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USE OF RAPD MOLECULAR MARKER TO IDENTIFY PARENTS FOR DROUGHT TOLERANCE BREEDING IN MUSTARD

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Abstract
Thirteen genotypes belonging to five species Brassica rapa, Brassica juncea, Brassica carinata, Brassica napus and Eureca sativa were evaluated for molecular traits to study the diversity among the genotypes and to identify the desirable and potential parents for mustard breeding programme during the year 2008-09 at College of Agriculture Nagpur. The molecular markers (RAPD) was found to show polymorphism. The genomic DNA from the mustard genotypes when amplified using eighteen random primers gave 279 scorable amplified product. The maximum number of bands were generated by the primer E07, E09 and IOP700 (19). The similarity index between the drought tolerant genotypes RTM-314 and T-27 belonging to Eureca sativa with the cultivated genotypes Pusa Bold and ACN-9 belonging to Brassica juncea were found to be 0.21 to 0.30 which indicates a high level of dissimilarity and diversity among these two species. Drought tolerant genotype RTM-314 and T-27 deviated from all other genotypes on the basis of molecular markers. This study, lead to conclusion that the cultivated genotype ACN-9 and Pusa Bold of Brassica juncea can be further improved by crossing with RTM-314 and T-27 (Eureca sativa) for drought tolerance.
Keywords: Mustard genotypes, molecular markers, RAPD, diversity

Introduction

Choice of parents influences the success of breeding programme in self pollinated crop like mustard and other crop also. The assessment for choice of parents were conducted by many workers in various crop species using different types of marker system like morphological, biometrical, biochemical and molecular. Till recently the assessment of genetic diversity and phylogenetic studies were conducted by many worker in various crop species, based on the quantitative and qualitative traits, using biometrical method such as Mahalonobis D2 statistic, metroglyph analysis, principal component analysis etc. Several biochemical and molecular approaches have been used to identify, diagnose and delimit species and access phylogenetic relationship between different species. Biochemical methods like seed protein, banding pattern, isozyme banding pattern and molecular methods like RAPD, RFLP etc. have been the most extensively applied technique for screening mustard species. Molecular markers are the best tools for determining genetic relationships. Neverthless, evaluation of genotypes at the subgenetic level using DNA sequence variation is best method because this procedure completely eliminates environmental influence. Brassica juncea is a cultivated species of Vidarbha region having high yielding capacity. However, as it is grown with the conserved moisture from monsoon rain it invariably suffers from moisture stress during different phases of growth. The unpredictable rainfall received also results in unfavorable residual moisture. This affects the yielding potential of the mustard varieties grown in the Vidarbha region. Therefore, there is a need for development of high yielding and early maturing mustard varieties along with the drought tolerant trait. In an attempt to fulfill this need partly this experiment was taken up to screen the available mustard species based on molecular (RAPD) markers with the objective to identify the parents for hybridization to develop drought tolerant varieties.

Material and Methods

This experiment was conducted during 2008-2009, at the farm and laboratory of Botany section, College of Agriculture, Nagpur. The experimental material consisted of thirteen genotypes of mustard belonging to five different species viz., Brassica rapa (toria) â€“ Bhavani, PT-303, T-9, Brassica rapa (yellow sarsan) â€“ YST-151 & Ragini, Brassica juncea â€“ Pusa Bold & ACN-9, Brassica rapa (brown sarsan) â€“ BSH-1 & KBS-3, Eureca sativa â€“ RTM-314 &

T-27, Brassica carinata â€“ PC-5 & Brassica napus â€“ GSL-1. The thirteen genotypes were subjected for molecular analysis by using RAPD markers. This molecular study based on randomly amplified polymorphic DNA (RAPD) were carried out in the marker aided selection laboratory of National central laboratory, Pune. Eighteen primers namely A07, D18, E07, E09, D07, D09, D08, E06, E07, E08, A08, B09, C01, G02, IOP576, IOP700, IPI178 & IPI701 were used in this study. Isolation of genomic DNA was done as per the method given by Krishna and Jawali (1997), quantification of genomic DNA was done using flurometer (Dyna Quant, USA), amplification of genomic DNA through polymerase chain reaction as per the method Saiki et al.(1998) and separation of DNA fragments by agarose gel electrophoresis as per the method of Sambrook et al. (1989). The gel was viewed in an alpha amager (alpha imager 1200, alpha innotech corporation), and the image was documented. All the electromorphs observed from the electrophoretic field were scored for their presence or absence of bands using binary codes as 1 and 0 respectively. The similarity indices were calculated using the formulae, Similarities (F) = 2(nxy) / (nx + ny) (Nei and Li, 1979) Where, nxy = number of bands is common to sample A and sample B, nx = number of bands for sample A and ny = number of bands for sample B. Based on these co-efficients UPGMA clustering was carried out and a cluster dendogram was constructed for the thirteen genotypes by neighbourhood joining method. The entire analysis was performed by using â€œRAPDistanceâ€ 1.04, a computer software developed by John Armstrong (Research School of Biological Science, Institute of Advanced studies, A.N.U., Australia.

