



Signatures of Osmotic Stress-Responsive Genes in Tomato (*Lycopersicon esculentum* L.) Somaclonal Variant Cells

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ABSTRACT

This study was conducted to analyze the osmotic stress-induced polymorphism in the two stress-responsive genes [TSW12 (non-specific lipid transfer protein1) and TAS14 (abscisic acid and environmental stress-inducible protein)] of somaclonal variant cells of tomato (*Lycopersicon esculentum* L.). The cells were subjected to the sublethal concentration of mannitol (250 mM) and the mutations of TSW12 and TAS14 genes were investigated by PCR using specific (upstream and downstream) primers for each gene. The amplification products were sequenced to uncover the mutations. Mutation effect on protein structure and function was predicted using SIFT PolyPhen-2, PANTHER, and PredictSNP tools. The results showed nine mutations in TSW12 and TAS 14 genes (6 and 3, respectively), and 7/9 were SNPs while the other two are frameshift mutations. Among them, a couple of SNPs mutations was novel; 104C>T on TSW12 and 146G>C on TAS14. Prediction analysis of the 3D structure of the amino acids showed that most SNPs were benign. The subjection of somaclonal variant cells to drought stress resulted in slight genetic modifications of the two investigated responsive genes, and therefore, neglected changes of corresponding proteins. This result demonstrated that mutations reported in the examined genes may have a minimal effect on the role of these genes in controlling drought injury in tomato.

1. Introduction

Drought is one of the most dominant environmental disastrous abiotic stresses that reduce the quantity and quality of crop yield, particularly in arid and semiarid areas. Drought entails water deprivation for a period long enough to cause moisture depletion in soil with a decrease of water potential in plant tissues [1]. Drought resistance is not well understood because it is a quantitative trait controlled by several genes and highly influenced by the environment [2]. Tomato plant is one of the most beneficial crops in the world and its fruit is widely consumed [3]. This plant usually requires a high water availability for ideal growth, as its

productivity and growth are considerably affected by drought stress [4, 5]. Many strategies have been used to develop tomato tolerant to abiotic stresses, including conventional breeding, genetic modification, and selection of somaclonal variants [6].

In vitro selection of somaclonal variants is beneficial as it diminishes greatly the time for the selection of favorable qualities without substantial environmental intervention [7]. Some reports were found on the use of *in vitro* selection of somaclonal variants to generate drought-tolerant tomato plants [8–11]. *In vitro* selection of somaclonal variants can be attained by subculturing the *in vitro* grown callus or cell suspension under

severe desired stressful conditions. The surviving cells under such an environment are somaclonal variants that will be able to develop genetic or epigenetic changes [12]. The problem with somaclonal variants is that when those changes are genetic it's heritable, and when it is epigenetic it is not heritable and the efforts invested in the selection for tolerance, in the latter case, are in vain. Therefore, it is necessary to assess the *in vitro* produced somaclonal variants to ensure their heritability to the next generations. Screening of different tomato cultivars selected *in vitro* for drought tolerance was achieved by measuring growth parameters, regeneration, shoots number, yield, proline content, peroxidase activity, isozymes, and RAPD analysis [8,10,11,13–15]. Yet the usage of specific primers to assess the type of mutation responsible for the resistance of *in vitro* developed tomato somaclonal variants has not been accomplished so far to the best of our knowledge.

Sacco et al. (2013) [16] sequenced the exons of stress-responsive genes and analyzed their polymorphism within 27 tomato genotypes in response to water deficit conditions. They studied polymorphisms in six stress-responsive genes (ARS2, MKP1, CIP1, TSW12, TAS14, and ERD15) in leaf samples of tomato plants subjected to different abiotic stresses. They acquired the sequences of these six genes from the Heinz 1706 complete tomato genome sequence at the Solgenomics website (www.solgenomics.net).

TSW12 gene was isolated and characterized by **Torres-Schumann et al. (1992)** [17]. They suggested that this gene encodes for one of the non-specific lipid transfer proteins (nsLTP) and added that it is expressed in stems of tomato in response to abiotic stress and abscisic acid (ABA). Non-specific lipid transfer proteins are short-chain polypeptides that facilitate the intermembrane transfer of different phospholipids, glycolipids, and sterols, with shreds of evidence for possible involvement in cutin transport during cuticle biosynthesis [18].

The sequence of wild type TSW12 gene, located on chromosome 10 (NC_015447.2) of tomato genome, has two exons; exon 1 (1-422) and exon 2 (687-916), and its ORF is spanning 343 base pairs (88-422 and 687-696) encoding for non-specific lipid transfer protein 1 precursor (NP_001234074.1) with 114 amino acids, in which the first 23 amino acids exhibit the sequence characteristic of a signal peptide [17].

