Molecular diversity analysis using Simple sequence repeats (SSRs) in ‘A’ and ‘R’ lines of Ogura CMS system in Indian mustard

Neeraj Kumar, Ram Avtar, Nisha Ahlawat and Rakesh

DOI: https://doi.org/10.22271/chemi.2020.v8.i4k.9778

Abstract
Fifty-eight SSR primers were used for molecular diversity analysis among 45 ‘A’ and ‘R’ lines (5 A lines and 40 restorers) of Indian mustard. Out of 58 primers 33 primers were found to be polymorphic. A total of 95 alleles were detected across all 45 genotypes. The PCR amplified products ranged from 70 bp to 350 bp in size. The number of alleles per locus varied from 2 to 5 with an average of 2.89. The PIC value varied from 0.206 to 0.749 with an average of 0.519. The highest PIC value was obtained for BG 132 (0.749) followed by BG 114 (0.744). The UPGMA cluster analysis suggested demarcation of 45 genotypes into six distinct groups in dendrogram wherein three groups had single genotype (restorer line) each. Cluster VI consisted of all five Ogura CMS lines only. In the remaining clusters, restorer lines were distributed. Based on similarity coefficient, cross combinations viz., RH 0749-OA & IOR-17, RH 0749-OA & IOR-20 and RH 119-OA & OR-17 were identified as diverse and might be suitable for using parent in heterosis exploitation in Indian mustard.

Keywords: Indian mustard, ‘A’ and ‘R’ lines, SSRs, molecular diversity

Introduction
Indian mustard [Brassica juncea (L.) Czern & Coss.], commonly known as raya, is one of the major oilseed crops cultivated in the Indian sub-continent. The predominant oilseed Brassica species plays a pivotal role in the vegetable oil economy. B. juncea (AABB; 2n=36) is a natural amphidiploid arisen from hybridization between B. rapa (AA; 2n=20) and B. nigra (BB; 2n=16) followed by chromosome doubling which led to the evolution of it (U, 1935). The wild form of B. rapa and B. nigra are found in the Middle East hence, this region is the most probable place of origin of B. juncea (Prakash and Hinata, 1980) [11]. Indian mustard plays a crucial role in the Indian oilseed economy and shares about 23.5% area with 24.2% production of total oilseeds in the country. The oil content in Indian mustard varies from 38-46% (Tomar et al. 2015) [19]. Mustard oil is low in saturated fat and high in monounsaturated and polyunsaturated fat along with a significant amount of omega-3 fatty acids. The mustard oil-cake is a valuable protein source and recommended feed of cattle. The mustard seed contains some of the essential nutrients such as selenium, magnesium, dietary fibre, omega-3 fatty acids, vitamin B3, calcium, protein and zinc (Kaur et al., 2019) [6].

In India during rabi, 2018-19 Indian mustard was grown in an area of 6.94 million hectares with an output of 7.20 million tons. Yet, India meets 57% of the domestic edible oil requirements through imports and ranked 7th largest importer of edible oils in the world. The further boom in human population and improved living standards has led to a rapid increase in the per capita oil consumption. Thus, there is an urgent need to increase the yield potential through genetic interventions to meet the present and future oil requirements. Heterosis breeding could be a potential alternative to substantially increase the productivity of Indian mustard (Ghosh et al., 2002) [14]. Attempts to exploit hybrid vigour have been stimulated by the availability of a large number of CMS sources. These are nap, polima, ogura, tournefortii, axyrrhina, stifolia, catholica, sinapis, trachystoma, moricandia and lyratus. Among these, Ogura (Ogu) CMS shows very stable and complete sterility suitable for hybrid development. Hybrid development depends upon the magnitude of heterosis. Heterosis in crosses involving diverse parents is generally higher than related parents. Hence, the selection of parents based...
on diversity estimates is a vital criterion to initiate a hybrid development program (Banga et al., 2015) [3]. Diversity analysis based on DNA markers is more reliable than conventional methods. DNA or molecular markers are unique DNA sequences that can be traced or identified using molecular biology techniques. These markers can be used for diversity analysis in different crops. Some of the currently used DNA molecular markers for diversity analysis include RFLP, RAPDs, AFLP, SSR etc. The SSR markers are more advantageous compared to other marker systems. They are reproducible, co-dominant in nature, abundant, widely distributed throughout the genome, highly polymorphic and easily scorables representing multi-allelic variation (Adhikari et al., 2017) [1]. Numerous SSR markers have been used in different Brassica species as in B. juncea (Vini et al., 2013; Pratap et al., 2015) [21, 12], B. napus (Wang et al., 2012; Li et al., 2013) and B. rapa (Suwabe et al., 2002; Ramchiary et al., 2011) [18, 13]. Hence, we used SSR markers for diversity analysis among the CMS and restorer lines.

