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Nutritional and Bread-Making Quality of Wheat as Influenced by Mineral Fertilization in a Compost-Amended Regosol soil

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***Abstract*— *This experiment was conducted to assess the effect of different levels of nitrogen (N), phosphorus (P) and potassium (K) fertilization on growth, grain yield, nutritional and bread-making quality of wheat in a compost-amended regosol soil. Wheat cultivar Minaminokaori was grown in containers containing a mixture of regosol and aerobic compost (2:1 v/v). This study comprised a non-fertilized control (F_1) and six levels of NPK fertilizers: F_2 (80 kg N + 40 kg P_2O_5 +40 kg K_2O ha^{-1}), F_3 (110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}), F_4 (140 kg N + 80 kg P_2O_5 + 70 kg K_2O ha^{-1}), F_5 (170 kg N + 100 kg P_2O_5 + 85 kg K_2O ha^{-1}), F_6 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}) and F_7 (230 kg N + 140 kg P_2O_5 + 115 K_2O kg ha^{-1}). A significant improvement in agronomic performance, grain nutritional and bread-making quality of wheat was observed with the F_6 treatment, it increased the grain yield (151.6%), crude protein (65.3%), water-soluble pentosan (40.5%), and dry gluten (4-fold) compared to the control. The F_6 treatment also increased grain total N, P, K, Mg, Ca, Zn and inorganic phosphorus contents by 65.2, 33.6, 8.9, 19.7, 165.9, 26.1 and 80.0%, respectively, compared to control. However, it slightly increased grain phytate P content. The results from this study suggest that agronomic performance, grain yield, nutritional and bread-making quality of wheat can be improved with an appropriate dose of NPK in a compost-amended regosol soil.*

Keywords*— *Crude protein, Gluten, NPK, Pentosan, Phytate P, Wheat.

I. INTRODUCTION

Wheat is an important cereal crop in the world and is primarily grown for its grain, which is consumed as human food. It contributes about 20 % of the total dietary calories and proteins worldwide (Shiferaw *et al*., 2013), and has gotten more attention for food security especially with fast growing populations in developing countries. Enhancing crop productivity and achieving food security is possible through a wise fertilizer application, particularly in regosol soil which is deficient in nutrient. NPK fertilization in balanced share at proper time has a great impact on wheat growth and yield (Malghani *et al*., 2010).

N is a key component of proteins, nucleic acids, enzymes, coenzymes, and chlorophyll and therefore contributes to the biochemical processes of the plant (Benin *et al*., 2012). N fertilization during anthesis has a positive effect on synthesis of high grain protein content than earlier application (Wuest and Cassman, 1992). An adequate level of N fertilization increases the number of fertile tillers (Wilhelm, 1998), number of spikes, number of grains per spike, and grains yield (Al-Abdulsalam, 1997). Application of P enhances seed maturity and development (Ziadi *et al*., 2008), and adequate supply of P can increase grain yield of wheat by 20 % (Ascher *et al*., 1994). More than 70 % of grain total P is stored as phytic acid in seeds (Rosa, 1999). K plays a significant role in plant biochemical functions such as activation of various enzymes, synthesis of protein and carbohydrates, enhancement of fat content, improving drought tolerance, and resistance to frost and lodging (Marschner, 1995). An optimum rate of K fertilization increases number of productive tillers, number of grain per spike, 1000 grain weight, grain yield, and protein content of wheat (Alam *et al*., 2009).

Phytic acid (phytate) is the main phosphorus storage form in most cereals, legumes and nuts (Lopez *et al*., 2002). It is considered as an anti-nutritional factor that complex with proteins and chelates with some nutritionally important micronutrients (Fe, Zn, Mg, and Ca) and resulting in a significant decrease in the bioavailability of these nutrients (Raboy, 2001). Pentosans are the major non-starch polysaccharides and is divided into water-soluble pentosan and water unextractable solids. Water-soluble pentosan has a positive effect on dough rheological characteristics and macaroni production processing (Menger, 1976). Pentosan added to the dough increased dough development time, water-binding capacity and viscosity of dough (Jelaca and Hlynka, 1971).

