

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.806.366>

Analysis of Allelic Differentiation and Prediction of Suitable Parents among *Brassica juncea* L. Genotypes using Microsatellite Markers

Mir Ahmad Habib, Manmohan Sharma, Punya*, R.K. Salgotra and Usha Kiran

School of Biotechnology, SKUAST-J, Chatha, Jammu (J&K), India

*Corresponding author

ABSTRACT

Information on allelic differentiation is very important to elucidate the nature and extent of divergence among *Brassica juncea* genotypes. In present study 20 *Brassica juncea* L. genotypes generated 79 polymorphic alleles from 15 SSR primer pairs with an average of 5.26 alleles per locus. The polymorphism percentage was recorded to be the maximum in the case of the primer Ni4- D04 (80%) and the minimum in the case of 3 different primers namely A06-20686249, Ni3-D03 and A05-20242013 (33.3%). The polymorphism information content (PIC) values ranged from 0.41 (A01-13393871) to 0.94 (A05-25290881), reflecting the presence of high allelic variation. Considering the number of alleles generated by different primer pairs in conjunction with the level of polymorphism detected in the present study, the primers A04-9627743 and A04-15440685 appeared to be more informative primers. The magnitude of similarity coefficient between RH749 and Kranti (0.368) was found to be the maximum amongst pair-wise combinations of genotypes under evaluation in the present study and considered as most similar genotypes. The dendrogram generated with UPGMA cluster analysis revealed six major clusters. Genotype combinations VNIK 405 and J/817/2, Miike Akachirimem and RH749, and VNIK 405 and RH749 exhibited significant genetic diversity and were consider as suitable parent combinations for breeding programmes. The genotype Vardan was found to be distinct from all other groups. SSR markers utilized in the present study proved efficient in discrimination and unambiguous classification of genotypes and for identifying diverse parental combinations.

Keywords

Brassica juncea L. genotypes, Alleles, SSRs, Genetic variability

Article Info

Accepted:

24 May 2019

Available Online:

10 June 2019

Introduction

Brassica species, commonly called as rapeseed-mustard, are the third most important oilseed crops of the world after soybean and palm. These are the second most important oilseed crops of India, next to soybean. India accounts for 19.29 percent area under rapeseed production and 11.70 percent share to the global production

(Kumarawat and Yadav, 2018). *B. juncea* (2n=36, AABB genome), an allopolyploid commonly called as Indian mustard, contributes more than 80 percent to the total rapeseed-mustard production in the country and is an important component in the oilseed sector. It 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. With the

increasing population and improving life standards, *per capita* oil consumption has increased tremendously. To meet out the present oil requirements, there is an urgent need to increase the yield potential of *B. juncea* through genetic interventions.

Genetic diversity in any crop species is an essential element for its improvement and adaptation to different environments. Knowledge of genetic distances between genotypes helps in selection of perspective parents for development of new high yielding varieties. These distances among parents may be attributed to their differences for number of genes and their functional relations in a given environment (Nei, 1976). Knowledge about genetic diversity in *B. juncea* could help breeders and geneticists to understand the structure of germplasm, predict which combinations would produce better offsprings (Hu *et al.*, 2007), and facilitate to widen the genetic base of breeding material for selection (Qi, Yang and Zhang, 2008). Evaluation of genetic diversity and relatedness in *B. juncea* using phenotypic parameters has been previously done by many researchers (Singh *et al.*, 2010; Alie *et al.*, 2009). Isozyme loci have been used as markers in various genetic studies including genetic diversity in *B. juncea* (Kumar and Gupta, 1985). However, environmental factors and the developmental stages of the plant are the two limiting factors associated with these markers. Among various types of markers used for genetic diversity estimation in plants, molecular or DNA markers are more specific, proficient, precise and consistent in distinguishing closely related cultivars or species (Mishra *et al.*, 2011). Amongst the various types of molecular markers used, microsatellite or simple sequence repeat (SSR) markers are preferred ones because of their higher reproducibility, co-dominance nature and abundance, wide distribution throughout the genome, easy storability and multi-allelic

variation (Powell *et al.*, 1996). They are highly polymorphic PCR-based markers and are expected to be very powerful in germplasm discrimination. More reliable SSR markers are now available which are present in abundance genome-wide in A and B genomes of *B. juncea* and are efficient in identification of polymorphism (Sharma *et al.*, 2018 and Sharma *et al.*, 2019). Keeping into consideration the potential of SSR markers in assessing the nature and extent of genetic diversity among germplasm, the present study was conducted to generate reliable information on the genetic relationship among germplasm of *Brassica juncea* and to identify suitable parents for breeding programme.

