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Short Communication

Inter and intra population diversity analysis in toria (*Brassica rapa* L.) using SSR marker

Aradhana Phukan*, P. K. Barua, R. N. Sarma and N. Borah

Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat 785 013

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Abstract

Six popular toria (*Brassica rapa* L.) lines of Assam were used to determine the intra and inter populations diversity using SSR markers. Application of 39, 28 loci SSR loci revealed a total of 80 alleles. The SSR marker BRMS-029 had the highest PIC value in each population. Genetic diversity within individuals was high and accounted for 86% of the total variation, while among population variation was accounting for 13%. So, intra population diversity was found higher than the inter population diversity. The population M27 showed the highest number of alleles (2.128), highest number of effective alleles (1.592), Shanon information index (0.463), expected heterozygosity (0.275) and unbiased expected heterozygosity (0.282) indicating the presence of more heterozygotes in the population. The dendogram showed three distinct populations (TS 36, TS 38 and Jeuti) among the six populations and other three populations are slightly overlapping indicating gene flow. The high genetic diversity within cultivars of *B. rapa* is required to avoid inbreeding and to enable high yield. This study provided a new insight into the exploitation of the genetically diverse toria lines as potential resources for future breeding programs.

Key words: *Brassica rapa*, genetic diversity, SSR marker,

Toria (*Brassica rapa* L.) is a predominant oilseed crop of Assam. It is a short duration crop and fits well in the rainfed rice fallow cropping system. Toria is distributed in the sub-Himalayan states from Assam in the east to Punjab in the west. Plants of Toria are characterized by weak and pithy stems, spreading branches, low siliqua density and non synchronous maturity having shallow root system resulting in low yield.

The utilization of species for breeding and its adaptation to different environments depends on the level of genetic diversity which has significant implications for the improvement of crop plants. The major objectives of brassica research programme in the country are to improve oil as well as seed yield and hybrid development. Hence, there is a need to study the genetic variation and relatedness among the released popular cultivars of rapeseed for crop improvement programmes in Assam. As toria is self incompatible, it holds importance to study the variation among the popular cultivars. Genetic diversity among individuals or populations can be determined by using morphological, biochemical and molecular approaches (Mohammadi and Prasanna 2003). Molecular marker systems such as microsatellites or SSR markers are the most popularly used PCR-based markers (Gupta and Varshney 2000; O'Neill et al. 2003). These markers are successfully utilized across plant species as they are co-dominant, locus specific, hyper variable, multi-allelic and robust. The objective of the current research was to estimate the intra and inter population genetic diversity among the toria lines grown in Assam using SSR markers.

The Breeder seeds of six genotypes (populations) of toria were collected and grown in field (Table 1). Fresh leaves of twenty individual plants per genotype were collected for DNA extraction.

The total genomic DNA from each genotype was extracted following the protocol of Murray and

*Corresponding author's e-mail: ara118aau@gmail.com

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Table 1. Name and origin of the toria lines

S.No.	Population	Origin
1	M27	RARS Shillongoni by mass selection in local landraces
2	TS46	RARS Shillongoni from M27 x TS29
3	TS38	Recurrent selection from M27, developed at RARS Shillongoni
4	Jeuti (JT-90-1)	Developed from B9 x M27 for late sown condition at AAU Jorhat. Seed was supplied by Department of Plant Breeding & Genetics, AAU, Jorhat
5	TS36	RARS Shillongoni from T9 x TS 29
6	TS67	A variety developed by compositing four varieties at RARS, Shillongoni

Thompson (1980) with slight modifications. To confirm the DNA extraction, samples were run on 1% agarose gel and DNA quantification and was measured by using Nanodrop ND-1000. DNA dilutions (50ng/µl) were prepared for PCR reaction.

SSR analysis was carried out by using fifty two pairs of primer. However, thirty nine primers showed results and among them only twenty eight showed polymorphisms. Standard PCR protocol was used (Koh et al. 2017). Bands were detected and photographed using a UVP gel documentation system.

Band scoring was done by using (1) for presence and (0) for absence of bands in order to obtain the genetic similarity matrix. To partition genetic diversity into intra and inter population components, an analysis of molecular variance (AMOVA) was computed with the software GenAlEx v. 6 (Peakall and Smouse 2006). Basing on the estimated fragment sizes of each marker and each genotype, the genetic diversity parameters of mean number of alleles (Na), effective number of alleles (Ne), Shannon information index (I) expected heterozygosity (He) and unbiased expected heterozygosity (uHe) were estimated for each *B. rapa* cultivar with GenAlEx v. 6 (Peakall and Smouse 2006). Similarly genetic similarities were also estimated through this software. The dendrogram was built based on the unweighted pair group method with arithmetic average (UPGMA) displaying genetic relationships between six populations. The polymorphic information content (PIC) of each SSR marker was also calculated using the following formula:

$PIC_i = 1 - \sum_{j=1}^{n} Pij^2$, where Pij is the frequency of the jth pattern for marker *I* and summation extends over n patterns.

