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Molecular Identification of Tomato Mutant Lines

Nasya Tomlekova[1*] • Svetla Yancheva[2] • Elena Balacheva[3] • Bistra Atanasova[3]

[1] Department of Breeding, Variety Maintenance & Introduction, Maritsa Vegetable Crops Research Institute, 32, Brezovsko shosse St., 4003, Plovdiv, Bulgaria
[2] Department of Genetics and Plant Breeding, Agricultural University, 12, Mendeleev Blvd., 4000, Plovdiv, Bulgaria
[3] Department of Applied Genetics, Institute of Plant Physiology and Genetics, bl. 21, Prof. G. Bonchev St., 1113, Sofia, Bulgaria

Corresponding author: * **nasia.tomlekova@gmail.com**

ABSTRACT

Molecular characterization of 7 tomato breeding lines (6 mutant and a parent line), maintained in the collection of Maritsa Vegetable Crops Research Institute, Bulgaria, was conducted. Irradiations on an initial genotype of *Solanum lycopersicum* L. performed by 250 Gy ^{137}Cs generated mutation causing late-flowering. Six tomato mutant lines were advanced to M_3 by induced mutation. In this study, both applied, AFLP and ISSR techniques were effective in assessing polymorphic patterns between the studied tomato mutant lines and the corresponding initial ones. The AFLP technique, which has the potential to provide valuable information in a number of areas, revealed discriminating polymorphism among tomato mutant lines and enabled their identification from the initial line with three primer combinations only. AFLP polymorphism was not related to the mutant character. Clear discrimination of the mutant in comparison with the initial tomato lines was revealed by using the ISSR technique. The bulk of the observed morphological evidence, corroborated by the molecular data in this study, indicated DNA variability in the tomato mutant lines.

Keywords: AFLP, flowering time, ISSR, mutagenesis, *Solanum lycopersicum*
Abbreviations: AFLP, amplified fragment length polymorphism; **cDNA**, complementary DNA; **EST**, expressed sequence tags; **FAO**, Food and Agriculture Organization of the United Nations; **IAEA**, International Atomic Energy Agency; **IBPGR**, International Board for Plant Genetic Resources; **ISSR-PCR**, inter simple sequence repeats-polymerase chain reaction; **KafTom**, (Kazusa full-length tomato cDNA database); **lf**, late flowering; **M**, mutant; **MiBASE**, micro-tom database; **SGN**, Solanaceae genomics network; **TBE**, tris/borate/ethylenediaminetetraacetic acid buffer

INTRODUCTION

Bulgaria is among the first ten countries in the world in vegetable production per capita of the population, based on the long-standing traditions of vegetable growing. The annual vegetable consumption per head is 200–215 kg, including 56% for fresh consumption in 2009. Many of the local varieties have been spread in the past by Bulgarian gardeners to other European countries – Hungary, Austria, Romania, Serbia, Russia, etc. Part of them were used as gene-carriers in the breeding programs for developing new varieties in these countries. Today researchers in the field of vegetable production are faced with more complex issues (Masheva *et al*. 2009; Mihov *et al.* 2009).

The tomato has become a model species for the study of developmental processes, particularly those related to fruit formation and ripening (Giovannoni 2004; Carrari *et al*. 2007; Giovannoni 2007; Seymour and Manning 2007; cited by Pineda *et al*. 2010).

Using available mutant genes, the knowledge about molecular biology and plant breeding is a solid basis for modifying the crop composition and developing superior genotypes in different crops (Ronen *et al*. 1999, 2000; Satomi 2012). Thus, several selective processes seem to determine the natural variation for flowering time in crop, but their relative significance and molecular targets are just beginning to emerge (Soppe *et al.* 1999; Jack 2004; Hanocq *et al*. 2007; Alonso-Blanco *et al*. 2009). The impact of the season on flowering time and the organization and morphogenesis of the reproductive structures are described in three tomato mutants: *compound inflorescence* (*s*), *single flower truss* (*sft*), and *jointless* (*j*), respectively, compared with their wild-type varieties (Quinet *et al.* 2006). In all environmental conditions, the *sft* mutant flowered significantly later, while the flowering time in *j* was delayed, and in *s* it was delayed in winter but not in summer, compared with the corresponding initial varieties, suggesting the existence of an environmentally regulated pathway for the control of floral transition. Over the past centuries, botanists have classified inflorescences based on their morphology, which has led to an unfortunate maze of complex botanical terminology. With the rise of molecular developmental biology, research has become increasingly focused on how inflorescences develop, rather than on their morphology. Inflorescence development depends on groups of stem cells at the growing tips of shoots, called meristems. Inducing and analysing mutants provide an excellent opportunity to study inflorescence development. The so-called transient model can produce broad inflorescence types: cyme, raceme, and panicle, into which most inflorescences found in nature can be classified (Castel *et al*. 2010).

