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Genetic diversity studies for drought tolerance among various genotypes of *Brassisa juncea* L. using SSR markers

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Abstract
Ten SSR primer were employed to generate polymorphism of 20 *Brassica juncea* genotypes. Primers with high polymorphic bands SSR Na14-G06 and SSR Na14-D09 were more efficient to discriminate the varieties. These primers are useful for fingerprinting in *Brassica juncea* lines as they were able to resolve all the genotypes. Polymorphism information content of each primer was calculated and ranged from 0.33 (primer SSR Na12_H09) to 0.79 (primer A01_268890) with an average of 0.58. Percentage of polymorphism ranged from 10.52% to 44.44% with an average of 19.85% per primer.

Keywords: *Brassica juncea*, SSR markers, Polymorphism, Genetic diversity

Introduction
Indian mustard (*Brassica juncea* (L.) Czern & Coss) is an economically significant oilseed crop across the globe with well-known nutritive values. Rapeseed and mustard are the major rabi oil seed crops of India. India produced 10.1 million tons of rapeseed and mustard in year 2020-21. The area under rapeseed and mustard in India is 6.7 million hectares, with a productivity of 1511 kg/ha during 2020-21. In the state of Jammu & Kashmir the area under rapeseed - mustard is 51870 ha with production of 59600 MT and 1149 kg per productivity during 2018-19. *B. juncea* (2n=36, AABB genome), an allopolyploid commonly called as Indian mustard, contributes more than 80% to the total rapeseed-mustard production in the country and is an important component in the oilseed sector due to its high oil content (37-42%). *B. juncea* is known to be more drought tolerant and shattering resistant than *B. napus* and *B. rapa*, therefore, has an enormous cultivation potential in semi-arid areas. The maximum utilization of any species for breeding and its adaptation to different environments depend on the level of genetic diversity it holds. Genetic distance among parents may be attributed to their differences for number of genes and their functional relations in a given environment (Nei, 1976) [9]. Knowledge on genetic diversity in *B. juncea* could help breeders and geneticists to understand the structure of germplasm, predict which combinations would produce the best off springs (Hu *et al*., 2007) [4], and facilitate to widen the genetic basis of breeding material for selection (Qi, Yang & Zhang, 2008) [10].
Genetic diversity among individuals or populations can be determined using morphological, biochemical and molecular approaches **(**Mohammadi & Prasanna, 2003) [7]. Among various markers available for genetic analysis in plants, molecular markers are more efficient, precise and reliable in discriminating closely related species and cultivars (Mishra *et al*., 2011) [6], even then, many breeding groups emphasize in morphological traits than molecular markers **(**Hu *et al*., 2007) [4]. Among different types of molecular markers, microsatellite also known as simple sequence repeats (SSRs) have been utilized for different purposes in plant breeding and genetics including genetic mapping, cultivar discrimination and detection of genetic diversity (Gupta &Varshney, 2000) [3]. They are co-dominant markers, reproducible, highly efficient, detect high level of polymorphism and are evenly distributed (Morgante & Olivieri, 1993).

Experimental material
The seed material of 20 genotypes of *Brassica juncea* species was procured from different institutes of India including SKUAST-Jammu. This experimental material was grown in Randomized Blocked Design with 3 replications under irrigated and rainfed conditions at SKUAST Chatha farm in 2018-19.

Table 1: Genotypes with their pedigree used for study

S. No	Genotypes	Pedigree	Source
1	RH-1209	RH-0555 X RH-O401B	CCSHAU, Hissar
2	DRMRIJ-15-85	(EC 39288 XPCR 11)X (B33X Sanjucta Asch)	DRMR, Bharatpur
3	DRMRIJ-15-5	Choupka x PWR	DRMR, Bharatpur
4	RGN-385	RL 99-27 X RGN-73	ARS, Ganganagar
5	RB-77	RH-819 X RH-8814	CCSHAU, Bawal
6	RLJEB-84	(*B. juncea* x *Diplotaxis erucoides*) x *B. juncea*	PAU, Ludhiana
7	Kranti	Selection from Varuna	CSAUA&T, Kanpur
8	Varuna	Selection from Varanasi local 786,02.021976	CSAUA&T Kanpur
9	RSPR-69	RLM 198 X Varuna	SKUAST-Jammu
10	RH-749	RH-781 X RH-7617	CCSHAU, Hissar
11	RH-749	RH-781 X RH-7617	CCSHAU, Hissar
12	NPJ-183	(NPJ-102 X Pusajaganath) x NPJ-102	IARI, Delhi
13	Giriraj	HB-9908 X HB-9916	DRMR, Bharatpur
14	RB-50	Laxmi x RH-9617	RRS, Bawal
15	RB-55	-	RRS, Bawal
16	NRCHB-101	BL4 X Pusa Bold	DRMR, Bharatpur
17	Pusa Bold	Varuna x BIC 1780	IARI, Delhi
18	RH-819	Prakash x Bulk Pollen	CCSHAU, Hissar
19	DRMRIJ-541-44	-	DRMR, Bharatpur
20	DRMRIJ-59	-	DRMR, Bharatpur

