INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 12, No. 3, p. 19-24, 2018

RESEARCH PAPER OPEN ACCESS

Physiological and biochemical changes during seed germination of wheat (*Triticum aestivum* L.) as influenced by mother plant NPK nutrition

Mohammad Safar Noori[*1,2]

[1]*Graduate School of Biosphere Science, Hiroshima University, Japan*

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

Article published on March 28, 2018

Key words: Germination, Phytase activity, Phytate, Soluble protein, Wheat

Abstract

High quality and vigorous seeds perform better physiological activities, and therefore play a key role in successful field crop production. This experiment was conducted to evaluate the effects of mother plant NPK nutrition on seed physiological activity and biochemical changes during seed germination in wheat. In this study three levels of NPK fertilizers (T_1: non-fertilized or control, T_2: 110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}, and T_3: 200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}) were applied to the wheat plants and the seeds which were obtained from these plants were kept for germination test, and the physiological parameters were analyzed. The results indicated that mother plant NPK nutrition has significantly enhanced seed germination percentage, seedling fresh weight, soluble protein, phytase activity and inorganic P, and accelerated phytate metabolism during the germination period. Phytase activity was maximum on 6^{th} day of germination and phytase level was increased by 10.4% in seeds obtained from T_3 treated plants compared to T_1. At the end of 7 days from germination the contents of inorganic P and soluble protein were increased by 91.7 and 41.0% with T_3treatment compared to T_1, respectively. Phytate breakdown resulted in increased inorganic P (4.6-fold) bioavailability on 7^{th} day of germination compared to 0-day. From the results of this study, it can be concluded that seed viability and physiological performance of seedlings can be improved with adequate NPK fertilization of the mother plants in wheat.

* **Corresponding Author:** Mohammad Safar Noori ✉ safar_noori@yahoo.com

Introduction

Chemical fertilizers play a vital role in increasing yield and improving the quality of crops. Nitrogen (N) is actively involved in many metabolic processes making a direct contribution to increasing productivity and improving crop yields (Barker and Pilbeam, 2007). N is a constituent of many fundamental cell components such as nucleic acids, amino acids, enzymes, and photosynthetic pigments (Bungard *et al.*, 1999). Phosphorus (P) is an essential component of most organic compounds in the plant including nucleic acids, proteins, phospholipids, sugar phosphates, enzymes and energy-rich phosphate compounds, such as adenosine triphosphate (Sylvia *et al.,* 2005; Brady and Weil, 2008). Application of P improves crop quality by increasing sugar, protein and P content, and improving feed value in wheat (Havlin *et al.*, 2005; Karl *et al.*, 2013). Potassium (K) plays a key role in regulation of metabolic processes such as photosynthesis; activation of enzymes that metabolize carbohydrates for synthesis of amino acids and proteins; facilitation of cell division and growth by helping to move starches and sugars between plant parts. It is reported that K stands out as a cation having a considerable influence on quality attributes, and the concentration of critically important human-health associated phyto-nutrients or bioactive compounds (Jifon and Lester, 2009; Lester *et al.*, 2010).

Production of high quality and vigorous seed is essential for the precise and effective agronomic practices. Utilization of high quality seed decreases the cost of production by reducing the seed rate and input wastage. High seed germination, seedling growth and physiological activity contributes for a better crop establishment in different soils, even under challenging environments. Therefore, this study was conducted to evaluate the effects of mother plant NPK nutrition on seed germination and physiological performance of the second-generation seedlings in wheat.

Materials and methods

Wheat cultivar Minaminokaori was grown in a greenhouse of the Faculty of Applied Biological Sciences, Hiroshima University. Wooden containers were used and filled with a mixture of regosol and nursery soil compost (2:1 v/v). This study comprised a control where no fertilizer was applied (T_1), and two levels of NPK fertilizer: T_2 (110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}), and T_3 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}). The source of NPK was urea, single super phosphate, and potassium chloride, 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. Wheat seeds were sown in the third week of November, then 10-day-old seedlings were transplanted into the containers with 10 cm distance, following a randomized complete block design with 4 replicates. All the recommended agronomic practices were followed for raising the crops during the experiment. Wheat plants were harvested after full maturity, and their seeds were used for germination test, soluble protein assay and phytase activity analysis.

