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CREATION OF GENETIC VARIABILITY THROUGH WIDE HYBRIDIZATION AND ITS ASSESSMENT USING SSR MARKERS IN SOYBEAN [*Glycine max* (L.) Merril.]

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ABSTRACT

Poor genetic variability is often considered as a serious bottleneck in improving the productivity of soybean in India. In this study, an accession of wild type (*G. soja*) soybean (DC2008-1) was crossed with a cultivated North Indian soybean variety (DS9712) to create genetic variability and introgressing useful gene(s). Seeds of the F_2 and subsequent generations were advanced through single seed descent (SSD) approach to isolate 206 recombinant inbred lines (RILs) in $F_{2:6}$ generation. Enormous genetic variability was observed among the RILs for plant type, seed size, seed color, maturity duration, growth habit, yield and other agro-morphological traits. Promising and early maturing transgressive segregants bearing more than 450 pods plant^{-1} were identified. Genetic polymorphism studied with 317 simple sequence repeat (SSR) markers spanning uniformly all over the 20 chromosomes (@16 markers chromosome^{-1}) indicated higher level of polymorphism (64.98%). Distribution of the polymorphism was not uniform across the genome; some chromosome showed higher polymorphism than others. Highest level of polymorphism (90%) was observed on chromosome number 10 and 20 (linkage group "O" and "I") and the least (50%) was on chromosome number 1 and 11 (linkage group "D1a" and "B1"). Molecular genotypic data of the 206 RILs segregated in expected 1:1 ratio. The polymorphic markers identified and the genetic variability created in this study will be useful in mapping QTL and genetic improvement of soybean through molecular breeding.

(Key words: Genetic variability, *G. soja*, RILs, SSR, soybean, wide hybridization)

INTRODUCTION

Soybean [*Glycine max* (L.) Merr. (2n = 40)], also known as "Miracle Bean", is the most important oilseed crop of the world. It contains high quality protein (~40-45%), oil (~18-22%), and phytochemicals essential for human health. India is occupying the 5th position in the world in terms of production and area, which is increasing ever since its commercial introduction during 1968-70. Currently, soybean is occupying an area of about 12.03 m ha with an average production of 12.45 m t (Annonymous, 2015). Despite its large scale cultivation in India, its productivity is still remains very low (1.0 t ha^{-1}) as compared to the world average (2.58 t ha^{-1}). Besides others, the key factor for low yield in soybean includes poor genetic diversity in the existing cultivars and rare use of wild type soybean in breeding program (Yashpal *et al*., 2015).

Cultivated soybeans [*Glycine max* (L.) Merr.] have much lower genetic diversity than their wild [*Glycine soja* Sieb. & Zucc.] counterparts (Carter *et al.,* 2004; Hyten *et al.,* 2006). Both the species differ in a number of agro-morphological traits (Table 1 and 2). The cultivated soybean genotypes are of determinate growth habit with stout primary and secondary branches; bears large seeds in non-shattering pods. The wild type genotypes are primarily climbing vine, procumbent with many small branches, bearing small, coarse black seeds. Seeds of the cultivated type are permeable (soft-seeded) but often short-lived. On the other hand, seeds of the wild type are poorly permeable (hard seeded), but lives longer. Both the species also differ for the yield and domestication-related traits (DRTs) (Bailey *et al*., 1997; Funatsuki *et al*., 2006), but cross-compatible as it belongs to the primary gene pool of *Glycine* (Hymowitz, 2004). Therefore, wild type soybean can be utilized as a resource of genes for adaptation, protection and production of soybean.

Yield is a quantitative trait and governed by a number of minor genes usually called as quantitative trait loci (QTL). In soybean, yield is the final product of several component traits such as number of primary and secondary branches plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, seed weight, etc. Therefore, analyzing and mapping QTL for yield and yield-related trait through conventional approach is cumbersome. In recent past, molecular markers

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have been deployed to map QTL for yield and yield related traits (Kumar *et al*., 2015). As reported in the SoyBase (www.soybase.org), more than 3624 QTLs for various agro-morphological traits, and 3621 molecular markers consisting of RFLP, AFLP, RAPD, SSR, SNP etc. have so far been mapped in soybean.

However, limited genetic studies have been carried out to map and understand the genetic basis of the DRTs (Liu *et al*., 2007). In this study, therefore, attempt was made to create genetic diversity through distant hybridization and perform molecular assessment for seed yield (SY) and domestication-related traits (DRTs) in soybean using SSR markers.