SSR Assay

Primers used for DNA Amplification

A set of 10 SSR primers were selected for use in amplification of genomic DNA. Based on earlier studies, SSR's were selected. These were got synthesized from IDT (Integrated DNA Technologies, USA).

Primer Dilution: Double distilled, autoclaved and deionized water was used to dilute primers at a concentration of 5pmol as shown in table 2.

Table 2: Arbitrary 10 SSR primers with their sequence selected for DNA amplification

S. No.	Markers	Sequences	Primer sequence	Annealing Temp (^{0}C)
1	SSR Na10-B04	Forward	GCGTCGAGAGAGATCGAGAG	52
		Reverse	CTCACCGTCACTGCTTCATC	
2	SSR Na12-D03	Forward	GGTAAGCCAAAAACCCTTCC	50
		Reverse	GGTAAGCCAAAAACCCTTCC	
3	SSR Na12-H09	Forward	AGGCGTCTATCTCGAAATGC	48
		Reverse	CGTTTTTCAGAATCTCGTTGC	
4	SSR Na14-D09	Forward	GATCAACGTAAGGTCGCCTC	48
		Reverse	GAATCCAACGGATCAGAAGC	
5	SSR Na14-G06	Forward	AAACGGCTTGCATTGTTCTC	48
		Reverse	GGCTTGCTTGATCCAGTCTC	
6	Ni4-F11	Forward	CGTAAGTTTCAATTGTCAACGG	53
		Reverse	TCGTACGAAACAATCAACGG	
7	A01_2688930	Forward	CAATGTAATGGGAAGAAAATG	51
		Reverse	GTACCTCTCCTGGTCCTGTAT	
8	A02_18870790	Forward	TACACCGTCTGATTCCATCT	50
		Reverse	GCCTGACTGCTGCTACTAAC	
9	A03_25410649	Forward	ATAGCCATACGCTGAAGAAA	51
		Reverse	GAGACGAATAATCCTCCAAAT	
10	A05_15183336	Forward	GTTACCTATGAGCTCTCCTT	52
		Reverse	CGTGCGGGTATTTATTTTTAT	

Components used for PCR Reaction

DNA amplification was carried out in PCR tubes containing 25 µl reaction mixture. Reaction mixture contained 2.5 µl of template DNA (25ng/µl), 2.5 µl of 10X PCR Buffer, $Mgcl_2$ (2mM), 0.2 mM of each dNTPs (dTTPs, dGTPs, dCTPs, dATPs), primer (1.67Mm) concentration, 5 units Taq DNA polymerase. The quantity of these components used in a reaction is given in Table 3.

Table 3: Reagents with their concentration and quantity used for single PCR reaction

S. No.	Reagents	Concentration	Quantity
1.	Template DNA	25 ng/µl	2.5 µl
2.	Sterile water		13.5 µl
3.	PCR Buffer	10 X	2.5 µl
4.	$Mgcl_2$	50 mM/ µl	1 µl
5.	dNTPs	2.5 mM/ µl	2µl
6.	Primer	5pmole	3µl
7.	Taq polymerase	5 U/µl	0.5µl
	Total		25 µl

PCR Amplification Program

PCR tubes containing master mix and DNA template were thoroughly mixed and subjected to the thermal profile given in table 6.The amplification reaction was carried out in a gradient master cycler. An initial denaturation step of 4 minutes was programmed in the thermo cycler, followed by a loop of 35 cycles each consisting of denaturation (at 94 ^{0}C for 1 minute), annealing (at 36 ^{0}C for 1 minute) and extention (at 72 ^{0}C for 2 minutes). The final extension was performed at 72 ^{0}C for 10 minutes. The PCR products were then stored at 4 ^{0}C.

