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Genetic Diversity Analysis Based on Molecular Level in Selected *Brassica compestris* L. var. toria Genotypes

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The present study was carried out at Dr R.H. Richharia Research Laboratory, Department of plant molecular biology and biotechnology, College of Agriculture Raipur (C.G.), Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during Rabi 2013-14. Experimental materials comprised 24 *Brassica compestris* L. var. toria genotypes with the objectives to estimate the genetic diversity analysis at molecular level. The present investigation genetic diversity at molecular level in 24 genotypes of toria, two major cluster with 53% genetic similarity in which two genotypes named GPT-13 and GPT-59 comes under one cluster and remaining 22 falls under second cluster. Genotypes GPT-1 and GPT-43 and genotype GPT-125 and GPT-126 exhibited 87% genetic similarity with each other. These results indicated the existence of sufficient Variability among genotypes and there are very much chance of improvement either through selection or through hybridization by getting heterosis.

Introduction

Rapeseed-Mustard group of crops is among the oldest cultivated plants in human civilization. Biologically, the rapeseed and mustard plants belongs to the family Crucifarae and under the genus *Brassica* with large number of species and sub species cultivated in India. The word 'rape' and 'mustard' have been derived from the word *rapum* meaning turnip and European practice of mixing the sweet 'must' of old wine with crushed seeds of black mustard (*Brassica nigra* (L.) Koch) to form a hot paste, respectively.

Rapeseed mustard crops in India comprise traditionally grown indigenous species namely toria (*Brassica campestris* L.*var. toria)*, brown sarson (*Brassica campestris* L. *var. brown sarson),* yellow sarson (*Brassica campestris L. var yellow sarson),* Indian mustard (*Brassica juncea* L. *Czern and Coss*), black

mustard (*Brassica nigra)* and taramira (*Eruca sativa* Mill), which have been grown since about 3500 BC along with non-traditional species like gobhi sarson *(Brassica napus L.*) and Ethopian mustard or Karan rai (*Brassica carinata A. Braun)* (Chouhan *et al.,* 2011). The rapeseed-mustard group is comprised of two distinct type (i) self pollinated – Indian mustard, raya and yellow sarson of which Indian mustard is the most important member of the group accounting for 75-80 per cent of the area under rapeseed-mustard and (ii) cross pollinated – brown sarson, toria and taramira.

Among rapeseed-mustard group toria is an important oilseed considered as a catch crop in early *rabi* season of India. Toria has a relatively dwarf plant frame, short duration (70-80 days), small seeded siliquae and mature early and susceptible to aphids, alternaria blight and frost. Toria seeds are spherical or ovoid in shape and are reddish or dark brown in colour, having slightly wrinkled surface. Seeds are slightly smaller than those of mustard. It is cultivated largely in Assam, Bihar, Orissa and West Bengal mainly as winter crop. In Chhattisgarh the productivity of toria is very low comparable to the national productivity and other states like Haryana (1609 kg), Gujrat (1577 kg), Rajshthan (1187 kg), Uttar Pradesh (1125 kg) and Madhya Pradesh (1108 kg) etc. The reasons for low productivity of toria due to local genotypes which have low yielder, dwarf in nature, bushy or trailing habit and susceptible to alternaria blight, powdery mildew and aphids etc. This results in a big gap between requirement and production of rapeseed and mustard in Chhattisgarh and India.

Molecular markers provided an important tool for the genetic diversity study of Indian and Chinese oilseed mustard. All Indian accessions grouped together as assayed by random amplified polymorphic DNA (RAPD) (Jain *et al.,* 1994; Khan *et al.,* 2008). As assayed by amplified fragment length polymorphism (AFLP) markers, all the Indian and Chinese *B. juncealines* were clustered together whereas lines from other sources formed another group (Srivastava *et al.,* 2001). RAPD analysis indicated that the genetic relationship of Chinese oilseed mustard accessions was mainly decided by the specific ecological environment as well as the local cultivation customs and high diversity of B. juncea was discovered in south-western and western China (Puet *et al.,* 2007). This is supported by the analysis using AFLP, sequence-related amplified polymorphism (SRAP) and simple sequence repeat (SSR) markers (Xu *et al.,* 2008). Analysis using SRAP markers revealed that the Chinese oilseed mustards were divided into different groups mainly according to their growth habitat (Wu *et al.,* 2009). SSR allelic diversity in the A genome and B genome supported a polyphyletic origin and secondary centres of genetic diversity of oilseed B. juncea in China and India (Sheng *et al.)*

In Chhattisgarh, large number of local toria germplasm is available. Hence, local genotypes of toria can be upgraded by crossing with improved varieties. This will lead to the higher yield and oil content in local genotypes of toria along with good adaptation.

