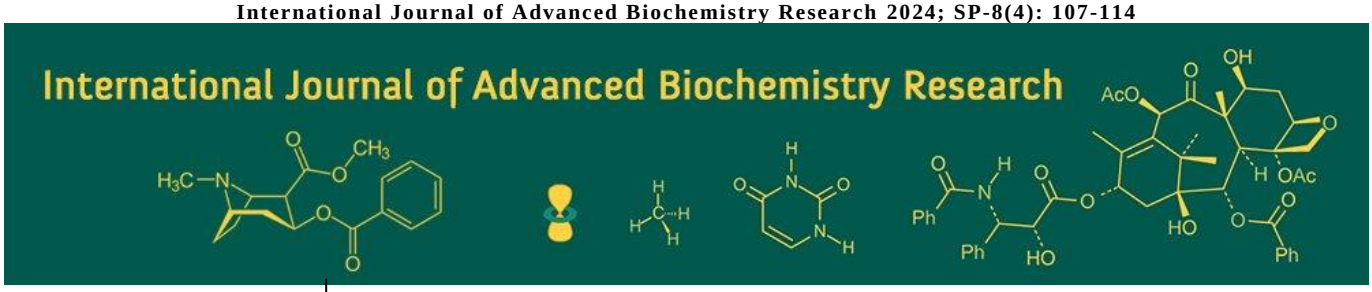

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LncRNA-SSRs based molecular diversity analysis in Indian mustard [*Brassica juncea* (L.) Czern & Coss

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Abstract
Indian mustard (*Brassica juncea* L. Czern & Coss.) is an important oilseed crop of rapeseed-mustard group. Understanding the nature and magnitude of genetic variation in conjunction with genetic gain of the traits is pertinent for a breeding programme aimed at developing high yielding stable varieties. Yield and oil content decreases progressively with the delay in planting from optimum time of sowing due to terminal heat during the reproductive phase. So, it is important to screen out the potential genetically diverse genotypes which will perform well during the late sown condition using molecular diversity analysis. We employed long non coding-RNA (LncRNA)-SSR markers, as these are highly polymorphic and useful in genetic diversity analysis. We analysed total thirty six genotypes of Indian mustard including four checks (NRCHB-101, Kranti, CS-56 and RGN-73) using LncRNA-SSR markers. Out of twenty five of these markers, fourteen were found to be polymorphic with an average PIC value 0.28 and maximum 0.56. The Jaccard's coefficient (similarity matrix), showed maximum similarity (1.0) between NRCHB101 and Kranti, followed by (0.94) between BRRM119 and BRRM 116, BRRM119 and BRRM 117, BRRM120 and BRRM-117, BRRM120 and BRRM 118, and the least similarity (0.17) was found between CMCNL22-14 and Kranti, followed by (0.24) BRRM118 and CMCNL22-15. Dendrogram prepared on the basis of molecular data showed two major clusters, I (sub-cluster IA and IB) and II (sub-cluster IIA and IIB), containing 29 and 7 genotypes, respectively. The genotypes like CMCNL22-14 and Kranti, BRRM118 and CMCNL22-15, and others having a large cluster distance could provide good heterotic combination and there is also possibility of getting desired transgressive segregants in the advance generations. The present study led to identification of suitable genotypes that could be used in future breeding programme.

Keywords: LncRNA-SSR, Indian mustard, *Brassica juncea*, Molecular marker, diversity analysis

1. Introduction
Indian mustard (*Brassica juncea* L. Czern & Coss.) is an important oilseed crop of rapeseed-mustard group, belonging to family *Brassica*ceae with a physical genome size of 922 Mb. It is an amphidiploid crop (AABB, 2n=36), which evolved by natural hybridization between two primary diploids, *B. Rapa* (AA, 2n=20) and *B. Nigra* (BB, 2n=18). Understanding the nature and magnitude of genetic variation in conjunction with genetic gain of the traits is pertinent for a breeding programme aimed at developing high yielding stable varieties. Yield and oil content decreases progressively with the delay in planting from optimum time of sowing due to terminal heat during the reproductive phase. So, it is important to screen out the potential genotype which will perform well during the late sown condition by their morpho-molecular characterization and diversity analysis. The crop improvement in mustard crop is quite complex in nature due to a complex nature due to a complex nature of inheritance of yield and its attributes. Inheritance of characters in mustard has evidenced both additive and non-additive gene action. The assessment of various genetic parameters like genotypic coefficient of variance, phenotypic coefficient of variance, heritability and genetic advance are also pre-requisite to carry out effective selection from diverse breeding materials. In Bihar, farmers mostly prefer medium and long duration nice varieties to be grown in their field. This prolongs the duration of rice crop in the field, which ultimately result in delay of mustard sowing in the state.

