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ORIGINAL ARTICLE

Alleviation of drought stress in wheat (*Triticum aestivum* L.) by mineral fertilization

Mohammad Safar Noori[1,2] *

[1] *Faculty of Agriculture, Takhar University Afghanistan*

[2] *Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, 739-8528, Japan*

*E-Mail: safar_noori@yahoo.com

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The present study was conducted to ascertain the effect of combined application of nitrogen (N), phosphorus (P) and potassium (K) fertilizers on productivity, and nutritional quality of wheat under drought stress conditions. Wheat cultivars Minaminakaori and Lalmi-2 were grown in pots in a greenhouse, and subjected to 3 levels of NPK fertilizer applications. Then the plants were exposed to 2 levels of drought stress and well-irrigated control at grain filling stage. The result of this study indicated that drought stress significantly decreased grain yield, grain starch content, and water-soluble pentosan content, but increased grain crude protein content, total pentosan content, and phytate phosphorus content of both cultivars. Lalmi-2 exhibited a greater tolerance to drought conditions, by higher grain yield, and higher total K and starch contents under both well-irrigated and drought stress conditions than Minaminakaori, while Minaminakaori recorded higher grain mineral concentration, crud protein, total pentosan, water-soluble pentosan and phytate phosphors content than Lalmi-2. It was suggested that increase in rate of NPK fertilization could ameliorate the adverse effects of drought stress and enhance plant productivity, and concentrations of minerals, crude proteins and water-soluble pentosan in the grain under drought stress conditions. Besides applying higher rates of NPK fertilizers, it is suggested that use of fertilizer responsive and drought-tolerant genotypes such as Lalmi-2 will be beneficial to minimize the risk of yield loss due to drought stress.

Key words: Wheat, Phytate phosphorus, Pentosans, Starch, NPK, Crud protein

Drought is a limiting factor that adversely affects wheat productivity, and reduces plant growth by influencing several vital processes in plants, such as transpiration, translocation, mineral uptake and the metabolism of nutrients (Farooq *et al*., 2009). Crop production limitation rising from drought stress might be increased in the upcoming decades due to climatic change which may reduce precipitation and accelerate evapotranspiration (World Bank, 2007; Lobell *et al.,* 2008). Under drought stress conditions, plant water potential and turgor decrease greatly, and therefore normal physiological functions cannot be performed (Zhu, 2002). Drought stress affects adversely on leaf growth (Galle *et al*., 2010), leaf chlorophyll concentration (Brevedan and Egli, 2007), stomatal conductance (Liang *et al.,* 2002), and therefore it reduces photosynthetic activity and speed up leaf senescence (Yang and Zang, 2006) which eventually results in declined growth and yield of crops *(*Ercoli *et al*., 2007*).* Plant survival or death under stress conditions, depends on severity of stress, duration and frequency of exposures, plant developmental stage, organ or tissues that expose to stress, and genetic characters (Farooq *et al*., 2009). Yang and Zang (2006) concluded that wheat is highly sensitive to drought stress during flowering and grain filling stages.

Increased doses of nitrogen (N) improved drought tolerance (Halvorson and Reule, 1994). N fertilization mitigates the adverse effects of drought stress on plant growth by improving water use efficiency (Saneoka *et al*., 2004). It also enhances leaf area and leaf chlorophyll content and thus improves photosynthetic efficiency and reduces photo-damage under drought stress conditions (Wu *et al*., 2008). Phosphorus (P) fertilization effectively promotes plant growth under drought stress by enhancing root growth (Singh and Sale, 1998), increasing water use efficiency (Garg *et al.,* 2004; and Waraich *et al*., 2011) increasing leaf area and photosynthetic activity (Singh et al., 2006), maintaining leaf stomatal conductance and nitrate reductase activity (Bruck *et al*., 2000; and Naeem and Khan, 2009**)** and increasing cell-membrane stability and water relations (Sawwan *et al*., 2000; and Kang *et al.,* 2014). Application of adequate level of potassium (K) enhances water use efficiency (Egilla *et al*., 2005), photosynthetic activity, plant growth and yield in different crops under drought stress conditions (Tiwari *et al.,* 1998; and Egilla *et al*., 2001). K fertilization alleviates detrimental effects of drought stress by improving many physiological processes such as regulation of turgor pressure and photosynthesis, enzymes activation and translocation of cations (Mengel and Kirkby, 2001). Marschner (2012) concluded that K^+ as an inorganic osmotica, plays a significant role in formation of the osmotic adjustment ability, even under drought conditions.

