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Evaluation of genetic diversity and relationships among tomato genotypes using morphological parameters and SRAP markers

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ABSTRACT

The exploration of genetic diversity is a pre-requisite in any breeding programme for effective selection of superior genotypes. Hence, the diversity analysis was carried out with respect to nine morphological traits of 16 tomato genotypes along with one check. For diversity analysis at DNA level, five forward and reverse sequence related amplified polymorphism (SRAP) primers were used. The 18 combinations of SRAP primers produced 177 amplified products among which 106 were polymorphic (59.92% polymorphism). Comparison of UPGMA dendrograms of morphological and SRAP markers using the Mantel's test indicated a non-significant correlation of r = 0.019 (P = 0.56). Among all tomato genotypes, SRAP similarities ranged from 72 to 98%, indicating the available genotypes as an important source of genetic diversity that can be exploited in future breeding programmes.

Key words: Genetic diversity, SRAP, tomato, Mantel's test.

INTRODUCTION

The reduction of genetic variation in tomato (*Solanum lycopersicum* Mill.) through domestication and breeding (Tanksley *et al*., 14) has resulted in the need for long term conservation and utilization of genetic resources. Heterogeneous landrace populations are among the most important sources of genetic variation (Zeven, 17) and have been and will continue to be utilized in plant breeding programmes. Commercial F_1 hybrids are very common in tomato and selection of newer parents for higher heterosis is a continuous process. Generally, diverse plants are expected to give high hybrid vigour (Harrington, 7).

Although phenotypic traits are classic and indispensable breeder's selection tools, they can be greatly affected by environmental factors. The molecular markers have certain advantages over morphological markers, *viz*., phenotypically neutral, neither influenced by environment nor by pleiotropic and epistatic interactions, expressions are not dependent on plant genotype and thus show a clear segregation. Researchers have studied genetic variation in tomato landrace and cultivar accessions using various molecular techniques, including restricted fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) (Miller *et al.*,11; Mazzucato *et al*., 9; Park *et al*., 12; Carelli *et al*., 1; Garcia-Martinez *et al*., 5). However, RFLPs are time-consuming and have low reproducibility, while AFLPs involves high cost and SSRs require the knowledge of the flanking sequences for the development of species-specific primers (McGregor *et al.*, 10). The sequence related amplified polymorphism (SRAP) technique can overcome the above limitations (Li and Quiros, 8; Ferriol and Nuez, 4; Gulsen *et al*., 6). SRAP markers are PCR-based markers that amplify open reading frames and produce a number of co-dominant markers per amplification (Li and Quiros, 8). Hence, a study was conducted to evaluate SRAP and phenotypic markers to determine diversity and relationships among tomato genotypes.

MATERIALS AND METHODS

The material for the study was obtained from the Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad. The investigation consisted of 16 advanced breeding lines, *viz.*, TS-1, TS-2, TS-3, TS-4, TS-5, TS-6, TS-7, TS-8, TS-9, TS-10, TS-11, TS-13, TS-14, TS-15, TS-16 and TS-17 obtained from biparental mating in F_2 generation of commercial F_1 hybrids of MHTM-256 and S-14-41 along with one check L-15 (Megha). The pedigree of all genotypes is mentioned in Table 1.

All the genotypes were evaluated for genetic diversity during *rabi* 2008-09. Data were recorded for nine quantitative characters, *viz.*, plant height, number of primary branches, day to 50 per cent flowering, number of flowers per raceme, number of locules, pericarp thickness, number of fruits, average fruit weight and yield per plant. Based on the evaluation of the morphological characteristics, a raw data matrix

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Table 1. Pedigree details of advanced breeding lines and the check used in experiment.

Genotype	Pedigree
TS-1	Derived from S-22 x L-15
TS-2	Derived from intra population mating in F_2 population of S-4-40
TS-3	Derived from intra population mating in F_2 population of S-4-40
TS-4	Derived from S-22 x L-15
TS-5	Derived from CO-3 x Solan Vajra
TS-6	Derived from CO-3 x Solan Vajra
TS-7	Derived from CO-3 x Solan Vajra
TS-8	Derived from CO-3 x Solan Vajra
TS-9	Derived from intra population mating in F_2 population of S-4-40
TS-10	Derived from Arka Vikas x Sivap
TS-11	Derived from Arka Vikas x Sivap
TS-13	Derived from intra population mating in F_2 population of MHTM-256
TS-14	Derived from intra population mating in F_2 population of MHTM-256
TS-15	Derived from inter population mating in F_2 population of MHTM-256 x S-4-40
TS-16	Derived from inter population mating in F_2 population of MHTM-256 x S-4-40
TS-17	Derived from the cross between S-22 x L-15
L-15	NTDR-1 x AVRDC breeding line

was created. A similarity matrix was derived by the UPGMA clustering procedure of the software NTSYS pc version 2.1 (Rohlf, 13).