Treatment and growth condition

To determine the effect of mother plant NPK fertilization on growth and physiological performance of the second generation, 200 seeds of 4 replicates were planted on germination wetted papers (top of paper method) and placed in a germinator at 23 ˚C for 7 days. The samples were taken every day, frozen with liquid N and stored under -80 ˚C. The data on phytase activity, phytate content, inorganic P and soluble protein content were recorded daily.

Germination test

Normal seedlings were counted on 7^{th} day of germination and the result was expressed in percent. Seedlings were harvested on 7^{th} day of germination and fresh weight was recorded and expressed as total fresh weight per plant.

Determination of phytase activity and soluble protein

In order to measure phytase activity, fresh samples were ground with liquid nitrogen, transferred to Erlenmeyer flasks, and buffer solution (Na-Phytate + Sodium acetate) was added. Then the samples were shaken for 30 minutes at 37 ˚C. Subsequently, aliquot of the sample was transferred to two sets of plastic tubes and placed in water bath at 37 ˚C.

The Phytase activity was stopped by adding Trichloroacetic acid (TCA) 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 solution (Ammonium Molybdate + Ferrous Sulfate Heptahydrate) were added. The absorbance was measured colorimetrically at 700 nm against control. Soluble protein was assayed using protein reagent G250 (Coomassie brilliant Blue G 250) as per method suggested by Bradford 1976. The absorbance at 595 nm was read on a UV-Spectrophotometer (U-3310, Hitachi Co. Ltd. Tokyo, Japan).

Determination of seedling phytate and inorganic P

Phytate was measured according to the method suggested by Raboy and Dickinson (1984), 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 using ammonium Molybdate reaction reagent. Grain inorganic Phosphorus (Pi) was extracted in TCA (12.5%) + MgCl2 (2 mmol/l) while stirring overnight, and Pi was measured colorimetrically, using a spectrophotometer following the molybdenum reaction regent method (Raboy and Dickinson, 1984).

Statistical analysis

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

Results

The final count of normal seedlings on the 7th day of germination showed that seedlings which were produced by seeds fromT_3 and T_2 treated plants recorded a higher germination percentage compared to T_1 (Fig. 1). The seedling fresh weight was higher in both T_3 and T_2 treated seedlings over the control (T_1) on the 7th day of germination. However, T_3 treatment produced a slightly higher fresh weight but the mean value did not differ from T_2 significantly (Fig. 2).

Germination resulted in a significant increase in the phytase activity of both NPK-fertilized and control (T_1) seedlings. 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 T_3 and T_2 seedlings compared to T_1 (Table 1). Germination enhanced the phytase level by 3.22-fold, 3.38-fold and 4.25-fold in T_3, T_2, and T_1, respectively, on the 6th day of germination compared to 0-day.

The phytate content of the seeds declined during the germination period significantly. The highest phytate content was recorded in the seeds of T_3, followed by T_2 and T_1 treated plants before germination (0-day).

The lowest phytate was observed in T_1, followed by T_2 and T_3 seedlings on the 7th day of germination. At the end of the 7th day from germination, the phytate content was decreased by 2.31-fold, 2.01-fold and 2.43-fold for T_3, T_2, and T_1 compared to 0-day, respectively (Table 1).

Seed germination enhanced the phytase activity and resulted in bioavailability of inorganic P (Pi). There was a liner increase in Pi content with increasein the time of germination (Table 1).

The highest Pi was recorded in T_3 (2.09 mg g^{-1} dry weight), followed by T_2 (2.03 mg g^{-1} dry weight), andT_1 (1.73 mg g^{-1} dry weight) on the 7th day of germination, while the lowest Pi was observed in T_1 before germination (0-day).