Determination of grain nutrients, pentosan, gluten and phytate P content of wheat under different soil fertility is important as they influence nutritional quality and bread-making characteristics of flour. Therefore, the present experiment was conducted to study the effects of different levels of NPK on yield, grain minerals, crude protein, pentosan, gluten and phytate P content of wheat in a compost-amended regosol soil.

II. MATERIALS AND METHODS

The experiment was conducted in a greenhouse of the Faculty of Applied Biological Sciences, Hiroshima University. Conditions in the greenhouse were 65% humidity, 20-25 ºC day/15-18 ºC night temperatures, and natural sunlight. Containers (1.5 m length, 30 cm width, and 18 cm in depth) were used, and filled with a mixture of regosol and aerobic compost (2:1). Chemical analysis of this mixture showed that it contained; 0.2 % total N, 6.8 mg kg^{-1} available P and 79.6 mg kg^{-1} available K. Soil pH (H_2O) was adjusted to 6.5 by adding 1 ton ha^{-1} of dolomitic calcium magnesium carbonate. This investigation comprised a control (F_1, non-fertilized) and six levels of NPK fertilizers: F_2 (80 kg N + 40 kg P_2O_5 +40 kg K_2O ha^{-1}), F_3 (110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}), F_4 (140 kg N + 80 kg P_2O_5 + 70 kg K_2O ha^{-1}), F_5 (170 kg N + 100 kg P_2O_5 + 85 kg K_2O ha^{-1}), F_6 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}) and T_7 (230 kg N + 140 kg P_2O_5 + 115 K_2O kg ha^{-1}). The source of NPK was urea, single super phosphate, and potassium sulfate, respectively. All P, K and half dose of N were applied before sowing, and the remaining N was applied in two equal splits at tillering and anthesis stages. Minaminokaori, a commonly grown wheat cultivar in Japan was sown in the third week of November, then, ten-day-old seedlings were transplanted into the containers with 10 cm distance, following a randomized complete block design with 4 replicates. During the experiment all agronomic management practices were performed uniformly as required.

2.1. Growth, Yield, and Yield component:

Twenty plants (5 from each replicates) were randomly taken and the following yield components were evaluated: Number of tillers per plant was obtained by counting all tillers produced in each plant before harvest. Number of spikes were counted in each plant by the time of harvest. Number of grains per spike was obtained by counting grains in 10 spikes which were randomly collected from each treatment. To measure 1000 grain weight, 500 grains were counted and weighted with a prescribed accuracy, and then the value was multiplied by 2. To measure grain yield, mature spikes were collected, oven dried at 80˚C for 48 hours, threshed, and the grain yield was recorded and expressed in kg per hectare. Crop growth rate (CGR) was calculated as plant's dry weight increase per unit of time (Nogueira *et al*., 1994).

$$CGR= (W_2 - W_1) / (T_2 - T_1)$$

Where: W_1 and W_2 = total dry weight of plant at first and second sampling; T_1 and T_2= time of first and second sampling.

2.2. Grain mineral content

Samples of mature seeds were ground finely with a vibrating sample mill (TI-100, Heiko, Japan) and grain minerals contents were measured. The grain powdered samples were digested by sulfuric acid and heating, then they were diluted with distilled water, and K content was measured using flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan). Total P was determined in the same digested samples by UV-Spectrophotometer (U-3310, Hitachi Co. Ltd. Tokyo, Japan), following the molybdenum reaction solution method suggested by Chen *et al*. (1956). Grain Ca, Mg and Zn concentration were measured by an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu, Japan). Total N was measured using the Kjeldahl method after digestion with concentrated H_2SO_4 and H_2O_2 (10:5, v/v). Grain inorganic Phosphorus (Pi) was extracted in trichloroacetic acid (12.5%) + $MgCl_2$ (2 mmol /l) while stirring overnight, and Pi was measured colorimetrically, using a spectrophotometer following the molybdenum reaction regent method (Raboy and Dickinson, 1984).