The effects of nutrients such as N, P, and K on enhancing plant growth, grain yield, and grain quality of plants under normal conditions has been well studied so far; however, little is known about their function, if applied in combination, in improving grain yield, grain nutritional quality, pentosans content and phytate P content of wheat under drought stress conditions. Therefore, the present experiment was conducted with the aim to evaluate the effects of combined application of NPK on growth, productivity, grain minerals, starch, crude protein, total and water-soluble pentosans, and grain phytate P of wheat, under drought stress condition.

MATERIALS AND METHODS

Experiment description

This experiment was designed to evaluate the effect of combined fertilization of different levels of NPK on wheat productivity and grain nutritional quality under late season drought stress conditions. A pot experiment was conducted in the greenhouse of the Faculty of Applied Biological Sciences, Hiroshima University. Two wheat cultivars, Minaminakaori a commonly grown winter wheat in Japan and Lalmi-2 a facultative bread wheat grown in rainfed lands of Afghanistan were used in this study. The experiment was carried out in a vinyl greenhouse with natural sunlight and temperature to prevent nutrient leachate and avoid interruption by rainfall. Pots with capacity of 10 liters (top and bottom diameters of 29 and 25 cm and a depth of 27 cm) were used. They were filled with 10 kg of a mixture of regosol, aerobic compost and vermiculite (2:1:1, v/v). Chemical analysis of this mixture showed that it contained: 0.16 % N, 5.74 mg kg^{-1} P, and 72.58 mg kg^{-1} K. Furthermore, (6.4 g pot^{-1}) of dolomitic calcium magnesium carbonate was mixed with the soil to adjust the pH (H_2O) to 6.5. The present experiment was designed with 3 levels of NPK fertilizers: F_1 (150 kg N + 100 kg P_2O_5 + 75 kg K_2O ha^{-1}), F_2 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}), and F_3 (250 kg N + 140 kg P_2O_5 + 125 kg K_2O ha^{-1}). The sources of N, P and K were urea, single super phosphate (SSP), and potassium sulfate, respectively. They were applied in pots, as F_1 (2g urea+3.76 g SSP+0.96 g potassium sulfate pot^{-1}), F_2 (2.8g urea+4.51 g SSP+1.28 g potassium sulfate pot^{-1}) and F_3 (3.5g urea+5.3 g SSP+1.6 g potassium sulfate pot^{-1}). All of P and K, and a half dose of N were applied before sowing, and the remaining N was applied in two equal splits at the time of tillering and anthesis stages. Wheat seeds were sown in the middle of November 2017. 15-day-old seedlings (2 plants pot^{-1}) were transplanted in the pots, and the pots were arranged in a randomized complete block design with 4 replications. The plants were irrigated with tap water regularly until imposing the drought stress. Drought treatments consisting of well-irrigated, mild stress and severe stress were applied during the grain filling stage. The soil water content was adjusted to 20%, 10% and 6% for well-irrigated, mild drought stress and severe drought stress conditions, respectively, and monitored by a Time Domain Reflectometry (TDR-341F model, Fujiwara, Japan) digital moisture meter regularly. The drought stress continued for 40 days until the crop reached harvesting maturity. The following parameters were recorded by the time of harvest and thereafter.

Growth, Yield, and Yield component:

Number of fertile tillers (spike bearer tillers) per plant was counted before harvest. To measure 1000 kernel weight, 500 grains were counted, and weighted with a prescribed accuracy, and then the value was multiplied by 2. To determine the grain yield, mature spikes were collected, oven dried at 80˚C for 48 hours, threshed, and the grain yield was recorded and expressed as grams per plant.

Grain mineral concentrations:

Samples of mature seeds were ground finely with a vibrating sample mill (TI-100, Heiko, Japan). 100 mg of ground samples were digested by sulfuric acid, diluted to 100 ml of distilled water, and K content was measured using a flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan). Total P was determined using the same digested sample by UV-Spectrophotometer (U-3310, Hitachi Co. Ltd. Tokyo, Japan) following the molybdenum reaction solution method suggested by Chen *et al*., (1956). Total nitrogen was measured using the Kjeldahl method after digestion of ground sample with concentrated H_2SO_4 and H_2O_2 (10:5, v/v). Grain Ca, Mg, and Zn concentration were measured by an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu, Japan). For grain inorganic P (Pi) determination, 10 ml of trichloroacetic acid (12.5%) + $MgCl_2$ (2 mMol /l) were added to plastic tubes containing 50 mg of ground samples and stirred for 24 hours at 4 ºC. The tubes were centrifuged at 7000 rpm under 4 ºC temperature, then a specific amount of supernatant was drawn and Pi was measured using a spectrophotometer by the molybdenum reaction regent method (Raboy and Dickinson, 1984).