**Materials and Methods**

**Plant Material**

Forty-five ‘A’ and ‘R’ lines (Table, 1), including five cytoplasmic male sterile lines (‘A’ line) and forty restorer lines (‘R’ line), of Indian mustard were grown in paired rows of 5m at the Oilseeds Research Area, Department of Genetics and Plant Breeding, CCS HAU, Hisar during Rabi, 2016-17. The row-to-row spacing was kept at 30 cm and the plant-to-plant spacing was maintained at 10 cm.

**Genomic DNA Isolation, Purification and Quantification**

Total genomic DNA was extracted from the fresh leaves of the four-week-old plants according to the CTAB method suggested by Saghai-Marooif et al. (1984) [15] with a slight modification. The precipitated DNA pellets were washed with cold ethanol (70%) and allowed to air dry overnight. These pellets were re-suspended into 100 μL of 10X T.E. buffer and stored at -20 °C. The quality and quantity of DNA were checked by gel electrophoresis using 0.8% agarose gel along with standard lambda DNA (50 ng/μL).

**SSR Markers**

A total of 58 SSR primers (Table, 2) were used for PCR amplification to determine genetic diversity in 45 Indian mustard ‘A’ and ‘R’ lines. The primers showing monomorphic banding patterns were excluded from further analysis. Finally, data produced by polymorphic SSR primers were used to analyze diversity and collect other information.

**PCR Amplification**

PCR amplification was carried out in a PCR machine (Bio Rad, USA). The total volume of PCR reaction mixture was 20 μL per sample, containing template DNA (1.0 μL), 10X PCR buffer (2.0 μL), reverse and forward primer (1.0 μL), 10 mM dNTP mix (0.4 μL), SUTaq DNA polymerase (0.5 μL) and nuclease-free water (13.1 μL). The PCR tubes were set on the wells of the thermal cycler. The machine was run as follows: Initial denaturation at 95.0 °C for 3 min; 40 cycles of denaturation at 94.0 °C for 1 min; annealing for 1 min at a particular temperature of primer; and extension at 72.0 °C for 1 min. Final extension was done at 72.0 °C for 7 min, and then the products were held at 4.0 °C. The amplified products were stored at -20 °C until further use. The PCR products were resolved by electrophoresis in TBE buffer through 3.5% horizontal electrophoresis containing ethidium bromide. A DNA ladder of 50 bp was run along with the PCR products. DNA bands were visualized in UV-trans-illuminator in the dark chamber of the Image Documentation System, which is linked to a computer that also runs the digital imaging system.

**SSR Data Analysis**

The size of the amplicons was measured by comparing the migration of amplified fragments with that of a known size DNA ladder (50 bp). All distinct bands (SSR markers) were given identification numbers according to their position on the gel and scored visually on the basis of their presence (1) or absence (0), separately for each primer. The scores obtained from all polymorphic markers in the SSR analysis were pooled to create a single data matrix and construct a UPGMA dendrogram using NTSYS–pc software (Rohlf, 1998).

