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Morphological and molecular characterization of *Brassica rapa* ssp *yellow sarson* mutants

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Abstract

Thirteen out of 55 gamma-rays induced promising mutants of *Brassica rapa* ssp *yellow sarson* along with their two parents namely, NDYS-2 (V1) and YST- 151 (V2) were investigated with a view to characterize the genetic variability through morphological as well as ISSR markers analysis. Mutants showed wide range of variability for seed yield, yield attributes, oil content (%) and Alternaria blight disease reaction. Five mutants, namely, V1M5, V1M20, V1M42, V1M43 and V1M54 showed significantly high seed yield and high oil content as well as improved level of tolerance to Alternaria blight as compared to parents. Investigation using ISSR analysis showed high degree of variation yielding 78 bands scored for the presence or absence of bands among genotypes. Three out of 13 oligonucleotide primers gave reproducible DNA bands suitable for establishment of genetic diversity. Genetic similarity ranging from 0.54 to 0.91 using UPGMA cluster analysis based on ISSR bands was noticed. The mutants were put into three major clusters; cluster II was represented by three sub-clusters IIAa, IIAb and IIB. The present investigation clearly indicated that morphological traits alone could not be considered as the true reflection of their genotypic characteristics and hence the need of molecular analysis using ISSR markers is proved.

Key words: *Mutants, B. rapa ssp yellow sarson, AUDPC, oil content, ISSR markers*

Introduction

Brassica rapa ssp *yellow sarson* is most important species of rapeseed-mustard group because of its high oil content (45-46%). Despite high oil content, productivity of the yellow sarson is declining due to its high vulnerability to biotic and abiotic stresses. Domestication and cultivation under limited area in India, probably, has led to genetic erosion of the existing genetic variability of this crop species. Inter varietal hybridization has also limited scope to wider the existing variability. Under such situations, induced mutagenesis appears to be the simplest tool to widen the genetic base of this species. At BHU, gamma irradiation in *B. rapa* ssp. *yellow sarson* has yielded very promising mutants.

For enabling better exploitation of genetic resources, it is desirable to know the genetic diversity at morphological as well as molecular levels. A modification of SSR-based marker system, i.e. ISSR (inter simple sequence repeat) analysis (Wolfe *et al.*, 1998) has wide applicability in a variety of plants as it provides highly reproducible results and generates abundant polymorphisms in many systems (Liu and Wendel, 2001). The present experiment, therefore, was undertaken to characterize the nature and magnitude of variability present in the 13 gamma-ray induced promising mutants of two genotypes of *B. rapa* ssp *yellow sarson*, namely, NDYS-2 and YST-151 at morphological and molecular levels using ISSR analysis.

Materials and methods

Thirteen out of fifty five mutants of *B. rapa* ssp. *yellow sarson* and their two parents, NDYS-2 (V1) and YST-151 (V2) selected from M_7 generation on the basis of morphological traits, were planted in RBD with three replications; each genotype was

grown in single row of 5 meter length; row to row distance was 45 cm and plants spaced at 10 cm apart during 2008-09. These mutants were further subjected to molecular analysis using ISSR markers. The mutants were morphologically characterized on the basis of seed yield, its component traits, oil content and reaction to Alternaria blight disease.

Plant genomic DNA was extracted by a CTAB (Cetyl trimethyl ammonium bromide) protocol (Bornet and Branchard, 2001). Leaf tissue (100mg) were grinded in 1000 µL of CTAB extraction buffer (100 mM Tris [pH-8.0], 1.4M NaC1, 20 mM EDTA [pH-8.0, 0.2% (p/v) mercaptoethanol, 2% [p/v] CTAB) and heated at 60°C for 30 minute. DNA was isolated with chloroform: isoamyl alcohol mixes (24:1) and precipitated in presence of isopropanol 40% (v/v). The DNA pellet was washed with 5mM ammonium acetate and 70% ethanol, dried and dissolved in 100µL of TE (100mM Tris-HCL [pH-8.0], 1mM EDTA [pH-8.0]). After addition of 1µL of RNase (10mg/ml) DNA concentration was determined with fluorometer (Hoefer TKO 100) using bisbenzimide (Hoechyst dye 33258) as the fluorescent dye. Isolated DNA was stored at 4°C for further use.