Materials and Methods

The molecular characterization of twenty five *Brassica juncea* genotypes, belonging to both Indian and European gene pools (Table 1), was carried out at the Plant Genomics Laboratory of School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha during 2018-19. The genotypes were grown for genomic DNA extraction from young and actively growing fresh leaves using modified cetyltrimethyl-ammonium bromide (CTAB) method (Doyle and Doyle, 1990). Amplification of DNA was carried out by using a panel of 15 SSR primer pairs (Table 2) in total volume of 10 µl containing 5.3 µl of nuclease free water, 2.2 µl 5X PCR buffer with 15mM (MgCl₂), 0.3 µl of 2.5 mM/ µl dNTP, 0.5 µl of each forward and reverse primers, 5 U of Taq polymerase. Amplification cycle comprised of initial denaturation for 5 min at 94°C; 30 cycles of 94°C for 1min, annealing at 58 °C for 1 min and extension at 72°C for 2 min.; followed by a final extension at 72 °C for 7 min. in Master Cycler Gradient (Eppendorf). The products of amplification were stored at 4°C and resolved

by electrophoresis in horizontal agarose gel system at 110 V for 1 h 30 min. on 2% agarose gel stained with ethidium bromide (10mg/ml) using 1X TBE buffer. The amplified products were visualized under gel documentation system and the size of amplicons was estimated with the help of 100bp ladder (Fermentas).

Allelic differentiation was determined on the basis of presence and absence of bands in different genotypes and data recorded in this respect were entered into binary matrix as discrete variables and this data matrix was subjected to further analysis for calculating, total alleles, number of alleles per locus, number of unique and shared alleles. The polymorphism information content (PIC) value was calculated for each SSR marker measuring the diversity of alleles at one locus by the formula:

$$PIC = 1 - \sum f_i^2$$

Where, f_i is the frequency of i th allele (Smith *et al.*, 1997).

The information regarding informativeness of the marker was obtained by computing the polymorphism percent as follows:

$$PP = (\text{Number of unique alleles} / \text{Total number of alleles}) \times 100$$

The degree of genetic similarity is the level of character similarity of the genotypes and is estimated from the number of allele data using the Jaccard coefficient (Rohlf, 2000) with the formula:

$$GS = \frac{m}{(n + u)}$$

Where, m = the number of DNA (allele) bands of the same position; n = total DNA band; u = the number of bands (alleles) of DNA that are not equal in position.

The dendrogram based on similarity indices was obtained by un-weighted pair-group method using arithmetic mean (UPGMA). Analysis was performed with the help of NTSYS-pc software (Rohlf, 1997). The nature of differentiation and diversity among brassica genotypes under evaluation in the present investigation was assessed by identifying the clusters at appropriate phenon levels. Principal component analysis is another multivariate approach utilized in present study to estimate the extent of genetic variability among *Brassica* genotypes evaluated

Results and Discussion

Amplification pattern of targeted genomic region of twenty genotypes of *Brassica juncea* using fifteen SSR markers revealed that there is significantly higher level of allelic differentiation among them. Genetic diversity assessment at molecular level was done by the presence or absence, relative position and size of bands (Figure 1). The efficiency of primer pairs was determined by calculating the number of allelic variants and the occurrence of unique allelic variant (Chen *et al.*, 2011 and Chandra *et al.*, 2013). Moreover, exclusive bands proved valuable to distinguish the genotypes at the molecular level without using field data (Sahu *et al.*, 2012 and Vinu *et al.*, 2013)

