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EVALUATION OF JAPANEASE SOYBEAN [*Glycine max* (L.) Merrill] GENOTYPES GROWN IN INDIAN CONDITIONS FOR PHYSICAL AND BIOCHEMICAL CHARACTERISTICS

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Increasing global awareness about soybean as a 'functional food' that reduce the risk of breast cancer, uterus cancer, osteoporosis, cardiovascular diseases, diabetes, the predominant fatal diseases of the century, has generated an interest in soy-foods in India too. However, efforts for genetic improvement of soybean in India, hitherto, were focused on crop yield rather than delivering food processors' beans suitable for food uses. Soybeans that are large in seed size, light seed coat color, clear hilum, high protein content and low in trypsin inhibitor and lipoxygenase isozymes are preferred by soy food processors (Liu, 2004). Large seed size and light colored beans with higher protein content not only give whiter soy milk and tofu but also tend to produce soy milk or tofu with higher protein content and improved yield (Liu, 1995; Liu 2004).

Lipoxygenase (EC1.13.11.12), constituting 1-2% of total soybean seed protein (Kitamura, 1984), catalyses the oxidation of polyunsaturated fatty acids containing *cis cis 1,4 pentadiene* moiety. Resultant volatile carbonyl compounds are the major contributors to off-flavour development in different soy-products (Rackis *et al.*, 1979) to which Indians are not accustomed to. Lipoxygenase in soybean seeds is present in the form of three isozymes *i.e.* Lox-I, Lox-II and Lox-III (Axlerod *et al.*, 1981) categorized into two classes. Class I is characterized by high pH optima of around 9.0 such as lipoxygenase-I (Lox-I) while class II designates pH optima of around 7.0 such as lipoxygenase-II and III. Trypsin inhibitor, an antinutritional factor present in soybean seed, affects protein digestibility and though, it is heat labile but the heat inactivation insolubilizes the much-valued soy proteins (Anderson, 1992), causing loss of essential amino acids in soy proteins (Rio-Iriarte and Barnes, 1996). In the back drop of unavailability of the above-mentioned desirable food-grade characteristics in the Indian cultivars, few Japanese genotypes which are commercially grown for food uses in Japan were procured under Indo-JICA (Japanese International Co-operative Agency) programme. In view of the fact that growing environment influences various biochemical constituents of seed (Nielson, 1996; Kumar *et al.*, 2003), these procured genotypes were evaluated for various quality characteristics after growing in Indian climatic conditions.

Of the thirteen Japanese genotypes, twelve genotypes viz. Akiyoshi, Akishengoku, Boiling type, Enerie, Fukuyutaka, Hakucho, Hatsataka, Hougyoku, Hyuuga, Kegone, Sapori Midori,Toyoshirome were procured from Japanese International Co-operative Agency and one Japanese genotype (PI 542044) was obtained from United States Department of Agriculture. Akiyoshi, Enerie, Fukuyutaka, Hyuuga and Hougyoku are the genotypes identified for tofu-making (Zhou *et al.* 2002) while Sapori Midori and Kegone are vegetable-type genotypes. All these 13 imported genotypes and JS 335, the most popular Indian soybean cultivar, were grown in the fields of National Research Centre for Soybean in Kharif 2003. On maturity, all the genotypes were hand harvested and were analysed for various physical parameters viz. seed size, hilum colour and biochemical parameters viz. protein content, oil content, fatty acid composition, tryspin inhibitor and lipoxygenase isozymes.

Physical Characteristics : 100 seed weight for each genotype was recorded on dry weight basis. Minimum twenty seeds were observed for seed coat and hilum color for each genotype.

Protein content : Protein content was determined as 5.71 times the micro-Kjeldahl nitrogen content.

