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Identification of QTLs for seed viability in soybean [*Glycine max* (L.) Merill]

Ashish Kumar, Akshay Talukdar*, Raju R. Yadav, Shatakshi Poonia, Rumesh Ranjan and S. K. Lal

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

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Abstract

Soybean seed loses viability rapidly during ambient storage in tropical and sub-tropical climate. Understanding genetic mechanisms would enable enhancing storage life of seeds through breeding approach. In this study, attempt was made to identify quantitative trait loci (QTL) controlling viability of seeds using an inter-specific recombinant inbred line (RIL) population and SSR markers. Significant variation was observed among the RILs for seed viability after one and two years of ambient storage. RILs with more than 90% seed viability after two years of storage were identified. Genetic polymorphism among the RILs was very high (52.9%). Two QTLs *viz*., *qSv2.1* and *qSv14.1* were mapped on Chromosomes, 2 and 14 after one year of storage that explained 10.65% and 5.35% of the phenotypic variation, respectively. Two more QTLs *viz*., *qSv2-1* and *qSv14-1* were mapped for seed viability after two years of storage of which *qSv2.1* (PVE=6.0%) was consistent and the *qSv14.1* (PVE=8%) was novel. QTL and linked markers identified in the study would be useful in soybean breeding for enhancing seed viability during ambient storage.

Key words: RILs, Germination, storability, SSR markers, Soybean

Introduction

Soybean is not only a great source of protein for human and animal consumption but also a major source of edible vegetable oil in the world. The two most important environmental factors that influence seed storage life of soybean are relative humidity and temperature which acts interdependently (Harrington 1972). High relative humidity increases seed moisture content leading to increase in hydrolytic enzyme activity, enhanced respiration and free fatty acids. High temperature on the other hand elevates rates of many enzymatic and metabolic reactions causing more rapid seed deterioration. High temperatures hasten deterioration of the high-moisture seeds and have minimal deteriorative effect on the low-moisture seeds (Copeland and McDonald 2001). The humid and warm climate prevailing in the tropical and sub-tropical regions of the world are the primary causes of reduction in vigour and viability of the seeds leading to poor germination and sub-optimal plant stand in the soybean field (Singh and Ram 1986). Soybean is therefore classified as the least storable crop in their relative storability index (Justice and Bass 1978). In India, germination level of soybean seeds for certification has been kept as low as 70%. Development of soybean cultivars resisting to such adverse climatic conditions is the most promising solution to the problem of seed deterioration in storage.

For effective breeding, knowledge of genetic control of the trait is important. Further, identification of molecular marker linked to the target trait would accelerate breeding program through marker-assisted selection (MAS). However, seed storability is considered to be a complex trait as it is controlled by several genes and hugely affected by environmental conditions during seed formation, harvest and storage (Clerkx et al. 2004). Genetic control of seed storability in soybean have been studied by many researchers *viz*., Kueneman (1983), Cho and Scott (2000) and other researchers. However, the findings of the studies are diverse and not consistent (Watanabe et al. 2004; Zhang et al. 2008; Dargahi et al. 2014). Singh et al. (2008) reported association of four SSR markers *viz*., Satt434, Satt538, Satt281 and Satt598 with seed coat permeability and electrolyte leaching in soybean. Another set of four SSR markers *viz*., Satt538, Satt285, Satt600 and Satt434 has been reported to

*Corresponding author's e-mail: akshay.talukdar1@gmail.com
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be significantly associated with seed-traits. Sooganna et al. (2016) indicated that the SSR marker Satt423 can effectively differentiate good storing genotypes from the poor storing ones. Kumar et al. (2019) studied 217 inter-specific RILs of soybean and reported identification of lines that were permeable and maintained more than 80% germination even after two years of ambient storage. Association of seed coat traits such as seed coat permeability, minute cracks in hilum and seed coat, breakage in hourglass cells, etc. with seed viability has also been reported. In this study, attempt was made to identify QTL and SSR markers associated with seed viability during ambient storage.