The availability of causal genes and polymorphisms enables the study of the evolutionary processes that generate and maintain flowering time variation within and among species (Mitchell-Olds 1998; Roux *et al*. 2006). Detailed analyses indicate that some loci affect not only the heading date but several other fitness components, as shown with the effects of *Ghd7* on plant height, panicle size, and seed production (Xue *et al.* 2008). Current multilocus analyses of cultivated varieties are elucidating the role of flowering time allelic variation in regional adaptation and crop expansion over the several thousand years after domestication (Alonso-Blanco *et al*. 2003). Similar studies have been initiated in *Arabidopsis thaliana*, where in contrast with crop plants, several of the natural alleles identified are found in a single accession. Therefore, it is possible that they are rare deleterious alleles and their allelic variation might be maintained by various types of selection

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(Olsen *et al*. 2004), e.g. irradiation induction. Nine genes have been isolated that contribute to *A. thaliana* natural variation for flowering time. Most of the large number of loss-of-function alleles have an adaptive value, as they contribute to latitudinal and local adaptation complex phenotypic pleiotropy (Alonso-Blanco *et al*. 2003).

Due to the new requirements of breeders, it is essential to introduce new useful attributes for further genetic improvement of the genotypes (Mustilli *et al*. 1999; Abushita *et al*. 2000; O'Hare *et al*. 2005). Induced mutations have the capability to solve some of the existing problems in cultivated tomato (Atanassova *et al*. 2001). Some mutants of this species with better quality were produced by irradiation (Chopra 2005). A particular progress which holds new significance to the application of mutation technologies is plant molecular biology, which can now be integrated with conventional techniques in target selection of generated mutations and faster development of new genotypes (Bennetzen *et al*. 2001; Walker 2002). Amplified Fragment Length Polymorphism (AFLP) markers were generated by Keygene n.v. (Wageningen, The Netherlands) using the procedure of Vos *et al*. (1995). At present, there are high-density genetic maps for *Solanum lycopersicum*, based on amplification techniques (Tanksley 1993; Tanksley and McCouch 1997; Park *et al*. 2004). Genetic maps in tomato were summarized in the review article of Foolad (2007). A great part of the tomato genome has recently been released and is best represented at the Solanaceae Genomics Network web site (SGN, http://solgenomics.net/). Moreover, draft genome sequences of some wild relatives, large numbers of tomato expressed sequence tags (ESTs; 320,000 clones) and the full-length cDNA sequence (13,722 unigene clones) have been released at the SGN, MiBASE (http://www.pgb.kazusa.or.jp/mibase/) and KafTom (http://www.pgb.kazusa.or.jp/kaftom/) databases (Takeshy *et al*. 2011). Molecular biology offers a number of techniques for increasing efficiency in breeding process making it far more targeted and faster for developing modern plant varieties. A wealth of sequence information about tomato is now available. New developments such as the use of saturated mutant populations and advanced methods for mutant detection are bridging the gap between tomato genes and their functions. AFLP is a powerful technique, allowing rapid and reliable analysis of multiple, potentially polymorphic sites in a single experiment, requires no *a priori* knowledge of genome structure or preparation of molecular probes, and is immediately useful for a wide variety of plant species. AFLP offers highly informative patterns and the obtained results can be useful for scientific and practical purposes. AFLPs are the preferred choice of marker for fingerprinting, marker 'infilling' (Kahl *et al*. 2001). The application of the AFLP method simultaneously generates fragments from many genomic sites that are separated by gel-electrophoresis and generally yield highly informative fingerprinting profiles (Treuren *et al*. 2001). The conversion of AFLP fragments to forms better adapted to large-scale, locus-specific applications greatly expands the usefulness of this molecular technique (Bradeen *et al*. 1998; Budiman *et al*. 2000). However, because AFLP markers are dominant, costly and technologically demanding, the technique has limited application for large-scale, locus-specific uses (Mohan *et al*. 1997).