Thus, a need arises for developing mustard varieties which is well adapted to late sown condition in Bihar coupled with high yield potential. For developing a variety or hybrid, exploiting genetic diversity in the population is a prerequisite as the hybrids between genetically divergent lines mostly show greater heterosis. Higher seed yield is the prime objective any crop improvement programme. The response to selection for yield is low since it is an end product of a chain of contributing characters as well as also influenced by prevailing environment to a certain extent. Keeping in view above facts, the current research was conducted with objectives of genetic diversity analysis using a panel of recent molecular markers. We used long non coding-RNA (LncRNA)-SSRs, as these are highly polymorphic and useful in genetic diversity analysis (Singh *et al*., 2023, Kumar, 2023) [60].

2. Materials and Methods

The field experiment was conducted at the experimental field, Department of Genetics & Plant Breeding, Bihar Agricultural College (BAC), Sabour, Bhagalpur (Bihar). It is situated between 25˚-50˚N latitude and 87˚-19˚E longitudes at an altitude of 52.73 meters above the mean sea level. The geographical location of Sabour comes under the Middle Gangetic plain region of Agro-climatic Zone III A of Bihar. The research trial was conducted in favourable ecosystem of heavy textured alluvial soil with no heterogeneity in the field.

Thirty six genotypes of *B. juncea* included in the present study were available in our Oilseed section, BAC, Sabour. The details of genotypes are as mentioned in the Table 1.

2.1 Experimental design and layout

The experimental details of trials followed for the conduct of field investigation of thirty six genotypes of *B. juncea* is shown in Table 2.

2.2 Molecular analysis using LncRNA-SSRs

Total DNA was extracted from the leaves (50-100 mg) of each sample of *B. juncea* (Table 1) following CTAB method (Doyle and Doyle, 1990) with some modification. LncRNA-SSR markers designed (Table 3) from the SSRs containing LncRNA transcript sequences using primer 3 online tool (https://primer3.ut.ee/). Twenty five LncRNA-SSR markers were used to amplify genomic DNA of thirty six genotypes of *B. juncea* by PCR. PCR was carried on a thermos cycler (Veriti R#9902, ABI, Singapore) as follows for 10µl PCR reaction: 4µl of 2X Premix *Taq* DNA polymersase (Xceleris Genomics, India), 0.5µl of primer, 3µl of distilled autoclaved water was added and further 2µl of DNA was added. The reaction was carried out in thermos cycler, initial denaturation at 94 °C for 5 m, 38 cycles of denaturation at 94 °C for 1 m, annealing at 52 °C for 30 s, extension at 72 °C for 2 m and final extension at 72 °C for 7 m. The separation of DNA amplification products was done using horizontal 2.5% agarose gel electrophoresis and the image was captured using gel documentation system (UVITEC, Cambridge, UK).

2.3 Allele scoring and LNC RNA-SSR analysis

The PCR products from LNC RNA-SSR analysis were scored visually for either presence of absence of bands. Based on the banding pattern, the datasheet was prepared as 0, 1 matrix 1 was taken for the presence of band (allele), while 0 was taken for absence of band (allele), PIC value and genetic distance based clustering and the dendrogram construction was performed with Un-weighted Pair Group Method for Average (UPGMA) method given by Sokal and Michener (1958) [24] and tool available online at http://genomes.urv.cat/UPGMA/UPGMAboot_v12.cgi.. The genetic dissimilarity identified by LNC RNA-SSR markers and taxonomic distance measured by mean genetic distance was estimated using Jaccard's similarity index.

3. Results

3.1 Molecular diversity analysis using Lnc RNA-SSR markers

The 36 genotypes of the Indian mustard have been used for molecular diversity analysis using 25 SSR markers. Out of 25 SSR markers used, only 14 markers turned out to be polymorphic. The amplification profiles of each of the 14 polymorphic markers run on gel across all the 36 genotypes were visualized on the 2% Agarose gel with the help of gel documentation system. The representative image of amplification with three LncRNA-SSR markers (LNC15563, LNC10290, LNC16304) is shown in Fig. 1. The 14 identified polymorphic markers yielded the 2 to 5 allele per marker. The allelic frequencies of the upper and lower alleles of all 14 polymorphic SSR markers were given in the Table 4. The sizes of amplicons ranged between 100 to 500 base pairs. The Polymorphic Information Content (PIC) value calculated was varied from 0.006 (LNC10290) to 0.560 (LNC15563) with an average of 0.28 (Table 4). SSR marker polymorphism level is generally measured in terms of PIC values. The discriminatory power of SSR marker can be defined as high for PIC values >0.50, moderate for PIC values in the range of 0.25 to 0.50 and low for PIC values <0.25. The average PIC values were calculated over all loci for better understanding the genetic diversity at the molecular level. The scored allelic data from the amplification of all the 14 polymorphic SSR markers were used to generate the dendrogram with the help of UPGMA clustering of simple matching dissimilarity indices.

