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Assessment of genetic diversity using DNA markers among *Brassica rapa* var. yellow sarson germplasm lines collected from Eastern Uttar Pradesh and Uttarakhand hills

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Abstract: The genetic diversity and the relatedness among thirty-one germplasm lines of yellow sarson collected from eastern UP were evaluated using morphological characters and Random Amplified Polymorphic DNA (RAPD) markers. Molecular parameters, viz. A total number of bands, average polymorphic band, average percent polymorphism, average polymorphic information content (PIC), Jaccard's similarity coefficient, Principal Coordinate Analysis (PCA) and dendrogram generated using RAPD markers. A total of 148 different polymorphic amplification products were obtained using 10 selected decamer primers. The Jaccard similarity coefficient ranged from 0.557-0.899. Maximum polymorphism detected was 100 %.The range of amplification was from 190bp to 9 kb. Some unique bands were also reported with different primers that can be used for the identification of particular accession. PYSC-11-11 and PYSC-11-36 genotypes showed a maximum number of unique loci of different size. 31 germplasm lines grouped into two major clusters I and II based on RAPD profiling. Morphological characterization was done on the basis of leaf, petal and beak characteristics. The similarity value among the germplasm lines ranged from 0.222 to 1.000 using morphological descriptors. The dendrogram generated grouped the germplasm accession into two major groups at 44% similarity value. The cluster analysis was comparable up to some extent with Principal Coordinate Analysis (PCA) of two and three-dimensional plots. The variability revealed by morphological and molecular profile were found to be non-comparable. This study indicated the presence of high genetic diversity among collected yellow sarson germplasm, which could be used for developing for breeding and germplasm management purposes.

Keywords: *Brassica rapa*, Genetic diversity, PCA, RAPD

INTRODUCTION

Rapeseed-mustard is an important crop with great economic value in worldwide. *Brassica juncea* (Indian mustard) and *B. rapa* are the important oilseed crops in India. The *B. rapa* is divided into three ecotypes, *viz.*, toria, brown sarson and yellow sarson which are grown in India. Among *Brassica rapa* var yellow sarson is grown since ancient time due to the presence of a comparatively higher percentage of oil in the seed, shorter crop duration and attractive seed coat colour. North-eastern India is considered as primary centre of origin, however, genetic diversity found in eastern Uttar Pradesh and West Bengal (Misra, 2011)

Assessment of genetic variability present in the available germplasm is the first step in any crop improvement programme. The collected information on the genetic diversity in *B. rapa* var yellow sarson could help breeders and geneticists to understand the structure of *B. rapa* var yellow sarson germplasm and help them to evaluate which combinations would produce the best offspring. Accurate assessment of the levels and patterns of genetic diversity have diverse applications, viz. (i) analysis of genetic variability in cultivars, (ii) identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and (iii) introgressing desirable genes from diverse germplasm into the available genetic base. There are various techniques available, which allow studying the genetic variability of crop germplasm. Morphological traits, total seed proteins, isozymes and several types of DNA markers are well-known examples. DNA-based molecular marker techniques are being used enormously for estimating the magnitude of diversity (Abbas *et al*., 2009). However, molecular markers reveal differences of natural sites at the DNA level. These variations are not seen in the phenotype and each might be single nucleotide differences in a gene or a piece of repetitive DNA (Chen *et al*., 2011). Moreover, molecular markers are not subject to any environmental fluctuations, making them specifically informative and superior to conventional methods of genotyping such as the use of morphological traits and biochemical markers (Gupta *et al*., 1999). Molecular marker techniques include restriction fragment length polymorphisms (RFLPs) (Beckman and

Soller, 1983), simple sequence repeats (SSRs) or microsatellites (Tautz, 1989), random amplification of polymorphic DNA (RAPDs) (Williams et al., 1990; Welsh and McClelland, 1990; Karp et al., 1997) and amplified fragment length polymorphisms (AFLPs) (Vos et al.,1995).

