Improvement of Grain Yield, Nutritional and Antinutritional Quality, and Seed Physiological Performance of Wheat by NPK Fertilization

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ABSTRACT

The present research was conducted to study the effect of NPK fertilization on wheat grain yield, minerals concentration, grain quality, gluten, pentosan, and phytate phosphorous (phy-P) content, and the influence of maternal plant NPK fertilization on the seed physiological attributes during the germination period. NPK treatments comprised a control, where no fertilizer was applied (T₀), and two levels of NPK fertilizer: T₁ (110 kg N+60 kg P₂O₅+55 kg K₂O ha⁻¹), and T₂ (200 kg N+120 kg P₂O₅+100 kg K₂O ha⁻¹). Winter wheat was grown in a greenhouse during the growing season of 2015-16, following randomized complete block design with 4 replicates. The results indicated that a high level of NPK (T₂) fertilization increased the grain yield, crude protein, water-soluble pentosan, and dry gluten, up to 151.6, 65.3, 40.5, and 40.8.9% compared to the control, respectively. It also enhanced the grain mineral concentration, but did not affect the grain starch significantly. Grain phy-P was increased with a high NPK fertilization and, interestingly, the level of phytase enzyme was also increased up to 46% in T₂ compared to the control. Moreover, maternal plant NPK fertilization enhanced seed germination percentage, seedling fresh weight, phytase activity, inorganic phosphorus, and phy-P metabolism during the germination period. From the results of this study, it was concluded that grain nutritional quality was improved with increasing NPK rates, but antinutritional compound phy-P was also increased, while it may enhance seed viability, germination, and seedling vigor.

Keywords: Germination, Pentosan, Phytate, Wheat.

INTRODUCTION

Sustainable agricultural productivity and ensuring food security is possible through a precise and wise management of nutrients. Application of fertilizer adequately enhances the yield per unit area, improves grain quality, and bread quality of wheat. Nitrogen is an important component of proteins, nucleic acids, enzymes, coenzymes, and chlorophyll, and it contributes to the biochemical processes of the plant (Benin et al., 2012). Nitrogen fertilization at anthesis, is more effective in the synthesis of a high grain protein content than an earlier application (Wuest and Cassman, 1992). Sufficient N fertilization results in the production of higher productive tillers and an increased number of spikes, number of grains per spike, grain yield, and biological yield. Phosphorus (P) fertilization of wheat crop significantly increased the plant height, number of tillers per plant, the straw and grain yield, and P uptake in grain over a control (Alam et al., 2003). More than 70% of the total P is stored in the grain as phytate (Rosa et al., 1999). The phy-P is an anti-nutritional factor that binds with proteins and some important micronutrients, such as iron and zinc, and significantly reduces the availability of these nutrients (Raboy, 2001). Accordingly,
it is necessary to reduce the concentration of such anti-nutrient compounds in wheat grains by proper nutrient management. Potassium (K) plays a vital role in the biochemical functions of the plants like improvement of protein and carbohydrates, activating various enzymes, enhancement of fat concentration, developing drought tolerance, and resistance to lodging and frost (Marschner, 1995). An optimum dose of K increases the number of effective tillers, grains per spike, 1,000-grain weight, grain yield, straw yield, and protein content of wheat (Alam et al., 2009).

Pentosan is a major fiber component of the non-starch polysaccharides in cereal, which is called flour gum or hemicellulose. It affects food absorption in the human body, and decreases absorption of lipids and cholesterol and, therefore, plays a key role in the human diet (Mohammadkhani, 2005). Pentosans are important components of dough in which they bind water and contribute to the formation of viscous dough (Buksa et al., 2010). Water-soluble pentosan has a positive effect on the bread-making quality of wheat flour (Courtin and Delcour, 2002). Gluten is composed of glutenins and gliadins, which play an important role in the baking quality of bread due to their influence on the water absorption capacity, elasticity, and extensibility of dough and, thus, affect the flour quality of wheat (Torbica et al., 2007). Phytase (myoinositol hexaphosphate phosphohydrolase) is an enzyme which catalyzes the hydrolysis of phytate to inositol and orthophosphate, and helps in the bioavailability of P. Seed germination increases phytase activity by de novo synthesis of this enzyme during germination (Sung et al., 2005).

