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Phenotypic and molecular assessments of newly derived F_4 recombinant lines of tomato

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Abstract

Seven F_4 recombinant lines of tomato (*Solanum lycopersicum* L.) were derived from the cross Peto-86 × Supermarmande following a rigid tandem multi-trait selection scheme. Selection was exercised for desirable traits such as fruit firmness, pericarp thickness, redness, total soluble solids (TSS) and fruit yield while discarding the fruit fasciation and deep ribs. These lines were assessed for field performance of horticultural traits with emphasis on fruit firmness. Diversity analysis and to study relationship among the parents and the derived lines based on phenotypic horticultural traits was carried out using RAPD, ISSR and SRAP molecular markers. Tansgressive segregation for fruit firmness was observed in all the lines exceeding the better parent in strength. Three lines (1/A, 2/A and 2/B) were notable for higher fruit yield. Recombinant line 2/B showed 44.7% increase in yield over the higher yielding parent, Supermarmande. This line also showed high TSS. The recombinant line 1/A was the best among the selected lines for fruit pericarp thickness having greater fruit firmness. Molecular markers data revealed a significant positive correlation with morphological traits indicator suggesting that these independent sets of data are likely to reflect the same pattern of genetic diversity.

Key words: Breeding, cluster analysis, fruit firmness, molecular markers, tandem selection scheme

Introduction

Tomato (*Solanum lycopersicum* L.) a diploid (2n = 2x = 24) belongs to family *Solanaceae*. This family *Solanaceae* is considered economically important since it comprises tomato, potato, pepper and eggplant (Van der Hoeven et al. 2002) and other vegetable crops. Tomato a self-pollinated plant is grown worldwide under diverse conditions and it comes only after potato as the most consumed vegetable (FAO 2005, http://faostat.fao.org). Egypt is the fifth among the major tomato producing countries.

Being an important crop, tomato received relatively a great research interest in Upper Egypt, particularly, for improvement in crop performance under prevailing adverse abiotic stress conditions (Mohamed 1997a; Mohamed et al. 2002a). No research efforts to enhance fruit firmness were reported, except the recent study by Mohamed et al. (2017). The heirloom tomato cultivar Supermarmande is highly adapted to Egyptian environmental conditions and is widely cultivated, especially in winter season in Upper Egypt region. In spite of possessing many advantageous traits, including high yield, fruit setting at relatively low temperatures and early fruiting, the farmers turned to other cultivars due to its poor shelf-life. The post-harvest fruit yield losses in Supermarmande are appreciably high. The fruit firmness is an overall estimation of fruit resistance to compression. Precisely, it is a combination of skin resistance and flesh firmness (Grotte et al. 2001). A number of reports have been published concerning different aspects of tomato fruit firmness (Schuelter 2003; Nadeem et al. 2013). Breeding of new lines or hybrids using locally adapted tomato with improved yield and fruit characteristics would be useful but efforts are lacking in Egypt (Mohamed et al. 2017). Recently, DNA markers have been used to detect genetic diversity and relationship in various plant species including tomato. The molecular markers such as RAPD, ISSR and SRAP are an important tool to study polymorphism, biodiversity, Qtl analysis and to distinguish closely related agro-morphological traits within tomato genotypes (Terzopoulos and Bebeli 2008;

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Hasssan et al. 2013).

The present study was therefore, conducted to assess the performance of some main phenotypic horticultural traits for seven newly derived F_4 recombinant lines of tomato with emphasis on fruit firmness and to study the diversity and relationship among these recombinant lines and their parental genotypes based on phenotypic horticultural traits indicator using different molecular markers.

Materials and methods

The present field study was conducted at experimental Research Center, Faculty of Agriculture, during the years 2013 to 2017 and molecular analysis was carried out in the Biotechnology Laboratory, Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Development of recombinant F_4 lines

Seeds of tomato (*Solanum lycopersicum* L.) cultivars 'Peto-86' and 'Supermarmande' (both exotics) were planted in the nursery and the seedlings were transplanted in the open field 30 days later. At early flowering, F_1 cross 'Peto-86' (weak ribs and firm) × 'Supermarmande' (deep ribs, fruit fasciation and fast softens fruits) was made following the hand emasculation and pollination technique. Seeds were extracted from fully ripe fruits following the fermentation method. The F_2 seeds were produced through self-pollination were planted and individual plants were selected following a tandem scheme. Five flowers were isolated at the blooming stage to produce self-pollinated seeds for F_3. Initially, F_2 plants producing fasciated fruits and reduced intense fruit redness and deep ribs were discarded. Ripe fruits were harvested separately from each of the remaining individual plants and transferred to the laboratory. Fruit firmness (kg/cm^2, using penetrometer) was recorded. Fruits with high firmness record were then horizontally cut and among them, fruits of greater pericarp thick were saved. Lastly, those with higher juice TSS (Brix, using a hand refractometer Model ATAGO) were marked and kept.