Soluble protein content of seeds was increased during the germination period and was maximum on 7th day of germination (Table 1).

Table 1. Effect of mother plant NPK nutrition on phytase activity, phytate, inorganic P, and soluble protein content of wheat seeds during germination. The same letters are not significantly different at the 0.05 probability level.

Days of Germination	NPK levels	Phytase (U g^{-1})	Phytate (mg g^{-1})	Inorganic P (mg g^{-1})	Soluble protein (mg g^{-1})
0	T_1	0.99^{i}	3.14^{bc}	0.25^{m}	26.06^{l}
	T_2	1.31^{i}	3.52^{ab}	0.37^{lm}	26.90^{l}
	T_3	1.45^{h}	3.86^{a}	0.45^{lm}	28.38^{kl}
1	T_1	2.18^{h}	2.56^{cd}	0.36^{lm}	28.91^{kl}
	T_2	$2.47g^{h}$	2.80^{cd}	0.44^{lm}	37.14^{ijk}
	T_3	2.83^{fg}	3.12^{bc}	$0.48k^{lm}$	40.62^{ij}
2	T_1	2.85^{fg}	2.31^{defg}	0.56^{kl}	33.24^{jkl}
	T_2	3.09^{efg}	2.54^{def}	0.65^{jkl}	43.07^{jk}
	T_3	3.52^{de}	2.79^{cd}	0.73^{ijk}	45.17^{i}
3	T_1	3.39^{def}	2.15^{efgh}	$0.86h^{ij}$	59.01^{h}
	T_2	3.74^{bcd}	2.36^{defg}	$0.96g^{hi}$	67.37^{gh}
	T_3	4.02^{abc}	2.58^{cd}	$1.03f^{gh}$	68.11^{gh}
4	T_1	3.70^{cde}	1.91^{ghij}	1.14^{e}	71.02^{g}
	T_2	4.01^{abc}	2.08^{defg}	1.25^{ef}	84.01^{f}
	T_3	4.31^{abc}	2.32^{defg}	1.35^{de}	85.99^{ef}
5	T_1	4.00^{abc}	1.78^{hij}	1.40^{cde}	83.69^{f}
	T_2	4.41^{ab}	1.92^{ghij}	1.59^{bcd}	94.55^{de}
	T_3	4.61^{a}	2.13^{efgh}	1.67^{bc}	99.29^{cd}
6	T_1	4.22^{abc}	1.62^{ijk}	1.65^{bc}	97.25^{d}
	T_2	4.43^{a}	1.77^{hij}	1.83^{ab}	120.36^{b}
	T_3	4.66^{a}	1.98^{fghi}	1.88^{ab}	128.11^{b}
7	T_1	3.52^{de}	1.29^{k}	1.73^{b}	107.75^{c}
	T_2	3.98^{abc}	1.50^{jk}	2.03^{a}	147.41^{a}
	T_3	3.99^{abc}	1.67^{ij}	2.09^{a}	150.82^{a}

A significant difference was observed between the treatments from 4th day until the end of germination period (7th day). T_3 treatment resulted ina higher soluble protein content (40.0%) compared to T_1 (control) on 7th day of germination.

Discussion

Mother 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_3 and T_2 seeds. Seeds which obtained more fertilizer and irrigation during the production stage can increase seedling establishment in comparison with other treatments (Hampton, 1992). Similarly, Bittman (1989) found that a difference in 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 a 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).