Variability can also be determined using different quick and simple DNA screenings such as Inter Simple Sequence Repeats (ISSR), based on tandem repeats, which represent a major part of variable regions of the plant genome. The power of AFLP and ISSR is based upon the molecular genetic variations that exist between closely related genotypes. These variations are exploited by both techniques such that 'fingerprints' of mutant genotypes can be generated showing changes occurred in DNA sequences (Noli *et al*. 1999; McGregor *et al*. 2000).

In this study we used induced mutations in tomato resources whose availability in the working collection promises to result in important developments. The implementation of molecular characterization with discriminating capability in tomato was the key issue in this research. The establishment of DNA polymorphism based on AFLP and ISSR patterns among mutant tomato lines and the corresponding initial lines was the aim of the present investigation.

MATERIALS AND METHODS

Experiment to obtain mutant lines

1. Plant material, irradiations

An initial tomato line 'HD', a local genotype from the working tomato collection of the Institute of Plant Physiology and Genetics, determined as mid-early, suitable for field production, with square-round fruit and a green ring. Seeds of the 'HD' line were irradiated in the Dobrudza Agricultural Institute by 150 Gy, 250 Gy, and 350 Gy ^{137}Cs gamma rays. The mutant used for this study was generated by the radiation dose of 250 Gy ^{137}Cs.

The selection of mutant characters was conducted in M_2 and verified in M_3 generation. The M_2 seeds for the next generation were gathered from the altered forms and sown at different time from the beginning of March to the end of April together with control seeds from the parent 'HD' line. Six different mutant lines, originating from the same mutant, were developed to M_3 by conventional breeding methods.

The morphological traits of irradiated populations were characterized following a descriptor list derived from the IPBGR for *Solanum* (IPBGR 1996) and the altered forms were selected. The parental and the mutant tomato lines were used for molecular study. Two additional genotypes of *S. lycopersicum* were used to confirm the radiation-induced nature of the mutation (line 'Adelina' and variety 'Odyssey') from the Bulgarian tomato collection, two Indian varieties (T1210 and T1224- Zuari Seeds Limited, Bangalore) and two commercial varieties ('Rosa' and 'Luch'), brought from Kazakhstan.

2. Sample preparation

2.1. Lyophilization

Leaves from the studied plants were lyophilized in the phase of first true leaf. Lyophilization for keeping longtime DNA native in the studied leaf material was performed under the following conditions – freezing of the material was at –18°C for 24 h, followed by increasing the temperature for 48 h under vacuum –0.2 to –0.4 Torr (26 – 53 Pa) to a maximum warming of 40°C. Drying dependencies - 2 g of dried material was obtained from 40 g fresh material (for 5% dry matter content).

2.2. Isolation of DNA

Isolation of DNA was performed using Nucleon PhytoPure kits (Amersham Biosciences (GE Healthcare), Uppsala, Sweden) from 0.033 g lyophilized leaves. The protocol was high yielding and assured 300-500 ng DNA per sample.

3. Molecular characterization

3.1. Molecular characterization by using AFLP

AFLP was conducted according to the protocol of Kashkush *et al*. (2001), involving:

Restriction/ligation: The reaction was performed on 0.5 µg genomic DNA using the enzymes: *Eco*RI (a 6-base rare cutter), *Mse*I (a 4-base frequent cutter), purchased from VBC-Genomic, Vienna, Austria. Adapter pairs were ligated onto the ends of the restriction sites: *Eco*RI adapter pair: *E*-adapter-olig1: 5′-CTCGTA GACTGCGTACC-3′, and *E*-adapter-olig2: 5′-AATTGGTACGCA GTCTAC-3′, and *Mse*I adapter pair: *M*-adapter-olig1: 5′-GACGAT GAGTCCTGAG-3′, and *M*-adapter-olig2: 5′-TACTCAGGACTC AT-3′. The adapters were purchased from VBC-Genomic, Vienna, Austria and designed by Kashkush *et al*. (2001).