F_3 progeny obtained from these plants were discretely planted in rows. Fifty plants were established from each F_3 family and ten plants from them spaced 30 cm apart per row were marked. The same aforementioned selection scheme was followed within F_3 families. In addition, plants showing heavier fruit yield within families of greater mean and homogeneity (lower coefficient of variation) were screened. Seven F_4 plants were obtained, two selected within each of two F_3 families and three selected within the third one. Seeds of F_4 progenies of these plants were planted along with the two parental cultivars in randomized complete blocks with 3 replicates. Each replicate comprised two rows of each entry and each row had 10 plants spaced 30 cm apart on the northern side of ridges 1 m wide and 3 m long. The recommended package of cultural practices was followed as in production of commercial tomato (Hassan 2008).

Data recording

Data were recorded on ten guarded plants per replicate for each of the parental cvs. and seven F_4 lines. Data were recorded on thirteen characters including the growth and yield traits such as, plant height (cm), number of branches/plant, fruit yield/plant (g) and number of fruits/plant. The fruit characters were determined considering the equatorial diameter of fruit (cm, using digital electronic render), polar diameter (cm), total soluble solids (TSS) (Brix, using a hand refractometer, Model: ATAGO), firmness (kg/cm^2, using penetrometer for full colored fruits), ribs depth (using a scale of 1 (deep ribs), 2 (moderate ribs) and 3 (ribs free fruits), fruit shape index (polar diameter/ equatorial diameter), intensity of redness (using a scale from 1 (light red), 2 (moderate) and 3 (dark red), number of locules/fruit (fruits horizontally cut) and fruit pericarp thickness (mm, using digital electronic render for horizontally cut fruits).

Statistical analysis

All data for the crop assessment of the derived F_4 recombinant lines and the parents of the initial cross were statistically analyzed using F-test. The treatment means were separated using Duncan's Multiple Range Test (DMRT) at $P < 0.05$ (Gomez and Gomez 1984). Additionally, the aforementioned phenotypic traits data were analyzed to produce a matrix of dissimilarity values. Euclidean distance for the phenotypic traits was calculated using NTSYS-pc ver. 2.1 (Rohlf 2000).

Molecular analysis

Total DNA of parents and their seven recombinant F4 lines was isolated from the fresh leaves (bulked from 5 different plants per genotype) using CTAB method with some modifications (Murray and Thompson 1980). Concentration and quality of DNA was measured at 260 nm using a spectrophotometer and checked by separating DNA on 0.8% agarose gel.

A total of five RAPD primers (OPA 08, OPA 10, OPA 09, OPA 15 and OPA 09), five ISSR primers (HB, HB 08, HB 09, HB 10 and HB 12) and five SRAP (Em1a-Me1b, Em1a-Me2, Em1a-Me3, Em2-Me2 and Em2-Me3) primer combinations obtained from Metabion International AG Company (Germany) were used in this investigation to amplify the template DNA. The reaction conditions were optimized and mixtures (25 μl total volume) were composed of 11.7 μl dH2O, 3.0 μl 10X reaction buffer, 3.0 μl dNTP's mix (2.5 mM each dNTP; Promega), 2.0 μl primer (2.5 μM) for (RAPD and ISSR), 1.0 μL forward primer, 1.0 μL reveres primer for (SRAP), 4.0 μl MgCl-2 (25 mM), 0.3 μl *Taq* DNA polymerase (5 U per μL; Promega) and 1 μl Template DNA (50 ng per μL). PCR procedures were carried out in a Lab Cycler (Model SensoQuest, GmbH, Germany).