2.3. Determination of grain starch and crude protein

To measure grain starch content, ethanol (80%) 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). To determine grain crude protein, the observed total N content from the Kjeldahl method was multiplied by 5.47 (Fujihara *et al*., 2008).

2.4. Determination of total pentosan and water-soluble pentosan

Total pentosan was measured using the orcinol-hydrochloric acid method, where finely ground samples were hydrolyzed with 2 N hydrochloric acid in a boiling water bath for 2.5 hours, and then centrifuged. A specific quantity of supernatants was transferred to the 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 water-soluble pentosan was extracted by hydrolyzing powdered samples in distilled water with shaking for 2 hours at 30 °C. Then, 4 N hydrochloric acid was added to the aliquots of the supernatant and placed in boiling water for 2 hours, and allowed to cool, and grain water-soluble pentosan content was measured by a spectrophotometer, using $FeCl_3$-orcinol reagents (Hashimoto *et al*., 1986).

2.5 Determination of gluten and phytate P content

Gluten content was measured according to (AACC) international approved method by hand washing with 30 minutes resting time, and the result was expressed as dry gluten percentage. Grain phytate P was measured according to the method suggested by Raboy and Dickinson (1984), where aliquots of flour were extracted in extraction media (0.2 M HCl: 10 % Na_2SO_4) overnight at 4 °C with shaking. Extracts were centrifuged and phytate was obtained as a ferric precipitate and assayed for P colorimetrically using ammonium molybdate reaction reagent.

2.6 Statistical analysis

All the collected data were subjected to analysis of variance using SPSS statistics package, Student Version 19, and means (n = 4) were separated using the Duncan Multiple Range Test at p = 0.05.

III. RESULT AND DISCUSSION

3.1. Agronomic performance and yield

Crop growth rate (CGR) was significantly affected by different levels of NPK fertilization (Figure 1). There was a linear increase in CGR with an increase in NPK levels but further increase in NPK (F_7) did not enhance CGR significantly compared to F_5. This result is in agreement with Laghari *et al*. (2010) and Asghar *et al*. (2010) who found that further increase in NPK levels had a non-significant response. The result of this study indicated that NPK fertilization significantly increased the number of tillers per plant. The highest number of tillers was observed in plants where a high NPK (F_7) was applied (Table 1). There was a progressive increase in the number of tillers with increased levels of NPK. Similar findings were reported by Kausar *et al*. (1993) and Niamatullah *et al*. (2011) indicating that high level of NPK significantly increased the number of tillers in wheat. The mean number of spike per plant was ranged from 5.5 to 13.35. NPK fertilization enhanced the number of spike per plant and the highest number of spike per plant was observed with F_6 treatment (Table 1). The high rate of NPK (F_7) did not increase the number of spikes per plant because of prolonged vegetative growth which resulted in the production of more number of infertile tillers. These findings are in agreement with Hussain *et al*. (2002) who reported a decrease in the number of fertile tillers due to the application of a high rate of NPK. Similarly, Ali and Yasin (1991) found that high dose of N and P reduced the number of the spike in wheat. The maximum number of grains per spike was obtained with F_7 treatment. However, the number of grains produced by F_6, F_5, F_4, and F_3 treatments were statistically similar (Table 1). The reason for high number of grains per spike in F_7 might be due to higher rate of N and P which enhanced seed set in the spike (Hussain *et al*., 2002; Alam, *et al*., 2007; Malghani *et al*., 2010). The 1000 grain weight as an important yield contributing parameter was higher in plants which were supplied with a high rate of NPK. F_7 and F_6 treatments recorded the higher value for 1000 grain weight compared to control (Table 1). The moderate fertilizer levels (F_5, F_4, F_3, and F_2) produced statistically similar 1000 grain weight. Significant effects of NPK fertilization on 1000 grain weight of cereals were also reported by Maqsood *et al*. (2001) and Asghar *et al*. (2010).

