

Nitrogen and Phosphorus Content, Hectoliter Weight and Yield Variations of Wheat Grain as Affected by Cropping Intensity

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Summary

Public concerns over the potential environmental hazards of intensive agriculture have renewed an interest in the low-input nitrogen (N) fertilization practices for wheat (*Triticum aestivum* L.). A two-year study (2001 and 2002) was conducted to determine the influence of cropping intensity, namely the low-N (67 kg N ha⁻¹) and high-N (194 kg N ha⁻¹) input levels on the grain N and phosphorus (P) content, phytate-P (phytic acid and its salts), hectoliter weight and grain yield of three bread wheat cultivars widely grown in Croatia. Growing conditions significantly affected grain yields averaging 6641 kg ha⁻¹ in the first growing season and 8295 kg ha⁻¹ in the following year. Despite an associated increase in the 1000-grain weights, the use of the low-N fertilization brought about a significant decrease in grain yield, hectoliter weight and grain N content in all cultivars by an average of 18.1%, 1.5% and 22.6%, respectively, when compared to the high-N fertilization level. The reductions in grain N content were consistent in both years regardless of variations in grain yields among three tested cultivars. Grain N content under the low-N fertilization averaged 17.1 g kg⁻¹ only, which may limit its use for breadmaking. In contrast, grain P content was not affected by N fertilization or growing season and averaged 4.70 g kg⁻¹ across all treatments. Absolutely small, but significant differences existed among cultivars for total grain P content, of which about 80% was in the form of phytate-P. A negative correlation between the 1000-grain weight and P content was found because of tendency toward lower P content in heavier grains. When compared to the high-N input, the low-N fertilization practices for wheat crop were associated with a significant decrease in grain yield and grain N content in all cultivars, but had no effect on grain P content regardless of cultivar.

Key words

wheat, quality, cultivar, nitrogen, phosphorus, cropping intensity

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Introduction

In Croatia, wheat producers rely on excessive N fertilization to achieve high grain yields. However, in recent years there has been increased concern about the detrimental effect of intensive agricultural practices on environmental quality, which has renewed an interest in the low-input production practices. Fertilization effects of N on wheat protein and yield have been well documented. Many authors (e.g. Szentpetery et al., 2005) demonstrated that intensified wheat fertilization with N resulted in better milling and baking quality through increased hectoliter weight, grain protein content and bread volume. Increasing N availability generally increases yield more than protein until a yield maximum is reached, whereupon protein levels increase if N is increased further (Varga, 1980; Varga and Svečnjak, 2006).

Among the complex factors affecting wheat grain nutritional quality, the N and P contents are highly important. The mineral contents of wheat grains may be affected by a number of factors including soil, climate and cultural practices. Miller (1958) has indicated that the amount of protein in hard red spring wheat may range from 9.6 to 21.8%. In like manner, the grain contents of P in wheat cultivars have been also shown to vary as much as 233% (Erdal et al., 2002). Limited information is available on grain P content of modern bread wheat cultivars in Croatia.

A comparison of grain from organic and conventional wheat production in South Australia found higher P in conventional grain, along with higher grain phytic acid concentrations (Ryan et al., 2004). Phytic acid is a compound found in most cereal seeds and it is present in wheat as a mixed insoluble salt of Mg, Ca and K, with phytate-P constituting 49-80% of the total P (Bassiri and Nahapetian, 1977). Phytic acid is a strong chelator of mineral nutrient elements (Raboy, 2001). The complex of phytic acid and mineral elements, in the form of phytate, leads to a marked reduction in bioavailability of these nutrient elements, and thus a consequent public health problem of iron and zinc deficiency for the populations whose diets are mainly cereals and legumes (Raboy, 2001).

Our objective was to evaluate the grain N and P content, hectoliter weight and yield variations of wheat breadmaking cultivars as affected by cropping intensity.

Materials and methods

Field experiment

A field experiment on a winter wheat-corn (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] crop rotation was conducted in northwestern Croatia at the Faculty of Agriculture Zagreb experimental field during the 2000-2001 (2001) and 2001-2002 (2002) growing seasons on a silt loam soil (Typic Udifluvents). Three widely grown winter

wheat cultivars ('Marija', 'Žitarka' and 'Renan') in Croatia were planted under the both low-N and high-N fertilization levels. Previous crops (corn and soybean) were also grown at these two N fertilization input levels. The experimental design for each production system consisted of five replications with two factors arranged in a randomized complete block in a split-plot design. Application of N fertilization (high-N and low-N inputs) was the whole-plot factor while cultivars were the sub-plot factor. The high-N input involved fertilization with 194 kg N ha⁻¹ including three top-dressing applications (54, 27, and 27 kg N ha⁻¹ at growth stages 22, 24, and 31, respectively) (Zadoks et al., 1974). The low-N input consisted of 67 kg N ha⁻¹ including one top-dressing application with 27 kg N ha⁻¹ at growth stage 24.

