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Phosphorus requirement of barley and wheat for seed and food quality

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Abstract: While geological phosphate reserves are continually depleting and effective phosphorus (P) recycling methods are still being developed, more efficient fertilisation of P can help to avoid unnecessary losses of this nutrient. In this way, environmental damage resulting from excessive P inputs to agricultural soils could also be reduced. The aim of this study is to identify a P concentration which is optimal for high seed quality of one cultivar of spring wheat and spring barley in addition to possessing a high nutritional value. While a critical threshold at 1.65 mg P/g grain for the studied barley cultivar has been identified, above which germination capacity and power were close to 100%, no such concentration was applicable to wheat. This concentration is well below the widely accepted doctrine, which calls for a P concentration of 2 mg/kg and could thus lead to a lower and thus more efficient use of the nutrient. Furthermore, in this study, an estimation of food quality by the molar ratio of phytate of the two micronutrients iron and zinc illustrates that such a concentration could only be found for zinc but not for iron.

Keywords: cereals; macronutrient; bioavailability; phytic acid; nutritional quality

Phosphorus (P) fertilisation is a key factor in securing high and constant yields in agricultural production. While the extraction of rock phosphates from geological deposits is limited, high P surpluses on agricultural land cause ecological problems (Stamm et al. 2021). Although recent publications have drawn a less dramatic estimation than those of the past (Scholz and Wellmer 2013), even the most optimistic claims extend the availability of geological reserves by a few centuries at the most. Even though recent developments in P-recovery techniques point towards decreasing dependence on geological reserves (Stamm et al. 2021), at this point in time, a reduction of application rates would appear to be the appropriate remedy. In addition, high P concentrations in foods can subsequently affect the bioavailability of micronutrients.

Furthermore, crops only poorly utilise P fertilisation. When using a different method to assess P use efficiency (PUE) across all cereals, only 17% of fertilised P was metabolised by the plants in the year of application (Dhillon et al. 2017).

Therefore, the aim of future agriculture should not only involve using P sparingly but also efficiently, e.g. *via* the use of efficient crops or by precision farming techniques (Srivastava et al. 2021), and thereby sustainably.

In cereals, P is stored primarily as phytate, the salt of phytic acid, complexing micronutrients, e.g. iron and zinc, which are essential for animal metabolism, rendering them useless for monogastric such as humans or pigs (Kumar et al. 2010).

Apart from causing physiologic deficiencies, this leads to increased P inputs in agricultural systems, as fed P passes through the animal without being internalised. It was shown that a reduction of the phytate content of fodder from 0.18% to 0.04% while maintaining P concentrations could lead to a decline in excreted P of approximately 75% (Veum and Raboy 2016).

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Consequently, we set out to assess whether a decreased phytate content of cereals could also be achieved by agricultural methods, especially *via* reduced P fertilisation.

We subsequently formulated the following hypotheses, which state that for spring barley as well as for spring wheat, critical P-concentrations in the kernel can be found for seed quality as well as for nutritional quality.

I. There is a critical P-concentration in the kernel for the seed and nutritional quality of barley;
II. There is a critical P-concentration in the kernel for the seed and nutritional quality of wheat.

MATERIAL AND METHODS

In order to generate the analysed seeds, 25 kernels of the spring barley (*Hordeum vulgare* L. cv. Propino) and the spring wheat (*Triticum aestivum* L. cv. Thasos) were sown in small Mitscherlich pots containing 6 kg of a mixture of 50% of a P-deficient subsoil and 50% quartz sand. After germination, the plants were thinned to 20 plants per pot. The experimental soil was a subsoil (0.6–1.2 m depth) from a Luvisol derived from loess (20.4% clay, 34.7% silt, 44.9% sand, 0.20% total carbon, 0.04% total N, pH 6.4 in 0.01 mol $CaCl_2$ and 6.3 mg CAL-extractable P/kg soil). Using $Ca(H_2PO_4)$ concentrations of 10 mg P/kg, 20 mg P/kg, 40 mg P/kg, 80 mg P/kg and 160 mg P/kg soil were established, resulting in doses of 60 mg P, 120 mg P, 240 mg P, 480 mg P and 960 mg P. For both crops, each fertiliser level was repeated three times, resulting in a total of 30 pots. Each pot was fertilised with 1.83 g K in the form of KCl and K_2SO_4, 0.378 g S in the form of K_2SO_4 and 0.84 g N in the form of NH_4NO_3. For micronutrient fertilisation, 2.76 mg B in the form of H_3BO_3, 0.66 mg Mo in the form of $(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$, 120 mg Mn in the form of $MnSO_4 \cdot H_2O$, 60 mg Zn in the form of $ZnSO_4 \cdot 7\,H_2O$, and 30 mg Cu in the form of $CuSO_4 \cdot 5\,H_2O$ were added. After 30 days, 0.5 g NH_4NO_3 were added to each pot. Irrigation was carried out manually based on the current water demand.

