

Mehlich 3 extractant used for the evaluation of wheat-available phosphorus and zinc in calcareous soils

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ABSTRACT

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Relation between wheat (*Triticum aestivum*) nutritional status determined at the beginning of stem elongation and during anthesis, respectively, and available content of phosphorus (P-M3) and zinc (Zn-M3) determined by the Mehlich 3 extractant was studied. Both one-year pot experiment with spring wheat and two-year on-farm trials with winter wheat were run on various calcareous soils (pH values of 7.18–7.94, median 7.80, P-M3 1–289 ppm, median 54, and Zn-M3 2–14 ppm, median 4), in the Czech Republic (Central Europe). Phosphorus nutrition index (ratio of phosphorus concentration in shoot biomass to critical phosphorus concentration – P_c) was calculated using the Belanger et al.'s model: $P_c = -0.677 + 0.221N - 0.00292N(2)$, where both phosphorus and nitrogen concentrations were expressed in g/kg shoot dry matter. Unlike phosphorus concentration in shoot biomass, phosphorus nutrition index significantly correlated with P-M3 content in soil. Optimal values of the phosphorus nutrition index were recorded if P-M3 was 51–68 ppm. Zinc concentration in shoot biomass more strongly correlated with P:Zn ratio (M3) in soil compared to Zn-M3 content in soil. P:Zn ratio in shoot biomass of 130:1 did not lead to phosphorus deficiency and corresponded to P:Zn (M3) ratio in soil of 9.3:1–14.3:1.

Keywords: biofortification; bioavailability; carbonate; soil test; zinc deficiency; soil test

Phosphorus (P) fertilization significantly affects grain yield of wheat plants (Zhou et al. 2017). Concurrently, both P and zinc (Zn) nutrition of wheat is reduced by increased soil Ca^{2+} content (Kizilgoz 2016). The Mehlich 3 (M3) method is suitable for the determination of available zinc content even in soils with pH > 6.5 (Wang et al. 2004). Furthermore, the Mehlich 3 method provides the advantage of multielement analysis (Iatrou et al. 2014). Zbíral (2016) expressed critical values of Zn-M3 content in soil. However, Zn concentration in cereal grains depends on available phosphorus content in soil (Nikolic et al. 2016) because the excess P rate decreases Zn uptake by plants (Konieczny and Kowalska 2016, Nikolic et al. 2016). Subclinical Zn deficiency is widespread (Rosado 2003). Zn bioavailability is often lower in vegetarian

diets mainly due to their low Zn and high phytic acid contents (Kristensen et al. 2006). A latent Zn deficiency in wheat grain poses a high risk for grain quality relevant to human health in regions where wheat bread is a staple food (Nikolic et al. 2016). Therefore, high levels of P supply should be avoided, and Zn application to high-P soils should be considered (Kizilgoz and Sakin 2015). Karaman et al. (2006) stated, that an important factor for maximum Zn utilization by wheat plants is a site-specific P:Zn ratio in high-P soils (Karaman et al. 2006). However, the P:Zn ratio has not been determined in the Mehlich 3 extractant simultaneously for both elements so far. Furthermore, optimal values of P:Zn in soil remain to express.

Jones et al. (2005) stated that adequate soil P levels can substantially offset the impact of drought

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on growth and yield of barley. This is especially important because of frequent drought occurrence and higher temperatures during the growing season in the conditions of changing climate in Central Europe as reported by Neugschwandtner et al. (2015) and Křen et al. (2015). Sensitivity to Zn deficiency stress also becomes more pronounced when plants are drought-stressed (Bagci et al. 2007). Remobilization of Zn from older tissues is then critical for Zn accumulation in wheat grain (Kutman et al. 2012).

The aim of this study was to define the optimal P:Zn ratio in calcareous soils using the Mehlich 3 multielement extractant. The optimal P:Zn ratio in soil is bound to respect optimal P:Zn ratio in shoot biomass and simultaneously avoid reduced P uptake by wheat plants. All these criteria are essential prerequisites for biofortification of wheat with Zn.

MATERIAL AND METHODS

A pot experiment was conducted in a greenhouse in the year 2015 with spring wheat of cv. Chamsin on 37 different calcareous soils (pH > 7.1). Each soil had three repetitions (pots). Four germinated seeds were sown in each pot filled with 400 g of soil with the moisture of 60% of field capacity. Each pot was thinned to two plants nine days after emergence. A dose of 0.1 g N/kg soil (NH_4NO_3 solution) and 0.1 g K/kg soil (KCl solution) was applied two times during a vegetation period: two weeks after emergence and six weeks after emergence. The experiment was harvested when most plants reached a beginning of anthesis (61 BBCH). Results of the pot experiment were accompanied by on-farm trials realized at 43 plots with calcareous soils in various regions of the Czech Republic. Sampling of shoot biomass of winter wheat in on-farm trials was done at the beginning of stem elongation (30–31 BBCH) and during anthesis (61–69 BBCH). Sampling of soils was done at the beginning of stem elongation of winter wheat to a depth of 30 cm. Soils studied in both pot experiment and on-farm trials achieved pH values of 7.18–7.94 (median 7.80), P-M3 of 1.0–289.0 ppm (median 54.0) and Zn-M3 of 2–14 ppm (median 4).