Table 1 AFLP selective primers used for the amplifications in the studied tomato lines.

Selective nucleotides for *Eco*RI and *Mse*I	Total sequences of selective primers
Eco-AAG	GACTGCGTACCAATTCAAG
Eco-AAC	GACTGCGTACCAATTCAAC
Eco-AGG	GACTGCGTACCAATTCAGG
Eco-AGC	GACTGCGTACCAATTCAGC
Eco-ACT	GACTGCGTACCAATTCACT
Eco-ACC	GACTGCGTACCAATTCACC
Eco-ACG	GACTGCGTACCAATTCACG
Eco-ACA	GACTGCGTACCAATTCACA
Mse-CAG	GATGAGTCCTGAGTAACAG
Mse-CTT	GATGAGTCCTGAGTAACTT
Mse-CAA	GATGAGTCCTGAGTAACAA
Mse-CAT	GATGAGTCCTGAGTAACAT
Mse-CTC	GATGAGTCCTGAGTAACTC
Mse-CAC	GATGAGTCCTGAGTAACAC
Mse-CTA	GATGAGTCCTGAGTAACTA
Mse-CTG	GATGAGTCCTGAGTAACTG

Table 2 ISSR primers assayed for amplifications in the studied tomato lines.

Primers explored	5` anchor	Tandem sequences	3` anchor
ISSR1		(CA)6	(AG)(CT)
ISSR2		(CA)6	(AG)G
ISSR3		(AGC)4	(CT)
ISSR4		(AGAC)6	
ISSR5	(AC)	(GACA)4	
ISSR6		(GACA)4	(AC)
ISSR7		(GACA)4	(CA)
ISSR8	(CAG)	(CT)8	
ISSR9	(CG)(CA)	(AC)10	
ISSR10	(GCC) (ACG)	(CTG)6	
ISSR11	(CGC) (ACA)	(AGA)5	
ISSR12		(AG)8	(CT)C

no – smeared DNA after ISSR-PCR
yes – bands with good resolution (polymorphic or monomorphic)

Preselective amplification: Primer pairs were used to amplify a subset of the restriction fragments using *E*-pre-primer: 5′-GAC TGCGTACCAATTCA-3′ and *M*-pre-primer: 5′-GATGAGTCC TGAGTAAC-3′ (purchased from USB chemicals, Cleveland, Ohio, USA).

*End labeling: Eco*RI-based primers were labeled with γ-ATP-P^{32} for radioactive visualization of the results.

Selective amplification: The pre-selective products of the parent and mutant lines were amplified with ACT/CAT; ACA/CAT; AGG/CTT; ACT/CAG; ACC/CAC; AAC/CTG; AAG/CAA listed in **Table 1**. Seven of the selective primer combination pairs of *Eco*RI/*Mse*I tested in previous studies (Tomlekova and Atanasova 2009) were chosen for the present study.

Electrophoresis: The results were visualized on Polyacrilamide gel (PAAG) (USB chemicals, Cleveland, Ohio, USA).

3.2. Molecular characterization by using ISSR-PCR

This technique was performed using DNA amplification primers, purchased from USB chemicals, Cleveland, Ohio, USA. Previously (Tomlekova 2006), ISSR primers were screened for amplifications in different genotypes of Solanaceous crop species from our working collection and twelve were selected for the present study of tomato genotypes. For each result, at least two repetitions were performed using DNA from different isolations. The ISSR primers (VBC-Genomic, Vienna, Austria) were used for screening for polymorphisms between the parental 'HD' and the mutant 'M_{lf}' genotypes (**Table 2**). ISSR-PCR reactions were performed in 25 µl volume containing 25 ng of template DNA. Amplification conditions were: an initial denaturation at 94°C for 4 min and 1 cycle of 94°C for 30 sec, 54°C for 45 sec and 72°C for 2 min. A touchdown PCR was performed by stepwise reduction of 1°C in annealing temperature from 55 to 50°C in the first 5 cycles. In the subsequent 30 cycles, the annealing temperature was maintained at 50°C. The final elongation was conducted at 72°C for 7 min.

