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Zinc and amino acids impact on nutrient status of maize during the 'critical window'

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ABSTRACT

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It has been assumed that zinc (Zn) fertilizers applied to maize simultaneously with amino acids (AA) at early stages of its growth may decrease the yield variability due to correcting its nutritional status during the 'critical window'. Two Zn carriers were evaluated (Zn-I – Zn chelate; Zn-II – Zn oxide); they were applied to maize at BBCH 14/15 with or without amino acids, based on two rates of nitrogen (80 and 160 kg N/ha). The precipitation deficiency in 2015 resulted in the grain yield decrease by 35% compared to 2014. An advantage of higher N rate was proved in 2014, whereas the influence of Zn and AA showed in 2015. In this year, the beneficial impact of Zn-oxide and AA combined application resulted in amelioration, at least partially, of the imbalance of certain macronutrient content (N, P, Mg) during the 'critical window'. These effects were revealed due to a boosted number of kernels in cob, and particularly higher thousand kernel weight. Consequently, the yield depression in 2015 was partly overcome. The results indicated that simultaneous application of Zn oxide and AA to maize at BBCH 14/15 corrected both its nutritional status during the 'critical window' and yield components, but had no effect on the yield itself.

Keywords: zinc fertilizers; amino acids; *Zea mays* L.; rainfall deficiency; DRIS analysis

In spite of a huge breeding progress resulting in new maize cultivars well adapted to the area-specific climate conditions, the grain yield still depends on the precipitation level and its distribution during the growing season (Sárvári and Pepó 2014). According to Nagy (2012), the unit productivity of water is lower during dry years than during the growing season with optimal precipitation. In Poland, a significant reduction of the summer rainfall (June–August) compared to the overall annual precipitation was frequently recorded between 1951 and 2010 (Czarnecka and Niezgolska-Lencewicz 2012). Soil moisture conditions significantly influence the nitrogen use efficiency (NUE). Melchiori and Caviglia (2008) findings indicate a much stronger response of maize to the nitrogen (N) fertilization in the year of rainfall deficiency. Water management and nutrition requirements of a plant should be examined through their impact on the yield components. In literature, the period between tasseling (BBCH 51) and blister stage (BBCH 71) is described as the 'critical window'. During that period, even a slight water deficiency in plant tissue results in a yield loss, which is a consequence of a lower number of kernels in the row (NKR) (Ritchie and Alagarswamy 2003, Nielsen et al. 2010).

Nitrogen management and plant abiotic stress resistance are assured by the zinc (Zn) supply (Potarzycki 2011). The yield-forming role of Zn relates to the entire maize growing season. It was documented that Zn increases plant resistance to soil water deficiency through the control of auxin synthesis, which has an influence on the growth of the root system (Barker and Eaton

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2015). Consequently, plant water and N use is more intense not only before flowering, but also during the grain filling period (Grzebisz et al. 2008a). Leaves of the Zn-fertilized maize have larger surface area, photosynthesis capacity and stay physiologically active much longer (Anees et al. 2016). In the agricultural practice, the use of products containing amino acids is increasing. Amino acids can affect numerous processes in plants. They are easily available sources of N, precursors of plant hormones, including auxins, anti-stress agents, in turn positively affecting plant growth and yield (Calvo et al. 2014). Thus, their application to crop plants may be considered as an alternative way to control abiotic stresses. It can be therefore assumed that a simultaneous application of zinc fertilizers and AA is a way to attenuate the effects of numerous stresses experienced by maize plants during the seed set up.

The purpose of this study is to evaluate the impact of two types of Zn fertilizers applied with and without amino acids, based on two N rates, on maize nutritional status during the 'critical window'. It was assumed that the recorded nutrient disorder during this period can be used as a predictor of yield structure and grain yield.

MATERIAL AND METHODS

The field experiments were carried out in Poznań (52'30°N, 16'84°E, Poland) on sandy soil, classified as Albic Luvisol. The soil contained a low level of organic carbon (0.31%). The content of available nutrients, measured each year before the application of fertilizers, was in the medium class for phosphorus (46–48 mg P kg/soil) and low for potassium (50–51 mg K kg/soil) (double lactate – Egner Riehm method; Grzebisz 2015). The Zn content was in the low class (3.6–3.8 mg Zn kg/soil; 1 mol/L HCl; Rinkis method; Gembarzewski and Korzeniowska 1990). The amount of mineral N (N_{min}) was 32–39 kg/ha (0.01 mol/L $CaCl_2$). Soil pH was 5.0 (1 mol/L KCl).