MATERIALS AND METHODS

The plant material used in the study comprised of 206 recombinant inbred lines (RILs) in $F_{2:6}$ and $F_{2:7}$ generations, which were developed from an inter-specific cross involving a wild type (*G. soja*) accession (DS2008-1) and a popular North Indian (*G. max*) cultivar (DS9712) of soybean (Fig. 1). Phenotypically, both the parental genotypes were highly diverse; salient features are presented in table 1. Segregating plants from F_2 and subsequent generations were advanced through single seed descent (SSD) method.

For phenotypic evaluation, the RILs in $F_{2:6}$ and $F_{2:7}$ generations along with the parental genotypes were evaluated in two consecutive years i.e. *kharif* 2014 and *kharif* 2015 in the experimental field of the Division of Genetics, ICAR-IARI, New Delhi following augmented block design (Federer, 1956). Geographically, IARI, New Delhi campus is located in $28^0$38'23" N and $77^0$09'27"E with an altitude of 228.61m above mean sea level. It represents sub-tropical climate with moderate annual rainfall 708.7 mm that spans nearly over 3 months (July-Sept.). The temperature and light duration during *kharif* season remain fairly optimum for growing soybean. Each RIL was grown in 3.0 m long row with a row-to-row spacing of 45cm and plant-to-plant spacing of 10 cm. Standard packages of practices were followed to raise a healthy crop. For collection of phenotypic data, 3 plants were selected from each row avoiding bordering plants, and averaged it. The morphological traits under study were days to flower, days to 50% flowering, days to maturity, plant height (cm), number of primary branches, number of nodes on main stem, maximum internodal length (cm), number of pods plant^{-1}, 100-seed weight (g) and yield plant^{-1} (g). The DRTs considered for the study were pod shattering, seed size and twining habit. The data were subjected to statistical analysis by using SPAS software of IASRI, New Delhi.

DNA extraction and SSR analysis

Genomic DNA was extracted from young leaves of the two parents and each $F_{2:6}$ lines derived from 206 RILs using CTAB (Cetyl-Tetra Methyl Ammonium Bromide) method (Murry and Thompson, 1980). The DNA was quantified against a lambda DNA on 1.0% agarose gel stained with Ethidium Bromide and diluted to 10 ngil^{-1} concentrations. Based on the sequences published on the Soy Base website (http://soybase.org), 317 SSR primer pairs were synthesized for genotyping the 206 F_{2-6} RIL populations. The polymerase chain reactions (PCR) were carried out using sterile 96 well PCR plates obtained from Axygene Scientific Inc. Union city, CA, USA. The master mix consists of 25ng of genomic DNA, 0.2 U of *Taq* DNA polymerase, 1X PCR assay buffer with 1.5 mM $MgCl_2$, 12 ng each of forward and reverse primer and 200µM of dNTP mix. The reaction volume was made up to 10µl using sterile double distilled water. The entire exercise was carried out over ice and the PCR plate was immediately loaded in the thermal cycler (Applied Biosystems, USA). The details of the PCR cycles are as follows:

Initial Denaturation		94^{0}C	5 min
Cycles 1-35	Denaturation	94^{0}C	1 min
	Primer annealing	49^{0}C	1 min
	Primer extension	72^{0}C	1 min
Final primer extension		72^{0}C	7 min

At the end of the run, PCR plate were taken out and kept at 4^0C for electrophoretic separation. The PCR amplified fragments products were separated on 3% MetaPhor ® agarose gel and visualized by Eethidium Bromide (EtBr) staining dye at the rate 2.5ul100ml^{-1}. Electrophoresis was carried out in 1x TBE buffer at 80 volts for 3.5 hours. For sizing the fragments 50bp ladder (MBI Fermentas, Lithuania) was run alongside the samples. Gels were photographed using CCD camera attached to gel documentation system (Alfa Imager™). Polymorphic marker scoring was done manually.

