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TOTAL AND FRACTIONAL CONTENTS OF PROTEINS IN BEAN SEEDS UNDER THE CONDITIONS OF VARIED FERTILISATION WITH MICROELEMENTS

ZAWARTOŚĆ I SKŁAD FRAKCYJNY BIAŁKA NASION FASOLI W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA MIKROELEMENTAMI

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ABSTRACT

Over 2003-2005 at the Experiment Station at Wierzchucinek at the University of Technology and Life Sciences in Bydgoszcz, there was performed a strict one-factor micro-plot experiment in split-splot design. The factor tested was a type of microelements [n=5: Cu, Zn, Mn, Mo, B]. The microelements were foliar sprayed in a chelated form, as the series of 'Symfonia' fertilizers.
The study aimed at comparing the effect of five agricultural-engineering basic microelements on the contents and protein composition of the seeds of 'Aura' cultivar. The fertilization applied, boron and manganese in particular, showed an effect on the increase in the contents of total protein in bean seeds. It also modified the fractional composition of the bean seed protein. There was observed a clear increase in the fraction of albumins and globulins in seeds as a result of the microelements applied, except for boron. The fertilization with molybdenum, boron, copper and zinc reduced the content of glutelins, and the sum of glulelins and prolamines in the bean seeds.

KEYWORDS: bean, foliar fertilization, fractional composition of protein, microelements, protein

STRESZCZENIE

W latach 2003-2005 przeprowadzono na terenie Stacji Badawczej Uniwersytetu Technologiczno-Przyrodniczego w Bydgoszczy ścisłe jednoczynnikowe doświadczenie mikropoletkowe w układzie split-plot. Za badany czynnik przyjęto rodzaj mikroelementu (n=5: Cu, Zn, Mn, Mo oraz B). Mikroelementy zastosowano dolistnie w formie schelatowanej, w postaci serii nawozów „Symfonia". Celem podjętych badań była ocena oddziaływania pięciu mikroelementów aplikowanych dolistnie w formie schelatowanej, na zawartość i skład frakcyjny białka nasion fasoli odmiany ‘Aura’.

Wykazano oddziaływanie zastosowanych mikroelementów, w szczególności manganu i boru, na zwiększenie zawartości białka ogólnego w nasionach fasoli. Zastosowane nawożenie modyfikowało również jego skład frakcyjny. Stwierdzono wyraźny wzrost udziału albumin i globulin w nasionach fasoli pod wpływem zastosowanych mikroelementów, z wyjątkiem boru. Nawożenie molibdenem, borem, miedzią oraz cynkiem obniżało zawartość glutelin, a także udział sumy glutelin i prolamin w nasionach.

SŁOWA KLUCZOWE: fasola, nawożenie dolistne, frakcje białkowe, mikroelementy, białko