TAS14 gene (abscisic acid and environmental stress-inducible gene) of *L. esculentum* is located on chromosome 2 (NC_015439.2) and contains two exons; exon 1 (1-278) and exon 2 (497-964), where its CDs is 390 base pair (48-278, and 497-658), two satellite sequences of 26 bases in exon 1. It encodes for abscisic acid and environmental stress-induced protein (NP_001234038.1) with 131 amino acids and starting ATG codon on exon 1 [19]. **Godoy et al. (1990)** [20] distinguished over-expression of TAS14 gene in the perivascular and vascular tissues of stems and leaves of tomato under treatment with salinity, mannitol, and ABA. They found that coding protein had highly hydrophilic properties and contained a glycine-rich (23.8 %) polypeptide. Moreover, the study conducted by **Muñoz-Mayor et al. (2012)** [21] showed that transgenic tomato over-expressing TAS14 gene exhibited improved long-term tolerance to drought and salinity without affecting plant growth under non-stressful conditions. Those plants were found to redistribute toxic Na⁺ between young and adult leaves and to achieve osmotic adjustment by accumulating solutes like sugars and K⁺ as mechanisms of stress tolerance.

The aim of the present work was to investigate genetic polymorphism in two stress-responsive genes (TSW12 and TAS14) in somaclonal variant cells of tomato (*Lycopersicon esculentum* L. var. super strain B). These cells were selected following the exposure of tomato plant to a sub-lethal dose of mannitol (250mM) as an osmotic stress agent. Moreover, the potential changes in protein structure and function associated with this polymorphism were predicted using SIFT PolyPhen-2, PANTHER, and PredictSNP.

2. Materials and Methods

2.1 Establishment of callus culture

Tomato (*L. esculentum* L. var. super strain B) seeds were obtained from Bonanza Seeds International, Inc., California, USA. All chemicals were of tissue culture grade, and all plant growth regulators were obtained from Sigma Chemical Company. Seeds of similar size, shape, and color were sterilized with commercial Clorox (50%) and washed three times with sterilized distilled water then inoculated on MS medium supplemented with 2.69 µmol naphthalene acetic acid (NAA), 2.84 µmol indole acetic acid (IAA), 8.88 µmol benzyl aminopurine (BAP), and 9.29 µmol kinetin (Kn)

(callus induction medium, CIM) [11].

The medium was supplied with 30g/L sucrose as a carbon source. The pH of the medium was adjusted to 5.8 ± 0.1 , using 0.1 N HCl or 0.1 N NaOH, before the addition of 10g/L agar as a gelling agent. Cultures were incubated for four weeks at $25 \pm 1^\circ\text{C}$ and continuous light of $35\mu\text{mol m}^{-2} \text{s}^{-1}$. Sub-culturing of the induced callus was carried out every 4 weeks on the same medium as a control callus line.

2.2 Selection of somaclonal variants tolerant to osmotic stress

The proliferated calli were sub-cultured on solidified MS medium containing the same growth regulators mentioned above (CIM) in addition to elevated concentrations of mannitol as an osmotic stress agent (50, 100, 150, 200, 250, and 300 mM). Cultures were incubated at $25 \pm 1^\circ\text{C}$ and continuous light for 3 weeks. The lethal concentration of mannitol which killed all the callus cells and the sub-lethal concentration which killed about 80% of cells (ID80, inhibition dose) were determined according to **Montaser et al. (2018)** [11]. All callus cells died at 300 mM mannitol (a lethal concentration). Whereas 250mM mannitol killed about 80% of callus cells (a sub-lethal concentration), the remaining survived cells (20% of cells) were considered somaclonal variant cells (SVC) tolerant to osmotic stress. Those cells were selected and sub-cultured on the same sub-lethal concentration, then transferred to a stress-free medium, and back again to the sub-lethal concentration, each passage took four weeks [11]. These steps were necessary to ensure that they are true mutant cells (somaclonal variant cells).

The somaclonal variant cells and control calli cells were photographed to compare the morphological characterization of each. The SVC cells were immediately collected and frozen at -20°C for genetic analysis. To evaluate the genetic polymorphism in TAS14 and TSW12 genes in response to osmotic stress induced by 250mM mannitol, DNA was extracted from twelve replicates of SVC and control calli.