Altogether 79 alleles were obtained from 15 SSR primer pairs with an average of 5.26 alleles per locus. The number of alleles per locus ranged from 3 in the case of primer A05-20242013 to 8 in the case of Ni4-G08 with a range between 80 bp to 295bp (Table 3). The polymorphism percent revealed in the form of percentage of unique alleles was recorded to be the maximum in the case of the primer Ni4- D04 (80%) and the minimum in the case of 3 different primers namely A06-20686249, Ni3-D03, & A05-20242013

(33.3%) with an average value of 44.9 percent. Remarkably greater magnitude of polymorphism percentage was obtained in the cases of primers A01-13393871, Ni2-F11, Ni2-D12, A04-9627743, Ni4-D04, A04-15440685 and A07-7758930. The results obtained in this study are comparable to those reported earlier (Salazar *et al.*, 2006; Abdelmigid, 2012; Ali *et al.*, 2007 and Khan *et al.*, 2011). The polymorphism information content (PIC) demonstrated the informativeness of primer pairs and their potential to detect differences among genotypes based on genetic differences. In the present study, PIC values ranged from 0.41 (A01-13393871) to 0.94 (A05-25290881) with mean of 0.73 which matched the findings of other workers (Shi *et al.*, 2014; Ali *et al.*, 2019; Avtar *et al.*, 2016), reflecting the presence of high allelic variation. Significantly greater magnitude of PIC value was obtained in the case of primers A02-18870790, Ni3-D03, Ni4-G08, A03-22221630, A04-9627743, Ni4-D04, A04-15440685 and A05-20242013. Among the primer pairs having higher PIC values, primers A02-18870790, Ni2-D12, Ni3-D03, Ni4-G08, A04-9627743 and A04-15440685 generated considerably greater number of allelic variants per primer due to variation in the length of simple sequence repeats based amplified products as a consequence of amplification of the simple sequence repeats flanked by these primer pairs. Further, the primer pairs A04-9627743 and A04-15440685 detected considerably greater percentage of unique alleles amongst these eight primer pairs which had higher polymorphism information content and generated greater number of alleles. Considering the number of alleles generated by different primer pairs in conjunction with the level of polymorphism detected in the present study, the primers A04-9627743 and A04-15440685 appeared to be more informative primers.

The estimates of similarity coefficients amongst *Brassica* genotypes, which were computed by a comparison on the basis of presence and absence of the amplified products generated with fifteen primer pairs, ranged from zero to 0.368. The magnitude of similarity coefficient between RH749 and Kranti (0.368) was found to be the maximum amongst pair-wise combinations of genotypes under evaluation in the present study and considered as the most similar genotypes. This was followed by remarkably higher magnitude of similarity coefficient between Yanagawa Shirokuki and Commercial Brown Mustard, Yanagawa Shirokuki and Vardann; and Kranti and RSPR-69. However, the magnitude of similarity coefficient between VIIMK 405 and J/817/2, Miike Akachirimen and RH749, VNIIMK 405 and RH749 was equal to zero. The results revealed ample diversity at the molecular level amongst the twenty genotypes under evaluation in the present study. Considering broad classification of *Brassica* genotypes as revealed by dendrogram, genotypes subjected to molecular characterization in the present study were classified into six clusters (Figure 2). Out of six clusters, three clusters, namely, the cluster II, cluster III and cluster VI were mono-genotypic consisting of SB-12-P4, VNIIMK 351 and Vardan respectively. Cluster I was di-genotypic comprising Neosypajusca 2 and, J/817/2, and cluster IV was tri-genotypic consisting of RH749, RSPR-69, Kranti whereas cluster V was multi-genotypic having Miike Skorospelka, J807/1/6, VNIIMK 405, Zeltoseminaja 230, Pusa Mehak, Primus, Skorospelka, IB1436, Raya, Brown Mustard and Yanagawa Shirokuki. Cluster IV and cluster V were further subdivided into sub-clusters, accommodating only the genotypes with increasingly similar pattern of marker genotyping. The genotype Vardan was found to be distinct from all other groups, as it was accommodated into a separate cluster

showing more divergence. Thus, present study, depicted that genotypes which were genetically similar were grouped together and explained the relationship between these genotypes. Genetic variation estimated among *B. juncea* genotypes evaluated will be further helpful in developing and planning breeding strategies for developing high yielding varieties and hybrids.

