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PHYTIC ACID CONTENT IN DIFFERENT DRY BEAN AND FABA BEAN LANDRACES AND CULTIVARS

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ABSTRACT

Phytic acid (inositol hexakisphosphate) is the principal storage form of phosphorus in many plant tissues. Phosphorus in this form is not available to non-ruminants, due to lack in the enzyme phytase that separates phosphorus from phytate molecules. Phytic acid is a strong chelator of important minerals such as calcium, magnesium, iron, and zinc, and can therefore contribute to mineral deficiencies in people whose diet rely on these foods as basic mineral intake. Phytic acid may be considered also as a phytonutrient, providing an antioxidant effect. Its content in plants is influenced by genotype, environmental, edaphic and agro-climatic factors, but also by species characteristics. Cereals and legumes are rich in phytic acid. The aim of this research was to examine the phytic acid content in seeds of diverse genotypes of common bean (*Phaseolus vulgaris* L.), runner bean (*Phaseolus coccineus* L.) and faba bean (*Vicia faba* L.), and to compare Serbian landraces and cultivars with widely distributed foreign cultivars. Faba beans had lower phytic acid content (mean 6.969 mg/g) in comparison to Phaseolus sp. (mean 8.095 mg/g). The lowest content was found in landrace from Temska (KVF-6-NS), 2.106 mg/g, and the highest in cultivar Panonski Gradištanac (10.47 mg/g) and Sarajevski zeleni (10.310 mg/g). According to phytic acid content, faba beans are grouped into two groups on one side of the dendrogram. Hierarchical cluster method of the multivariate analysis grouped dry beans genotypes into two larger and one small group (with only two members).

Key words: dry bean, faba bean, runner bean, genetic diversity, phytic acid.

INTRODUCTION

Plants from the *Leguminosae* family, i.e. grain legumes or pulses, have a long history in the nutrition of humans and domestic animals. In Serbia, dry bean (*Phaseolus vulgaris* L.) is the most common pulse in human diet (Todorović et al., 2008). Modern systems for cultivating of high-value agricultural products for human and animal consumption are going back to other traditional leguminous plants. This includes a return to faba bean (*Vicia faba* L.) as a traditional plant and to the landraces grown in this climatic region (Vasić et al., 2008). Runner bean (*Phaseolus coccineus* L.), used to be known as "Turkish bean", is grown in mountain regions of the Balkan Peninsula. Recently, new modern cultivars were produced in Bosnia and Herzegovina (Ćota et al., 2008).

Legumes are very important in human diet, especially in developing societies. They are the main meal for people with low income, but also a very good source of nutrients. Dishes made from dry beans are part of the national cuisines in all countries of the Balkan Peninsula, as well as in many other countries around the world. Dry beans are often considered as the “poor man’s meat” because they provide a high amount of food protein at a lower cost than most animal food protein sources (Sathe, 2002). Dry beans have been one of the best non-meat sources of iron (Shimelis and Rakshit, 2005), due to its amino acid composition. They contain large amount of lysine, significantly low concentrations of methionine, tryptophan and cysteine.

Starch is the most abundant carbohydrate in grain legumes, with 60-80% of total carbohydrates. Legumes are known as the cause of flatulence, due to incapability of the

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human intestine to digest oligosaccharide, as the result of the lack of α-1,6-galactosidase in intestinal mucosa (Múzquiz et al., 2006). Dietary fibers content in dry beans vary in the range 1.798-3.376 g/kg of dry matter (Vasić et al., 2009). Insoluble dietary fibers represent 92% to 100% of the total amount of dietary fibre for various beans, soluble fibers being the remaining percentage (0.0-3.2%) of the total dietary fibre (Bednar et al., 2001). Dry beans are also an excellent source of minerals (Geil and Anderson, 1994; Anderson et al., 1999; Tepić et al., 2007), including calcium, copper, iron, magnesium, phosphorus, potassium, and zinc.