The plants grew in climate chambers with 16 h light at 250 µE/s/m^2 and 20 °C and 8 h night at 16 °C and were harvested upon maturity by separating straw and ears. The ears were counted and threshed, straw and husk were dried at 105 °C for dry matter yield determination. A part of the kernels was also dried at 105 °C for dry matter, 1 000 kernel weight and phytate and total P determinations. Total P concentrations and contents were assessed *via* the molybdate-vanadate method in the fine ground grain and straw samples.

For phytate analysis, the enzyme assay K-PHYT by Megazyme (Bray Business Park, Bray, Ireland) was employed. Phytate was extracted from flour using hydrochloric acid in the process. The resulting solution was neutralised and centrifuged. The supernatant was then separated into two portions, one to assess free P-concentration in the sample and the other to be degraded by a phytase and an alkaline phosphatase. When comparing the extinctions of the solutions coloured with molybdenum blue, the difference marked the P formerly bound in phytate.

As a micronutrient example, Fe and Zn concentrations were analysed *via* atom-absorption-spectrometry after wet ashing in concentrated nitrous acid as a modification of the methods described in VDLUFA III 11.1.2 and 11.5.2 (1983).

Germination capacity and velocity of barley and wheat seeds were measured *via* a modified method recommended by Eggenbrecht (1949). In three biological replications, 50 kernels of each treatment were placed in quartz sand moisturised to 80% of the maximal water holding capacity and incubated without light for 10 days at 20 °C. After a period of three days, germinated kernels were counted for germination velocity and after 10 days for germination capacity.

To gain further insight into the performance of the seeds, germination power was measured by a modification of the Hiltner method (Eggenbrecht 1949), where 50 kernels of each treatment were placed in a bed of brick granulate and covered with a 4 cm layer

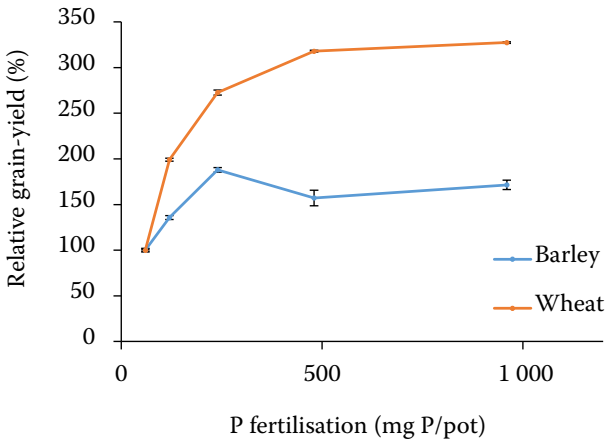

Figure 1. Relative grain yield growth as related to phosphorus fertilisation. The yield of the lowest fertiliser level was set as 100%. Values are means of three biological replications ± standard error

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Table 1. Yield parameters of the investigated cereals. Only grain- and straw-yield showed significant effects of phosphorus application, while all other parameters were effected in no significant way

