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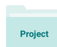

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Genotypic Variation in Lipoxygenase Isozymes' Activity Among Indian and Imported Food Grade soybean accessions

Lipoxygenase (EC 1.13. 11. 12), an iron containing dioxygenase, that catalyses the oxidation of polyunsaturated fatty acids containing *cis cis* 1,4 pentadiene moiety constitutes about 1-2% of total soybean seed protein (Kitamura, 1984). Lipoxygenase in soybean seeds is present in the form of three isozymes i.e. Lox-I, Lox-II and Lox-III (Siedow *et al.*, 1991) which have been categorized into two classes. Class I is characterized by high pH optima of around 9.0 and formation of large amounts of 13-hydroperoxides such as lipoxygenase - I (Lox-I) while class II designates pH optima of around 7.0 and formation of equal amounts of 9 and 13-hydroperoxides such as lipoxygenase- II and III.

Globally, consumption of soy-foods is being encouraged as one of the most economical, nutritious and functional food, however, off-flavour associated with soy products, ascribed to soybean seed lipoxygenases, is the prime deterrent in their wider acceptance of soy-foods in many countries including India. Major contributors to this off-flavour are volatile carbonyl compounds formed as a result of oxidation of linoleic and linoleic acid by seed lipoxygenases (Rackis *et al.*, 1979). However, the ability of lipoxygenases to bleach carotenoid pigments to produce whiter bread and to catalyze oxidation and subsequent cross linking of wheat gluten thereby enhancing bread texture does underline the importance of these isozymes in bread and bakery industry (Eskin *et al.*, 1977). Furthermore, the role of soybean seed lipoxygenase in seed deterioration has also been implicated (Bewley *et al.*, 1986). During storage and transport, due to slight mechanical or bacterial damage, the polyunsaturated fatty acids in the membrane bound and storage lipids in seed become favorable substrates for lipoxygenase to catalyze oxidation. Free radicals so generated set the chain reaction of oxidation of membrane lipids ultimately disrupting membrane integrity (Vick and Zimmerman, 1987).

Heat treatments traditionally used to inactivate lipoxygenases in soy-food processing is not only expensive but also results in insolubilisation of other soybean proteins (Macleod and Ames, 1988). Thus, barring bread and bakery industries where soybean varieties with higher lipoxygenases are preferred, genotypes with genetically lower lipoxygenases are desired not only for producing soy products with reduced beany flavour but for better storability of soybean seeds as well.

Though, lipoxygenase levels of some of the released soybean cultivars was reported earlier (Kumar *et al.*, 2002), it was considered pertinent to evaluate all the released Indian soybean cultivars (75) and some of the recently imported food-grade accessions to identify soybean lines with low and high lipoxygenases for breeding purpose.

Seeds of all the released Indian cultivars (75) maintained at National Research Centre for Soybean, some of the important food-grade accessions (PI 133226, PI 408251, PI 596540, PI 417458, PI 086023) imported from USDA through National Bureau of Plant Genetic Resources, New Delhi and two Japanese vegetable cultivars Sapori Midori and Kegone obtained from Japanese International Cooperation Agency were grown in the fields of National Research Centre for Soybean., Indore on 24th June 2003. For determination of lipoxygenase isozymes, freshly harvested seeds were ground. The ground soy flour was defatted with petroleum ether at 0-4°C following Marczy *et al.* (1995) until soy flour became fat-free to ascertain the absence of fats interfering in the analysis. The air dried samples were sieved through 150 mesh size.

The soybean extract was prepared by homogenizing sieved soy flour with 100 volumes of phosphate buffer (0.2 M, pH 6.8) in a microtissue homogenizer for 20 min at 0-4°C. The homogenized solution so obtained was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant so obtained was used as the crude extract for assaying lipoxygense isozymes following the standard method (Axelrod *et al*, 1981). Reaction mixture for lipoxygenase-I consisted of crude extract as enzyme source (25 micro litre), boric acid borax buffer (0.2 M, pH 9.0) and 10 mM sodium linoleate as a substrate. Lipoxygenase - II and III were

analysed collectively with the reaction mixture consisting of crude extract as enzyme source (50 microlitre), phosphate buffer (0.2 M, pH 6.8) and 10 mM sodium linoleate as a substrate. The change in absorbance was recorded in Shimadzu UV-160 pectrophotometer at 234 nm. One unit of enzyme was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 per min at 234 nm.

Analysis reflects a large variation for lipoxygenases among released soybean cultivars and imported accessions. In all the released cultivars and advanced Indian soybean lines lipoxygenase-I varied from 492 to 3233 units g^{-1} defatted soy flour while lipoxygenase II+III varied from 136 to 852 units g^{-1} of defatted soy flour. The lowest activity for lipoxygenase-I was observed in MACS 58 while that for lipoxygenase II+III was observed in Birsa soya 1. The highest activity for lipoxygenase-I was observed in JS 90-41 while the highest lipoxygenase II+III activity was observed in VLSI. Total lipoxygenase activity was observed to range from 714-3825 units per gram of defatted soy flour and was found to be the highest in JS 90-41 while MACS 58 showed the lowest level. Cultivars JS 90-41, NRC2, PK262, Pusa 40, Pusa 24, MAUS 61-2, ADT-1, JS 93-05, MAUS 32, MAUS1 and PK1042 showed comparatively very high activity of total lipoxygenase isozymes while MACS 58, Pbl, JS2, MACS 450, Hardee, Shilajeet, KHSb2 exhibited comparatively lower levels of total lipoxygenases. Cultivar, JS 335, the most popular variety with wide adaptability in different soybean growing regions in the country showed moderate levels of lipoxygenase activity. MAUS 61-2 and JS 93-05 are newly released varieties of soybean. Pbl and Shilajeet, which showed lower levels of lipoxygenases in the present study are in consonance with earlier report (Kumar *et al.*, 2002), however, the activities' levels are different. This may be because of varying climatic conditions prevailing during developmental stages in two cropping years as earlier studies have indicated the influence of growing environment on lipoxygenase isozymes' activity (Kumar *et al.*, 2003).

Furthermore, varieties Pusa 40, PK262, Pusa 20 which showed comparatively very high level of lipoxygenases in our study have also been observed to possess lower seed longevity (an index of seed deterioration) under accelerated aging while varieties with lower level of total lipoxygenase activity observed in our study *viz.* Pbl, Hardee has been reported to possess high longevity (Bhatia, 1996).

Japanese vegetable-type cultivars *viz.* Sapori Midori and Kegone did not show significant lower levels of lipoxygenases. Among imported accessions, PI 133226 showed the lowest activity for lipoxygenase-1 and total lipoxygenases activity whereas PI 596540 and PI 686023 showed very low activity for lipoxygenase II +III. In general, lipoxygenase-I showed a greater variation as compared to lipoxygenase II +III among different varieties.

In view of the results obtained, PI 596540, PI 086023 and PI 133226 may be considered suitable genetic material for development of varieties with low levels of lipoxygenases while some of the varieties showing comparatively very high lipoxygenases activity *viz.* NRC2, PK262, Pusa-40 may be used in bread making and bakery industries where high lipoxygenases activity is desirable.

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