At seeding, plots consisted of 10 rows 11 cm apart and 8.0 m in length. Weeds and diseases were controlled throughout vegetation. The grain was harvested at maturity using a small plot combine, and grain samples were dried in a forced air drier at 40°C. Hectoliter weight was determined from two grain samples taken at harvest from each plot using standard procedures (Schopper chondrometer). Average 1000-grain weight was determined by counting and weighing two 100-grain samples. Grain yields and 1000-grain weights are expressed as a kilogram per hectare basis at a 130 g kg⁻¹ moisture basis. Grain samples for nutrient analysis were ground in a coffee mill and stored in plastic bags until analyses.

Laboratory analyses

Grain N was measured by Kjeldhal analysis (ICC 105/2, International Association for Cereal Chemistry, 1994). Grain P was analyzed by the AOAC method 931.01 (AOAC International, 2000). Phytate-P (phytic acid and its salts) was determined on a composite sample from all replications. The method used for phytate-P determinations was described by Haug and Lantzsch (1983). Ten milliliters of 0.2 M HCl were added to 100 mg of sample, and the mixture was shaken for 3 h at room temperature and then filtered. Then, 0.5 mL of distilled water and 2 mL of ferric solution were added to 0.5 mL filtrate and the mixture was boiled for 30 min. The resulting solution was centrifuged at 2400 x g, and 1.5 mL of 2,2'-bipyridine solution was added to a 1-mL aliquot of the supernatant. The absorbance of the mixture was read against distilled water at 519 nm using a Pharmacia Ultrospec 2000 spectrophotometer.

Statistical analysis

Data were analyzed using the PROC MIXED procedure (SAS Inst., 1997). Analysis of variance was computed with N fertilization and cultivar considered fixed. Means separation was calculated using the LSD values if the F-test was significant at $P=0.05$. Direct relationships

among grain and flour quality traits were analyzed with simple Pearson correlation coefficients.

Results and discussion

Growing season significantly affected grain yields (Table 1), which averaged 6641 kg ha⁻¹ in the growing season of 2001 and 8295 kg ha⁻¹ in the following year (Table 2). These significantly lower grain yields produced in 2001 were partly associated with a 5.0% decrease in 1000-grain weights (40.0 g) compared with those in 2002 (42.0 g). The low-N fertilization rate significantly decreased grain yields, which averaged 6725 kg ha⁻¹ compared with 8211 kg ha⁻¹ at high-N rate. In contrast, the 1000-grain weight showed an opposite pattern of response because it was significantly lighter at the high-N (38.6 g) than at the low-N fertilization rate (43.5 g). In Slovenia, Bavec et al. (2002) reported similar results, whereas N fertilization had no effect on 1000-grain weight in a research by Simon et al. (2002) and Olesen et al. (2003). The absence of year × N rate interaction for grain yield (Table 1) indicated that yield reductions associated with the low-N rate were consistent in both growing seasons despite relatively large variations in grain yields across two experimental years (Table 2). Averaged over all treatments, cultivars significantly differed in grain yield responses (Table 1). The largest average grain yield produced cultivar Marija (7715 kg ha⁻¹), which surpassed 'Žitarka' (7446 kg ha⁻¹) and 'Renan' (7243 kg ha⁻¹). Cultivar-specific responses for grain yields were not affected by N fertilization inputs (Table 1). Moreover, these differences in grain yields among tested cultivars were evident in both growing seasons as indicated by an absence of year × cultivar interaction (Table 1).