The RAPD and ISSR amplification conditions were initial denaturation for 3 min at 94°C, 45 cycles of 1 min denaturation at 92°C, 1 min annealing at (32°C-34°C for RAPD and 38°C-44°C for ISSR) and 2 min extension at 72°C, 10 min final extension at 72°C, then followed by a final hold at 4°C. The SRAP amplification program was followed as initial denaturation for 4 min at 94°C, 10 cycles of 1min denaturation at 92°C, 1 min annealing at 35°C and 2 min extension at 72°C, 35 cycles of 1 min denaturation at 92°C, 1 min annealing at 50-55°C and 2 min extension at 72°C, 10 min final extension at 72°C, then followed by a final hold at 4°C. Amplification products were separated on agarose gel 1.4%, 2% and 2.5% for RAPD, ISSR and SRAP, respectively. Gels were stained with ethidium bromide (EB) (0.5 μg/ml) and DNA fragments were visualized using GelDoc-It®[2] Imager

For each primer the presence (1) or absence (0) of DNA bands in each genotype was visually scored and entered into a binary matrix. The pairwise comparisons between the tested genotypes were used to calculate the coefficient of genetic similarity matrix (Gs) according to Dice (1945). A dendrogram was constructed based on similarity estimates using NTSYS-pc version 2.11T (Rolhf 2000). The correlation between the different molecular marker systems as well as between molecular markers and phenotypic traits were calculated using mantel test (Mantel 1967). The three parameters *viz*., Polymorphic information content (PIC), Marker index (MI) and Resolving power (Rp), were calculated as follows: "PIC" = 1-[$(p)^2$ + $(q)^2$] (Ghislain et al. 1999), "MI" = PIC x ηβ (Powell et al. 1996) and "Rp"=ΣIb, (Prevost and Wilkinson 1999).

Results and discussion

Tomato fruit firmness greatly affects post-harvest performance and crop loss (Brummell and Harpster 2001). The present study was planned mainly to conduct selection for increased fruit firmness of the locally adapted cv 'Supermarmande' as it suffers fast ripe fruit softness derived from the cross 'Peto-86' × 'Supermarmande'. All new lines derived from the cross 'Peto-86' × 'Supermarmande' were found to perform better (Table 1) than the parent of the higher fruit firmness (Peto-86). The results of the generation mean analysis and frequency distribution investigation for the progenies of cross 'Peto-86' × 'Supermarmande' presented by Mohamed et al. (2017) were supported by this study. The authors observed results of the present study showed tendency towards increased fruit firmness in F_3 progenies and suggested a solid appearance of transgressive segregations with the increase of homozygosity in latter generations. As shown in Fig. 1, F_4 generation and selected lines surpassed the maximum segregate values of F_2. It is indicated that preceded assessment of genetic/breeding aiding parameters is a prerequisite and indispensable for directing breeding programs.

It is well known that crop yield is the ultimate goal of breeding programs regardless of the initial target trait(s). However, the fruit quality in tomato is a crucial factor for the outcome of a breeding program to be accepted by the consumers. Most important challenge facing the production of improved cultivars is, therefore, a compromise on a specific level of fruit quality and fruit yield to develop an economically accepted cultivar is to be reached by the plant breeder. Therefore, association among different traits and its direction are useful. Correlation estimates are important to determine traits to be used as indirect selection criteria for more effective selection program. Indirect selection would be effective if heritability estimate of the secondary character was greater than that of the primary one (Hallauer and Miranda 1988). Also, it supplements the information on undesirable traits associated with the desirable ones.

Positive significant correlation coefficient for fruit firmness with each of the number of fruits/plant and number of locules/fruit, but negative (r) with fruit diameter was observed and the earlier results reported by Mohammed et al. (2017) were corroborated. However, no significant correlation coefficient was found between fruit firmness and TSS. Parents and F_1 showed no significant correlation coefficients for

Table 1. Performance of various phenotypic horticultural traits for seven recombinant F_4 lines derived from progeny of cross Peto-86 (P_1) × Supermarmande (P_2)