Grain yield was significantly affected by different levels of NPK fertilization. Application of F_6 and F_7 resulted in increased number of fertile tillers, number of grains per spike and maximum 1000 grain weight which eventually contributed to the production of a higher grain yield. F6 treatment resulted in 151.64 % increase in grain yield over control. There was a slight decrease in grain yield of plants with F_7 treatment, compared to F_6 that might be due to the N interaction with P and K (MacLeod, 1969). Niamatullah *et al.* (2011), Khursheed and Mahammad (2015), and Abdul-Aziz *et al*. (2016) also concluded that grain yield of wheat and cereal crops can be increased with application of N, P, and K fertilizers.

3.2. Grain mineral content

Grain minerals contents were significantly influenced by NPK fertilization. Plants with F_6 treatment recorded higher grain total N, P, K, Pi, Mg, and Zn content. It was observed that further increase in NPK dose (F_7) only increased Ca content but the contents of N, P, K, Pi and Zn were slightly reduced in F_7 treated plants (Table 2). Gain Zn content was enhanced with application of high and moderate levels of NPK fertilization. In general, F_6 treatment increased grain total N, P, K, Mg Ca, Zn and Pi contents by 65.2, 33.6, 8.9, 19.7, 165.9, 26.13 and 80%, respectively, compared to control. Laghari *et al*. (2010) also found that NPK fertilization increased mineral uptake particularly, N, K and P in wheat. Application of high N fertilizer enhanced K accumulation in wheat grains (Sheoran *et al*., 2015). Saha *et al*. (2014) found that application of P fertilizer enhanced P content in wheat grains. While, application of very high dose of N did not enhance grain total P content of wheat (Akhtar *et al*. (2011). In this study the decrease in grain mineral content with F_7 treatment might be due to prolonged vegetative growth and excessive biomass production that reduced grain mineral content in wheat grains.

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Table 1. Effect of NPK fertilization on number of tillers plant-1, number of spikes plant-1, number of grains spike^{-1}, 1000 grain weight, and grain yield of wheat. The same letter indicates no significant difference ($p \leq 0.05$).

NPK levels	Number of tillers	Number of spikes	Number of grains	1000 grain	Grain yield
	(plant^{-1})	(plant^{-1})	(spike^{-1})	(g)	(ton ha^{-1})
F_1	5.55^{e}	5.45^{d}	36.85^{b}	42.03^{c}	2.77^{c}
F_2	13.96^{d}	9.60^{c}	37.15^{b}	45.82^{ab}	4.52^{bc}
F_3	14.10^{d}	9.67^{c}	39.30^{ab}	46.29^{ab}	5.03^{b}
F_4	18.30^{c}	10.80^{bc}	40.35^{ab}	46.33^{ab}	5.22^{b}
F_5	19.40^{bc}	11.30^{bc}	40.41^{ab}	46.91^{ab}	5.53^{b}
F_6	21.30^{ab}	13.35^{a}	41.00^{ab}	47.57^{a}	6.97^{a}
F_7	23.40^{a}	12.20^{ab}	43.10^{a}	47.90^{a}	6.9^{a}

Table 2. Effect of NPK fertilization on grain mineral content in wheat grain. The same letter indicates no significant difference ($p \leq 0.05$).