Among molecular markers, random amplified polymorphic DNA (RAPD) has been employed in genetic research owing to their speed and simplicity (Welsh and McClelland 1990) RAPD analysis has been widely used in recent studies on Brassica crops: (1) for determining the genetic relationships between different related species (Demeke *et al*., 1992, Thormann *et al*., 1994 and Ren *et al.,* 1995), (2) for the identification of cultivars (Quiros and Hu 1991) and the percentage of hybridity (Marshall *et al*. 1994), (3) for the estimation of genetic relationships and diversity among crop germplasm (Kresovich *et al*. 1992, Mailer *et al*. 1994, Santos *et al*. 1994, Divaret and Thomas 1998). RAPD marker has been extensively used for diversity analysis in various crops (Saha *et al*., 2008 studied the Genetic relationship between four *Brassica* varieties using RAPD markers, Ghosh *et al*., 2009 analysed the Genetic diversity through RAPD markers in *Brassica* varieties in Bangladesh, Khan *et al*., (2011) used RAPD in *Brassica juncea*, Gupta *et al*., (2014) used RAPD markers in Indian Mustard for analysis of Genetic variation, and Yousuf *et al*., (2012) also used RAPD marker for comparative studies between Indian mustard varieties.

It is, therefore, the aim of this work was to provide genetic variation and relatedness among the yellow sarson germplasm lines by PCR-based RAPD technique, as it is important particularly for selection of diverse parents to be used in a hybridization programme.

MATERIALS AND METHODS

Plant material: The experimental material comprised of 31 yellow sarson germplasm lines including two checks viz; PPS-1 and B-9. The germplasm lines were collected from Eastern Uttar Pradesh and Uttrakhand hills. Various parameters related to morphology characterization viz; like petal length, petal width, leaf length, leaf width, leaf lobes, the number of leaf lobes, leaf hairiness and beak length were recorded as per the DUS (Distinctness, Uniformity and Stability) guidelines during rabi season 2012-13 following random block design (RBD) with three replications.

Total DNA isolation and RAPD fingerprinting: Total cellular DNA was isolated from freshly germinated young leaves following the CTAB method of Murry and Thompson (1980) with following modification: about 3 gm of 4-5 days fresh germinated seedlings tissue was crushed to powder in liquid nitrogen and transferred to pre-warmed (65ºC) 2xCTAB buffer. The DNA was purified by phenol-chloroform extraction and ethanol precipitated. The final DNA pellet was dissolved in TE buffer was stored in -20ºC until use. An initial screening of 15 random primers was performed in order to evaluate their capacity to produce polymorphic, reproducible and reliable RAPD patterns. Ten primers were selected among them for molecular diversity analysis.

DNA amplification: Polymerase chain reaction was performed based on the protocol of Williams *et al*. (1990), with minor modification. Amplification was carried out in 25µl of reaction mixture containing 2.5 µl reaction buffer (10X), 2.0 µl dNTPs mixes (200µM each), 2 µl (40 ng μl^{-1}) decamer primer, 2 µl (40 ng μl^{-1}) genomic DNA and 0.5 µl (3Uμl^{-1}) *Taq* DNA polymerase was used for the amplification of template DNA. PCR reactions were performed with Bio-Rad thermocycler. PCR programme had an initial denaturation at 94^0C for 4 min, denaturing at 94^0C for 1 min, annealing at 36^0C for 60 sec and polymerization at 72^0C for 2 min and final extension 72^0C for 10 min. After amplification, the PCR product was resolved on 1.5 % agarose gel in 1X TBE buffer. The gel was visualised under UV using gel documentation system.

Data analysis: All polymorphic bands were scored as (1) for presence and (0) for absence. Only reproducible and reliable bands were scored for all the genotypes and primer combinations. The data was analysed using NTSYS pc 2.02 (**N**umerical **T**axonomy **Sys**tem) software. Similarity Jaccarrd coefficient computed. Cluster analysis was done and a dendrogram was constructed using unweighted paired group of the arithmetic mean (UPGMA) algorithm. The structure of the genetic diversity was further analysed by Principal Coordinate Analysis (PCA) from the correlation matrix among the markers. Both cluster analysis and PCA were performed using NTSYS pc 2.02 statistical software.