STRESZCZENIE SZCZEGÓŁOWE

Podstawą badań było jednoczynnikowe ścisłe doświadczenie mikropoletkowe, przeprowadzone w latach 2003-2005 na terenie Stacji Badawczej Uniwersytetu Technologiczno-Przyrodniczego, zlokalizowanej w Wierzchucinku (Borys et al. 1996). Założono je metodą losowanych podbloków w układzie split-plot w trzech powtórzeniach na glebie płowej, klasy bonitacyjnej III b, kompleksu żytniego dobrego. Zasobność gleby w przyswajalne formy fosforu, potasu oraz cynku, manganu i boru była średnia, natomiast zawartość miedzi oszacowano jako niską. Badanym czynnikiem był rodzaj mikroelementów (n=5: Cu, Zn, Mn, Mo i B), zastosowanych w postaci serii nawozów ‘Symfonia’, zawierających schelatowaną formę tych pierwiastków. Celem badań była ocena wpływu aplikowanych dolistnie mikroelementów na zawartość i skład frakcyjny białka nasion fasoli odmiany ‘Aura’. W nasionach fasoli oznaczono zawartość azotu ogólnego metodą Kjeldahla, natomiast skład frakcyjny białka - metodą Michaela-Bluma w modyfikacji Łoginowa i in, poddając je ekstrakcji kolejno: wodą destylowaną (albuminy i azot niebiałkowy), 5% roztworem siarczanu [VI] potasu (globuliny) oraz 0,1 M roztworem NaOH w 70% etanolu (prolaminy i gluteliny). Stwierdzono, że zastosowane nawożenie dolistne roztworami manganu i boru, wpływało istotnie na zawartość białka ogólnego w suchej masie nasion fasoli. W warunkach prowadzonych badań wykazano istotny wpływ zastosowanych mikroelementów, z wyjątkiem boru, na udział albumin w białku nasion fasoli. Najwyższą zawartość albumin uzyskano na obiektach, na których aplikowano dolistne cynk oraz mangan. Szczególnie korzystny wpływ na zawartość globulin, frakcji o największym ilościowym udziale w białku nasion fasoli, miało dolistne zastosowanie molibdenu i cynku. Średnia zawartość sumy albumin i globulin była istotnie modyfikowana przez zastosowane nawożenie mikroelementami. Stwierdzono wyraźny wzrost zawartości sumy tych frakcji na wszystkich obiektach nawozowych w odniesieniu do obiektu kontrolnego. Przeprowadzone badania wykazały, że pod wpływem dolistnej aplikacji mikroelementów średnia zawartość sumy glutelin i prolamin na ogół obniżała się w porównaniu z obiektem nieopryskiwanym. Stwierdzone zmiany składu frakcyjnego białka fasoli pod wpływem nawożenia mikroelementami mogą odgrywać znaczącą rolę w kształtowaniu jego wartości biologicznej.

INTRODUCTION

Vegetables are one of the main sources of proteins in human food, with bean being one of the most important vegetable crops containing highly valuable protein, which is a precious supplement of animal protein (Martín-Cabrejas et. al 1997). Producing a high qualitative bean crop depends on the effectiveness of symbiosis and satisfying the increasing nutritional requirements of the plants throughout increased photosynthesis and seed-filling. A developed root system enabling the plant to collect nutrients from sparingly soluble compounds (Jasińska and Kotecki 1993) lowers the effectiveness of fertilisation of the Fabaceae with macro-components, compared to other crops. A significant factor limiting the bean yielding and lowering the quality of biological crop may be shortage of microelements, which is due to the reduced content of their forms assimilable by plants in soil (Grzyś 2004), the increased demand, or impeded uptake caused by precipitation shortage in the vegetation period (Sienkiewicz-Cholewa (2002); Szewczuk and Michałojć 2003). An adequate supply of leguminous plants with the most important microelements enhances the adequate pattern of photosynthesis and the processes connected with nitrogen transformation and the synthesis of amino acids (Ziółek and Kulig 1999). Since foliar fertilisation, as opposed to soil fertilisation, is an agrotechnical procedure that acts almost immediately (Szewczuk and Michałojć 2003), it seems to be the best way of complementing nutrient deficits in plants. According to the assumed research hypothesis, a suitable selection of microelements and their use in the right proportions should enhance not only the contents but also the fractional distribution of the bean seed proteins, thus improving its biological quality.

The study aimed at assessing the effect of five agricultural-engineering basic microelements on the contents and protein composition of the seeds of ‘Aura’ cultivar.

MATERIAL AND METHODS

Over 2003-2005 at the Experiment Station at Wierzchucinek, of the University of Technology and Life Sciences in Bydgoszcz, there was carried out a strict one-factor microplot experiment in split-splot design, in three replications, on fallow land classified as 3b productivity class, a good rye complex. The abundance of assimilable forms of phosphorus, potassium and zinc, manganese and boron was average, however, the content of copper – low. The type of microelements [n=5: Cu, Zn, Mn, Mo, B] constituted the experimental factor. The microelements were foliar sprayed in a chelated form, as the series of ‘Symfonia’ fertilisers, at the dose of 200 $g \cdot ha^{-1}$ for each element.