	Phosphorus application (mg P/kg soil)	Grain yield	Straw yield	Ears per pot	Kernels per ear	Weight per thousand kernels (g)
		(g/pot)		(Qty)		
Hordeum vulgare L.	10	32.84 ± 1.03	44.42 ± 2.66	44.67 ± 4.51	15.51 ± 0.24	47.64 ± 2.16
	20	44.60 ± 2.08	53.76 ± 2.64	56.00 ± 5.00	15.49 ± 1.00	51.74 ± 1.83
	40	61.74 ± 2.46	56.31 ± 0.50	69.33 ± 3.21	16.32 ± 0.36	54.70 ± 2.97
	80	51.62 ± 8.60	54.32 ± 3.21	63.67 ± 9.81	15.08 ± 0.32	53.00 ± 3.43
	160	56.34 ± 5.15	53.62 ± 3.48	62.67 ± 14.15	15.69 ± 0.66	57.87 ± 1.35
Triticum aestivum L.	10	19.31 ± 2.04	33.69 ± 3.42	23.33 ± 4.04	23.65 ± 1.80	35.34 ± 2.53
	20	38.47 ± 1.62	54.07 ± 2.38	34.33 ± 1.53	32.22 ± 1.62	34.84 ± 0.56
	40	52.66 ± 2.79	63.15 ± 2.90	42.67 ± 4.93	33.21 ± 1.06	37.28 ± 0.66
	80	61.40 ± 1.01	66.93 ± 1.97	45.00 ± 1.73	34.36 ± 0.81	39.75 ± 0.74
	160	63.23 ± 0.92	75.75 ± 5.76	45.67 ± 2.31	34.49 ± 1.74	40.33 ± 1.57

of the same material. After 14 days of incubation at 20 °C without light, penetrating shoots were counted.

Statistically significant differences were calculated by applying a one-way ANOVA followed by Tukey's tests, while correlations were tested by the Pearson product-moment correlation using Microsoft R Open (3.5.1, Redmond, USA).

RESULTS AND DISCUSSION

Both crops showed a yield response to increased P fertilisation, and while no significant effect could be shown for barley, the wheat cultivar displays significant gains. When related to the sample with the minimal P application of 60 mg P, wheat realised a yield gain of over 300%, while barley hardly doubled (Figure 1). The results for wheat were comparable to Ifran et al. (2018), where similar yield gains in a similar experiment were observed.

No significant effects could be shown for ears per pot, kernels per ear or weight per thousand kernels (not depicted). The fact that yields exhibited significant gains, whereby the number of kernels per ear, the number of ears per pot, and the weight per thousand kernels did not, can be attributed to the multiplication of the three factors (Table 1).

While P, as well as phytate concentrations, displayed increased values in both crops by increased P fertilisation consistently (Figure 2), contents of P and phytate increased only in barley, suggesting a dilution effect in wheat (Figure 3). In addition, the P-concentrations in wheat were mostly comparable to the results of

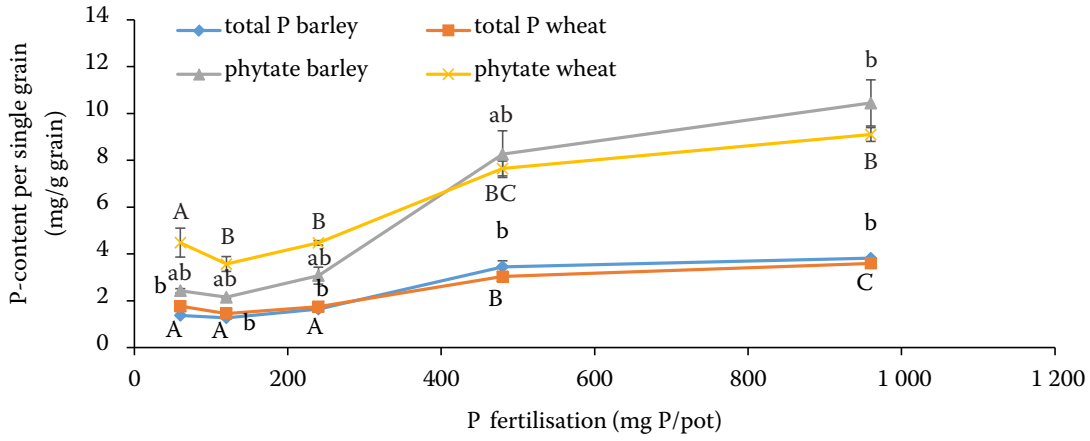

Figure 2. Concentrations of total phosphorus (P) and phytate as related to phosphorus fertilisation. Values are means of three biological replications ± standard error. Different letters indicate significant differences at α = 0.05, where only letters of the same size and colour are compared

