DETECTION OF SOMACLONAL VARIATIONS IN TOMATO USING RAPD MARKERS

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Tomato (*Solanum lycopersicum* Mill.), 2n=2x=24; is the most important vegetable crop in the world after potato, being cultivated in many regions of the world. In terms of medical science, it encompasses antioxidant lycopene (alkaloids) a health promoting compounds whose consumption reduces the incidence of many types of cancer (Pohar *et al.*, 2003). It is one of the most important protective foods as it possesses appreciable quantities of vitamins and minerals and sometimes rightly referred to as poor man's orange (Devi *et al.*, 2008). Tomato was also used as bioreactor in biopharming for the production and oral delivery of vaccines (Jiang *et al.*, 2007).

Somaclonal variation; a common phenomenon in plant cell cultures, includes all types of variations among plants or cells that derives from all kinds of tissue cultures (Larkin and Scowcroft, 1981; Evans *et al.*, 1986; Filipecki and Malepszy, 2006). When plants are regenerated from somatic cells via cell culture, they show genetic variability. The variations can be genotypic or phenotypic, which in the latter case can be either genetic or epigenetic in origin. This variability may be epigenetic and transmitted through meiosis. However, stable genetic changes are common and, if useful, can be of interest to plant breeders. These genetic changes are usually due to any of the following causes: changes in chromosome numbers (polyploidy and aneuploidy), chromosome structure (translocations, deletions, insertions and duplications) and DNA sequence (base mutations), cytoplasmic gene change, chromosome rearrangements, mitotic crossing over and activation of transposable elements (Leva *et al.*, 2012). The best application of somaclonal variation to conventional plant breeding lines is introducing the best available varieties into cell culture and selecting among regenerated plants or their progeny for the desired changes.

DNA markers provided valuable tools in various analyses ranging from phylogenetic to the positional cloning of genes. Scoring of morphological and biochemical changes in plant can be useful in some studies, but variations generated from them are limited diversity and trait may be affected by environmental influences. Molecular techniques such as Random Amplified Polymorphic DNA

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(RAPD) is often favored over traditional phenotypic, cytological and biochemical analysis, and generally assess even if small variations were introduced to the genome. Detection of somaclonal variations using RAPD markers has several advantages. RAPD markers are technically simple, quick to perform with small amount of DNA and do not require previous information about genome or radioactive labeling (Michelmore *et al.*, 1991). RAPDs are usually dominant and are inherited in a simple Mendelian fashion. Thus RAPD analysis is a useful tool for determining genetic relationships among regenerated plant and their original cultivars. The use of the PCR-based RAPD technique to detect somaclonal variations has been applied successfully to several plant species, such as *Lolium* (Wang *et al.*, 1993) and *Allium sativum* L. (Al-Zahim *et al.*, 1999) it has also been applied for tomato (Soniya *et al.*, 2001) and potato (Khatab, 2000). The aim of the present study was to identify polymorphic RAPD markers in tomato cultivar to detect the somaclonal variations in plants generated from tissue culture.

MATERIALS AND METHODS

1. Plant Materials

Four commercial varieties of tomato (*Solanum lycopersicum* Mill.) were used in the present investigation; namely, Super Strain B, Castle Rock, Advanttage II and Edkawy as well as sixteen somaclones which were regenerated through tissue culture technique using cotyledon and hypocotyl explants from them (Table 1).

2. Molecular analysis

2.1. DNA extraction

For DNA extraction, approximately 150 mg fresh leaves of sixteen somaclones randomly selected from their original cultivars (Super Strain B, Castle Rock, Advanttage II and Edkawy) were frozen in liquid nitrogen and ground to a fine powder using mortar and pestle. DNA was extracted by the CTAB method according to Doyle and Doyle (1990). Each sample was then washed with chloroform: isomyl alcohol (24:1) precipitated with isopropanol and resuspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). RNA was removed by adding of 0.5 mg of RNase per sample.