In mechanically disintegrated bean seeds, a total nitrogen content was determined with the Kjeldahl’s method, and the fractional contents of proteins – using the method of Michael-Blum (1960). The protein fractions were extracted, in turn, with: distilled water, 5% solution of potassium sulfate (VI), and 0.1 M NaOH in 70% ethanol, isolating, respectively: nitrogen non-proteinous compounds and albumins, globulins and glutelins, as well as prolamines. Albumins were separated from nitric non-proteinous compounds as a result of precipitation with 20% solution of trichloroacetic acid. Glutelins were separated from prolamines by alcoholic extraction with pH=5.5. The contents of the individual fractions were calculated by determining nitrogen in the respective precipitates or extracts, using the Kjeldahl’s method and expressed as a fraction of their nitrogen in a total content of this element ($g\ N \cdot kg^{-1}$ of the protein). The results were verified with the analysis of variance for randomized block designs and the Tukey test at the significance level of $\alpha = 0.05$ was used to assess the significance of differences.

RESULTS AND DISCUSSION

The present research demonstrated that the use of foliar fertilisation with solutions of boron and manganese affected the content of total protein in dry matter of bean seeds (Table 1).

Table 1. Total protein content in bean seeds in relation to fertilisation with microelements [g·kg^{-1}d.m.]
Tabela. 1. Zawartość białka ogólnego w nasionach fasoli w zależności od nawożenia mikroelementami [g·kg^{-1}s.m.]

Year/Rok	0	Mn	Zn	Cu	Mo	B	$LSD_{0,05}$/$NIR_{0,05}$
2003	328,3	329,0	325,0	341,3	337,0	347,0	15,91
2004	302,3	326,0	311,0	294,3	302,7	329,0	10,16
2005	326,7	337,3	335,0	338,7	331,0	325,3	n.s/n.i.
2003-2005	319,1	330,8	323,7	321,4	323,6	336,1	10,14

n.s. – n.i. – non significant differences – różnice nieistotne

Czuba et al. (1999) also report on a clear reaction of leguminous plants on foliar fertilization with microelements, particularly with magnesium. Depending on the micro-element used, the content of total protein, on average, ranged from 319.1 g·kg^{-1} to 336.1 g·kg^{-1} for three years of the experiment. The highest value of total protein was found in the seeds sprayed with boron and manganese solutions. This procedure induced an increase in the mean content of protein for bean seeds: 5.3% and 3.6%, respectively, as compared with the control. A favourable effect of boron was found particularly in the second year of the experiment, when the increase in total protein content in bean seeds reached 8.8%, as compared with the non-spraying. After applying zinc, copper and molybdenum, the mean content of protein in seeds generally increased, as compared with the control, however, the increase was not significant. Manganese, zinc molybdenum, boron and copper are essential parts of living tissues and perform essential and specific functions. They are components of many enzymes controlling metabolic processes in organisms. The lack of micro-elements in soil and plants disturbs biochemical processes, inhibiting their growth and development. A physiological role of micro-elements has been already quite well recognised. Manganese, responsible for increasing the intensity of photosynthesis, stimulating nitrogen assimination and protein biosynthesis (Cakmak 2000; Henriques 2003; Prusiński and Kotecki 2006), demonstrated in our experiment a very favorable effect on the increase in total protein content in the bean seeds. Boron, participating in the formation of wall structures, cell divisions as well as in the protein synthesis (Brown et al. 2002; Matsunaga et al. 2003), showed the most favorable effect of all the nutrients investigated on the increase in the content of protein in the seeds. The element, according to Ruiz et al. (1998), also plays an essential role in the process of reducing nitrates (V) in the plant. Copper, zinc and molybdenum play also an important role in the transformations of nitrogen in plants, however, as shown by the present results, the elements did not modify the content of total protein significantly. The effect of micro-elements on the plant metabolism is a high-complex process resulting from their direct and indirect effects on the individual transformations, which makes the present results difficult to account for.