Fatty Acid Analysis : Oil was extracted from freshly ground seed flour using petroleum ether (boiling point 40-60^{0}C) and transesterified in methanol with 1N sodium methoxide as catalyst (Ludy *et al.*, 1968). Fatty acid methyl esters (FAMEs) prepared were separated and analyzed in gas liquid chromatograph (GLC), Shimadzu GC 17A, using capillary column with length and diameter of 30 meter and 0.32 millimeter, respectively. Data given in Table 2 for different fatty acids are mean of triplicate determinations.

Sample preparation for trypsin inhibitor and lipozygenase isozymes : Freshly harvested seeds were ground with pestle and mortar and defatted with pertoleum ether (40-60°C) until soy flour becomes fat free to ascertain the absence of fats interfering in the analysis. The air-dried samples were sieved through 150 mesh size.

Quantitative estimation of tryspin inhibitor : One gram of defatted sieved soy flour was extracted with 50 ml NaOH (.01 N) for 4 hrs with constant stirring at 125 rpm in an orbital shaker so as to keep the samples in suspension. The suspension so obtained was appropriately diluted so that 2 ml of the sample extract inhibited 40-60% of the trypsin used as a standard in the analysis. Trypsin inhibitor activity was determined by standard procedure (Kakade *et al.*, 1974) as modified by Hammerstrand *et al.* (1981).

Table 2. Fatty acid composition of Japanese soybean genotypes

Genotype	Percent Fatty Acids				
	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3
Akiyoshi	13.5	4.3	17.6	58.5	6.1
Akishengoku	10.7	3.1	37.5	41.9	5.6
Boiling	13.0	2.6	27.6	49.7	7.1
Enerei	10.4	1.9	37.8	42.7	6.0
Fukuyutaka	10.4	2.6	36.3	45.1	4.8
Hyuuga early	12.2	2.8	23.3	55.5	6.2
Hougyoku early	11.6	3.5	16.4	60.2	7.3
Hakucho	13.1	3.4	23.7	52.2	7.5
Hatsataka	11.5	2.9	27.1	50.4	7.0
Kegone	12.3	2.5	34.1	43.6	6.4
Sapori Midori	11.3	3.0	35.5	42.9	6.4
Toyoshirome	12.1	2.8	26.5	50.4	7.1
PI 5420044	10.6	1.9	22.0	60.7	4.8
JS 335	13.1	2.3	37.4	41.7	5.5