Electrophoresis: For visualization of the ISSR profiles, the amplified products were separated on 2% agarose, high gel strength in 1x TBE buffer (USB chemicals, Cleveland, Ohio, USA) at 70 V. Lambda DNA-*Hind* III Digest (USB chemicals, Cleveland, Ohio, USA) was used for molecular weight standards. The gels were stained with ethydium bromide solution (0.0005%) (VBC-Genomic, Vienna, Austria) and photographed with a gel documentation system.

Fig. 1 Time flowering mutant 'M_{lf}' in M_3 generation obtained using ^{137}Cs.

RESULTS AND DISCUSSION

Inducing of a late-flowering mutation by irradiation

As a result of the mutagenic treatments of tomato seeds, irradiation with 250 Gy ^{137}Cs, a 14-day late-flowering mutant plant was observed in the M_2 population, when selection of mutations was performed (**Fig. 1**). The mutant plant did not show any morphological differences in comparison to the parental plants. From the 'M_{lf}' flowering time, mutant plant observed in the M_2, and 6 mutant lines were developed in the M_3 generation that flower later than the parental genotype. The radiation showed a strong effect on flowering time with no other visible effects on morphology or development. All the results obtained in the M_3 plants showed a delay in flowering that did not depend on the sowing time.

Two widely utilized marker techniques were assessed for their efficacy in detecting polymorphism between the ^{137}Cs-induced mutants and the parental genotype. As a result of the conducted molecular study, DNA polymorphism was established between the tomato mutants and the corresponding parental line.

Detecting DNA polymorphism by the obtained AFLP patterns

By using the AFLP technique, seven selective primer combinations were tested. The four primer combinations with the highest number of polymorphic bands and the best reproducibility were tested in order to compare the DNA samples. The selective primer combinations of AFLP, which showed polymorphic patterns in the late flowering mutant plants and differentiated between them and from the plants of the initial line, were:

I-ACT/CAT, II-ACA/CAT, III-AGG/CTT and IV-ACT/CAG (**Fig. 2**).

The selected mutant lines also differed between one another by 1-3 DNA fragments per each pattern. The total number of DNA fragments within the polymorphic patterns that differentiated the 'M_{lf}' mutants from the initial line was from 8 to 13 (**Table 3**).

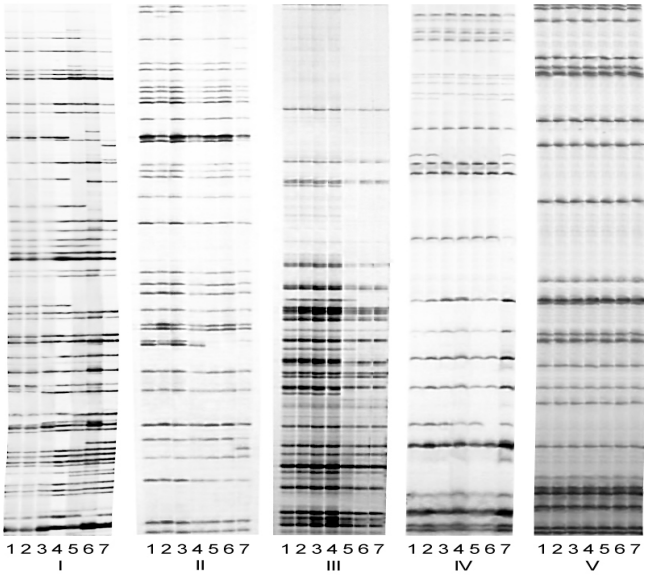

Fig. 2 AFLP patterns in tomato lines obtained by selective primer combinations I - ACT/CAT; II - ACA/CAT; III - AGG/CTT; IV - ACT/CAG; V - ACC/CAC. Sample order on each gel: 1. 1st 'M_{lf}' line 2. 2nd 'M_{lf}' line, 3. 3rd 'M_{lf}' line, 4. 4th 'M_{lf}' line, 5. Parental line 'HB', 6. 5th 'M_{lf}' line, 7. 6th 'M_{lf}' line.