2.2. Polymerase Chain Reaction (PCR)

Eleven RAPD random primers were used to identify polymorphism between the four parental genotypes and their somaclones. The details of primer sequences are given in (Table 2).

DNA amplification

Somaclones and their original cultivars were subjected to RAPD-PCR analysis. Polymerase Chain Reaction (PCR) was carried out in the presence of 12.5 µl master mix, 1.0 µl template DNA, and 2.0 µl primer (15 pM/µl) and 9.5 µl sterile distilled water in a total volume of 25 µl. PCR amplification was performed in au-

tomated thermal cycler (MJ-Mini, Bio Rad) programmed as follow: 95°C for 4 min. followed by 40 cycles of 1 min. for denaturation at 94, 30 sec. for annealing at 37°C and 1.30 min. for polymerization at 72°C, followed by a final extension step at 72°C for 7 min. The amplification products were resolved by electrophoresis in 1.5% agarose gels in 0.5 X TBE buffer. The gels were stained with ethidium bromide and photographed by gel documentation system (UVITEC, UK). The amplified bands were scored for RAPD marker based on the presence (+) or absence (-) of bands.

RESULTS AND DISCUSSION

RAPD analysis for somaclones

The aim of the present study was to detect the somaclonal variation in plants generated from tissue culture. RAPD analysis was used to determine the genetic variability among sixteen somaclones and their original cultivars; Super Strain B, Castle Rock, Advanttage II and Edkawy (Table 1). Out of eleven random primers used, only six primers (OPA01, OPA02, OPA03, OPA04, OPA05 and OPB11) successfully produced scoreable RAPD bands for all the tested genotypes (Tables 3, 4, 5, 6, 7 and 8). The patterns of DNA amplification using different primers are shown in (Figs 1 a, b, c, d, e, f). Among the primers used, OPA05 produced the highest number of bands (16 bands) while primers OPA04 produced the lowest number (12 bands). DNA amplification with six different primers generated 285 bands (total No. of band for the sixteen somaclones and their original cultivars), 130 of them were polymorphic and 155 monomorphic bands (Table 9). Regarding original cultivars and their somaclones, Table (9) illustrated that all primers produced 73 bands in Super Strain B cultivar and its somaclones, out of them 40 bands were polymorphic with polymorphism percentage of 54.8%. Primer OPA02 produced the highest number of polymorphic bands (11 bands) while primer OPA05 produced the lowest number (one band).

Regarding Castle Rock cultivar and its somaclones, all primers produced 77 bands out of them 33 bands were polymorphic with 42.9% polymorphism. Primer OPA02 produced the highest number of polymorphic bands (nine bands) while primers OPA05 and OPB11 produced the lowest number of bands (three bands).

As for Advanttage II cultivar and its somaclones, all primers produced a total of 71 bands out of them 30 bands were polymorphic with 42.3% polymorphism. Primer OPA02 produced the highest number of polymorphic bands (eight bands) while primer OPA05 produced the lowest number (two bands).

Edkawy cultivar and its somaclones, exhibited a total of 64 bands were produced from all primers out of them, 27 bands were polymorphic with polymorphism percentage of 42.2%. Primer OPA02 produced the highest number of polymorphic bands (nine bands) while primer OPA05 produced the lowest number of bands (three bands).