Trait	P_1	P_2	1/A	1/B	1/C	2/A	2/B	3/A	3/B
Fruit shape index	1.2^{A}	0.66^{B}	0.61^{B}	0.69^{B}	0.71^{B}	0.68^{B}	0.67^{B}	0.72^{B}	0.7^{B}
Fruit ribbing	2.84^{A}	1.39^{E}	2.11^{D}	2.25^{C}	2.2^{CD}	2.6^{B}	2.25^{C}	2.08^{D}	2.52^{B}
Fruit redness	1.8^{A}	1.29^{E}	1.50^{BCD}	1.64^{ABC}	1.48^{CD}	1.4^{DE}	1.4^{DE}	1.6^{BC}	1.65^{AB}
Total soluble solids (T.S.S)	5.02^{E}	4.8^{E}	6.11^{D}	6.91^{A}	6.52^{BC}	6.72^{AB}	6.84^{A}	6.48^{BC}	6.25^{CD}
Fruit firmness (kg/cm^2)	2.97^{C}	2.05^{D}	5.63^{A}	5.0^{AB}	4.5^{B}	4.6^{B}	4.9^{AB}	4.86^{AB}	5.03^{AB}
Fruits/ plant (no.)	35.43^{E}	47.46^{C}	44.0^{D}	24.0^{G}	28.0^{F}	55.0^{B}	64.0^{A}	20.0^{H}	24.0^{G}
Fruit yield / plant (kg)	2.85^{EF}	5.17^{D}	6.6^{B}	3.08^{E}	3.04^{E}	6.04^{C}	7.48^{A}	2.48^{F}	3.2^{E}
Branches/plant (no.)	6.2^{AB}	5.3^{BC}	7.0^{A}	6.0^{ABC}	6.0^{ABC}	7.0^{A}	7.0^{A}	6.0^{ABC}	5.0^{C}
Plant height (cm)	74^{B}	87^{A}	61^{CD}	58^{D}	60^{CD}	77^{B}	84^{A}	54^{E}	63^{C}
Fruit polar diameter (cm)	6.36^{A}	4.82^{BC}	4.9^{B}	4.55^{CD}	4.89^{B}	4.33^{D}	4.46^{D}	4.5^{D}	4.51^{CD}
Fruit equatorial diameter (cm)	5.3^{D}	7.3^{AB}	8.0^{A}	6.55^{BC}	6.89^{BC}	6.4^{BC}	6.68^{BC}	6.21^{CD}	6.4^{BC}
Fruit pericarp thickness (cm)	0.65^{C}	0.51^{F}	0.81^{A}	0.53^{EF}	0.71^{B}	0.54^{E}	0.56^{DE}	0.58^{D}	0.65^{C}
Locules / fruit (no.)	4.02^{D}	8.97^{A}	7.87^{AB}	7.32^{B}	6.87^{BC}	5.81^{C}	5.7^{C}	5.71^{C}	5.67^{C}

Means followed by the same letter(s) are not significantly different at P = 0.05 using Duncan's Multiple Range Test

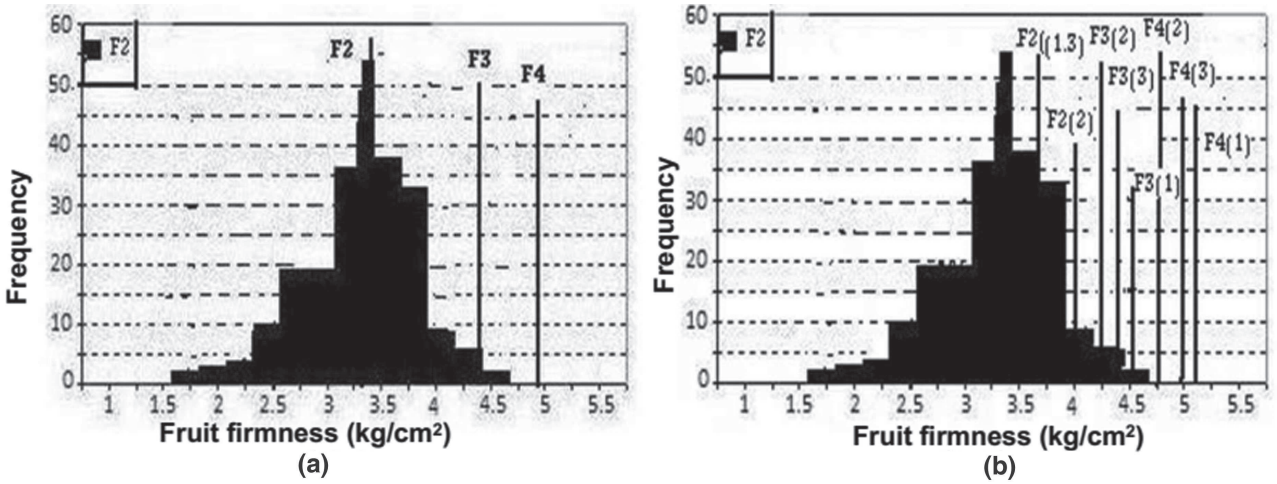

Fig. 1. Histogram showing phenotypic distribution of fruit firmness for the F_2 individuals derived from the trait cross 'Peto-86' × 'Supermarmande' and the position of the means of selected F_3 families and F_4 lines (A) in addition to mean position of the selected individual plants from F_2 and of each F_3 families and F_4 lines during the progress of the selection program (B)