RESULTS AND DISCUSSION

Morphological description: During the present study morphological characterization was done on yellow sarson germplasm lines and the data was recorded as per the DUS(Distinctness, Uniformity and Stability) guidelines. Similarity matrix and a dendrogram were generated using NTSYS pc 2.02 software. The value of similarity matrix for morphological characterization ranged from 0.222 to 1.000. B-9(1.000) was found to be most diverse from PPS-1(0.222) and PYSC-11-29 (0.222). The dendrogram generated using NTSYS 2.02 grouped the germplasm accession into two major groups at 44% similarity value. Cluster I have four lines namely PYSC-11-5, PYSC-11-29, PYSC-11-39 and PPS-1. Rest all the lines fall in the cluster II (Fig.3.). Yassein *et al*. (2013) also used the morphological traits for the purpose of genetic diversity and heritability analysis in *B.napus* in Egypt.

Molecular profiling: Ten primers were selected to amplify total DNA from samples of all genotypes which showed 100% polymorphism. A total 148 repro-

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Fig. 1. *Jaccard similarity coefficients of 31 germplasm lines of Yellow sarson using RAPD profiling.*

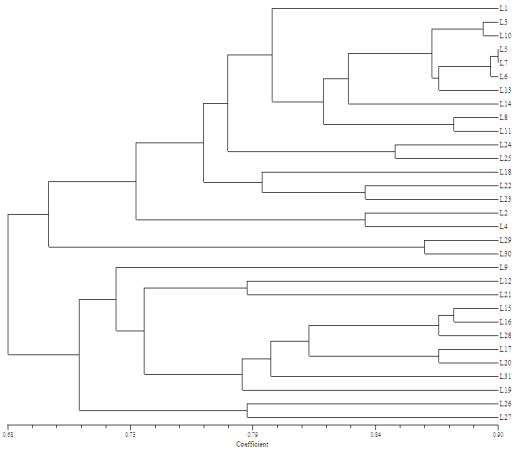

Fig.2. *Dendogram derived from UPGMA cluster analysis using RAPD profile.*

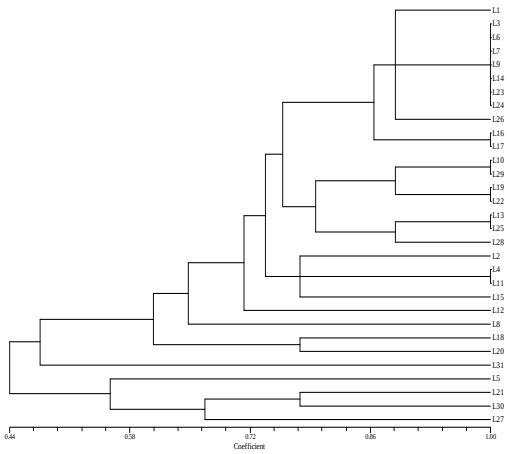

Fig.3. *Dendogram derived from UPGMA cluster analysis using morphological descriptors.*