Determination of grain starch and crud protein:

For the determination of grain starch, 80% ethanol was added to the powdered samples to remove sugars and then starch was extracted with perchloric acid. Anthrone reagent was added to the test tubes containing extracted samples and then heated in a boiling water bath for 7.5 minutes. The absorbance of the extract was measured at 630 nm (Nag, 2016). Grain crude protein content was calculated by multiplying grain total N content by 5.47 (Fujihara *et al*., 2008).

Determination of total pentosan (TP) and water-soluble pentosane (WSP):

Grain TP was measured following the orcinol-HCl method where finely ground samples were hydrolyzed with 2 N HCl in a boiling water bath for 2.5 hours, and then centrifuged. A specific amount of supernatants was transferred to new test tubes and reaction regents ($FeCl_3$ and Orcinol) were added and vortexed. The tubes were heated in boiling water for 30 minutes, cooled, and the absorbance was measured using a spectrophotometer. Grain WSP was extracted by hydrolyzing powdered samples in distilled water by shaking for 2 hours at 30˚C. Then, 4 N HCl was added to the aliquots of the supernatant and placed in boiling water for 2 hours, and allowed to cool, and WSP was estimated by a spectrophotometer, using $FeCl_3$-orcinol reagents (Hashimoto *et al.,* 1986).

Estimation of phytate phosphorus (Phy-P)

Phy-P was measured following the method suggested by Raboy and Dickinson (1984) where aliquots of ground samples were extracted in an extraction media (0.2 M HCl: 10% Na_2SO_4) overnight at 4°C while shaking. Extracts were centrifuged, and phytate P was obtained as a ferric precipitate and assayed for P using ammonium molybdate reaction reagent.

Statistical analysis

All the collected data were subjected to analysis of variance using SPSS statistics package, Version Student Version 22, and means (n = 4) were separated using the Duncan Multiple Range Test at $p \leq 0.05$.

RESULTS

Grain yield and yield components

Statistical analysis of data showed that drought stress significantly reduced the number of fertile tillers, 1000 kernel weight and grain yield in both cultivars (Table 1). Application of a high rate of NPK (F_3) resulted in the production of a higher number of fertile tillers in both cultivars under well-irrigated conditions, while F_1 treatment recorded the lowest number of fertile tiller in Minaminakaori cultivar under severe drought stress conditions. F_3 treatment enhanced the production of fertile tillers in both cultivars under mild and severe stress conditions. Cultivar Lalmi-2 recorded higher number of fertile tillers under all levels of NPK and drought stress treatments compared to that of Minaminakaori.

The 1000 kernel weight was significantly different between cultivars, NPK treatment levels and drought stress levels. Cultivar Lalmi-2 recorded higher 1000 kernel weight under all levels of NPK fertilization and drought stress conditions compared to Minaminakaori. NPK treatments slightly increased 1000 kernel weight in Minaminakaori under all levels of drought stress levels however, the mean values did not differ significantly. F_3 and F_2 treatments recorded the highest 1000 kernel weight in Lalmi-2 cultivar under well-irrigated conditions. NPK fertilization significantly increased 1000 kernel weight in Lalmi-2 under all levels of stress conditions. In comparison with F_1, F_3 treatment increased 1000 kernel weight in Lalmi-2 cultivar by 7.7% and 16.8 % under mild stress and severe-stress conditions respectively. In Minaminakaori, increase in 1000 kernel weight due to the high rate of NPK (F_3) fertilization was 9.1% under mild stress and 4.75% under severe stress compared to F_1 treatment.

Grain yield was negatively affected by drought stress in both cultivars. Lalmi-2 recorded higher grain yield under all levels of drought stress conditions compared to Minaminakaori. NPK treatment significantly increased grain yield. F_3 treatment recorded higher grain yield in both cultivars under both drought stress conditions. It was observed that mild drought stress had less effect on grain yield of Lalmi-2 cultivar. F_3 treatment recorded the highest grain yield in Lalmi-2 under well-irrigated and mild stress conditions. Grain yield was the lowest in cultivar Minaminakaori under severe drought stress conditions with the lowest NPK fertilizer (F_1) level. F_3 treatment increased grain yield by 38.2% under mild stress and 24.0% under severe-stress conditions compared to F_1 treatment in Lalmi-2 cultivar. However, in Minaminakaori increase in grain yield due to high NPK (F_3) fertilization under mild and severe stress conditions compared to F_1 treatment was 29.9 and 35.0% respectively.