Some new additional bands/loci occurred in some somaclones and absent in their original cultivars example, bands with sizes 800, 500, 300, 275 and 225 bp amplified by OPA01 for Super Strain B somaclones (Table 3 and Fig. 1 a), also bands with size 200 bp amplified by OPA02 for some Castle Rock somaclones (Table 4 and Fig. 1 b). Also bands with size 1350, 1150, 1000, 900, 800 and 500 bp which amplified by OPA03 for Advanttage II somaclones (Table 5 and Fig. 1 c). On the other hand, some bands present in the original cultivars and absent in one or more of their respective somaclones, for example, bands with size (1500, 1350 and 200 bp) amplified by OPA03 for Castle Rock cultivar (Table 5 and Fig. 1c). The results showed that, most of the used primers revealed unique bands in most regenerated somaclones. These unique bands were absent in their original cultivar for example, band with size 500 bp which amplified by OPA04 for Super Strain B somaclones (SS10) (Table 6 and Fig. 1 d). Also bands with size 2000 and 100 bp which amplified by OPB11 for Advanttage II somaclones (SA9 and SA2), respectively (Table 8 and Fig. 1 f). Also band with size 1600 bp amplified by OPA05 for Edkawy somaclones (SE2) (Table 7 and Fig. 1 e).

These results were in agreement with those obtained by Abd El-Hady *et al.* (2010) who examined eight tomato varieties using RAPD markers by seven random primers and the results revealed a high level of polymorphism among the studied genotypes. Our results were also agreement with Tabassum *et al*. (2013) who obtained 94.16% polymorphism among the tomato varieties. The results were also in agreement with earlier reports on the application of RAPD in describing genetic polymorphism among regenerated plants in several other plants, *viz.,* rice (Khai and Lang, 2005), oil palm (Sanputawong and Techato, 2011) and in tomato (Shalaby and El-Banna, 2013).

It could be concluded that RAPD can be successfully used to detect somaclonal variations among *in vitro* regenerated tomato plants. Numerous researches proved that the sensitivity of RAPD was adequate enough to detect genetic changes in many tissue culture derived plants; for instance, sugar cane (Devarumath *et al*., 2007), sorghum (Singh *et al*., 2006) and apple (Bernardo and Itoiz, 2004). The relatively high frequency of variations (ranged between 42.2% for Edkawy to 54.8% for super strain B) detected here might bias stable plant propagation. However it could be regarded as a novel source of tomato improvement.

SUMMARY

Eleven random primers were used to study somaclonal variation among sixteen somaclones derived from four parental genotypes (Super Strain B, Castle Rock, Advanttage II and Edkawy). Out of eleven random primers used, only six primers (OPA01, OPA02, OPA03, OPA04, OPA05 and OPB11) successfully produced scoreable RAPD bands for all the tested genotypes. DNA amplification with

the six different primers generated 285 bands 130 of them were polymorphic and 155 monomorphic bands in all the genotypes studied. Among the primers used, OPA05 produced the highest number of bands (16 bands) while primers OPA04 produced the lowest number (12 bands). RAPD patterns generated by these primers achieved high polymorphic percentage, indicating high level of genetic variations among somaclones and their parental genotypes. Super Strain B cultivar showed the highest number of polymorphic percentage 54.8%, while Advanttage II and Edkawy cultivars showed the lowest percentage of somaclonal variations 42.3% and 42.2%, respectively. The relatively high frequency of variations detected here might bias stable plant propagation. However it could be regarded as a novel source of tomato improvement.

REFERENCES

Abd El-Hady, E. A. A., A. A. A. Haiba, N. R. Abd El-Hamid and A. A. Rizkalla (2010). Phylogenetic Diversity and Relationships of Some Tomato Varieties by Electrophoretic Protein and RAPD analysis. Journal of American Science, 6: 434-441.

Al-Zahim, M., B. Ford-Lloyd and H. Newbury (1999). Detection of somaclonal variation in garlic (*Allium sativum* L.) using RAPD and cytological analysis. Plant Cell Reports, 18: 473-477.

Bernardo, R. and R. J. Itoiz (2004). Evaluation of the discriminance capacity of RAPD, isoenzymes and morphologic markers in apple (Malus 9 domestica Borkh.) and the congruence among classifications. Genet. Resour. Crop Evol., 51: 153-160.

