Research Article

Genetic Variability for the Activities of Soybean Seed Lipoxygenase-1, Lipoxygenase-2 and Lipoxygenase-3 Isozymes

Poorva Bajpai, Anita Rani, Aseem Kumar Anshu, Trupti Tayalkar, Pramod Pimpale and Vineet Kumar*

Crop Improvement, ICAR-Indian Institute of Soybean Research, Indore, India

Abstract

Soybean seed lipoxygenases are undesirable as they generate off-flavor in soy products and affect seed viability. Conversely, the bakery industry makes use of their bleaching property. The studies concerning the quantification of individual lipoxygenase isozymes are scarce. In the present investigation, 102 soybean genotypes were analyzed for activities of 3 lipoxygenase isozymes, namely, lipoxygenase-1, lipoxygenase-2 and lipoxygenase-3. Lipoxygenase-1, lipoxygenase-2 and lipoxygenase-3 showed 86, 12.9 and 71 fold genetic variation, respectively. Soybean genotypes with high lipoxygenases and low individual isozymes were identified, which can be the potential raw material for different end users in the industry and can be useful for breeding high and low lipoxygenase soybean varieties.

Keywords: Lipoxygenase-1, lipoxygenase-2, lipoxygenase-3, soybean

***Correspondence**

Author: Vineet Kumar

Email: Vineet.Kumar@icar.gov.in

Introduction

Soybean seeds contain three lipoxygenase (Lox) isozymes, namely, lipoxygenase-1 (Lox-1), lipoxygenase-2 (Lox-2) and lipoxygense-3 (Lox-3). Lox-1 shows maximum activity at pH 9.0 while Lox-2 and Lox-3 have maximum activity at pH 6.8 and 7.0, respectively. Lox-1 and Lox-3 are the most abundant lipoxygenases and Lox-2 is the least abundant lipoxygenase in soybean seeds. These lipoxygenase isozymes are responsible for the catalytic oxidation of unsaturated fatty acids containing *cis- cis 1,4 pentadiene* moiety i.e. linoleic and α-linolenic acid. This results in the release of hydroperoxides which are further acted upon by hydroperoxide lyases, thereby synthesizing aldehyde and ketone compounds. These compounds lend off-flavour associated with the soy products which constrain the utilisation of soybean in food uses. Lox-2 is the principal contributor to the off-flavour.

Besides, lipoxygenases-induced catalytic oxidation of the unsaturated fatty acids present in the cell membrane generates free radicals which impact the seed viability/seed germination. This necessitates the development of soybean genotypes with very low level of lipoxygenases or their genetic removal from the soybean seeds. On the other hand, bleaching action of soybean lipoxygenase is exploited in the bakery industry for whitening the bread. Therefore, plant breeding programme for the development of both null/low and high lipoxygenases activities in soybean genotypes are sought after for different end users in the industry.

Genetic variability of lipoxygenase isozymes is the pre-requisite for the development of high and low lipoxygenase soybean. In literature, several reports pertaining to the genotypic variation of lipoxygenases in soybean are available [1-3]. Kumar *et al.* [1] estimated lipoxygenase-1 and lipoxygenase-2+3 activities in soy flour of 51 soybean genotypes and reported genotypes with low lipoxygenase isozymes activities. Kumar *et al.* [2] evaluated 9 soybean genotypes raised at different locations for lipoxygenases activities. In these investigations, all the 3 lipoxygenase isozymes have not been individually quantified as activities of Lox-2 and Lox-3 were collectively determined. Only in one study individual estimation of 3 soybean seed lipoxygenases has been reported [4], however, the authors investigated only 3 genotypes. Furthermore, genotypes have been developed for either of the 3 individual lipoxygenase isozymes or all three null lipoxygenases in several countries [3, 5-8].