fruit firmness with other main key traits. The selection for increased fruit firmness was practiced in tandem fashion with visual exclusion of plants showing fruit fasciations, less intensive fruit redness and deep fruit ribs. Among the remaining plants a tandem selection was followed as plants of the firmer fruits were identified and then those having fruit of greater TSS were saved. Considering the above traits, the seven derived F_4 lines appeared recombinants as they showed differential assortment among the 13 assessed traits suggesting absence or week associations between these traits. Practicing rigid selection for TSS led to producing lines with higher solids of the fruit juice the higher the value of tomato crop yields. High soluble solids means removal of less water to produce tomato based food products. Such fruit are also likely to be sweeter as sugar is a major constituent of total soluble solids. Therefore, considerable interest exists in manipulating the soluble solids content of tomato (Baxter et al. 2005) and increasing fruit solids content has been the focus of numerous tomato breeding programs (Foolad 2007).

From crop production view point, three lines (1/A, 2/A and 2/B) were identified concerning the total fruit yield. The enhanced fruit yield for these F_4 lines can occur due to significant positive correlation coefficients (r) between fruit firmness and fruit number and positive tendency of association for fruit firmness with total yield. All three lines resembled Marmande type as they produce oblate fruit and increased number of locules/fruit but with elevate fruit firmness, TSS and fruit redness while having reduced fruit ribbing. The highest fruit yield was produced by recombinant line 2/B giving 44.7% increase over the higher yielding parent (Supermarmande). Line 2/B showed high TSS. The recombinant line 1/A was the best for fruit pericarp thickness having great fruit firmness. From viewpoint of germplasm resources, all the seven recombinant lines may serve as useful material in other breeding programs.

Phenotype-based genetic relationships among the recombinant lines and their parents

The phenotypic distance of the genotypes (Euclidean distance, ED) using 13 phenotypic horticultural traits varied from 2.15 in lines 2/A and 2/B to 7.03 in Peto-86 and Supermarmande (Table 2). This indicates a relatively high amount of genotypic variation existed among the lines. These values, which are assumed to reflect the genetic diversity of the loci controlling these traits, indicate the possibility of selecting varieties having a diverse genetic background and the prospect of obtaining broad segregation for the characters. Cluster analysis of the parents and their seven F_4 lines based on the 13 phenotypic traits was performed. Cluster-1 contained Supermarmande parent which displayed higher mean values for plant height and number of locules/fruit, whereas lowest mean values for fruit ribbing, fruit redness, T.S.S, fruit firmness and fruit pericarp thickness. The super-marmande was distinguished from other genotypes within 6.87 branched-off genetic distances, reflecting a relatively longer genetic distance from the other genotypes. Cluster-2 consisted of two lines (2/A and 2/B) characterized by low mean value of polar diameter. The genetic distance value of these varieties was 2.15. Cluster-3 had one line (1/A) having the highest mean values of equatorial fruit diameter, fruit firmness and pericarp thickness, whereas lowest mean values for fruit shape index. Cluster-4 divided into two sub-clusters, sub-cluster-1 had parent Peto-86 and this cluster could be characterized by the highest mean value of fruit shape index, fruit ribbing, fruit redness and polar diameter and the lowest mean values of fruit diameter and the number of locules/fruit. Sub-cluster-2 consisted of four lines (1/B, 3/A, 1/C and 3/B) and represented 44.4% of the total number of genotypes. This cluster had a characteristic of having lowest mean value for the number of fruit/plant. The genetic distance between these genotypes ranged from 2.56 (between 1/B and 3/A) to 4.75 (between 1/B and 3/B) with an average of 3.65 (Table 2).