Materials and Methods

The present study entitled "Genetic diversity analysis based on molecular level in selected *Brassica compestris* L. var. toria genotypes*"* was carried out at Dr R.H. Richharia Research Laboratory, Department of plant molecular biology and biotechnology, College of Agriculture Raipur (C.G.), Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) during Rabi 2013-14. Experimental materials comprised 24 *Brassica compestris* L. *var. toria* genotypes with the objectives to estimate the genetic diversity analysis at molecular level.

Genomic DNA isolation

DNA was isolated by modified CTAB method of DNA extraction for Rapeseed as suggested by Jonathan and Wendel (1990).

The leaf bits were cut in an eppendrof tube. Added 700 µl CTAB buffer in this and kept it at 4^0 C for 3-4 hrs. Grinded the leaf and added some more CTAB buffer. Kept it in water bath at 65^0C for 20 min. Added 700 µl of chloroform: isoamyl alcohol (24:1). Then vortex the sample. Centrifuged it for 20min. at 14000 rpm. Transferred the upper clear layer in new 1.5 ml Eppendrof tube (Repeat twice from the step 5-8). Added 175 µl of 3M sodium acetate and 400 µl of ice cold isopropanol in this and kept it for incubation at -20^0C for overnight. Centrifuged at 14000 rpm for 20 min. and discarded the supernatant. Then washed the pellet with 70% ethanol (50 µl). Centrifuged it for 10 min. Air dried the pellet. Added 100µl of TE buffer and dissolved the pellet.

DNA quantification

The concentration and quality of the genomic DNA sample were estimated on spectrometer ND-2000 (Nanodrop, USA). Finally, all the genomic DNA samples were diluted to a final concentration of 40 ng/µl with autoclaved distilled water and stored at -20^0C for further use.

PCR amplication of ISSR markers

A total of 4 ISSR primers of toria were used in this study. Polymerase chain reaction (PCR) for amplification of DNA preparation was carried out in a 25 µl for ISSR. All PCR reaction were carried out in a Veriti Thermal Cycler-96 mells (Applied Biosystem, USA). PCR products were separate using 5% PAGE, stained with ethidium bromide and photographed under UV light using Image Gel Doc software Version 2.0.1(Bio-Rad, USA)

Data analysis

The band profiles were scored only for distinct, reproducible bands as present (1) or absent (0) for each ISSR primer pair. Jaccard's similarity coefficient values were calculated and dendrogram based on similarity coefficient values were generated using unweighted pair-group method with arithmetic mean (UPGMA) by the NTSYSpc 2.10e software (Rohlf, 2000). The polymorphism information content (PIC) value of ISSR markers was calculated using the following formula (Anderson *et al.,* 1993).

$$PIC = 1 - \sum_{i=1}^{k} Pi2$$

Where k is the total number of alleles (bands) detected for one ISSR locus and P_1 is the frequency of the i^{th} allele (band) in all the samples analyzed.

Results and Discussion

Genetic diversity on the molecular markers in selected toria genotypes

Four ISSR primers were used to study the genetic diversity among twenty four genotypes (Fig. 1 and 2). All were showed polymorphic. The similarity coefficient ranged from 0.53 to 0.87. Turi *et al.,* (2012) at Peshawar, Pakistan investigated genetic diversity among 120 different accessions of *Brassica* species were characterized with the help of SSR markers. 39 SSR primers were used and they produced 162 scorable bands in which 105 were polymorphic. The average rate of polymorphic loci was 46%, which indicates high genetic diversity among the accessions. The UPGMA cluster analysis revealed two main clusters and nine sub-clusters. The dandogram indicated that based on above maker study formed two major clusters namely 'A' and 'B'. Two genotypes named GPT-13 and GPT-59 comes under one