Primers displayed a different level of polymorphism among all the six populations. Twenty eight primer pairs appeared to detect more than one locus. These SSR primers revealed a total of 80 alleles. The number of alleles amplified by each primer pair (locus) varied from 2-6, with an average value of 2.85 alleles per locus. The primer pair BRMS-029 amplified the highest number of alleles (6). The PIC values of markers for each population are given in Table 2. The SSR marker BRMS-029 had the highest PIC value in each population, which was expected as it produced the highest number of alleles. In M27, PIC values ranged from 0.095 to 0.766 and classified 9 SSR loci as informative markers (PIC>0.5). In TS46 also, 9 SSR loci were found informative whereas in TS38, 7 loci were found informative. In Jeuti, 6 SSR loci, 5 SSR loci in TS36 and in TS67 10 SSR loci were found informative.

The diversity parameters such as number of alleles (Na), effective number of alleles (Ne), Shanon's information index (I), expected heterozygosity (He) and unbiased heterozygosity (uHe) were computed and presented in Table 3. The unbiased heterozygosity was observed highest in M27 and lowest in Jeuti. Analysis for molecular diversity was done to know the detail estimates of intra and inter population genetic diversity. Genetic diversity within population was high and accounted for 86% of the total variation, while among population variation was moderate, accounting for the remaining 13%. So, intra population diversity was found higher than inter population diversity.

The similarity matrix showed that population 1 (M27) and population 2 (TS 46) were closely related (96.2%) followed by population 4 i.e., Jeuti (94%), population 6 i.e TS 67 (92.7%), population 3 i.e., TS 38 (92.2%). The population 1(M27) and population 5 (TS 36) were found the most dissimilar (89.9%). This is also indicated by dendogram (Fig. 1). This clustered the genotypes into three distinct groups and other three populations are slightly overlapping indicating gene flow. TS36, TS38 and Jeuti formed distinct clusters.

Twenty eight SSR loci used in this study were significantly polymorphic and useful for differentiating the toria lines studied. Moreover, the 28 SSR loci revealed a total of 80 alleles with an average value of

Table 2. Polymorphic information content of each primer for each population

Primer	M27	TS46	TS38	Jeuti	TS36	TS67
BRMS-001	0.139	0.540	0.665	0.500	0.524	0.614
BRMS-006	0.505	0.335	0.320	0.320	0.320	0.255
BRMS-007	0.399	0.289	0.255	0.375	0.255	0.320
BRMS-008	0.658	0.555	0.554	0.605	0.339	0.565
BRMS-011	0.570	0.399	0.504	0.265	0.320	0.320
BRMS-014	0.610	0.595	0.701	0.505	0.480	0.560
BRMS-018	0.320	0.375	0.489	0.320	0.420	0.495
BRMS-020	0.434	0.584	0.184	0.489	0.261	0.545
BRMS-024	0.180	0.139	0.420	0.000	0.095	0.180
BRMS-026	0.541	0.555	0.000	0.455	0.454	0.524
BRMS-029	0.766	0.621	0.524	0.788	0.611	0.564
BRMS-030	0.180	0.139	0.139	0.095	0.320	0.289
BRMS-031	0.095	0.180	0.180	0.500	0.499	0.399
BRMS-036	0.375	0.320	0.320	0.375	0.349	0.399
BRMS-037	0.180	0.000	0.000	0.180	0.180	0.180
BRMS-040	0.660	0.684	0.661	0.455	0.654	0.594
BRMS-042	0.095	0.095	0.139	0.180	0.320	0.320
BRMS-042-2	0.454	0.465	0.320	0.180	0.000	0.180
BRMS-044	0.495	0.499	0.499	0.499	0.499	0.500
BRMS-049	0.500	0.500	0.500	0.500	0.500	0.500
BRMS-050	0.320	0.320	0.000	0.000	0.320	0.289
BRMS-054	0.265	0.095	0.000	0.000	0.000	0.000
BRMS-088	0.000	0.000	0.095	0.000	0.000	0.000
BRMS-096	0.340	0.265	0.255	0.180	0.480	0.000
Na10-D09	0.180	0.255	0.455	0.180	0.180	0.255
Na10-F06	0.560	0.485	0.375	0.499	0.500	0.500
Na10-G10	0.455	0.585	0.495	0.485	0.580	0.500
Ni2-B01	0.455	0.439	0.480	0.469	0.439	0.420

2.85 alleles per locus. Ofori et al. (2008) reported higher average number of allele per locus in *B. rapa* with 16 SSR primers. Cui et al. (2008), El- Esawi et al. (2016) and Thakur et al. (2018) also reported higher mean number of alleles per locus (2.91, 3.92 and 3.41, respectively). This could be due to difference in the SSR loci or the populations assessed. The Shanon's information index and expected heterozygosity in our results were lower than those reported by Ofori et al. (2008). This could be due to difference in genotypes assessed. Fu and Gugel (2009) reported the estimates of mean heterozygosity ranged from 0.126 to 0.197 and averaged 0.165 in turnip rape. Since the populations were self incompatible, the unbiased heterozygosity gave the actual level of heterozygosity in a population. Unbiased heterozygosity avoids the relatedness among cultivars. The unbiased heterozygosity was observed highest in M27 and lowest in Jeuti.