Table 2. Euclidean distance matrix of two tomato parents P_1 and P_2 and their derived seven F_4 recombinant lines using thirteen phenotypic horticultural traits

	P_1	P_2	1/A	1/B	1/C	2/A	2/B	3/A	3/B
P_1	0								
P_2	7.03	0							
1/A	5.47	6.17	0						
1/B	5.77	5.63	5.14	0					
1/C	3.95	5.92	4.12	3.7	0				
2/A	5.61	5.45	5.2	4.23	4.87	0			
2/B	6.09	5.99	5.25	5.43	5.33	2.15	0		
3/A	4.69	6.63	5.08	2.56	2.65	4.62	5.5	0	
3/B	5.01	6.28	6.04	4.75	3.02	4.8	5.32	3.49	0

P_1 = Peto-86; P_2 = Supermarmande

These results agree with Rodríguez-Gustavo et al. (2006), who evaluated plant and fruit traits of seventeen recombinant lines of tomato with their parental genotypes. Significant differences were found among parental genotypes and recombinant lines. Many of the recombinant lines were similar to one of the two parents. In the cluster analysis, shelf life was an important discriminatory trait for these lines and the parental genotypes. The authors proposed some of the new genotypes as new source of useful variability in tomato breeding programs. In view of the considerable genetic diversity found in the present study, genotypic improvement through hybridization between the genotypes from divergent clusters may be gainful.

Molecular-based assessment of genetic relationships among the recombinant lines and their parents

A total of 150 DNA fragments were obtained from the parents and the seven derived F_4 lines, with an average of 10 bands/primers, using 5 RAPD, 5 ISSR and 5 SRAP primers (Fig. 2a-c). Out of 150 fragments,

76 (50.67%) showed polymorphism and 74 (49.33%) bands were common (monomorphic), in all the genotypes. The line 2/B displayed the highest number of DNA fragments (127 bands) followed by 1/A (124 bands), while the parent 'Supermarmande' revealed the least number of bands (107 bands). Variations in the number of DNA fragments generated by different primers can be influenced by one or more factors such as primer sequence and number of matching sites in the genome (Kernodle et al. 1993).

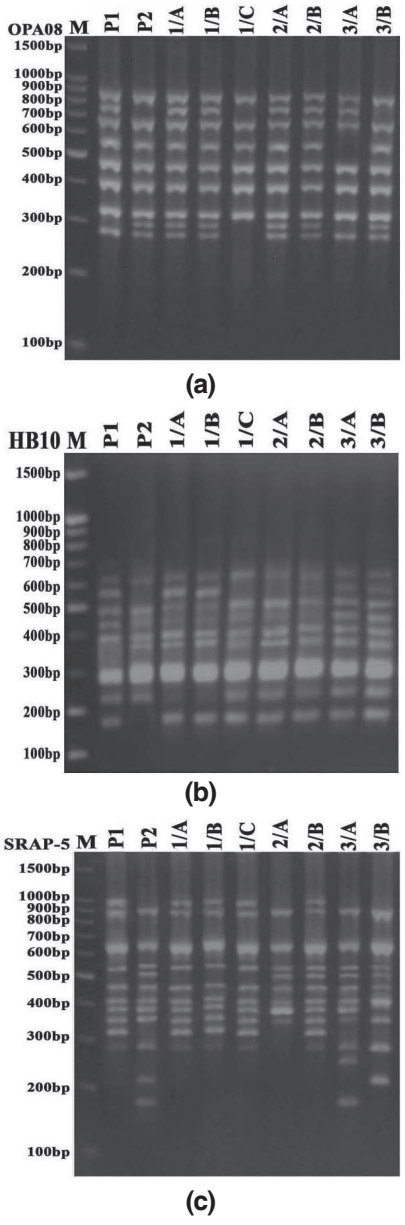

Fig. 2. RAPD, ISSR and SRAP banding profile of two tomato parents (Peto-86 and Supermramande) and their derived seven F_4 lines. Lane M represented the DNA ladder

Polymorphism information content (PIC) value, an indicative parameter of the informative degree of a marker, ranged from 0.05 (ISSR-HB12) to 0.34 (SRAP-1) with an average value of 0.15 (Table 3). The marker index (MI) may be used to evaluate overall utility of a marker system (Powell et al. 1996). Marker index ranged from 0.10 for ISSR-HB12 primer to 4.75 for SRAP-1 and OPA08 primers (Table 3). The resolving power (RP) estimates ranged from 0.44 to 8.44 with an average of 2.5 per primer (Table 3). In the present study, SRAP-1 primer possessed high RP value (8.44) and, therefore, appears to be the best primer that have the information for distinguishing tomato genotypes. The high level of polymorphism indicates existence of genetic diversity and support the efficiency of the three molecular markers in detecting polymorphism among tomato genotypes. Polymorphism is considered as a useful selection tool in monitoring alien genome introgression in tomato breeding programs.