However, in the genotypes free from any of the three lipoxygenases, the other two isozymes have not been quantitatively determined. Kumar *et al.* [5] developed lipoxygenase-2 (Lox-2) free soybean genotype NRC109 and NRC110 from the cross Samrat × PI086023. Valeria Carpentieri-Pipolo [6] developed Kunitz trypsin inhibitor free and lipoxygenase-free soybean variety. Ciabotti *et al.* [3] estimated lipoxygenase-1 and lipoxygenase-2+3 activities in soybean genotypes, taking BRS213 as standard which is a lipoxygenase free cultivar. Kumar *et al.* [7] developed lipoxygenase-3 free soybean lines and similarly lipoxygenase-1 free soybean lines have also been developed in plant breeding programme focussing on the development of food-grade soybean. Rawal *et al.* [8] employed marker assisted

backcross breeding approach for introgression of a null allele of lipoxygenase-2 (Lox-2) from PI596440 into a high yielding Indian soybean variety JS97-52 and developed several lipoxygenase-2 free soybean lines. Wang *et al.* [9] developed triple null lipoxygenase through Crisper-cas9. However, in all these genotypes free from any of the 3 lipoxygenase isozymes, the activities of other 2 isozymes have not been reported. Therefore, it was thought worthwhile to determine the activities of individual lipoxygenase isozymes in large number of soybean genotypes which included genotypes wherein all the three lipoxygenases are present and also the genotypes developed for null activity of any of the 3 lipoxygenase isozymes.

Experimental

Material

One hundred two soybean genotypes were raised in a single row plot of 3-meter length, with row-to-row distance of 45 cm, in triplicate in the randomized block design. Among these genotypes, PI133226 was a germplasm accession free from lipoxygenase-1. PI596540, PI086023 and NRC109 were lipoxygenase-2 free. PI596540 and PI086023 were the germplasm lines, while NRC109 was developed by marker assisted forward breeding through Samrat × PI086023 [5]. Samrat was a farmer's variety. PI417458, J313-2, J313-3 and J313-4 were lipoxygenase-3 free. J313-2, J313-3 and J313-4 were the advanced breeding lines developed from JS335 × PI417458. PI417458 was a germplasm line. Freshly harvested seeds were analysed for lipoxygenase isozymes activities. Before analysis, soybean seeds were finely ground and defatted with petroleum ether (bp 40-60° C).

Methods

Quantitative Estimation of Lipoxygenase isozymes activities

Lipoxygenase activity was estimated using the enzymatic assay. Crude extract was prepared by homogenization of 0.5 g of the defatted soy flour in 50 ml of sodium phosphate buffer (0.2 M, pH 6.8) in a micro-tissue polytron homogenizer (PT2100, Luzern, Switzerland) at 20,000 rpm for 20 min at 0-4 °C. The homogenized solution was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant so obtained was used as the crude extract for the enzymatic assay following Axelrod *et al.* [10]. Lipoxygenase-1 was assayed under alkaline conditions with 2.8 ml sodium borate buffer (0.2 M, pH 9.0), suitable volumes of crude extract, and 10 mM sodium linoleate.

The reaction mixture for lipoxygenase-2 consisted of 2.8 ml sodium phosphate buffer (0.2 M, pH 6.1), suitable volumes of crude extract and 10 mM sodium arachidonate; while assay conditions for estimating lipoxygenase-3 consisted of 2.8 ml sodium phosphate buffer (0.2 M, pH 6.5), suitable volumes of crude extract and 10 mM sodium linoleate. The reaction was initiated by the addition of sodium arachidonate/ linoleate. Changes in the absorbance were recorded every 15 sec at 234, 238, and 280 nm for lipoxygenase-1, -2, and -3, respectively.

One unit of enzyme activity was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 min^{-1}. For conversion of the values to full fat soy flour, oil content in all the genotypes was determined and values obtained were used for the conversion of lipoxygenase isozymes activities from unit/g of defatted soy flour to units/g full fat soy flour.