RESULTS AND DISCUSSION

Morphological characterization of the parental genotypes and RILs

The parental genotypes (DS9712 and DC2008-1) varied significantly for the traits under consideration. Brief statement about the morphological variations between the two parental genotypes is given in table 2. The RILs also found to vary significantly for a number of agro-morphological traits under consideration. Morphological variation between the RILs and seeds is shown in fig. 2. The analysis of variance (ANOVA) of the data was calculated following procedure for augmented design (Rathor *et al*., 2005). The ANOVA indicated that there are significant variations between the RILs and the parental lines for the yield and domestication-related traits under consideration (Table 3). The summary descriptive statistics of yield and domestication-related traits (DRTs) for *kharif* 2014 and *kharif* 2015 is presented in table 4, respectively. Trait-wise,

performance of the RILs and their parental lines is presented below:

Days to flowering (DF):

Days to flower or flowering time was estimated by counting the actual number of days required from the date of sowing to the initiation of first flower in each entry. DS 9712, a cultivated variety, took 44 days to flower during *kharif* 2014, while it took only 42 days during *kharif* 2015. However, the *G. soja* accession DC-2008-1 took 68 and 67 days to flower during *kharif* 2014 and 2015, respectively (Table 4). The range for days to flower in the RIL population was 30-97 days with a mean of 62 days in *kharif* 2014. Similarly, the range for days to flower during *kharif* 2015 was 29-75 days with a mean of 56 days. Thus, the flowering range was more dispersive during *kharif* 2014. Transgressive segregation was observed for days to flower. More number of transgressive segregants was observed during 2015 (55.16%) than 2014 (51.61%). RILs that matured earlier to early parent (DS9712) and later to late parent (DC2008-1) were observed. More transgressive segregates were recorded for earliness than lateness.

Days to 50% flowering (DFF):

The number of days required from sowing date to start of flowering in 50% of the plants in a RIL was recorded and presented as days to 50% flowering. The parental line DC-2008-1 came to 50% flowering in about 73 days during *kharif* 2014, while it took about 76 days during *kharif* 2015. Similarly, the other parental genotype DS9712 came to 50% flowering stage in about 59 and 55 days during *kharif* 2014 and *kharif* 2015, respectively. The RILs attained the 50%-flowering stage in 35-101 days (average 67 days) during *kharif* 2014 and 33-79 days (average 60 days) *during kharif* 2015.

Days to maturity (DM):

Days to maturity was recorded by counting the days from date of sowing to the days when the seeds attained physiological maturity. The DC2008-1 was relatively a late maturing genotype and showed physiological maturity only in about 134-140 days during *kharif* 2014 and 124-139 days during *kharif* 2015. Similarly, the DS9712 matured in 118-120 days during *kharif* 2014 and 109-118 days during *kharif* 2015. The days-to-maturity among the RILs ranged from 56 to 124 days and from 85.50 to 134 days with mean days-to-maturity of 110 and 112 days during *kharif* 2014 and *kharif* 2015, respectively.

Plant height (PH):

Height of a plant was measured at the maturity stage. It was recorded from base of the plant at ground to the tip of the main stem. The plant height of DS9712 ranged from 62 to 84 cm across the two years. On the other hand, height of the wild type genotype DC2008-1 was much variable and attained about 117 cm and 150 cm during 2014 and 2015, respectively. Height of the plants in the RILs was more during 2014 than 2015 (Table 4). The weather during 2014 was more favorable with optimum rain during *kharif* season. It might have affected overall growth and height of the plants. Transgressive segregates with shorter plant type were observed. The skewness and Kurtosis was positive and normal.

Number of primary branches (PB) plant^{-1}:

Branches coming out of the main stem were counted and recorded as the number of primary branches plant^{-1}. In DS9712, there were a few branches plant^{-1}; it ranged from average 2.14 to 3.33 over both the years. On the other hand, the number of primary branches plant^{-1} in DC2008-1 varied from 6.67 to 11.13 during the years of testing. In the RILs, the number of primary branches plant^{-1} ranged from 0.67 to 23 with an average of 6.09 during *kharif* 2014. Similarly, during *kharif* 2015, the account of branches plant^{-1} was 0.33-14.33 with a average of 5.72 (Table 4).

Number of pods plant^{-1} (NP):

Average number of pods plant^{-1} in DS9712 was 82-144 while in DC2008-1, it was 52-78 over both the years (Table 4). Among the RILs, a huge variation was observed for the number of pods plant^{-1}. It exhibited transgressive segregation both for higher (451 during 2014, 551 during 2015) and fewer (7 during 2014, 24 during 2015) number of pods plant^{-1}. During *kharif* 2014, the number of pods plant^{-1} ranged from 7 to 451 with an average of 210.29. There was more pods plant^{-1} during *kharif* 2015, which ranged from 24.03 to 515.67 with an average of 224.78 number of pods plant^{-1}.