The application of marker analysis yielded three molecular marker systems of 150 DNA fragments, out of which 86 were common for the two parents, while 54 bands were polymorphic. Polymorphic bands clearly distinguished the Peto-86 from the Supermarmande genotypes. Therefore, three molecular marker systems were successful in characterizing 6 out of the 7 F_4 recombinant lines by unique positive and/or negative markers. Line 1/C was distinguished by two negative bands at 283 bp (OPA08) and 318 bp (HB08) in addition to positive unique bands at 322 bp (SRAP-4). Line 3/A was distinguished by negative unique marker at 564 bp (OPA08) and one positive marker at 268 bp SRAP-5. Line 3/B was distinguished by two negative unique markers at 636 bp (HB10) and 395 bp (SRAP-5). Line 1/B was identified by one positive unique marker at molecular weight 177 bp (SRAP-1). Line 2/A was characterized by one positive unique marker at molecular weight 302 bp (SRAP-1) in addition to one negative unique marker at 282 bp (SRAP-5). Line 2/B was distinguished by one positive unique marker at 239 bp SRAP-2.

The similarity coefficient varied from a minimum of 0.76 between Peto-86 and Supermarmande to close similarity (GS = 0.972) between 1/A and 1/B with an average value of 0.87 (Table 4). The dendrogram generated based on a combined RAPD, ISSR and SRAP data sets shows that the parent Supermarmande was separated in a single branch from the other genotypes within 80.6% branched-off genetic similarity, reflecting relatively far genetic distance from the other genotypes, while the other genotypes grouped

Table 3. Marker information using three molecular marker systems

	Primer	TB	PB	PPB	PIC	MI	RP
RAPD	OPI09	6	3	50.00	0.19	0.57	1.56
	OPA08	9	4	44.44	0.14	0.57	1.78
	OPA10	8	3	37.50	0.10	0.31	1.11
	OPA09	4	1	25.00	0.11	0.11	0.67
	OPW15	7	2	28.57	0.13	0.25	1.33
ISSR	HB15	7	3	42.86	0.14	0.42	1.33
	HB08	6	2	33.33	0.09	0.18	0.67
	HB12	8	2	25.00	0.05	0.10	0.44
	HB	8	4	50.00	0.10	0.40	0.89
	HB10	9	4	44.44	0.12	0.48	1.56
SRAP	SRAP-1	16	14	87.50	0.34	4.75	8.44
	SRAP-2	18	9	50.00	0.21	1.93	6.22
	SRAP-3	16	9	56.25	0.18	1.58	3.78
	SRAP-4	14	8	57.14	0.18	1.47	3.56
	SRAP-5	14	8	57.14	0.20	1.58	4.22
	Average	10.00	5.07	45.95	0.15	0.98	2.50

TB = Total bands, PB = Polymorphic bands, PPB = % Polymorphic bands, PIC = polymorphism information content, MI = Marker index, RP = Resolving power

into two major clusters. The first cluster comprised four genotypes Peto-86, 1/A, 1/B and 1/C. The second cluster included four lines, 2A, 2B, 3/A and 3/B. This cluster divided into two sub clusters. The first sub-cluster included lines 2/A and 2/B, which is closely related with genetic similarity of 0.94. The second sub-cluster includes closely related lines 3/A and 3/B with 0.92 genetic similarity. Effectiveness of molecular tools to distinguish tomato lines with specific markers are well recognized and the findings have been reported to connect marker data with prediction of heterosis (Abd El-Aziz et al. 2016). RAPD and ISSR primers were successfully employed in this context distinguishing individual lines of tomato among widespread varieties in Egypt with specific markers and divide them into groups in cluster analysis.