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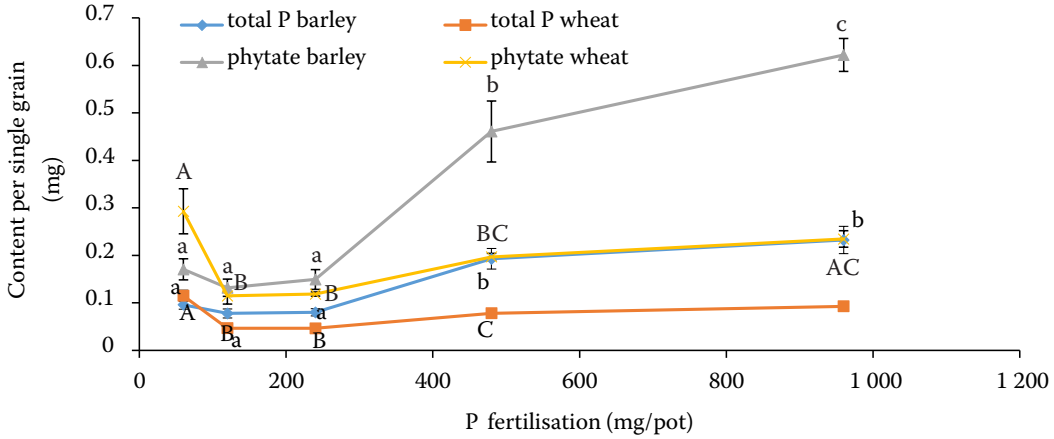

Figure 3. Contents of total phosphorus (P) and phytate in the grain as related to the amount of applied phosphorus fertiliser. Values are means of three biological replications ± standard error. Different letters indicate significant differences at α = 0.05, where only letters of the same size and colour are compared

Ifran et al. (2018). Both cereals showed a prominent decrease in P-concentrations in the second lowest fertilisation level, which can be attributed to dilution effects. In general, P-concentrations largely reacted analogously in both cereals. However, considering P-contents (Figure 3), it is evident that barley, due to its higher weight per thousand kernels, is remarkably better supplied with phosphorus.

It is widely known that P is stored in the kernel as phytate. Brinch-Pedersen et al. (2014) state that approximately 70% of P in the kernel is stored as phytate. This statement is consistent with the findings of this study (Figure 3). Even though there is a significant increase in the phytate content in barley (Figure 3), contents were largely stable in wheat at about 70% of the total phosphorus content. This effect could also be observed in studies by Cossa et al. (2000), Noack et al. (2014) and Safar-Noori et al. (2018). Both total P concentration in the grain (Figure 4A) and phytate-P concentration (Figure 4B) were closely correlated to the applied P, indicating a direct effect of culture management on this trait.

The studied wheat cv. Thasos exhibited no reaction of germination capacity with regard to increased P-fertilisation (Figure 5A). Germination capacities of ca. 90% were already observable in the lowest fertilisation level of 10 mg P/kg soil. The barley cv. Propino, however, showed two discreet levels of germination capacity:above and including 40 mg P/kg soil and below 40 mg P/kg soil (Figure 5A). Tests of germination velocity showed curve progressions in both crops, albeit at different levels, with peaks at 80 mg P/kg soil but no differences in 40 mg P/kg soil and the highest level at 160 mg P/kg soil (Figure 5B). Due to similarities in the methods, results for germination power were widely analogous to those of germination capacity, though the wheat cultivar displayed similar discreet levels to barley in this

