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Short Communication

Analysis of molecular diversity in Indian and Exotic genotypes of *Brassica juncea* using SSR markers

Jebi Sudan, Prakriti Khajuria, S. K. Gupta[1] and Ravinder Singh*

School of Biotechnology, [1]Department of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu 180 001 (J & K)

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Abstract

Molecular diversity analysis of Indian and Exotic genotypes of *Brassica juncea* genotypes was carried out using simple sequence repeat (SSR) markers. Sixteen out of 32 SSR markers were found to be polymorphic and amplified 54 alleles in 23 genotypes with an average of 2.37 and 0.31 for alleles per locus and polymorphic information content (PIC), respectively. The genotypes were grouped into three distinct clusters based on unweighted neighbour joining and population structure analyses. The molecular analysis revealed that markers obtained from diploid progenitors were useful for molecular diversity studies in *Brasicca juncea.*

Key words: *Brassica juncea,* genetic diversity, population structure, dendrogram

Brassica juncea, commonly known as Indian mustard, is a dominant species grown in Indian subcontinent on a total area of about 64.54 lakh hectares with an approximate yield of 72.82 lakh tonnes of seed (Mustard Crop Survey Report 2014-15; www.religarecommodities.com). Realizing the potential of this crop for its use as oilseeds, vegetables, forage, green manure and condiments, a number of genetic diversity analyses were conducted to identify genetic differences between the genotypes of *B. juncea* (Vinu et al. 2013; Pratap et al. 2015). The studies have indicated that alloploid *B. juncea* has narrow genetic base and DNA markers developed from diploid progenitors will be an additional source to uncover polymorphic regions in *B. juncea* (Grewe et al. 2014). The present study was conducted to analysis the molecular diversity and population structure among a collection of Indian and Exotic genotypes using SSR markers developed from diploid progenitors and closely related species of *Brassica.*

A total of 23 genotypes consisting of 11 Exotic [Zem 1 (Australia), Skorospieka II and Donskaja IV (Russia), Cutlas (Canada), EC 287711 and EC 491584 (Sweden), *B. juncea* from Turkey, EC 206712 and EC 699059, EC 699038-I and EC 699038-II (Spain)], 10 indigeneous (Pusa Tarak, RSPR-01, Urvashi, DRMR2017, Pusa Mehak, Pusa Karishma, Varuna, Kranti, RSPR-69 and Pusa Bold) and two progenitors (*B. nigra* and *B. rapa*) were used in the present study. For PCR analysis, high quality genomic DNA was isolated using Doyle and Doyle (1990) with slight modifications. The SSR markers used for PCR amplification and diversity analysis were obtained from genomic DNA (gDNA-SSRs with prefix *BR, SSR* and *Bni*) and ESTs (EST-SSRs with prefix *Brapa*). Of 32 SSR markers, 20 markers were obtained from genome sequence of A-genome diploid progenitor *B. rapa* and twelve SSR markers were obtained from public databases (Lowe et al. 2004). The molecular data was scored as per 'allelic format' as required for diversity and population structure analysis using DARwin5 software (Perrier and Collet, 2006) and STRUCTURE software (Pritchard et al. 2000) respectively.

All 32 SSR markers used in the present study were found functional as they all amplified PCR product

*Corresponding author's e-mail: rssandey@gmail.com
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in the expected size range. Sixteen markers detected polymorphism by amplifying 54 alleles among 23 genotypes (Table 1). The number of alleles per SSR marker ranged from 2 to 4 with an average of 2.37 alleles per locus, with eleven, four and one SSR marker detecting two, three and four alleles, respectively. PIC (Polymorphism Information Content) values in the present study ranged from 0.12 to 0.61 with an average of 0.314. The values for average number of alleles and PIC were different between gDNA-SSR and EST-SSR markers. The average values for number of alleles for gDNA-SSR and EST-SSR markers was 2.5 and 2, respectively; while the average PIC value for gDNA-SSR and EST-SSR markers was 0.353 and 0.195, respectively. The average PIC value observed in the present study was comparable to other diversity studies in *B. juncea* (Vinu et al. 2013; Pratap et al. 2015). The diversity within a collection of germplasm depends upon the relatedness and origin of individual genotypes included in the study and the types of markers used to estimate allelic information at different loci. The gDNA-SSR markers obtained from non-coding DNA tend to uncover higher allelic diversity than EST-SSR markers that are obtained from highly conserved coding regions of a genome.