One of the oldest and most frequently used criteria for the evaluation of milling quality for bread wheat is hectoliter weight since it may indicate potential flour yield (Finney et al., 1957). Hectoliter weigh was significantly affected by growing conditions in our research (Table 1). Contrary to grain yield responses, hectoliter weights were significantly smaller in the higher-yielding growing season of 2002 (78.7 kg hl⁻¹ on average) when compared to those in the previous growing season (81.1 kg hl⁻¹). Hectoliter weight was also affected by N fertilization inputs (Table 1). Although absolutely and relatively small, significantly higher hectoliter weight was found at the high-N input, which averaged 80.3 kg hl⁻¹ compared to 79.6 kg hl⁻¹ at the low-N rate. These results are in full contrast with those for the 1000-grain weights (Table 2), demonstrating that the hectoliter weight may vary depending not only on grain weight but also on grain shape and texture, as also shown by Pushman and Bingham (1976). However, a significant year × N rate interaction existed (Table 1) because improved hectoliter weights following the high-N input existed in the dryer than average growing season of 2001 only (Table

Table 1. Analysis of variance for grain yield and grain quality traits

Source of variation	df	Grain yield	Hectoliter weight	1000-grain weight	Grain N content	Grain P content
Year (Y)	1	***	***	*	***	NS
R / Y	8	—	—	—	—	—
N rate (N)	1	***	**	***	***	NS
Y × N	2	NS	**	NS	*	NS
Error a	8	—	—	—	—	—
Cultivar (C)	2	*	***	***	***	*
Y × C	2	NS	*	**	***	NS
N × C	2	NS	NS	NS	NS	NS
Y × N × C	2	NS	NS	NS	NS	NS
Error b	32	—	—	—	—	—

*, **, ***, NS: Significant at $P = 0.05, 0.01,$ and 0.001 levels, and not significant, respectively.

Table 2. Average grain yields and grain quality traits of bread wheat cultivars grown at low-N and high-N fertilization rates. Zagreb, 2001–2002

Year	N rate	Grain yield (kg ha ⁻¹)	Hectolite r weight (kg hl ⁻¹)	1000-grain weight (g)	Grain N content (g kg ⁻¹)	Grain P content (g kg ⁻¹)
2001	Low	5796	80.5	42.6	16.6	4.66
	High	7486	81.8	37.4	21.0	4.81
2002	Low	7655	78.7	44.7	17.6	4.61
	High	8935	78.7	39.8	23.2	4.71
LSD (0.05)†		NS‡	0.63	NS	0.7	NS
LSD (0.05)§			0.73		0.8	

† LSD values for comparing means within the same growing season; ‡ Not significant for N rate × growing season interaction at $P = 0.05$; § LSD values for comparing means across N rates and growing seasons.

2). In the higher-yielding growing season of 2002, hectoliter weights were not affected by N fertilization levels and averaged 78.7 kg hl⁻¹. Cultivars significantly differed for hectoliter weight responses. The highest hectoliter weights had cultivar 'Žitarka' (80.9 kg hl⁻¹ on average) followed by 'Renan' (79.7 kg hl⁻¹). The smallest hectoliter weights were found for the highest yielding cultivar 'Marija', which averaged 79.1 kg hl⁻¹. Consequently, a negative correlation existed between grain yields and hectoliter weights in our research (Table 3). Cultivar specific responses for hectoliter weights were not affected by growing season or N fertilization, as indicated by the absence of cultivar × year and cultivar × N rate interactions (Table 1). Grain N content was inversely related to hectoliter weight (Table 3), as also reported by Syltie and Dahnke (1983).

Previous findings, reviewed by Evans et al. (1975), suggests that there may be differences between cultivars in the relations between N and P contents of wheat grains as they mature, and that these appear to be influenced by

Table 3. Simple correlation coefficients among the grain yield and grain quality traits of bread wheat cultivars (n = 60)

	GY	HW	TGW	N	P
Grain yield (GY)	–				
Hectoliter weight (HW)	–0.34*	–			
1000-grain weight (TGW)	0.16	–0.24	–		
Grain N content (N)	0.67*	–0.16	–0.23	–	
Grain P content (P)	–0.03	0.06	–0.47*	0.18	–

* Significant at $P = 0.05$.**Table 4.** Grain N and P content of bread wheat cultivars across two growing seasons, Zagreb, 2001–2002

Cultivar	Grain N (g kg ⁻¹)		Grain P (g kg ⁻¹)	
	2001	2002	2001	2002
Marija	19.7	20.4	4.89 (4.01)†	4.87 (4.03)
Žitarka	17.8	19.5	4.71 (3.75)	4.68 (3.82)
Renan	18.8	21.4	4.61 (3.64)	4.43 (3.79)
LSD (0.05)‡	0.07		NS§	
LSD (0.05) ¶	0.06			

† Numbers in parentheses show phytate-P content; ‡LSD values for comparing means within the same growing season; § Not significant for cultivar × year interaction at $P = 0.05$; ¶ LSD values for comparing means across N rates and growing seasons.