3.2 Molecular diversity and relationships among Indian mustard genotypes

The mean genetic similarity index for all the 36 Indian mustard genotypes was calculated as the Jaccard's similarity indices between the genotypes with the help of scored LncRNA-SSR markers data, which were varied from 0.17 between the genotypes like CMCNL-22-19 & BRRM-118 to 0.88 between the genotypes like CS-56 & BRRM-120 (Table 5).

The 36 genotypes of Indian mustard were grouped on the basis of SSR amplification profiles of 14 polymorphic markers by neighbour-joining UPGMA cluster analysis of the pair-wise simple matching similarity coefficients matrix into two major clusters *viz*., cluster I and cluster II and four sub-clusters *viz*., sub-cluster I'A, sub-cluster I'B and sub-cluster II' A, sub-cluster II'B as shown in (Table 6). Sub-cluster I'B was the highest of all four sub-clusters found on basis of molecular scoring which comprised of seventeen genotypes *viz* CMCNL-22-15, CMCNL-22-13, CMCNL-22-17, CMCNL-22-18, CMCNL-22-16 BRRM-118, BRRM-114, BRRM-115, BRRM-113, CS-56, BRRM-117, BRRM-116, BRRM-119, BRRM-120, BRRM-121, BRRM-112, RGN-73. Followed by sub-cluster I'A which comprises

twelve genotypes *viz*; CMCNL-22-5, CMCNL-22-1, CMCNL-22-12, CMCNL-22-11, CMCNL-22-6, CMCNL-22-4, CMCNL-22-3, CMCNL-22-2, CMCNL-22-10, CMCNL-22-7, CMCNL-22-9, CMCNL-22-8. Sub-cluster II'A comprises two genotypes *viz*; CMCNL-22-19, CMCNL-22-14 and Sub-cluster II'B comprises five genotypes *viz*; BRRM-110, BRRM-111, CMCNL-22-20, Kranti, NRCHB-101.

4. Discussion

Simple Sequence Repeat (SSRs) markers are the most preferred molecular markers because of their higher reproducibility co-dominance nature, wide distribution throughout the genome, easy scorability, and multi-allelic variation. Microsatellites surpass other DNA based markers such as RFLPs, RAPDs, AFLPs, etc. It had been documented that SSRs can analyse better genetic diversity than other molecular markers. Also, improved techniques that is more simple and efficient to find polymorphism in SSR marker makes SSR marker more useful (Kumar *et al.* 2015) [49]. SSR markers have been widely used in genetic diversity analysis in various *Brassica* spp. (Vinu *et al*., 2013; Prajapati *et al*., 2012; Sudan *et al*., 2016) [56, 30, 47, 49].

Recently, LncRNA derived SSRs (LncRNA-SSRs) were identified as a new generation of molecular markers with higher efficiency (Singh *et al*., 2023) [60]. LncRNA-based SSR markers were developed from RNA-seq data of *B. juncea* available with us and used in the present study. In our study, out of twenty five LncRNA SSR markers used for molecular diversity, fourteen markers were found to be polymorphic, and rest were found to be monomorphic. The size of amplified regions for the polymorphic primers was present between 100 to 500 base pairs. The PIC value calculated was varied from 0.006 (LNC10290) to 0.560 (LNC15563) with an average of 0.28 (Table 4). Though, there are only few reports of non-coding RNA or LncRNA-based SSRs use in *B. juncea* for genetic diversity analysis, but genic/genomic SSRs have been widely used for this purpose. We have discussed here some of the relevant SSRs-based work in *B. juncea*. Used 58 SSR primers for molecular diversity analysis among 45 lines of Indian mustard. Out of 58 primers used. 33 primers were found to be polymorphic. The number of alleles per locus varied from 2 to 5 having an average of 2.89. The PIC value ranged from 0.206 to 0.749 with an average of 0.519.

The genetic similarity matrix generated with the help of scored LncRNA-SSR marker data was ranged from 0.17 to 0.88 (Table 5). The 36 Indian mustard genotypes were grouped into two major clusters *viz*., cluster I and cluster II and four sub-clusters *viz*., sub-cluster I A, sub-cluster I B and sub-cluster II A, sub-cluster II B on the basis of LncRNA-SSR scoring amplification profiles of fourteen polymorphic primers by neighbour-joining UPGMA cluster analysis. The dendrogram (Fig. 2) and the similarity coefficient matrix (Table 5) indicated that less amount of genetic diversity was present in the Indian mustard germplasm. Further, on the detailed observation of the dendrogram, it was found that all the clusters were containing the genotypes being recommended and adapted to different agro-climatic zones of India (Fig. 2, Table 6) indicating that the geographical distribution had very less effect in the molecular clustering of Indian mustard genotypes. The experiment also suggests the need of screening some more number of polymorphic primers for further understanding the molecular diversity.