Table 1. Polymorphic SSR markers used for genetic diversity analysis

Primer name	Motif	T_m (°C)	No. of alleles	PIC
BR_A01_5347462[*]	$(CT)_{13}$	55	2	0.48
BR_A01_24105503[*]	$(TA)_{11}$	55	3	0.47
BR_A05_22761470[*]	$(CA)_8$	55	2	0.23
BR_A06_22686930[*]	$(ATG)_7$	55	3	0.56
BR_A07_5198196[*]	$(GGT)_5$	55	3	0.61
BR_A07_23288577[*]	$(AGA)_6$	55	2	0.20
BR_A10_14752995[*]	$(AAG)_5$	55	2	0.12
Brapa-GR723528[#]	$(GA)_5$	59	2	0.23
Brapa-GR723865[#]	$(CCT)_9$	64	2	0.16
Brapa-GR723869[#]	$(CAG)_6$	58	2	0.23
Brapa-GR723735[#]	$(CAA)_7$	65	2	0.17
SSRNa12-C06[##]	$(CT)_9$	55	2	0.15
SSRNa14-D07[##]	$(CCG)_5$	53	2	0.14
BniB.Ni2-B01.1[##]	$(GT)_{13}$	56	2	0.23
BniB.Ni2-E12.5[##]	$(GCT)_8$	56	3	0.45
SSRRa3-D02B[##]	$(GA)_9$	54	4	0.60

[*]*BR_A01_5347462* means a $(CT)_{13}$ SSR at position 5347462 on chromosome A01 of *B. rapa*.
[#]*Brapa-GR723528* means a $(GA)_5$ SSR in *B. rapa.* EST with Accession No. GR723528 (www.ncbi.nom.nih.gov)
[##] SSR markers from related species (Lowe et. al. 2004)

A dendrogram based on the unweighted neighbour joining (UNJ) method grouped all 23 genotypes into three major clusters (Fig. 2). In cluster I, six out of eight genotypes were of Indian origin (Pusa Mehak, Pusa Karishma, Varuna, Kranti, Urvashi and DRMR-2017) indicating that the marker data was able to identify the related genotypes from a geographical region. The grouping of Kranti (a selection of Varuna) with Varuna in Cluster I indicate that the molecular markers have greater tendency to discriminate among individuals with high level of accuracy. Cluster II mainly comprised of exotic genotypes (EC206712, EC699038-II, EC491584, EC699038-I, *B. juncea* from Turkey and EC699059). However, some of the Indian genotypes (Pusa Tarak, RSPR-01, RSPR-69 and Pusa Bold) were also grouped with Exotic genotypes. The clustering of Indian and Exotic genotypes in the cluster II could be due to the fact that the allelic composition among these genotypes was identical at some of the SSR loci that were considered in present study. Cluster III consisted of two sub clusters with three genotypes of European origin genotypes *viz*., Skorospieka II, Donskaja-IV and Cutlas in the sub-cluster IIIa and *B. nigra* and *B. rapa* (being parental progenitor) in the sub-cluster IIIb. The population structure of all 23 genotypes was estimated under Hardy-Weinberg Equilibrium by using STRUCTURE V2.3.4 software. Based on maximum likelihood and delta K (ΔK) values, an optimal number of three sub-populations were identified (Fig. 2). While diversity analysis has been used to identify patterns of sub-populations and genetic relatedness; the population structure is a measure to estimate patterns of genetic differentiations among a population. Euclidean index calculated from polymorphic SSRs identified the highest dissimilarity value between EC 206712 and Donskaja IV (0.46) followed by the similar values between Kranti and *B. juncea* from Turkey (0.43) suggesting that these genotypes could be subjected to further analysis with additional markers to determine their suitability in future breeding programmes.