Dry beans storage their phosphorus in a form of phytic acid (potassium and magnesium salts). Phytic acid has been known as anti-nutrient, as it binds proteins, minerals and starch, leading to decreased availability of these nutrients in the digestive tract. However, in low amounts, phytic acid may have some beneficial nutritive effects, like the reduction of starch assimilation, decreasing the blood glucose level, teeth caries control, colon cancer suppression, antioxidant (Graf et al., 1987; Thompson and Zhang, 1991), hypocholesterolemic and hypolipidemic effect in human organism (Coelho et al., 2002; Vucenik and Shamsuddin, 2003; Chen, 2004). Besides, phytic acid is a natural waste water cleaner, which is used on industrial scale, since it bounds three times more heavy metals, than the similar amount of conventionally used means (Veličković et al., 1999; Šćiban et al., 2010).

The aim of this research was to examine the phytic acid content in seeds of diverse genotypes of common bean (Phaseolus vulgaris L.), runner bean (Phaseolus *coccineus* L.) and faba bean (*Vicia faba* L.), and to compare Serbian landraces and cultivars with widely distributed foreign cultivars.

MATERIAL AND METHODS

The studied material consisted of 38 grain legumes genotypes: 24 dry bean, 2 runner bean and 12 faba bean. They represent a part of the working collection of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Their names, origin and genetic status are given in Tables 1 and 2.

A small-plot trial was carried out at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi (Novi Sad, Serbia) on a carbonated chernozem soil. All dry and runner bean genotypes were sown with an average crop density of 40 viable seeds m^{-2}. All faba bean genotypes were sown with an average crop density of 45 viable seeds m^{-2}.

Habitus and grain colour for dry and runner bean are presented in Table 1. Additionally, purpose and 100 seeds weight (g) were presented for faba bean (Table 2). Phytic acid content (mg g^{-1}) was determined in all genotypes of grain legumes seeds.

Samples were freshly powdered and homogenized for determination of phytic acid content, according to modified Haug and Lantzsch (1983) method. The determination was based on indirect spectrophotometric determination of phytic phosphorus in dry bean extracts. Phytic acid was precipitated by addition of ferric ammonium sulfate. Part of iron forms insoluble ferric phytate, and the remaining iron was determined spectrophotometrically. Calibration curve was prepared by series of standard solutions of sodium salt of phytic acid. All reagents were of analytical grade.

0.5 g of powdered sample was extracted with 100 ml of 2.4% HCl during 3 h with constant stirring. The extract was filtered through Whatman No 41 filter paper. 0.5 ml of extract was transferred into a glass tube with stopper, ammonium iron (III) - sulfate solution (0.2 g of $NH_4Fe(SO_4)_2 \times 12\ H_2O$ dissolved in 100 ml of 2 mol/L HCl and filled to mark with distilled water) was added. Closed glass tube was held in boiling water bath for 30 min., cooled in ice bath for 15 min. and left to attain room temperature. Tube was centrifuged at 3000 r/min. 1 ml of supernatant was transferred to another glass tube and 1.5 ml of 2,2'-bipyridine solution (10 g 2,2'-bipyridine dissolved in 10 ml thioglycolic acid and filled to mark with distilled water) was added. After exactly defined time, absorbance was measured at 519 nm.

Variability of the phytic acid content was expressed via minimum, maximum and average values and variation. The genotypes were partitioned into groups by the method of clustering. The hierarchical cluster method of the multivariate analysis was used to classify the tested genotypes. The genotypes were partitioned into groups by the single linkage method (nearest neighbour) of hierarchical clustering by Euclidean distances. Statistical processing was done with statistical packages Statistica ver. 9.1 (StatSoft, Inc. USA).

RESULTS

Constant population migrations, the intersection of major trading routes, and diverse soil and climatic conditions have been the main contributing factors leading to a great divergence of many crop species in Serbia, particularly edible and feed grain legumes (Vasić et al., 2008). The divergence between genotypes, concerning the phytic acid content of the three species was investigated (Tables 1 and 2).