Markers were classified as informative when PIC was ≥0.5. Polymorphic information content (PIC) values for each SSR were estimated by determining the frequency of alleles per locus using the following formula

\[
\text{PIC} = 1 - \sum x_i^2
\]

Where, \(x_i\) is the relative frequency of the \(i^{th}\) allele of the SSR loci

**Results and Discussion**

**SSR Marker-Based Divergence and Allelic Diversity Analysis**

Out of 58 primers used in the study, three primers were not amplified, 22 were monomorphic and 33 primers generated polymorphic bands (Table 3 & 4). The level of genetic diversity among 45 lines was evaluated by calculating allele amplified and PIC value for each polymorphic marker. A total of 95 alleles were detected with 33 polymorphic SSR markers across 45 lines. The overall size of the PCR amplified products ranged from 70 bp (BG93) to 350 bp (BG100, BG132, and BG133). The molecular size difference between the smallest and largest allele at a locus varied from 10 bp (BG126) to 220 bp (BG93). The number of alleles per locus varied from 2 to 5 with an average of 2.89 (Table 5). This was consistent with some earlier reports of 2.79, 2.9 and 3 average alleles per locus (Prajapat et al., 2014; Avtar et al., 2016; Singh et al., 2016) [10, 2, 17]. The highest number of alleles (5) was recorded with primers BG41 and BG132, followed by 4 alleles detected with primers BG48, BG100, BG111, BG114, BG129, and BG157. The lowest number of alleles (2) was detected at several loci. The average number of alleles per locus observed in this study was 2.89; in comparison, Sudan et al. (2016) [17] reported slightly lower value of 2.37 alleles per locus. The variability in the number of alleles detected per locus might be due to the use of diverse lines and different SSR primers. The calculated PIC value of each marker varied widely from 0.206 to 0.749 with an average of 0.519. The highest PIC value was obtained for BG132 (0.749), followed by BG114 (0.744), BG100 (0.733) and BG41 (0.729). However, BG119 (0.206), BG116 (0.234) and BG135 (0.278) had low PIC values and could be considered as the least powerful markers. The average PIC value (0.519) observed in our study was consistent with previous estimates of SSR marker analysis in Indian mustard by Avtar et al. (2016) [2] and Patel et al. (2018) who reported average PIC values of 0.529 and 0.555, respectively. On the other hand, the observed value was higher than the values of 0.46, 0.28 and 0.31 reported by Turi et al. (2012) [20], Gupta et al. (2014) [5], and Sudan et al. (2016) [17], respectively.
Similarity Coefficients and UPGMA Cluster Analysis

In this study, the dendrogram is constructed according to the UPGMA cluster analysis based on the similarity coefficient by using SAHN subprogram of NTSYS-pc. The range of similarity coefficients was found to be 0.49 to 0.91. The cluster dendrogram analysis revealed six clusters demarcated at a cut-off value of 72% of similarities, below which the similarity values narrowed conspicuously (Fig 1). Clusters I, II and III consisted of a single line, i.e., OR-11, OR-9 and IOR-11 respectively. Cluster IV consists of the maximum number of lines, i.e., 27 ‘R’ lines. Cluster V is comprised of 10 ‘R’ lines only. Interestingly, Cluster VI consisted of five ‘A’ lines only. The details about the clustering pattern of genotypes are given in Table 6. Based on this cluster analysis, we conclude that all A-lines were derivatives of a single parent that must be different from the R line. So, there may be a chance for heterosis exploitation. Among the different combinations of ‘A’ and ‘R’ lines, the minimum similarity coefficient value of 0.53 was found between RH 8812-OA & IOR-2 followed by 0.54 between RH 8812-OA & IOR-3 and RH 8812-OA & IOR-14. There were also other combinations having low similarity coefficient value such as 0.57 between RH 0749-OA & IOR-17, RH0749-OA & IOR-20, and RH 119-OA & OR-17. Among all CMS-lines, RH 8812-OA was the most diverse. Thus, it can be concluded that the above parental combinations, based on similarity coefficient values can be utilized successfully for the hybrids development in Indian mustard.