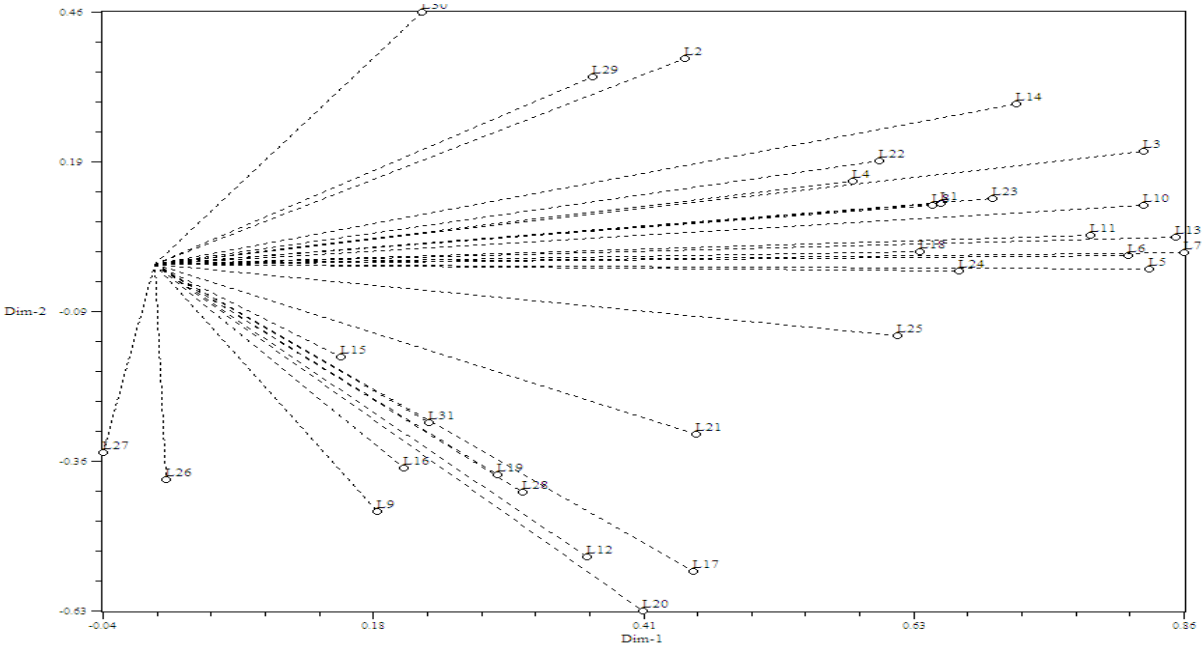

Fig.4. *Two dimensional plot (with vector) of 31 germplasm lines of yellow sarson obtained using principal coordinate analysis (RAPD profiling).*

ducible bands amplified against thirty-one yellow sarson germplasm lines. The total number of bands, average polymorphic bands, average percentage polymorphism and average polymorphic information content (PIC) was determined (Table 1).Molecular fingerprinting using RAPD marker is widely used for detecting genetic polymorphism between genotypes at the molecular level in many crop species viz oilseed crops, pulses and cereals crops. The number of polymorphic amplicons generated varied from 7(OPA-8) to 22(OPG -2) and presented molecular weight between 190bp to 9kb. From the RAPD primers, OPG-02 (Fig.7.) , OPC-09 and Operon-6 showed a higher level of genetic polymorphism among the germplasm line based on amplification of maximum no. of reproducible bands 22, 22 and 19 respectively while OPA-8 and Operon-8 showed the minimum no. of reproducible bands 7 and 9 respectively. Shengwu *et al*. (2003) also evaluated the genetic diversity of *Brassica napus* germplasm using 10 selected RAPD primers generated a total of 79 different polymorphic amplification in China.

In present study on molecular diversity analysis in yellow sarson germplasm lines, all the primer showed some unique loci (band) at a particular base pair (bp) size and differentiated the genotype from the rest of genotype present in germplasm. OPG-02 produced two unique bands in the PYSC-11-20 genotype of 190 bp and in PYSC-11-30 genotype of 5kb (Fig.7.). OPC-9

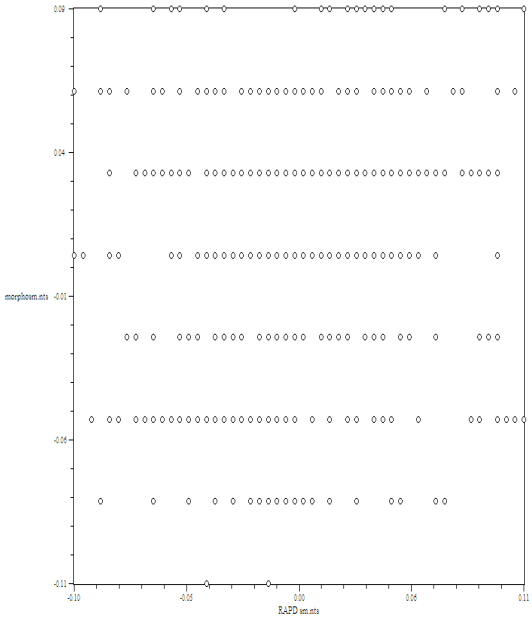

Fig. 5. *Three dimensional plot (with vector) of 31 germplasm lines of yellow sarson obtained using principal coordinate analysis (RAPD profiling).*