Table 1. Phytic acid content (mg/g) in dry and runner (*) bean seeds

Dry bean	Status	Origin	Habitus	Grain colour	Phytic acid content
Zlatko	cultivar	Serbia	det	gold yellow	8.724 ± 0.275
Palanački zlatnožuti	cultivar	Serbia	det		7.747 ± 0.366
Belko	cultivar	Serbia	det	white	8.369 ± 0.383
Dvadesetica	cultivar	Serbia	det		7.980 ± 0.370
Maksa	cultivar	Serbia	det		7.161 ± 0.066
Balkan	cultivar	Serbia	det		8.884 ± 0.108
Aster	cultivar	Serbia	det		8.244 ± 0.305
Galeb	cultivar	Serbia	det		6.886 ± 0.190
Panonski gradištanac	cultivar	Serbia	det		10.470 ± 0.270
Panonski tetovac	cultivar	Serbia	det		8.625 ± 0.655
Levač	cultivar	Serbia	ind, IV	white	7.368 ± 0.276
Medijana	cultivar	Serbia	det		9.039 ± 0.142
Oplenac	cultivar	Serbia	det		8.654 ± 0.230
Sremac	cultivar	Serbia	det	greenish yellow	8.478 ± 0.225
Slavonski zlatno-žuti	landraces	Croatia	det		7.519 ± 0.157
Studenica	landraces	Serbia	det		7.026 ± 0.286
Žuto-zeleni Stepanovićevo	landraces	Serbia	det		8.504 ± 0.331
Sarajevski zeleni	landraces	Bosnia and Herzegovina	det		10.310 ± 0.279
Jovandeka	landraces	Serbia	det	seed coat patterns	8.662 ± 0.166
Butmirski trešnjo	landraces	Bosnia and Herzegovina	det		7.019 ± 0.182
Naya Nayahit	cultivar	USA	ind, II	black	7.564 ± 0.418
C-20	cultivar	USA	ind, II	white	6.832 ± 0.302
Ludogorje	cultivar	Bulgaria	ind, II		5.324 ± 0.279
Prelom	cultivar	Bulgaria	ind, II		7.653 ± 0.154
Igman *	cultivar	Bosnia and Herzegovina	det		8.898 ± 0.246
Darko*	cultivar	Bosnia and Herzegovina	ind, IV		8.530 ± 0.190
min					5.324
max					10.470
mean					8.095

Habitus: det = determinate; I type
ind, II = indeterminate; II type, upright
ind, IV = indeterminate; IV type, climbing

In this research, 26 genotypes (6 landraces and 20 cultivars) of dry beans and runner beans of different habitus and seed colour were included (Table 1). One dry bean genotype, Levač, and one runner bean genotype, Darko, had an indeterminate climbing habitus. The cultivars with white seed colour were prevailing (12 accessions). The phytic acid content ranged from 5.324 mg/g (cultivar Ludogorje from Bulgaria) to 10.470 mg/g (cultivar Panonski Gradištanac from Serbia). The mean value of the phytic acid content was 8.095 mg/g in the dry bean and runner bean genotypes studied.

Table 2. Phytic acid content (mg/g) in faba bean seeds

Name	Status	Purpose	Country of origin	100 seeds Weight (g)	Phytic acid content
KVF-1-NS	landrace	food	Serbia	125.3	3.834 ± 0.193
KVF-2-NS	landrace	food	Serbia	121.8	4.177 ± 0.514
Aštar	cultivar	food	Czech Rep.	58.0	4.434 ± 0.157
Piešťansky	cultivar	food	Czech Rep.	121.2	4.377 ± 0.338
Aquadulce	cultivar	food	Serbia (from Italy)	138.6	3.841 ± 0.255
Šarac	cultivar	feed	Serbia	48.6	5.276 ± 0.114
Gema	cultivar	feed	Serbia	43.7	4.234 ± 0.193
Irena	cultivar	food	France	58.1	3.634 ± 0.185
Diva	cultivar	food	France	38.4	5.262 ± 0.156
Göttingen Winter Bean Population	population	food	Germany	5.2	6.969 ± 0.268
KVF-6-NS (Temska)	landraces	food	Serbia	106.3	2.106 ± 0.325
KVF-7-NS	landraces	food	Serbia	197.2	3.449 ± 0.233
Min.					2.106
Max.					6.969
Mean					4.304