Earlier, reported non-coding RNA based SSR marker, Out of 623 ncRNA SSRs, 120 (including 60 each miRNA SSRs and lncRNA SSRs) were used for genotyping of 96 *Capsicum* accessions belonging to *C. annuum*, *C. chinense*and *C. frutescens*; and 75% SSRs were found to be polymorphic. Model-based and distance-based cluster analyses identified three species specific clusters, i.e. cluster-I (*C. annuum*), cluster-II (*C. frutescens*) and cluster-III (*C. chinense*); therefore, these SSRs may have a potential role to play in inter-specific *Capsicum* breeding. Verma *et al.* (2021) [55] also analysed of genetic diversity among *B. juncea* genotypes using morpho-physiological and LncRNA-SSR markers.

Performed genetic diversity analysis among 96 germplasm lines of *B. juncea* using 83 SSR primers and 20 phenotypic variables. 16 primers were found to be polymorphic which gave a total of 47 alleles which varied from 2 to 5 with an average of 2.9 alleles per primer. The mean PIC value from all the polymorphic primers was 0.529. In the dendrogram all the 96 germplasm lines were divided in to 2 main clusters at similarity coefficient of 0.65. Molecular diversity analysis of 38 Indian mustard genotypes was also carried out by employing 18 SSR. From 18 markers used for amplification, 130 alleles were amplified across 18 genotypes. Among them 128 alleles were scored polymorphic with 77 percent average polymorphism. These SSRs grouped the genotypes into five major clusters at similarity coefficient of 0.001 which indicated considerable dissimilarity existed among the clusters. Baghel *et al*. (2020) [13] carried out molecular categorization of 48 mustard (*Brassica* spp.) genotypes by employing 20 SSR markers, the best eight amplified markers were picked. In total 50% polymorphism was observed and major allele frequency ranged from 0.3750 to 0.52 having a mean value of 0.43. Heterozygosity ranged between 0 and 20. The PIC ranged from 0.69 to 0.992 and genetic similarity varied between 0.478 and 1.000. The cluster analysis disclosed two main clusters with highest 57% similarity percentage.

Sharma *et al*. (2020) [13] carried out molecular analysis on 55 genotypes of Indian mustard with 155 SSRs which resulted in 482 alleles and the number of alleles varied from 1 to 8 with an average of 3.11 alleles per marker a total of 122 SSRs resulted into polymorphic amplicons. PIC value varied from 0.38 to 0.77 with an average value of 0.45 per SSR locus. The UPGMA based dendrogram analysis divided all the 59 accessions into two major groups which were on the basis of both agro-morphological traits and SSR markers. Genetic diversity was studied in forty germplasm lines and eight cultivars of Indian mustard using 50 SSR markers, out of which 7 SSR molecular markers were found to be highly polymorphic between all the genotypes of mustard. All seven SSR primers exhibited PIC value above 0.5 (50%) demonstrating high genetic diversity. Genotypic correlations were higher than phenotypic ones in magnitude for all the characters. Studied on 87 Indian mustard varieties using 200 genomic-SSR markers for genetic diversity analysis. Out of 200 SSRs evaluated, 189 SSR markers produced clear and scorable bands, while remaining 11 exhibited no amplification at all. The PIC value ranged from 0.10 to 0.68 with 0.39 as mean PIC value.

