been well documented (Olgun et al. 2006; Monreal et al. 2007; Atanasova 2008). Moreover, increased ROS activity also triggers stress signal influencing adaptive responses such as accumulation of proline and other osmolytes to mitigate the negative effects of water stress (Maggio et al. 2002; Verbruggen and Hermans 2008).

The concentration of leaf TSS, TSP, and TFA increased in plants applied with NPK_c under drought stress. These results imply that limited supply of water escalates the breakdown of proteins (Rodriguez et al. 2002), resulting in enhanced production of amino acids useful in osmoregulation (Nayyar and Walia 2003). The high concentration of TSS might be attributed to starch decomposition as a result of amylase activity under drought stress (Nawaz et al. 2013). The supply of nutrients in the form of NPK is essential for increased production of TSS, TFA, and proteins (Monreal et al. 2007; Atanasova 2008) to sustain growth under normal and water stress conditions because these are the main constituent of hormones, enzymes, and proteins. The greater accumulation of TFA by NPK_c suggests that these nutrients might cause disorder in amino acid metabolism due to increased soluble protein content and nitrate reductase activity (Djanaguiraman et al. 2004). The literature indicated significant effect of nutrients applied either in the form of N (Mehrabdi and Mohassel 2000), P (Rahim et al. 2010), or K (Abd EL-Latif et al. 2011) on protein content of crop plants due to improved enzymatic activity and role in the transportation and restoration of protein synthesis.

The increased ROS production can be detrimental to plants, but it also acts as signals for the activation of antioxidant machinery by production of enzymes such as CAT, POX, superoxide dismutase (SOD), glutathione reductase (GR), and APX (Meharg and Hartley-Whitaker 2002; Cao et al. 2004;

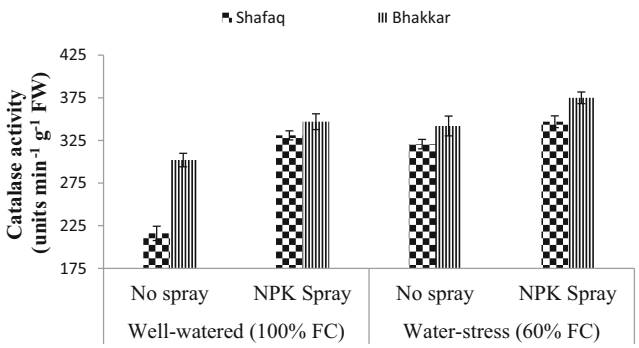

Fig. 7 Effect of supplemental foliar NPK application on catalase activity (U min^{-1} g^{-1} FW) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

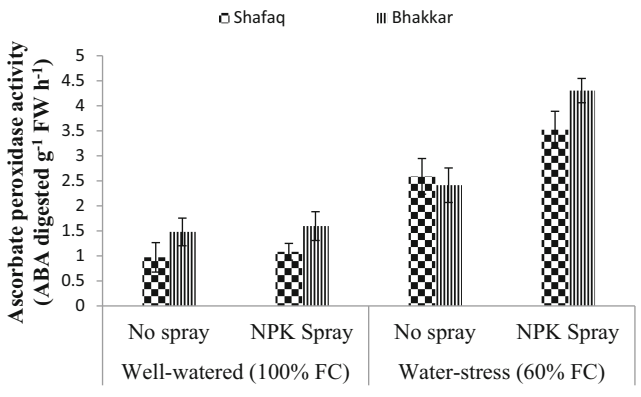

Fig. 9 Effect of supplemental foliar NPK application on ascorbate peroxidase activity (ABA digested g^{-1} FW h^{-1}) of two wheat genotypes in well-watered (100 % FC) and water stress (60 % FC) conditions (means±SE)

Table 2 Mean values of yield parameters and nutrient concentrations for main effects of genotypes, water stress levels, foliar applied NPK, and their interactions