2.3 DNA extraction and quantification

Genomic DNA was extracted and isolated according to the CTAB method [22], with some optimization for plant material. Approximately, 200 mg of calli was ground to a fine paste with 500 μl of CTAB buffer using a mortar and pestle. The CTAB/plant extract mixture was

transferred to a microcentrifuge tube and was incubated for 15 min at 55°C in a circulating water bath (Water Bath Shaker, TFT Display, SHK-W100, Bioveopeak, China). After incubation, the mixture was spun at 12000g for 5 min, and 300 μl of the supernatant was transferred to clean microcentrifuge tubes. To each tube, 300 μl of chloroform: isoamyl alcohol (24:1 v/v) was added and the solution was mixed by inversion, the tubes were then spun at 13000g for 1 min. The upper aqueous phase (containing the DNA) was transferred to a clean microcentrifuge tube, and 30 μl of 7.5 M ammonium acetate was added followed by the addition of 750 μl of ice-cold absolute ethanol. DNA was precipitated by inverting the tubes slowly several times and placed for 1 h at -20°C , the tubes were then centrifuged at 13000g for a minute to form pellets. The supernatant was then removed, and the DNA pellets were washed by adding two changes of ice-cold 70% ethanol, finally centrifuged at 13000g for 1 min, and the supernatant was discarded. Purified DNA was resuspended in 100 μl sterile DNase-free water, and stored at 4°C . DNA was quantified by double-beam UV-vis spectrophotometry and agarose gel electrophoresis according to **Sambrook et al. (1989)** [23].

The concentration and purity of DNA were measured spectrophotometrically (double beam spectrophotometer, Thermo Fisher Scientific, USA) according to manufacturers' instructions [24]. The purity of the samples was determined by measuring the absorbance at wavelengths 260 and 280 nm. The concentration was automatically calculated based on the optical density (OD) of 1 corresponding to approximately 50 $\mu\text{g}/\text{ml}$ for double-stranded DNA, and confirmed with the following equation:

$$\text{DNA concentration } (\mu\text{g}/\mu\text{l}) = \text{Absorption} \times \text{dilution factor} \times 0.05$$

The DNA was considered pure when OD260/OD280 ratio is ≥ 1.8 .

2.4 DNA amplification and purification

Amplification of all DNA fragments of the TSW12 and TAS14 genes was done using specific (the upstream and downstream) primers designed by the Primer-BLAST Table 1. The amplification of the exons of each of the two genes was performed, according to **Saiki et al. (1985)** [25], in a 25 μl volume containing 10 mmol

Tris-HCl, pH 8.3; 50 mmol KCl; 1.5 mmol MgCl₂; 200 μmol dNTPs; 250 μmol each primer; 0.25 U Taq polymerase (Fermentas); and 2 ng DNA. The cycling conditions were 5 min at 94 °C followed by 35 cycles consisting of 30 s at 94 °C, 45 s at 55 °C and 1 min at 72 °C, finally, post-extension cycle for 10 min at 72 °C. Amplification was carried out using (Perkin-Elmer-USA) thermocycler.

PCR reaction products were purified by using a PureLink™ PCR purification kit (Invitrogen, USA) following the manufacturer's protocol. Amplified products were analyzed by electrophoresis in 1.5% agarose gel run in 1X TAE buffer containing 0.7% ethidium bromide and viewed under a UV transilluminator and photographed (UView™ Mini Transilluminator #1660531. Bio-Rad USA).

Table 1 Oligonucleotide sequences of primers used for mutation screening

Gene	Primer's sequence	Primer Length	Ta (°C)	Position	PCR (bp)
TSW12 F	5`CATACTATACTCTTTACTATAAT3`	23	57 °C	47-69	420
TSW12 R	5`TCTCCTCAAATGGTTCCATATTCT3`	24		467-444	
TAS14 F	5`ACTTGAATGTTATCTGCAGTAGCC3`	24	57 °C	9-32	500
TAS14 R	5`CATGAAATAGAATCAAGCACTACCT3`	25		506-482	

2.5 DNA sequencing and analyzing

The purified amplicons were sequenced with chain-terminating inhibitors, according to **Sanger et al. (1977)** [26] on an ABI 3730xl DNA Sequencer (Applied Biosystem, USA). All amplicons were sequenced in both forward and reverse directions. Sequencing was done in 96-well plates in 10 μl sequencing reactions containing; 2 μl of purified PCR product, 0.5 μl of BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), 2 μl of 5X sequencing buffer, 5 μl of water, and 0.5 μl of sense or anti-sense primer. Analyses of sequences among the entire region of TSW12 and TAS14 genes between the control and the somaclonal variant sequences of the corresponding region were done using MEGA6 program and "Basic Local Alignment Searching Tool" (BLAST algorithm; [27]) database. The fragments were searched and compared for the mismatch sequences

2.6 DNA data and statistical analyses

TSW12 and TAS14 gene mutations were annotated using the IBM Reporter Suite software (version 5.4.0 Life Technologies). The reference sequences for TSW12 and TAS14 were NM_001247145.2 and NM_001247109.1, respectively, and variation analysis was performed without bias. Because there is no unique SVC population database, all reported known variations in TSW12 and TAS14 were submitted to the LDlink tool for linkage disequilibrium (LD) and

haplotype analysis [28].