Table 1: List of genotypes used in the study

S. No.	Genotype	Source	S. No.	Genotype	Source
1.	BRRM-110 (IC-520769*IC-426311)	Oilseed section, BAC, Sabour	19.	CMCNL-22-3	AICRP-Rapeseed-mustard trial (2022-23)
2.	NRCHB-101(C)	Oilseed section, BAC, Sabour	20.	CMCNL-22-4	AICRP-Rapeseed-mustard trial (2022-23)
3.	BRRM-111 (IC-371721*IC-638803)	Oilseed section, BAC, Sabour	21.	CMCNL-22-5	AICRP-Rapeseed-mustard trial (2022-23)
4.	Kranti (c)	Oilseed section, BAC, Sabour	22.	CMCNL-22-6	AICRP-Rapeseed-mustard trial (2022-23)
5.	BRRM-112 (IC-426388*IC-491543)	Oilseed section, BAC, Sabour	23.	CMCNL-22-7	AICRP-Rapeseed-mustard trial (2022-23)
6.	BRRM-113 (IC-426392*IC-520769)	Oilseed section, BAC, Sabour	24.	CMCNL-22-8	AICRP-Rapeseed-mustard trial (2022-23)
7.	BRRM-114 (IC-371721*IC-264131)	Oilseed section, BAC, Sabour	25.	CMCNL-22-9	AICRP-Rapeseed-mustard trial (2022-23)
8.	CS-56(C)	Oilseed section, BAC, Sabour	26.	CMCNL-22-10	AICRP-Rapeseed-mustard trial (2022-23)
9.	BRRM-115 (Varuna*PusaJaikisan)	Oilseed section, BAC, Sabour	27.	CMCNL-22-11	AICRP-Rapeseed-mustard trial (2022-23)
10.	BRRM-116 (P. BOLD*Laxmi)	Oilseed section, BAC, Sabour	28.	CMCNL-22-12	AICRP-Rapeseed-mustard trial (2022-23)
11.	BRRM-117 (P. Agrani*Varuna)	Oilseed section, BAC, Sabour	29.	CMCNL-22-13	AICRP-Rapeseed-mustard trial (2022-23)
12.	BRRM-118 (P. Bold*Varuna)	Oilseed section, BAC, Sabour	30.	CMCNL-22-14	AICRP-Rapeseed-mustard trial (2022-23)
13.	BRRM-119 (IC-371721*IC-347949)	Oilseed section, BAC, Sabour	31.	CMCNL-22-15	AICRP-Rapeseed-mustard trial (2022-23)
14.	BRRM-120 (Rajat*P.Bold)	Oilseed section, BAC, Sabour	32.	CMCNL-22-16	AICRP-Rapeseed-mustard-2022-23
15.	BRRM-121 (IC-264131*IC-426388)	Oilseed section, BAC, Sabour	33.	CMCNL-22-17	AICRP-Rapeseed-mustard trial (2022-23)
17.	CMCNL-22-1	AICRP-Rapeseed-mustard-2022-23	35.	CMCNL-22-19	AICRP-Rapeseed-mustard trial (2022-23)
18.	CMCNL-22-2	AICRP-Rapeseed-mustard-2022-23	36.	CMCNL-22-20	AICRP-Rapeseed-mustard trial (2022-23)

Table 2: Details of experimental layout

Particular of trial	Description
Design	Randomized Block Design
Number of treatments	36 including 4 checks (NRCHB-101(C), Kranti (c), CS-56(C), RGN-73(C)
Number of replications	3
Gross plot size	1.8 m x 5.0 m =9 m2
Planting geometry (R x P)	3 0 cm x10 cm
Number of rows/entries	6
Planting time	3rd week of November
Seed rate	3-4 kg ha^{-1}
Recommended dose of fertilizers(kg ha-1)	80 : 40: 40 (N:P:K)

Table 3: List of LncRNA-SSR markers used in the study

S. No.	Primer's name	Primer Sequence (5′-3′)		Average Tm (˚C)
1.	LNC15563	F	GAAGCTCATCCATCTGAGC	49
		R	ACTCAGTTAAAAAAAAAACCCT	
2.	LNC10290	F	TCTCGGTGGTTCTCCTCGGC	55
		R	AAGTACCGAGAGAGAGAGAG	
3.	LNC16304	F	ATGACTACTCAGCAATAATC	46
		R	GATGTTGATTATATGCAGTC	
4.	LNC49637	F	ACGCTCTTTCATAGAGAAG	46
		R	CACCATCATAGTAATAGATAT	
5.	LNC35372	F	TGAACTAAATTTTTCTGTTCG	47
		R	ATCAGATCGTCTTCGGAC	
6.	LNC54412	F	TCTTTAGCTCCCATTTTCTC	48
		R	GCATAAACAACAAAGTCCTG	
7.	LNC50279	F	ACTTCAAGCAAAGCATATG	45
		R	TAGTATCTATCTATGACAATG	
8.	LNC139492	F	GAACATGTGAAGATTAGACAG	50
		R	CCCAAAGATCCAATCAAACG	
9.	LNC36791	F	CGTTAAGCCTGTTACCAATAC	53
		R	CTCGGTACCTCCCTCTCGT	
10.	LNC52070	F	ATCAGAAAGACTGGTCAGAG	49
		R	GGCAAACTTTAACAGTTCAC	
11.	LNC2501	F	GAGGCGATGAGAACGATC	50
		R	GGTGTCTAGTCCTAATTTCG	
12.	LNC18852	F	TTAGGTTGCGTACTATAG	41
		R	AAATACATGATATATATATATA	
13.	LNC131289	F	CTAGACAGCAAATAGGATT	45
		R	CATGTTTGTTCAAGAAAATC	
14.	LNC22585	F	AGATATGGCCGGTGTGAC	47
		R	CATCATCTTTAGATTTATGTT	
15.	LNC4443	F	CCTTCTCCTTCTCCTTTTCTT	55
		R	AGTCTGATGAAGCTTACAAGA	
16.	LNC63494	F	GCT TTC TTC AAT TTC AGA GC	52
		R	GTT ACA TGT AAC ATT TCT ATA	
17.	DN8435	F	CTG ACA GAA GAA AGG GGT TTG	60