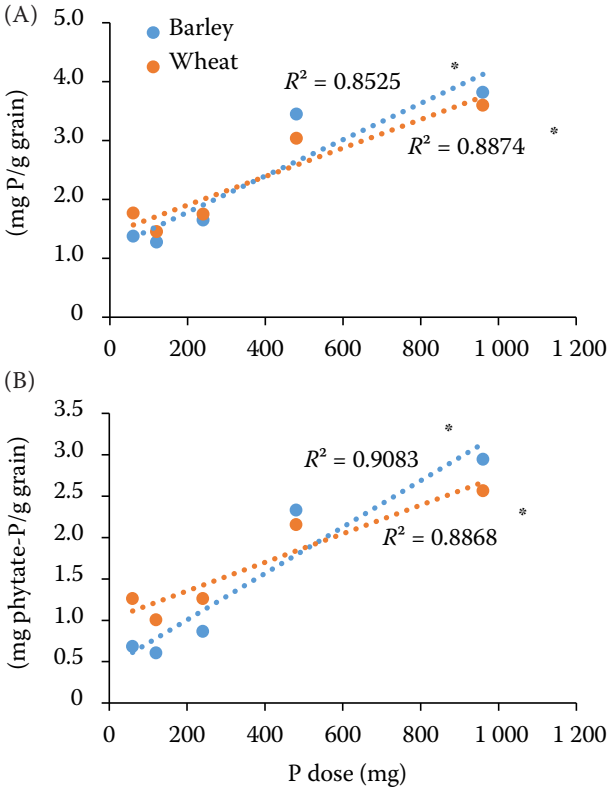

Figure 4. Correlations between the applied phosphorus (P) fertiliser and the concentration of (A) total P and (B) phytate-P in the grain. Values are means of three biological replications. *Significant correlations

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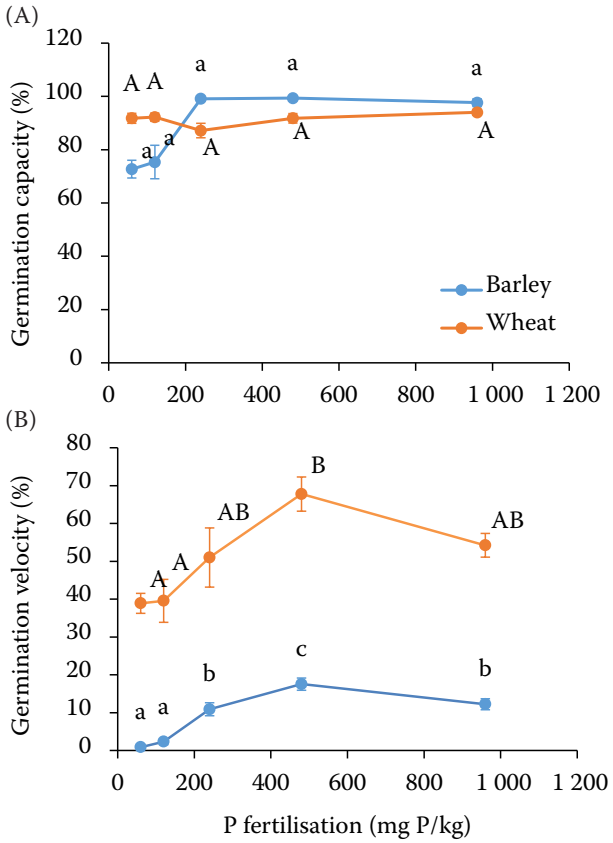

Figure 5. (A) Germination capacity and (B) velocity in relation to the amount of applied phosphorus fertiliser. Values are means of three biological replications ± standard error. Different letters indicate significant differences at α = 0.05, where only letters of the same size and colour are compared

analysis (Figure 6). In general, there would appear to be a relatively low demand for P for this trait.

Following these analyses, the first hypothesis can be upheld in part. Above a concentration of about 1.5 mg P/g grain, corresponding to a fertilisation of 40 mg P/kg soil, no further increase in germination capacity and power could be detected. These results imply that the analysed barley cultivar is significantly more dependent on exogenous P sources than the analysed wheat cultivar, which can be attributed to 62% higher phytase activity in wheat kernels compared to barley kernels (Brinch-Pedersen et al. 2014).