The present research showed a significant effect of the fertilization applied on the presence of albumins in the bean seed protein, the mean content of which was 7.01 $g \cdot kg^{-1}$ of seeds for the three years of the experiment (Table 2).

Table 2. Participation of protein fractions in bean seeds in relation to fertilisation with microelements [g kg^{-1} of seeds]
Tabela. 2. Udział frakcji białkowych w nasionach fasoli w zależności od nawożenia mikroelementami [g kg^{-1} nasion]

Muicroelements Mikroelementy	Albumins Albuminy	Globulins Globuliny	Glutelins Gluteliny	Prolamins Prolaminy	$LSD_{0,05}$/$NIR_{0,05}$
			2003		
0	5,79	15,24	2,20	0,75	Alb.-0,726 Glob.-1,724 Glut. -0,260 Prol. – n.s./n.i.
Mn	6,41	17,41	2,18	0,70	
Zn	6,50	18,20	1,92	0,84	
Cu	6,10	16,30	2,02	0,78	
Mo	6,72	18,99	1,71	0,76	
B	6,00	15,59	1,81	0,73	
			2004		
0	5,98	15,88	3,18	0,67	Alb.-0,379 Glob.-2,511 Glut. -0,407 Prol. – n.s./n.i.
Mn	6,92	16,55	3,32	0,68	
Zn	7,12	18,96	2,59	0,65	
Cu	6,31	16,42	2,43	0,62	
Mo	6,82	18,39	2,59	0,59	
B	6,18	15,84	2,99	0,62	
			2005		
0	7,62	16,25	2,50	0,87	Alb.-0,452 Glob.- n.i./n.s Glut. -0,320 Prol. – 0,174
Mn	8,90	16,74	2,35	1,02	
Zn	8,73	16,79	2,45	1,12	
Cu	8,71	16,30	2,61	0,89	
Mo	7,91	17,15	2,25	0,81	
B	7,52	15,75	2,22	0,92	
			2003-2005		
0	6,46	15,79	2,63	0,77	Alb.-0,136 Glob.-0,540 Glut. -0,098 Prol. – 0,054
Mn	7,41	16,9	2,62	0,80	
Zn	7,45	17,98	2,32	0,87	
Cu	7,04	16,34	2,35	0,76	
Mo	7,15	18,17	2,19	0,72	
B	6,57	15,73	2,34	0,76	

n.s. – n.i. – non significant differences – różnice nieistotne

Interestingly, the increase in the content of this fraction in proteins was found under the effect of each of the micro-elements applied, except for boron. Albumins are the structural and enzymatic proteins forming complex compounds with carbohydrates, lipids and nucleic acids (Jasińska and Kotecki 1993). This fraction is characterised by the most balanced composition of exogenous amino acids and determines the nutritional value of the seeds to a considerable extent. In comparison to the other proteins of bean seeds, albumins contain a substantial amount of tryptophan which, besides methionine, is the second amino acid limiting the nutritional value of the legumes proteins. This fraction also contains significant amounts of lysine and threonine (Flaczyk 1995). The highest content of albumins was reported for the treatment where foliar fertilization with zinc was applied. As compared with the control, the difference was on average 15.3%. The foliar application of the other elements resulted in slightly lower increases in the content of that fraction, with the respective differences being on average: for manganese – 14.7%, for copper – 9.0%, for molybdenum – 10.7%. Interestingly the reaction after the cooper application was recorded only in 2005.