Devarumath R. M., R. B. Doule, P. G Kawar, S. B. Naikebawane and Y. S. Nerkar (2007). Field performance and RAPD analysis to evaluate genetic fidelity of tissue culture raised plants vis-à-vis conventional setts derived plants of sugarcane. Sugar Tech., 9: 17-22.

Devi, M., M. S. Dhaliwal, A. Kaur and S. S. Gosal (2008). Effect of growth regulators on *in vitro* morphogenetic response of tomato. Indian J. Biotechnol., 7: 526-530.

Doyle, J. J. and J. L. Doyle (1990). Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Evans, N., D. Foulger, L. Farrer and S. Bright (1986). Somaclonal variation in explant derived potato clones over three-tuber generation. Euphytica, 35: 353-361.

Filipecki, M. and S. Malepszy (2006). Unintended consequences of plan transformation: a molecular insight. J. Appl. Genet., 47: 277-286.

Jiang, X. L., Z. M. He, Z. Q. Peng, Y. Qi, Q. Chen and S. Y. Yu (2007). Cholera toxin B protein in transgenic tomato fruit induces systemic

immune response in mice. Transgenic Res., 16: 169-175.

Khai, T. H. and T. N. Lang (2005). Using SSR markers to identify allele variation of somaclonal mutants in indica rice. Omonrice, 13: 121-125.

Khatab, A. I. (2000). Genetical and Molecular effects of pathogenic fungal filtrate on somaclonal variation in potato (*Solanum tuberosum* L). MSc. Thesis Fac. of Agriculture, Tanta University Egypt.

Larkin, P. and W. Scowcroft (1981). Somaclonal variation a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., 60: 197-214.

Leva, A. R., R. Petruccelli and L. M. R. Rinaldi (2012). Somaclonal Variation in Tissue Culture: A Case Study with Olive. Recent Advances in Plant *in vitro* Culture, 10-17.

Michelmore, R. W., I. Paran and R. Y. Kesseli (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating population. Proc. Natl. Acad. Sci., USA, 88: 9828-9832.

Pohar, K. S., M. C. Gong, R. Bahnson, E. C. Miller and S. K. Clinton (2003). Mutation induction and tissue culture in improving fruits. Plant Cell Tissue Organ Cult., 64: 185-210.

Sanputawong, S. and S. Techato (2011). Analysis of somaclonal variation of callus, somatic embryo and plant regeneration of *in vitro* oil palm (*Elaeis guineensis* Jacq.). J. Agric. Technol., 7: 531-545.

Shalaby, T. A. and A. S. El-Banna (2013). Molecular and horticultural characteristics of *in vitro* induced tomato mutants. J. Agric. Sci., 5: 155-163.

Singh, M., K. Chaudhary, H. Singal, C. Magill and K. Boora (2006). Identification and characterization of RAPD and SCAR markers linked to anthracnose resistance gene in sorghum (*Sorghum bicolor* L., Moench). Euphytica, 149: 179-187.

Soniya, E. V., N. S. Banerjee and M. R. Das (2001). Genetic analysis of somaclonal variation among callus-derived plants of tomato. Research communications. Current Science, 80: 1213-1215.

Tabassum, N., K. S. Sonia, K. B. Sujay and N. I. Mohammad (2013). Analysis of genetic diversity in eleven tomato (*Lycopersicon esculentum* Mill.) varieties using rapd markers. Plant Tissue Cult. and Biotech., 23: 49-57.

Wang, Z., J. Nagel, I. Potrykus and G. Spangenberg (1993). Plants from cell suspension derived protoplasts of *Lolium* species. Plant Science, 94: 179-193.

Table (1): Name of parental genotypes and its somaclones.

Somaclones	Parents			
	Super strain B	Castle Rock	Advanttage II	Edkawy
1	SS1	SC1	SA2	SE2
2	SS4	SC4	SA3	SE3
3	SS6	SC6	SA7	SE5
4	SS10	SC9	SA9	SE7

Table (2): Details of the used primers and their nucleotide sequences.