Principal component analysis (PCA) reveals the major contributor of the total variability at each distinct point. Results of PCA along the two principal axes in two dimensional plots of fifteen SSR primers dependent genetic profile are depicted in Figure 3. The first principal components showed Eigen value more than one and cumulatively explained 13.3 percent variability. Twenty *Brassica*

juncea genotypes studied here exhibited significant genetic diversity; the highest being between genotypes VNIIMK 405 and J/817/2, Miike Akachirimem and RH749; and VNIIMK 405 and RH749, while minimum genetic diversity between RH749 and Kranti was reflected. The most important attribute of SSR marker is their high level of allelic variation, making them valuable and informative genetic markers (Fayyaz *et al.*, 2014; Hassan *et al.*, 2006 and Subaran *et al.*, 2017). The results of this investigation clearly indicated that utilization of fifteen SSR primer pairs in the analysis of *Brassica juncea* genotypes revealed a remarkably higher level of genetic polymorphism, which allowed unique genotyping of *Brassica juncea* included in the analysis.

Table.1 *Brassica juncea* genotypes used in the study

Sl. No.	Genotypes	Country
1	J/817/2	United Kingdom
2	Neosypajuscajasia 2	Russia Federation
3	Miike Akachirimem	Japan
4	VNIIMK 351	Russia Federation
5	Skorospelka	Russia Federation
6	Skorospelka 2	Russia Federation
7	Pusa Mehak	India
8	J807/1/6	United Kingdom
9	VNIIMK 405	Russia
10	Primus	Canada
11	IB-1436	India
12	SB-12-P4	India
13	Zeltosemiannaja 230	Russia
14	Raya (L.23)	Pakistan
15	Vardan	India
16	Commercial Brown Mustard	Canada
17	Yanagawa Shirokuki	Japan
18	RSPR-69	India
19	Kranti	India
20	RH749	India

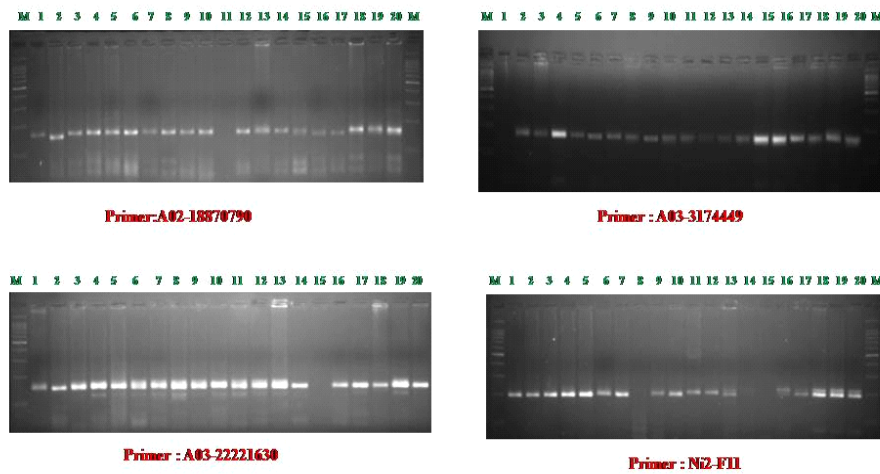
Table.2 SSR primers used for analysing genetic diversity in *Brassica juncea* germplasm