cluster 'A' with 53% genetic similarity while, remaining 22 genotypes falls under second cluster B with 66% genetic similarity. Shiran *et al.,* (2004) at studied Shahre Kord, Iran the genetic diversity among twenty seven rapeseed (*Brassica napus*) cultivars and one cultivar of *Brassica rapa* was investigated using Random Amplified Polymorphic DNA (RAPD) molecular marker. A set of twenty four ten-mer arbitrary primers was used which produced reliable polymorphic DNA bands ranging in molecular weight from 440 to 3299 bp. A total of 133 polymorphic bands out of 173 reproducible bands were obtained. Genetic similarity matrix based on Jaccard's index detected coefficient ranging from 0.38 to 0.78. Coefficients were used to contrast a dendrogram using unweighted paired group of arithmetic mean (UPMGA) algorithm. Cultivars were clustered into two major groups.

In major cluster 'B' showed two sub-clusters as 'B1' and 'B2' near the 70% similarity level. Genotypes GPT-1 with GPT-43 and genotype GPT-125 with GPT-126 exhibited 87% genetic similarity with each other. Genotype PT 507, PT 30 and GPT-48, GPT-54 exhibited 79% genetic similarity. Hasan *et al.,* (2006) at Giessen, Germany studied set of ninety six genotypes was characterised using publicly available mapped SSR markers spread over the *Brassica napus* genome. Allelic information from 30 SSR primer combinations amplifying 220 alleles at 51 polymorphic loci provided unique genetic fingerprints for all genotypes. UPGMA clustering enabled identification of four general groups with increasing genetic diversity as follows (1) spring oilseed and fodder; (2) winter oilseed; (3) winter fodder; (4) vegetable genotypes (Table 1).

Table.1 Detail about ISSR markers used for diversity analysis in toria

Marker	Sequence	# of bp	Tm	Yield
UBC 807	AGAGAGAGAGAGAGAGT	17	42.5	14.6
UBC 810	AGAGAGAGAGAGAGAGT	17	42.9	21.8
UBC 812	AGAGAGAGAGAGAGAGA	17	44.3	18
UBC 815	CTCTCTCTCTCTCTCTG	17	44.9	83.3

Fig.1 Dendrogram constructed using UPGMA based on Jaccard's coefficient of 24 *Brassica compestris var. Toria* genotypes (ISSR Markers)