Primer name	Primer sequence (*5′-3′*)	Annealing temperature (°C)	Primer name	Primer sequence (*5′-3′*)	Annealing temperature (°C)
OPA01	5′ CAGGCCCTTC 3′	34	OPA07	5′ GAAACGGGTG 3′	32
OPA02	5′ TGCCGAGCTG 3′	34	OPA08	5′ GTGACGTAGG 3′	32
OPA03	5′ AGTCAGCCAC 3′	32	OPA09	5′ GGGTAACGCC 3′	34
OPA04	5′ AATCGGGCTG 3′	32	OPA10	5′ GTCATCGCAG 3′	32
OPA05	5′ AGGGGTCTTG 3′	32	OPB11	5′ GTAGACCCGT 3′	32
OPA06	5′ GGTCCCTGAC 3′	34			

Table (3): Survey of RAPD markers selected to detect somaclonal variations in Super Strain B (SB), Castle Rock (CR), Advanttage II (AII), Edkawy (ED) and their somaclones for primer OPA01.

Size (bp)	SB	SS_1	SS_4	SS_6	SS_{10}	CR	SC_1	SC_4	SC_6	SC_9	AII	SA_2	SA_3	SA_7	SA_9	ED	SE_2	SE_3	SE_5	SE_7
1500	+	+	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	-	-	-
1380	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	+	-	-	-
1200	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+
1000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
900	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
700	-	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+
600	-	+	+	+	-	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-
500	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
450	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
300	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
275	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
225	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	-	+	+	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-
Total	7	15	15	12	11	14	10	15	15	15	11	12	13	12	12	14	14	11	11	10

Table (4): Survey of RAPD markers selected to detect somaclonal variations in Super Strain B (SB), Castle Rock (CR), Advanttage II (AII), Edkawy (ED) and their somaclones for primer OPA02.

Size (bp)	SB	SS_1	SS_4	SS_6	SS_{10}	CR	SC_1	SC_4	SC_6	SC_9	AII	SA_2	SA_3	SA_7	SA_9	ED	SE_2	SE_3	SE_5	SE_7
1500	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
1200	-	+	+	-	-	+	-	+	+	+	-	-	+	+	-	+	+	-	-	-
1000	-	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	+	-	-	-
900	-	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+	-	-	-
800	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
700	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
600	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	-	-	-
550	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
500	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	-
400	-	+	+	-	-	+	+	-	+	+	-	-	+	+	-	+	+	-	-	-
365	-	+	+	-	-	+	+	-	+	+	-	-	+	+	-	+	+	-	-	-
300	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	-	-
250	-	+	+	+	-	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-
200	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Total	1	12	12	6	4	9	6	7	13	10	2	5	8	8	1	10	10	2	1	1

Table (5): Survey of RAPD markers selected to detect somaclonal variations in Super Strain B (SB), Castle Rock (CR), Advanttage II (AII), Edkawy (ED) and their somaclones for primer OPA03.

Size (bp)	SB	SS_1	SS_4	SS_6	SS_{10}	CR	SC_1	SC_4	SC_6	SC_9	AII	SA_2	SA_3	SA_7	SA_9	ED	SE_2	SE_3	SE_5	SE_7
1500	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1350	-	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-
1150	-	+	-	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-
1000	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-
900	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+
800	-	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+
700	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
600	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
500	-	+	-	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+
460	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
355	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
300	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
200	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Total	6	14	9	13	15	15	7	12	12	11	7	14	13	13	13	7	7	7	7	7

Table (6): Survey of RAPD markers selected to detect somaclonal variations in Super Strain B (SB), Castle Rock (CR), Advanttage II (AII), Edkawy (ED) and their somaclones for primer OPA04.