In the present research, foliar fertilization with molybdenum and zinc showed an especially favorable effect on the contents of globulins, the quantitatvely highest fraction in the bean protein, which resulted in the mean increase in their content by 15.1 and 13.9%, as compared with the non-fertilized control. Fertilisation with manganese and copper resulted in an average increase in globulins, respectively, by: 7.0 and 3.5%. In 2003 molybdenum (a 24.6% increase) and zinc (a 19.4% increase) were most effective. A similar reaction of globulins on the fertilization with those microelements was also recorded in 2004, while in the successive year there was noted no clear effect on the contents of that fraction in bean seeds.

In leguminous plant seeds the proteins are mainly represented by globulins (Lampart-Szczapa 1997), which make 80-90% of the total protein, whereas the other proteins represent albumins, the level of which ranges from 10 to 25% of total protein in seeds (Jasińska and Kotecki 1993). The globulins present in codyledons of fabaceae plant embryos are storage proteins, consisting of legumins and vicilins. Legumins are characterised by higher contents of methionine and cysteine – basic sulphur amino acids (SAA), the deficiency of which in protein of leguminous plants is a factor limiting its biological value.

Zinc, molybdenum and manganese showed the greatest favorable effect on the contents of albumins and globulins in the bean seeds. The bean is a plant susceptible to the deficiency in these microelements (White and Broadley 2001). Besides several biochemical reactions they participate in, zinc and manganese play an important role in the transformations of nitrogen compounds (Zimmer and Mendel 1999; Cakmak 2000; Henriques 2003). Foliar fertilization, rapidly enhancing the supply of plants with nutrients, primarily affects the elements the deficiency of which the plant is most sensitive to. A lower susceptibility of the bean to copper deficiency, also taking part in the transformations of nitrogen compounds (Sienkiewicz-Cholewa and Wróbel 2004), resulted in a relatively lower yet significant increase in the contents of albumins and globulins in the seed protein after applying this microelement.

Based on the present results, it was demonstrated that the mean content of the total albumins and globulins was significantly modified by the fertilization with microelements applied (Table 3).

Table 3. Participation of protein fractions in bean seeds in relation to fertilisation with microelements [g kg^{-1} of seeds]

Tabela. 3. Udział frakcji białkowych w nasionach fasoli w zależności od nawożenia mikroelementami [g kg^{-1} nasion]

Microelements Mikroelementy	Albumins+Globulins Albuminy+Globuliny	Glutelins+Prolamins Gluteliny+Prolaminy	N-nonprotein N-niebiałkowy	$LSD_{0,05}/NIR_{0,05}$
		2003		
0	21,03	2,97	4,84	Alb.+ Glob.-
Mn	23,83	2,89	5,02	4,602
Zn	24,70	2,76	4,47	Glut.+Prol.-0,391

Cu	22,38	2,81	4,61	N-n. -0,665
Mo	25,70	2,47	4,75	
B	21,58	2,53	5,11	
		2004		
0	21,86	3,85	4,34	
Mn	23,47	4,00	4,58	Alb.+ Glob.-
Zn	26,08	3,24	4,22	1,951
Cu	22,73	3,05	4,27	Glut.+Prol.-0,418
Mo	25,21	3,18	4,41	N-n. -0,375
B	23,36	3,61	4,62	
		2005		
0	23,87	3,37	4,02	
Mn	25,64	3,37	5,03	Alb.+ Glob.-
Zn	25,53	3,57	3,55	1,368
Cu	25,01	4,50	4,06	Glut.+Prol.-0,453
Mo	23,26	3,06	3,89	N-n. -0,472
B	23,36	3,14	4,18	
		2003-2005		
0	22,25	3,40	4,40	
Mn	24,31	3,42	4,88	Alb.+ Glob.-
Zn	25,44	3,19	4,08	0,414
Cu	23,37	3,12	4,32	Glut.+Prol.-0,138
Mo	24,98	2,90	4,35	N-n. -0,144
B	22,73	3,10	4,64	

