THE EFFECTS OF FERTILIZATION AND VARIETY ON THE ISOFLAVONES OF SOYBEANS

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Abstract: *The aim of this work was to evaluate the influence of year condition, variety and fertilization treatments on content of isoflavones genistein and daidzein in soy seeds. Four soybean varieties Korada, Supra, Alma Ata and OAC Vision were growing at experimental farm Oponice (south-western Slovakia) in 2007-2008. The experimental site belongs to warm and moderate arid climatic region. Soybean was growing after sugar beat forecrop each year. The conventional tillage practices were used. Fertilization treatments were as follows: I. Non-fertilized control, II. LAV 27 % (40 kg ha^{-1} N) in growing stage of first pair of true leaves (BBCH 101), III. Humix komplet (rate 8 L ha^{-1}) applied in growing stage of first pair of true leaves (4 L ha^{-1}) and before flowering (4 L ha^{-1}), IV. DAM 390 (20 kg ha^{-1} of N) applied in growing stage before flowering (BBCH 501). Seeds were inoculated by HiStick preparation. Concentration of daidzein and genistein were determined using a high-performance liquid chromatography. All nitrogen fertilization treatment significantly increases the yield of soy seeds. High significant correlation between daidzein concentration and genistein was confirmed. The positive correlation of daidzein with seed yield suggests that daidzein concentration significantly increased as seed yield went up. In contrast, application of different fertilizers at two development stage of soy plant (BBCH 101 and BBCH501) consistently decreased the daidzein content, except OAC Vision variety with very low content of daidzein, with comparison to variety Supra. Averaged over fertilization treatments content of daidzein and genistein were decrease by nitrogen input relative to zero nitrogen as follows: in LAV treatment 27% of daidzein and 21.9% of genistein; after Humix application daidzein/ genistein content decrease amount 22.3/36.8% and DAM application have decreased the content of daidzein of 8.0% and genistein content of 20.9% soy seeds. On the other hand nitrogen input increased the total production of isoflavones due to significant increasing in soy seeds yield. Appropriate nitrogen management may be among the important factors controlling isoflavones concentration and production.*

Key words**:** *soybean, variety, fertilization, yield, daidzein, genistein, correlation*

INTRODUCTION

Text Soybean [*Glycine max* (L.) Merr.] contents many beneficial components. The consumption of soy products has many health benefits, including protection against breast cancer, prostate cancer, menopausal symptoms, heart disease and osteoporosis. Many of the health benefits of soy are derived from its isoflavones (CHEN, YU-MING ET AL., 2004; ORHAN ET AL ., 2007; LECERF, 2008). Soy contains many types of isoflavones, but the most beneficial are genistein and daidzein mainly in seeds. In soybean, isoflavones are principally found in roots and seeds; however, the metabolite has also been isolated from leaf and stem tissue (ROMANI ET AL., 2003). Isoflavones composition of some American and Japanese varieties were studied previously by WANG AND MURPHY (1994 a, b). Daidzein and genistein, which are isoflavone aglycones having a 3-phenylchroman skeleton, are mainly found in soybeans and soy products (MURPHY AND BARR, 2005). For food nutrition the changes in isoflavones content of soybeans during heating process have to be taken into consideration (KASUGA ET AL., 2006). Because soybean supplies a preponderance of the world's protein and oil and consumption of soy-based foods has been associated with multiple health benefits, the effects of this agronomic

practice on seed quality traits such as protein, oil, and isoflavones should be investigated (LECERF, 2008; CHANG ET AL., 2009).

The aim of this work was to evaluate the influence of year condition, variety and fertilization treatments on content of isoflavones genistein and daidzein in soya seeds.