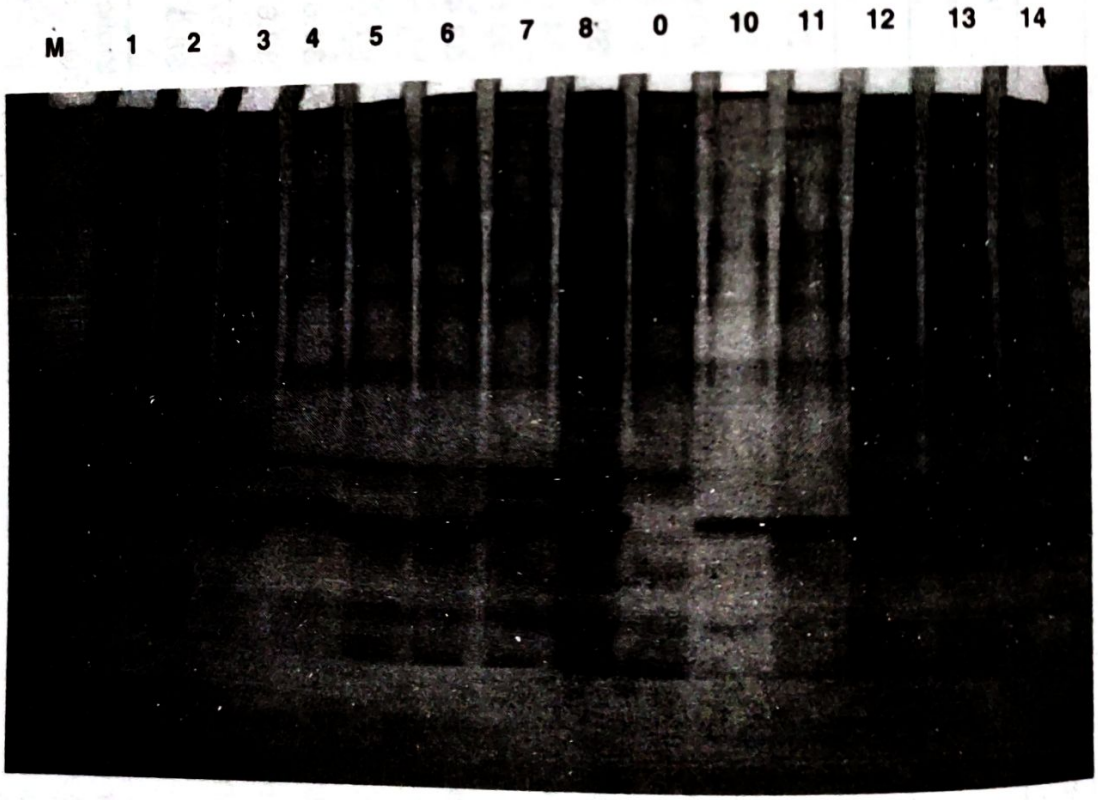

Fig. 1. Lane M represents marker for Kunitz inhibitor and lanes 1-14 represent Akiyoshi, Akishengoku, Boiling type, Enerie, Fukuyutaka, Hougyoku, Hyuuga, Hakucho, PI 542044, Kegone, Sapori Midori, Hatsataka, Toyoshirome and JS 335 respectively

Table 1. Physical and biochemical characteristics of Japanese cultivars

Genotype	Physical parameters			Biochemical parameters				
	100 SW g	Seed coat	Hilum color	Protein %	Trypsin inhibitor[a]	Lipoxygenases[b]		
						Lxl	Lxll+lll	Total lox
Akiyoshi	20.2	Yelow	light brown	38.8	104.4	1348	412	2160
Akishengoku	16.7	Yellow	light brown	39.0	81.1	1156	370	1526
Boiling type	21.3	Yellow	clear	40.0	49.0	1132	250	1382
Enerei	22.6	Yellow	clear	38.6	40.72	1184	346	1530
Fukuyutaka	24.3	Yellow	clear	39.4	74.2	1224	424	1648
Hyuuga early	27.4	creamy yellow	clear	34.6	109.3	1624	860	2484
Hougyoku	18.7	Yellow	light brown	38.8	117.5	1868	396	2264
Hakucho	27.6	Green	black	33.4	70.83	1952	728	2680
Hatsataka	19.5	Green	black	33.0	57.3	1352	788	2140
Kegone	20.8	Yellow	Clear	37.0	60.0	880	525	1405
Sapori Midori	22.1	Green	light brown	34.6	67.5	1220	358	1578
Toyoshirome	24.5	creamy yellow	clear	33.6	100.2	1160	336	1496
PI 5420044	15.5	yellow	black	39.57	23.0	1396	392	1788
JS 335	14.1	yellow	black	38.0	45.8	1360	239	1599

[a]mg per gram of defatted soy flour and values are man of triplicate observations
[b]units per g of defatted soy flour and values are mean of triplicate observations

Lipoxygenase-I was observed to range from 880-1952 units/g defatted soy flour for cultivars Kegone and Kakucho respectively while lipoxygenase II+III was observed to range from 250-860 units/g of defatted soy flour for cultivar boiling type and Hyuuga early respectively. Total lipoxygenase activity was observed to range from 1382-2680 units/g of defatted soy flour for genotype Hakucho and Boiling type respectively. Averaged over thirteen genotypes, lipoxygenase-1 and lipoxygenase II+III showed values of 1368 and 480 units/g defatted soy flour respectively. Sapori Midori and Kegone, the two Japanese vegetable-type cultivars, didn't show lower levels of lipoxygenases.