A clear increase in the sum of these fractions was observed for all the fertilization-applied treatments as compared with the control. The mean content of total albumins and globulins was the highest in the seeds from the treatment which involved spraying with zinc (a 14.3% increase), however, the increase for the treatments which involved spraying with molybdenum, manganese, copper and boron was 12.3, 9.3, 5.0 and 2.2%, respectively. In the respective experiment years, spraying with zinc and manganese resulted in the fractions increasing by 7.0 and 7.4 in 2005, as well as 19.3 and 15.3 following the application of zinc and molybdenum in 2004. In 2003 the foliar application of molybdenum increased in total of albumins and globulins by 22.2%, as compared with the control.
The mean content of glutelins for three research years was 2.41 $g \cdot kg^{-1}$ (Table 2). Foliar application of molybdenum, zinc, copper and boron reduced this fraction in bean seeds, as compared with the control, by 16.7; 11.8; 10.6; and 11.0%. Fertilization with microelements in 2005 did not differentiate the contents of glutelins in the bean seeds significantly. In 2004 fertilization with molybdenum and zinc reduced the glutelins fraction by 18.6%. In 2003 foliar application of molybdenum and boron reduced the contents of glutelin by 22.3% and 17.8%, as compared with the treatments without spraying.
The content of prolamines in the bean seeds, quantitatively the lowest fraction, was modified by microelement fertilization to a low extent. Foliar zinc application was the only one which increased the contents of prolamines in been seed proteins, on average for three years by 13.0%. The favorable effect of this micro-element was observed especially in 2005, when their significant increase accounted for 28.7%.
The present research indicated that foliar fertilisation with microelements generally resulted in lowering the mean total content of the sum of glutelins and prolamines, as compared with the treatment without spraying (Table 3). Under the effect of spraying with zinc and boron, the fraction of the sum of glutelins and prolamines decreased by 6.2% and 8.8%, however, after the foliar application of copper and molybdenum - by

8.2% and 14.7%. In 2004 the reduction after the application of the microelements was in 15.8% – for zinc, 20.8 – for copper and for 17.4 – after the application of molybdenum. In the other studies, the effect of the manganese applied on the content of the sum of glutelins and prolamines was inconsiderable.
In 2003 the application of molybdenum and boron decreased the share of the mean sum of glutelins and prolamins by 16.8% and 14.8%, as compared with the control. The content of non-protein nitrogen changed clearly only under the effect of fertilisation with manganese in 2005 (a 25.1% increase). In 2003 the only differences in the contents of this fraction were observed in the bean seeds from the treatments where molybdenum, copper and zinc were foliar applied. On average, for three years of the experiment, spraying with manganese and boron increased the contents of non-protein nitrogen by 10.9% and 5.5%.
The protein of leguminous plants, in comparison to the protein of cereals, contains relatively high amounts of lysine and threonine, however, the factor limiting its biological value is a low content of sulphur amino acids (methionine and cysteine) (Jasińska and Kotecki 1993). Bean proteins are mainly represented by globulins which contain legumins with a higher share of those amino acids, and albumins, demonstrated by more balanced contents of exogenous amino acids. The present research demonstrated that the foliar application of copper, zinc, manganese, molybdenum and boron has shown a favorable effect on the content of the fractions in seeds and, at the same time, may contribute to increasing the biological value of the crop by enhancing the content of amino acids in seeds of this plant. Considering a high protein efficacy per area unit, characteristic for leguminous plants, the changes in the fractional content of the bean protein due to fertilisation with microelements seem to play a considerable role in forming their nutritive value.

CONCLUSIONS

1. The foliar application of boron and manganese increased the total protein in the bean seeds significantly.
2. A clear increase in the fraction of albumins and globulins in the bean seeds was observed under the effect of all the micro-elements applied (manganese, zinc, copper and molybdenum), except for boron.
3. Fertilization with copper, zinc, molybdenum and boron reduced the content of glutelins and total glutelins and prolamins in the bean seeds, as compared with the control.

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