Grain mineral concentrations

Mineral concentration was highly affected by drought stress and NPK treatments (Table 2). Drought stress increased total N content in both cultivars. Minaminakaori recorded a higher total N content compared to Lalmi-2. NPK fertilization enhanced total N content in both cultivars. F_3 treatment recorded the highest total N content in Minaminakaori under severe-stress conditions. The lowest total N content was recorded in Lalmi-2 cultivar under well-irrigated conditions where the lowest rate of NPK (F_1) was applied. Grain K content was also increased under drought stress conditions. The highest value of grain K content was observed under severe drought stress condition in F_3 treatment of cultivar Lalmi-2. NPK treatment enhanced total K content in both cultivars, however the response was greater in cultivar Lalmi-2. The lowest K content was observed in Minaminakaori cultivar under well-irrigated condition with the lowest rate of NPK (F_1) treatment. Grain total P content was recorded higher in Minaminakaori cultivar under severe drought stress conditions. Grain total P content was greater with F3 treatment under well-watered as well as drought stress conditions. Minaminakaori recorded higher total P content under mild and sever stress conditions than Lalmi-2. F_1 treatment recorded the lower total P content in both cultivars under well-irrigated conditions. Drought stress increased Pi concentration in Minaminakaori cultivar. Pi content was reduced in Lalmi-2 cultivar due to severe stress conditions. The highest value of grain Pi content was recorded in Minaminakaori cultivar under severe drought stress conditions with high rate of NPK fertilizer application. F_1 treatment in Lalmi-2 cultivar recorded the lowest Pi content under severe stress condition. NPK fertilization enhanced Mg content and the highest Mg content was observed in Minaminakaori cultivar with F_3 treatment under sever condition. The lowest Mg content was observed in well irrigated Lalmi-2 with low NPK (F_1) application. Minaminakaori recorded a higher Mg content under all levels of stress conditions compared to Lalmi-2. NPK fertilization enhanced Ca content and the highest value of Ca was recorded with F_3 treatment in Minaminakaori under mild-stress condition. The lowest Ca content was observed in Lalmi-2 cultivar under severe-stress conditions with the lowest level of NPK (F_1) application. Minaminakaori recorded a higher Ca content irrespective of NPK treatments and drought stress conditions compared to Lalmi-2. Zn content was found to be higher in Minaminakaori cultivar irrespective of drought stress and NPK levels compared to Lalmi-2. F_3 treatment slightly enhanced Zn content in both cultivars but the mean values did not differ significantly within the same cultivar. F_3 treatment increased N, K, P, Pi, Mg, Ca and Zn contents in grain by 9.1%, 6.4%, 9.8%, 9.4%, 5.2%, 26.37% and 14.60% compared to F1 treatment under mild stress respectively, and by 10.1%, 5.1%, 3.1%, 36.4%, 3.0%, 13.2 and 7.5% respectively under severe stress condition in Minaminakaori. However, in cultivar Lalmi-2, the high level of NPK fertilization (F_3) increased N, K, P, Pi, Mg, Ca and Zn content in grain by 5.1%, 3.6%, 6.3%, 33.5%, 5.1%, 8.4% and 8.5% respectively under mild stress and by 15.2%, 4.1% 7.0%, 31.0%, 1.7%, 15.3% and 6.4% respectively under severe stress condition**.**

Grain Starch content

Grain starch content decreased by drought stress (Table 3). The highest grain starch content was recorded in cultivar Lalmi-2 under well-irrigated condition, while the lowest was observed in Minaminakaori under severe stress condition. Grain starch content was decreased with increase in NPK rate. In comparison with well-irrigated condition, mild and severe drought stress decreased grain starch content by 1.5% and 2.6%, respectively in cultivar Minaminakaori, and by 2.7% and 3.4% respectively in cultivar Lalmi-2.

Grain crude protein content

Grain crude protein content was increased under drought stress conditions, and the higher crude protein was observed under severe-stress conditions. Minaminakaori recorded higher crude protein content compared to Lalmi-2 irrespective of NPK levels and drought stress conditions (Table 3). NPK fertilization significantly enhanced crude protein content in both cultivars, and F_3 treatment recorded the highest crude protein content in Minaminakaori under severe-stress conditions. However, the lowest crude protein content was recorded in Lalmi-2 cultivar under well-irrigated condition where the lowest rate of NPK (F_1) was applied. In comparison to F_1, F_3 treatment increased grain crude protein in cultivar Minaminakaori by 9.1% and 10.1% under mild and severe stress conditions respectively. For the cultivar Lalmi-2 the increase due to high NPK (F_3) fertilization was 4.8% and 15.2% under mild and severe stress conditions, respectively.