The present study concluded that gDNA-SSR markers were more polymorphic than EST-SSRs, which is in agreement with the previous reports (Vinu et al. 2013). Both gDNA-SSR and EST-SSR markers developed from progenitors and closely related species is an important genomic resource for diversity analysis of *B. juncea* and other allopolyploid crop species. The allelic information derived from SSRs indicated that the genotypes used in the present study have distinct

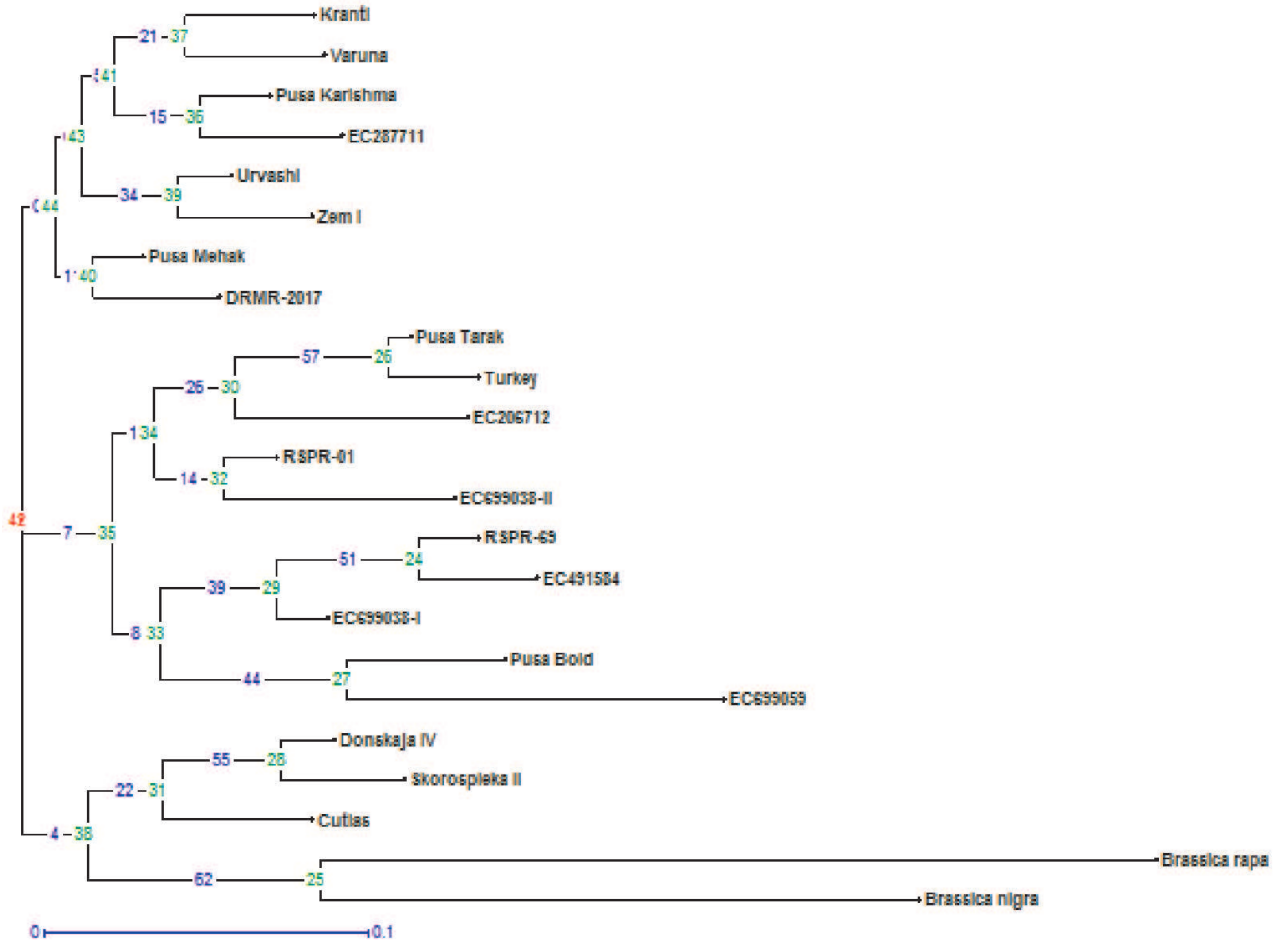

Fig. 1. Dendrogram showing genetic relationship among 23 different Brassica genotypes

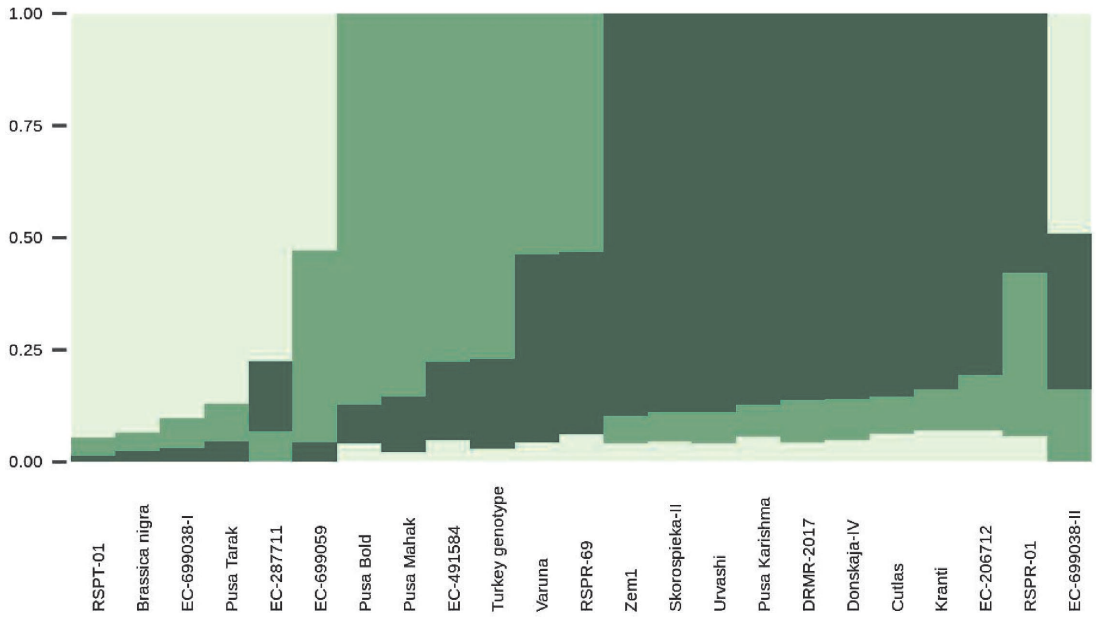

Fig. 2. STRUCTURE analysis showing the formation of three groups (G1, G2 and G3). The vertical coordinate of each group indicates the membership coefficients for each genotype

genetic patterns as both dendrogram based on UNJ method and population structure were able to group the genotypes into distinct clusters with high level of similarity between results.

Authors' contribution

Conceptualization of research (RS); Designing of the experiments (RS, JS); Contribution of experimental materials (RS, SKG); Execution of field/lab experiments and data collection (RS, JS, PK); Analysis of data and interpretation (RS, JS, PK); Preparation of the manuscript (RS, JS).

Declaration

The authors declare no conflict of interest.

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