The effect of coding non-synonymous mutation on protein function was predicted using different computational tools like SIFT [29], PolyPhen-2 [30], PANTHER [31], and PredictSNP [32]. The effect of coding non-synonymous mutation on protein stability was also studied using computational algorithms like MUpro [33].

3. Results

3.1 Morphology of somaclonal variant cells (SVC)

The selected SVC at the concentration of 250 mM mannitol showed a distinct morphology Fig. 1, as it showed necrotic regions of callus and pale color. This change might indicate a potential genetic modification to be confirmed by sequencing.

3.2 PCR amplification products

Specific one set of primers covering the entire coding sequence for the TSW12 gene (chromosome 10, NC_015447.2), and TAS14 gene (Chromosome 2, NC_015439.2), through two exons, were used for PCR reaction. The amplified DNA products were loaded on 1.5% agarose gel for electrophoresis. PCR conditions were adjusted to optimize annealing temperature and salt concentration; the best condition used for the appearance of a clear single band was used for photographing and sequencing Fig. 2.

3.3 Detection of mutation by PCR-sequence products

Data of purified PCR products sequencing were

subjected to nucleotide blast program on the National Center for Biotechnology (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for alignment with the TSW12 gene and TAS14 gene reference DNA strand with their accession number to detect homozygous and heterozygous new/reported mutations. The DNA sequence for 12 different samples revealed 7 SNPs in both genes, (5 SNPs in TSW12 and 2 in TAS14), in addition to two frameshift mutations; one on each gene.

Sequence analysis of the six detected mutations concerning TSW12 gene of SVC tolerant to 250mM

mannitol included five missense mutations in exon 1, two transversion, and three transition types (100T>G; 101T>G; 102G>A; 103 G>A, and 104 C>T) Fig. 3, in addition to a frameshift deletion (105-106 delTC) mutation Fig. 3. Four SNPs of them were previously reported and one mutation was novel (104C>T).

Pairwise sequence alignment by ClustalW2 program for DNA multiple sequence alignment for sequences of TSW12 reference DNA (NC_015447.4) with SVC treated callus are shown in Fig. 4. In which 4/12 samples with 101T>G, 2/12 samples each with 102G>A, 104 C>T, 105-106delTC, and 1/12 for the remaining the mutations.

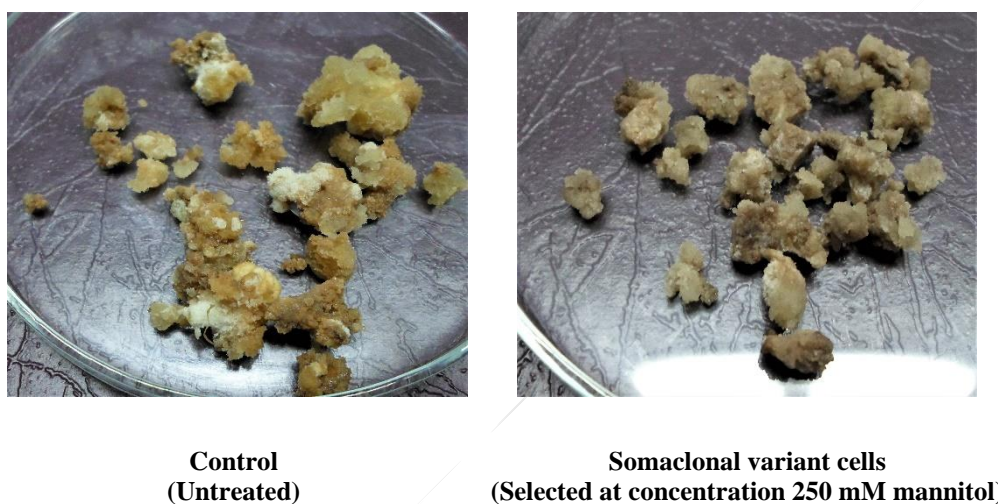


Fig. 1 Morphology of control (untreated) callus and selected somaclonal variant cells (SVC)

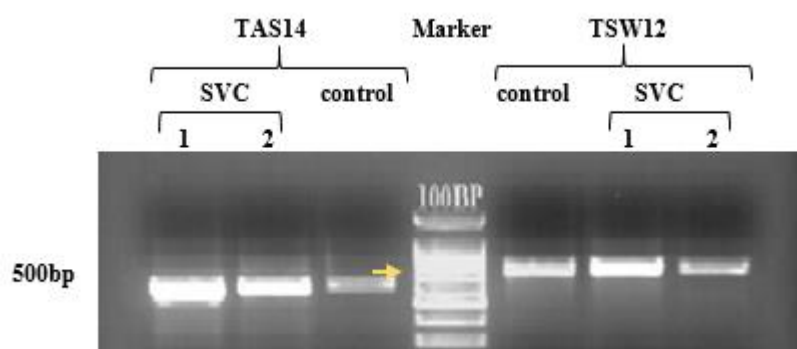


Fig. 2 Agarose gel electrophoresis (1.5%) for amplification products from TSW12 and TAS 14 genes of selected somaclonal variant cells (SVC), two samples each (1 and 2), compared with control untreated cells. DNA marker was 100 bp