Grain TP and WSP content

Grain TP was recorded higher under mild stress condition in both cultivars. Increased levels of NPK (F_3) reduced TP content, while low NPK (F_1) treatment under mild stress condition resulted in the highest grain TP content in cultivar Minaminakaori. The lowest TP content was observed in cultivar Lalmi-2 under severe-stress condition where a high level of NPK (F_3) was also applied (Table 3). Grain WSP was negatively affected by drought stress conditions. Different from TP, WSP was recorded higher under well-irrigated conditions. F_3 treatment resulted in the highest grain WSP content in cultivar Minaminakaori under well-irrigated conditions. NPK fertilization enhanced WSP content and F_3 treatment recorded higher WSP under both levels of drought stress conditions in both cultivars (Table 3). Cultivar Minaminakaori recorded a higher WSP content under will-irrigated as well as drought stress conditions. Compared to F_1, F_3 treatment increased grain WSP content in Minaminakaori by 9.7%, 14.0% and 8.7% under well-irrigated, mild stress and severe stress conditions respectively. These values for the cultivar Lalmi-2 were 11.3%, 13.4% and 22.5%.

Grain Phy-P content

Drought stress significantly increased grain phy-P content and the highest Phy-P content was recorded under severe stress conditions. NPK fertilization enhanced the accumulation of Phy-P in both cultivars under well-irrigated as well as drought stress conditions. Cultivar Minaminakaori recoded a higher Phy-P content under severe-stress conditions than cultivar Lalmi-2. F_3 treatment recorded the highest Phy-P content in Minaminakaori cultivar under severe-stress conditions (Table 3). However, F_1 treatment resulted in lower level of accumulation of Phy-P in both cultivars under will-irrigated conditions. F_3 treatment increased grain Phy-P content in Minaminakaori cultivar by 11.0%, 8.9% and 4.1% under well-irrigated, mild stress and severe stress conditions respectively, compared to F_1 treatment. These values for the cultivar Lalmi-2 were 3.4%, 3.8% and 3.5%. Severe drought stress increased grain Phy-P content by 10.7 % in Minaminakaori and by 11.3 % in Lalmi-2, compared to well-irrigated conditions.

Table 1: Average number of fertile tillers per plant, 1000 kernel weight and grain yield of two wheat cultivars influenced by different levels of NPK fertilization under drought stress conditions. Means with different letters are significantly different from each other at $p \leq 0.05$.

Cultivars	Drought stress levels	NPK levels	Fertile tillers/plant	1000 Kernel weight (g)	Grain yield (g/plant)
Minaminakaori	Well-irrigated	F_1	19.50^{efg}	39.79^{f}	33.79^{gh}
		F_2	22.75^{abcde}	39.89^{f}	42.34^{e}
		F_3	25.25^{ab}	40.44^{f}	47.11^{d}
	Mild stress	F_1	19.75^{efg}	36.45^{f}	31.61^{hi}
		F_2	23.50^{abcd}	37.41^{f}	40.62^{ef}
		F_3	25.25^{ab}	39.78^{f}	41.05^{ef}
	Severe stress	F_1	18.00^{g}	36.61^{f}	27.52^{i}
		F_2	20.75^{defg}	37.09^{f}	31.99^{hi}
		F_3	22.50^{bcdef}	38.35^{f}	36.97^{fg}
Lalmi-2	Well-irrigated	F_1	21.25^{cdef}	55.40^{abc}	50.16^{d}
		F_2	24.00^{abc}	56.96^{a}	63.04^{b}
		F_3	24.75^{ab}	57.79^{a}	69.68^{a}
	Mild stress	F_1	20.75^{defg}	52.09^{cd}	50.33^{d}
		F_2	23.00^{abcd}	52.53^{bcd}	57.46^{c}
		F_3	25.75^{a}	56.11^{ab}	69.55^{a}
	Severe stress	F_1	20.75^{defg}	47.50^{e}	48.45^{d}
		F_2	22.50^{acdef}	50.21^{de}	56.42^{c}
		F_3	23.75^{abcd}	55.50^{abc}	60.07^{bc}

Table 2: Average grain mineral contents in two cultivars of wheat influenced by different levels of NPK fertilization under drought stress conditions. Means with different letters are significantly different from each other at $p \leq 0.05$.