A two-factorial experiment, replicated four times, was arranged as follows: (i) N rate of 80 and 160 kg N/ha in the form of ammonium nitrate; (ii) foliar applied Zn fertilizer (Zn-I and Zn-II) with or without the addition of free L-amino acids (93 g/L) (AA) at BBCH 14/15. The Zn-I contained Zn EDTA-chelate at the rate of 100 g/L (rate of Zn 400 g/ha). In the Zn-II, the Zn carrier was Zn oxide with 5% blend of EDTA-chelate in the total amount of 500 g/L (rate of Zn 1000 g/ha). Based on Mortvedt et al. (1999), a 2.5 higher efficiency of Zn chelate versus Zn oxide was assumed. Together with Zn fertilizer, an amino acid solution was applied to maize foliage in accordance to the experimental design at the rate of 2 L/ha together with Zn fertilizer. Phosphorus and potassium fertilizers were applied before sowing at the rates of 40 kg P/ha and 133 kg K/ha.

Maize cv. ES Fortran (FAO 210–220) was sown at the end of April in a number of 85 000 seeds/ha. At the beginning of the flowering stage sub-samples of ear leaves were taken from 10 plants per each plot. At each field, an area of 10 m^2 of fully matured plants was harvested. The yield was adjusted to 86% of dry matter content. The yield structure, including numbers of: rows (NR); kernels per row (NKR); kernels per cob (NKC), and thousand kernel weight (TKW) were determined based on 16 randomly chosen cobs per plot.

Sub-samples of leaves were first dried at 65°C. Nitrogen concentration was determined using the standard macro-Kjeldahl procedure. Plant material for other nutrients was mineralized at 600°C and the obtained ash was then dissolved in 33% HNO_3. Phosphorus concentration was measured by the vanadium-molybdenum method, while potassium by the flame-photometry, and magnesium by the atomic-absorption spectrometry – flame type.

Partial factor productivity indices of the applied fertilizer N (PFP_N) were calculated by dividing the harvested grain yield (GY) by the N rate (80 or 160 kg N/ha):

$$PFP_N = GY/N \text{ rate (kg grain/kg N)}$$

The study adopts the method of diagnosis and recommendation integrated system (DRIS). The primary purpose of this method is the assessment of plant nutrition status based on the analysis of elements interactions (Elwali et al. 1985). DRIS indices are the relative measures of the plants nutrients deficiency or excess in relation to the adopted norm. The assessment objective is to find one or more elements, deficiency (–) or excess (+) responsible for the nutritional balance of a plant. The interpretation of plant nutritional conditions, with the use of the DRIS index values, is based on the assessment that for every two elements included in the analysis, the sum of indices always equals zero. A high level of the absolute sum of indices (ASI) demonstrates a plant nutritional imbalance.

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DRIS indices were then calculated according to the following formulae, example for nitrogen:

$$I(N) = [f(N/P) + f(N/K) + f(Mg/N)]/3$$

$$\text{when } N/P > n/p \text{ then } \quad f\left(\frac{N}{P}\right) = \left[\frac{N/P}{n/p} - 1\right] \times \frac{1000}{CV}$$

$$\text{when } N/P < n/p \text{ then } \quad f\left(\frac{N}{P}\right) = 1 - \left[\frac{n/p}{N/P}\right] \times \frac{1000}{CV}$$

Where: N/P – nutrient ratio of N to P contents in the studied crop; n/p – nutrient ratio of N to P in the DRIS norm (Elwali et al. 1985); *CV* – coefficient of variation for n/p ratio for the DRIS norm; 1000 – coefficient of recalculation.

The obtained data were subjected to the conventional analysis of variance (Statistica 10, StatSoft. Inc., Tulsa, USA). Differences between the mean values of treatments were compared with the Tukey's *HSD* (honest significant difference) test, with the significance level of α = 0.05.

RESULTS AND DISCUSSION

The study was carried out in two years of extremely diverse amount and distribution of precipitation. The strong differences occurred during the most critical stages of maize growth (Table 1). In comparison to 2014, during the 2015 growing season, quite different conditions were recorded in May and August. Water supply to plants was disturbed first at the early stages of maize growth (BBCH 15–19), as well as at the early stage of kernel growth (BBCH 69–75). The primary period is very sensitive to N supply. Its shortage impacts negatively the cob structure (Grzebisz et al. 2008b).