Total DNA was extracted from 40–50 mg young leaf tissue of individual genotypes following mini pre-rapid method given by Edwards *et al.* (3) with few modifications. The 25 combinations of five forward and five reverse SRAP primers (Table 2) previously evaluated in *Brassica* by Li and Quiros (8) were used in this study. Each 20 µl reaction consisted of 5 pM/µl of each of primer pairs, 200 µM of each of dNTPs (Bangalore Genei Pvt. Ltd., Bengaluru), 2 µl of $(NH_4)_2SO_4$ 10X *Taq* buffer, 2 mM of $MgCl_2$ as a final concentration, 6 µl ddH_2O, 3 U/µl of *Taq* polymerase and 25 ng of template DNA. Eppendorf gradient thermal cycler was used and cycling parameters included: one step of 4 min. at 96°C, 35 cycles of 1 min. at 94°C, 1.15 min. at 35°C, 1.15 min. at 50°C 1 min. at 72°C, and for extension, one step of 5 min. at 72°C. PCR products were separated on 1.5% agarose gel at 80 V for 5 or 6 h.

Table 2. SRAP primers used and their sequences.

Sl. No.	Primer	Sequence (5' to 3')
A	Forward	
1	me1	TGAGTCCAAACCGGATA
2	me2	TGAGTCCAAACCGGAGC
3	me3	TGAGTCCAAACCGGAAT
4	me4	TGAGTCCAAACCGGACC
5	me5	TGAGTCCAAACCGGAAG
B	Reverse	
1	em1	GACTGCGTACGAATTAAT
2	em2	GACTGCGTACGAATTTGC
3	em3	GACTGCGTACGAATTGAC
4	em4	GACTGCGTACGAATTTGA
5	em5	GACTGCGTACGAATTAAC

The SRAP bands were scored based on the presence (coded as 1) or absence (coded as 0) of polymorphic fragments for each primer, and a similarity matrix was constructed based on Dice's coefficient (Dice 2). Cluster analysis was performed on similarity matrix for molecular data with the unweighed pair group method using the arithmetic means algorithm (UPGMA), from which dendrogram depicting similarity among genotypes was drawn and plotted with the NTSYS-pc 2.1 software.

Comparison between SRAP and morphological data was performed for the accessions for which morphological data was available calculating the correlation of the two data sets by means of the Mantel's test using MXCOMP module of the NTSYS-pc 2.1 software.

RESULTS AND DISCUSSION

The UPGMA dendrogram obtained using morphological characters is shown in Fig. 1. The dendogram separated all the tomato genotypes into two main-clusters. The genotypes TS-1, TS-11, TS-8, TS-6 and TS-17 grouped into a cluster, whereas, the genotypes TS-2, TS-13, TS-14, TS-15 and TS-16 formed another main cluster. The genotypes TS-15 and TS-16 were found closely related, which were derived from the same ancestors. On the contrary, genotypes TS-1 and TS-4 were diverse from each other though both are derived from same parental combinations. This divergence could be due to different gene combinations. The similarity matrix showed that TS-1 and TS-11 as well as TS-15 and TS-16 genotypes