Material and methods

An inter-specific RIL was developed by hybridizing DC2008-1, an accession of wild type soybean (*G. soja*), and DS9712, a popular variety of soybean (*G. max*.). Seeds of DC2008-1 live longer in ambient storage than DS9712. Contrasting features of both the genotypes are shown in Table 1. Seeds of 188 RILs in F_7 generation was used for viability and mapping study.

Phenotyping

About 150g of untreated fresh seeds were collected during 2015 from each of the 188 RILs and the parental lines and packed in water proof seed envelop and kept in ambient storage environment (average 25±2°C and 65±5% RH). Viability of the seeds were tested immediately after harvest and subsequently after one and two-years of ambient storage using between-paper method at 25°C in two replications of 50 seeds each following International Seed Testing Association (ISTA) rules (Anonymous 2013). The germination percentage was recorded on 8^{th} day by counting the number of normal seedlings. The data on germination (%) were analyzed following Complete Randomized Design (CRD) with the software package OPSTAT (http://hau.ac.in/about/opstat.php).

Molecular genotyping, map construction and QTL mapping

Genomic DNA was extracted from tender soybean leaves using CTAB procedure (Saghai-Maroof et al. 1984). Quality and quantity of the DNA extracted from the genotypes was ascertained through spectrophotometer analysis. For uniformity of amplification through polymerase chain reaction (PCR), the DNA samples were diluted to a concentration of 20ng/µl of solution.

For surveying the level of polymorphism between the parents, 310 simple sequence repeat (SSR) markers were picked up from the consensus genetic map of soybean. The mapping population comprising of 188 RILs was genotyped using 164 polymorphic markers. Band pattern of each SSR marker was scored as '2' for DC2008-1 (P1), '0' for DS9712 (P2), '1' for heterozygotes (F_1) and '-1' for missing (M) band data as per the guidelines (Wang et al. 2016). Genetic distance between markers was calculated using Kosambi map function (Kosambi 1944). A set of 27 markers showing distorted segregation was culled out and only 137 markers were used to map the QTL for seed viability using the average germination data of 188 RILs. Critical threshold for the Inclusive Composite Interval Mapping of each trait in each year was determined by performing test with 1000 permutations.

Results

Seed viability

Germination (%) in the fresh seeds of DC2008-1 and DS9712 was comparable i.e. 99% and 97%, respectively; however, it declined with the period of ambient storage more rapidly in the DS9712 than DC2008-1. Germination in the seeds of DC2008-1 was 96% and 92% after one and two years of ambient storage as compared to 70% and 51% in DS9712, respectively (Table 1). Viability of the seeds of 188

Table 1. Morphological variation between parents

Particular	*Glycine soja*	*Glycine max*
Genotype	DC 2008-1	DS 9712
Seed coat colour	Black	Yellow
100 seed weight (g)	0.56	9.12
No. of Primary Branches /Plant	9	2.8
No. of Nodes /Plant	21	11
No. of Pods /Plant	69	110
Plant Height (cm)	140	77
Susceptibility to Shattering	Present	Absent
Twining Habit	Present	Absent
Yield/Plant (gm)	0.78	20.2
Seed coat permeability (%) at 6 hrs.	0	100
Seed coat permeability (%) after 7 days	36	100
Germination of fresh seeds (%)	99	97
Germination (%) after 1 year storage	96	70
Germination (%) after 2 year storage	92	51

RILs varied significantly during storage and found to be 29-97% and 1-93% with a mean of 74.78% and 53.84% after one and two years of storage, respectively (Table 2).