The divergence of phytic acid content was also examined in four landraces and eight faba bean cultivars - four Serbian food faba bean local landraces, two cultivars from Czech Republic, one food faba bean old domestic cultivar from Italia (Aquadulce), two Serbian feed faba bean cultivars (Gema and Šarac), two French faba bean cultivars and one German faba bean population (Table 2). The phytic acid content varied between 2.106 mg/g in the local landrace KVF-6-NS to 6.6969 mg/g in Göttingen Winter Bean Population. The mean value of phytic acid content was 4.304 mg/g for the examined faba bean genotypes.

The phytic acid content is also presented in the Figure 1, where genotypes are arranged by increasing values of phytic acid content. All genotypes of faba bean with the lowest values, except one (Göttingen Winter Bean Population), are placed at the bottom of the Figure 1. According to their phytic acid content, runner bean varieties Darko and Igman are highly rated, as well as dry beans.

The grouping of the genotypes of the examined grain legume species is presented in the dendrogram made by hierarchical cluster method of the multivariate analysis (Figure 2). In the upper part of dendrogram, 8 genotypes of faba bean are clearly distinguished.

The second group consists of 24, mostly dry bean genotypes. This large group includes two sub-groups. One faba bean genotype is present in the first sub-group. In the second sub-group, both cultivars of runner bean are present. Separated, but close to the first group of 8 faba beans, are two more cultivars of faba bean, and, almost independent, the cultivar Ludogorje.

At the bottom of dendrogram, one pair of dry bean genotypes is separated, closer to the other dry beans than to faba beans.

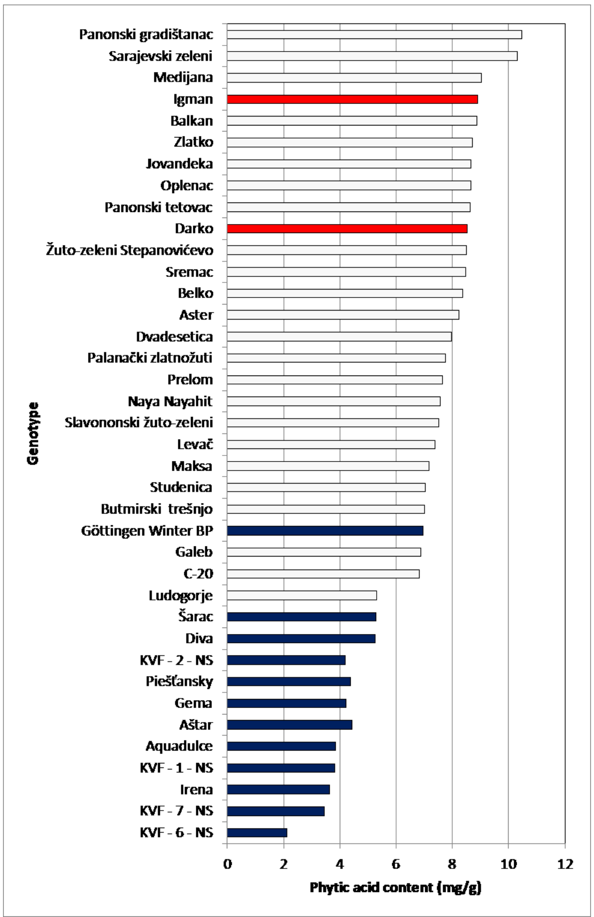

Figure 1. Phytic acid content in 38 grain legume genotypes (mg/g)