The Mantel test values between Dice similarity matrices of molecular markers show significant correlations, RAPD + ISSR (r = 0.734), RAPD+ SRAP (r = 0.56614), ISSR + SRAP (r= 0.42311) and (RAPD+ISSR+SRAP (r = 0.51721) which indicate good agreement between RAPD, ISSR and SRAP markers. Several researchers reported that SRAP markers have high potential for identification and characterization

Table 4. Genetic similarity for the parents (Peto-86 and Supermarmande) and their seven derived F_4 recombinant lines based on combined RAPD, ISSR, SRAP data analyses

	P_1	P_2	1/A	1/B	1/C	2/A	2/B	3/A	3/B
P_1	1.00								
P_2	0.76	1.00							
1/A	0.96	0.80	1.00						
1/B	0.97	0.77	0.97	1.00					
1/C	0.92	0.81	0.94	0.93	1.00				
2/A	0.84	0.85	0.86	0.85	0.85	1.00			
2/B	0.88	0.85	0.88	0.88	0.88	0.94	1.00		
3/A	0.90	0.80	0.90	0.91	0.89	0.88	0.90	1.00	
3/B	0.87	0.81	0.88	0.89	0.87	0.89	0.89	0.92	1.00

P_1 = Peto-86; P_2 = Supermarmande

compared with other molecular markers (Ruiz et al. 2005). In the present study, the phylogenetic analysis on the basis of RAPD derived dendrogram revealed relatively similar clustering pattern to that obtained from the ISSR and SRAP markers. This confirms the phylogenetic relationship among parents and their inbred F_4 tomato lines and congruence among the three marker systems. Thus utilizing these marker systems to determine the genetic variations among the genotypes are useful prior to conducting crop breeding programs (Munazza et al. 2009).

Correlation between phenotypic horticultural traits and molecular markers

The fragment size 743 bp generated by OPA09 primer appeared in Supermarmande and line 1/A which had the highest average fruit equatorial diameter and number of locules/fruit as compared to other genotypes and hence could be used as positive marker. Two fragments at molecular size 177 bp (SRAP-1) and 239 bp (SRAP-2) appeared only in line 1/B which had the highest average for T.S.S and fruit yield/plant and could be used as positive marker. Three DNA fragments at molecular size 214 bp (SRAP-1), 150 bp (SRAP-3) and 474 bp (SRAP-4) appeared only in lines 2/A and 2/B which had the highest number of fruit/ plant and hence these fragments may be useful.

Fragments of molecular size 439 bp, 134 bp (HB) and 373 bp (HB10) appeared in Supermarmande and seven lines which had the highest average number of locules/fruit and fruit equatorial diameter (cm), but absent in Peto-86 having lowest values for these traits.

Hence, these fragments are considered as positive specific markers for number of locules/fruit and fruit equatorial diameter (cm). On the other hand, fragments at molecular size 569 bp and 292 bp (OPA10), 529bp (HB15), 713 bp and 442 bp (HB12), 334 bp and 224 bp (HB), 179 bp (HB10), 648 bp (SRAP-1), 514 bp, 485 bp, 169 bp and 93 bp (SRAP-3) and 702 bp, 218 bp (SRAP-4) appeared in Peto-86 and the seven recombinat lines which had the highest average for fruit ribs and fruit firmness. However, these fragments were absent in Supermarmande which had the lowest values for these traits, so these fragments are considered as positive specific markers for these traits using such tested primers. Fragments at molecular size 636 bp (HB10) and 395 bp (SRAP-5) may contribute to control of no. of branches/plant. These fragments appeared in all the genotypes except in 3/B having lowest no. of branches/plant. Also, fragment of molecular size 564 bp (OPA08) was found in all genotypes except 3/A which had the lowest averages for no. of fruit/plant, fruits yield/plant and no. of branches/plant. These fragments may be related to these traits.

Clusters developed based on phenotypic horticultural traits had a close similarity with those clustered based on molecular markers. The three molecular markers revealed a highly significant positive correlation with phenotypic horticultural indicators ($r = 0.51$, $p < 0.01$). The significant correlation indicates that the independent sets of the data are likely to reflect the same pattern of genetic diversity and validates the use of phenotypic horticultural traits data to calculate the different diversity statistics in the studied tomato genotypes (Figueiredo et al. 2016; Rai et al. 2016). In conclusion, all recombinant lines exceeded the parents for fruit firmness and up to 44.7% increase over the higher yielding parent. There was a good agreement between molecular markers data and the phenotypic horticultural trait descriptors. Thus phenotypic indicators here may provide fundamental diversity information in tomato.

Authors' contribution

Conceptualization of research (MFM, BES, AGH); Designing of the experiments (MFM, BES); Contribution of experimental materials (MFM, AGH); Execution of field/lab experiments and data collection (BES, DSM); Analysis of data and interpretation (MFM, BES, AGH, DSM); Preparation of manuscript (MFM, BES).

Declaration

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