Table 3 Established AFLP polymorphism between parental and mutant tomato lines.

Start No on gel	Lines	Polymorphic bands between mutant line in M_3 greneration and parent per pattern				Total polymorphic bands
		Patterns				
		I-ACT /CAT	II-ACA /CAT	III-AGG /CTT	IV-ACT /CAG	
1	1st 'M_{lf}'	7	2	2	1	12
2	2nd 'M_{lf}'	7	3	2	1	13
3	3rd 'M_{lf}'	6	2	2	2	12
4	4th 'M_{lf}'	5	1	2	2	10
5	Parent					
6	5th 'M_{lf}'	5	0	2	1	8
7	6th 'M_{lf}'	4	1	2	1	8
Total scored bands		50	37	34	26	

No variations in the AFLP patterns using the rest of the assayed primer combinations were established. An example for monomorphic pattern is shown with V-ACC/CAC primer combination in **Fig. 2**. The selective combinations produced for all lines identical patterns to the initial line used for irradiations.

In total, 1890 bands generated by 7 selective primer combinations were scored, and 63 polymorphic bands were established in all studied mutant lines. The percentage of polymorphic AFLP bands was 3.3 % in the mutant lines as compared to the parental line.

The analyses performed were effective for obtaining unique fingerprints for each mutant line and assessing variability in the AFLP patterns. Nonetheless, the established polymorphism in this study, using the obtained AFLP data, could not be related to the mutant character.

Detecting DNA polymorphism by the obtained ISSR patterns

The ISSR-PCR technique was performed on all genotypes included in this study and polymorphism was shown in only one of the patterns of the studied mutant that can be related to the observed character altered after irradiation. A polymorphic band present in the initial 'HD'line and absent in the mutant 'M_{lf}' line was established with one of the applied primers 5'-$(CA)_6$(G/A)(C/T)-3' within all studied mutant

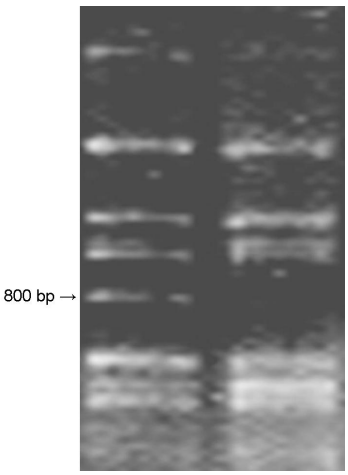

Fig. 3 ISSR1 polymorphism of 800 bp band in tomato lines obtained by primer 5'-$(CA)_6$(G/A)(C/T)-3'. Sample order: 1. 'HD' parent, 2. 'M_{lf}' line.

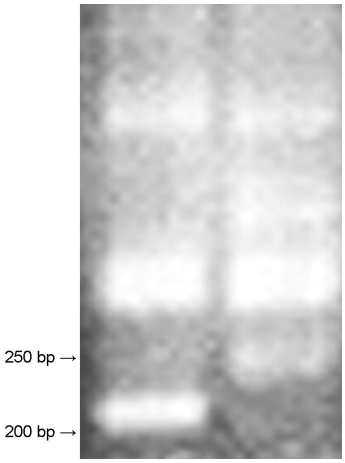

Fig. 4 ISSR1 polymorphism of 200 bp and 250 bp bands in tomato lines obtained by primer 5'-$(CA)_6$(G/A)(C/T)-3'. Sample order: 1. 'HD' parent, 2. 'M_{lf}' line.

lines. 'M_{lf}' differed from the initial line in all studied M_2 plants by one band, with approximate length of 800 bp, among 11 well reproducible bands that could be scored. The pattern of the late-flowering mutant line 'M_{lf}' differed from the non-irradiated 'HD' initial line by other DNA fragments, too, as shown in **Table 3**, however, their reproducibility was not stable within the studied mutant lines. Polymorphism between the late flowering mutant and the initial line was revealed.