Results and Discussion

Table1 shows the concentration of 3 lipoxygenase isozymes in the soy flour prepared from the seeds of 102 soybean genotypes. Lipoxygenase-1 activity ranged from 80 (PI133226) to 6920 units/g of soy flour (RVS2001-4), with an average value of 3126.6 units/g soy flour, revealing 86 times variation for the lipoxygenase-1 activity. Four genotypes, namely, PI133226, Palam Soya, Punjab1, J313-4 showed lipoxygenase-1 activity below 1000 units/g soy flour. PI133226 which is devoid of functional Lox-1 gene but still showed some Lox-1 activity, though the activity was very low (80 units/g soy flour) which may be attributed to the presence of lipoxygenases like enzyme in soybean reported in the earlier study [11] or any other metabolic pathway involved in the generation of n-hexanal [12]. Eighteen genotypes showed Lox-1 activity in the range of 1001-2000 units/g soy flour and 57 genotypes in the range of 2001-4000 units/g soy flour. Twenty three genotypes showed very high Lox-1 activity, exceeding 4000 units/g soy flour. RVS2001-4, Pratap Soya1, J97-52, Type49, AGS191, SL525 were the top five genotypes for Lox-1 activity. **Figure 1A** gives the distribution of genotypes according to Lox-1 activity. The values for skewness and kurtosis for lipoxygenase-1 activity were 0.0175 and 0.104018, respectively, representing the symmetric distribution of data.

Table 1 Lox-1, Lox-2 and Lox-3 activity (unit/g defatted soy flour) in soybean genotypes

Genotype	Lox-1	Lox-2	Lox-3	Genotype	Lox-1	Lox-2	Lox-3
PI 133226[#]	80±4	808±48	184±12	Gujrat soya 1	3480±278	808±56	256±23
PI 596540[#]	1280±76	80±9	24±2	JS 71-05	3680±294	344±20	328±29
PI 086023[#]	4160±291	90±7	184±8	JS 76-205	3280±262	296±17	160±14
NRC 109[$]	1560±176	80±4	80±4	PS 1029	4160±332	528±32	264±18
PI 417458[#]	3240±162	192±19	8±0.7	PS 1024	1280±102	584±35	192±13
J313-2[$]	3240±176	312±27	8±0.8	NRC 7	2320±185	600±38	272±19
J313-3[$]	3680±180	200±15	ND	PRS 1	1760±140	592±35	216±15
J313-4[$]	839.79±54	183.95±16	15.9±2	Maus 47	4120±288	808±48	296±20
Palam soya	319.92±15	399.9±34	55.98±5	Bragg	3960±277.	616±36	200±13
Punjab 1	799.68±40	127.9±16	39.98±3	Pusa 98-14	3000±210	768±46	368±25
PK-262	4240±170	288±39	104±8	Type 49	5280±369	664±39	256±17
PS 1092	3200±149	191.95±20	111.97±9	Maus-158	2360±165	592±35.5	256±16
PS 1042	3040±198	560±59	368±31	Monetta	3640±254	776±46	232±17