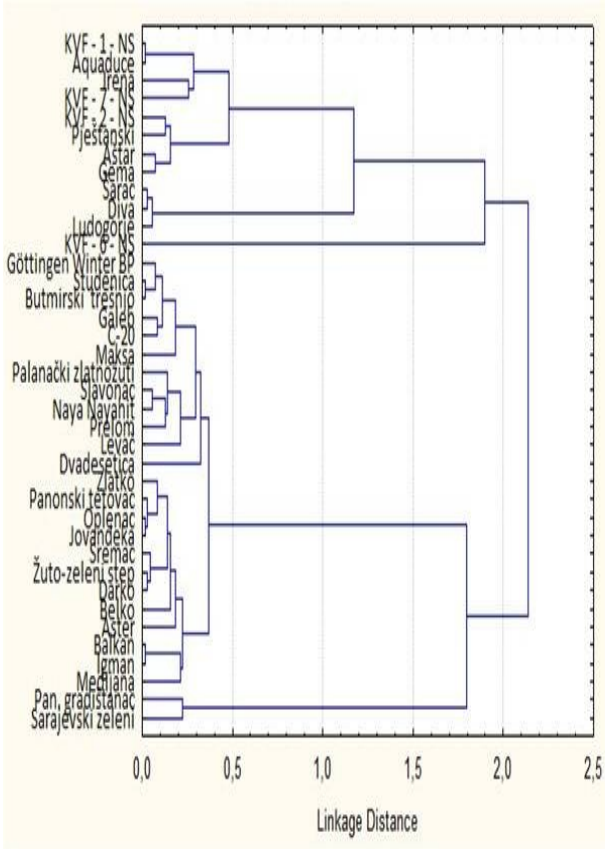

Figure 2. Dendrogram for 38 genotypes of grain legumes according to phytic acid content (mg/g)

DISCUSSION

In the examined samples, only two genotypes were with an indeterminate climbing habitus. Pole, climbing genotypes are present in lower number in the collections (Todorović et al., 2008; Ćota et al., 2008), although they are frequently found among landraces (Vasić et al., 2009).

The phytic acid content in various dry bean genotypes is in accordance with previous investigations of Tepić et al. (2007). Namely, they found that five Serbian dry bean varieties contained 7.20 - 13.20 mg/g. The lower values of the mentioned results are also in accordance with the results of Siddhuraju et al. (2005), who found 9.5 ± 0.5 g/kg. The phytic acid content in dry beans, determined in this research, is somewhat higher than reported by El Maki et al. (2007). Alonso et al. (2000) reported 15.9 g/kg of phytic acid on dry matter basis. Kyriakidis et al. (1998) found an increase in the phytase activity and a decrease in phytate during the germination of four common legumes, reporting a similar phytic acid content in the seeds of runner and faba beans from local markets, similar to our genotypes.

The results about faba beans genotypes obtained in this research are somewhat lower than reported by Fernandéz et al. (1997). Múzquiz et al. (2006), on the basis of the previous research, concluded that faba bean is much richer in phytates in comparison to other grain legume crops. According to the results obtained in this research, faba bean had the lowest values of phytic acid. High content of phytic acid was found only in the Göttingen Winter Bean Population, with a different agronomy, as a consequence of its prominent winter hardiness (Link et al., 2010).

In this research, the phytic acid content varied according to plant species and genotype, from 10.470 (highest in dry bean, Table 1) to 2.106 mg/g (lowest in faba bean,

Table 2). However, the variability of phytic acid content in the same genotype can be caused by other factors as well. Thavarajah et al. (2010) reported that the phytic acid content in lentil seeds was influenced by the temperature during seed filling period, varying from 8.8 mg/g in rising and 6.7 mg/gin the decreasing temperature regime.

As there is a tendency to breed genotypes with low phytic acid in legume seeds (Raboy, 2009; Perrone et al., 2009), the diversity identified among the examined genotypes of the three different grain legume species could be used in the future breeding programs.