MATERIAL AND METHODS

Field experiment was established to evaluate the effect of different fertilization treatments on yield and content of isoflavones genistein and daidzein in soya seeds of four soybean varieties at experimental farm Oponice (West Slovakia, 48° 28´N, 18° 9´E)) in 2005-2007. The experimental site belongs to warm and moderate arid climatic region. The average rainfall is 607 mm. The average temperature is 9.5 °C, for the growing season 16.1°C. The main soil type is loamy Haplic Luvisols on loess. Forecrop was sugar beet and conventional tillage practices were used. Mouldboard ploughing in autumn and harrowing in the spring were used. During seedbed preparation pre-emergent herbicides were applied. The sowing/harvest data of growing soybean are as follows: 2007 24 April/1 October, 2008 29 April/3 October. The sowing pattern was 0.6 million of fertile seeds per hectare into the depth 0.05m, row space was 0.125m. The agrochemical soil characteristics before sowing are in table 1.

Table 1

The agrochemical soil characteristics (0.3m soil layer) before sowing in 2007-2008

Year	Content of available nutrients in soil (mg kg^{-1})							Humus %	pH
	N_{an}	N- NH_4^+	N- NO_3^-	P	K	Mg	Ca		
2007	32.90	18.65	14.25	50.0	255.0	300.0	3010	3.31	6.62
2008	27.00	14.82	12.18	60.0	232.0	260.0	2 310	2.42	7.48

Seeds were inoculated by HiStick preparation. The fertilization treatments were as follows: (I) unfertilized treatment; (II) application of nitrogen fertilizers LAV 27 % (40 kg ha^{-1} nitrogen) in the growth stage of first pair of true leaves unfolded – BBCH 101; (III) Humix komplet in split application of total dose 4+4 L ha^{-1} applied in growth stage of first pair of true leaves unfolded – BBCH 101 and in growth stage of first flower buds visible – BBCH 501, (IV) DAM 390 - 20 $kg.ha^{-1}$ N – BBCH 501. Growth stage of soybean was described according (MUNGER ET AL., 1997). Humix complet contain bioactive ingredient and nutrients for plant nutrition with 2.5% of humic acids, 4.0% total N, 0.5 P_2O_5, 3% K_2O, and micronutrients: Fe, Cu, B, Co, Zn, Mn, Mo and pH of 11-13. Grain yield and yield of aboveground biomass and harvest index were determined. The variety Korada, Supra, Alma Ata and OAC Vision were tested. Concentration of daidzein and genistein were determined using a high-performance liquid chromatography. Approximately 1 g of soybean seed from each cultivar was ground to a fine powder. The powder was extracted with 60% methanol. Daidzein and genistein were separated on a HPLC using the following instruments conditions: HPLC, Waters BREEZE with: Waters binary pump 1525, UV-VIS detector 2487, column heater, in-line degasser and Column: PUROSPHER STAR C18 250x4.6 mm, 5 micron particle size. Solvent: A: was 02 % H3PO4 and solvent B: was 60% HPLC grade methanol.

RESULTS AND DISCUSSIONS

The yield of soya seeds was statistically influenced by year condition, growing variety and different type of fertilization. Significantly higher yield in 2007 (3.13 t ha^{-1}) with comparison to 2.85 t ha^{-1} in 2008 was noted. Evaluated soya varieties can be divided according yield potential into two groups. Variety Korada and Supra reached significantly higher yield than Alma Ata and OA Vision (Table 2). All fertilization treatments also significantly increase

the yield of soya seeds in an average. Yield gain range between 0.51–0.82 t ha^{-1}. Previous research in soil fertility and nutrient management has focused mainly on the optimum levels on nutrients for high crop yield. Further understanding of the relationships between isoflavones concentrations and soil and crop management factors is essential. Only few investigations have evaluated nutrient management effects on individual and total isoflavones responses or the relationships of isoflavones with other seed attributes such as yield (VYN ET AL., 2002).

Table 2

The impact of fertilization on yield of soybean seeds 2007-2008. The means between yield of varieties and fertilization followed by the same letter are not significant at P=0.05 probability level

Variety	Control	LAV	Humix	DAM	Yield of variety
Korada	2640	2960	3580	4120	3325b
Supra	2970	3610	2950	3950	3370b
Alma Ata	2220	2860	2610	2750	2610a
OAC Vision	2270	2750	3180	2570	2680a
Fertilization	2525a	3045b	3080bc	3348c	

The relationships between evaluated factors are shown on the Table 3. The positive relationship between yield and daidzein was noted ($r=0.22^{*}$). The positive correlation of daidzein with seed yield suggests that daidzein concentration significantly increased as seed yield went up. This positive relationship between daidzein and seed yield is very encouraging, as it suggest that high soybean yield could be compatible with high quality seeds from an isoflavones based functional food perspective (VYN ET AL., 2002).