PK 327	2720±173	400±37	104±7	Pusa 37	3640±247	1000±64	208±14
PS 564	3400±128	352±25	104±8	JS 20-34	4600±322	552±33	136±9
Shilajeet	1240±80	832±66.5	224±18	Maus 32	4520±316	784±47	288±20
SL 96	3000±177	512±30	256±19	TAMS 98-21	2200±154	680±42	280±19
VLS 63	2880±167	520±40	112±11	JS 2	1160±81.2	592±36	280±17
VLS 65	1440±96	400±34	352±35	MACS 13	4040±282	536±31	296±19
VLS 2	2000±140	880±67	160±13	Birsa soya 1	2920±204	272±16	168±11
Pusa 16	1840±130	880±59	160±14	LSB 1	3120±217	808±48	504±35
JS 20-29	4560±190	512±28	240±28	Samrat	3160±221	568±34.8	424±29
JS 80-21	3039.2±160	700±56	143.9±10	NRC 108	1400±97	200±12	136±9
Davis	1560±108	496±26	104±7	NRC 106	3760±263	496±29.76	400±28
Ankur	1640±113	456±28	272±27	SL 688	1120±78.4	352±21	128±8
VLS-1	4080±202	840±62	220±22	NRC 107	2400±168	592±35	568±39
JS 79-81	2798.8±160	600±30	191.9±10	New teripen	2360±165	640±38	344±24
Gujrat soya 2	3040±180	304±20	176±16	JS 97-52	5280±475	784±47	352±24
JS 75-46	4000±268	600±40	136±8	NRC 102	3640±327	416±24	376±26
MACS 450	4800±278	600±45	144±12	SL744	3760±338	544±32	288±20
CO-2	1880±159	600±29	272±24	PZ 2	1920±134	544±29	336±23
Maus 1	3160±186	264±19	160±12	P3-20	4880±341	352±21	176±12
NRC 12	2320±165	200±23	120±9	Hardee	4720±330	688±41	400±28
RKS 24	3040±145	272±29	136±10	PUSA 20	1960±137	576±34	200±14
KHSB 2	1960±159	704±58	336±28	NRC 86	3080±215	728±43	400±28
SHIVALIK	3480±170	144±8	128±11	JS 95-60	3440±240	640±38	296±20
MACS 57	2440±174	672±40	192±16	NRC 77	3640±254	680±40	224±15
CO 1	3319.1±157	192±24	159.9±14	MAUS 61	4480±	792±47	440±30
KB 79	3480±178	184±16	112±10	CO 3	3840±268	920±82	344±24
MACS 58	3280±196	704±40	272±25	SL 525	5000±350	528±47	416±29
Alankar	2600±200	432±30	159.96±14	AGS 191	5040±352	744±64	280±19
JS 90-41	3280±196	752±46	279.9±24	Hara soya	3600±252	408±36	152±10
Improved pelican	4200±267	592±47.6	167.8±16	PS 1347	1920±134	288±25	136±9
Kalitur	3280±142	480±39	120±12	PUSA 9712	3600±245	912±67	424±37
Durga	4800±327	552±43	272±24	Pratap soya 1	5560±412	672±62	280±24
ADT 1	2920±170	584±54	176±16	RVS 2001-4	6920±510	1032±89	520±46
Lee	3480±278	424±47	200±20	VLS-59	2240±130	600±35	176±15
VLS 47	5200±489	928±74	392±34	Pusa 22	2440±120	376±26	160±14
PK 472	4960±358	656±68	304±29	Pusa 40	1080±98	352±26	288±25
PK 308	3400±201	440±47	216±19.44	VLS 21	3880±156	536±49	368±33.12
JS 335	2440 ±190	296±23	320±19	Indira soya 9	2640±211	536±32	304±21