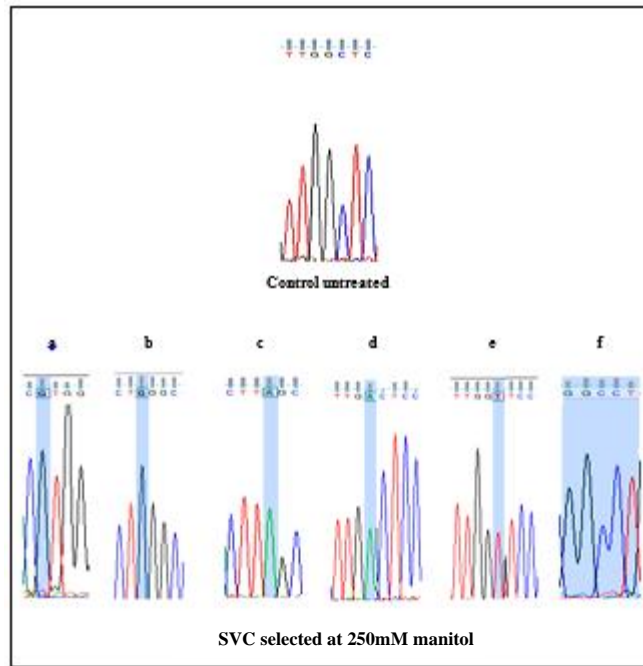


Fig. 3 Genogram of region 100 to 106 of TSW12 gene (NC_15447.2) (upper panel) and corresponding region of six unrepeated mutations in twelve SVC cells (lower panel), where a) 100 T>G; b) 101 T>G; c) 102 G>A; d) 103 G>A; e) 104C>T (novel mutation), and f) 105-106 delTC.

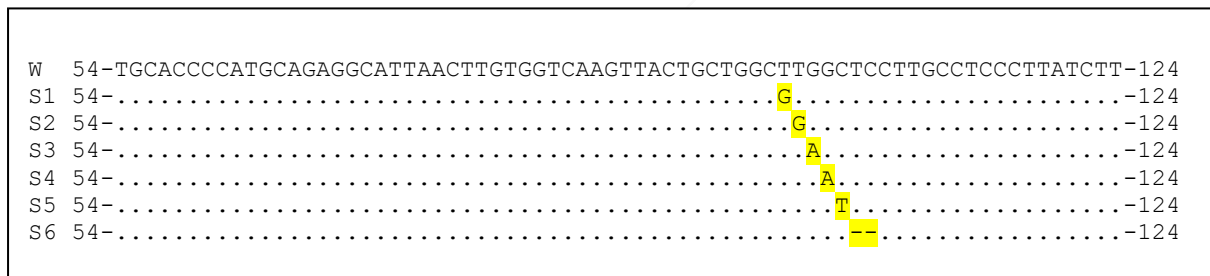


Fig. 4 DNA sequences in FASTA format of TSW12 gene (located in the hot spot region on DNA sequence (100-106)) of the mutant SVC tolerant to 250 mM mannitol, showing the six mutations in yellow color alignment with wild type sequence (NC_001247145.2)

Our results of PCR sequences revealed that there were two different SNPs (one transition and one transversion) and one frameshift DNA mutation in exon 1 of the TAS14 gene in SVC selected at 250mM mannitol Fig. 5. Two of them were previously reported (82G>A was repeated in 3/12 samples and 130-132 delATG repeated in 4 /12 samples) and one mutation was recorded as a novel (146G>C, in the coding sequence, was repeated in 3/12 samples) and 2/12 samples have no detected mutations in the coding region of this gene. Based on an alignment of TAS14 sequence (NC_015439.2) with the corresponding region among SVC, three detected mutations in SVC callus samples using pairwise alignment byClustalW2

program was detected Fig. 6.

All detected DNA mutations were examined to evaluate the effect of DNA mutation on the amino acids sequence of both TSW12 and TAS14 peptides using the blast program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The seven mutations detected on the DNA level were homozygous mutations leading to six different missense mutations, and one synonymous. Concerning amino acid mutations ofTSW12 protein, three hotspots (34-36 aa) were detected in SVC samples. Leucine amino acid at position 34 was replaced with either valine or tryptophan, followed by position 35 where amino acid alanine was replaced with either threonine

or valine, and the last position is the replacement of proline at position 36 with phenylalanine due to indel frameshift mutation to generate a stop codon after 17 amino acids producing a truncated protein with only 54 amino acids. Regarding the three amino acid

mutations of TAS14 protein, the first one resulted in the substitution of amino acid glycine by serine at position 28, the second caused a dropping of methionine at position 43, and the third mutation led to the exchange of amino acid glycine by alanine Table 2.