Table 1: List of genotypes used for molecular diversity analysis

| Sr. No. | Genotype   | Sr. No. | Genotype   |
|---------|------------|---------|------------|
|        | ‘A’ lines  |         | ‘R’ lines  |
| 1.      | RH 8812-OA | 18.     | IOR-18     |
| 2.      | RH 0749-OA | 19.     | IOR-19     |
| 3.      | RH 0406-OA | 20.     | IOR-20     |
| 4.      | RH 0119-OA | 21.     | IOR-21     |
| 5.      | RH 30-OA   | 22.     | OR-1       |

Table 2: A brief description of SSR primers used during the present investigation

| Sr. No. | Primer Name | Forward primer (5’ to 3’) | Reverse primer (5’ to 3’) | Ann. Temp |
|---------|-------------|---------------------------|----------------------------|-----------|
| 1.      | BG41        | TCTCTCCGCAACAAACAATCTAA  | ATCTAACCCTCTTCGGAATCTG     | 67.5°C    |
| 2.      | BG48        | CACGAAAGCTGTAAGAGGATGCA  | TCTTTTCTGTCCTATGAGATCTCA   | 64.5°C    |
| 3.      | BG89        | TGCCAAACAAATACAGGATGC    | CGGAACTGACTTGTGATTTCCCA    | 65.0°C    |
| 4.      | BG90        | TGCTTAAATGCTTATGTTACCTTA | AAATTTTCTACAGATACAAACACCA  | 59.5°C    |
| 5.      | BG91        | ACCTGAGCTTCTCATATTTACCC  | GAGAAAGAAGATCTCTAGCACCA    | 64.5°C    |
| 6.      | BG92        | GACAGCTGGCTATCTTAAAAGC   | CTTCCTACGGCAACACCCAGAA     | 65.5°C    |
| 7.      | BG93        | TGATAGCTCAGCTGTTGTTGCT   | AGGCACTGTATGAGATGATGAGTGA  | 68.0°C    |
| 8.      | BG94        | CCTCTAAAGAAGAGGAGGGAAA   | GAGAGAGAGAGGCCATATAAAAGG   | 66.0°C    |
| 9.      | BG95        | AGCTGCCAAGAAGGGCACA     | ATGGCCGAACCTACTCTCAT       | 67.5°C    |
| 10.     | BG96       | TTGTGAGAGATGTGCTTTGTT    | CCAAATTAGAGAATAATGATGGG     | 59.5°C    |
| 11.     | BG97        | AATCTGCTTCTCTGCTGAAA     | GCTTACAGAATGAGGTGAGGCG     | 66.5°C    |
| 12.     | BG98        | CTTGCTGCCAGGGGAAAGATGT   | GATAGTACGGCTCCAGATCCC    | 70.5°C    |
| 13.     | BG99        | CAGAGCGGAGCTGCAAGACAG    | CATTGGCCAGCTACTCTCAT       | 67.5°C    |
| 14.     | BG100       | TGTGAATGCTACTTCAATATATGTGTT | TGTGAATGATGATGAGATGGCGG | 62.5°C   |
| 15.     | BG102       | GGACGCGATTTAGCAAGTCCA    | GGTGAGTATGAGATGATGAGGCGA   | 67.0°C    |
| 16.     | BG104       | GTGCTTGCAAGGTTTAAAAGA    | AAAGGACACCAACCCACATCA      | 65.5°C    |
| 17.     | BG105       | TCTGATCTTTTTTGGGTTTGGAT  | TGGCAGATGCTACATCCCATTAAA   | 65.5°C    |
| 18.     | BG106       | TTCTTCTCTTAAATTCAGTCTGTG | GGAAGTGAAGGTTAGAAGGAGTGA  | 60.0°C    |
| 19.     | BG108       | TGTGGGCATTACCCCGATCTCT   | CAAAATAAAGAAAGGCAGAAGCTGCA | 65.5°C |
| 20.     | BG109       | AAGCCGCTTCTGCAAGGTGGT    | CATGGACATGCTACATCCCATTAAA | 65.5°C |
| 21.     | BG110       | GCATACTCTACATTTGGAACCA   | GCAGATCTACATTTAGGGATGGA   | 66.0°C    |
| 22.     | BG111       | ACCGGAGATAGAATGGCCTCTG   | ACAGTGGGCTAGTGTTGGG       | 67.5°C    |
| 23.     | BG112       | TTCTCGACCAGTATGAGGCTT    | AGCATGCTACCCAGTCCTAAA     | 66.0°C    |
| 24.     | BG113       | ATGTCCTCCTTCTGTTGACTT    | GGCATCGACAGGGCTTATT       | 67.0°C    |
| 25.     | BG114       | GCAAAATCCATTTGGTAAATCGGA | TGGCGACTGTACACTCTAATCA    | 64.5°C    |
| 26.     | BG115       | GAGAGGGAGGAGGAGGAGGGA    | CACCTTCTTAAAGACCCACCAT    | 62.0°C    |
| 27.     | BG116       | ATGCGGGCGTCTCAGGAAAAG    | CCTTCAATTTGAGTTCTGTTAGTC   | 64.5°C    |
| 28.     | BG117       | CTGGAGAAGATCATCTGTGTTG   | CAAAGGATTTCTCTGTGATC      | 63.0°C    |
| 29.     | BG118       | TGTGCTTCTTCTTAAAGAGGGA  | CGAAGAACAAAGCTAGTCA      | 63.5°C    |
| 30.     | BG119       | TGTGAAATGTTTCTGCTGAC     | CAGCAGAATGCAAAGTGA         | 64.5°C    |
| 31.     | BG120       | TTGCAAGAGTACATCCACCACA   | GCTCATCTTCTTACTATGTTTTT    | 63.5°C    |
| 32.     | BG121       | CAACTGTCATTTTGAGATGAATGGT| GAATGGTTGTTGATGCGGTA     | 64.5°C    |
| 33.     | BG122       | CCATATTGGAAATACATCTTGTTG | AAATACTAATGAGTTGGAATGGA   | 60.5°C    |
| 34.     | BG123       | CCAACGGAGAGGATGTTGAAAG  | TTATCCGTGCAACACACACA        | 63.0°C    |
| 35.     | BG124       | TAGATCTTTTACACAGGTTGAT   | TCTATGGCAAAAGATTGACAGG     | 64.0°C    |
| 36.     | BG125       | CAGACCAGGGAACACTAGTGA    | TAACTGCGGACACCTTCTCATG     | 65.0°C    |
| 37.     | BG126       | GAAGACGATGTCGCGAGATT    | AGTTGGTGGAAGATGTGGTTA     | 67.8°C    |
Table 3: Allelic variation and PIC values for 33 polymorphic SSR loci identified among 45 Indian mustard genotypes