Both P and Zn content in soil in the Mehlich 3 extractant, 1:10 w/v, and in shoot biomass were determined using the inductively coupled plasma with optical emission spectroscopy (ICP-OES Varian Vista Pro, Melbourne, Australia). Available

P content in soil was also determined in 0.5 mol/L NaHCO_3 solution, pH 8.5 (Olsen et al. 1954) by segmented flow colorimetric analysis (Skalar San plus System, Breda, the Netherlands).

Due to the connection between phosphorus nutrition index and plant nitrogen (N) status, only wheat samples with nitrogen nutrition index (NNI) calculated according to Ziadi et al. (2010) of $\text{NNI} = 0.84\text{--}1.30$ (median 1.12) were assessed in the pot experiment. The nitrogen nutrition index was expressed as a ratio of the nitrogen concentration measured in shoot biomass to critical nitrogen concentration (N_c) calculated according to the model of Ziadi et al. (2010):

$$N_c = 38.5(\text{DM})^{-0.58} \quad (1)$$

In on-farm trials, only wheat plants with N concentration in shoot biomass ranging around 4.1% at the beginning of stem elongation (Balkcom and Burmester 2015) and 1.8% during anthesis (Lopez-Bellido et al. 2004) were assessed, which should indicate N optimal conditions. Total nitrogen concentration in shoot biomass of wheat plants was determined by the Kjeldahl method (Gerhardt Vapodest 50s, Königswinter, Germany).

Plant P status at the beginning of stem elongation as well as during anthesis were assessed using the calculation of shoot critical P concentration (P_c) according to the model of Belanger et al. (2015):

$$P_c = -0.677 + 0.221N - 0.00292N^2 \quad (2)$$

with both P and N concentrations expressed in g/kg of shoot dry matter (DM). Analogously to nitrogen nutrition index calculated by e.g. Ziadi et al. (2010), phosphorus nutrition index (PNI) was calculated as a ratio of measured total P concentration in shoot biomass – P_m (g/kg) and the critical phosphorus concentration in shoot biomass – P_c :

$$\text{PNI} = \frac{P_m}{P_c} \quad (3)$$

Values of PNI equal to or greater than 1.0 indicated that the crop is in situation of non-limiting P, while the values lower than 1.0 would indicate P deficiency.

A statistical analysis of the data was carried out using the Statistica 13 (Dell Inc., Reston, USA). Spearman's rank correlations were used to analyse relations among variables studied in the pot experiment; each variable consists of 111 cases. A probability value of 0.05 or less ($P \leq 0.05$) was taken to be statistically significant. Correlations among the selected variables in on-farm trials were studied using a linear regression analysis.

Table 1. Spearman's rank correlations between the traits determined in pot experiment

	P-M3	Zn-M3	P-Olsen	P plant	Zn plant	PNI	P:Zn soil	P:Zn plant	Shoot DM
Zn-M3	0.42**								
P-Olsen	0.75**	0.35**							
P plant	0.18	0.20	0.34*						
Zn plant	-0.21*	0.38**	-0.25*	0.46**					
PNI	0.48**	0.20	0.60**	0.88**	0.21				
P:Zn soil	0.69**	-0.27*	0.49**	-0.04	-0.58**	0.28*			
P:Zn plant	0.39**	-0.26*	0.52**	0.24*	-0.70**	0.46**	0.63**		
Shoot DM	0.78**	0.41**	0.74**	0.00	-0.32*	0.28*	0.54**	0.36**	
% shoot DM	0.49**	0.12	0.41**	-0.36**	-0.52**	-0.05	0.49**	0.26*	0.73**

The *r*-values marked with asterisks are significant at the levels of significance **P* < 0.05 and ***P* < 0.001. M3 – Mehlich 3; PNI – phosphorus nutrition index; DM – dry matter

RESULTS AND DISCUSSION

Although Olsen P method is usually used for determination of available soil P in calcareous soils (Iatrou et al. 2014), a strong correlation was recorded between P-M3 content and P-Olsen content in soil in the pot experiment (Table 1), which is in accordance with the findings of Iatrou et al. (2014). Therefore, in our study, the content of available P in soil was determined solely using the Mehlich 3 extractant.