Table 3

The correlation coefficients of daidzein and genistein concentration with relationship to yield year condition and fertilization at Oponice (2007-2008). Significance * and **, indicate the treatment effect is statistically significant at P =0.05 and P 0.01, respectively

Factor	Daidzein	Genistein
Yield	0.2182^{*}	0.1171
Year	0.0109	0.0175
Fertilization	-0.0592	-0.2817^{*}
Daidzein		0.7719^{**}

High significant correlation between determined isoflavones content was also revealed. In spite of this, negative correlation between total content of genistein with nitrogen fertilization into soil or plants could be taken into consideration for adoption of appropriate nutrient management from an isoflavones based functional food perspective.

High-performance liquid chromatography was utilized to determine accrual of isoflavones in soybean seed as affected by variety and fertilization. The amount of daidzein and genistein in four soybean varieties under different fertilization and two year conditions are shown in Figure1. A significant difference in isoflavones content was not evident among the seeds harvested in different year 2007 or 2008. The daidzein contents ranged from 132 mg kg^{-1} to 651 mg kg^{-1} of soybean, and the genistein contents ranged from 27.10 mg kg^{-1} to 89.140 mg kg^{-1}.

Contribution of genetic background of varieties on assimilation of isoflavones was evaluated on control treatments without fertilization. Similarly as in yield potential, we recognized two group of germplasm according content of isoflavones. Korada with higher level of daidzein 5.24 – 5.98 mg kg^{-1} and genistein 58.45-60.89 mg kg^{-1} and Supra with higher level of daidzein 7.80 – 8.09 mg kg^{-1} and genistein 59.36-60.31 mg kg^{-1} belongs to first group. The

second group comprises from Alma Ata with lower level of daidzein 4.19-4.20 mg kg^{-1} and genistein 33.63–34.10 mg kg^{-1} and OAC Vision with content of daidzein amount 3.49-3.73 mg kg^{-1} and genistein 42.28-42.54 mg kg^{-1}. In an average of these groups the daidzein content of Korada and Supra varieties is 73% higher and genistein content is about 57% higher than Alma Ata and OAC Vision group of varieties.

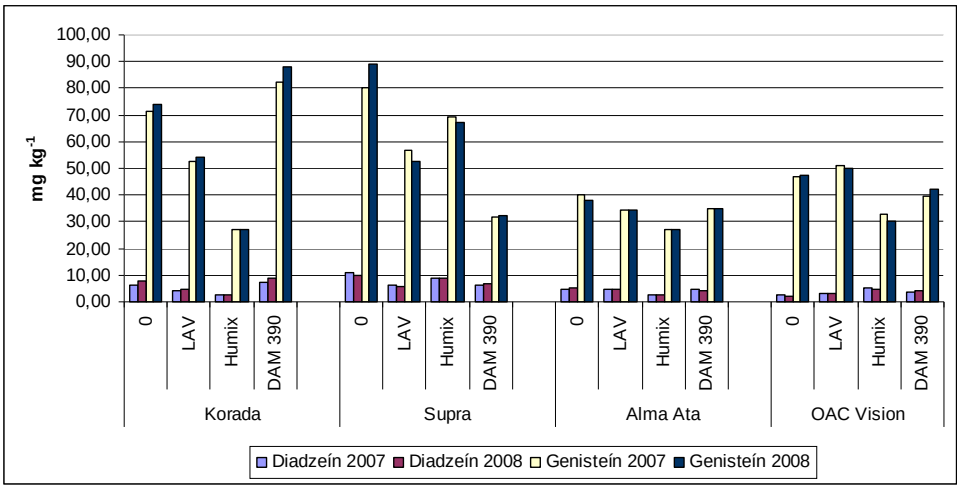

Figure 1 - Assimilation of daidzein and genistein in soybean varieties in seeds under different fertilization treatments. For description of fertilization treatments please see material and method

In soybean, isoflavones are principally found in roots and seeds (HO ET AL., 2002). The increase in isoflavones content, as the result of high yield potential, could arise solely from increased synthesis in the seed components or possible be translocated from distal production sites (BENETTT ET AL., 2004).