| Sr. No | Primer | Amp. Range(bp) | Total no. of alleles | PIC |
|--------|--------|----------------|----------------------|-----|
| 1.     | BG41   | 160-250        | 5                    | 0.729 |
| 2.     | BG48   | 230-260        | 4                    | 0.658 |
| 3.     | BG89   | 130-150        | 2                    | 0.499 |
| 4.     | BG91   | 200-250        | 2                    | 0.473 |
| 5.     | BG93   | 70-320         | 3                    | 0.298 |
| 6.     | BG94   | 230-300        | 3                    | 0.549 |
| 7.     | BG95   | 80-100         | 2                    | 0.316 |
| 8.     | BG96   | 280-300        | 2                    | 0.485 |
| 9.     | BG99   | 120-270        | 3                    | 0.690 |
| 10.    | BG100  | 220-350        | 4                    | 0.733 |
| 11.    | BG105  | 190-240        | 3                    | 0.430 |
| 12.    | BG109  | 130-200        | 3                    | 0.540 |
| 13.    | BG111  | 120-320        | 4                    | 0.727 |
| 14.    | BG114  | 160-300        | 4                    | 0.744 |
| 15.    | BG116  | 140-160        | 2                    | 0.234 |
| 16.    | BG119  | 200-300        | 2                    | 0.206 |
| 17.    | BG121  | 200-250        | 3                    | 0.509 |
| 18.    | BG123  | 220-250        | 3                    | 0.480 |
| 19.    | BG125  | 140-190        | 3                    | 0.654 |
| 20.    | BG126  | 140-150        | 2                    | 0.483 |
| 21.    | BG129  | 200-300        | 4                    | 0.464 |
| 22.    | BG132  | 180-350        | 5                    | 0.749 |
| 23.    | BG133  | 250-350        | 3                    | 0.657 |
| 24.    | BG135  | 270-300        | 2                    | 0.278 |
| 25.    | BG136  | 220-240        | 2                    | 0.278 |
| 26.    | BG156  | 180-220        | 3                    | 0.486 |
| 27.    | BG157  | 150-250        | 4                    | 0.643 |
| 28.    | BG158  | 250-300        | 2                    | 0.497 |
| 29.    | BG160  | 150-200        | 2                    | 0.413 |
| 30.    | BG161  | 160-250        | 3                    | 0.606 |
| 31.    | BG162  | 140-300        | 3                    | 0.665 |
| 32.    | BG164  | 250-330        | 3                    | 0.617 |
| 33.    | BG167  | 130-150        | 2                    | 0.363 |