Table 4: Thermal profiles used for DNA amplification

Steps	Cycles	Temperature	Duration
Denaturation	1	94^{0}C	4 min
Denaturation	35	94^{0}C	1 min
Annealing		36^{0}C	1 min
Extension		72^{0}C	2 min
Final Extension	1	72^{0}C	10 min

The same reaction mixture without genomic DNA was run for each reaction to serve as a negative control.

Results and Discussion

Genomic DNA isolation and quantification of 20 *Brassica juncea* genotypes was done and subjected to PCR amplification using 10 SSR primers. The amplified bands were scored and dendrogram constructed to assessment genetic variability.

Scoring of Bands

The banding patterns of 20 genotypes against each primer were compared and used to generate Bi-nomial data matrix. Variability was assessed on the basis of polymorphic bands (present in one genotype and absent in another genotype). The capability of SSR primers to discriminate genotypes was assessed based on Polymorphic information content (PIC) and percentage of polymorphism. Polymorphic information content of each primer was calculated and ranged from with an average of 0.62.

Table 5: Total number of Bands, number of polymorphic bands, number of monomorphic bands and calculated parameters for the 10 primers used.

S. No.	Primer	TNB	NPB	NMB	PPB	PIC
1	SSR Na10_B04	17	2	15	11.76	0.48
2	SSR Na12_D03	16	3	13	18.75	0.67
3	SSR Na12_H09	16	1	15	6.25	0.58
4	SSR Na14_DO9	15	4	11	33.36	0.69
5	SSR Na14-G06	13	4	9	44.44	0.33
6	Ni4-F11	13	3	10	23.07	0.42
7	A01_2688930	8	2	6	25	0.79
8	A02_18870790	24	4	20	16.67	0.54
9	A03_25410649	19	2	17	10.52	0.68
10	A05_15183336	16	3	13	18.75	0.62
Average		15.7	2.8	12.9	19.85	0.58

Where,

TNB= Total number of Bands
NPB =Number of polymorphic Bands
NMB=Number of monomorphic Bands
PPB= Percentage of polymorphic bands
PIC= Polymorphism Information content

Data Analysis

The PIC values for each primer were evaluated. Polymorphism information content of each primer was calculated and ranged from 0.33 (primer SSR Na12_H09) to 0.79 (primer A01_268890) with an average of 0.58. The percentage polymorphism was evaluated by the SSR –PCR amplification profile of 20 genotypes produced by 10 primer.

PIC values of present study were found to be higher than that reported by Turi *et al.*, (2012) [12] in *B. juncea* (0.46). Gupta *et al.*, (2014) [2] reported low PIC value 0.281; Sudan *et al.*, (2016) PIC values ranged from 0.12-0.61 with an average to 0.314 but lower than that, observed by Nanjundan *et al.*, (2015) [8]. The PIC values (0.38-0.96) observed by Avtar *et al.*, (2016) [1] were found to be higher than that of our study. Lower number of alleles per locus and lower PIC values may be attributed either to the use of less informative SSR markers, or the presence of lesser genetic diversity among the tested genotypes.

Dendrogram Analysis

Dendrogram was constructed based on DAR win 5.0 and all the genotypes were clustered in two major groups. The cluster I includes 4 genotypes. The cluster II includes 16 genotypes which are grouped in two sub-clusters, that are further divided into many sub-sub clusters. The genotypes present in II cluster *viz*., RSPR-69 and DRMRIJ-15-85 are more diverse from the genotypes present in the cluster I. To exploit heterosis, the hybridization programme postulated on the basis of genetic divergence may be successful between the genotypes *viz*, RH-406 and DRMRIJ-15-85 or RSPR-69 and RH-406.

However, Kumar *et al.*, (2013) [5] observed eight clusters with a range of 1-18 genotypes in each cluster (cluster III being the largest) in the genetic diversity study on forty six Indian mustard genotypes. The presence of less number of clusters in the present study may be because lesser number of genotypes considered in the study.