NPK levels	N	P	K	Pi	Mg	Ca	Zn
	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g $^{-1}$)	(mg g $^{-1}$)	(µg g^{-1})	(µg g^{-1})
F_1	16.01^{d}	3.42^{c}	4.72^{b}	0.247^{c}	1.32^{e}	71.04^{c}	74.66^{b}
F_2	18.07^{cd}	4.01^{b}	4.68^{ab}	0.349^{b}	1.35^{de}	74.61^{c}	81.47^{ab}
F_3	21.10^{bc}	4.11^{b}	4.89^{ab}	0.369^{b}	1.43^{cd}	107.89^{bc}	87.97^{a}
F_4	21.40^{bc}	4.25^{ab}	4.92^{ab}	0.383^{ab}	1.48^{bc}	147.98^{ab}	88.87^{a}
F_5	22.93^{ab}	4.31^{ab}	4.98^{ab}	0.396^{ab}	1.52^{abc}	150.43^{ab}	90.99^{a}
F_6	26.45^{a}	4.57^{a}	5.14a	0.445^{a}	1.58^{a}	188.95^{a}	94.17^{a}
F_7	24.95^{ab}	4.35^{ab}	5.00^{ab}	0.407^{ab}	1.54^{ab}	197.14^{a}	92.29^{a}

3.3. Grain starch and crud protein content

Analysis of data showed that NPK fertilization did not affect grain starch content significantly. Control (F_1) recorded a higher value for grain starch, while with increase in NPK level grain starch content was slightly decreased. Kindred *et al.* (2008) observed that application of N fertilizer decreased the starch content of wheat grain. There is a negative relationship between crude protein and starch as the grain yield and crude protein increases with higher rate of fertilizers, the starch content decreases (Hlisnikovsky and Kunzova, 2014). A decrease in grain starch content due to fertilizer application was also reported by Crista *et al*. (2012) indicating that starch content was higher in plants with no fertilizer application. The highest grain crude protein (%) was observed with F_6 treated plants followed by F_7 and F_5.

However, the lowest crude protein was recorded in F_1 where no fertilizer was applied. There was a linear increase in crude protein with increase in NPK level up to F_6, but further increase in NPK rate (F_7) slightly reduced crude protein content of wheat grain (Table 3). F_6 treatment increased grain crude protein by 65.3% compared to control. Application of excessive NPK fertilizers reduced grain crude protein content. This result agrees with Sameen *et al.* (2002) who found a reduction in crude protein content of wheat cultivar V-94091 and V-94105 due to the highest rate of NPK fertilizers. Similarly, Crista *et al*. (2012) found that NPK fertilization enhanced the synthesis of the raw protein in wheat. N fertilizer plays a significant role in total N and crude protein accumulation in wheat grains, while application of high dose of N beyond the optimum level may have a negative effect on grain crude protein (Abedi *et al*., 2011).

3.4. Grain total and water-soluble pentosan content

Grain total pentosan content was decreased with increase in NPK level the highest value of total pentosan recorded with F_1 treatment where no fertilizer was applied. Plants supplied with different levels of NPK fertilizers, recorded statistically similar total pentosan content. The effect of NPK fertilization on the grain total pentosan and water-soluble pentosan contents of wheat has not been reported sufficiently by earlier researchers. The major proportion of total pentosan is water-unextractable pentosan which forms physical barriers for the gluten network during dough development. Unlike to total pentosan, grain water-soluble pentosan content was enhanced with application of NPK, and F_6 treatment recorded the highest value of grain water-soluble pentosan content. The F_6 treatment increased grain water-soluble pentosan content by 40.5% compared to control. Water-soluble pentosan plays a key role in bread-making quality of dough. It increases the viscosity

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and the stability of dough foam structure, which helps in a bigger loaf volume and a finer homogeneous bread crumb (Courtin and Delcour, 2002).

3.5. Grain dry gluten content

Gluten is responsible for the unique elasticity and stickiness of wheat dough, the properties that make it so useful in bread-making. In this study, grain dry gluten content was increased with increase in NPK level, and among the various levels of NPK F_6 and F_7 treatments recorded a higher dry gluten content. The F_6 treatment increased grain dry gluten by 4-fold compared to control. A slight decrease was observed with application of the highest NPK level (F_7) that might be because of decrease in protein content due to excess NPK application, as explained earlier in case of crude protein content. Mineral fertilization increased the gluten content of wheat compared to a control, but various levels of P and K did not affect grain gluten significantly (Gaj *et al*., 2013).