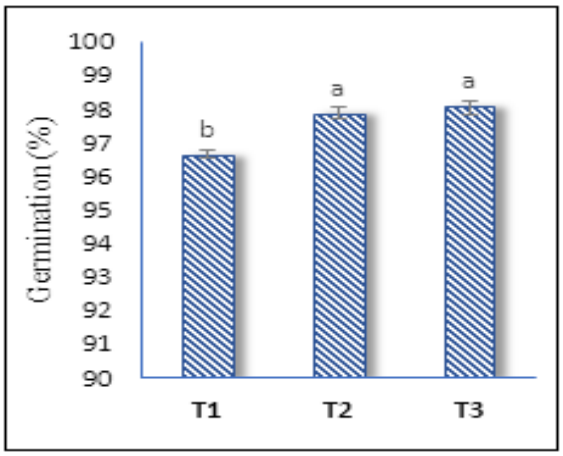

Fig. 1. Effect of mother plant NPK nutrition on seed germination percentage of wheatcounted at 7 days from germination. The same letters are not significantly different at the 0.05 probability level.

In this study, NPK fertilization of the mother plant improved grain food reserves and helped with a better growth of seedlings and contributed to a high seedling fresh weight compared to the control. Phytase activity reached a maximum level on the 6th day of

germination, as a result T_3 and T_2 recorded higher values of phytase. Ma and Shan (2002) reported that seed germination significantly increased phytase activity by 2.04-fold on 3rd the day of germination in wheat.

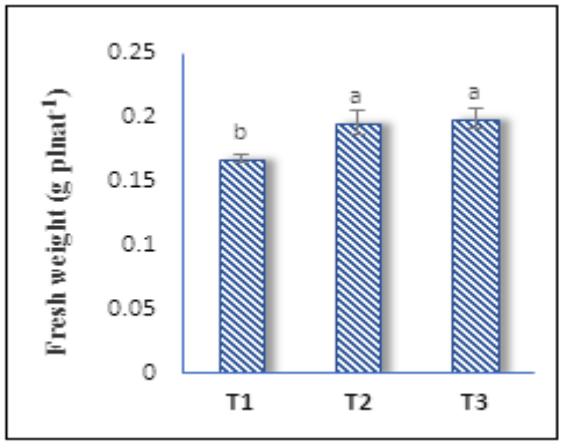

Fig. 2. Effect of mother plant NPK nutrition on the seedling fresh weight (g plant^{-1}) of wheat at 7 days from germination. The same letters are not significantly different at the 0.05 probability level.

The effect of NPK fertilization on the phytase activity of germinating seeds has not been much studied before, and the high phytase level of T_3 and T_2 during the germination period might be due to a high phosphorus and protein content in the mother plant grains compared to the control (T_1). 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 phytate content; as phytase activity increased the phytate content decreased.

The effect of NPK fertilization on the seed phytate content during germination has not been studied so far. Phytate is degraded by the phytase enzyme during the seed germination of cereals, (Kumar *et al.*, 2010). The same trend in the reduction of phytate content in germinating seeds of cereals has already been reported by other researchers (Azeke *et al.*, 2010; Sokrab *et al.*, 2012).

Inorganic phosphorus was increased during the germination period and it was at maximum on the 7th day of germination.

The influence of NPK fertilization on Pi concentration during seed germination has not been reported before. 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.*, 2010). There was a liner increase in soluble protein content with increase in germination period, and mother plant NPK nutrition significantly enhanced the level of soluble protein in T_3 and T_2 treated seeds. Application of NPK to the mother plants resulted in a higher protein content in seeds, and consequently increased soluble protein in germinating seeds which may help the plants to germinate and grow vigorously compared to T_1treatment where no fertilizer was applied.

Conclusion

In general, it can be concluded that NPK fertilization of the mother plant enhances seed germination, seedling growth, and improves the physiological performance of the germinating seeds compared to the control. Soluble protein content, phytase activity, phytate degradation, and the release of Pi during seed germination was highly influenced by mother plant NPK fertilization that consequently may improve seed viability, vigor, and seedling establishment.

References

Azeke MA, Samuel Jacob E, Mary Ugunushe E, Godwin Ihimire I. 2010. Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). Journal of Food Science and Technology **48,** 724–729. http://dx.doi.org/10.1007/s13197-010-0186-y

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-biniding. Annual of Biochemistry **72,** 248-254.