Investigation on the effects of macronutrients on the grain yield, quality, anti-nutrient compounds, and seed quality attributes, deserves more attention. On the other hand, the effects of NPK fertilization on the grain pentosan, gluten, phy-P, and physiological changes during seed germination have not been much studied. Therefore, the present experiment was conducted with the aim to study the effect of NPK fertilization on the wheat yield, grain quality, and anti-nutrient content, and the influence of maternal plant NPK nutrition on the seed germination, establishment, and physiological changes during seed germination.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The wheat cultivar Minaminokaori was grown in a vinyl greenhouse in Hiroshima University with natural sunlight and temperature to prevent nutrient leaching due to rainfall. Containers (30 cm in width, 1.5 m in length, 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: 0.20% total N, 6.84 mg kg$^{-1}$ available P, and 79.85 mg kg$^{-1}$ available K. Furthermore, 1 ton ha$^{-1}$ of dolomitic calcium magnesium carbonate was mixed with the soil to adjust the pH (H$_2$O) to 6.5.

This study comprised a control, where no fertilizer was applied (T$_0$), and two levels of NPK fertilizer: T$_1$ (110 kg N+60 kg P$_2$O$_5$+55 kg K$_2$O ha$^{-1}$), and T$_2$ (200 kg N+120 kg P$_2$O$_5$+100 kg K$_2$O ha$^{-1}$). The sources of NPK were urea, single super phosphate, and potassium chloride, respectively. All amount 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 tillering and anthesis stages. Wheat seeds were sown in the third week of November 2015, then, 10-day-old seedlings were transplanted into the containers at a 10-cm spacing, following a randomized complete block design with 4 replicates. All the recommended agronomic practices were followed for raising the crops during the experiment.

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.

**Determination of Grain Minerals**

Samples of mature seeds were ground finely with a vibrating sample mill (TI-100, Heiko, Japan) and the concentrations of grain minerals were measured. Finely ground samples were digested by H\textsubscript{2}SO\textsubscript{4}-H\textsubscript{2}O\textsubscript{2} and the K content was measured using a flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan). Ca, Mg, and Zn were measured by an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu, Japan). The total P was determined by a UV-Spectrophotometer (U-3310, Hitachi Co. Ltd. Tokyo, Japan) following the molybdenum reaction solution method suggested by Chen et al. (1956). Grain inorganic P (Pi) was extracted in trichloroacetic acid (12.5%)+MgCl\textsubscript{2} (2 mmol L\textsuperscript{-1}) while stirring overnight, and Pi was measured colorimetrically (Raboy and Dickinson 1984). The total nitrogen was measured using the Kjeldahl method after sample digestion with concentrated H\textsubscript{2}SO\textsubscript{4} and H\textsubscript{2}O\textsubscript{2}.

**Determination of Grain Quality**

Starch was measured using the anthrone-sulfuric acid method (Spiro, 1966). Crude protein was calculated by multiplying the total N content by 5.47 (as proposed by Fujihara et al., 2008). Grain total pentosan was measured following the orcinol-HCl method, where finely ground samples were hydrolyzed with 2N HCl in boiling water for 2.5 hours, and centrifuged. Then, a specific amount of supernatant was transferred to new test tubes and reaction regents (FeCl\textsubscript{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. Water-soluble pentosan was extracted by hydrolyzing flour samples in distilled water with shaking for 2 hours at 30\textdegree C. Then, 4N HCl was added to the aliquots of the supernatant and placed in boiling water for 2 hours, and allowed to cool, and water-soluble pentosan was estimated by a spectrophotometer, using FeCl\textsubscript{3}-orcinol reagents (Hashimoto et al., 1986). Gluten was measured according to (AACC) international approved method 38-10, by hand washing with 30 minutes resting time, and the result was expressed as dry gluten percentage. Phy-P was measured following the method suggested by Raboy and Dickinson (1984) where aliquots of flour were extracted in extraction media (0.2M HCl: 10% Na\textsubscript{2}SO\textsubscript{4}) overnight at 4\textdegree C with shaking. Extracts were centrifuged, and phytate P was obtained as a ferric precipitate and assayed for P using ammonium molybdate reaction reagent.