On the basis of P content per single grain, it should be noted that P-fertilisation had no effect on the performance of the used wheat cultivar. Consequently, the consistently high germination capacity could be attributed to the fact that the analysed wheat cultivar could metabolise given phytate reserves efficiently and was not reliant on exogenous P sources. The second hypothesis must consequently be rejected with regard to the seed quality.

While barley showed no reaction to increased P-fertilisation regarding micronutrient contents in the kernel (Figure 7), wheat did, however, react with a strong decrease of Fe and Zn contents (Figure 7). These findings matched those of Zhang et al. (2015) and Sánchez-Rodríguez et al. (2017), where dilution effects or an increased fixation in the root tissue are mentioned as possible explanations. Xia et al. (2020) reported in a review article that high P application to wheat resulted in an increase of P concentration in grains but a strong decrease of Zn concentration in grains. According to Sánchez-Rodríguez et al. (2017), barley, however, should have shown similar effects. The reason the used cultivar did not show this effect remains to be assessed. To secure the bioavailability of iron, Gibson et al. (2010) state a critical molar ratio of phytate and iron of < 1. With minimal phytate-to-iron ratios of 7.33 for the studied barley cultivar and 10.82 for the studied wheat cultivar, none of the studied cereals met this requirement at any of the investigated fertiliser levels (Figure 8). The study by Gibson et al. (2010) demanded a ratio of < 18 with regard to zinc. Again, for barley, the fertiliser level of 40 mg P/kg soil, or a concentration of about 1.65 mg P/g grain, corresponding to a phytate to zinc ratio of 13.37, was to be determined as the critical threshold above which zinc bioavailability could no longer be considered to occur (Figure 8). For wheat, the threshold is reached at the fertiliser level of 20 mg P/kg soil or a concentration of

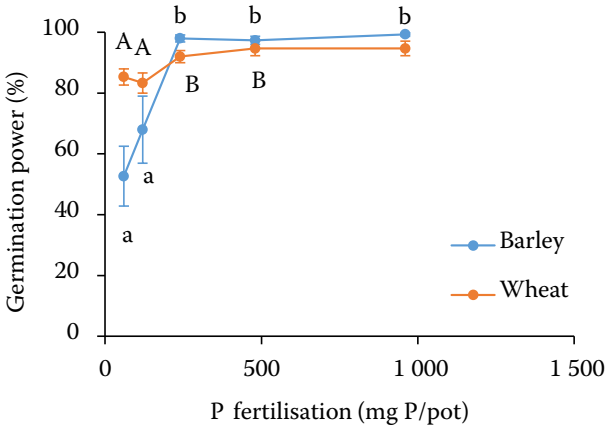

Figure 6. Germination power in relation to the amount of applied phosphorus (P) fertiliser. Values are means of three biological replications ± standard error. Different letters indicate significant differences at α = 0.05, where only letters of the same size and colour are compared

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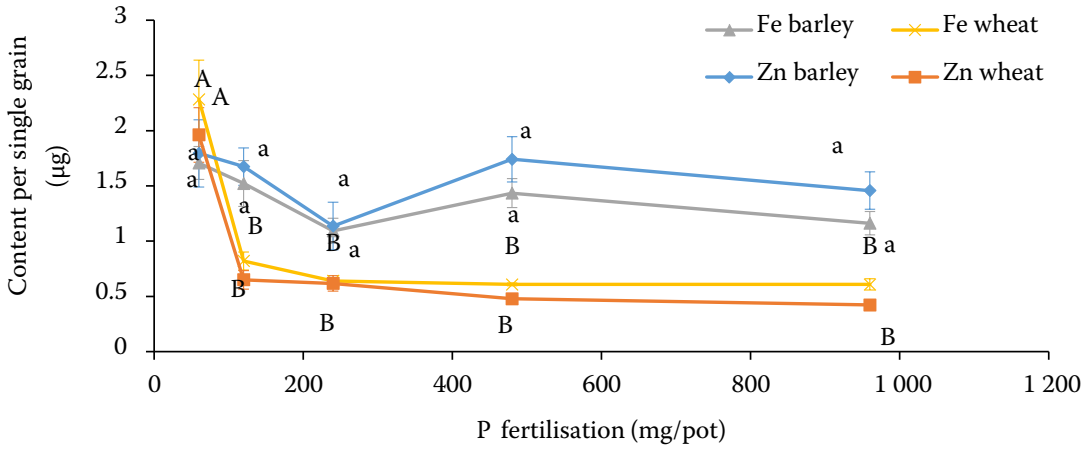

