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Article · January 2007

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Isoflavones Concentration in Popular Soybean Genotypes of Malwa Region in Central India

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Received : 30.12.2007

ABSTRACT

Nine popular soybean genotypes of the Malwa region were grown in the fields in kharif 2007. The seeds of different genotypes were harvested at their respective maturity and subjected to estimation of isoflavones using High Performance Liquid Chromatography (HPLC). Genotypic variability was observed for daidzein, glycitein, genistein and total isoflavones contents and the range for these were: 95.5-624.4; 154.5-517.1, 79.7-817, 477.9-1731.4 µg/g soy flour, respectively. 'Samrat' and 'NRC 7' exhibited the lowest levels of daidzein and total isoflavones while 'JS 97 - 52' exhibited the lowest value for genistein. Genotypes 'JS 80 - 21', 'JS 71 - 05' and 'MAUS 61 - 2' were the top 3 cultivars for total isoflavones content. Correlation studies indicated that breeding soybean genotypes with early maturity as well as with low isoflavones content may be easier.

Key words: Daidzein, genistein, glycitein, *Malwa* region, soybean

Isoflavones are flavonoids compounds, with two benzyl rings joined by three-carbon chain (C_6-C_3-C_6). They differ from flavones in the site of attachment of second benzyl ring (B) which is joint at position 3 instead at position 2 (Fig 1). Soybean owes much of its 'functional food' sobriquet to the versatility of isoflavones present in its seeds. Isofavones as antioxidant compounds reduce the risk of prostrate and colon cancer (Sarkar and Li, 2004) and cardiovascular diseases (Clarkson *et al.*, 2002); and as estrogen-like compounds mitigate the post-menopausal blues and prevent the incidence of osteoporosis and hormone-related breast, ovarian and uterine cancers. Specially designed dietary supplements with soy isoflavones concentrate have already arrived in the market for this purpose. However, concerns have been raised about the possible adverse effects of isoflavones on foetal development and infants fed on soy-based formulae (Mendez *et al.*, 2002; Chen and Rogan, 2004) so much so that some

Fig. 1. Structure of isoflavones

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countries have recommended safe upper limit for daily intake of isoflavones (Morandi *et al.*, 2005). Besides, high level of isoflavones in certain genotypes is considered to be responsible for astringency associated with soy products (Kudou *et al.*, 1991). Therefore, the development of soybean genotypes with low as well as high levels of isoflavones is important breeding objective to meet the requirement of different end users. The isoflavones in soybean exist as free aglycones (daidzein, glycitein, genistein, daidzein), as glucoside (genistein, daidzein and glycitein) when sugar is conjugated to aglycones and as malonyl and acetyl derivatives of glucoside (Griffth and Collison, 2001). However, free alglycone forms, *i. e.* daidzein and genistein are stable and have physiological activities superior to other isoflavones (Akiyama *et al.*, 1987).

Soybean is being increasingly sought as health food across the globe, going beyond the traditional expanses of China, Japan and Southeastern countries. In India too, the dismal scenario of soybean in food uses has been constantly improving as people are becoming increasingly aware of the health benefits of soybean. *Malwa* is the hub of soybean cultivation in India and the seeds and defatted meal of the soybean genotypes grown by the farmers in this region reach soy food processing units located in the regions and neighbouring states as well. Therefore, isoflavones levels in the seeds of soybean cultivars of the region would also help to indicate the concentration of isoflavones in different soy-products processed in the region. Recently, genotypic variability for isoflavones concentration has been reported in the major soybean growing countries (Seguin *et al.*, 2004; Craig *et al.*, 2005); however, the investigation of Indian soybean genotypes for isoflavone concentration has not yet been conducted. Therefore, it was felt pertinent to analyze the popular soybean genotypes of the *Malwa* region for isoflavones content.

MATERIAL AND METHODS

Nine soybean cultivars, *viz.* 'JS 335', 'JS 93 05', 'NRC 7', 'JS 71 05', 'JS 97 52', 'JS 80 21', '*Samrat*', 'MAUS 61 2' and 'NRC 37'of *Malwa* region were raised in the fields of National Research Centre for Soybean, Indore, in *kharif* 2007 following recommended agronomic practices. Barring '*Samrat*', which is a farmers' selection, remaining all 8 genotypes are released varieties. Standards of the isoflavones (daidzein, glycitein and genistein) were procured from Sigma Aldrich.