The PCR mix for ISSR analysis consisted of 1µL DNA, 2.5µL buffer (l0X, 100mM Tris-HCL pH-8.0, 500mM KCL, 20mM MgCl and 0.2% gelatin), 0.2µL dNTPs (100mM), 0.33 µL primer, 0.l µL Taq pol (5µ/ml, sigma) and H_20 to a final volume of 25µL. A total of 13 primers (table 1) were used for the analysis of genetic diversity in the 13 mutants. The first amplification cycle consisted of initial denaturation at 94 ^oC for 4 min, primer annealing at 48^0C for 1 min and primer extension at 72 ^0C for 1 min. This was followed by 35 cycles with 1 min at 94 ^0C, 1 min at 48 ^0C, and 1 min and 72 ^0C; the final extension was allowed for 5 min at 72 ^0C (Charters *et al*.1996). PCR amplification was performed on a PTC 225 peltier thermal cycler from MJ Research. The amplified fragments were resolved by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis using pre-cast clean Gel 48 S and stained using a DNA silver staining (Charters *et al*., 1996). The amplified products were scored on the basis of presence or absence of ISSR markers. Amplified bands were recorded as present: 1 or absent: 0 and only polymorphic bands were scored. The 0-1 data set was entered into the program Popgene version 1.31. Similarity matrix and genetic distance were calculated based on Nei and Li (1979). A dendogram was prepared using UPGMA (unweighted pair-group method with an arithmetic average) based on Jaccard similarity coefficient.

Table 1: Name, sequence and specific annealing temperature for each of the 13 primers

Primer name	Sequence	Amplification temperature (^0C)
BV-11	CTC TCT CTC TCT CTC TAT	45.4
BV-17	CAC ACA CAC ACA GT	44.7
BV-26	GAGAGAGAGAGAGG	41.9
BV-29	GAG AGA GAG AGA CC	42.3
BV-35	GTG TGT GTG TGT GG	46.6
BV-38	CAC CAC CAC GC	44.7
BV-41	GAGGAGGAGGC	41.0
BV-53	GAGAGAGAGAGAGAGAA	45.7
BV-47	GTG GTG GTG GC	44.0
BV-50	AGAGAGAGAGAGAGAT	47.0
BV-44	CTC CTC CTC GC	41.8
BV-59	CAG CAG CAG CAG CAG	54.3
BV.-62	CAA CAA CAA CAA CAA	40.7

Results and discussion

Data on 11 morpho–physiological traits, such as days to 50% flowering, days to maturity, days to reproductive period, plant height, number of primary branches per plant, siliqua length, number of seeds per siliqua, seed yield per plant (g), 1000 seed weight, AUDPC for leaf blight and percent oil content on 13 mutants along with 2 parental checks were subjected to analysis of variance; treatment variance for each character was significantly indicating the presence of variability among the treatments. Mutants showed wide range of variability from their respective parent for each of the 11 traits as evident from range and CV. Promising mutants identified on the basis of seed yield, oil content and reaction to leaf blight were V1M5, V1M20, V1M42, V1M43 and V1M54. Mutants V2M37 and V2M38 showed improved level of tolerance to leaf blight as evident from low values of AUDPC but were at par with seed yield and oil content. Most of the mutants showed increased reproductive period (table 2) as compared to their respective parent. Component traits, such as, enhanced reproductive period, plant height and number of primary branches per plant coupled with low AUDPC for leaf blight were noted to be main contributors towards increased seed yield. In this respect mutants V1M42, V1M43 and V1M53 were very promising. Increased variability due to mutagenic treatments were amply demonstrated in several crops including oilseeds *Brassica* (Labana *et al*., 1980; Kumar and Rai, 1998; Khatri *et al*., 2005l; Muhammad *et al.*, 2007 and Khan *et al*., 2008). Positive contribution of plant height, number of primary branches per plant, seeds per siliqua and 1000-seed weight as noted in the present case were also reported by several workers (Mishra and Kashyap, 2004; Singh, 2004; Rai *et al*., 2005; Mitra *et al*., 2006; Muhammad *et al*., 2007; Marjanovic *et al*., 2008).