Sl.no.	Primers	Sequences
1	A04-9627743	F 5'ATGGAATCTGCTCATCTCAC3' R 3'TAAGCTGCAATGATCAAAGAT5'
2	A04-15440685	F 5'TTTGAACGATACACAACAACA3' R 3'GTTGGTCCACGAGTAAAAGAT5'
3	A05-20242013	F 5'AGAAGCTTTTTCTCTTGTTGC3' R 3'TGATGTAAGAGCGTGAAAGAT5'
4	A05-25290881	F 5'ATAAAGATTTGATGGGAGGAG3' R 3'GGTGGAGGAGGATAGTTGTAG5'
5	A07-20686249	F 5'AGGAAAAGACTGGAAAGTGTC3' R 3'CTTCTGCTCCTCTTGTTGTTA5'
6	A01-13393871	F 5'CCGTTTTTATGTCACAAATCT3' R 3'AAACAAAACGAACTTTGTCAG5'
7	A02-188870790	F 5'TACACCGTCTGATTCCATCT3' R 3'GCCTGACTGCTGCTACTAAC5'
8	AO3-3174449	F 5'AAAGAAGAGCTTTGAAGAGGA3' R 3'TTGATTCACAACACACATACC5'
9	AO7-7758930	F 5'AGGTGATATGCTCAAACACAC3' R 3'TACGGTGAAAAATCGCTAATA5'
10	AO3-22221630	F5'ATCGTCTCTTTCGTCTTGTCT3' R 3'CGTAAACTGAAACCATTAC5'
11	Ni2-F11	F 5'AAAGGGTTTCAATTTACGC3' R 3'GGGAAACATACTCACCACGC5'
12	Ni2-D12	F 5'GAGATGAGGATTTGCTTTTGC3' R3'ACAGTATGAGAGAGAGAGAGAG5'
13	Ni3-D03	F 5'ACCGGAGACGAAACTACCG3' R 3'CCTCTTCGACGTTTTTGGTG5'
14	Ni4-G08	F 5'ATTTGACGGACTCCTCTTGC3' R3'CACTTGGTAACTCTATGGATGCC5'
15	Ni4-D04	F 5'TTTAAAGTGTGTTTCACAAACTGG3' R 3'GAGTTCACAGGGGCTACAGG5'

Table.3 Analysis of allelic differentiation in *Brassica juncea* genotypes based on the amplification of SSR markers

Sl. No.	Primer	No. of alleles	Allele Size (bp)	PP	PIC
1	A01-13393871	4	135-160	50	0.41
2	A02-18870790	7	160-198	42.8	0.807
3	A05-25290881	4	125-150	40	0.94
4	A06-20686249	5	160-175	33.3	0.62
5	Ni2-F11	4	150-170	50	0.53
6	Ni2-D12	5	145-175	60	0.73
7	Ni3-D03	6	110-125	33.3	0.76
8	Ni4-G08	8	80-110	37	0.80
9	A03-22221630	5	160-175	40	0.735
10	A03-3174449	5	170-190	40	0.72
11	A07-7758930	5	135-160	60	0.63
12	A04-9627743	6	195-220	50	0.75
13	Ni4-D04	5	270-295	80	0.83
14	A04-15440685	7	170-192	57.14	0.91
15	A05-20242013	3	170-160	33.3	0.86

Fig.1



Sl. No.	Genotypes	Sl. No.	genotypes	Sl. No.	genotypes	Sl. No.	genotypes
1.	J8172	6.	Skorospelka 2	11.	IB-1436	16.	Commercial Brown Mustard
2.	Neosypajuscjasia 2	7.	Pusa Mehak	12.	SB-12-P4	17.	Yanagawa Shirakuki
3.	Milke Akachirimen	8.	J807/1/6	13.	Zeltosemianaja 230	18.	RSPR-69
4.	VNIIMK 351	9.	VNIIMK 405	14.	Raya (L.23)	19.	Kranti
5.	Skorospelka	10.	Primus	15.	Vardan	20.	RH749

Fig.1. Primer pair dependent amplification of region of DNA extracted from leaves at seedling stage in different brassica genotypes.