**Germination Experiment**

To determine the effect of maternal plant NPK fertilization on the growth and physiological performance of produced seeds, 200 seeds of 4 replicates were planted on germination wetted papers and placed in a germinator at 23\textdegree C for 7 days. Samples were taken every day, frozen with liquid N, and stored under -80\textdegree C. The data on phytase activity, phy-P content, and Pi content were recorded daily in the laboratory of plant nutritional physiology, faculty of applied biological science, Hiroshima University as follow.

**Seed Germination Test and Determination of Physiological Attributes**

Normal seedlings were counted on the 7\textsuperscript{th} day of germination and the result was expressed in percent. Seedlings were harvested on the 7\textsuperscript{th} day of germination and fresh weight was recorded. To measure phytase activity, fresh samples were ground with liquid nitrogen, transferred to Erlenmeyer flasks, and a buffer solution...
(Na-Phytate+Sodium acetate) was added. Then, the samples were shaken for 30 minutes at 37°C. Subsequently, aliquots of the sample were transferred to two sets of plastic tubes and placed in a water bath at 37°C. The Phytase activity was stopped by adding TCA (Trichloroacetic Acid) to the first set of test tubes to act as a control, then, TCA was added to the second set of test tubes after 30 minutes to stop enzyme activity. The test tubes were centrifuged, then, supernatant was transferred to new test tubes, and reagent solutions (ammonium molybdate+ferrous sulfate heptahydrate) were added. The absorbance was measured colorimetrically at 700 nm against the control (Eeckhout and De Paepe 1994). Determination of phy-P and Pi was carried out following the procedures suggested by Raboy and Dickinson (1984), and the result was expressed based on the dry weight.

### Statistical Analysis

All the collected data were subjected to analysis of variance using SPSS statistics package, Student Version 18, and means (n= 4) were separated using the Duncan multiple range test at P= 0.05.

### RESULTS

#### Grain Yield

Grain yield was significantly affected by the various levels of NPK fertilization. Application of a high rate of NPK (T₂) resulted in the production of a higher grain yield. It was observed that NPK fertilization, increased grain yield by 151.6% in T₂ and 81.59% in T₁ compared to T₀ (Table 2).

#### Grain Minerals Concentration

Statistical analysis of the data showed that the concentrations of grain minerals (N, P, K, Pi, Mg, Zn, and Ca) were highly affected by NPK fertilization (Table 1). Grain minerals were found to be significantly higher in plants supplied with a high dose of NPK (T₂), while the lowest grain mineral content was observed in the control plants.

### Table 1. Effect of NPK fertilization on the grain minerals content of wheat.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N (mg g⁻¹ in dry matter)</th>
<th>P (mg g⁻¹ in dry matter)</th>
<th>K (mg g⁻¹ in dry matter)</th>
<th>Pi (µg g⁻¹ in dry matter)</th>
<th>Mg (µg g⁻¹ in dry matter)</th>
<th>Zn (µg g⁻¹ in dry matter)</th>
<th>Ca (µg g⁻¹ in dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>16.01 c</td>
<td>4.04 a</td>
<td>4.72 b</td>
<td>0.247 b</td>
<td>0.132 c</td>
<td>74.66 b</td>
<td>71.04 b</td>
</tr>
<tr>
<td>T₁</td>
<td>21.10 b</td>
<td>4.87 b</td>
<td>4.89 ab</td>
<td>0.369 a</td>
<td>0.143 b</td>
<td>87.97 a</td>
<td>107.89 ab</td>
</tr>
<tr>
<td>T₂</td>
<td>26.45 a</td>
<td>5.41 a</td>
<td>5.14 a</td>
<td>0.445 a</td>
<td>0.158 a</td>
<td>94.17 a</td>
<td>188.95 a</td>
</tr>
</tbody>
</table>

