Comparative Evaluation of RAPD, ISSR and Anchored-SSR Markers in the Assessment of Genetic Diversity and Fingerprinting of Oilseed Brassica Genotypes

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Forty-two genotypes representing oilseed Brassica species were analyzed for the level of genetic diversity and molecular identity using Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR) and 5'-Anchored Simple Sequence Repeat (ASSR) markers. DNA profiles revealed high degree of interspecific polymorphism, while the level was considerably low within a species, particularly in B. juncea. The UPGMA clusters clearly delineated genotypes of the respective Brassica species. Comparison of cophenetic matrices indicated a high degree of correspondence between dendrograms generated by different marker systems. A minimum of 10 random primers (approximately 105 bands) were required for the RAPD profiles to generate the expected cluster. Comparatively less number of primers was required to do the same in case of ISSR (4 primers) and ASSR (3 primers). The principal component analysis revealed similar genetic relationship among the genotypes as in cluster analysis. Although none of the DNA profiles could individually identify all the B. juncea genotypes, a combined DNA profile consisting 125 markers from the informative primers of all the three DNA marker systems could do the same. A positive correlation was found among the marker utility parameters (calculated for individual primers of different marker systems) such as marker index (MI), resolving power (Rp) and discrimination coefficient (D) with the number of genotypes identified by each primer with a few exceptions. Single plant analysis for a set of five B. juncea varieties revealed absence of intra-varietal heterogeneity in case of ASSR profiles, thereby suggesting its utility in varietal identification and differentiation.

Key words: Brassica, RAPD, ISSR, ASSR, resolving power, marker index, discrimination coefficient.

Assessment of genetic diversity and identification of crop genotypes are essential for efficient conservation and utilization of germplasm resources. Morphological characters have been traditionally used for germplasm characterization. Such characters are, however, limited in number, and show growth stage and environment dependent expression. In contrast, molecular markers based on difference in the DNA sequence are large in number. The stage of plant growth and the environment in which the plant grows, do not influence the differences in DNA sequence. Therefore, molecular markers are currently being used for an accurate estimation of genetic diversity and determination of unique identity of crop genotypes (1). In Brassica, many efforts have been made to characterize germplasm using different molecular markers (2-6). There is a lack of information on comparative evaluation of molecular markers in oilseed Brassica species, which constitute a single gene pool due to cross compatibility.

Different molecular marker systems have been evaluated for their efficiency in detecting polymorphism and assessing genetic diversity using various statistical parameters (7). There are many issues pertaining to suitability of a marker system, number of marker combinations to be employed and relevance of appropriate marker utility parameters, which need to be addressed in every crop species prior to a large-scale use of molecular markers in germplasm characterization and variety identification (8-10). To our knowledge no such effort has been reported in oilseed Brassica species as yet. In the present study, a comparative evaluation of three PCR based DNA marker systems namely, Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR) and 5'-Anchored Simple Sequence Repeat (ASSR) was carried out for their potential application in diversity analysis and fingerprinting of oilseed Brassica genotypes. Various aspects of the study included: (i) analysis of diversity, (ii) evaluation of individual primers of different
marker systems using parameters such as discrimination coefficient (D), marker index (MI) and resolving power (Rp) and their correlation with the number of genotypes identified, and (iii) single plant analysis using informative DNA profiles of each marker system to find out the level of intra-varietal heterogeneity.

Materials and Methods

Plant material — Forty-two genotypes of different oilseed Brassica species including 28 of B. juncea, four of B. carinata, three of B. napus, one of B. nigra, five of B. campestris and one of Eruca sativa were used in the present study (Table 1).

DNA isolation and PCR amplification — Total genomic DNA was isolated from bulked young leaves collected from 10 field-grown plants of each genotype as described earlier (11). Purified genomic DNA was subjected to PCR amplification using random primers (RAPD), microsatellites of tri- and tetra-nucleotide repeats (ISSR) and simple sequence repeats having trinucleotide anchors at the 5'-end (ASSR). ISSR and ASSR were consciously chosen as separate methods to evaluate their relative efficiency and utility in Brassica. A 25μl PCR mixture contained 25 ng of genomic DNA, 0.5U (RAPD) or 0.7U (ISSR and ASSR) of Taq DNA polymerase (Bangalore Genei, India), 1x PCR buffer containing 1.5 mM MgCl₂, 0.2 mM of each dNTPs (Perkin Elmer, USA) and 0.2μM (RAPD) or 0.3μM (ISSR and ASSR) of primer. PCR amplifications were done in a Perkin Elmer Cetus GeneAmp 9600 thermal cycler along with a control reaction containing all the above components except genomic DNA for each primer. Temperature profiles in RAPD were as given by Jain et al (12). In case of ISSR and ASSR, a 30 cycle programme of 1 min at 94°C, 2 min at 55°C and 30 sec at 72°C was followed with a final extension of 5 min at 72°C. Electrophoresis of PCR products was done on 1.5% (RAPD) and 2.5% (ISSR and ASSR) agarose gels at 75V for 3 h. Gels were photographed after ethidium bromide staining.

Data analysis — Only clear and reproducible DNA fragments were scored as 1 - 0 matrix for the presence and absence of a band, respectively. Dendograms were constructed based on UPGMA clustering of a similarity matrix generated by Jaccard’s coefficient. Principal components were derived for each genotype using eigen vectors and eigen values extracted from a correlation matrix among markers that was obtained from a standardized data matrix. A test of significance for explaining the proportion of variance among the genotypes was done using the Broken-Stick Model (13). Cophenetic matrices were generated using the similarity matrix of the corresponding marker systems and tested for correlation between them using mantel – matrix correspondence test. All the above analyses were done using NTSYS-pc (version 1.70) program. Bootstrap analysis (500 iterations) of the binary data was done using the WINBOOT programme (14) to determine the confidence limits of the UPGMA clusters. Discrimination coefficients (D) were calculated for all the genotype pairs with band differences

Table 1. Details of the oilseed Brassica genotypes used in the study

<table>
<thead>
<tr>
<th>Species</th>
<th>Varieties/Accessions</th>
<th>Area of adoption</th>
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<tbody>
<tr>
<td>B. juncea (Mustard)</td>
<td>Pusa Bold, Pusa Bahar, Pusa Barani, Pusa Agrani (Sej2), Pusa Basant RLM-198, Varuna Pusa Jaikisan RH-781, RH-819, RH-8812, RH8813, RH-30 Bio-772, D313XBio8, Ej-1, Ej X Pusa Bahar1-2, VX9-5, WR X (313XYs), BJWF-1, EF-1, EF-2, D, T, PPY, PVS, Bio-YSR BEC-144, BEC-286</td>
<td>North-east part on India Punjab All the Brassica belts North-west regions of India Rainfed areas of North-west India Genotypes under trial Exotic collections Punjab, Himachal Pradesh Rainfed areas of Haryana, Punjab, Himachal Pradesh and parts of Uttar Pradesh Bihar, West Bengal and parts of Orissa North-west, North-east and central India Mostly grows wild North-west India and Uttar Pradesh Dry regions of North-west India</td>
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