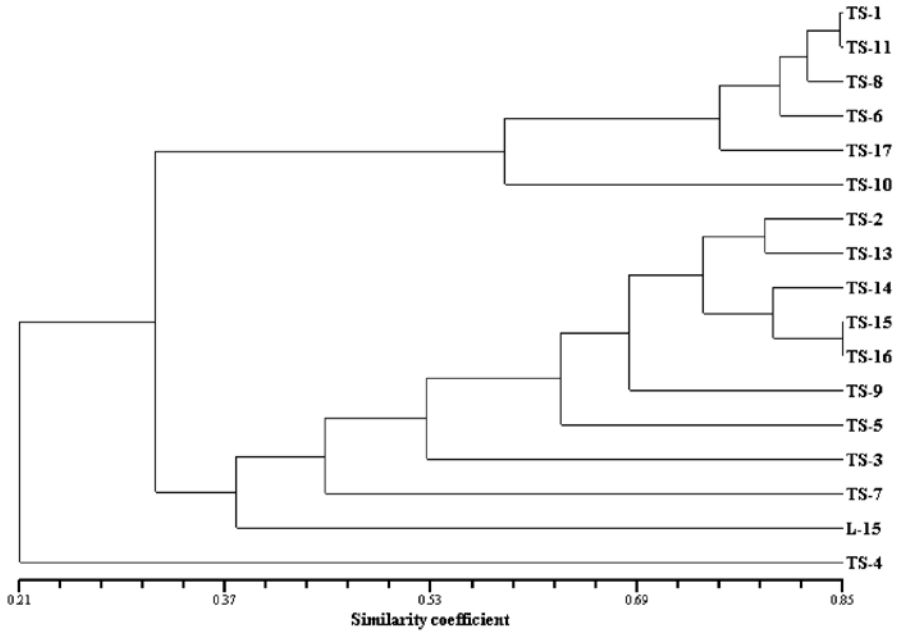

Fig. 1. Dendogram obtained from pooled data of nine quantitative characters in seventeen tomato genotypes.

showed the highest similarity with 0.85 similarity coefficient each (Table 3).

The 18 pairs of SRAP primers out of 25 pairs which showed amplification were used for diversity analysis among seventeen tomato genotypes. The eighteen primer pairs produced 177 amplified products among which 106 were polymorphic (Table 4), thus producing 59.92 per cent polymorphism. In previous findings on okra genotypes also gave 50 per cent polymorphism using SRAP primers (Gulsen *et al*., 6). The primer pair me3-em4 produced the highest polymorphism (100%). While, the primer pair me2-em4 showed the least polymorphism (10%) among all primer pairs.

Table 3. Genetic similarity values of tomato genotypes using Dice's coefficient with morphological markers.

Genotype	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	TS10	TS11	L-15	TS13	TS14	TS15	TS16	TS17
TS1	1.00																
TS2	0.41	1.00															
TS3	0.27	0.70	1.00														
TS4	0.33	0.11	0.17	1.00													
TS5	0.60	0.65	0.50	0.30	1.00												
TS6	0.81	0.43	0.29	0.24	0.62	1.00											
TS7	0.32	0.46	0.32	0.13	0.30	0.17	1.00										
TS8	0.83	0.47	0.26	0.32	0.65	0.79	0.15	1.00									
TS9	0.24	0.69	0.63	0.13	0.59	0.25	0.36	0.31	1.00								
TS10	0.60	0.00	0.00	0.20	0.27	0.53	0.24	0.57	0.00	1.00							
TS11	0.85	0.30	0.30	0.38	0.57	0.80	0.26	0.81	0.17	0.67	1.00						
TS12	0.38	0.45	0.13	0.00	0.61	0.60	0.11	0.45	0.33	0.15	0.32	1.00					
TS13	0.44	0.79	0.54	0.27	0.71	0.45	0.41	0.55	0.69	0.08	0.40	0.48	1.00				
TS14	0.47	0.69	0.43	0.33	0.61	0.42	0.58	0.51	0.58	0.15	0.44	0.37	0.79	1.00			
TS15	0.27	0.71	0.42	0.10	0.56	0.28	0.59	0.32	0.74	0.00	0.07	0.43	0.76	0.78	1.00		
TS16	0.26	0.75	0.48	0.10	0.67	0.40	0.57	0.38	0.71	0.00	0.28	0.50	0.74	0.81	0.85	1.00	
TS17	0.76	0.37	0.09	0.32	0.45	0.71	0.38	0.80	0.08	0.57	0.74	0.36	0.48	0.57	0.39	0.38	1.00

Table 4. SRAP banding patterns generated using eighteen pairs of primers for different tomato genotypes.

Primer (F - R)	No. of bands		Polymorphism (%)
	Total	Polymorphic	
me1 - em1	09	5	55.56
me1 - em2	08	5	62.50
me1 - em3	08	6	75.00
me1 - em4	16	9	56.25
me1 - em5	13	10	76.92
me2 - em1	10	7	70.00
me2 - em2	06	4	66.67
me2 - em3	10	7	70.00
me2 - em4	10	1	10.00
me2 - em5	10	8	80.00
me3 - em4	03	3	100.00
me4 - em1	09	6	66.67
me4 - em3	04	2	50.00
me4 - em5	14	8	57.14
me5 - em1	11	7	63.64
me5 - em2	15	7	53.33
me5 - em3	10	6	60.00
me5 - em4	11	5	45.45
Total	177	106	
Average	9.83	5.89	59.92