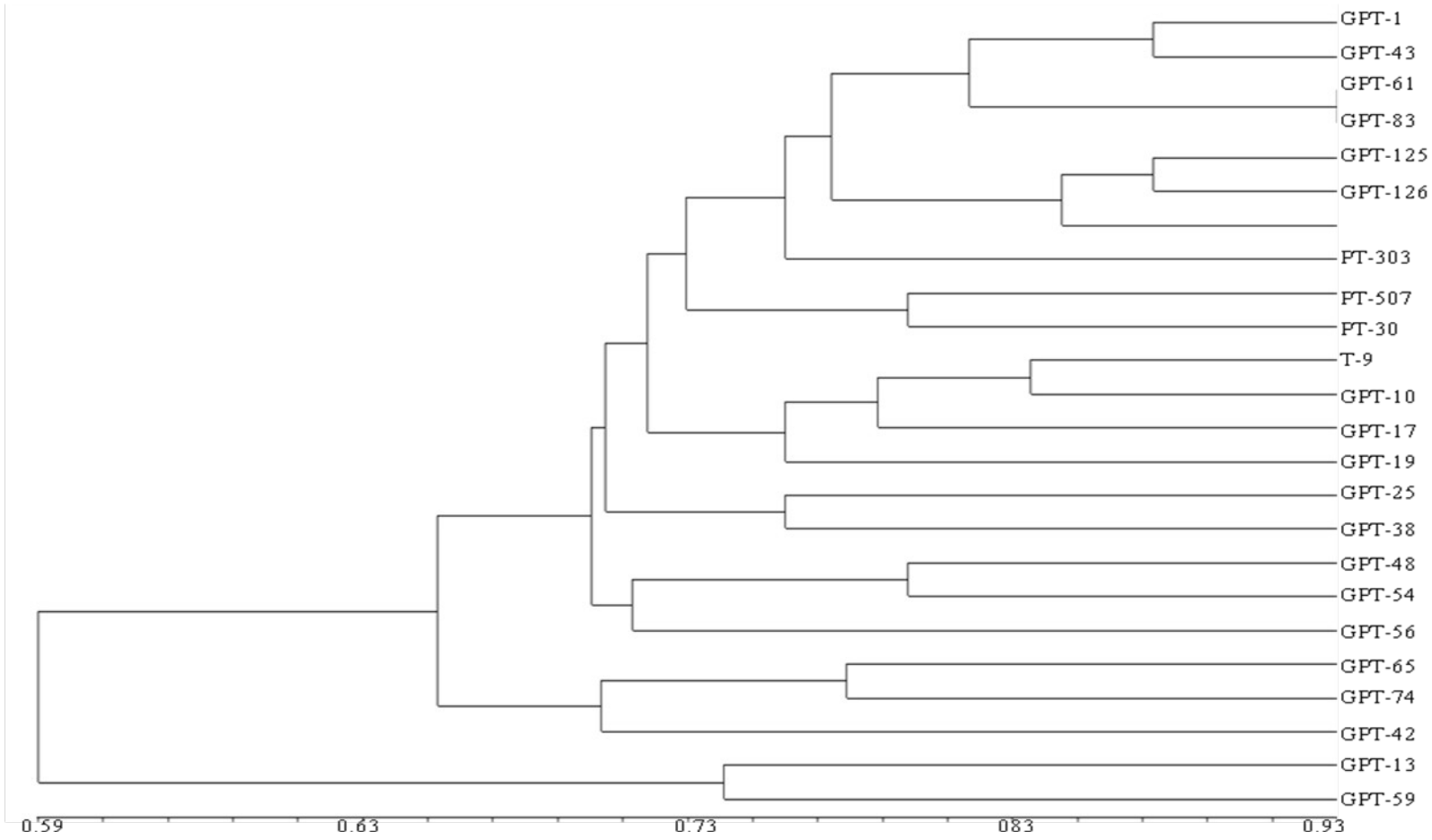
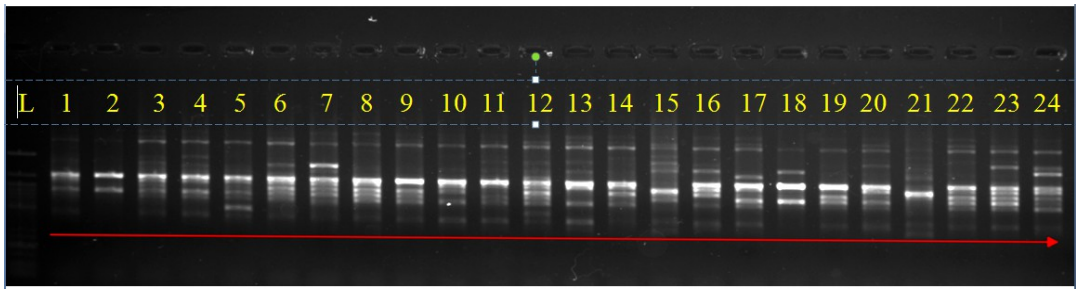
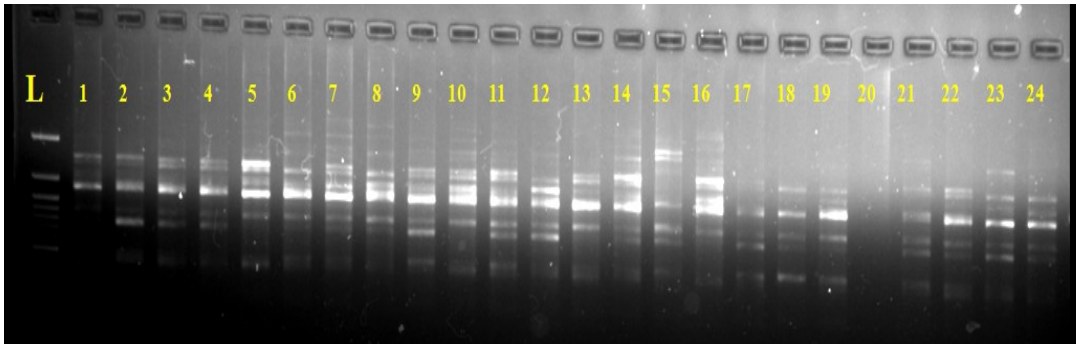