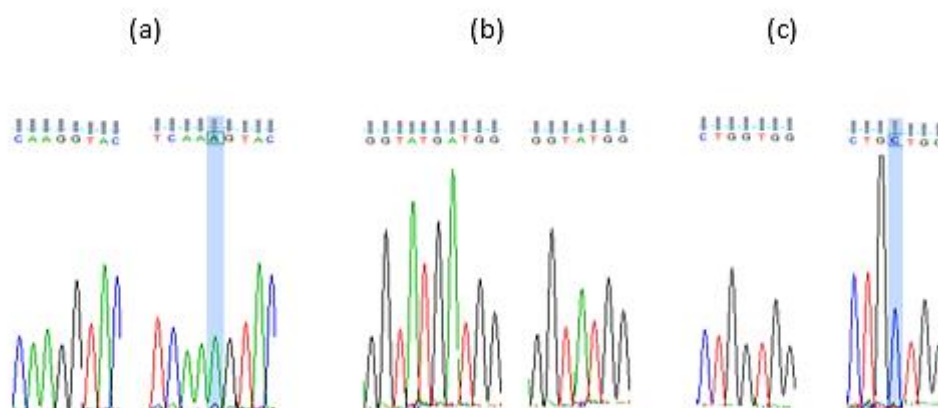


Fig. 5. Genogram of wild type DNA sequence of TAS14 gene (NC_015439.2) and three recorded mutations of SVC selected at 250 mM mannitol for a) 82G>A, b) 127-129 del ATG and, c) 144G>C mutation respectively compared with corresponding region (NC_015439.2) reference gene

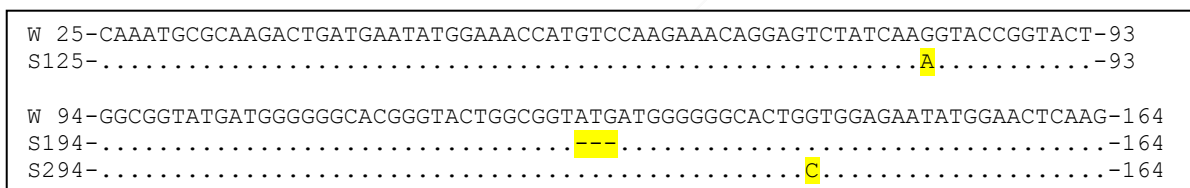


Fig. 6. DNA sequences of TAS14 gene (NC_001247145.2) of untreated *L. esculentum* (region from 25 to 164) in alignment with the mutant SVC tolerant to 250 mM mannitol representing three mutations in yellow color

Table 2 Mutations detected in the TSW12 and TAS14 genes of SVC selected at 250 mM mannitol and its related amino acid change

No	Gene	Mutation	Type of mutation	AA Change	Polyphen-2	SIFT,mutpred2*
1		100T>G	Missense	p.L34V	tolerated	0.15
2		101T>G	Missense	p.L34W	tolerated	0.30
3		102G>A	Silent	p.L34=	tolerated	0.34
4	TSW 12	103 G>A	Missense	p.A35T	tolerated	0.53
5		104 C>T	Missense	p.A35V	Possible damaging	0.25
6		105-106 delTC	Frameshift deletion	(p.P36Ffs*17)	deleterious	0.0290*
7		82G>A	Missense	p.G28S	tolerated	0.15
8	TAS14	127-129 del ATG	Frameshift deletion	p. delM43	tolerated	0.0319*
9		144G>C	Missense	p.G48A	tolerated	0.45

*MutPred2 algorithm

3D structure of predicted amino acids of control TSW12 and missense mutations were summarized in Fig. 7a and b together with polyphen2 data for SNPs analyses, which ranged from benign to possibly damage mutations with its score of (0.545) with a sensitivity of 0.88 and specificity of 0.91. On the other side, frameshift mutation assigned by MutPred-2 algorithm for its truncated protein, which proves that the deduced polypeptide chain was considered as harmful with a score of 0.27801, but not confident harmful and with mechanism iron-binding ($p=0.022843$); disulfide linkage ($p=0.034136$); Fig. 7c.

Regarding TAS14 protein, the 3D structure of predicted amino acids of wild type and missense mutations together with the deletion of methionine amino acid were summarized in Fig. 8a. Analyses of data by polyphen2 data for SNPs analyses (Figure 8b), which is benign with its score of (0.00) with a sensitivity of 1.00 and specificity of 0.00. Frameshift mutation assigned by Polyphen2, which proves that the deduced polypeptide chain was a score of (0.204) with a sensitivity of 0.92 and specificity of 0.88 Fig. 8b and was also considered as harmful with MutPred-2 algorithm for its deduced protein, of a score (0.28093), and with mechanism signal cleavage ($p=0.031976$).