F: Forward primer; R: Reverse primer

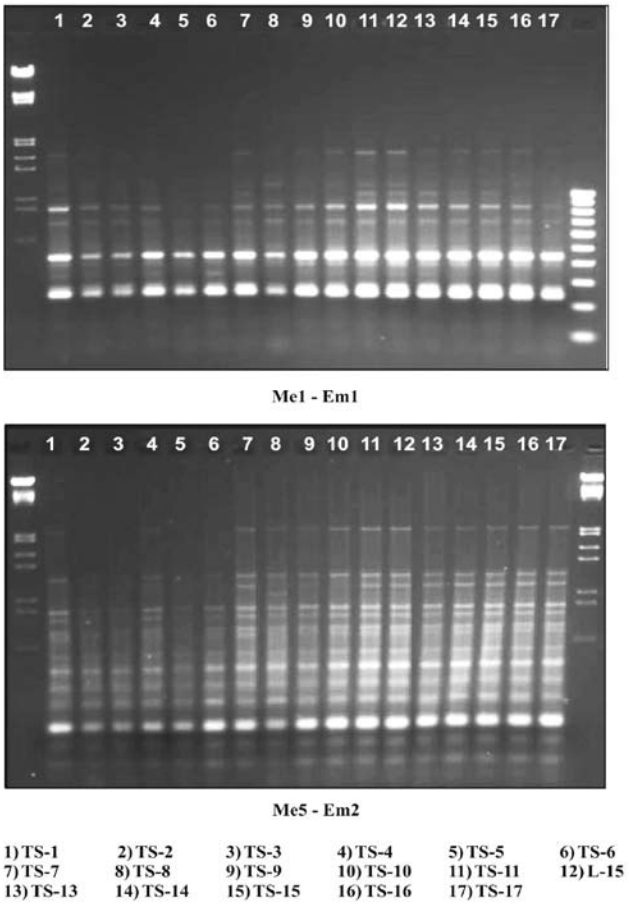

Fig. 2. Banding pattern of SRAP primer pairs Me1- Em1 and Me5-Em2. The arrow indicates the polymorphic SRAP bands.

The dendogram using SRAP data obtained from UPGMA analysis grouped all genotypes in two main clusters (Fig. 3). Cluster-I was divided into 3 sub clusters, *viz.*, a (TS-1, TS-4, TS-11, L-15, TS-13 and TS-14), b (TS-7, TS-15, TS-16 and TS-17) and c (TS-8, TS9 and TS-10). The genotypes TS-2, TS3 and TS-5 formed another main cluster. Since tomato is a self pollinating crop and does not show so much polymorphism within cultivated species, the similarity coefficient ranged from 0.72 to 0.98 indicating substantial diversity existing in the genotypes (Table 5). Also SRAP markers target the open reading frames and often targets coding regions which are more conserved, results in moderate polymorphism. The genotype TS-7 showed highest divergence from TS-2 and TS-3 with least similarity coefficient 0.72. The genotypes with same pedigree showed more similarity, *viz.*, TS-2 and TS-3; TS-13 and TS-14, TS-15 and TS-16 showed 0.98, 0.96, 0.95 similarity coefficients, respectively.

To provide an objective comparison, matrices of similarity coefficients, generated from SRAP and morphological data, were compared using the Mantel's test. Non-significant and quite low correlation between the dendrograms was obtained (r = 0.019, P = 0.56) through the MXCOMP procedure from NTSYS-pc program. The graph obtained through comparison of similarity matrices contain scattered points indicating there is poor correlation (Fig. 4). As per our knowledge, there are no studies on comparison between SRAP and morphological parameters through Mantel's tests in case of tomato. Poor correlation between ISSR and morphological parameters was also found during study on diversity among Greek tomato landraces (Terzopoulos and Bebeli, 15).

The absence of a significant relationship between the similarity matrices derived using morphological and SRAP data could be attributed to different levels of similarity being assessed by the two marker systems. SRAP markers measure diversity at the DNA level, thus no interaction with environment is expected. On the contrary, morphological traits are known to be affected by environmental conditions.