Parameters Spray (*F*)	Interactions	Genotypes CV		Water stress levels (*W*)		Foliar
		B	S	N	D	NS
FS						
Spike length (cm)		11.99 a	11.28 b	12.28 a	11.0 b	11.22 b
12.06 a	NS	6.39				
Spikelets spike^{-1}		16. 0 a	14.83 b	17.33 a	13.50 b	15.0 b
15.83 a	NS	6.56				
Grains spike^{-1}		35.92 a	34.50 a	38.17 a	32.25 b	33.33 b
37.08 a	NS	4.65				
Thousand grains weight (g)		43.75 a	39.42 b	46.33 a	36.83 b	40.08 b
43.08 a	NS	4.01				
Grain yield (g pot^{-1})		4.27 a	3.86 b	4.53 a	3.60 b	3.89 b
4.23 a	NS	4.85				
Nitrogen (mg g^{-1} DW)		18.92 a	14.76 b	17.53 a	16.15 b	15.93 b
17.76 a	NS	6.22				
Phosphorous (mg g^{-1} DW)		4.62 a	3.71 b	4.75 a	3.58 b	3.86 b
4.47 a	NS	8.93				
Potassium (mg g^{-1} DW)		37.82 a	34.15 b	40.62 a	31.35 b	33.38 b
38.59 a	NS	4.96				

Different letters indicate significant differences at $P \leq 0.05$, $P \leq 0.001$

B Bhakkar-02, *S* Shafaq-06, *N* normal supply of water (100 % field capacity), *D* drought stress (60 % field capacity), *NS* no NPK spray, *FS* NPK foliar spray, *CV* coefficient of variation

Asada 2006) to balance the elevated ROS levels (Mittler 2002). A significant increase in the activity of antioxidant enzymes by NPK_c was noted in the present study. It may be speculated that reactivation of CAT and POX helped in the removal of excess ROS, whereas APX acted as a signaling substance for the fine modulation of ROS (Mittler 2002) particularly H_2O_2. Reports indicated significant effect of N nutrition on enzymatic activity of *Catharanthus roseus* (Misra and Gupta 2006) and *Zea mays* (Lü et al. 2012). Likewise, Soleimanzadeh et al. (2010) observed marked increase in CAT, POX, and SOD activity in sunflower plants applied with K under water-deficit conditions. The increased antioxidant activity might be attributed to the spontaneous dismutation of O^{2-} into H_2O_2 (Cartes et al. 2010) or the direct quenching of O^{2-} and OH^- (Xu and Hu 2004) by NPK_c. Although the non-significant effect of NPK_c was recorded on APX however, drought-tolerant genotype, i.e., Bhakkar-02, exhibited higher APX activity than sensitive one (Shafaq-06). Khanna-chopra and Selote (2007) reported upregulation of APX in shoot and leaves of drought-tolerant wheat genotype and suggested that overexpression of a gene for APX provides protection against excessive photorespiratory H_2O_2 protection under limited water conditions.

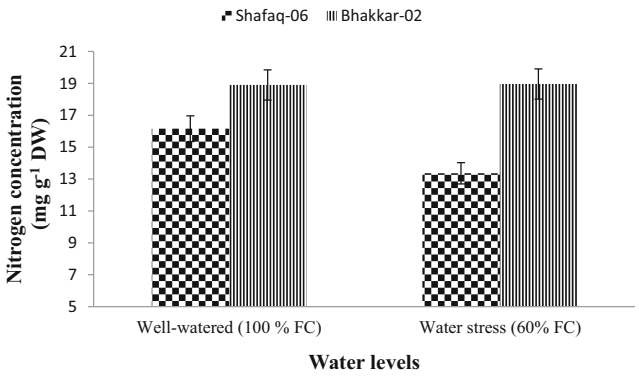

Fig. 10 Interaction between two wheat genotypes and water levels for nitrogen concentration (mg g^{-1} DW) in leaves (means±SE)

Water stress negatively influences N assimilation through reduction in NiR and NR activity in crop species. NR activity is markedly influened by NO_3^- concentration in cell sap which modulates and stabilizes NR transcripts. The decrease in NR activity under drought stress might be due to decline in NR transcript levels and is in line with the findings of Azedo-Silva et al. (2004) who reported significant changes in the behavior of NO_3 assimilatory enzymes under water stress conditions and suggested that these enzymes participate in a crucial process involving uptake and translocation of NO_3 within and between the cells. It was noted that NR activity was more influenced by water stress (Fig. 6) which suggests that NiR is more stable than NR under environmental stresses like drought (Naik and Karadge 2015) which might be attributed to its chloroplastic localization. The plants sprayed with NPK_c showed enhanced activity of NiR (Fig. 5) and NR (Fig. 6) under both normal and drought stress conditions. Kathju

et al. (1990) observed increased activity of NiR and NR in response to N application under water-deficit conditions, whereas Jabeen and Ahmad (2011) reported positive effect of K on NiR activity in sunflower and safflower. NR and NiR are the most important enzymes that play a critical role in nitrate assimilation in plants, and their enhanced activation by supplemental NPK_c indicated that it improves resistance to water stress through stabilizing NR transcripts and reduction in toxic NO_2 concentrations in leaves (Naik and Karadge 2015).