Values given are mean of triplicate samples ± standard deviation. # = germplasm line, $ = advance breeding line and remaining are released soybean varieties. One unit of enzyme activity was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 min^{-1}. ND corresponds to 'not detected'

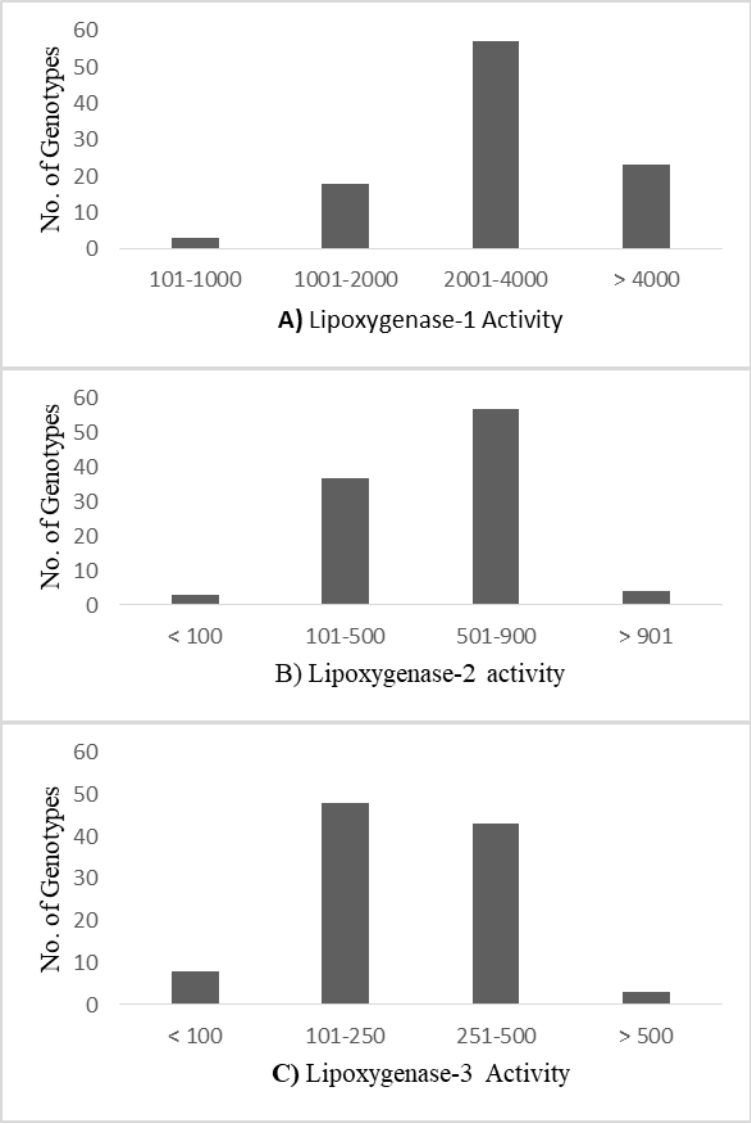

Figure 1 (A) correspond to the distribution of soybean genotypes according to Lipoxygenase-1 activity. (B) correspond to the distribution of soybean genotypes according to Lipoxygenase-2 activity and (C) corresponds to the distribution of soybean genotypes according to Lipoxygenase-3 activity.

Lipoxygenase-2 activity ranged from 80 (PI596540, NRC109) to 1032 units/g soy flour (RVS2001-4), with an average of 531.3 units/g soy flour, exhibiting 12.9 fold genotypic variation. PI596540, PI086023 and NRC109 despite having dysfunctional Lox-2 gene possessed some level of Lox-2 activity. Among the evaluated genotypes, 37 genotypes showed Lox-2 activity in the range of 101-500 units/g soy flour. PI596540 and NRC109 showed Lox-2 activity to the magnitude of 80 units/g soy flour while PI086023 exhibited 90 units/g soy flour. This low activity of Lox-2 enzyme in lipoxygenase-2 free genotypes, namely, PI596540, NRC109 and PI086023 may be attributed to the reason as explained above for the low Lox-1 activity noticed in Lox-1 free genotypes. Fifty seven genotypes exhibited Lox-2 activity in the range of 501-900 units/g soy flour whereas 4 genotypes showed lipoxygenase-2 activity above 901 units/g soy flour. RVS2001-4 which showed very high activity of Lox-1 also ranked first for the Lox-2 activity. Figure 1B shows the distribution of 102 genotypes according to the lipoxygenase-2 activity. The skewness and kurtosis value for lipoxygenase-2 activity were -0.07819 and -0.44469, respectively, representing the symmetric distribution of data.

Lox-3 activity could not be detected in J313-3 which was developed from the cross JS335 × PI417458. In the remaining 101 genotypes, Lox-3 activity ranged from 8 (PI417458) to 568 units/g soy flour (NRC107), with an average of 233.37 units/g soy flour, exhibiting 71 times variation. The negligible activity (8 units/g of soy flour) in PI417458 despite having dysfunctional Lox-3 gene may be attributed to the lipoxygenase like isozyme present in soybean as explained above for the negligible activities of Lox-1 and Lox-2 in Lox-1 and Lox-2 free soybean genotypes. Eight genotypes showed Lox-3 activity below 100 units/g soy flour. The Lox-3 activity in the range of 101- 250 units/g soy flour included 48 genotypes, the range 251-500 units/g of soy flour included 43 genotypes and 3

genotypes viz. LSb1, RVS2001-4, and NRC107 showed activity more than 501 units/g soy flour. Figure 1C shows the symmetric distribution of 102 genotypes according to the lipoxygenase-3 activity. The skewness showed positive value i.e. 0.362928 whereas kurtosis was negative -0.026182.