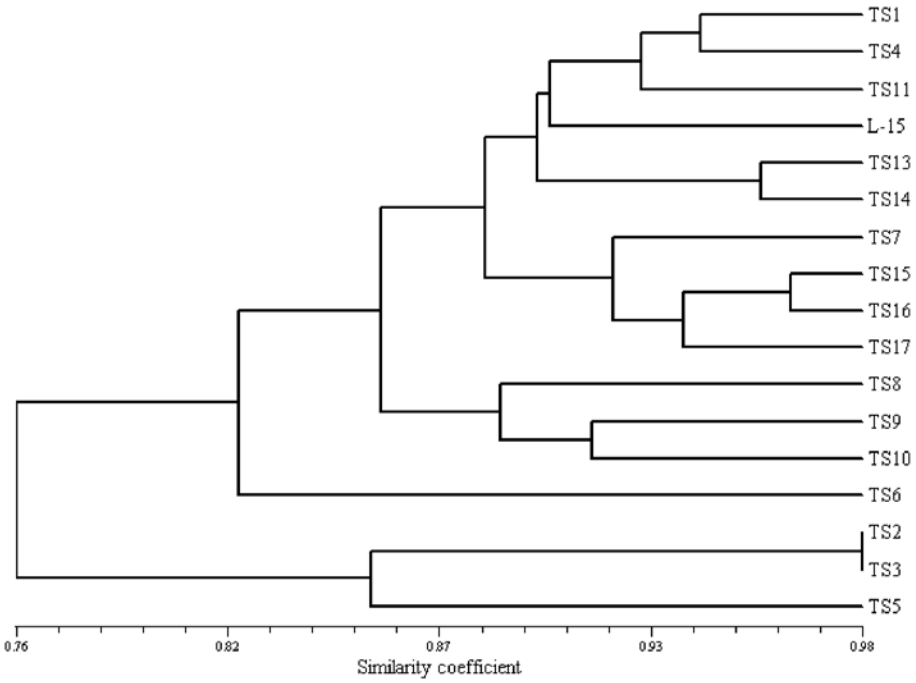

Fig. 3. Dendogram obtained from pooled data of eighteen pairs of SRAP profiles in seventeen tomato genotypes.