		R	GGA GGAGGAGGAGGAGGA	
18.	DN8935	F	ACT TAT TAT AGC TTC GTC GC	56
		R	ACG AGA GAC GGT TGA GCA	
19.	DN28978	F	TCT ATG TCT ATG TTC GAC AT	52
		R	TAT GTT CGG TTT GAG TGA AT	
20.	DN20845	F	AGT TGC AAT CAT TCT AAT GGA	55
		R	GAC ATG GCA GCC ACA AAG	
21.	DN8130	F	ATT GTC AGC CTT CGC GTT T	54
		R	CAC TGT TAC CCT ATC AGT	
22.	DN12512	F	TCC ACA AAT TTC ACG ATC CT	54
		R	TGC AAC CAA TGG AAA CGT TA	
23.	DN97034	F	AGG TTT ATT ATTATT CTG CT	51
		R	AGA ATC GGA GCA GCT CTA	
24.	DN12417	F	TTG TTC AGA ATC AAT TCA GA	52
		R	ACA CAT GGT CGG TCA	
25.	DN119044	F	ATA ATAATA AGA GAA ACA GTA	48
		R	TAT ATT AAT AGG GTT TTG TAT	

Table 4: Polymorphism-related information of LncRNA-SSR markers used in the present study

S. No	Primer Name	No of alleles	Allelic frequency		Range of alleles (bp)	PIC Value
			Up	Low		
1.	LNC15563	3	0.889	0.02	200-100	0.560
2.	LNC10290	2	0.923	0.077	200-150	0.006
3.	LNC16304	2	0.627	0.373	100-175	0.394
4.	LNC49637	2	0.615	0.385	150-100	0.148
5.	LNC35372	3	0.700	0.100	150-100	0.427
6.	LNC54412	4	0.773	0.0	250-100	0.373
7.	LNC50279	2	0.553	0.447	150-100	0.200
8.	LNC139492	3	0.679	0.038	180-100	0.343
9.	LNC36791	3	0.696	0.087	200 – 100	0.408
10.	LNC52070	4	0.538	0.026	500-100	0.426
11.	LNC2501	5	0.452	0.081	700-150	0.326
12.	LNC18852	3	0.528	0.167	150-140	0.166
13.	LNC131289	3	0.806	0.083	200-100	0.066
14.	LNC22585	2	0.917	0.083	200-180	0.076