Size (bp)	SB	SS_1	SS_4	SS_6	SS_{10}	CR	SC_1	SC_4	SC_6	SC_9	AII	SA_2	SA_3	SA_7	SA_9	ED	SE_2	SE_3	SE_5	SE_7
1500	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1450	-	-	-	-	-	+	-	+	+	-	-	+	-	+	-	+	+	+	-	-
1250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
1000	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
900	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	-
800	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
700	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
500	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-
400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
350	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	+	+	+
300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Total	1	3	4	1	5	6	3	5	8	4	4	4	4	6	2	8	9	9	2	2

Table (7): Survey of RAPD markers selected to detect somaclonal variations in Super Strain B (SB), Castle Rock (CR), Advanttage II (AII), Edkawy (ED) and their somaclones for primer OPA05.

Size (bp)	SB	SS_1	SS_4	SS_6	SS_{10}	CR	SC_1	SC_4	SC_6	SC_9	AII	SA_2	SA_3	SA_7	SA_9	ED	SE_2	SE_3	SE_5	SE_7
1600	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	+	-	-	-
1500	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1150	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
900	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
800	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
700	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
600	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
550	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+
500	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+
400	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-	-	-	-
340	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	-	-	-	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
150	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Total	13	13	13	14	14	13	11	14	14	12	12	13	14	13	14	14	14	12	13	13

Table (8): Survey of RAPD markers selected to detect somaclonal variations in Super Strain B (SB), Castle Rock (CR), Advanttage II (AII), Edkawy (ED) and their somaclones for primer OPB11.

Size (bp)	SB	SS_1	SS_4	SS_6	SS_{10}	CR	SC_1	SC_4	SC_6	SC_9	AII	SA_2	SA_3	SA_7	SA_9	ED	SE_2	SE_3	SE_5	SE_7
2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
1500	-	-	+	+	-	+	+	+	-	+	-	+	+	+	+	-	-	-	-	+
1250	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1000	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
900	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
800	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
700	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
600	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
500	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
400	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
300	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
200	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
100	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
Total	7	8	9	12	8	11	11	11	10	11	10	12	12	11	12	5	7	7	8	9

Table (9): Distribution of RAPD markers among the four tomato cultivars and their sixteen selected somaclones.

Primer	Cultivar									
	Super Strain B					Advanttage II				
	T	M	P	U	P%	T	M	P	U	P%
OPA01	15	6	9	-	60.00	14	9	5	1	35.70
OPA02	12	1	11	-	91.60	9	1	8	1	88.89
OPA03	15	6	9	-	60.00	14	7	7	1	50.00
OPA04	5	1	4	1	80.00	7	2	5	2	71.43
OPA05	14	13	1	-	7.14	14	12	2	-	14.28
OPB11	12	6	6	2	50.00	13	10	3	2	23.57
Total	73	33	40	3	54.20	71	41	30	7	42.30
Primer	Castle Rock					Edkawy				
	T	M	P	U	P%	T	M	P	U	P%
OPA01	15	10	5	-	33.30	14	10	4	-	28.60
OPA02	13	4	9	3	69.23	10	1	9	-	90.00
OPA03	15	7	8	-	53.33	7	7	0	-	0.00
OPA04	8	3	5	-	62.50	9	2	7	-	77.78
OPA05	14	11	3	-	21.43	15	12	3	1	20.00
OPB11	12	9	3	1	25.00	9	5	4	1	44.44
Total	77	44	33	4	42.90	64	37	27	2	42.20

T- Total No. of band, M- Monomorphic, P- Polymorphic, U- Unique, P%- Polymorphism percentage.