100-seed weight (SW):

Seeds of the cultivated genotype DS9712 were larger than that of the wild type DS2008-1. Range of weight of 100-seeds of DS9712 was 7.90 - 9.50 g and 7.40 - 8.97 g during 2014 and 2015, respectively. Similarly, the 100-seed weight for DC2008-1 seeds ranged from 0.52 g to 0.56 g during 2014 and 0.57 g to 0.58 g during 2015. The RILs showed huge variations for seed weight. During *kharif* 2014, 100-seed weight of the RILs was 1.09-12.20 g with a mean of 2.65 g. Similarly, during *kharif* 2015, the seed weight ranged from 1.17-12.10 g with a mean of 2.65 g.

Yield plant^{-1} (YP):

The mean seed yield plant^{-1} for DC 2008-1 was comparatively less variable and it ranged from 0.59 g to 0.84 g across the years, while seed yield for the other parent DS9712 ranged from 9.33 g to 23.20 g and, 9.40 g to 24.09 g during *kharif* 2014 and *kharif* 2015, respectively. Among the RILs, the seed yield ranged from 1.09g to 29.33 g and from 1.50 g to 16.00 g with a mean of 6.96 g and 7.57 g during *kharif* 2014 and *kharif* 2015, respectively.

Number of nodes (NN):

Mean number of nodes on the main stem for DC 2008-1 was comparatively higher than that in DS9712. In DC2008-1, it ranged from 15 to 27, while the same in DS9712 ranged from 9 to 16 across the years. Among the RILs, large variations were observed for this trait. On average, the

number of nodes on main stem of the RILs ranged from 3.00 to 33.00 and from 7.30 to 29.33 with mean of 16.68 and 16.83 during *kharif* 2014 and *kharif* 2015, respectively.

Maximum inter-nodal length (MIL):

The length of the internodes was more in the wild type genotype DC2008-1 as compared to the DS9712. It ranged from 21 to 31 cm in DC2008-1 and in DS9712, it ranged from 2.90 to 4.40 cm. The RILs also showed variations for this trait. During 2014, it ranged from 2.00 to 9.50 cm with a mean value of 5.28 and during 2015 it ranged from 1.67 to 10.80 cm with mean of 5.61 cm.

Enormous phenotypic and genotypic variability was observed among the RILs (Fig. 2). It varied significantly for all the traits under study (Tables 2, 3 and 4). Transgressive segregates were observed for 5 of the 10 traits. RILs with > 451 number of pods plant^{-1} were observed in this study, which is a record by itself. Lines with erect plant type and synchronous maturity have also been identified.

Molecular characterization of parental lines and RIL population

For surveying the level of genetic polymorphism between the two parents i.e. DC2008-1 and DS9712, a set of 317 simple sequence repeat (SSR) markers were selected at random from across the soybean genome with about 16 markers chromosome^{-1} from soybase genome website (Cregan *et al*., 1999). Among the 317 SSR markers tested, 206 were found to be polymorphic between the parents i.e. the level of genetic polymorphism between the two parents were 64.98%. Such higher level of polymorphism was observed due to existence of genetic diversity between the two species. However, distribution of the polymorphic markers was not uniform across the chromosome; some chromosome had more polymorphic markers than others. Distribution of the markers over the chromosome and level of polymorphism is given in table 5. Chromosome numbers 10 and 20 had the highest level of polymorphism (90.91%), while the chromosome number one had the least (50.00%).

Power of the SSR markers in detecting the polymorphism level found to vary with the type of motif and the number of repeats in a particular motif. The tri-nucleotide motifs were more polymorphic (54.06%) than dinucleotide (37.79%) or polynucleotide motifs (8.13%). The pi-diagram showing variations in polymorphism level with respect to SSR motif size is depicted in fig. 3. Further it was observed that the level of polymorphism detected between the two species also varied with the number of times a particular motif is repeated. Out of 111 monomorphic SSR markers, 66 had repeat motif with less than 20 repeat units. On the other hand, among 206 polymorphic markers, 111 had motifs with 20-30 or more repeats. Thus, it appeared that the SSRs with 20-30 repeats, i.e. say ATT20, ATT25, ATT30, etc. are relatively more polymorphic than others. Similar finding was also reported earlier by Yashpal *et al*. (2015). A representative gel picture showing amplification pattern of the markers between the two parental genomes is presented in fig. 4.