A representative gel profile of three polymorphic DNA fragments using primer 5′-$(CA)_6$(G/A)(C/T)-3′ is shown in **Fig. 3** and **Fig. 4**.

Six polymorphic amplicons were observed in the case of the ISSR reactions conducted for the six M_3 mutant lines: with primer ISSR1 (3 polymorphic bands of 11 scored, shown in **Fig. 3** and **Fig. 4**), ISSR5 (2 polymorphic bands of 11 scored) and with ISSR7 (1 polymorphic band of 6 scored). Eighty bands generated by nine selective primer combinations were scored per each line, and 2 polymorphic bands within all were established. Thus, the ISSR polymorphism observed for the six analysed mutant lines compared to the parental one is higher (7.5%) as compared to the AFLP technique (**Table 4**).

In order to confirm that the polymorphism detected in ISSR1 and ISSR7 patterns was related to the mutation and it was not due to a spontaneous variation, the polymorphic ISSR primers were tested on all six lines produced in M_3 generation. Six to ten individuals from each line were analyzed.

Table 4 ISSR primers assayed for amplifications in the studied tomato genotypes.

Primers explored	Successful amplification in the mutant tomato lines and parent	Number of polymorphic bands per line	Total number of reproducible bands	Successful amplification in other tomato genotypes from our previous study	Successful amplification in pepper from our previous study
ISSR1	yes	3	11	yes	no (yes)*
ISSR2	no	-	-	no	no (yes)*
ISSR3	no	-	-	(yes) *	yes
ISSR4	yes	0	8	yes	yes
ISSR5	yes	2	11	yes - main in tomato	yes
ISSR6	yes	0	11	yes	yes
ISSR7	yes	1	6	yes	yes
ISSR8	yes	0	11	yes	yes
ISSR9	yes	0	6	yes	yes
ISSR10	yes	0	8	yes	no (yes)*
ISSR11	yes	0	8	yes	no (yes)*
ISSR12	no	-	-	no	yes

no – smeared DNA after ISSR-PCR
yes – bands with good resolution (polymorphic or monomorphic)
(yes)* - Data established in *Capsicum annuum* in our previous study were with different anchors of the primers (unpublished results)

In addition, the same polymorphic primers were tested on another six, randomly chosen tomato accessions collected from different regions – a variety and a line from Bulgaria, two commercial varieties from Kazakhstan and two lines from India. No polymorphism observed between the *S. lycopersicum* genotypes. In contrast, a reproducible polymorphism between the radiation-induced mutants and the initial line, as well as between the mutant lines and the genotypes collected from different locations, was established. Thus the existence of variations of microsatellites in the M_3 ^{137}Cs radiation-induced mutants was observed. The mutant lines will be further developed to the next mutant generations. The stability of the polymorphic ISSR markers over generations is to be studied in a genetic cross between the initial line 'HD' and the advanced mutant lines 'M_{lf}', and cloned for developing a SCAR marker.

Microsatellites have an inherent spontaneous instability due to slippage during replication and unequal recombination involved in the susceptibility of these loci to irradiation-induced mutagenesis (Bridges 2001). The genetic stability of the tandem replications decreases after radiation induction, an event whose nature is not yet clarified (Bouffler *et al*. 2006). In some cases the instability within the replications is found to influence gene function (Armour 2006). Deletions that frequently occur after radiation also involve tandem replications (Joshi-Saha *et al*. 2007). At this stage of the study, a deletion of microsatellite loci can be hypothesized in the late-flowering ^{137}Cs irradiation-induced mutant. Another study using ISSR a late-flowering mutant of *Sesbania rostrata* indicated that an ISSR amplicon was linked to the flowering time trait (Joshi-Saha *et al*. 2007). The non-coding tandemly repeated regions are functionally neutral, as they are not associated to coding regions of the genome (Morgante *et al*. 2002), but they can be effectively used in mutants to indicate the mutation events. As an advantage, the ISSR compared with the AFLP is an uncomplicated technique for large populations screening.