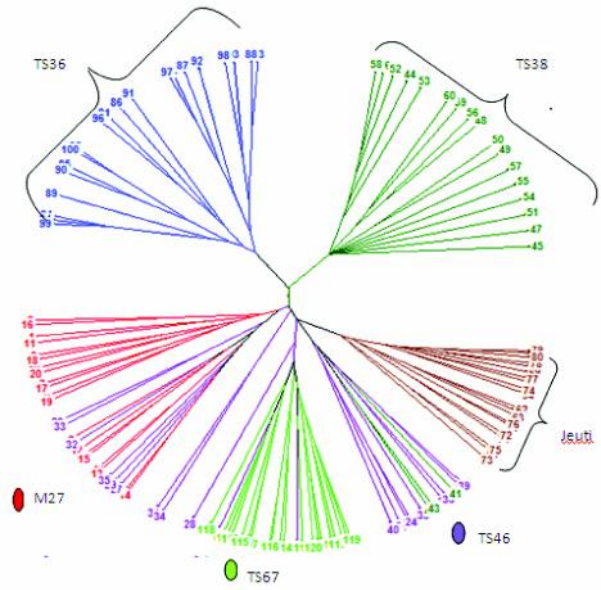

Fig. 1. Association among six populations based on UPGMA at 39 SSR loci

Table 3. Mean genetic diversity parameters in different populations

Parameters	M27	TS46	TS38	Jeuti	TS36	TS67
Na	2.128±0.165	2.000±0.137	1.897±0.151	1.795±0.133	1.897±0.146	1.821±0.115
Ne	1.592±0.114	1.535±0.093	1.503±0.099	1.458±0.084	1.482±0.081	1.508±0.082
I	0.463±0.066	0.435±0.062	0.394±0.062	0.371±0.056	0.400±0.056	0.408±0.057
He	0.275±0.039	0.264±0.038	0.244±0.038	0.238±0.036	0.254±0.036	0.263±0.037
uHe	0.282±0.040	0.271±0.039	0.251±0.039	0.244±0.037	0.261±0.037	0.270±0.038

In the present study, intra population diversity was found more than inter population diversity. Ofori et al. (2008) reported that the genetic diversity within cultivars was high and accounted for 83% of the total variation revealed by AMOVA while between-cultivar variation was moderate but still significant (P = 0.001), accounting for the remaining 17% in three open pollinated cultivars of *B. rapa*. Similar findings were reported by (Lazaro and Aguinagalde 1998; El-Esawi et al. 2016; Watson-Jones et al. 2006; Hintum et al. 2007). The low level of genetic differentiation among population (Fst = 0.128) was probably indicative of a relatively high gene flow and that most of the genetic diversity of *B. rapa* resided within cultivars rather than between cultivars which were collected from the same area (Persson et al. 2001; Zhao et al. 2005, Thakur et al. 2018). It is believed that genetic diversity of a crop is influenced by the breeding system (Loveless and Hamrick 1984).The results indicated that population 1(M27) and population 5 (TS36) were the most dissimilar (89.9%).

Polymorphic information content (PIC) is an important parameter used for assessing the differentiation ability of the molecular markers (Junjian et al. 2002). The SSR marker BRMS-029 had the highest PIC value in each population. There were very limited studies on diversity analysis in *B. rapa* cultivars of Assam. The high genetic diversity within cultivars of *B. rapa* is required to avoid inbreeding for enabling high yield. Continuous plant breeding activities are often expected to narrow down the genetic diversity of crop germplasm sometimes referred to as 'genetic erosion' (Harlan1972). However, Ofori et al. (2008) reported that continuous breeding efforts for quality improvement did not reduce the genetic diversity in the cultivars studied. This study provided an insight for use of toria lines for future breeding to develop more productive crops.

Authors' contribution

Conceptualization of research (AP, PKB); Designing of the experiments (PKB, RNS, AP); Contribution of experimental materials (PKB); Execution of field/lab experiments and data collection (AP, NB); Analysis of data and interpretation (RNS, AP, PKB); Preparation of manuscript (AP, NB).

Declaration

The authors declare no conflict of interest.

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