The days-to-flower (DF) and the days-to-maturity (DM) of each of the 9 genotypes were recorded. The harvested seeds were ground into fine flour with metallic pestle and mortar and passed through a sieve of 500μ. The finely ground soy-flour was used subsequently for the extraction of the isoflavones.

Extraction of isoflavones: 125 mg of the finely ground soy-flour was extracted with 80 per cent ethanol (8 ml) and concentrated hydrochloric acid (2 ml) for 2 h in a boiling water bath (Vyn *et al.*, 2002), which relies on acid hydrolysis of

Table 1. Isoflavone concentration* in 9 popular soybean genotypes of *Malwa* region with their respective days-to-flowering (DF) and days-to-maturity (DM)

Genotype	DF	DM	Isoflavones			
			Daidzein	Glycitein	Genistein	Total
JS 335	47	105	317.6 ± 10	517.1 ± 13	615.2 ± 19	1449.9
JS 93-05	47	95	236.3 ± 15	252.3 ± 18	398.4 ± 22	887.2
NRC 7	36	88	97.2 ± 12	240.0 ±11	153.2 ± 27	490.4
JS 71-05	45	95	377.4 ± 9	493.6 ± 10	817.0 ± 21	1688.0
JS 97-52	48	96	417.9 ± 12	378.0 ± 10	79.7 ± 24	875.6
JS 80-21	51	110	452.5 ± 8	507.9 ± 15	770.8 ± 21	1731.2
Samrat	35	87	95.5 ± 12	154.5 ± 16	228.0 ± 15	478.0
MAUS 61-2	57	103	484.0 ± 18	436.8 ± 14	757.3 ± 13	1678.1
NRC 37	53	115	624.4 ± 10	270.0 ± 14	739.0 ± 36	1633.4

**µg/g ± SD of soy flour (dry weight basis); the value given are the mean values of duplicate samples*

Table 2. Significant correlations among isoflavones, total isoflavones, days-to flowering (DF) and days-to-maturity (DM)

	Glycitein	Genistein	Total isoflavones	DF	DM
Daidzein	-	0.655*	0.829**	0.750*	0.897**
Glycitein	-	-	0.773*	-	-
Genistein	-	-	0.938***	0.658*	0.676*
Total isoflavones	-	-	-	-	0.822**
DF	-	-	-	-	-
DM	-	-	-	-	-

, **, * indicate the significance for P < 0.05, < 0.01 and < 0.001, respectively*

ranged from 1160 to 3090 µg per gram in 4 soybean cultivars in 4 environments. Wang and Murphy (1994) analyzed total isoflavone concentration in 3 Japanese cultivars and 8 American cultivars. In Japanese cultivars, total isoflavones concentration ranged from 1261 to 1417 µg per gram soy-flour while in 7 American cultivars, it ranged from 2053 to 4216 µg per gram soy-flour. Hoeck *et al.* (2000) studied the influence of genotype on isoflavone concentration in 6 cultivars grown at 6 different locations for 2 consecutive years. Genotypic variation was observed for total isoflavones at all 6 different locations. Lee *et al.* (2003)

13 endogeneous isoflavones isomers to their respective aglycone form, i.e. daidzein, glycitein and genistein. The suspension resulted after the extraction was centrifuged at 10,000 rpm.

HPLC analysis of isoflavones: The supernatant obtained after centrifugation was passed through the PTFE syringe filter (Whatman 0.5 micron, 13 mm diameter) before loading into the HPLC system. A 20 µl of the syringe-filtered sample was injected into a Shimadzu chromatographer (LC-10AT *VP*), equipped with a UV detector (SPD 10AT VP) and oven (CTO-10) housing a C-18 silica column (Phenomenex; 5 µM with dimension of 250 mm x 4.6 mm), which was preceded by a guard column (Phenomenex 4.0 mm x 3.0 mm). The column was maintained at 40°C. The separation and elution of isoflavones was accomplished by employing binary gradient mode with solvent A (10 % Acetonitrile) and solvent B (38% Acetonitrile) at a flow rate of 0.8 ml per minute for 25 minutes. The solvent system was run as follows: [(% solvent A/ solvent B; 0 min (0/100), 5 min (10/90), 20 min (0/100) and 25 min (0/100)]. The isoflavones were detected at a wavelength of 260 nm. Standard curves for daidzein, glycitein and genistein were prepared by injecting varying concentrations of isoflavones standards into the HPLC system. The relative concentration of individual isoflavone in the sample was calculated by Spinco software CSW version 1.7 after superimposing the chromatograph of the sample on the standard curve. Individual isoflavone concentration was expressed as µg per gram of soy-flour on dry weight basis. Concentrations of aglycones were summed up to compute the total isoflavone concentration.