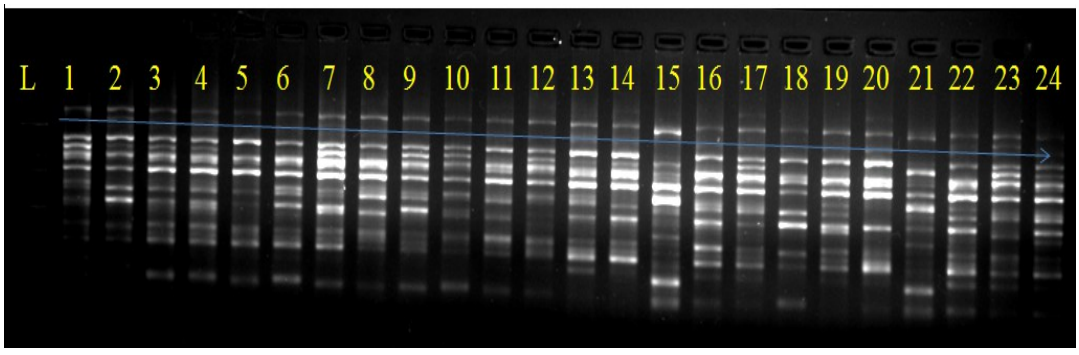
Fig.2 ISSR Profile generated by UBC 810, UBC 807, UBC 812 and UBC 815 primers of 24 Toria germplasm including varieties



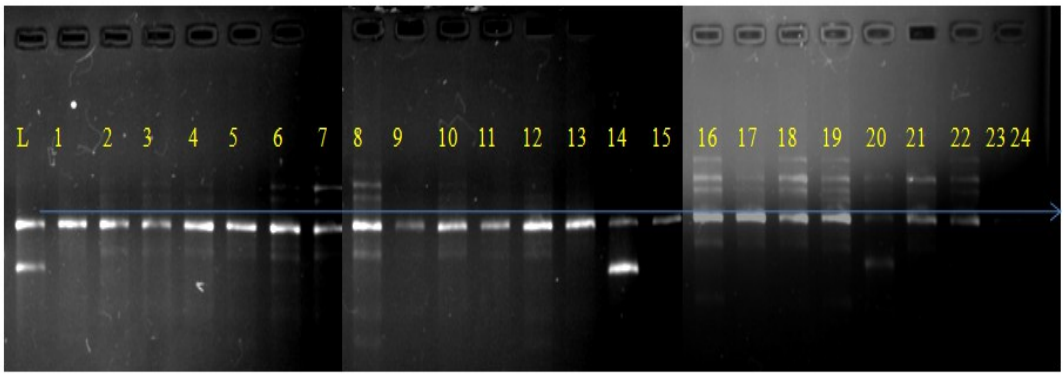
UBC 810



UBC 807



UBC 812



UBC 815

These results indicated the existence of sufficient Variability among genotypes and there are very much chance of improvement either through selection or through hybridization by getting heterosis. This result also indicated that ISSR analysis is effective for the assessment of genetic diversity among *Brassica compestris* genotypes. A similarity matrix based on the proportion of shared ISSR fragments was used to establish the level of relatedness between the various collected toria genotypes. These results were further strengthened by the earlier findings of Yu *et al.,* (2007), Abbas *et al.*(2009), Ghosh *et al.,* (2009), Moghaddam *et al.,* (2009), Villa *et al.,* (2009), Ali *et al., (2011*), Das *et al.,* (2011), Molla *et al.,* (2011), Abtahi and Arzani (2013), Vinu *et al.,* (2013), Tahira *et al.,* (2014), Ahmad *et al.,* (2014) and Iqbal *et al.,* (2015).

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