In India, soybean was adopted as a commercial crop only during 1968-70s. Barring a few local germplasm available in the Northern hilly states, most of the soybean genotypes available in India are introduced from China (Taiwan) and USA. Basically, soybean is domesticated nearly ~6000-9000 years ago in China (Kim *et al.,* 2010*)* and introduced to India nearly ~ 4000 years ago through Indo-China silk route which posses single gene pool. It is therefore considered that the genetic variability among the soybean genotypes in India is very low. Further, the soybean breeders were biased in involving only a few genotypes in hybridization programs keeping the variability at low. It thus, makes sense to involve diverse genotypes along with the wild types in the breeding program so as to widen the genetic base of Indian soybean. The wild type genotypes also harbor genes/alleles useful for various traits including biotic and abiotic challenges. Wide hybridization not only can widen the genetic base, but can enrich the gene pool with useful gene/alleles too (Concibido *et al*., 2003). In this study, huge genetic variability was created through wide-hybridization. Lines with some important traits have also been identified. Advanced lines with more than 450 pods plant^{-1} have been obtained from the segregating lines. Such line will contribute towards enhancement of yield of soybean.

It is expected that in F_6 or F_7 generation RILs, the lines would be homozygous at all the loci and would segregate at 1:1 ratio. In this study, 47.25% of the alleles in $F_{2:6}$ and $F_{2:7}$ generations resembled *G. soja* and 47.88% resembled *G. max* parents. Thus, the alleles segregated in the expected 1:1 ratio. Average heterozygosity content of the RIL population was 1.01% corresponding to 1.2 cM genetic length across twenty chromosomes, which is less than expected heterozygosity content of F_6 generation. It indicated that the population has got nearly stabilized and is fit for mapping QTL for the important traits. Similar kind of results on heterozygosity in RILs was reported by Liu *et al*. (2007) in a set of 96 RIL population developed from interspecific crosses using wild accession. In three different independent studies involving *G. soja* as donor parent and with three different *max* recurrent parents, polymorphism level was reported to range from 18 to 39% (Mansur *et al*., 1993; Lark *et al*., 1994; Maughan *et al*., 1996)

Lines with improved traits developed in this study will pave the way for identification of high yielding soybean genotypes. Similarly, the polymorphic SSR markers identified in this study will be useful in mapping, analysing and deployment of QTL for genetic improvement of soybean. Further assessment and utilization of these lines in breeding program would be improving yield, quality and stability in soybean.

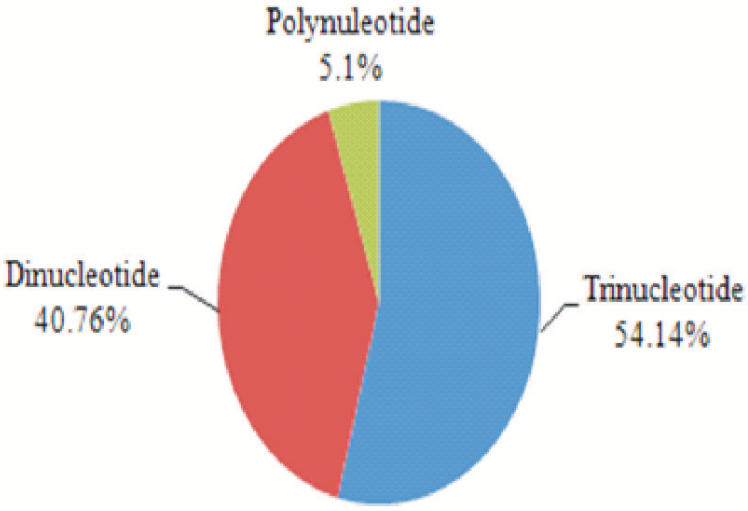

Fig. 3. Pi-diagram showing power of SSR marker motifs in detection of polymorphism.(The trinucleotide repeat motifs could detect more polymorphism than others)