A highly significant variation was also noted between genotypes in the present study. Wheat genotype Bhakkar-02, categorized as drought-tolerant in our previous study, accumulated more proline, TSS, and TFA and showed high activity of NiR, NR, and antioxidant enzymes as compared to Shafaq-06 (drought-sensitive). Khan and Naqvi (2012) also observed more accumulation of reducing sugars in drought tolerant than sensitive genotypes and were of the view that the level of reducing sugar could be useful for the selection of drought-tolerant species. Such variations between genotypes might be due to differences in their genetic makeup (Rhodes and Samaras 1994) or ability to activate antioxidant machinery against ROS species (Sairam et al. 2000). Plant species with low antioxidant activity might not adapt to stress condition due to loss of membrane permeability by lipid peroxidation (Bhardwaj et al. 2009). Moreover, the accessibility to substrate also affects NiR and NR activity and may define drought tolerant and sensitive genotypes (Chen and Gallie 2004).

The uptake of nutrients like N, P, and K improved the growth and development of wheat plants particularly under water stress conditions. Leaf N, P, and K uptake decreased with increased in water stress. So, foliar application of NPK_c increased the availability and uptake of nutrients which might be helpful for wheat plants to cope with water stress conditions. In the present study, foliar application of NPK_c increased the uptake of N, P, and K. This might be due to direct availability of N, P, and K to the leaves of wheat and requires less energy for the absorption of these nutrients. These results are also in agreement with the findings of Afifi et al. (2011), who reported increase in N, P, and K contents with supplemental foliar application of urea in maize. Ouda et al. (2005) reported that the application of P under water stress conditions proved to be effective in increasing N, P, and K percentages in barley grains in all tested varieties.

In the present study, foliar application of NPK_c significantly increased the yield and yield components. On the other hand, water stress causes growth retardation (Baser et al. 2004), consequently affecting the yield and yield components. This might be due to that nutrient uptake by roots is limited and which, in turn, affects protein synthesis, cell structures, enzymatic activity, and metabolism. These plants may have less and smaller leaves (Fricke et al. 1997), which is the main site of photosynthesis. All these damaging effects of reduced nutrient supply besides other foremost effects of drought stress on plant growth and development could be responsible for the decline in yield and yield components. Foliar application of growth enhancers helps to alleviate the drastic effects of water stress through activation of antioxidant machinery and increased concentration of plant secondary metabolites that ultimately improves wheat yield (Yasmeen et al. 2013). Application of foliar NPK_c to water-stressed wheat plants helped to mitigate the negative effects of water limitations by improving several plants' physiological and metabolic processes, therefore ultimately improving the yield and yield components. These results are in agreement with (Liu et al. 2011), who reported increased grain yield, largest grain number, and grain weight through NPK application. Bruns and Ebelhar (2006) also reported increased corn yield and grain weight with N fertilization but not with K fertilizer on soils with high K in a cotton-corn rotation. In contrast, Amal et al. (2011) recommended that foliar K and N spray increased the plant height, number of spikes m^{-2}, weight of grains, and biological yield in sandy soil. Baque et al. (2006) reported that dry matter accumulation was negatively affected by water stress in leaf, root, and stem which was due to lower uptake of N, P, and K. Likewise, Hammad and Ali (2014) reported significant reduction in %NPK and uptake under water-deficit conditions. Application of K in high amounts significantly increased the concentration of NPK, particularly under water stress conditions (Baque et al. 2006) which suggests that supplemental soil and foliar application of macronutrients might be helpful in mitigating the adverse effects of water stress on wheat yield (Karim and Rahman 2015). Humayun et al. (2011) reported a marked increase in seed yield of lentil (*Lens culinaris* Medic) by combined foliar NPK application as compared to alone application of N, P, and K.

Conclusion

The results of the present study conclude that foliar spray of NPK_c is an effective approach that attenuates the drastic effects of drought stress through increased accumulation of osmoprotectants and high antioxidant enzyme activity. The limited availability of water at later growth stages can cause deleterious effects to wheat growth and development, so the application of NPK_c at anthesis stage is crucial for improved growth and yield under water-deficit conditions. Furthermore, the use of drought-tolerant genotypes is critical for obtaining high yields under environmental stress conditions like drought.

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