No significant correlation was recorded between the P-M3 content in soil and P concentration in shoot biomass of wheat plants (Table 1). In contrast, the P-M3 content significantly correlated with phosphorus nutrition index. The phosphorus nutrition index can be then considered as a more reliable indicator of wheat P status on calcareous soils compared to the P concentration in shoot biomass during anthesis. Bélanger et al. (2015) recommend this predictive model of critical P concentration to quantify the degree of P deficiency during the wheat growing season mostly for high shoot N

concentrations, which is in accordance with our results because nitrogen nutrition index ranged between the values of NNI = 0.84–1.30 (median 1.12). Simultaneously, a very strong correlation (*r* > 0.80) was recorded between the phosphorus concentration in shoot biomass and PNI.

The phosphorus nutrition index correlated significantly both with the P-M3 content in soil and P:Zn ratio in shoot biomass. A strong correlation was also found between the P:Zn ratio in shoot biomass and P:Zn ratio in soil, which is in accordance with the results of Karaman et al. (2006). However, the P:Zn ratio in soil weakly correlated with the PNI. Therefore, the P:Zn ratio in soil itself cannot be used as an indicator of available P content in soil. As a result, relations of P-M3 content in soil vs. PNI, P:Zn ratio in shoot biomass vs. PNI and P:Zn (M3) ratio in soil vs. P:Zn ratio in shoot biomass of winter wheat plants were further studied in on-farm trials (Figures 1–3).

A strong correlation was recorded between the PNI determined at both growth stages and P-M3 con-

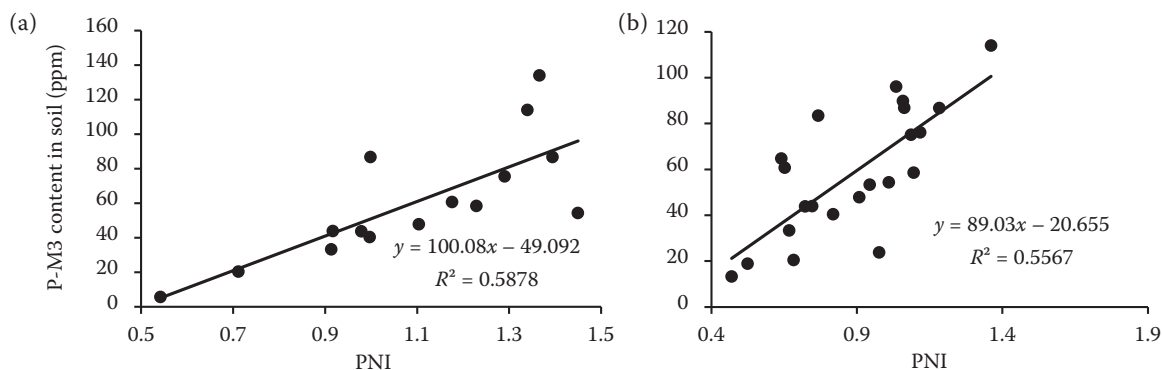


Figure 1. Correlation between P-M3 content in soil and phosphorus nutrition index (PNI) of winter wheat (a) at the beginning of stem elongation and (b) during anthesis, respectively, in on-farm trials

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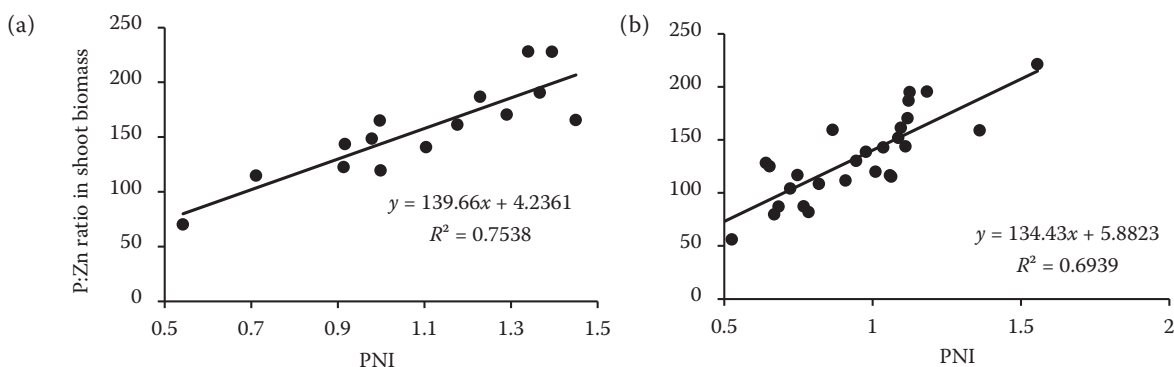