growing conditions. One objective of our research was to analyze the effects of growing season, as well as of cultivar, on the N and P content in whole grains. Thus, tested cultivars were chosen on the basis of various 1000-grain weights. The heaviest grains in our research had 'Renan' (47.0 g on average), which exceeded 'Žitarka' (38.9 g) and 'Marija' (37.3 g). Growing season significantly affected grain N content (Table 1) because of higher grain N in the higher-yielding growing season of 2002 when compared to that in 2001 (Table 2). Thus, a strong positive correlation existed between grain N and grain yields ($r = 0.67^*$, Table 3). As expected, the low-N input brought about a relatively large decrease in grain N content, which was on average 22.6% lower than that at the high-N fertilization (Table 2). These responses for grain N were consistent in both growing seasons, as indicated by an absence of year × N rate interaction (Table 1). The highest average grain N content had cultivars 'Renan' (20.1 g kg⁻¹) and 'Marija' (20.0 g kg⁻¹), which were significantly larger than that for 'Žitarka' (18.7 g kg⁻¹). However, cultivar responses for grain N were affected by growing conditions (Table 1). This was mainly because 'Marija' and 'Renan' showed similar grain N content in the dry growing seasons of 2001, whereas 'Renan' exceeded 'Marija' in the following year (Table 4).

In contrast to grain N responses, growing season and N fertilization rate showed no important effect on grain P content (Table 1), which averaged 4.70 g kg⁻¹ across all treatments. Consequently, the amount of P removed in the

grain (grain yield × grain P content), on a cropping intensity basis, followed a pattern identical to that of grain production and varied between 27 kg P ha⁻¹ at the low-N rate in 2001 and 42 kg P ha⁻¹ at the high N rate in 2002. Despite the large variations in grain N content across years and N fertilization rates (Table 4), an absence of year × N rate interaction (Table 1) indicated that grain P content is rather constant in wheat crop produced under contrasting growing and cultural conditions. Similar results were reported by Zebarth et al. (1992). On the contrary, grain P content was significantly reduced when wheat cultivars were grown under dryland conditions when compared to irrigated wheat in a research by Bassiri and Nahapetian (1977). Peterson et al. (1983) reported close and positive correlations between grain N and P concentrations, whereas in our research we found a small positive correlation between grain N and P (Table 3). Absolutely small, but significant differences in grain P content existed among tested cultivars (Table 1), as also reported by Bassiri and Nahapetian (1977). 'Žitarka' and 'Marija', two cultivars characterized by smaller grain size, had larger grain P content than 'Renan' (Table 4), a cultivar with large grain size. This resulted in a negative correlation ($r = -0.47^*$) between grain P and 1000-grain weight. Cultivar effects for grain P content were uniform across both years and fertilization rates (Table 1) and despite grain N and yield variations among themselves (Table 4).

Phytate-P averaged 3.84 g kg⁻¹, which was 82% of total average grain P content. Similar results were reported by Syltie and Dahnke (1983) and Zebarth et al. (1992), whereas Liu et al. (2006) found larger values ranging from 5.16–9.87 g kg⁻¹. O'Dell et al. (1972) reported that a major part of the phytate in wheat grain is found in the aleurone layer. During the milling process, most of the aleurone cells remain with particles of pericarp; hence phytate becomes concentrated in the bran fractions. Therefore, whole wheat may contain about 0.3–0.4% phytate and the bran may contain up to 5% (O'Dell et al., 1972). In our research, tested cultivars had similar proportion of phytate-P in total grain P content (Table 4). However, Bassiri and Nahapetian (1977) reported that the ranges for the proportion of phytate in total grain P may vary 38–77% for cultivars grown under dryland conditions and 73–94% for those grown under irrigation.

Conclusions

Despite an associated increase in grain weights, the use of the low-N fertilization rate resulted in a relatively large decrease in grain yield (18.1%) as well as grain N (22.6%) content in all cultivars which may limit their baking quality. In contrast, grain P content was not affected by N fertilization or growing conditions. Absolutely small, but significant differences existed among cultivars

for total grain P content, of which about 80% was in the form of phytate.