The present study helped us in identifying the highly polymorphic SSR, and in order to explore the diversity among huge number of genotypes, these informative primers can be of great help. However, there is further need of involving larger number of genotypes as well as markers to have better picture of germplasm structure to exploit for crop improvement programs.

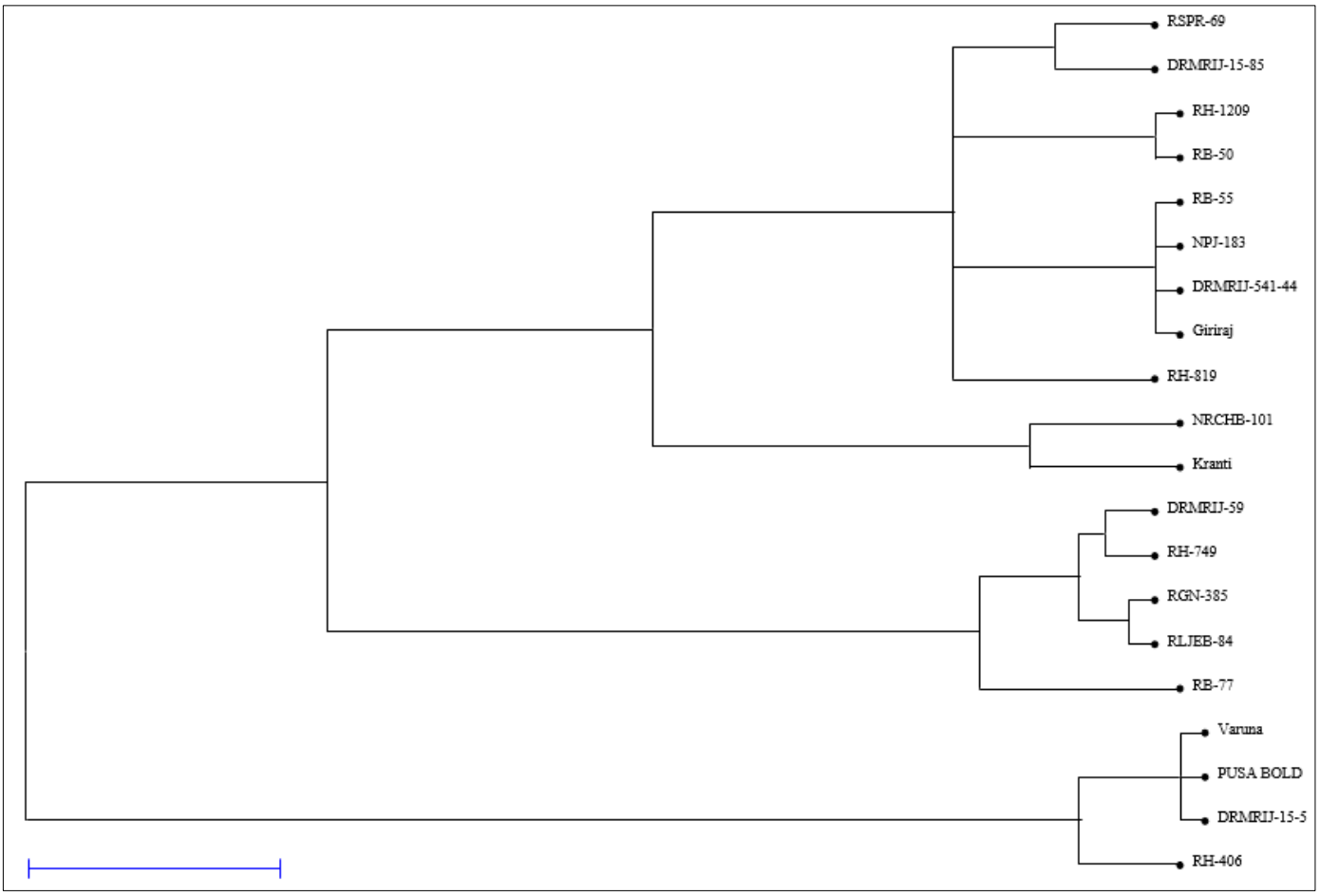

Fig 1: Dendrogram of 20 genotypes obtained by SSR markers analysis using DAR win 5.0

Conclusions

Primers with high polymorphic bands SSR Na14-G06 and SSR Na14-D09 were more efficient to discriminate the mustard varieties.

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