Fig.2 Dendrogram based on Jaccard similarity coefficients for fifteen SSR primer pairs among 20 *Brassica juncea* genotypes

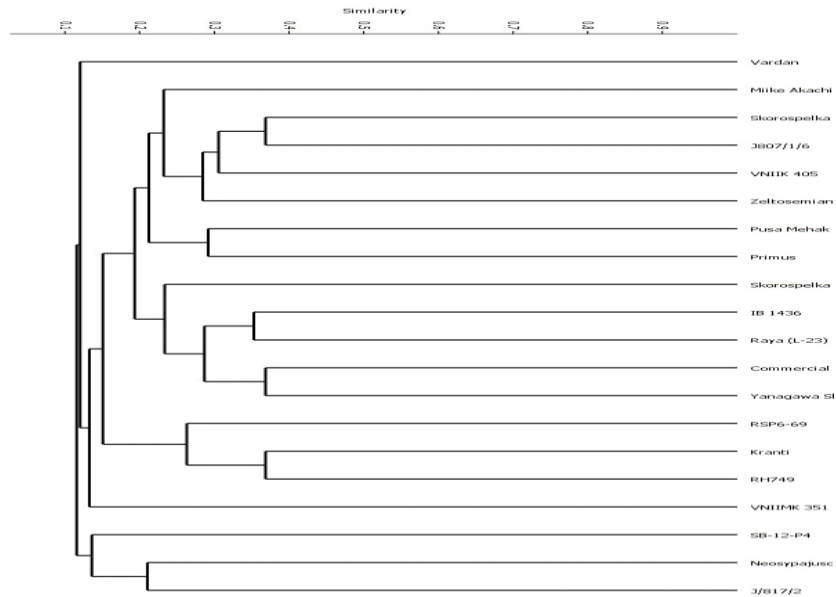
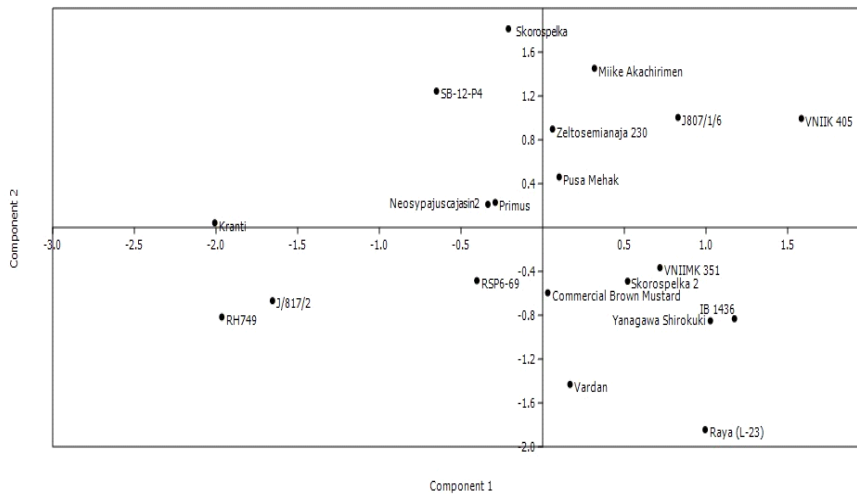


Fig.3 Principal component analysis based on fifteen SSR primer pairs among of twenty *Brassica juncea* genotypes



Selection of appropriate parents to be used in any crop improvement programme is one of the main decisions taken by plant breeders, which facilitates the exploitation of minimum

genetic similarity and production of superior recombinant genotypes. Based on the values of similarity coefficients observed, parental combinations identified included VNIK 405

and J/817/2, Müike Akachirimén and RH749, and VNIK 405 and RH749 which are predicted to yield better F₁s and recombinants. It has been reported previously that SSR is an important tool for germplasm characterization in a variety of crops, including oilseed *Brassica* (Saal *et al.*, 2001). A similar result regarding effectiveness of SSR markers in monitoring genetic diversity have also been reported by Hopkins *et al.*, (2006) and Fu and Gugel, (2010). Thus, SSR markers utilized in the present study were found efficient in discrimination and unambiguous classification of genotypes and for identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection.

Acknowledgements

The authors acknowledge Department of Biotechnology, New-Delhi for financial support and School of Biotechnology, SKUAST-Jammu for providing facilities to carry out the work.

References

Abdelmigid, H.M., 2012. Efficiency of random amplified polymorphic DNA (RAPD) and Inter-simple sequence repeats (ISSR) markers for genotype fingerprinting and genetic diversity studies in canola (*Brassica napus*). *African J. Biotech.* 11: 6409-6419.