Figure 7. Micronutrient contents as related to the amount of applied phosphorus (P) fertiliser. Values are means of tree biological replications ± standard error. Different letters indicate significant differences at α = 0.05, where only letters of the same size and colour are compared

1.47 mg P/g grain, corresponding to a phytate-to-zinc ratio of 17.37. In cereal-heavy diets, however, none of the studied cereals in any of the tested fertiliser levels would positively affect micronutrient supply and, therefore, further malnutrition.

Consequently, it can be noted that both hypotheses must be rejected in regard to nutritional quality. While critical thresholds for zinc bioavailability could be determined, none was applicable to iron bioavailability.

The results of these experiments showed that for the studied spring barley cultivar, a concentration of 1.65 mg P/g grain, corresponding to a dose of 40 mg P/kg soil, is sufficient for an efficient germination. Such a concentration could not be observed in the grain of the studied summer wheat cultivar since wheat had efficient germination at 1.46 mg P/g grain, corresponding to 10 mg P/kg soil. Although pot experiments tend to imply higher P use efficiencies due to a denser root system than field experiments, Wang et al. (2005) could show that there is a significant correlation between the PUE measured in pot experiments and the PUE measured under field conditions. Nevertheless, the results of the pot experiment cannot be easily transferred to field conditions and require further validation by future field trials.

Even though PUE and phytate turnover is genetically determined (Gao et al. 2018), it was nonetheless shown that a change in cultivation conditions, by reducing the P supply, did not reduce the seed quality of the resulting grains immediately (Figure 5A).

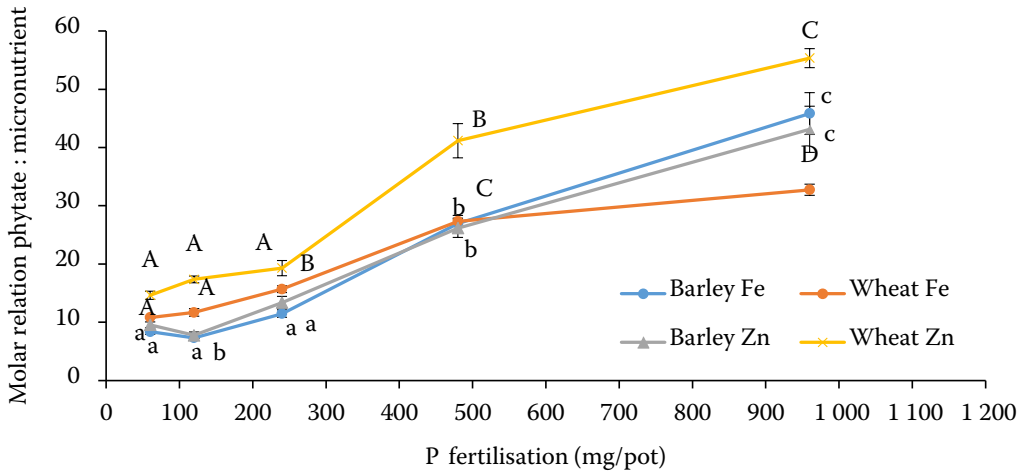

Figure 8. Molar relation of zinc (Zn) and iron (Fe) to phytate in relation to the applied phosphorus (P) fertiliser. Values are means of three biological replications ± standard error. Different letters indicate significant differences at α = 0.05, where only letters of the same size and colour are compared

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It is, therefore, plausible that for the studied cultivars, the commonly estimated minimum concentration of total P in the grain of 2 mg P/g is not always required for efficient germination. Fertilisation could therefore be reduced, leading to more efficient nutrient use.

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