*The same letter indicates no significant difference (p ≤ 0.05). T₀ (control), T₁ (110 kg N + 60 kg P₂O₅ + 55 kg K₂O ha⁻¹), and T₂ (200 kg N + 120 kg P₂O₅ + 100 kg K₂O ha⁻¹).*

### Table 2. Effect of NPK fertilization on the grain yield and quality of wheat.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Grain yield (ton ha⁻¹)</th>
<th>Starch (%)</th>
<th>Crude protein (%)</th>
<th>Total pentosan (mg g⁻¹)</th>
<th>Water-soluble pentosan (mg g⁻¹)</th>
<th>Dry gluten (%)</th>
<th>Phytate phosphorus (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>2.77 b</td>
<td>65.08 b</td>
<td>8.87 b</td>
<td>8.58 b</td>
<td>1.11 b</td>
<td>3.7 b</td>
<td>3.14 b</td>
</tr>
<tr>
<td>T₁</td>
<td>5.03 b</td>
<td>62.98 b</td>
<td>11.69 bc</td>
<td>7.75 ab</td>
<td>1.34 ab</td>
<td>8.5 b</td>
<td>3.52 b</td>
</tr>
<tr>
<td>T₂</td>
<td>6.97 a</td>
<td>63.02 a</td>
<td>14.66 a</td>
<td>7.24 a</td>
<td>1.56 a</td>
<td>18.9 a</td>
<td>3.86 a</td>
</tr>
</tbody>
</table>

*The same letter indicates no significant difference (p ≤ 0.05). T₀ (control), T₁ (110 kg N + 60 kg P₂O₅ + 55 kg K₂O ha⁻¹), and T₂ (200 kg N + 120 kg P₂O₅ + 100 kg K₂O ha⁻¹).*
Figure 1. Effect of maternal plant NPK nutrition on the seed germination percentage (left) and fresh weight (right) of wheat at 7 days after germination. The same letter indicates no significant difference (P ≤ 0.05).

*T0 (control), T1 (110 kg N+60 kg P2O5+55 kg K2O ha−1), and T2 (200 kg N+120 kg P2O5+100 kg K2O ha−1).
The seed phytase content significantly increased during germination period. A lower phytase activity was recorded in 0-day seeds before germination. The level of phytase activity was highest on the 6th day of germination and the phytase level was recorded as being higher in T2 and T1 seedlings compared to T0 (Figure 2). Germination enhanced the phytase level by 3.22-fold, 3.38-fold, and 4.25-fold in T2, T1, and T0, respectively, on the 6th day of germination compared to 0-day.

The phy-P of the seeds declined during the germination period significantly. The highest phy-P content was recorded in the seeds of T2, followed by T1 and T0 plants before germination (0-day). The lowest phy-P was observed in T0, followed by T1 and T2 seedlings on the 7th day of germination. At the end of the 7th day of germination, the phy-P content decreased by 2.31-fold, 2.34-fold, and 2.43-fold for T2, T1, and T0, respectively, on the 7th day of germination compared to 0-day (Figure 2).

The phytase activity was enhanced during seed germination, and resulted in bioavailability of inorganic Pi. There was a liner increase in Pi with increased time of germination (Figure 3). The highest Pi was recorded in T2 (2.09 mg g⁻¹ dw), followed by T1 (2.03 mg g⁻¹ dw), and T0 (1.73 mg g⁻¹ dw) on the 7th day of germination, while the lowest Pi was observed in T0 before germination (0-day).