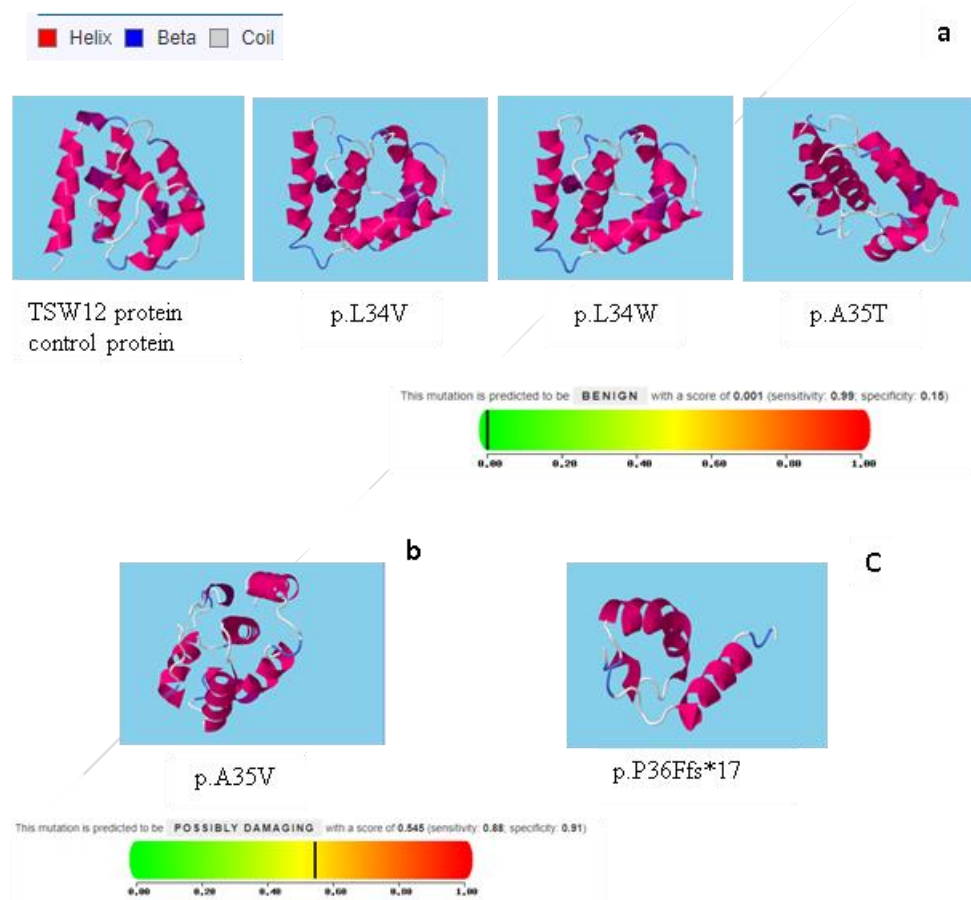


Fig. 7 3D secondary structure of deduced control and mutant TSW12 proteins. Panel A is missense mutations with benign mutations, panel B is a missense mutation with possibly damaging mutation and panel C is a frameshift mutation with truncated protein