In contrast, application of different fertilizers at two development stage of soy plant (BBCH 101 and BBCH 501) consistently decreased the seed isoflavones content, except DAM application in Korado variety. An averaged over two year and four varieties, nitrogen fertilization decreased daidzein and genistein concentrations compared with zero N is shown on the table 4.

Averaged over fertilization treatments content of daidzein and genistein were decrease by nitrogen input relative to zero nitrogen as follows: in LAV treatment 27% of daidzein and 21.9% of genistein; after Humix application daidzein/ genistein content decrease amount 22.3/36.8% and DAM application have decreased the content of daidzein of 8.0% and genistein content of 20.9% soy seeds. On the other hand nitrogen input increased the total production of isoflavones due to significant increasing in soy seeds yield. Nutrient management effects on individual and total isoflavones responses or the relationship of isoflavones with seed yield have to be discus.

In spite of an ambiguous effect of nitrogen on isoflavones concentration total hectare yield of genistein and daidzein was markedly higher in Korada and Supra variety than in Alma Ata and OAC Vision. Appropriate nitrogen management may be among the important factors controlling isoflavones concentration and production.

Table 4

Daidzein and genistein concentration and soya seed yield with relationship to nitrogen fertilization of four varieties at Oponice, averaged over 2007 to 2008

Variety	Year/Fertilization	2007-2008	2007	2008	2007	2008
		Seed yield (kg ha^{-1})	Daidzein (mg kg^{-1})	Daidzein (mg kg^{-1})	Genistein (mg kg^{-1})	Genistein (mg kg^{-1})
Korada	0	2 640	6.50	7.75	71.45	74.00
	LAV	2 960	4.30	4.80	52.70	54.30
	Humix	3 580	2.85	2.60	27.10	27.15
	DAM	4 120	7.30	8.75	82.55	88.10
	Average	*3 320*	*5.24*	*5.98*	*58.45*	*60.89*
Supra	0	2 970	10.85	9.95	79.95	89.10
	LAV	3 610	6.05	5.65	56.55	52.80
	Humix	2 950	9.10	9.00	69.35	67.20
	DAM	3 950	6.35	6.60	31.60	32.15
	Average	*3 370*	8.09	7.80	59.36	60.31
Alma Ata	0	2 220	*4.65*	*5.35*	*40.10*	*38.15*
	LAV	2 860	4.65	4.45	34.30	34.15
	Humix	2 610	2.85	2.60	27.10	27.15
	DAM	2 750	4.65	4.35	34.90	35.05
	Average	*2 610*	*4.20*	*4.19*	*34.10*	*33.63*
OAC Vision	0	2 270	2.60	2.20	47.10	47.40
	LAV	2 710	3.25	3.25	50.90	49.80
	Humix	3 180	5.20	4.50	32.80	29.95
	DAM	2 570	3.85	4.00	39.35	41.95
	Average	*2 680*	3.73	3.49	42.54	42.28
	Total average	*3 013*	*5.31*	*5.36*	*48.61*	*49.28*

CONCLUSIONS

The evaluate varieties were divided according their yield and isoflavones content into two separate groups. Korada and Supra varieties (as first group) have significantly high yield potential and also considerably higher content of isoflavones than second group of Alma Ata and OAC Vision on an average. Significant yield responses to different N fertilization manner were observed at all treatments relative to those of zero nitrogen. Positive relationship between daidzein and seed yield suggest that high soybean yield could be compatible with high quality seeds from an isoflavones based functional food perspective. Negative correlation between total content of genistein with nitrogen fertilization into soil or plants could be taken into consideration for adoption of appropriate nutrient management from an isoflavones based functional food perspective.

Acknowledgement: This paper was supported by VEGA project N 1/0816/11 "Production process of field crops at different tillage systems, application of fertilizers and plant residues to maintenance and increasing of soil fertility.

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