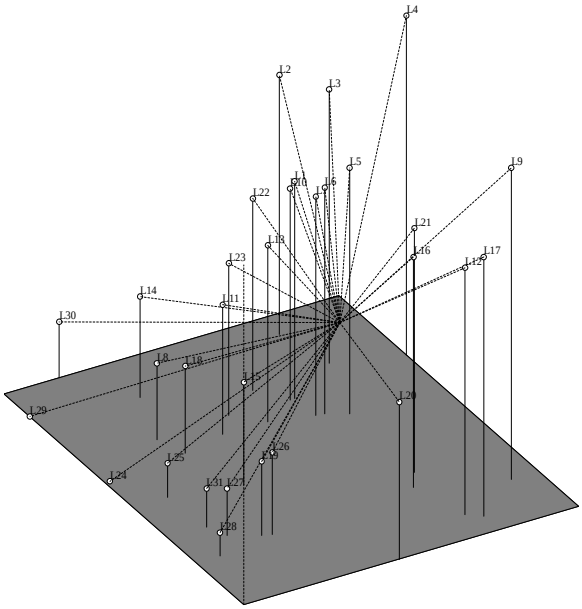

Fig. 6. *Correlation matrix between morphological descriptors and RAPD profiling.*

primer produced maximum numbers of the unique loci (6) present at the specific position in the germplasm lines were PYSC-11-36 (9000 bp and 6800 bp), B-9 (8000 bp), PYSC-11-25 (4000 bp), PPS-1 (2000 bp) and PYSC-11-35 (350 bp) (Fig.8). OPB-14 produced a single band of 900 bp in PYSC-11-11and primer OPA-8 also produced unique band size of 300bp in PYSC-11-35 genotype. PYSC-11-11 and PYSC-11-36 genotypes showed a maximum number of unique loci of different size. Ahmad *et al*. (2009) used RAPD marker for the study of DNA polymorphism in *Brassica juncea* at Pantnagar Uttrakhand. Gupta *et al.* (2012) also reported polymorphism among twenty varieties of *Brassica juncea* L. using ten RAPD markers. Ten RAPD primers generated 62 loci with 50 polymorphic and 12 monomorphic showing 6.2 loci per primer. Gupta *et al.* (2014) also used DNA-based primers RAPD and EST SSRs to identified Genetic variation in Indian mustard collected from different institutes of Northern India.

UPGMA cluster analysis using RAPD: The distance matrices based on the Jaccard similarity coefficient ranged from 0.557 to 0.899. Based on the Jaccard similarity coefficient from the thirty-one yellow sarson germplasm lines PYSC-11-7 genotype showed maxi-