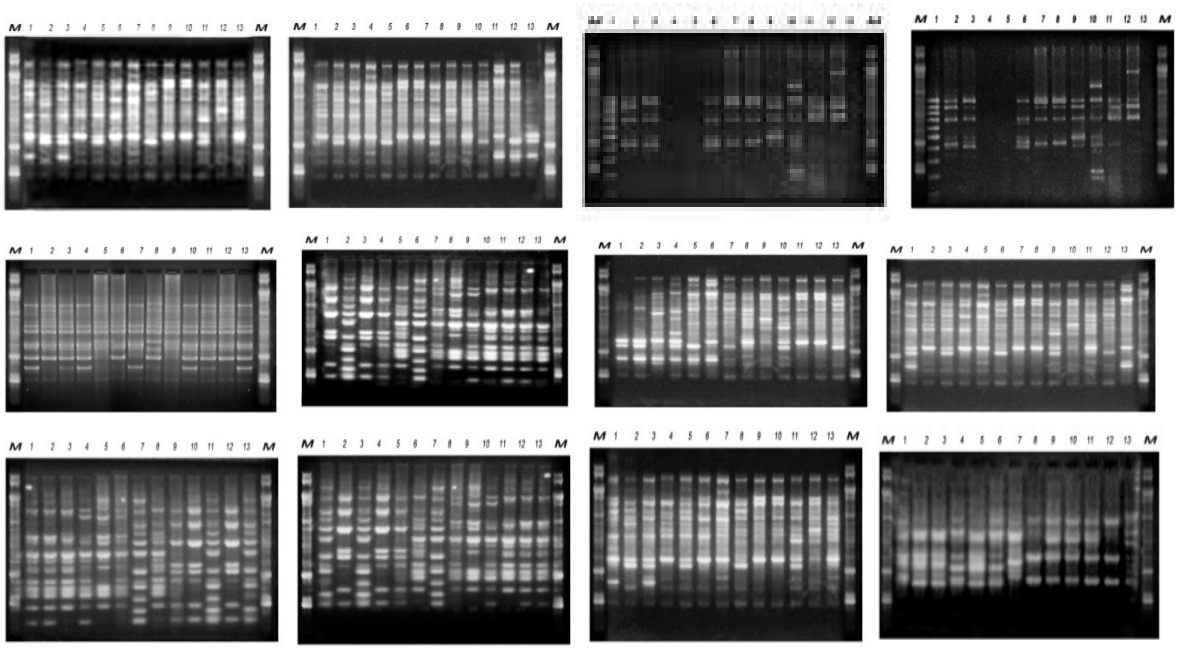
Result and Discussion

Attempt was made to study the genetic diversity among thirteen genotypes taken from five different species by scanning the entire genome using 18 orbitrary primers through RAPD analysis. All the eighteen primers revealed polymorphism between the thirteen genotypes taken for study. The amplified products varied in number and intensity among the selected genotypes. Variation in the intensity of the same band in different genotypes were noticed which may be due to the fact that specific site choosen by the primer could have been found in abundance when compared with other genotypes. Similarly various authors like Mailer et al. (1994), Qiao et al.,(1998) and Mahla et al. (2005) have also reported the variation in the band intensities due to the abundance of specific site choosen by the primer in one genotype compared to the others. The RAPD profiles of thirteen genotypes lines for eighteen primers were presented in plate 1. A total of 279 scorable bands were identified as a result of amplification by eighteen random primers out of which 275 were polymorphic for the thirteen genotypes studied (table 1). Among eighteen primer tested, the number of RAPD bands generated were more for the primer E07, E09 and IOP700 (19) followed by A07 and B09 (18). Amplification with the primer D07 yielded the least number of amplified fragments (9). When the primers A07, D18, E07, E09, D07, D08, E06, E08, A08, B09, C07, G02, IOP576 and IOP700 were used for amplification, all the fragments identified 18, 16, 19, 19, 9, 14, 13, 18, 17, 17, 18, 17, 15, 13 and 19 respectively were polymorphic. The genomic DNA samples amplified with D09, IPI178 and IPI701 gave varying number of polymorphic bands. In D09, 12 out of 13, in IPI178, 11 out of 12 and in IPI701, 10 out of 12 were polymorphic and the remaining were monomorphic in nature. The degree of polymorphism detected by different primers varied and thus, there was considerable variation in the ability of individual primers to detect DNA polymorphism. Similar to this result Mahla et al. .(2005) also reported a total of 205 amplicons of which 162 were polymorphic when genetic diversity among 45 genotypes of Brassica juncea were studied using 37 decamer primers. The binary data obtained from 18 random primers for thirteen genotypes were analysed and the similarity index between the genotypes was derived and presented in figure 1. The maximum similarity coefficient value ranging from 0.61 to 0.70 was observed between BSH-1 with Bhavani, PT-303 with BSH-1, Ragini with BSH-1, T-27 with ACN-9. The least similarity coefficient value (<= 0.20) was observed between T-27 and YST-151. The similarity index between the drought tolerant genotypes RTM-314 and T-27 belonging to Eureca sativa with the cultivated genotypes Pusa Bold and ACN-9 belonging to Brassica juncea were found to be 0.21 to 0.30 which indicates a high level of dissimilarity and diversity among these two species. In accordance to this result Mahla et al. (2005) reported the range of genetic similarity indes from 0.60 to 0.97 in Brassica juncea. In contrary minimum and maximum molecular genetic distance were found to be 0.12 and 0.78 respectively in Pea as reported by Handerson et al.(2014). Based on the similarity index, a