Ali, W., I. Munir, M. A. Ahmad, W. Muhammad, N. Ahmed, A.S. Durrishahwar, and Swati, Z.A. 2007. Molecular characterization of some local and exotic *Brassica juncea* germplasm. *African J. Biotech.* 6: 1634-1638.

Alie, F. A., T. Singh, Tariq and Sharma, P.K. 2009. Genetic diversity analysis in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. *Progr. Agri. Internl. J.*

9: 50-53.

Avtar, R., B. Rani, M. Jattan, M.N. Kumari and Rani, A. 2016. Genetic diversity analysis among elite gene pool of Indian mustard using SSR markers and phenotypic variations. *The Bioscan.* 11(4): 3035-3044.

Chandra, V., U. Pant, R. Bhajan and Singh, A.K. 2013. Studies on genetic diversity among *Alternaria* blight Tolerant Indian mustard genotypes using SSR markers. *The Bioscan.* 8(4):1431-1435.

Chen, F. B., K.C. Yang, G. F. Zhou, Y. H. Fan, Z.Y. Zhang, J. J. Shen, H. Zhang, and Jiang, L. L. 2011. Analysis of Heterosis, Combining Ability and Genetic Diversity in Tuber Mustard (*Brassica juncea* var. *tumida* Tsen & Lee) Inbred Lines Based on SSR Markers and Combining Ability Estimates. *Philipp agric scientist* ISSN 0031-7454. 94(2): 124-131.

Doyle, J.J., and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. *Focus.* 12:13-15.

Fayyaz, L., Farhatullah, M. A. Rabbani., S. Iqbal., M. Kanwal and Nawaz, I. 2014. Genetic diversity analysis of *Brassica napus/Brassica campestris* progenies using microsatellite markers. *Pakistan Journal of Botany.* 46(3):779-787.

Fu, Y. B. and Gugel, R. K. 2010. Genetic Diversity of Canadian elite summer rape (*B. napus* L.) Cultivars from the pre to post canola quality era. *Can. J. Plant Sci.* 90: 23-33.

Hasan, M., F. Seyis, A. G. Badani, J. Pons-Kuhnemann, W. Friedt, W. Lu hs and Snowdon, R.J. 2006. Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. *Genetic Resources and Crop Evolution*, 53: 793–802. DOI 10.1007/s10722-004-5541-2