Genetic diversity through ISSR analysis

A phyllo-genetic relationship was investigated using ISSR in 13 mutants with their respective check. A high degree of variation was noticed among mutant lines and a total of 78 bands were scored for the presence or absence of bands among genotypes (table 3). Out of 13 oligonucleotide primers used, 3 primers gave reproducible DNA bands suitable for the establishment of genetic diversity among 15 lines (13 mutants and 2 parents). These three primers were BV-38 (CAC CAC CAC GC), BV-44 (CTC CTC CTC G) and BV-62 (CAA CAA CAA CAA CAA). Moreover, one primer, namely, BV-38

Table - 2: Mean, Range & Coefficient of variation of promising mutants of *B. rapa* ssp. *yellow sarson*

Genotype	Days to 50% flowering	Days to maturity	Reprod -uctive period	Plant height	Primary branch	Siliquae length	Seeds per siliquae	Seed yield per plant (g)	1000 -seed wt.(g)	AUDPC	Oil content (%)
V1M2	44*	107	63*	136.0	9.0	4.8	29.3	6.35	3.57	1625	38.1
V1M5	40*	110	70*	159.0*	5.9	3.8	32.8*	10.99*	5.06	975*	38.1
V1M15	40*	110	70*	133.5	7.1	5.0	15.3	4.12	4.17	1555	40.3
V1M19	50	110	60*	141.7*	5.7	4.3	25.5	6.95*	4.97	1825	42.8
V1M21	48*	107	59*	119.0	4.2	4.6	25.5	3.7	4.17	698*	42.8
V1M27	52	110	56*	144.0*	9.0*	4.6	28.0	9.90*	5.59*	1538	43.2*
V1M42	44*	106	62*	159.0*	7.8*	4.9	25.0	10.34*	5.26	989*	43.1*
V1M43	44*	110	56*	152.0*	7.7*	5.8*	35.0	8.85*	5.06	978*	42.7*
V1M53	52	110	58*	156.0*	8.0*	5.4*	31.0	10.3*	5.56*	1081	42.2
V2M33	44*	107	63*	128.0*	7.5	5.1*	39.0*	8.68*	4.42*	2862	42.1
V2M36	54	108	54	149.0	8.6*	4.1	34.0*	8.47*	3.89	2862	43.9
V2M37	52	107	55	166.0*	6.3	4.7*	33.0*	7.48	4.88*	838*	42.0
V2M38	55	110	55	142.0	11*	3.0	18.0	7.40	3.77	808*	41.5
V1(NDYS-)	55	105	50	132.0	6.2	4.1	24.1	5.56	4.98*	1885	40.3
V2(YST-51)	53	109	56	143.0	7.3	3.7	24.0	7.27	3.69	2162	42.2
CV (%)	2.3	1.4	1.9	2.22	5.1	7.4	9.0	3.3	2.5	16.3	2.1
CD (p=0.0 5)	2.5	2.2	3.3	9.3	1.2	1.1	7.5	0.9	0.5	840	2.1

showed maximum polymorphism among genotypes. A total 15 polymorphic bands obtained; average number of bands per primer was 6 and average number of polymorphic bands per primer were 1.15 (table 3).

Table 3: Bands obtained in the ISSR analysis

ISSR analysis	Number of bands
Total number of bands obtained from 13 primers	78
Total number of polymorphic bands obtained from 13 primers	15
Total number of unique bands obtained from 13 primers	2
Average number of bands per primer	6
Average number of polymorphic bands per primer	1.15

Genetic similarity among samples was estimated using UPGMA cluster analysis based on ISSR bands scored using Jaccard's similarity coefficient. Sample was divided into different clusters by their distribution on the dendogram (figure 1). In the dendogram, genetic similarity among mutants ranged from 0.54 to 0.91. The mutants were grouped into three major clusters; the longest branch separate mutant V1M2 from the other mutants and parent at a similarity coefficient of 0.54 and this mutant was lone member of the cluster I. At the similarity coefficient 0.57, the next node resolve rest of 12 mutants and 2 parents into two major clusters; the III cluster V1M15 separated lonely. Cluster II showed two sub clusters (IIA and IIB) at 0.68 similarity coefficient; sub-cluster IIB had only one mutant V2M38, while sub-clusters IIA showed two groups at 0.76 similaritycoefficient. Sub-cluster IIA again divided into two groups (IIAa and IIAb). Sub-cluster IIAa was major group having three mutants. The sub-cluster IIAb consisted of 9 mutants along with 2 parents. Mutants V1M27 and V1M42 showed maximum similarity coefficient 0.91. The present investigation clearly indicated that morphological characters alone could not be considered as the true reflection of their genotypic characteristics and hence need further screening using more random primers to simplify their genetic relationships. DNA markers are preferable to morphological ones