Table 2. Germination (%) in fresh and stored seeds

Seed type	Range	Mean	SE(±)	SD	CV (%)
Fresh	75-100	90.74	0.38	5.26	5.8
1 year stored	29-97	74.78	0.98	13.46	18.01
2 years stored	1-93	53.84	1.57	21.52	40.35

SE = Standard error; SD = Standard deviation; CV = Coefficient of variation

Mapping of QTL for seed viability

Among the 310 SSR markers used, 164 markers turned out to be polymorphic between two parents indicating the level of polymorphism between them to be 52.9% (Table 3). It was observed that not all the chromosomes had equal number of polymorphic markers; some had more than others. Highest level of polymorphism (66%) was observed on Chr. 18, while the least (40%) was observed on Chr.10 (Table 3). Molecular genotyping of the RILs with the polymorphic markers showed that only 137 out of 164 markers segregated in the expected 1:1 ratio, rest 27 markers showed distorted segregation and hence excluded from map construction. The framework linkage map developed with the 137 SSR markers covered a total map length of 2287.87 cM with an average marker distance of 16.6 cM. The 20 linkage groups (LGs) formed were equivalent to the haploid (n = 20) chromosome number of soybean. The longest map was constructed for Chr. 1 (171.78cM) followed closely by Chr.20 (169.44cM).

The two-year mean germination data of the 188 RILs and the molecular genotypic data point of 137 markers were subjected to mapping of QTL for viability with the software QTL IciMapping V4.0, which applied Inclusive Composite Interval Mapping of ADDitive (and dominant) QTL (ICIM-ADD) method for the analysis. Seed viability was not an issue for the fresh seeds. Correspondingly, no QTL for seed viability were mapped for the fresh seeds.

QTL mapping after 1 year of storage

Significant variations were observed between the parental lines and the RILs for seed viability after one year of ambient storage. Analysis of marker trait association for seed viability identified and mapped two QTL for seed viability *viz*., *qSv2.1* and *qSv14.1* on Chr.2 and Chr.14, respectively. The phenotypic variation explained (PVE) by the QTL *qSv2.1* was 10.65%, and by QTL *qSv14.1* was 5.35%. The LOD score for the two QTL was 2.63 and 3.55, respectively. Map position of the identified QTL, markers bracketing them, PVE, LOD and additive effect of the QTL are presented in Table 4. The linkage maps showing map position of the QTL have been depicted in Fig. 1.

Table 3. Chromosome-wise distribution of SSR markers used and their level of polymorphism

Chr. No. (LG)	SSR used (No.)	SSR Poly-morphic (No.)	SSR used in mapping (No.)	Polymo-rphism Level (%)	Remarks[#]
1 (D1a)	15	9	8	60	High
2 (D1b)	19	9	7	47	High
3 (N)	19	9	7	47	High
4 (C1)	16	9	8	56	High
5 (A1)	14	8	6	57	High
6 (C2)	15	9	8	60	High
7 (M)	18	9	8	50	Medium
8 (A2)	16	9	6	56	High
9 (K)	13	7	7	53	High
10 (O)	15	6	7	40	Low
11 (B1)	15	8	8	53	High
12 (H)	17	9	8	53	High
13 (F2)	13	7	5	53	High
14 (B2)	19	9	8	47	Medium
15 (E2)	15	8	6	53	High
16 (J)	15	7	5	46	Medium
17 (D2)	14	7	6	50	Medium
18 (G)	12	8	6	66	High
19 (L)	14	7	5	50	Medium
20 (I)	16	10	8	62	High
Total	**310**	**164**	**137**	**52.9**	

[*]Figure in parentheses indicates linkage group; [#]High: > 50%, Medium = 45-50%; Low = <45%; Chr. = Chromosome

QTL mapping after 2 years of storage

The seed viability data of the parental and the RILs found to vary significantly after 2 years of ambient storage. Analysis of the viability and molecular genotypic data through appropriate software mapped

two QTL for the trait; one QTL was mapped on Chr.2 (*qSv2-1*) and the other on Chr.14 (*qSv14-1*). Both the QTL appeared to be moderate in its effect. The phenotypic variation explained (PVE) by the QTL *qSv2-1* and qSv14.1 was 8.67% and 6.61%, respectively. Linkage map of the QTL has been depicted in Fig 1. Map positions of the QTL, linked markers, additive effect, etc. have been presented in Table 4. Of the eight markers linked to four QTL, the minimum distance (~5.0cM) between marker and QTL was observed between Sat_230 and *qSv14.1* (Table 4, Fig 1). The LOD score of QTLs represented in Table 4.