RESULTS AND DISCUSSION

Concentration of individual isoflavones in 9 soybean cultivars was determined by HPLC (Table 1). Individual and total isoflavones were expressed as µg per gram soy-flour. Daidzein concentration ranged from 95.5 (*Samrat*) to 624.4 (NRC 37) with mean value of 344.7 µg per gram soy-flour. The ratio between the lowest and the highest value of daidzein was computed to be 1:6.5. Glycitein concentration ranged from 154.5 (*Samrat*) to 517.1 (JS 335) with mean value of 361.1 µg per gram soy-flour. The ratio between the lowest and the highest was 1:3.3. Genistein concentration ranged from 79.7 (JS 97 52) to 817 (JS 71 05) with mean value of 506.5 µg per gram soy-flour. The ratio between the lowest and the highest value was 1:10.3. Total isoflavones concentration ranged from 478 (*Samrat*) to 1731.2 (JS 80 21) with a mean value of 1212.3 µg per gram soy-flour. The ratio between the lowest and the highest value of total isoflavones worked out to 1:3.62. Of the 9 genotypes analyzed, 4 genotypes exhibited total isoflavones concentration more than 1500 µg per gram soy-flour, 1 cultivar between 1000-1500 µg per gram soy-flour and 2 cultivars each for 500-1000 and less than 500µg per gram soy-flour. Eldridge and Kwolek (1983) reported that total isoflavone concentrations in soybean seeds

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evaluated 15 Korean soybean cultivars in 3 consecutive years and observed a range of 188.4-483.0 mg per 100 grams in 1998; 218.8- 948.9 mg per 100 grams in 1999 and 293.1-483.0 mg per 100 grams in 2000. Seguin *et al.* (2004) reported isoflavone concentration in 20 soybean cultivars grown in eastern Canada region across two years ranging from 360 to 2241 μg per gram of soy-flour. Recently, Craig *et al.* (2005) analyzed isoflavones concentration in the seeds of 17 soybean cultivars and found that the total isoflavone concentration ranged from 2038 to 9514 μg per gram with an average value of 5644 μg per gram of the soy flour.

A negative correlation between oil content and isoflavones concentration has earlier been reported (Craig *et al.*, 2005). In the present study, 'NRC 7' which exhibited the lowest concentration of genistein and also ranked second lowest for daidzein concentration and total isoflavones concentration has earlier been reported for high oil content (Kumar *et al.*, 2007). In addition, 'NRC 7' and '*Samrat*', which exhibited comparatively low level of individual isoflavones and total isoflavones in the study, are both early maturing genotypes. This is in consonance with the earlier study (Wang *et al.*, 2000) that indicated a lower isoflavones concentration in early maturing varieties of soybean.

Significant correlations were observed among individual isoflavones, total isoflavones, days-to-flowering (DF) and days-to-maturity (DM) of the different soybean genotypes (Table 2). Both daidzein and genistein exhibited significant ($P < 0.05$) positive correlation with DF. DM was significantly correlated with daidzein, genistein and total isoflavones, with daidzein exhibiting the strongest ($r = 0.897$; $P = 0.001$) followed by total isoflavone ($r = 0.822$, $P = 0.006$) and genistein ($r = 0.676$; $P = 0.041$). All the three individual isoflavones exhibited significant correlations with total isoflavones; however, highly significant correlation ($P < 0.001$) was observed between genistein and total isoflavones content. Furthermore, significant correlation was observed between individual isoflavones, *i.e.* between daidzein and genistein, which was expected as the isoflavones are synthesized *de vovo via* a common phenylpropanoid pathway.

Conclusively, among the major soybean genotypes grown in *Malwa* region, soy-food industries located in the region and interested to manufacture foods with low levels of isoflavones may select 'NRC 7 'and '*Samrat*' as raw seed material for processing into soy-foods while seeds of 'JS 80 - 21' and 'JS 71 - 05' would constitute suitable raw material for pharmaceutical industries to realize maximum isoflavones extraction. Correlations presented in the study also suggest the convenience to develop soybean varieties with early maturity and low isoflavones levels by the plant breeder.