Our results were in commensurate with Kumar *et al.* [4] who estimated lipoxygenase-1, lipoxygenase-2 and lipoxygenase-3 isozyme activity in 3 soybean genotypes. i.e. IC210, NRC107 and JS95-60. The authors showed Lox-1 activity in the range of 1638 units/g soy flour (NRC107) - 4061 units/g soy flour (IC210), Lox-2 activity in the range of 490 (JS95-60)-675 units/g soy flour (IC210) while Lox-3 activity in the range of 328 units/g soy flour (JS95-60) - 430 units/g soy flour (NRC107). These ranges were similar to the results obtained in the present study, barring the low activities of corresponding isozymes in the individual isozyme free genotypes. Ciabotii *et al*. [3] reported Lox-1 activity in the range of 127-1880 units/g soy flour, while the activity of Lox-2+3 in the range of 85-246 units/g soy flour i.e. the range of Lox-1 observed by the authors was narrow than observed in our study. In another study, Kumar *et al.* [2] reported lipoxygenase-1 activity from 450 -2042 units/g soy flour which was about 4.53 fold. In both these studies, the authors did not determine Lox-2 and Lox-3 activities separately. Further, Punjab1 which has been reported to have low Kunitz trypsin inhibitor and total trypsin inhibitor activity in earlier studies [1, 13] showed relatively low Lox-2 activity (127.9 units/g soy flour) and low Lox-3 activity (39.9 units/g soy flour). Even the Lox-1 activity in this genotype was below 1000 units/g soy flour. PI596540 and PI086023 both were lipoxygenase-2 free germplasm, however, the former contained very low lipoxygenase-3 activity as well.

Conclusion

Wide genetic variability for all the three soybean seed Lox-1, Lox-2 and Lox-3 activities was observed, which can be exploited for breeding programme focussing on development of high or low lipoxygenase soybean genotypes. The study also showed that the genetic variation observed for Lox-2 was less than Lox-1 and Lox-3. The minor activity was observed for the lipoxygenase isozyme in genotype for which it has the dysfunctional Lox gene, which may be attributed to the lipoxygenase like isozymes or any other metabolic pathway involved in the generation of n-hexanal as suggested in the earlier studies. Among soybean varieties/advanced breeding lines, RVS2001-4 identified for high lipoxygenases may be suitable for its bleaching property in bakery while low lipoxygenases soybean genotypes, namely, NRC109, Punjab1, J313-4 may serve as excellent raw material for processing soy products.

References

[1] V. Kumar, S. Patra, A. Rani, S.P. Tiwari, J. Food Sci. Tech., 2002, 39, 78.
[2] V. Kumar, A. Rani, C. Tindwani, M. Jain, Food Chem., 2003, 83, 79.
[3] S. Ciabotti, A. Juhasz, J. Mandarino, L. Costa, A. Correa, A. Simmao, E. Santos, Braz. J. Food Tech., 2019, 22, e2018003.
[4] V. Kumar, A. Rani, P. Jha, L. Hussain, V. Pal, V. Petwal, P. Kumar, J. Dwivedi, J. Am. Oil Chem. Soc., 2017, 94, 457.
[5] V. Kumar, A. Rani, R. Rawal, S. M. Husain, Curr. Sci., 2013, 104, 586.
[6] V. Carpentieri-Pipolo, Crop Breed. Appl. Biot., 2015, 15, 191.
[7] V. Kumar, A. Rani, Indian J. Exp. Biol., 2019, 57, 540.
[8] R. Rawal, V. Kumar, A. Rani, S. M. Gokhale, Plant Breed. Biot., 2020, 8, 163.
[9] J. Wang, K. Huaqin, Z. Zhihui, Y. Yongqing, Y. Long, Z. Mengchen, S. Shikui, G. Yuefeng, Crop J., 2019, 8, 432.
[10] B. Axelrod, T. M. Cheesebrough, S. Laakso, Method Enzymol., 1981, 71, 441.
[11] D. R. Iassonova, A. Lawrence, Johnson, E. G. Hammond, S. E. Beattie, J. Am. Oil Chem. Soc., 2009, 86, 59.
[12] N. Mellor, F. Bligh, I. Chandler, C. Hodgman, J. Food Sci., 2010, 75, 131.
[13] V. Kumar, A. Rani, M. Shuaib, P. Mittal, Food Anal. Method., 2018, 11, 2431.

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