3.6. Grain phytate P content

Analysis of variance showed that F_6 and F_7 treatments recorded a higher grain phytate P content compared to F_1 (Table 3). Phytate is the major storage form of phosphorous in cereals, therefore the content of phytate P mostly depends on total grain P. Phytate P can contribute to the nutritional deficiencies when seeds are used as food (Rosa 1999; Raboy 2001). It binds with proteins and important minerals such as Fe, Ca and Zn, and reduces their availability (Raboy, 2001). There was no sufficient review on the effect of NPK fertilization on phytate P content of wheat grain. The increase in grain phytate P content with a higher dose of NPK fertilization might be associated with grain total P content which is enhanced by a higher rate of P fertilizer.

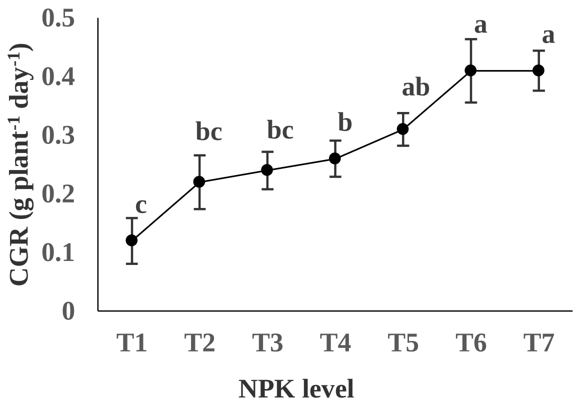

Fig. 1: Effect of NPK fertilization on crop growth rate (CGR). The same letter indicates no significant difference ($p \leq 0.05$).

Table 3. Effect of NPK fertilization on grain starch, crude protein, total pentosan, water-soluble pentosan and phytate P content of wheat. The same letter indicates no significant difference ($p \leq 0.05$).

Treatment	Starch	Crude protein	Total pentosan	Water-soluble pentosan	Dry gluten	Phytate P
	(%)	(%)	(mg g^{-1})	(mg g^{-1})	(%)	(mg g^{-1})
F_1	65.08^{a}	8.87^{d}	8.58^{a}	1.11^{b}	3.7^{c}	3.14^{c}
F_2	64.23^{a}	10.00^{cd}	7.78^{ab}	1.23^{b}	6.7^{bc}	3.26^{bc}
T_3	62.98^{a}	11.69^{bc}	7.75^{ab}	1.34^{ab}	8.5^{b}	3.52^{b}
F_4	62.93^{a}	11.85^{bc}	7.00^{b}	1.41^{ab}	12.3^{b}	3.64^{ab}
F_5	63.00^{a}	12.72^{ab}	6.94^{b}	1.49^{a}	16.5^{ab}	3.75^{ab}
T_6	63.02^{a}	14.66^{a}	7.24^{b}	1.56^{a}	18.9^{a}	3.86^{a}
F_7	63.00^{a}	13.82^{ab}	6.93^{b}	1.51^{a}	18.7^{a}	3.82^{a}

IV. CONCLUSION

The present results showed that different levels of NPK fertilization significantly influenced growth, yield, and grain nutritional and bread-making quality of wheat in a compost-amended regosol soil. F_6 treatment resulted in the production of higher grain yield, increased grain minerals contents, crude protein, water-soluble pentosan, and dry gluten content. F_6 treatment reduced the total pentosan, but did not significantly affect the level of grain starch content. By increasing the rate of NPK fertilization beyond F_6 treatment, only vegetative growth was enhanced. Therefore, F_6 treatment might have been an appropriate and economical rate of NPK to obtain maximum grain yield and improve grain nutritional and bread-making quality of wheat cultivar Minaminokaori in a compost-amended regosol soil.

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