References

- AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Gaithersburg, MD.
- Bassiri, A., A. Nahapetian. 1977. Differences in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, zinc, and iron in wheat varieties grown under dry land and irrigated conditions. *J. Agric. Food Chem.* 25:1118–1122.
- Bavec, M., F. Bavec, B. Varga, V. Kovačević. 2002. Relationships among yield, its quality and yield components, in winter wheat (*Triticum aestivum* L.) cultivars affected by seeding rates. *Bodenkultur* 53:143–151.
- Erdal, I., A. Yilmaz, S. Taban, S. Eker, B. Torun, I. Cakmak. 2002. Phytic acid and phosphorus concentrations in seeds of wheat cultivars grown with and without zinc fertilization. *J. Plant Nutr.* 25:113–127.
- Evans, L.T., I.F. Wardlaw, R.A. Fischer. 1975. Wheat. In *Crop Physiology*, L.T. Evans (Ed.), pp. 101-149, Cambridge University Press.
- Finney, K.F., J.W. Meyer, F.W. Smith, H.C. Fryer. 1957. Effect of foliar spraying on Pawnee wheat with urea solutions on yield, protein content and protein quality. *Agron. J.* 49:341–347.
- Haug, W., H.J. Lantzsch. 1983. Sensitive method for the rapid determination of phytate in cereal and cereal products. *J. Sci. Food Agric.* 34:1423–1427.
- International Association for Cereal Chemistry. 1994. Standard methods of the ICC. Verlag Moritz Schafer, Detmold, Germany.
- Liu, Z.H., H.Y. Wang, X.E. Wang, G.P. Zhang, P.D. Chen, D.J. Liu. 2006. Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (*Triticum aestivum* L.). *J. Cereal Sci.* 44:212–219.
- Miller, D.F. 1958. Composition of cereal grains and forages. National Academy of Sciences, National Research Council, Washington, DC. Publ. 585.
- O'Dell, B.L., A.R. de Boland, S.R. Koirtyohann. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. *J. Agric. Food Chem.* 20:718–721.
- Olesen, J.E., L.N. Jorgensen, J. Petersen, J.V. Mortensen. 2003. Effects of rate and timing of nitrogen fertilizer on disease control by fungicides in winter wheat. I. Grain yield and foliar disease control. *J. Agric. Sci.* 140, 1–13.
- Peterson, C.J., V.A. Johnson, P.J. Mattern. 1983. Evaluation of variation in mineral element concentration in wheat flour and bran of different cultivars. *Cereal Chem.* 60:450–455.
- Pushman, F.M., J. Bingham. 1976. The effects of a granular nitrogen fertilizer and a foliar spray of urea on the yield and breadmaking quality of ten winter wheats. *J. Agric. Sci.* 87:281–292.
- Raboy, V. 2001. Seeds for a better future: 'Low phytate' grains help to overcome malnutrition and reduce pollution. *Trends in Plant Science* 6:458–462.
- Ryan, M.H., J.W. Derrick, P.R. Dann. 2004. Grain mineral concentrations and yield of wheat grown under organic and conventional management. *J. Sci. Food & Agric.*, 84:207–216.
- SAS Institute. 1997. SAS/STAT software: Changes and enhancements through release 6.12. SAS Inst., Cary, NC.
- Simon, M.R., A.E. Perello, C.A. Cordo, P.C. Struik. 2002. Influence of *Septoria tritici* on yield, yield components, and test weight of wheat under two nitrogen fertilization conditions. *Crop Sci.* 42:1974–1981.
- Syltje, P.W., W.C. Dahnke. 1983. Mineral and protein content, test weight, and yield variations of hard red spring wheat as influenced by fertilization and cultivar. *Qual. Plant.* 32:37–49.
- Szentpetery, Z., Jolankai M., Kleinheincs C., Szollosi G. 2005. Effect of nitrogen top-dressing on winter wheat. *Cereal Res. Commun.* 33:619–626.
- Varga, B., 1980. Investigation of wheat varieties reaction upon quantity, time of application, and form of N-fertilisers in various agroecological conditions. (In Croatian, with English abstract). *Poljopr. Znan. Smotra* 52, 285–297.
- Varga, B., Z. Svečnjak. 2006. The effect of late-season urea spraying on grain yield and quality of winter wheat cultivars under low and high basal nitrogen fertilization. *Field Crops Res.* 96:125–132.
- Zadoks, J.C., T.T. Chang, C.F. Konzak. 1974. A decimal code for growth stages of cereals. *Weed Res.* 14:415–421.
- Zebarth, B.J., C.J. Warren, R.W. Sheard. 1992. Influence of the rate of nitrogen fertilization on the mineral content of winter wheat in Ontario. *J. Agric. Food Chem.* 40:1528–1530.

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