Cultivars	Drought stress levels	NPK levels	N (mg g⁻¹)	K (mg g⁻¹)	P (mg g⁻¹)	Pi (mg g⁻¹)	Mg (mg g⁻¹)	Ca (µg g⁻¹)	Zn (µg g⁻¹)
Minaminakaori	Well-irrigated	F_1	23.95^{ef}	5.23^{g}	3.91^{e}	0.174^{bcde}	1.22^{cdefgh}	177.69^{i}	64.41^{a}
		F_2	25.29^{de}	5.31^{fg}	4.18^{bcde}	0.181^{bcde}	$1.31^{abcdefg}$	286.98^{def}	65.87^{a}
		F_3	26.40^{cd}	5.51^{efg}	4.27^{abcd}	0.185^{bcde}	1.40^{a}	338.65^{cd}	66.62^{a}
	Mild stress	F_1	24.94^{de}	5.28^{g}	4.17^{bcde}	0.180^{bcde}	1.34^{abcde}	335.20^{cde}	64.04^{a}
		F_2	26.19^{cd}	5.53^{efg}	4.37^{abcd}	0.190^{bcde}	1.39^{ab}	394.73^{abc}	65.71^{a}
		F_3	27.22^{bc}	5.62^{efg}	4.58^{ab}	0.197^{bcd}	1.41^{a}	423.60^{a}	73.39^{a}
	Severe stress	F_1	26.36^{cd}	5.34^{fg}	4.52^{ab}	0.184^{bcde}	1.32^{anbdef}	365.45^{abc}	61.92^{a}
		F_2	28.54^{ab}	5.55^{efg}	4.53^{abc}	0.192^{bcde}	1.35^{abcde}	409.93^{ab}	62.50^{a}
		F_3	29.01^{a}	5.61^{efg}	4.66^{a}	0.251^{a}	1.36^{abc}	413.85^{ab}	66.56^{a}
Lalmi-2	Well-irrigated	F_1	20.48^{h}	5.79^{def}	$3.90d^{e}$	0.160^{de}	1.11^{h}	258.82^{fgh}	39.16^{b}
		F_2	21.10^{gh}	5.94^{cde}	$4.02d^{e}$	0.214^{abc}	1.17^{fgh}	271.28^{efg}	48.41^{b}
		F_3	21.53^{gh}	6.20^{bcd}	$4.13cd^{e}$	0.221^{ab}	1.19^{efgh}	353.47^{bc}	49.01^{b}
	Mild stress	F_1	21.41^{gh}	6.34^{bc}	$3.97d^{e}$	0.152^{de}	1.18^{efgh}	209.59^{ghi}	38.71^{b}
		F_2	21.49^{gh}	6.51^{ab}	4.13^{cde}	0.158^{de}	1.20^{defgh}	216.26^{ghi}	41.32^{b}
		F_3	22.51^{fg}	6.57^{ab}	4.22^{bcde}	0.203^{bcd}	$1.24^{bcdefgh}$	227.09^{fghi}	42.00^{b}
	Severe stress	F_1	21.64^{gh}	6.57^{ab}	4.28^{abcd}	0.145^{e}	1.15^{gh}	187.74^{i}	44.32^{b}
		F_2	22.27^{g}	6.61^{ab}	4.51^{abc}	0.169^{cde}	1.16^{efgh}	196.49^{hi}	46.11^{b}
		F_3	24.93^{de}	6.84^{a}	4.58^{ab}	0.190^{bcde}	1.17^{fgh}	216.48^{ghi}	47.17^{b}

Table 3: Starch content, crud protein content, total protein content, water soluble protein content and Phy-P content in grains of two wheat cultivars influenced by NPK fertilization under drought stress conditions. Means with different letters are significantly different from each other at $p \leq 0.05$.