cluster dendrogram was constructed which is presented in figure 2. Dendrogram obtained from the data of molecular data analysis revealed that the thirteen genotypes were distributed in two major and one minor cluster. The thirteen genotypes were grouped into two major clusters, comprising Bhavani, BSH-1, YST-151, PT-303, Ragini, and KBS-3, in one cluster and Pusa Bold, T-9, GSL1, ACN-9 and PC-5 in the other. Genotype RTM314 and T-27 was observed to fall in a separate minor cluster which was found to be distinct from that of the other two groups. The first major cluster was further partitioned into two subclusters one including Bhavani, BSH-1, and the other included YST-151, PT-23, Ragini, and KBS-3. Similarly the second major cluster comprised of two subclusters one including Pusa Bold, T-9, and GSL-1 the other subcluster included ACN-9 and PC-5. Interesting thing observed from this study is that the drought tolerant genotypes RTM314 and T-27 fell in a separate minor cluster which was found to be distinct from that of the other two groups. The cultivated genotypes Pusabold and ACN-9 even though belonging to Brassica jucea grouped in two different subgroup of the second major cluster which indicates the that these two varieties are distinct from one another in some aspects. It can be concluded from this study that the cultivated genotypes ACN-9 and Pusa Bold of Brassica juncea can be further improved by crossing with RTM-314 and T-27 of Eureca sativa for drought tolerance.

Table 1. RAPD products generated using 18 random primers in thirteen genotypes

Sr. no.	Primer number	Prime sequence (5' to 3')	No.of polymorphic bands	No. of monomorphic bands	Total no. of RAPD fragments
1	A07	GAAACGGGTG	18	-	18
2	D18	GAGAGCCAAC	16	-	16
3	E07	AGATGCAGCC	19	-	19
4	E09	AGGCATCTCD	19	-	19
5	D07	CGGGACCCGA	9	-	9
6	D09	GCCCAGGTCC	12	1	13
7	D08	CTAAAACGGC	14	-	14
8	E06	GGGCTGCTCA	13	-	13
9	E07	CCCGTACTGC	18	-	18
10	E08	ATTTGCCTCT	17	-	17
11	A07	GAAACGGGTG	18	-	18
12	B09	CCCGTAGTGA	18	-	18
13	C01	CCCTCGTAGA	17	-	17
14	G02	CCCGTAGTCG	15	-	15
15	IOP576	CCCGCTGACC	13	4	13
16	IOP700	CGTAGGCTGA	19	-	19
17	IPI178	CCCGTAGCTG	11	1	12
18	IPI701	GTGCGAATGT	10	2	12
		Total	275	4	279