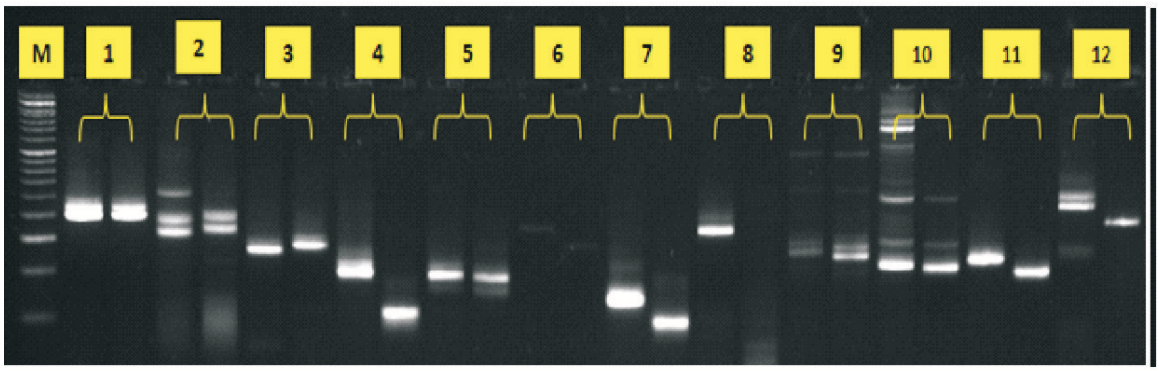

Fig. 4. Amplification pattern of 12 SSR markers between the two parental genotypes. In each pair of lanes, left and right side lanes represent DS9712 (*G max*) and DC2008-1 (*G. soja*) genotypes respectively. The markers in the gel image includes (from left to right) Satt418 , Satt063 , Satt601, Satt663, Satt070, Satt656, Sat_238, Satt460, Satt685, Satt618, Satt308, and Satt687. M - 100bp ladder.

Table 1. Silent features of two contrasting parents wild type (DC2008-1) and cultivated (DS 9712) used in wide hybridization

Traits	DC 2008-1	DS 9712
Species	*G. Soja*	*G. Max*
Domestication	Wild	Cultivated
Source	USDA	Delhi
Plant type	Indeterminate	Determinate
Growth habit	Procumbent	Erect
Plant height	120-150 cm	60-80 cm
Stem	Weak	Strong
Leaf type	Oval	Ovate
100-seed weight (g)	0.57- 0.60	8.00 -10.00
Pod pubescence	Present	Very rare
Days to 50% flowering	70-80 days	35 – 45 days
Pod colour	Black	Brown
Pod shattering	Susceptible	Resistant
Seed coat colour	Black	Yellow
Disease reaction	Resistant	Susceptible
Node number	25-30	10-13
Branching	Profuse	Few
Pri. branches	7-10	2-3
Stress	Resistant	Susceptible
Internodal length	High >9 cm	Low <4.0 cm
Pod number	55 – 75	120-140
Days to maturity	135-145 days	110-120 days
Flower colour	Purple	White
Yield (g)	1.00- 2.00	10-24
Hard seededness	Hard seeded	Soft seeded
Seed size	Small	Bold/large

Table 2. Variations between parental genotypes for yield and domestication-related traits (DRTs) used in wide hybridization

Sl. No.	Traits	Short name	Parent		t-value
			DC2008-1 (*G. soja*)	DS-9712 (*G. max*)	
1	Days to flower/Flowering time	DF/FT	89.00	44.00	53.80**
2	Days to 50% flowering	DFF	97.00	54.48	49.40**
3	Days to maturity	DM	137.0	109.0	58.97**
4	Plant height (PH)	PH	203.46	61.42	32.24**
5	No. of primary branches	PB	7.48	1.28	21.42**
6	Number of pods	NP	81.00	128	12.70**
7	100-seed weight (g)	SW	0.62	10.57	9.58**
8	Seed yield plant^{-1} (g)	YP	2.24	29.42	7.71**
9	Number of nodes	NN	23.31	13.22	4.12**
10	Maxi. inter-nodal length	MIL	13.59	3.45	6.65**

*, ** Significant at 0.05 and 0.01 per cent level of significance; FT: Flowering time, DF: Days to flowering, DFF: Days to 50% flowering, DM: Days to maturity, PH: Plant height (cm), PB: No. of primary branches, NP: No. of pods plant^{-1}, SW: 100-seed weight (g), YP: Seed Yield plant^{-1} (g), NN: No. of nodes on main stem, MIL: Maximum inter-nodal length.