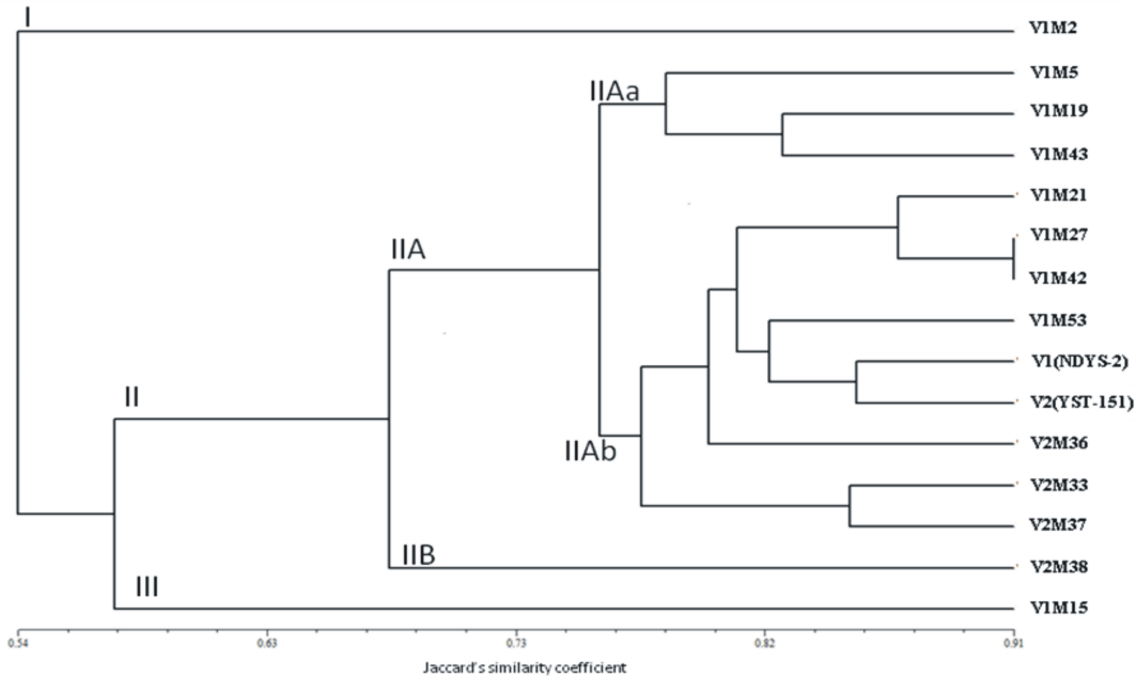

Fig. 1: Dendrogram derived from banding pattern ISSR analysis of 13 derived mutants and 2 parents.

because they relate variability directly at the genetic level and provide reliable and enormous data that permit a reproducible estimate of genetic diversity in the germplasm. Genetic diversity among different accessions and mutants using ISSR were reported in crops like sugarcane (Srivastava *et al*., 2008), mulberry (Vijayan, 2006) and cotton (Liu and Wendel, 2001). The results obtained from ISSR analysis did not conform to the conventional classification merely relying on the forms of yield contributing traits in Indian mustard (Mishra and Kashyap, 2004 and Mitra *et al*., 2006). In the present study, it was observed that mutants and its parents possess an intensive polymorphism as revealed by analysis (table 3). Result of morphological and ISSR analyses showed that mutants with similar phenotypes were not necessarily had closer relationships (figure 1 and table 2). The mutants V1M5, V1M19 and V1M43 differed significantly among them self but fall in sub-cluster IIA. Similarly mutants V1M27 and VM42 showed clear similarity by ISSR analysis but they differed significantly at morphological level. The markers like ISSR might accurately assay the degree of genetic change differentiating two genomes, but they might not necessarily reflect the divergence in terms of changes in traits of agronomic important. In addition, the expression of most of the phenotypic traits is markedly influenced by the environment, while detection of molecular markers is not confounded by the environmental effects. Ortiz (1997) also reported poor correlation in the diversity based on morphological traits and molecular markers, if a large number of morphological traits were used.

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