Cultivars	Drought stress levels	NPK levels	Starch (%)	Crud protein (%)	TP (mg g⁻¹)	WSP (mg g⁻¹)	Phy-P (mg g⁻¹)
Minaminakaori	Well-irrigated	F_1	66.76^{bcde}	13.26^{ef}	8.02^{ab}	1.85^{abc}	2.72^{bc}
		F_2	66.42^{bcde}	14.01^{de}	7.92^{ab}	1.93^{ab}	2.97^{abc}
		F_3	65.34^{cdef}	14.63^{cd}	7.88^{ab}	2.03^{a}	3.02^{abc}
	Mild stress	F_1	66.37^{bcde}	13.82^{de}	8.37^{a}	1.64^{abcd}	2.93^{abc}
		F_2	65.13^{def}	14.51^{cd}	8.30^{ab}	1.75^{abcd}	3.07^{abc}
		F_3	63.95^{ef}	15.08^{bc}	8.09^{ab}	1.87^{abc}	3.19^{abc}
	Severe stress	F_1	65.86^{cdef}	14.60^{cd}	8.17^{ab}	1.62^{abcd}	3.16^{abc}
		F_2	65.34^{cdef}	15.81^{ab}	7.87^{ab}	1.49^{cd}	3.19^{abc}
		F_3	62.20^{f}	16.07^{a}	7.69^{ab}	1.76^{abcd}	3.29^{a}
Lalmi-2	Well-irrigated	F_1	71.23^{a}	11.34^{h}	7.95^{ab}	1.68^{abcd}	2.71^{c}
		F_2	70.17^{ab}	11.93^{gh}	7.87^{ab}	1.79^{abcd}	2.84^{abc}
		F_3	69.55^{abc}	11.69^{gh}	7.84^{ab}	1.87^{abc}	2.91^{abc}
	Mild stress	F_1	69.13^{abcd}	11.90^{gh}	8.29^{ab}	1.57^{bcd}	2.87^{abc}
		F_2	68.45^{abcd}	11.86^{gh}	8.21^{ab}	1.68^{abcd}	2.96^{abc}
		F_3	67.63^{abcde}	12.47^{fg}	8.05^{ab}	1.78^{abcd}	2.98^{abc}
	Severe stress	F_1	68.49^{ancd}	11.99^{gh}	8.12^{ab}	1.38^{d}	3.11^{abc}
		F_2	67.79^{abcde}	12.34^{g}	7.85^{ab}	1.59^{bcd}	3.16^{abc}
		F_3	67.51^{abcde}	13.81^{de}	7.66^{b}	1.69^{abcd}	3.22^{ab}

DISCUSSION

The results of this study indicated that drought stress adversely affected grain yield and yield components irrespective of NPK treatment levels in both cultivars. Higher levels of combined NPK fertilization improved wheat performance under drought stress condition by enhancing plant productivity, grain mineral concentration, and grain quality. A high rate of NPK (F_3) fertilization improved photosynthetic activity by enhancing water and nutrient absorption that resulted in producing more assimilates. As a result, increase in number of fertile tillers, 1000 kernel weight and grain yield. This result could be contributed to the genetic potential of Lalmi-2 compared to Minaminakaori. Ati *et al*., (2016) concluded that application of NPK fertilizer increased number of spikes and grain yield of wheat under drought stress conditions. Akram *et al*., (2014) found that application of a higher rate of N improves water use efficiency and enables the plants to survive and thus produce high grain yield under drought stress conditions. Increase in grain yield and yield components of wheat through NPK fertilization under well-watered condition was reported by many researchers (Laghari *et al.*, 2010; and Abdel-Aziz *et al*., 2016), however there is insufficient information on effects of combined application of NPK on wheat yield and yield components under environmental stress conditions including drought.

The grain mineral concentration was significantly influenced by drought stress as well as the level of NPK fertilization and the cultivar differences. It was observed that concentrations of N, K, P, Pi, Mg and Ca in grain were increased under drought stress conditions. Concentrations of minerals in grains were significantly increased with an increase in NPK rate with an exception of Zn. Drought stress induced leaf senescence (Sarwat *et al*., 2013), and therefore minerals might be transported from leaves to the meristematic tissues and developing grains. The increase in N content in plants under drought stress is due to accumulation of free amino acids that are not synthesized into proteins (Alam, 1999). Under drought stress conditions, most of the N and P was accumulated in grains (Patel and Singh, 1998). F_3 treatment in our study consisted of a high rate of N, P and K fertilizers that enhanced N, P, K concentrations in wheat grains. Raza *et al.,* (2013) reported that application of a high dose of K, improved K, N, P and Ca uptake of wheat under drought stress condition. In this study, it was found that grain Pi content was increased under drought stress conditions. The effect of drought stress and NPK fertilization on grain Pi concentration was not sufficiently reported, however a positive relationship was observed between total P and Pi concentration in this study, which could be a reason for a high Pi concentration. P uptake is enhanced when the dose of P fertilizer was increased (Saha *et al*., 2014). Grain Mg concentration was higher in Minaminakaori cultivar under well-irrigated and mild stress conditions, however it was lower under severe drought stress condition. F_3 treatment recorded a higher Mg content under well-irrigated and mild stress conditions in both wheat cultivars. Slamka *et al*. (2011) concluded that N fertilization exhibits more expressive effect on plant Mg uptake under optimal water supply conditions. Cultivar Minaminakaori recorded higher Zn concentration compared to Lalmi-2 irrespective of drought stress and NPK treatments. It could be assumed that Lalmi-2 produced a higher grain yield compared to Minaminakaori and thus grain Zn concentration was decreased in Lalmi-2.