Table 3. Analysis of variance (ANOVA) for yield and other important traits in RILs during *kharif* 2014 and *kharif* 2015

Source/Year		DF	DFF	DM	PH	PB	PN	SW	YP	NN	MIL
Treatment adjusted (degree of freedom: block: 3, treatment: 211, error: 9)											
TMS	*kh* 2014	43.87**	54.41**	47.95**	196.23**	9.03*	646.56**	8.49	15.87*	13.54	8.31
	kh 2015	56.18**	53.21**	49.52**	264.54**	6.21	11208.56**	9.34*	48.25*	11.23	9.09
EMS	*kh* 2014	3.12	2.02	2.94	19.16	0.67	54.34	0.34	5.19	5.05	0.88
	kh 2015	5.32	2.20	2.74	29.16	1.63	204.34	4.28	5.28	4.47	0.77
F	*kh* 2014	23.76	26.98	16.28	10.24	11.38	81.08	24.87	3.06	12.73	13.67
	kh 2015	21.12	25.68	19.81	9.61	8.18	55.458	9.29	6.06	13.53	14.19
P>F	*kh 2014*	0.000010 at 0.009 and *kh* 2015			0.000013 at 0.089						
Block adjusted (degree of freedom: block:3, treatment: 211)											
BMS	*kh* 2014	2.19	2.31	9.18	49.10	1.10	21.28	0.48	6.87	11.41	2.08
	kh 2015	4.49	3.61	5.08	42.34	1.16	102.28	1.65	7.201	11.41	2.61
F	*kh* 2014	1.36	1.14	3.12	2.57	1.64	0.76	1.41	1.32	1.79	1.46
	kh 2015	1.16	1.74	1.78	1.33	1.45	0.79	1.41	1.35	1.79	1.24
P>F	*kh* 2014	0.56	0.36	0.06	0.09	0.22	0.39	0.28	0.30	0.35	0.24
	kh 2014	0.55	0.26	0.16	0.24	0.26	0.54	0.28	0.33	0.35	0.46

*, ** Significat at 0.05 and 0.01 percent level of significance

Table 4. Descriptive statistics for yield and domestication-related traits (DRTs) in RILs during *kharif* 2014 and *kharif* 2015

Traits	Year	Parents		RILs (n=206)			
		DC2008-1	DS9712	Range		Mean	SEd±
				Min.	Max.		
FT	*kh* 2014	68.00-79.00	44.00-51.00	30.00	97.00	62.00	8.54
	kh 2015	67.00-81.00	42.00-52.00	29.00	75.00	56.00	6.83
DFF	*kh* 2014	73.00-91.00	59.00-62.00	35.00	101.00	67.00	8.37
	kh 2015	76.00-89.00	55.00-61.00	33.00	79.00	60.00	7.08
DM	*kh* 2014	134.00-140.00	118.00-120.00	56.00	124.00	110.00	8.59
	kh 2015	124.00-139.00	109.00-118.00	85.00	134.00	112.00	9.64
PH	*kh* 2014	120.00-150.00	66.00-84.00	16.33	147.67	58.78	20.29
	kh 2015	117.00-141.00	62.00-82.00	14.67	122.33	57.66	20.31
PB	*kh* 2014	06.67-10.33	02.33-03.33	0.67	23.00	6.09	2.77
	kh 2015	07.60-11.13	02.14-03.09	0.33	14.33	5.72	2.73
NP	*kh* 2014	52.00-75.00	82.00-144.00	7.00	451.00	210.29	98.34
	kh 2015	55.00-78.00	89.00-121.00	24.00	515.67	224.78	108.48
SW	*kh* 2014	00.52-00.56	07.90-09.50	1.09	12.20	2.65	1.87
	kh 2015	00.57-00.58	07.40-08.97	1.17	12.10	2.65	1.88
YP	*kh* 2014	00.68-00.84	09.33-23.20	1.09	29.33	6.96	4.87
	kh 2015	00.59-00.81	09.40-24.09	1.50	16.00	7.57	3.62
NN	*kh* 2014	17.00-27.00	09.00-16.00	3.00	33.00	16.68	4.59
	kh 2015	15.00-26.00	09.15-15.28	7.30	29.33	16.83	4.89
MIL	*kh* 2014	24.00-31.00	02.90-04.40	2.00	9.50	5.28	1.64
	kh 2015	21.00-27.00	02.94-04.11	1.67	10.80	5.61	2.03

Note: FT-Flowering time, DFF-Days to 50% flowering, DM- Days to maturity, PH- Plant height (cm), PB- No. of primary branches, NP- No. of pods plant^{-1}, SW- 100-seed weight (g), YP- Seed Yield plant^{-1} (g), NN- No. of nodes on main stem, MIL- Maximum inter-nodal length