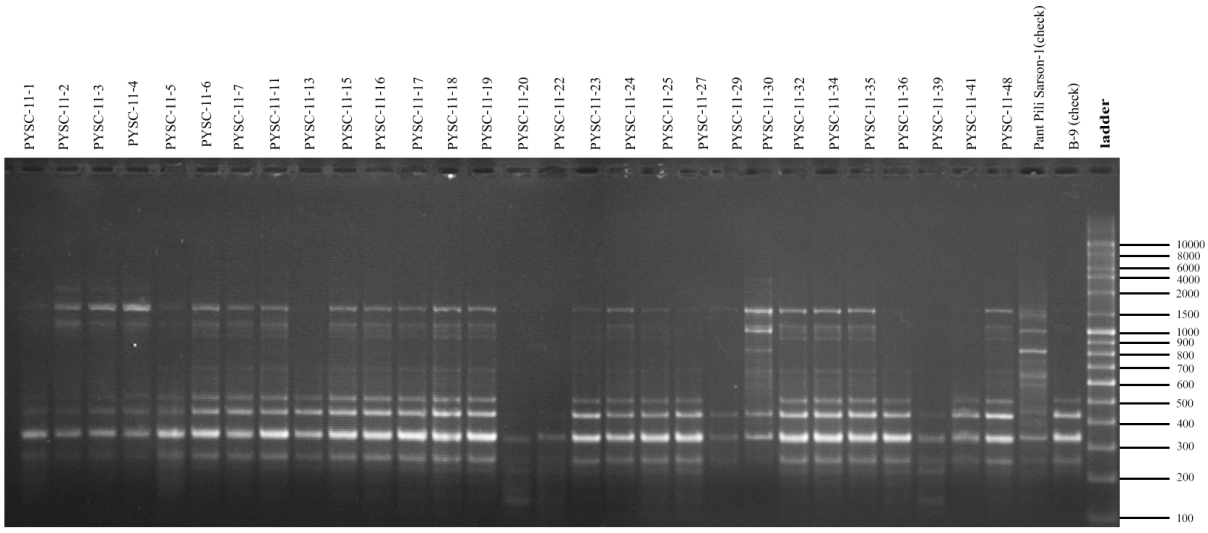

Fig. 7. *PCR amplification of 31 yellow sarson genotypes with RAPD primer OPG 2.*

Table. 1:-Range of RAPD loci scored, number and size of exclusive loci amplified in Brassica rapa var. yellow sarson germplasm.

S. No.	Primer code	No. of loci amplified	No. of polymorphic loci	Polymorphism (%)	Polymorphic Information Content (PIC)	Range of amplified loci (bp)	Exclusive loci		Size of distinguished loci
							Number	Name of genotype	
1	Operon- 6	19	19	100%	0.2060	250 – 4500	3	PYSC11-11 PYSC-11-20 PYSC-11-3	4500 810 250
2	Operon-8	9	9	100%	0.2338	290 - 1500	2	PPS-1 PPS-1	800 600
3	OPG-2	22	22	100%	0.2718	190 - 5000	2	PYSC-11-30 PYSC-11-20	5000 190
4	OPB-14	18	18	100%	0.2232	300 - 7000	1	PYSC-11-11	900
5	OPA-10	14	14	100%	0.2060	380 - 7000	3	PYSC-11-23 PYSC-11-19 PYSC-11-30	7000 720 410
6	OPC-09	22	22	100%	0.1974	290 - 9000	6	PYSC-11-36 B-9 PYSC-11-36 PYSC-11-25 PPS-1 PYSC-11-35	9000 8000 6800 4000 2000 350
7	OPA-8	7	7	100%	0.2417	300 - 4000	1	PYSC-11-35	300
8	OPG-1	14	14	100%	0.2276	250 - 4000	5	PYSC-11-27 PPS-1 PYSC-11-15 PYSC-11-11 PYSC-11-11	1600 650 500 330 280
9	OPB-10	10	10	100%	0.2106	500 - 3000	2	PYSC-11-34 PYSC-11-36	2000 750
10	Operon-18	15	15	100%	0.1831	200 -2100	2	PYSC-11-36 PYSC-11-25	2000 210