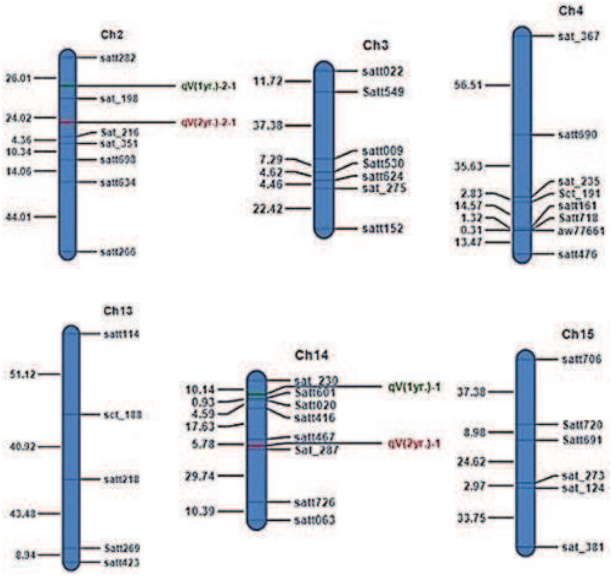

Fig. 1. Linkage maps of 20 soybean chromosomes depicting QTL along with their map position. *qV*(1yr.)2-1 = *qSv*2.1; *qV*(2yr.)2-1 = *qSv*2.1; *qV*(1yr.)14.1 = *qSv*14.1; *qV*(2yr.)14.1 = *qSv*14-1

The QTL on Chr.14 had negative additive effects in the range of –3.34 to –6.81 (Table 4) indicating that these allele were contributed by the poor storing genotype i.e. DS9712. On the other hand, QTLs mapped on Chr. 2 had positive effect ranging from 4.82 to 7.42. These alleles might have contributed by the good-storing genotype i.e. DC2008-1, which enhanced viability of the seeds during ambient storage.

Discussion

Identifying molecular marker(s) tightly linked to the gene/QTL governing the trait of interest is a pre-requisite for marker assisted selection (MAS). It has been routinely done in many crops including soybean by utilizing genetic linkage map developed from various mapping populations and molecular markers. However, for mapping of gene/QTL, SSR or microsatellite markers are preferred over others because of its co-dominant nature of inheritance, high reproducibility, random distribution in genome, abundance and multi allelic nature (Saghai-Maroof et al. 1994; Gupta and Varshney, 2000). In this study, SSR markers were chosen for construction of linkage map and mapping of QTL for seed viability.

Mapping population varies in its resolution or precision in mapping the QTL. RILs are often preferred over other mapping population for mapping of QTL. Among the RILs, the inter-specific RILs are found to be more powerful than the intra-specific RILs as it exhibits higher polymorphism and captures higher phenotypic variations enabling detection of large effect QTL for effective deployment in breeding program. In the present study, 188 RILs developed from an inter-specific crosses were used in mapping QTL for seed viability. It had higher level of polymorphism (52.9%) and thus allowed detection of QTL even with minor effect. Liu et al. (2007), reported detection of 64.38% polymorphism between Tokai-780 (*G. max*) and Hidaka-4 (*G. soja*). Kumar et al. (2011) reported up to 48.38% polymorphism in a set of *G. max* genotypes of diverse origin. However, the distribution of polymorphism was not uniform across the genome. The highest level of polymorphism (66%) was observed on Chr. 18, while the least (40%) was observed on Chr. 10. The polymorphic loci also sometime do not follow expected 1:1 ratio in the RIL, which is referred to as segregation distortion. It is nearly common in all the mapping populations with varying intensities; however, RILs often exhibit more distorted markers than others (Xu et al. 1997). The reasons for segregation distortion are many *viz*., scoring error, gametic or zygotic selection, chromosome rearrangement, genetic incompatibility, pollen competition, preferential fertilization, etc. However, differential gametophytic selection is considered to be the primary cause of segregation distortion in rice (Xu et al. 1997). In the inter-specific RILs used in this study, 27 out of 164 polymorphic markers exhibited distorted segregation (16.4%). These markers were therefore excluded from mapping studies.