Table 2 indicates the fatty acid composition of genotypes studied. Percent palmitic acid, stearic acid, oleic acid, linloleic and linolenic acid among Japanese cultivars ranged from 10.4-13.5, 1.9-4.3, 16.4-37.8, 41.9-60.7 and 4.8-7.5 respectively. Highest value for oleic acid was observed in Enerie while the lowest value for oleic acid was observed in Hougyoku. Hakucho and Fukuyutaka showed maximum and minimum values for linolenic acid. As evident from Table 2, neither any Japanese genotype nor JS 335 exhibited linolenic acid value less than 4% and oleic acid higher than 50% as desired for improved oxidative stability of soybean oil and better storability of full fat soy-flour products.

PI542044 is suggested to be used for introgressing null kunitz inhibitor character into popular Indian cultivars while Toyoshirome, Hyuuga early, Enerie and Fukuyutaka may be used for breeding for bolder seed size and clear hilum. Sensory evaluation of various soy products viz. soy milk and tofu prepared from Toyoshirome and Hyuuga early which possess bolder seed size, creamy seed coat and clear hilum need to be undertaken.

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Qualitative analysis of Kunitz inhibitor : Kunitz inhibitor from mature seed was extracted in Tris-C1 buffer (100 mM, pH 6.8) containing 0.23 M $CaCl_2$ and 5 mM phenyl methyl sulfonyl chloride (PMSF) following Kollipara *et al.*, (1991) and was resolved using non denaturing discontinuous polyacrylamide slab gel consisting of 5% stacking gel (pH 6.8) and 10% resolving gel (pH 8.8) as given by (Laemmli, 1970). Image was captured in GeneGenius bio-imaging system of Syngene.

Estimation of lipoxygenase isozymes : Soybean extract was prepared by homogenising sieved defatted soy flour with 100 volumes of phosphate buffer (0.2 M, pH 6.8) in a microtissue homogenizer for 20 min at 0.4^0C. The supernatant was used as the crude extract for assaying lipoxygense isozymes using the standard method (Axelord *et al.*, 1981).

Table 1 represents the physical characteristics of seeds of different genotypes studied. Among 13 Japanese geotypes, 100 seed weight was observed to range from 16.7-27.6 g with an average value of 21.6g which is much higher than the 100 seed weight of JS 335 (14.1g). Maximum and minimum 100 seed weight was observed for Hakucho and Akishengoku, respectively. Hyuuga early, Toyoshirome, Enerie, Fukuyutaka were observed to possess not only bolder seed size but clear hilum as well, an important characteristic of food-grade soybean (Liu, 2004). Of these four genotypes, Toyoshirome and Hyuuga early also possessed creamy seed coat color, a preferred characteristic for soy milk and tofu-making. None of Indian cultivars has been reported to possess clear hilum.

Percent protein content was observed to range from 33.0-40.0 respectively with an average value of 33.9 which is less than the protein content of JS 335 (38%), the variety which covers about 70% of the cultivated area under soybean in India. Trypsin inhibitor content ranged from 23-117.5 mg per gram of defatted soy-flour for genotype P1542044 and Hougyouku respectively with an average value of 73.3 mg/g.When all the genotypes were tested electrophoretically (PAGE) using kunitz inhibitor as marker protein, PI542044 did not exhibit kunitz inhibitor band (Fig. 1). Trypsin inhibitor activity has been ascribed to two polypeptides viz. kunitz inhibitor (20kD) and bowman birk factor (8 kD) (Liu, 1997). The former constitutes about 80% of total trypsin inhibitor activity (Moreira, 2004). The trypsin inhibitor activity observed in genotype PI542044 may be attributed to the presence of bowman-birk factor, the polypeptide with anti carcinogenic property. As evident from Fig. 1, two electrophoretically distinguishable variants of kunitz inhibitor were also observed. Genotypes Akiyoshi, Boiling type, Fukuyutaka, Hougyouku and JS 335 were observed to possess fast moving variant (Rm=0.77) while remaining showed slow moving variant (Rm=0.75).

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