Figure 2. Correlation between phosphorus nutrition index (PNI) of winter wheat and P:Zn in shoot biomass of winter wheat (a) at the beginning of stem elongation and (b) during anthesis, respectively, in on-farm trials

tent in on-farm trials with winter wheat (Figure 1). According to regression equations, optimal plant phosphorus status, i.e. PNI = 1, corresponded to P-M3 content of 51 ppm and 68 ppm at the beginning of stem elongation and during anthesis, respectively. This result is in accordance with the findings of Buondonno et al. (1992), who expressed optimal P-M3 content in calcareous soils in Italy as 37–77 ppm. Buondonno et al. (1992) determined P-deficient soil if the P-M3 content is lower than 30 mg P/kg. After fitting into the regression equations in Figure 1, the P-M3 content of 30 ppm corresponded to PNI = 0.79 and PNI = 0.57 at the beginning of stem elongation and during anthesis, respectively. These values of phosphorus nutrition index indicate P deficit in plants.

Zinc concentration in shoot biomass correlated weakly with Zn-M3 content in soil (Table 1), which is in accordance with the results of Junus and Cox (1987). However, the Zn concentration in shoot biomass correlated moderately with P:Zn (M3) in soil in the pot experiment.

Mai et al. (2011) stated that P:Zn ratio in shoot biomass of the wheat grown 30 days with addition of Zn into nutrient solution was at the level of ca. 120:1 which approximates to the ratio of 140:1 stated by Dennis (1971) as necessary for balanced P and Zn nutrition of wheat plants. As follows from a very strong linear correlation showed in Figure 2, P:Zn ratio of 140:1 in shoot biomass of winter wheat at the beginning of stem elongation and during anthesis corresponded to PNI = 0.97 and PNI = 1.0, respectively. The P:Zn ratio in shoot biomass of 120:1 corresponded to phosphorus nutrition index determined at the beginning of stem elongation and during anthesis of PNI = 0.82 and 0.85, respectively. Compromisingly, the P:Zn ratio in shoot biomass of 130:1 corresponded to the phosphorus nutrition index determined at the beginning of stem elongation and during anthesis of PNI = 0.90 and 0.92, respectively, indicating a sufficient phosphorus supply of winter wheat.

A correlation between P:Zn (M3) ratio in soil in on-farm trials and P:Zn ratio in shoot biomass of

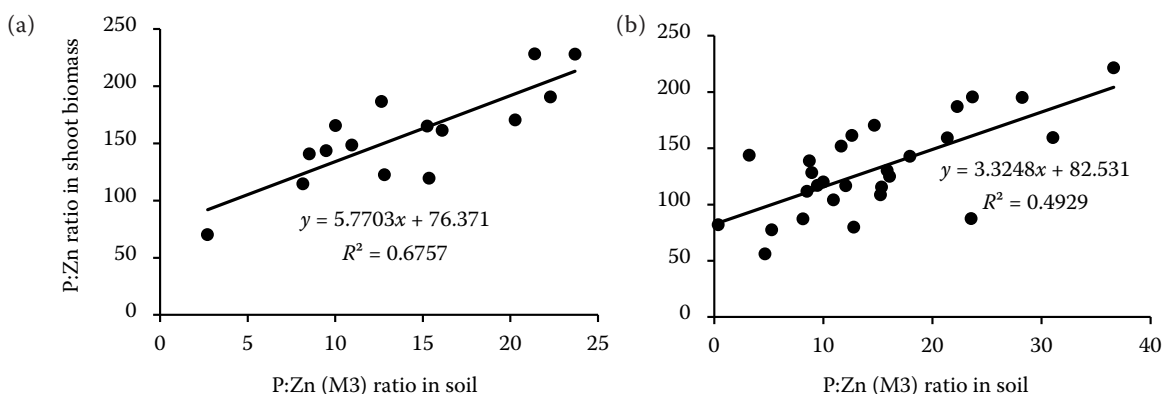


Figure 3. Correlation between P:Zn (M3) ratio in soil and P:Zn in shoot biomass of winter wheat (a) at the beginning of stem elongation and (b) during anthesis, respectively, in on-farm trials

winter wheat at the beginning of stem elongation and during anthesis was very strong and strong, respectively (Figure 3), which complies with the findings of Karaman et al. (2006). According to the regression equation, P:Zn ratio in shoot biomass of winter wheat at the beginning of stem elongation and during anthesis of 140:1 corresponded to P:Zn (M3) ratio in soil of 11.0:1 and 17.3:1, respectively. However, the P:Zn ratio in shoot biomass of winter wheat at the beginning of stem elongation and during anthesis of 130:1 corresponded to P:Zn (M3) ratio in soil of 9.3:1 and 14.3:1, respectively.

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