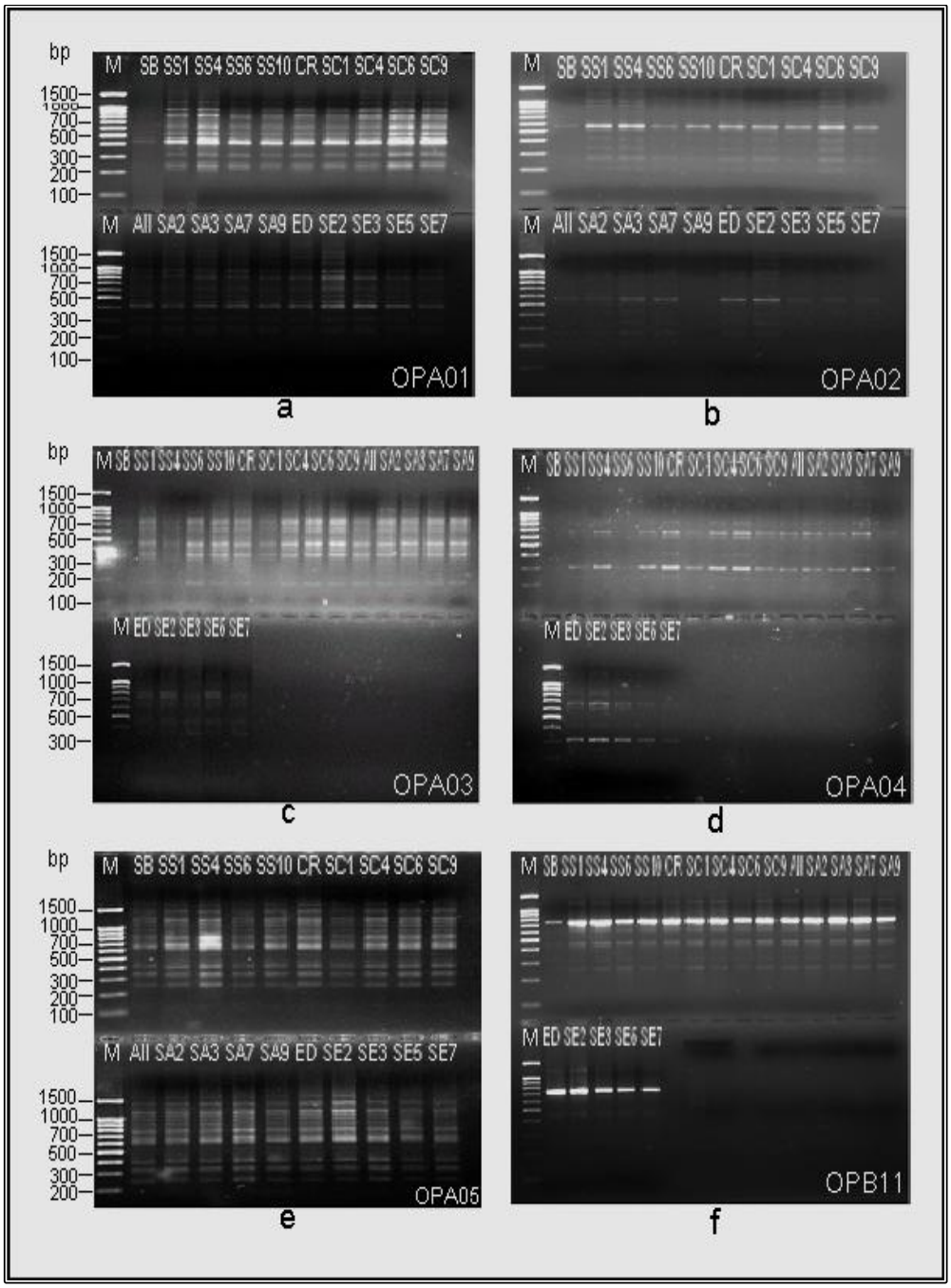

Fig. (1): RAPD banding patterns for: Super Strain B (SB) and its somaclones (SS1, SS4, SS6, and SS10), Castle Rock (CR) and its somaclones (SC1, SC4, SC6 and SC9), Advanttage II (AII) and its somaclones (SA2, SA3, SA7 and SA9) and Edkawy (ED) and its somaclones (SE2, SE3, SE5 and SE7). M: 100 bp ladder marker.