CONCLUSIONS

The results of this research, demonstrate large differences in phytic acid content in examined samples of common bean, runner bean and faba bean. *Phaseolus* sp. had higher phytic acid content in comparison to faba beans. Phytic acid content in dry beans varied from 5.324 (Ludogorje, modern Bulgarian variety) to 10.470 and 10.310 mg/g (Panonski Gradištanac, modern Serbian cultivar, and Sarajevski zeleni, BiH landraces, respectively). The two runner bean samples had 8.530 (Darko) and 8.898 mg/g (Igman) phytic acid. Phytic acid content in faba bean samples varied from 2.106 mg/g (landrace Temska, KVF-6-NS, Pirot) to 6.969 mg/g (Göttingen Winter Bean Population). Samples differentiated according to their status (cultivar or landrace), origin, habitus, colour of grain (*Phaseolus* sp.), purpose (*Vicia faba*) and 100 seeds weight, proved to be close in phytic acid content.

Considering consumers consciousness of healthy and functional diet, breeders are required to breed cultivars with lower phytic acid content. The collection examined in this research contains samples that could serve as basis for breeding suitable cultivars of these crops.

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REFERENCES

Alonso, R., Aguirre, A., Marzo, F., 2000. *Effects of extrusion and traditional processing methods on antinutrients an in vitro digestibility of protein and starch in faba and kidney beans*. Food Chem., 68: 159-165.

Anderson, J.W., Smith, B.M., Washnock, C.S., 1999. *Cardiovascular and renal benefits of dry bean and soybean intake.* Am. J. Clin. Nutr., 70: 464S-474S.

Bednar, G.E., Patil, A.R., Murray, S.M., Grieshop, C.M., Merchen, N. R., Fahey, G.C., 2001. *Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model.* J. Nutr., 131: 276-286.

Chen, Q., 2004. *Determination of phytic acid and inositol pentakisphosphates in foods by high-performance ion chromatography*. J. Agric. Food Chem., 52, 15: 4604-4613.

Coelho, C.M.M.C., Santos, J.C.P., Tsai, S.M., Vitorello, V.A., 2002. *Seed phytate content and phosphorus uptake and distribution in dry bean genotypes*. Braz. J. Plant. Phys., 14, 1: 51-58.

Ćota, J., Delilović, M., Vasić, M., Gvozdanović-Varga, J., 2008. *First selectionFirst selection of cultivar Phaseolus coccineus var. nanus in Bosnia and Hercegovina*. Proceedings of the International Conference on Conventional and Molecular Breeding of Field and Vegetable Crops (Ed.: B. Kobiljski), Institute of Field and Vegetable Crops, Novi Sad, Serbia: 452-455.

El Maki, H.B., Abdel Rahaman, S.M., Idris, W.H., Hassan, A.B., Babiker, E.E., El Tinay, A.H., 2007. *Content of antinutritional factors and HCl-extractability of minerals from white bean (Phaseolus vulgaris) cultivars: Influence of soaking and/or cooking*. Food Chem., 100: 362-368.

Fernandéz, M., Aranda, P., López-Jurado, M., García-Fuentes, M.A., Urbano, G., 1997. *Bioavailability of phytic acid phosphorus in processed Vicia faba L. var. major.* J. Agric. Food Chem., 45: 4367-4371.

Geil, P.B., Anderson, J.W., 1994. *Nutritional and health implications of dry beans: a review*. J. Am. Coll. Nutr., 13: 549-58.

Graf, E., Empson, K.L., Eaton, J.W., 1987. *Phytic acid a natural antioxidant*. J. Biol. Chem., 262: 11647-11650.

Haug, W., Lantzsch, H.J., 1983. *Sensitive method for rapid determination of phytate in cereals and cereal products.* J. Sci. Food Agric., 34, 12: 1423-1426.