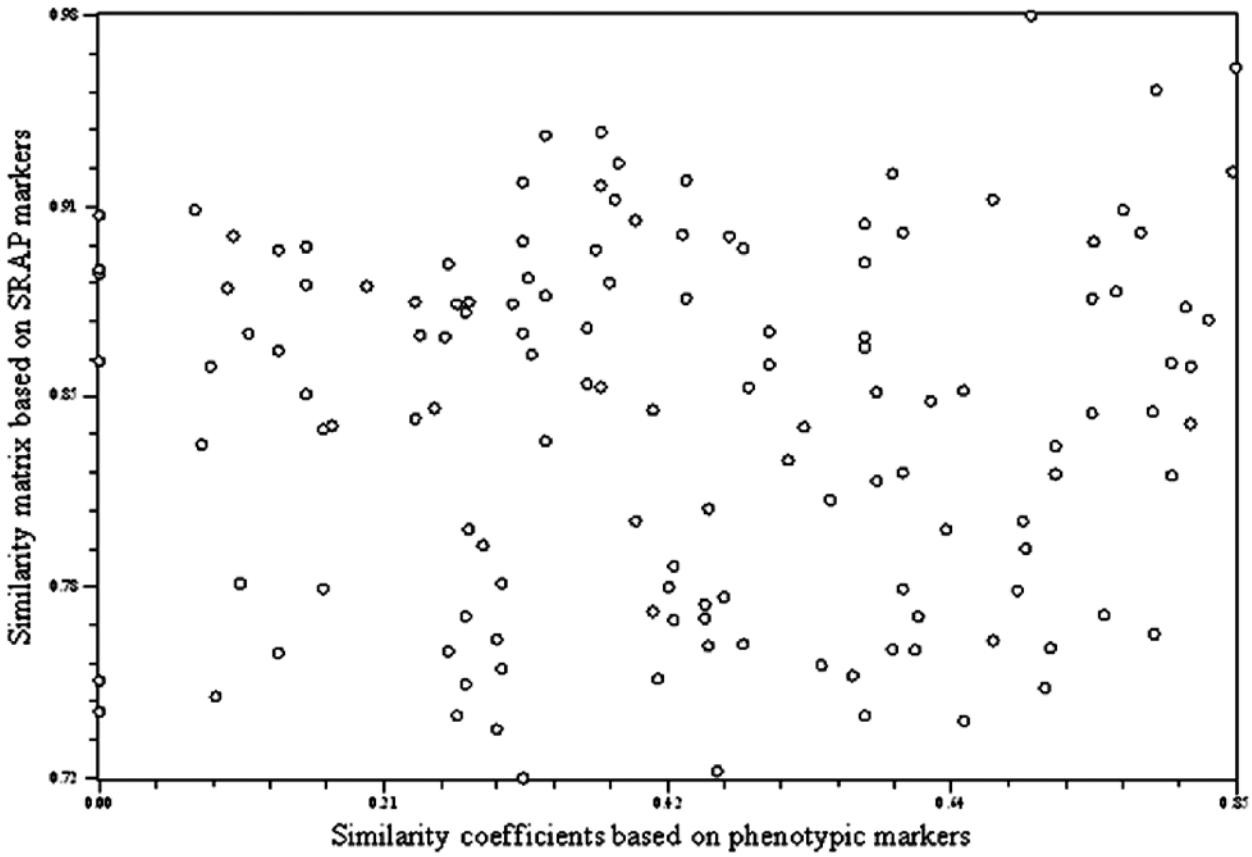

Fig. 4. Comparison of similarity matrices derived from SRAP and phenotypic markers.

Table 5. Similarity matrix of seventeen different tomato genotypes based on SRAP profiles.

Genotype	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	TS10	TS11	L-15	TS13	TS14	TS15	TS16	TS17
TS1	1.00																
TS2	0.77	1.00															
TS3	0.77	0.98	1.00														
TS4	0.94	0.78	0.78	1.00													
TS5	0.78	0.85	0.86	0.78	1.00												
TS6	0.84	0.79	0.80	0.84	0.85	1.00											
TS7	0.89	0.72	0.72	0.90	0.73	0.84	1.00										
TS8	0.87	0.78	0.76	0.87	0.74	0.84	0.90	1.00									
TS9	0.87	0.79	0.80	0.86	0.76	0.84	0.85	0.88	1.00								
TS10	0.90	0.75	0.74	0.89	0.75	0.84	0.88	0.89	0.91	1.00							
TS11	0.93	0.76	0.75	0.92	0.74	0.82	0.89	0.86	0.84	0.92	1.00						
L-15	0.89	0.76	0.76	0.89	0.76	0.82	0.87	0.81	0.83	0.85	0.92	1.00					
TS13	0.88	0.77	0.75	0.88	0.75	0.78	0.84	0.81	0.80	0.86	0.91	0.90	1.00				
TS14	0.90	0.78	0.77	0.88	0.77	0.78	0.85	0.83	0.82	0.89	0.92	0.90	0.95	1.00			
TS15	0.88	0.76	0.75	0.90	0.75	0.80	0.92	0.86	0.84	0.89	0.91	0.90	0.91	0.90	1.00		
TS16	0.87	0.77	0.76	0.88	0.76	0.80	0.91	0.85	0.83	0.86	0.88	0.87	0.90	0.88	0.96	1.00	
TS17	0.88	0.74	0.74	0.90	0.77	0.82	0.92	0.86	0.83	0.87	0.88	0.87	0.85	0.86	0.93	0.94	1.00

Discordance between morphological and molecular markers might occur if the morphological traits considered for comparison are under polygenic regulation. It would also occur if only a single or few genes controlling the expression of morphological traits were not detected by molecular markers. Hence, we can conclude that the diversity study on morphological basis needs to be supported through molecular diversity and the detailed knowledge of the genetic diversity within advanced breeding lines would facilitate a more efficient utilization. In the present study, most of the genotypes, *viz*., TS-2 and TS-3 with TS-4, TS-7, TS-10, TS-11 and TS-17 are diverse from each other at both morphological as well as molecular level, which can be used for further breeding programmes as diverse parental combinations. Here in this study we have used few combinations of SRAP markers but use of more number of combinations will help in detecting more polymorphism in future work.

In a plant-breeding programme, estimates of genetic relations among parental lines may be useful for determining which material should be combined in crosses to maximize genetic gain. Different genetic background among parental lines provided a large supply for allelic variations that can be used to create new favourable gene combinations. Sequence-related amplified polymorphism (SRAP) was the most effective, followed by inter-simple sequence repeats (ISSR), and morphological traits proved the least reliable for accessing the genetic diversity.

The SRAPs is a simple and efficient marker system that can be adapted for a variety of purposes in different crops, including map construction, gene tagging, genomic and cDNA fingerprinting, and map based cloning. It has several advantages over other systems. It is simple, has reasonable throughput rate, discloses numerous co-dominant markers, targets open reading frames (ORFs), and allows easy isolation of bands for sequencing (Li and Quiros, 8). SRAP markers could be more advantageous over SSR markers due to occasional loss of amplification sites of SSR primers in distant crop relatives and its relative simplicity.

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