The evaluation of the comparison methodology as well as the screening procedures revealing polymorphism highly depends upon the genetic potential of the material. In applying ^{137}Cs irradiation as a mutagenic factor, point mutations frequently occur. Among the great range of routine techniques, ISSR is applied as a quick, inexpensive method. AFLP is more powerful exhibiting the restriction sites polymorphism. Applied to the studied material, both techniques revealed polymorphism in the mutants. The AFLP technique, having the potential to provide valuable information in a number of areas, made possible to establish DNA variability among tomato lines and enabled their identification with four primer combinations only. The technique is sensitive and capable to detect differences among genetically closely related lines even among individuals.

Induced mutation factors usually affect more regions all over the genome. Only a small part of the mutations is expressed as a change of a character, which can be detected as a different phenotypic trait. The polymorphism between the different mutant lines established by the AFLP patterns was not related to the mutant character. The polymorphic fragments amplified in the 'M_{lf}' that differentiated them from the parental genotype, changes at DNA level after irradiation were established. Most mutant individual plants possessed identical AFLP patterns and differed from the initial non-irradiated plants with the same bands.

Genetic variation is essential to the plant breeder as the basis for crop germplasm improvement and development of new varieties (Petkova *et al*. 2009; Saito *et al*. 2011). Unique fingerprints were obtained by four out of seven AFLP primer pairs, despite the fact that all investigated lines were closely related. The polymorphism estimated by applying AFLP between the initial parent line and different mutants and the appearance of altered characters, showed the potential of irradiation for induction of genetic variability in the initial tomato line by using the selected dose of ^{137}Cs radiation.

The physiological evidence observed, corroborated by molecular data obtained in the present study, indicated polymorphism of the tomato mutant lines. The genetic diversity in breeding materials can be assessed by morphological, biochemical, and horticultural traits, pedigree record analysis, and DNA fingerprinting with molecular markers (Park *et al*. 2004).

DNA fingerprinting is an ideal tool for assessing induced genetic diversity in early mutant generations as it was done in this study. It can be useful in aiding genotype identification at DNA level without being influenced by environmental conditions, epistatic and pleiotropic effects or complex pedigree records like characters that are highly dependent on the environment.

The narrow genetic basis of cultivated tomato is due to a combination of genetic bottlenecks during domestication and high selection pressure for desired phenotypes within a limited germplasm pool, coupled with a high degree of self-pollination (Rick 1976; Miller *et al*. 1990). Traditionally, crop breeding is conducted using specific parental matings, followed by selection of superior recombinant individuals among the segregating progeny. These methods have been widely used to group germplasm with similar genetic constitution and identify parental combinations that could potentially create maximum genotypic variability in their progenies to achieve selection advance. However, the combination of available genes for target characters in germplasm pool restraints the genetic basis for variety improvement through conventional methods. When the genetic basis is reduced or even lost, one way to recover it is by the induced mutagenesis approach. The results obtained in this study proved that the genetic make-up of the tomato collection was amenable to change by induced mutagenesis.

The ISSR polymorphism estimated between the paren-

tal and different mutant lines and the appearance of changed characters revealed the potential of induced mutagenesis to create genetic variability in *S. lycopersicum* species. The established DNA polymorphism in the late-flowering mutant is useful for future investigations of the tomato genome towards SCAR marker (Joshi-Saha *et al*. 2007). The tomato has been selected as a core model plant for accelerating genomic studies in the Solanaceae family, and its genome is being sequenced by The International Solanaceae Genomics Project (SOL) (Mueller *et al*. 2005; Mueller *et al*. 2009; cited by Saito *et al*. 2011). The accumulation of new substantial information for assessing changes in the mutant genomes will impact the development of advanced mutant lines and varieties. The molecular genetic data of tomato will be further applied in other members of solanaceous food crop family.

CONCLUSIONS

A flowering time altered trait was induced by 250 Gy ^{137}Cs mutagenic treatment and 6 mutant lines were developed to M_3 generation. Polymorphism between mutant lines and the parental line was revealed by AFLP and ISSR markers.

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