Table 5. Chromosome wise distribution of SSR markers and level of polymorphism observed between two parental genotypes

Chr. No./ (Linkage Group)	Markers tested (No.)	Mono-morphic markers	Poly-morphic markers	Poly-morphism (%)	Name of the polymorphic SSR markers used for parental polymorphism survey and genotyping 206 recombinant inbred lines (RILs)
Chr. 1/(D1a)	20	10	10	50.00	Satt531, Satt368, Satt482, Satt342, Sat_201 , Satt383, Sat_110, AW781285, Sat_414
Chr.2/(D1b)	24	9	13	54.17	*BE021153, BE475343,* Sat_211, Satt698, Satt701, Satt634, Sat_ 254, *AI856415,* Satt296, Satt266, Satt282, Satt546, Sat_192
Chr. 3/(N)	17	7	10	58.82	Sat_379, Satt009, Satt530, Satt624, Satt584, Sat_166, Sat_275, Sat_266, Satt549, Satt022
Chr. 4/(C1)	18	7	11	61.11	Satt690, Satt396, Sat_367, Satt161, Satt718, Sat_404, *AW277661,* Sat_322, Sct_191, Sat_235, Satt524
Chr. 5/(A1)	18	8	11	61.11	Satt593, Satt591, Sat_356, Satt619, Satt545, Satt174, Satt200, Satt236, Satt511, Satt225,,Sat_271
Chr. 6/(C2)	15	5	9	60.00	Satt640, Satt170, Satt286, Satt277, Sat_238 , Satt460, Sat_263, Sat_252
Chr. 7/(M)	17	6	9	52.94	Sat_244, Satt463, Satt245, Sat_148, Sat_256, Sat_121, Satt618, Sat_276, Satt308
Chr. 8/(A2)	17	6	11	64.71	Sct_067, Satt589, *BE820148* , Sat_181, Sat_215, *AW132402,* Sat_199, Sat_377, Satt409, Satt538, Satt429
Chr. 9/(K)	16	3	14	87.50	Sat_087, Sat_119, Satt178, Satt381, Satt337, Sat_116, Sat_043, Satt499, Satt260, Sat_243, Sat_352, Satt196
Chr. 10/(O)	11	3	10	90.91	Satt358, Satt492, Satt347, Sat_291, Sat_282, Satt331, Satt581, Sat_274, Sat_231, Sat_190
Chr. 11/(B1)	16	8	8	50.00	Sat_261, Satt251, Satt197, Sat_149, Satt415, Satt665, Sat_123, Satt453, Satt467, Sat_287, Satt083, Satt168, Satt601, Sat_230, Satt020, Satt063
Chr. 12/(H)	16	3	13	81.25	Satt666, Sat_214, *U08405,*Satt353, Satt192, Satt442, Satt541, Satt676, Sat_175, Sat_216, Satt317,Satt181 Satt434,
Chr. 13/(F)	12	4	7	58.33	Sat_390, Satt325, Satt252, Satt114, Sct_188, Satt522
Chr. 14/(B2)	12	4	8	66.67	Satt467, Sat_287, Satt083, Satt168, Satt601, Sat_230, Satt020, Satt063
Chr. 15/(E)	12	5	7	58.33	Sat_112, Satt691, Satt720, Satt212, Sat_107, Satt483
Chr. 16/(J)	17	6	11	64.71	Satt674, Sct_046, Satt686, Satt529, Sat_255, Sct_001, Sat_366, Satt620, Sat_224, Sat_394, Sat_144
Chr.17/(D2)	16	9	9	56.25	Satt154, Sat_092, Satt311, Sat_338, Sat_365, Satt464, Sat_001, Sat_326, Sat_220
Chr. 18/(G)	15	2	11	73.33	Satt038, Sat_210, Sat_141, Satt235, Satt130, Sat_403, Sat_223, Satt199, Satt012, Satt517, Satt288
Chr. 19/(L)	17	3	14	82.35	Sat_408, Satt182, Satt652, Sat_187, Satt418, Satt284, Sat_150, Satt481, Sat_113, Sat_286, Satt006, Sat_245
Chr. 20/(I)	11	3	10	90.91	Satt451, Satt367, , Satt239, Satt049, Satt330, Satt292, Sat_324, Satt162, Sat_420, Sat_299
Total	**317**	**111**	**206**	**64.98**	

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