Table 5: Jaccard's Similarity Matrix

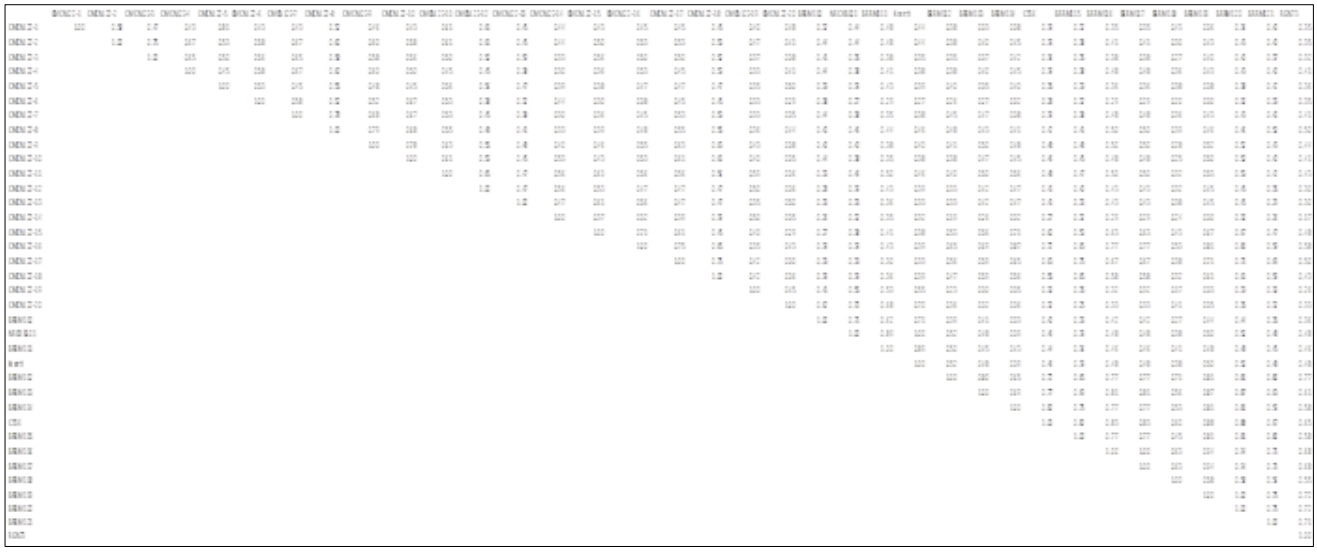

Table 6: Clusters based on UPGMA

S. No.	Number of cluster	Number of sub-cluster	Number of genotype	Name of the genotype
1	I	I'A	12	CMCNL-22-5, CMCNL-22-1, CMCNL-22-12, CMCNL-22-11, CMCNL-22-6, CMCNL-22-4, CMCNL-22-3, CMCNL-22-2, CMCNL-22-10, CMCNL-22-7, CMCNL-22-9, CMCNL-22-8
		I'B	17	CMCNL-22-15, CMCNL-22-13, CMCNL-22-17, CMCNL-22-18, CMCNL-22-16 BRRM-118, BRRM-114, BRRM-115, BRRM-113, CS-56,BRRM-117, BRRM-116, BRRM-119, BRRM-120, BRRM-121, BRRM-112, RGN-73
2	II	II'A	2	CMCNL-22-19, CMCNL-22-14
		II'B	5	BRRM-110, BRRM-111, CMCNL-22-20,Kranti, NRCHB-101

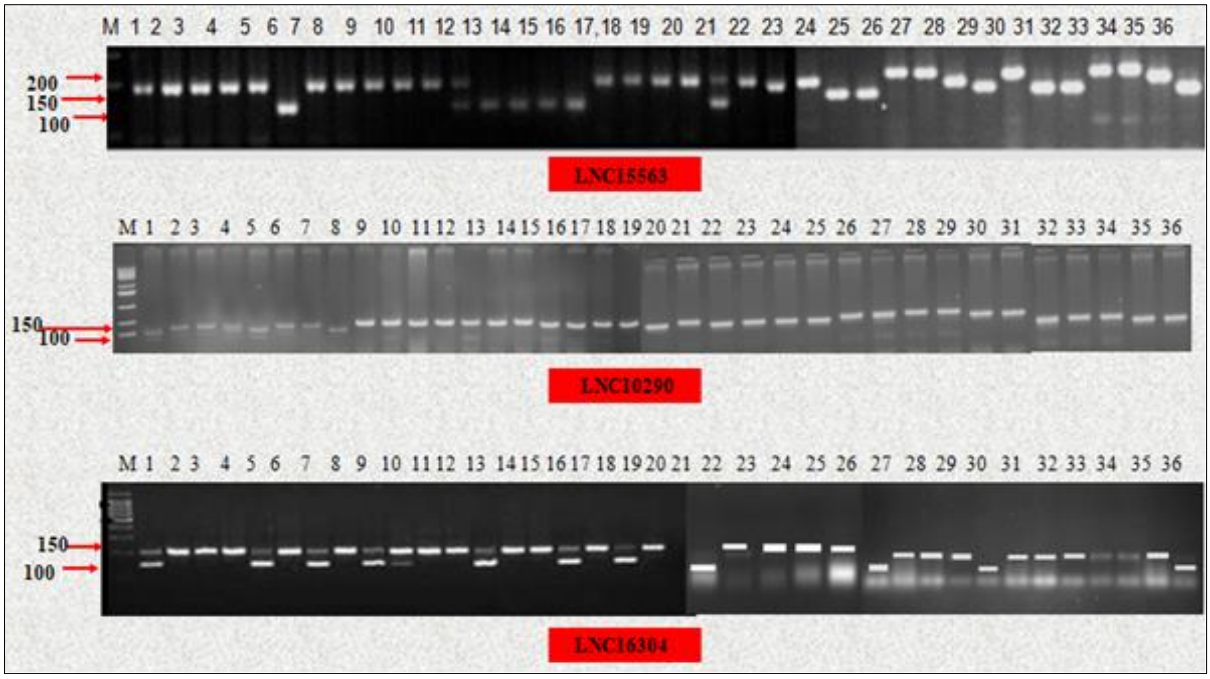

Fig 1: A representative agrose gel electrophoresis image of PCR amplification products with LncRNA-SSR markers (LNC15563, LNC10290, LNC16304) of 36 genotypes of B. juncea (1-36 as shown in Table 4)