Table 1. Rainfalls (mm) for the years 2014–2015

Month	2014	2015	Long-term mean
April	42.4	23.8	30.0
May	81.0	15.2	45.0
June	31.4	27.0	64.1
July	48.4	55.4	78.0
August	93.6	36	59.2
September	36.6	25	46.8
October	31.0	14.4	36.9
Total (IV–X)	364.4	196.8	360.0

The grain yield was significantly affected by the interaction of experimental factors and years (Figure 1). The rainfall deficiency in 2015 resulted in the yield decrease by an average of 35% compared to 2014. A positive response to the N rate was recorded in 2014, whereas the impact of Zn and AA fertilization was stronger in 2015. In 2014, the best yield was obtained in treatments with the N rate of 160 kg N/ha and fertilized with Zn-II and Zn-I applied with AA. In comparison with Zn-I, the yield increase on Zn-II amounted to 0.23 t/ha. In 2015, the yield increase due to Zn-II or Zn-II with AA application compared to Zn-I was 1.58, and 1.48 t/ha, respectively. The pronounced effect of Zn-II could be explained by its higher rate due to application zinc as Zn oxide. In many experiments carried out around the world, mineral the compounds of Zn (oxides and sulphates) were described as effective carriers of that particular microelement (Barker and Eaton 2015). Analogically, the values of the partial factor productivity indices of the applied fertilizer N were also closely related to weather variations in 2014 and 2015 (Table 2). In 2015, the unit N productivity of 80 kg N/ha decreased by 32.5%, whereas for 160 kg N/ha by 41.4%, compared

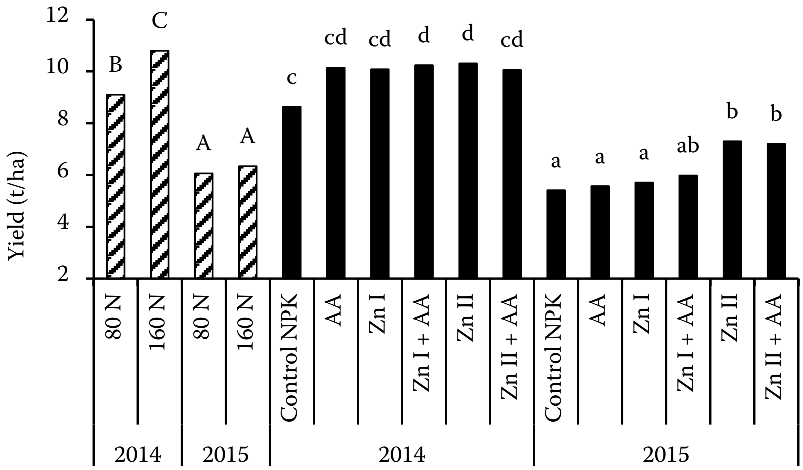

Figure 1. Grain yield response to the interaction of nitrogen (N) rate and foliar fertilization with years (numbers marked with the same letter are not significantly different at $P < 0.05$). AA – amino acids; Zn-I – Zn chelate; Zn-II – Zn oxide

Table 2. Partial factor productivity indices of the applied fertilizer nitrogen (PFP_N, kg/kg)

Experimental factor	PFP_N 2014	PFP_N 2015
N rate (kg N/ha)		
80	113.8^{d}	75.8^{c}
160	67.5^{b}	39.6^{a}
F-test	**	
Foliar fertilization		
Control NPK	81.4^{de}	50.7^{a}
AA	92.1^{e}	52.0^{a}
Zn-I	92.3^{e}	53.1^{ab}
Zn-I + AA	93.0^{e}	56.0^{abc}
Zn-II	93.6^{e}	66.3^{bc}
Zn-II + AA	91.4^{e}	67.9^{cd}
F-test	*	

$^{**}P < 0.01$; $^{*}P < 0.05$; AA – amino acids; Zn-I – Zn chelate; Zn-II –Zn oxide

to 2014. Moreover, comparison with the NPK control, confirmed a positive impact of the studied treatments on PFP_N, but only in 2015. A significant increase by 31% and by 34% was recorded for Zn-II and Zn-II with AA, respectively. The obtained results comply with the studies by Potarzycki and Grzebisz (2009), who related PFP_N increase directly to the increasing rates of Zn applied to maize at BBCH 15.

In this study, the yield forming effect of Zn and AA application was revealed in the higher degree of yield components' development such as NKC and TKW. In both years, the highest NKC was recorded on the plot treated with Zn-II. It was by 7.8%, and by 20.4% higher compared to the NPK plot in 2014 and 2015, respectively. However, it was lower by 9.8% in 2015 compared to 2014 (Figure 2). The impact of the studied treatments on TKW was recorded only in 2015 as recorded for Zn-II and AA (Figure 3). It was higher by 10.3% with respect to the NPK plot, but at the same time lower by 18.2% compared to 2014. The conducted stepwise regression analysis showed that these two elements explained 98% of the grain yield (GY) variability:

$$GY = -7.84 + 0.018NKC + 0.031TKW \text{ for } R^2 = 0.98.$$

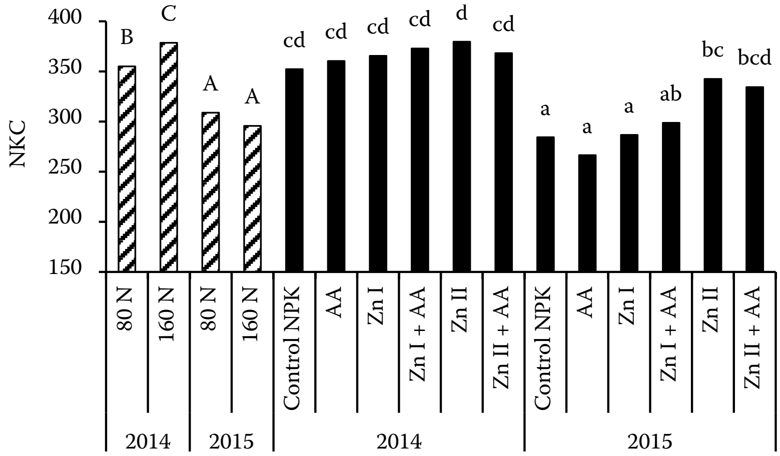

Figure 2. Number of kernels per cob (NKC) response to the interaction of nitrogen (N) rate and foliar fertilization with years (numbers marked with the same letter are not significantly different at $P < 0.05$). AA – amino acids; Zn-I – Zn chelate; Zn-II – Zn oxide

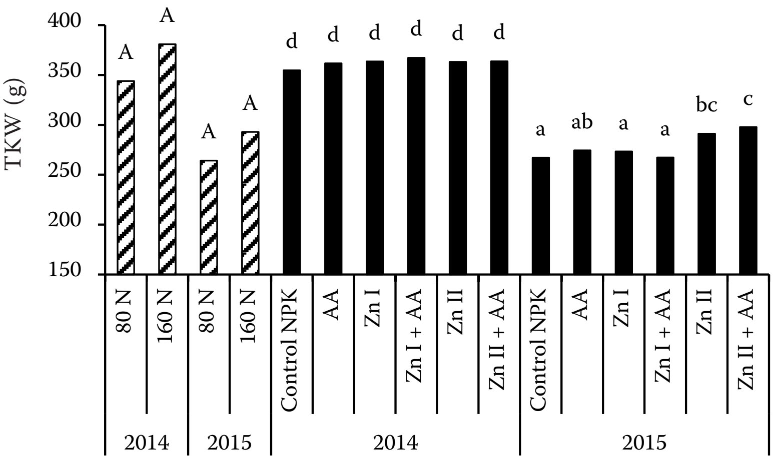

Figure 3. Thousand kernel weight (TKW) as affected by the interaction of foliar fertilization and years (numbers marked with the same letter are not significantly different at $P < 0.05$) AA – amino acids; Zn-I – Zn chelate; Zn-II – Zn oxide

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Table 3. Nutrient content (g/kg dry matter) in the ear leaf at BBCH 65

Statistical evaluation	2014				2015			
	N	P	K	Mg	N	P	K	Mg
Range	26.0–31.9	2.68–3.12	17.3–20.8	1.60–2.30	23.3–37.9	2.33–3.02	16.4–20.0	1.52–2.01
Mean	28.7	2.92	18.7	1.84	28.7	2.68	18.2	1.76
Standard deviation	3.50	0.19	1.11	0.18	4.94	0.20	0.99	0.13
Coefficient of variation (%)	12.2	6.7	5.9	9.8	17.2	7.3	5.4	7.2

Maintaining the NKC and further growth of kernels is closely related to the plant nutritional status of maize at the onset of flowering. The increased NKC in response to applied zinc or AA, as recorded in both years but especially in 2015, indicates the semi-flex type or the ear plasticity of the cultivar used in the study (Haegele et al. 2014). During the grain-filling period, nutrients accumulated in vegetative maize organs are remobilized and then transported to developing kernels. The effectiveness of these processes depends on the supply of soil N to maize plant (Ning et al. 2017) and Zn nutritional status in leaves (Grzebisz et al. 2008a, Potarzycki 2011, Barker and Eaton 2015). The observed responses of both yield elements suggest a potential use of the semi-flex type of maize hybrids in regions with a temporary water stress during 'the critical window'.