**DISCUSSION**

The results indicated that combined NPK fertilization increased grain yield and mineral concentration, improved grain quality, and enhanced seed germination and the physiological performance of germinating seedlings. Application of a high rate of NPK (T2) enhanced plant growth and productivity, and resulted in a higher grain yield. These results are in accordance with Hussain et al. (2002), Laghari et al. (2010).
and Abdel-Aziz et al. (2016) who concluded that the grain yield of wheat and cereal crops increased with the application of NPK fertilizers.

The grain mineral concentration was significantly influenced by NPK fertilization. It was observed that the mineral concentration was increased with an increase in NPK rate. Laghari et al. (2010) and Campillo et al. (2010) concluded that application of N, P, and K enhanced the concentrations of these minerals in wheat. Saha et al. (2014) reported that the application of phosphorus fertilizer (single superphosphate) enhanced the total P concentration in wheat grain.

Grain quality, except for starch, was highly influenced by NPK fertilization. The starch content was not affected significantly by NPK fertilization, and a slight decrease in the starch content was observed with an elevated rate of NPK. There is a negative relationship between crude protein and starch content, and N fertilization decreases the starch content of wheat grain (Kindred et al., 2008). Crista et al. (2012) and Hlisnikovsky and Kunzova (2014) reported similar findings that grain starch was higher in control plants where no fertilizer was applied. NPK fertilization was found to enhance the synthesis of the raw protein in wheat (Crista et al., 2012). Sameen et al. (2002) found that the crude protein of wheat grain was increased by application of a high level of NPK fertilizers in the wheat variety Inqulab 91. The effect of NPK fertilization on the grain total and water-soluble pentosan content of wheat has not been reported sufficiently in earlier research. However, the influence of ecological environment was found to be significant on the pentosan content of wheat grain (Chunxi et al., 2002). Increased pentosan content was found with additional N fertilization under water logging condition in waxy wheat (Jing et al., 2010). In this study, total pentosan was decreased with high NPK fertilization, whereas water-soluble pentosan was significantly increased. Courtin and Delcour (2002) reported that water-soluble pentosan had a positive impact on the bread-making quality of wheat, and that water-unextractable pentosan had a negative effect. NPK fertilization significantly influenced the gluten content of wheat flour and the highest gluten was recorded in T2, where high rate of NPK was applied. Tanacs et al. (2005) also found that application of NPK fertilizers significantly increased the gluten content of 4 tested wheat varieties in all 3 years of investigation. Gaj et al. (2013) also found that mineral fertilization increased the gluten content of wheat compared to the control, but different levels of P and K did not affect grain gluten significantly.

Phy-P is the major storage form of P in cereals, therefore, the concentration of phy-P mostly depends on grain total P. The phy-P content of many crops was determined by researchers (Garcia-Estepa et al., 1999; Rosa et al., 1999) but the effect of NPK fertilization on the phy-P content of wheat grain have not been much studied so far. Application of a high rate of P fertilizer might be one of the reasons for high phytate content (Raboy and Dickinson, 1984). In another study, Ali et al. (2014) found that application of P increased the grain total P in wheat. Similarly, Laghari et al. (2010) revealed that NPK fertilization resulted in a higher P uptake of wheat. Phytase helps phy-P metabolism and Pi bioavailability and activity of this enzyme was increased with NPK fertilization.