Table 4: Description of primers based upon amplification

| Nature of Primer | Number of primers | Primer |
|------------------|-------------------|--------|
| Polymorphic primer | 33                | BG41, BG48, BG89, BG91, BG93, BG94, BG95, BG96, BG99, BG100, BG105, BG109, BG111, BG114, BG116, BG119, BG121, BG123, BG125, BG126, BG129, BG132, BG133, BG135, BG136, BG156, BG157, BG158, BG160, BG161, BG162, BG164 and BG167 |
| Monomorphic primer | 22                | BG90, BG92, BG97, BG98, BG102, BG104, BG108, BG110, BG112, BG113, BG115, BG117, BG118, BG120, BG122, BG124, BG127, BG128, BG134, BG159, BG165 and BG166 |
| Non-amplified     | 3                 | BG106, BG131 and BG163 |
Table 5: Summary of molecular analysis

|                          |        |
|--------------------------|--------|
| Total number of genotype | 45     |
| Total number of primer tested | 58     |
| Total Number of polymorphic primer | 33     |
| Total number of monomorphic primer | 22     |
| Total number of polymorphic bands | 83     |
| Total number of monomorphic bands | 12     |
| Total number of bands | 95     |
| Size of amplified product (bp) | 70 bp to 350bp |
| Per cent polymorphism | 87.37 % |
| Number of alleles per locus | 2-5     |
| Average alleles per locus | 1.64    |
| PIC value range | 0.206 to 0.749 |
| Average PIC value | 0.519   |

Table 6: Distribution of 45 Indian mustard genotypes in different clusters based on SSR markers analysis

| Cluster No. | Number of genotypes | Genotypic details |
|-------------|---------------------|-------------------|
| I           | 1                   | OR-11             |
| II          | 1                   | OR-9              |
| III         | 1                   | IOR-11            |
| IV          | 27                  | IOR-1, IOR-2, IOR-6, IOR-7, IOR-3, IOR-4, IOR-5, IOR-13, IOR-16, IOR-8, IOR-17, IOR-10, IOR-12, OR-1, OR-3, IOR-20, IOR-21, IOR-9, OR-7, IOR-15, IOR-18, OR-12, IOR-19, OR-2, OR-5 and OR-6 |
| V           | 10                  | OR-13, OR-14, OR-15, OR-16, OR-23, OR-18, OR-21, OR-22, OR-24 and OR-17 |
| VI          | 5                   | RH 8812-OA, RH 0749-OA, RH 30-OA, RH 0406-OA and RH 0119-OA |

Fig 1: Dendrogram showing the clustering pattern of 45 Indian mustard genotypes based on 58 SSR markers
Fig 2: Agarose gel showing allelic polymorphism among 45 Indian mustard genotypes using SSR primer BG 160

Fig 3: Agarose gel showing allelic polymorphism among 45 Indian mustard genotypes using SSR primer BG 99