Assuming that maize nutritional status during flowering is essential for maintaining the initial structure of yield, the content of macronutrients (N, P, K, Mg) was evaluated at BBCH 65, adopting the ear leaf as the indicator (Table 3). The highest year-to-year variability, however, was recorded for N. According to the criterion set by Schulte and Kelling (2000), the content of the analysed macronutrients was within the optimal range. The applied DRIS analysis indicates that in plants fertilized with 160 kg N/ha there was an excess of N in the ear leaf. It was particularly evident in 2015 (Table 4), when P and Mg proved to be the nutrients limiting N productivity. In 2015, amino acids applied simultaneously with Zn oxide ameliorated the limiting impact of Mg shortage on yield. It was one of the main results of the studies, as the highest TKW in 2015 was recorded in the particular combination of AA + Zn-II, which significantly affected the grain yield compared to the NPK control. Plant nutrition status is well

Table 4. Maize nutritional status as assessed by the diagnosis and recommendation integrated system (DRIS) indices – ear leaf at BBCH 65

Nitrogen rate (kg N/ha)	Foliar fertilization	2014					2015				
		nutrients indices DRIS				limiting nutrients	nutrients indices DRIS				limiting nutrients
		N	P	K	Mg		N	P	K	Mg	
80	control NPK	2.18	−1.88	−0.59	0.29	P > K	−3.08	−2.85	4.09	1.83	N > P
	AA	−0.86	−2.15	4.62	−1.61	P > Mg > N	0.69	−3.03	2.18	0.16	P
	Zn-I	−2.01	−0.73	2.94	−0.20	N > P > Mg	−1.57	0.21	2.30	−0.94	N > Mg
	Zn-I + AA	−3.31	−1.77	3.65	1.43	N > P	1.13	−7.01	6.28	−0.40	P > Mg
	Zn-II	−1.76	−1.27	2.15	0.89	N > P	−5.34	−0.72	6.53	−0.47	N > P > Mg
	Zn-II + AA	0.93	−1.22	−0.53	0.83	P > K	4.84	−8.90	2.62	1.45	P
	mean	−0.81	−1.50	2.04	0.27		−0.56	−3.72	4.00	0.27	
160	control NPK	1.64	−1.40	−0.87	0.63	P > K	15.52	−6.36	−3.23	−5.93	P > Mg > K
	AA	5.52	−2.71	2.08	−4.88	Mg > P	10.03	−8.62	0.54	−1.95	P > Mg
	Zn-I	7.89	−4.07	−1.44	−2.37	P > Mg > K	16.57	−8.23	−1.64	−6.70	P > Mg > K
	Zn-I + AA	8.64	−1.66	−3.14	−3.84	Mg > K > P	1.69	0.13	−0.60	−1.21	Mg > K
	Zn-II	8.77	−2.99	−0.38	−5.40	Mg > P > K	11.67	−3.48	−3.86	−4.37	Mg > K > P
	Zn-II + AA	4.86	−1.85	−0.21	−2.80	Mg > P > K	5.98	−4.84	−0.44	−0.68	P > Mg > K
	mean	6.22	−2.45	−0.66	−3.11		10.24	−5.23	−1.54	−3.47	

AA – amino acids; Zn-I – Zn chelate; Zn-II –Zn oxide

Table 5. Absolute sum of indices (average values)

Experimental factor		Absolute sum of indices (± standard deviation)
Year	2014	9.99 ± 4.7
	2015	16.07 ± 6.6
Nitrogen rate (kg N/ha)	80	9.04 ± 3.4
	160	17.02 ± 5.2
Foliar fertilization	control NPK	13.1 ± 11.8
	AA	12.9 ± 1.0
	Zn-I	15.0 ± 5.8
	Zn-I + AA	11.5 ± 3.8
	Zn-II	15.0 ± 4.5
	Zn-II + PA	10.7 ± 5.8

AA – amino acids; Zn-I – Zn chelate; Zn-II –Zn oxide

described by the absolute sum of DRIS indices (ASI), which, in optimal conditions, should display close-to-zero values (Elwali et al. 1985). The water deficiency in 2015 caused a considerable variability of ASI, which, in comparison with 2014, increased by 60% (Table 5). A similar effect was achieved by increasing the rate of N from 80 to 160 kg N/ha. A positive effect of AA on ASI was revealed for treatments with combined application of both compounds. For plants grown on the Zn-II plot, the AA addition resulted in ASI decrease by 28.7%.

The obtained results confirm the purpose and benefits of the simultaneous application of Zn fertilizers and amino acids in order to ameliorate the influence of abiotic stress as indicated by the ASI. Foliar application of Zn fertilizers simultaneously with amino acids may therefore be an effective way to alleviate nutritional distress in plants during the 'critical window'. However, an advantage of this combination over the Zn oxide with respect to the grain yield was not found during this study.

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