- Hopkins, C., R. Mogg, N. Gororo, P. Salisbury, W. Burton, C. Love, G. Spangenberg, D. Edwards, and Batley, J. 2006. An Assessment of Genetic Diversity within and between *Brassica napus* and *Brassica juncea* lines from International Germplasm Collections. Proc. Joint Meeting 14th Crucifer Genetics Workshop 115 and 4th ISHS Symposium on Brassicas. Acta. Hort. 706: 115-119.
- Hu, S., C. Yu, H. Zhao, G. Sun, S. Zhao, M. Vyvadilova and Kucera, V. 2007. Genetic diversity of *Brassica napus* L. Germplasm from China and Europe assessed by some agronomically important characters. Euphytica 154: 916.
- Khan, W.M., I. Munir, Farhatullah, M. Arif, A. Iqbal, I. Ali, D. Ahmad, M. Ahmad, A. Mian, J. Bakht, Inamullah and Swati, Z. A. 2011. Assessment of genetic diversity of *Brassica juncea* germplasm using Randomly Amplified Polymorphic DNA (RAPD) markers. African J. Biotech. 10:3654-3658.
- Kumar, R., and Gupta, V.P. 1985. Isozyme studies in Indian mustard (*Brassica juncea* L.). Theor Appl. Genet. 69: 1-4.
- Kumarawat, M. and Yadav, M. 2018. Trends in area, production and yield of mustard crop in Bharatpur region of Rajasthan. Int. J. of Eng. Dev Res. 6(1): 315-321.
- Mishra, M. K., N. Suresh, A.M. Bhat, N. Suryaprakash, S. S. Kumar, A. Kumar and Jayarama 2011. Genetic molecular analysis of *Coffea arabica* (Rubiaceae) hybrids using SRAP markers. Rev. Biol. Trop. 59: 607-617.
- Nei, M., 1976. Analysis of gene diversity in subdivided populations. Pro Nat. Acad. Sci. USA 70: 3321-3323.
- Powell, W., G. C. Machray and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. Trends Plant. Sci. 1: 215-222.
- Qi, X., J. Yang and Zhang, M. 2008. AFLP based genetic diversity assessment among Chinese vegetable mustards (*Brassica juncea* (L.) Czern.). Genet. Resour Crop Evol. 55: 705-711.
- Raza, A., S. S. Mehmood, F. Ashraf and Khan, R. S. A. 2019. Genetic Diversity Analysis of *Brassica* Species Using PCR-Based SSR Markers. Gesunde Pflanzen, 71(1): 1-7.
- Rohlf, F. J. 2000. NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.1. Applied Biostatistics Inc.
- Saal, B., J. Plieske, J. Hu, C. F. Quiros and Struss, D. (2001) Microsatellite markers for genome analysis in Brassica II. Assignment of rapeseed microsatellite to the A genomes and genetic mapping in *Brassica oleracea* (L.) Theor Appl. Genet. 102:695-699.
- Sahu, P., D. Khare, N. Tripathi, A. N. Shrivastava and Saini, N. 2012. Molecular screening for disease resistance in soybean. J. Food Leg. 25(3):200-205
- Salazar, B., H. Laurentin, M. Davila and Castillo, M. A. 2006. Reliability of the RAPD technique for germplasm analysis of sesame (*Sesamum indicum* L.) from Venezuela. Interciencia 31:456-460.
- Sharma, D., M. Sharma, R. K. Salgotra, S. Hangloo, S. K. Gupta and Punya 2019 Screening of microsatellite markers for establishing parental polymorphism in *Brassica juncea*. Indian Res. J. Gene. & Biotech. 11(1): 18-24.
- Sharma, M., Punya, R. K. Salgotra, S. K. Gupta, S. K. Rai, U. Kiran, and Singh

- A. 2018. Molecular profiling of *Brassica juncea* genotypes using SSR marker derived polymorphism in Indian mustard. *India Res. J. Gene. & Biotech.* 10(3): 342-346.
- Shi, J., S. Huang, J. Zhan, J. Yu, X. Wang, W. Hua, S. Liu, G. Liu and Wang H. 2014. Genome-wide microsatellite characterization and marker development in the sequenced *Brassica* crop species. *DNA Res.* 21:53-68.
- Singh, D., R. K. Arya, N. Chandra, R. Niwas and Salisbury, P. 2010. Genetic diversity studies in relation to seed yield and its component traits in Indian mustard (*Brassica juncea* L. Czern & Coss.). *J. Oil. Brass.* 1:19-22.
- Singh, S., V.V. Singh, S. Ambawat, M. Dubey and Singh, D. 2017. Screening and Estimation of Allelic Differentiation in Indian mustard Using SSR Markers for Background Selection *Int. J. Curr. Microbiol. App. Sci.* 6(9): 2506-2516
- Smith, J.S.C, E.C.I. Chin, H. Shu, O.S. Smith and Wall S. J. 1997. An evaluation of utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree, *Theor Appl Genet* 95:163-173.
- Vinu, V., M. Singh, S. Vasudev, D. K. Yadava, S. Kumar, S. Naresh, S. R. Bhat and Prabhu K.V. 2013. Assessment of genetic diversity in *Brassica juncea* (Brassicaceae) genotypes using phenotypic differences and SSR markers. *Rev. Biol. Trop.* 61(4): 1919-1934.

How to cite this article:

Ahmad Habib, Mir, Manmohan Sharma, Punya, R.K. Salgotra and Usha Kiran. 2019. Analysis of Allelic Differentiation and Prediction of Suitable Parents among *Brassica juncea* L. Genotypes using Microsatellite Markers. *Int.J.Curr.Microbiol.App.Sci.* 8(06): 3071-3081. doi: <https://doi.org/10.20546/ijcmas.2019.806.366>