Barker AV, Pilbeam DJ. 2007. Handbook of Plant Nutrition. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Bittman S, Simpsan GM. 1989. Drought effect on water relation of tree cultivated grasses. Crop Science **29,** 992-999.

Brady NC, Weil RR. 2008. The Nature and Properties of Soils. Revised 14th Ed. Pearson Prentice Hall. New Jersey.

Bungard RAA, Wingler, Morton JD, Andrews M. 1999. Ammonium can stimulate nitrate and nitrite reductase in the absence of nitrate in *Clematis vitalba*. Plant Cell Environment **22,** 859–866. http://dx.doi.org/10.1046/j.1365-3040.1999.00456.x

Debnath D, Sahu NP, Pal AK, Baruah K, Yengkokpam S, Mukherjee SC. 2005. Present scenario and future prospects of phytaseinaqua feed. Asian-Australian Journal of Animal Science **18,** 1800–1812.

Doddagoudar SRBS, Vyakaranahal, Shekhargouda M. 2004. Effect of mother plant nutrition and chemical spray on seed germination and seedling vigour of China Aster Cv. Kamini. Karnataka Journal of Agricultural Science **17,** 701-704.

Hampton JG. 1992. Report of the Vigor Test Committee, 1983-1986. Seed Science and Technology**15,** 507-522.

Havlin JL, Beaton JD, Tisdale SLWL. 2005. Nelson Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 7th (Ed.). Pearson Prentice Hall. New Jersey.

Houde RL, Alli I, Kermasha S. 1990. Purification and characterization of canola seed phytase. Journal of Food Biochemistry **14,** 331–351. http://dx.doi.org/10.1111/j.17454514.1990.tb00846.x

Jifon JL, Lester GE. 2009. Foliar potassium fertilization improves fruit quality of field grown muskmelon on calcareous soils in South Texas. Journal of the Science of Food and Agriculture **89,** 2452-2460. http://dx.doi.org/10.1002/jsfa.3745

Karl A, Wyant, Jessica R, Corman, James Elser J. 2013. Phosphorus, Food and Our Future. Oxford University press. http://dx.doi.org/10.1093/acprof:osobl/9780199916832.001.0001

Kumar V, Sinha AK, Makkar HPS, Becker K. 2010. Dietary roles of phytate and phytase in human nutrition: a review. Food Chemistry **120,** 945–959. https://doi.org/10.1016/j.foodchem.2009.11.052

Lester GE, Jifon JL, Makus DJ. 2010. Impact of potassium nutrition on postharvest fruit quality: melon (*Cucumis melo* L.) Case study. Plant Soil **335,** 117-131. https://doi.org/10.1007/s11104-009-0227-3

Ma X, Shan A. 2002. Effect of germination and heating on phytase activity in cereal seeds. Asian-Australian Journal of Animal Science **15,** 1036-1039. https://doi.org/10.5713/ajas.2002.1036

Shimizu MM, Mazzafera P. 2000. Compositional changes of proteins and amino acids in germinating coffee seeds. Brazilian Archives of Biology and Technology **43,** 259-265. www.dx.doi.org/10.1590/S151689132000000300003

Sokrab AM, Isam A, Mohamed A, Elfadil Babiker E. 2012. Effect of germination on antinutritional factors, total, and extractable minerals of high and low phytate corn (*Zea mays* L.). Journal of Saudi Society of Agricultural Science **11,** 123–128. https://doi.org/10.1016/j.jssas.2012.02.002

Sung HG, Shin HT, Ha JK, Lai HL, Cheng KJ, Lee J. 2005. Effect of germination temperature on characteristics of phytase production from barley. Bioresource Technology **96,** 1297–1303. https://doi.org/10.1016/j.biortech.2004.10.010

Sylvia DM,Furhmann JJ, Hartel PG, Zuberer DA. 2005. Principles and Application of Soil Microbiology. 2nd (Ed.). Pearson Prentice Hall. New Jersey.