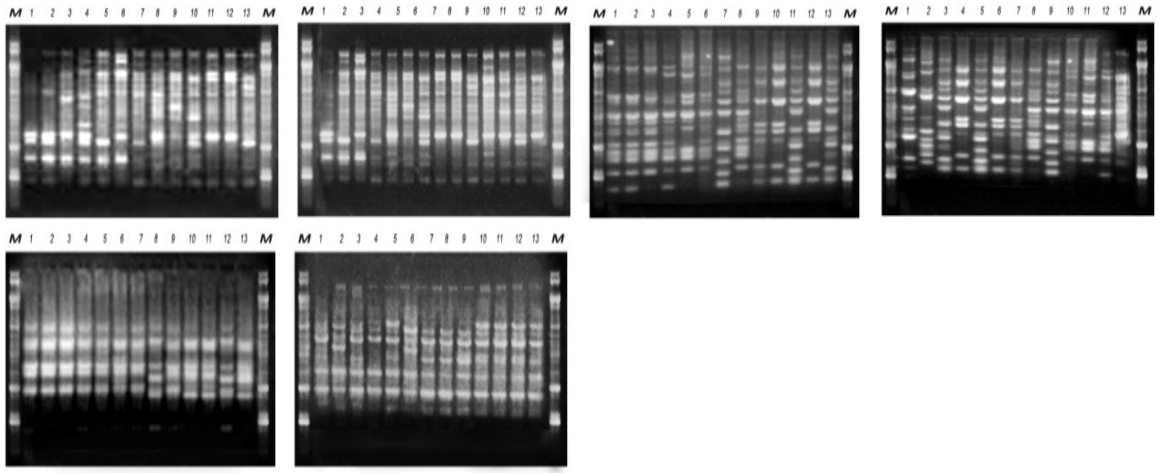


Plate 1. RAPD banding pattern between genotypes for the primer. Primers are arranged serially according to plates A07, D18, E07, E09, D07, D09, D08, E06, E07, E08, A08, B09, C01, G02, IOP576, IOP700, IPI178 & IPI701

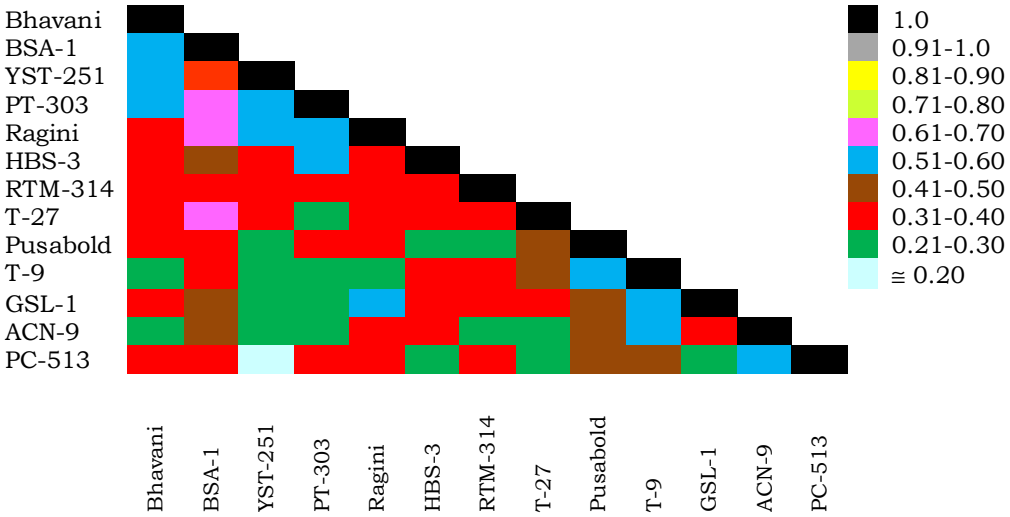


Figure 1. Similarity matrix of 18 random primer for 13 mustard genotypes

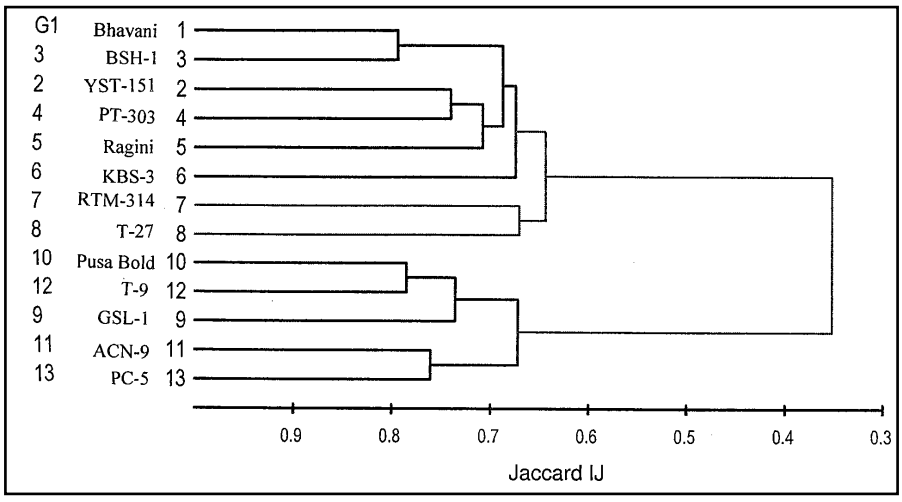


Figure 2. Dendrogram showing grouping of 18 random primer of thirteen mustard genotypes

Conclusion

It can be concluded from this study that the cultivated genotypes ACN-9 and Pusa Bold of Brassica juncea can be further improved by crossing with RTM-314 and T-27 of Eureca sativa for drought tolerance.

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