Maternal plant NPK nutrition improved the seed germination, seedling growth, and physiological performance. High nutrient reserves in seeds produced by NPK fertilized plants might be the reason for better physiological activity and a high germination percentage in $T_2$ and $T_1$ seeds. Seeds of plants which received more fertilizer and irrigation during the production stage can increase seedling establishment in comparison with other treatments (Hampton, 1992). Similarly, Bittman (1989) found that difference in the final germination percentage of seeds could be due to the amount of stored nutrient in the endosperm. Doddagoudar et al. (2004)
concluded that application of a higher rate of NPK improved seed quality and resulted in higher seed germination percentage in China aster (*Callistephus chinensis* Nees.L.). During seed germination, the nutrients present in the endosperm are hydrolyzed to guarantee seedling establishment (Shimizu and Mazzafera 2000). In this study, NPK fertilization of the maternal plant improved grain food reserves and helped with a better growth of seedlings and contributed to high seedling fresh weight compared to the control. Phytase activity reached a maximum level on the 6th day of germination, as a result, T2 and T1 recorded higher values of phytase. Ma and Shan (2002) reported that seed germination significantly increased phytase activity by 2.04-fold on the 3rd day of germination in wheat. The effect of NPK fertilization on the phytase activity of germinating seeds has not been much studied, and the high phytase level of T2 and T1 during the germination period might be due to a high P and protein content in the maternal plant grains compared to the control. Sung et al. (2005) revealed that the increase in phytase level may be due to de novo synthesis of the enzyme during germination. It was observed in this study that the phytase level started decreasing slightly on the 7th day of germination. The decrease in phytase activity might be due to the degradation of this enzyme by active protease (Houde et al., 1990). There was a negative relationship between phytase activity and phy-P content; as phytase activity increased the phy-P content decreased. The effect of NPK fertilization on the seed phytate content during germination has not been reported. Phytase in germinating seeds removes orthophosphate groups from the inositol ring of phytate to produce free Pi, and a chain of intermediate myo-inositol phosphates (Debnath et al., 2005). The increase in the phytase activity of germinating seeds, which coincides with a decrease in the phytate content, may enhance phosphorus availability and utilization (Azeke et al., 2011).

**CONCLUSIONS**

In this study, significant differences (P≤0.05) were observed in the grain yield, yield component, grain minerals, grain quality, and anti-nutrient content of wheat. A higher rate of NPK (200 kg N+120 kg P2O5+100 kg K2O ha⁻¹) produced high grain yield, and increased the content of grain minerals, crude protein, water-soluble pentosan, and dry gluten, and reduced total pentosan, but did not affect the level of starch in wheat grain. NPK fertilization of the maternal plant enhanced seed germination, seedling growth, and improved the physiological performance of germinating seeds compared to the control. Phytase activity, phy-P degradation, and the release of Pi during seed germination were highly affected by maternal plant NPK fertilization.

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بهبود عملکرد دانه و کیفیت تغذیه‌ای و ضد تغذیه‌ای و عملکرد فیزیولوژیکی بذر گندم NPK در اثر کوددهی با phy-P

م. صفرنوری، ک. دوتنگ، و ه. سانیوکا

چکیده

هدف از اجرای این پژوهش مطالعه اثر کوددهی با NPK بر عملکرد دانه و کیفیت تغذیه‌ای و عملکرد فیزیولوژیکی بذر گندم، در اثر تغذیه با فسفر (phy-P) و بررسی تأثیر کوددهی گیاهان مادربی روزهای صفات فیزیولوژیکی بذر در طی جوانه زنی بود. تیمارهای کودکی به صورت تیمار شاهد ( بدون NPK (کوددهی‌ای) (T0)، و دو سطح کوددهی (T1) (110 kg N + 60 kg P2O5 + 55 kg K2O ha⁻¹)، (T2) (200 kg N + 120 kg P2O5 + 100 kg K2O ha⁻¹) بود. گندم زمستانی این آزمایش در شرایط بلوک های کامل تصادفی در 4 تکرار در فصل زراعی 14-15-16-17 ماه بوشهر آغاز و به پایان کشاورزی که کوددهی ر horm و گیاهان مادربی بوده در آزمایش عملکرد دانه و برخی صفات اقتصادی کاکرهای آن در اثر تغذیه با NPK در تیمار کودکی (phy-P) در دو سطح کوددهی (T2) زنی، با جایگزینی زنی، و نزد کوددهی گیاهان مادربی با phy-P یا در طی جوانه زنی بهتر از اثر کوددهی با NPK در مصرف phy-P بهبود یافت و دارای این رفتارهای ای افزایش داشت مصرف phy-P بذر جوانه زنی، و قوت گیاه‌های را بهتر کرد.