References
1. Adhikari S, Saha S, Biswas A, Rana TS, Kumar TB, Ghosh P. Application of molecular markers in plant genome analysis: a review. The nucleus. 2017; 60:283-297.
2. Avtar R, Rani B, Jattan M, Manmohan, Kumari N, Rani A. Genetic diversity analysis among elite gene pool of Indian mustard using SSR markers and phenotypic variations. The Bioscan. 2016; 11:3035-3044.
3. Banga S, Kumar PR, Bhajan R, Singh D, Banga SS. Brassica Oilseeds: Breeding and Management. In Kumar A, Banga SS, Meena PD, Kumar PR. Eds, CAB International, Oxfordshire, UK, 2015, 11-41.
4. Ghosh SK, Gulati SC, Raman R. Combining ability and heterosis for seed yield and its components in Indian mustard (Brassica juncea L. Czern & Coss). Indian Journal of Genetics. 2002; 62:29-33.
5. Gupta N, Zargar S, Gupta M, Gupta S. Assessment of genetic variation in Indian mustard [Brassica juncea (L.)] using PCR based markers. Molecular Plant Breeding. 2014; 5:10-17.
6. Kaur R, Sharma AK, Rani R, Mawlong I, Rai PK. Medicinal qualities of mustard oil and its role in human health against chronic diseases: A review. Asian Journal of Dairy and Food Research. 2019; 382:98-104.
7. Li H, Younas M, Wang X, Li X, Chen L, Zhao B et al. Development of a core set of single-locus SSR markers for allotetraploid rapeseed [Brassica napus (L.)] Theoretical and Applied Genetics 2013; 1264:937-947.
8. Nagaharu U. Genome Analysis in Brassica with Special Reference to the Experimental Formation of B. Napus and Peculiar Mode of Fertilization. Journal of Japanese Botany. 1935; 7:389-452.
9. Patel R, Yadav R, Avtar R, Jangra S, Boken G, Singh B, Yadav NR. Genetic Diversity Analysis for Salinity Tolerance in Indian mustard [Brassica juncea (L.)] Using SSR Markers. International Journal of Current Microbiology and Applied Sciences. 2018; 7:1776-1785.
10. Prajapati P, Sasdharan N, Kumar M, Prajapati. Molecular characterization and genetic diversity analysis in four Brassica species using microsatellite markers. The Bioscan. 2014; 9:1521-1527.
11. Prakash S, Hinata K. Taxonomy, cytogenetics and origin of crop brassica, a review. Opera Botanica Societate Botanice Lundensi. 1980; 55:1-57.
12. Pratap P, Thakur AK, Meena P, Meena H, Sharma P, Singh D et al. Genetic diversity assessment in Indian mustard [Brassica juncea (L.)] for Alternaria blight tolerance using SSR markers. Journal of Oilseed Brassica. 2015; 6:175-182.
13. Ramchiary N, Nguyen VD, Li X, Hong CP, Dhandapani V, Choi SR et al. Genic microsatellite markers in Brassica rapa: development, characterization, mapping, and their utility in other cultivated and wild Brassica relatives. DNA Research. 2011; 18:305-320.
14. Rohlf FJ. NTSYS-pc Numerical taxonomy and multivariate analysis system, version 2.02. New York: Exeter Publication, 1998.
15. Saghai-M0061roof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population. Proceedings of the National Academy of Sciences. 1984; 81:8014-8019.
16. Singh V, Bhajan R, Pant U. Molecular diversity analysis of Indian mustard genotypes through SSR markers. Environment and Ecology. 2016; 34:1212-1217.
17. Sudan J, Khajuria P, Gupta SK, Singh R. Analysis of molecular diversity in Indian and Exotic genotypes of Brassica juncea using SSR markers. Indian Journal of Genetics. 2016; 76:361-364.
18. Suwabe K, Iketani H, Nunome T, Kage T, Hirai M. Isolation and characterization of microsatellites in [Brassica rapa (L.)]. Theoretical and Applied Genetics. 2002; 104:1092-1098.
19. Tomar A, Singh M, Singh SK. Genetic analysis of yield and its components based on heterotic response and combining ability parameters in Indian mustard [Brassica juncea (L.) Czern & Coss.]. Progressive Agriculture. 2015; 151:85-91.
20. Turi NA, Farhatullah, Rabbani MA, Shinwari ZK. Genetic diversity in the locally collected Brassica species of Pakistan based on microsatellite markers. Pakistan Journal of Botany. 2012; 44:1029-1035.
21. Vinu V, Singh N, Vasudev S, Yadava DK, Kumar S, Naresh S et al. Assessment of genetic diversity in Brassica juncea (Brassicaceae) genotypes using phenotypic differences and SSR markers. Revista De Biologia Tropical. 2013; 61:1919-1934.
22. Wang F, Wang X, Chen X, Xiao Y, Li H, Zhang S et al. Abundance, marker development and genetic mapping of microsatellites from unigenes in Brassica napus. Molecular Plant Breeding. 2012; 30:731-744.