Drought stress significantly reduced grain starch content in both cultivars. This reduction was due to the adverse effects of drought stress that decreased plant photosynthetic activity, and therefore carbohydrate formation and accumulation of starch was declined. Drought stress during anthesis and grain filling remarkably decreases starch content of wheat (Saeedipour, 2011). The starch content was decreased with increase in the rate of NPK fertilization. Lalmi-2 cultivar recorded higher starch content and low crud protein compared to Minaminakaori. Kindred *et al.* (2008) observed a negative relationship between crude protein and starch content, and N fertilization decreased the starch content of wheat grain. Hlisnikovsky and Kunzova (2014) also found a higher grain starch content in control plants where fertilizer was not applied.

In this study grain crude protein content was enhanced under drought stress conditions, and an elevated level of NPK fertilization greatly increased the crud protein content under both well-irrigated and drought stress conditions. Under well- irrigated conditions, grain protein content may decrease by dilution of N with carbohydrates (Guttieri *et al*., 2005). Singh *et al*. (2012) also observed a significant increase in protein content of wheat grown under rainfed conditions. NPK fertilization increased the synthesis of the raw protein in wheat (Crista, 2012). A high level of NPK fertilization enhanced grain crude protein content in the wheat variety Inqulab 91 (Sameen *et al*., 2002). In our study, F_3 treatment consisted a high level of N and K fertilizers that resulted in a higher crud protein content. Many researchers have found that adequate amount of K fertilization improves plant N uptake and protein synthesis (Alam *et al*. 2009; Lakudzala,2013; Daniel *et al*., 2016). Lalmi-2 recorded a lower grain crude protein content compared to Minaminakaori, and it might be due to the higher yield capacity of Lalmi-2 cultivar and varietal difference in uptake of N and protein synthesis.

Grain TP content was increased under mild drought stress condition. However, a higher level of NPK fertilization slightly decreased TP content in both cultivars under both levels of drought stress conditions. It was assumed that under drought stress, plants produced less grain yield, and most of the non-starch polysaccharides were accumulated in these grains. Effect of NPK fertilization on grain TP and WSP under drought stress conditions in wheat, has not been reported sufficiently by earlier research studies. The result of this investigation indicated that WSP content was reduced under drought stress conditions and NPK fertilization significantly increased WSP content under both well-irrigated and drought stress conditions. Water-unextractable pentosan had a negative effect, while water-soluble pentosan had a positive impact on the bread-making quality of wheat (Courtin and Delcour, 2002).

Phy-P serves as seed storage for phosphorus, but this compound can contribute to a nutritional problem, since it binds with proteins and some important micronutrients, such as Fe and Zn, and significantly reduces their availability (Rosa 1999; and Raboy, 2001). In this study, grain Phy-P content was increased with severity of drought and increased rate of NPK fertilization. The effect of NPK fertilization on the Phy-P content of wheat grain under drought stress conditions was not sufficiently reported by other researchers so far. Phy-P is the major storage form of P in cereals, therefore the concentration of this antinutrient compound mostly depends on the grain total P content. Application of a high rate of P fertilizer may results in a high phytate content (Raboy and Dickinson, 1984). The reason for a higher Phy-P synthesis and accumulation in wheat grains in F_3 treatment may be contributed to the higher rate of P fertilizer application.

CONCLUSION

In this study, drought stress significantly reduced grain yield and negatively affected grain nutritional quality of both wheat cultivars by increasing anti-nutrient compound such as grain phytate P content. Drought-induced variation in grain yield and yield components was smaller in cultivar Lalmi-2 than Minaminakaori. Cultivar Lalmi-2 maintained high grain yield, starch content, and total K content under all levels of stress conditions, while, Minaminakaori recorded higher grain minerals, crud protein, total pentosan, water-soluble pentosan and phytate P content. A higher rate of NPK fertilization found to be an effective approach that attenuates the deleterious effects of drought stress by improving wheat productivity, and nutritional quality under drought stress conditions.

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