Kyriakidis, N.B., Galiotou-Panayotou, M., Stavropoulou, A., Athanasapoulos, P., 1998. *Increase in phytase activity and decrease in phytate during germination of four common legumes.* Biotechnol. Lett., 20, 5: 475-478.

Link, W., Balko, C., Stoddard, F.L., 2010. *Winter hardiness in faba bean: Physiology and breeding.* Field Crop Res., 115: 287-296.

Múzquiz, M., Goyoaga, C., Pedrosa, M., Guillamon, E., Varela, A., Cuadrado, C., Burbano, C., 2006. *Nutritionally active factors in faba bean*. International Workshop on Faba Bean Breeding and Agronomy, M.T. Moreno, A.M. Torres (eds.), IFAPA, Córdoba: 15-18.

Perrone, D., D'Onofrio, B., Campion, B., 2009. *Low phytate beans in the field: first results*. Actas SAL, 4: 56-57.

Raboy, V., 2009. *Seeds for a better future: 'low phytate' grains help to overcome malnutrition and reduce pollution.* Trends Plant Sci., 6, 10: 458-462.

Sathe, S. K., 2002. *Dry bean protein functionality*. Crit. Rev. Biotechnol., 22, 2: 175-223.

Šćiban, M.B., Vasić, M.A., Prodanović, J.M., Antov, M.G., Klašnja, M.T., 2010. *The investigation of coagulation activity of natural coagulants extracted from different strains of common bean*. APTEFF, 41: 141-147.

Shimelis, E.A., Rakshit S.K., 2005. *Proximate composition and physico-chemical properties of improved dry bean (Phaseolus vulgaris L.) varieties grown in Ethiopia*. Swiss Soc. Food Sci. Technol., 38: 331-338.

Siddhuraju, P., Becker, K., 2005. *Nutritional and antinutritional composition, in vitro amino acid availability, starch digestibility and predicted glycemic index of differentially processes mucuna beans (Mucuna pruriens var. utilis): an under-utilized legume*. Food Chem., 91: 275-286.

Tepić, A., Vujičić, B., Vasić, M., Lučić, A., 2007. *Amino acids and phytic acid in some Serbian varieties of dry beans (Phaseolus vulgaris).* Proceedings of the 2nd International Congress on Food and Nutrition, Istanbul, Turkey: 180.

Thavarajah, D., Thavarajah, P., Seea, C.-T., Vandenberg, A., 2010. *Phytic acid and Fe and Zn concentration in lentil (Lens culinaris L.) seeds is influenced by temperature during seed filling period*. Food Chem., 122, 1: 254-259.

Thompson, L.U., Zhang, L., 1991. *Phytic acid and minerals: effect on early markers of risk for mammary and colon carcinogenesis.* Carcinogenesis, 12, 11: 2041-2045.

Todorović, J., Vasić, M., Todorović, V., 2008. *Dry and spring bean.* Grafomark, Institute of Field and Vegetable Crops, Novi Sad, Faculty of Agriculture, Banja Luka, Serbia and BiH., 333 p.

Vasić, M. A., Vujičić, B.L., Tepić, A.N., Gvozdanović-Varga, J.M., Šumić, Z.M., 2009. *Dietary fiber content in some dry beans*. APTEFF, 40: 103-110.

Vasić, M., Mihailović, V., Mikić, A., Mladenović, D., Gvozdanović-Varga, J., Jovićević, D., Nikolić, Z., 2008. *Genetic resources of edible grain legumes in Serbia*. Acta Hort., 830: 715-718.

Veličković, D., Vučelić-Radović, B., Blagojević, S., Barać, M., Stanojević, S., Ljubičić, S., 1999. *A modification of a method for phytic acid determination.* J. Serb. Chem. Soc., 64: 303-310.

Vucenik, I., Shamsuddin, A.M., 2003. *Cancer inhibition by inositol hexaphosphate (IP6) and inositol: from laboratory to clinic.* Am. Soc. Nutr. Sci., International Conference on Food, Nutrition, and Cancer, J. Nutr.: 3778S-3748S.