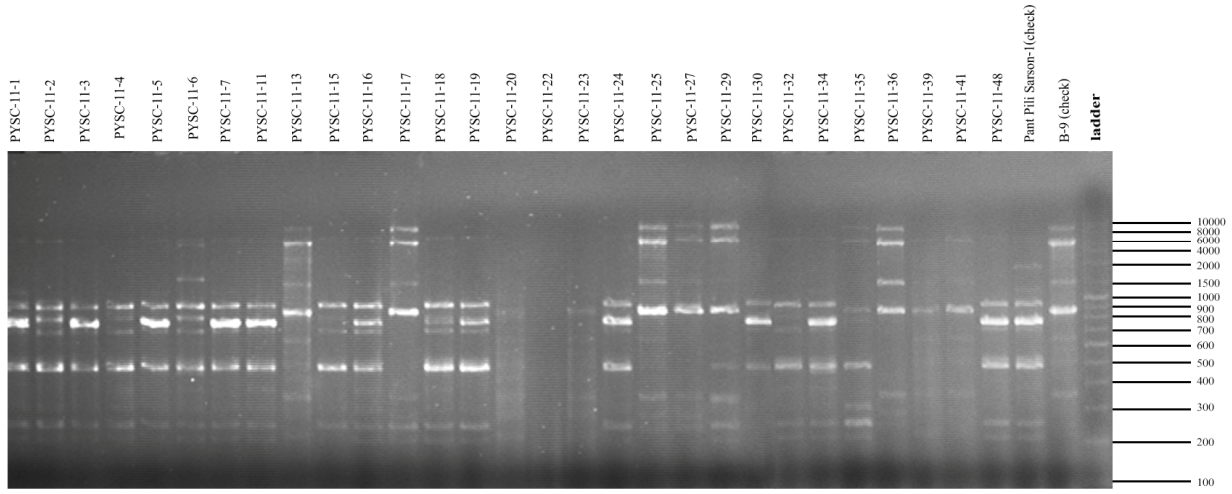

Fig. 8. *PCR amplification of 31 yellow sarson genotypes with RAPD primer OPC 09.*

mum resemblance (89.9%) with the PYSC-11-5and PYSC-11-76 genotypes. PYSC-11-5 and PYSC-11-6 genotypes were 89.3% similar to each other. PYSC-11 -3 showed 89.3% and 88.6% similarity with the PYSC-11-15 and PYSC-11-5 respectively. Minimum resemblance (57.7%) found between PYSC-11-36 genotype with the PYSC-11-1 and PYSC-11-30 genotypes and 56.4% similarity with PYSC-11-18 genotypes. PYSC-11-32 and PYSC-11-39 genotypes were found to 57% similar (Fig.1.). Cluster analysis was done using UPGMA method and dendrograms were constructed (Fig.2.). Dendrogram based on the J distance matrix defined two major clusters for thirty-one yellow sarson germplasm lines based on 58% similarity. Cluster I comprised 12 yellow sarson genotypes viz; PYSC-11-36, PYSC-11-39, PYSC-11-13, PYSC-11-25, B-9 (check), PYSC-11-23, PYSC-11-27, PYSC-11-41, PYSC-11-20, PYSC-11-22, PYSC-11-29 and PYSC-11-17. Cluster II comprised of 19 yellow sarson genotypes viz; PYSC-11-48, PPS-1(check), PYSC-11-2, PYSC-11-4, PYSC-11-32, PYSC-11-30, PYSC-11-24, PYSC-11-34, PYSC-11-35, PYSC-11-16, PYSC-11-11, PYSC-11-19, PYSC-11-15, PYSC-11-3, PYSC-11 -18, PYSC-11-6, PYSC-11-7, PYSC-11-5, and PYSC-11-1. The germplasm lines present in the different cluster were diverse enough hence can be used as parental lines in a hybridization programme. RAPD data also analysed using principal coordinate analysis (PCA). Associations among the yellow sarson genotypes revealed by PCA were presented in two and three-dimensional plot and have shown the comparable result to some extent with the cluster analysis using UPGMA (Fig. 4 and 5).

Correlation between genetic variability of morphological descriptors and RAPD markers: The correlation between genetic variability of the morphological descriptor and RAPD analysis was found to be non-significant (Fig. 6) similar reports were also reported by Geleta and Labuschagne (2005), found a non-significant correlation between morphological characters and AFLP markers in sorghum. Gupta *et al.* (2012) studied the genetic relatedness among seventeen lines of Indian mustard at IARI, New Delhi using RAPD marker and morphological traits. For the study on genetic variability from the morphological descriptor and molecular analysis found a difference in the pattern of the cluster, might be due to the fact that molecular marker measure variation mainly in a non-coding sequence which probably have a minor impact on phenotype. Morphological descriptors, on the other hand, are affected by environmental conditions and show considerable variation (Gupta *et al*. 2012).

Conclusion

In conclusion, this study demonstrated that RAPD markers provide an effective tool for the assessment of genetic variation among yellow sarson germplasm and in line identification. This information could be used for selection of most divergent pairs of parents, which could be used for genetic linkage map construction in this species. Information on the genetic diversity in *B. rapa* var. yellow sarson germplasm can help breeder to select the best combinations, which produce a high level of heterosis. The observed genetic variation among investigated yellow sarson germplasm found in analysed loci should alter the breeders for introducing new genetic sources in a hybridization programme. Investigated collection includes a collection of yellow sarson germplasm lines from Eastern UP and Uttrakhand Hills. However, direct effort to diversify the genetic base of breeding material and to seek new sources of favourable alleles would be still necessary.

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