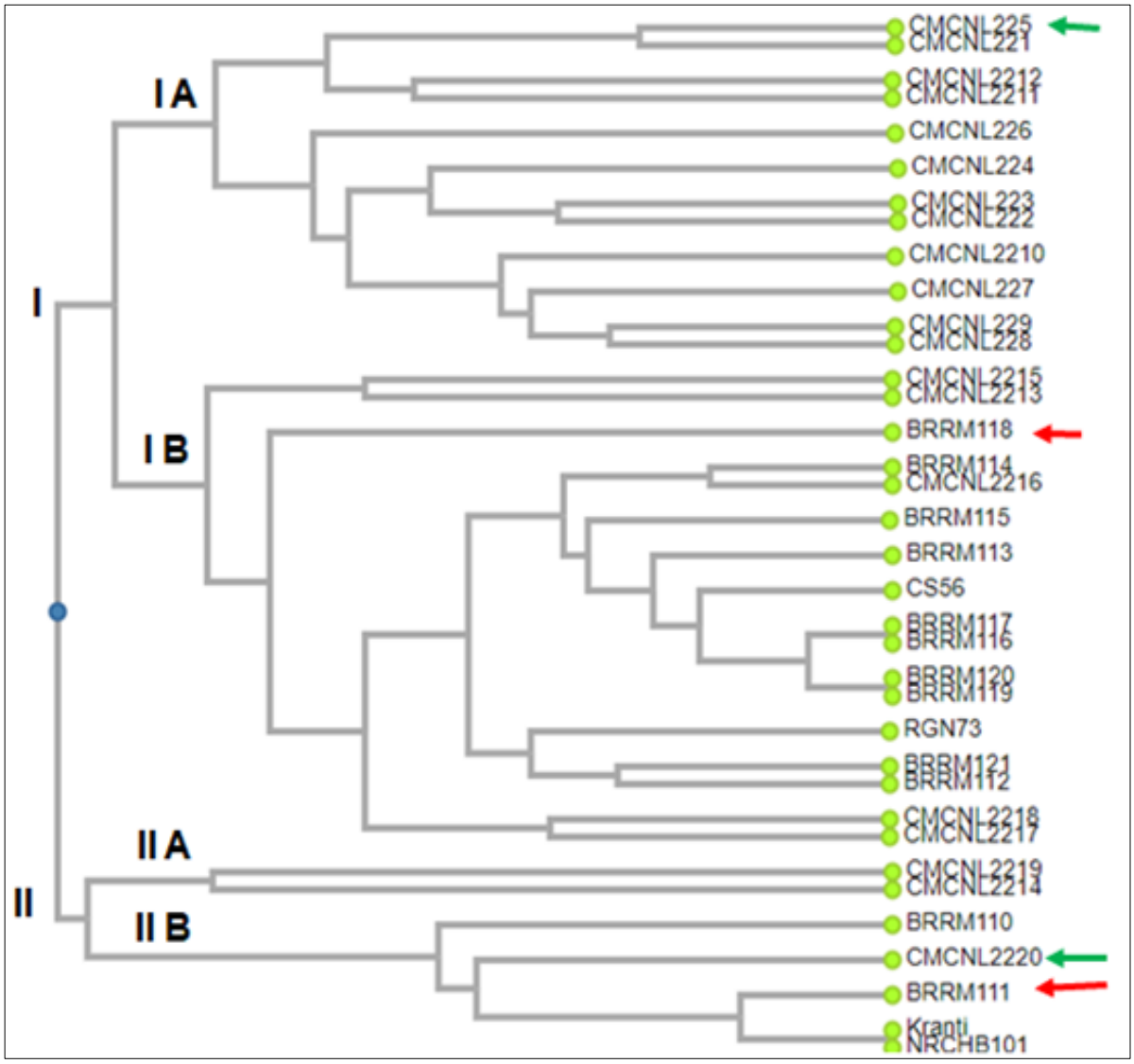

Fig 2: LncRNA SSR-based UPGMA dendrogram showing clustering of 36 B. juncea genotypes (Green and Red arrows indicate genotypes from CMCNL series and BRRM series, respectively, identified as promising ones for heterosis breeding).

5. Conclusion

The genotypes like CMCNL22-5, MCNL22-14, CMCNL22-15, CMCNL22-20. Kranti, BRRM110, BRRM 111, BRRM118 and others having a large cluster distance between any two of them could provide good heterotic combination and there is also possibility of getting desired transgressive segregants in advance generations. The present study led to identification of suitable genotypes that could be used in future breeding programmes.

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