High density linkage map is useful in identifying genes/QTLs controlling various traits in a wide range of species (Tanksley et al. 1989; Mohan et al. 1997). In the present study, linkage map was constructed using 137 markers that were distributed on the 20 linkage groups of soybean. Total length of the genetic

Table 4. QTL for seed viability mapped under various period of ambient storage

Storage period(year)	Chr. No.	QTL	Map position (cM)	Marker interval	LOD	PVE (%)	Add. effect
1	2	*qSv2.1*	18.0	Satt282- Sat_198	3.55	10.65	4.82
	14	*qSv14.1*	8.0	Sat_230- Satt601	2.63	5.35	-3.34
2	2	*qSv2-1*	41	Sat_198- Sat_216	3.10	8.67	7.42
	14	*qSv14-1*	37	Satt467- Sat_287	3.42	6.61	-6.81

LOD = LOD score, PVE (%): Phenotypic variation explained by QTL, Add = Estimated additive effect of the QTL; Chr. = Chromosome

map constructed in this study was 2287.87 cM with an average marker distance of 16.6 cM. This density is comparable with others used in similar studies in soybean. While studying seed viability, Zhang et al. (2008) used 148 markers to construct a linkage map of 1363.7cM length. The linkage maps constructed by various workers *viz*., Molnar et al. (2012), Liu et al. (2007) and Li et al. (2008) differed in map-length and marker densities. Such variations are the result of a number of factors including number of markers used for the linkage map construction, segregation pattern of the markers, missing values, accuracy of the linkage analysis, marker density, etc. Density of molecular markers on the linkage map also depends on level of homozygosity and recombination frequencies between the parental lines used to develop the mapping population (Castiglioni et al. 1999). The linkage map developed by using large mapping population and more number of co-dominant markers is generally considered as precise (Kumawat et al. 2012). The linkage map developed in this study had good number of markers and nearly uniform distribution of markers across the chromosomes and hence suit mapping.

Seed viability is a very complex trait. It is affected not only by the seed characters but also by the environmental factors. The seed size, color and permeability of the seed coat, oil and moisture content in the seed, storage condition etc. found to influence durability for which seed remain viable (Kumar et al. 2019). Therefore, it is hard to pin point precisely the sole factor controlling the viability of the seeds during ambient storage. In this study, seeds were stored in ambient condition for one and two years. The viability of the seeds in general decreased with the period of storage. However, a few RILs were identified that maintained viability better than others. Through molecular marker analysis, 2 QTL were mapped for seed viability after one and two years of storage. Of the 4 QTL mapped for seed viability, only one QTL i.e. *qSv2.1* appeared over the years indicating it to be a consistent QTL. The genomic region on Chr.2 bracketed by the markers Satt282 and Sat_216 found to harbor QTL governing viability of seeds during storage. Thus, the QTL identified in the study with higher LOD, PVE and consistency may be deployed through molecular breeding approach for enhancing storage life of the seeds of soybean.

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Authors' contribution

Conceptualization of research (AT); Designing of the experiments (AT, AK); Contribution of experimental materials (AT); Execution of field/lab experiments and data collection (AK, RRY, SP); Analysis of data and interpretation (AK, RR, SKL, AT); Preparation of the manuscript (AK, AT).

Declaration

The authors declare no conflict of interest.

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