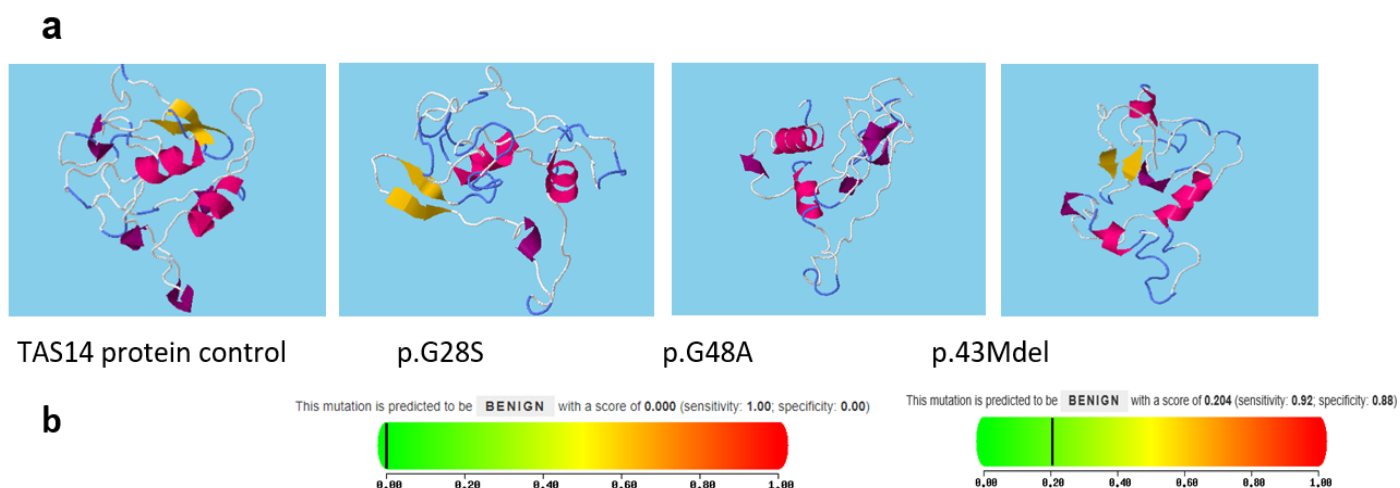


Fig. 8 Panel a is showing the 3D structure of control TAS14 protein and three detected mutations, and panel b is showing the polyphen2 analysis of two SNPs and frameshift mutations

4. Discussion

The source of variation in *in vitro* cultures could be due to pre-existing genetic differences among explant cells or can be induced by exposing the explant to stressful conditions [34]. Somaclonal variance aims to generate new genotypes tolerant to stressful conditions, in our case, drought. The drought in the current study was prompted by exposure of calli to different concentrations of mannitol. Somaclonal variant cells (SVC) of tomato were selected at the sub-lethal concentration of mannitol (250 mM). The visual examination of SVC cultures showed a slight phenotypic variation as compared with the control untreated cells. This observation was supported by our previous findings that selected SVC of tomato exhibited enhanced dry weight, osmolality, antioxidant capacity, reduced glutathione, ascorbic acid content, and ABA content [11]. Since drought is a multigenic trait [35], the question was whether the selection of SVC of tomato under drought stress, induced by mannitol, caused mutations in TAS14 and TSW12 genes and consequently on the produced proteins, which might affect their activity. This work was conducted to explore the answer to this inquiry and investigate the participation of these two stress-induced genes in drought tolerance.

It is established that mutation in genes regulating a specific developmental process will ultimately lead to the interruption of this process [36]. For this reason, genomic DNA of 12 samples of SVC and the control

untreated cells were prepared for PCR using a specific one set of primers covering the entire coding sequence of the TAS14 gene and TSW12 genes. The purified PCR products of both genes were subjected to sequencing and the results were subjected to the BLAST program at the National Center of Biotechnology (NCBI). The analysis revealed that SVC cells exhibited nine mutations on both of TAS14 and TSW12 genes (4 transitions, 3 transversions, and 2 frameshifts). TSW12 gene showed six mutations in exon 1, five of them were previously reported (100T>G; p. L34V, 101T>G; p. L34W, 102G>A; p.L34=, 103G>A; p. A35T, and 105-106 del TC) [16] and one mutation was newly recorded in our study (104C>T; p. A35V). While TAS14 gene exhibited three mutations in their structure at exon 1. Two of them were previously reported (82G>A; p.G28S and 130-132 del ATG; p.) [16], and one SNP was a new mutation recorded in this work (146G>C; p. G48A).

Both genes play a significant role in the determination of drought stress tolerance and have been found to be highly conserved among various genera [16,37-39]. Deficient mutants in the function of these genes might result in the formation of plants unresponsive to the stress, hence affecting their survival. In our previous study, though many of the measured physiological parameters were significantly different, no clear differences were observed in some parameters (like the relative water content, electrolyte leakage, and malondialdehyde) between the control and SVC callus exposed to mannitol [11].

Therefore, a single nucleotide mutation that did not lead to determinacy could have resulted in a spinoff of indirect damage to DNA by abiotic stress factors. In corroboration with other previous reports, it has been shown that the determinate mutants were characterized by reduced plant height and growth [40,41].

Moreover, all the SNPs recorded (7/9) in both genes were neutral the same as the chemical nature of unmutant amino acid, but in TSW12 gene mutants were localized in a certain hotspot region (position 34-36), suggesting that the point of mutation could be a highly mutable region within the gene and might explain the reduced development of callus. In contrast, mutations in TAS14 gene dispersed in different positions (from position 82 to position 146). Functional and translational genomics in *L. esculentum* L. are limited by its recalcitrance to genetic transformation. The computational and bioinformatics tools offer means for making *in silico* predictions and inferences about protein structure and function. However, SIFT, Polyphen2, and MutPred2 algorithms are significantly better at predicting loss-of-function mutations than gain-of-function mutations (SIFT, $P = 0.001$; PolyPhen2 and MutPred2P, ≤ 0.0001 ; with about 70% sensitivity [32]. Therefore, most data of these SNPs including novel mutations (6/7) were benign and did not have a dramatic effect on the amino acid sequence and protein structure, while p.A35V is a possible damaging mutation Table 2.

On the other side, deletion mutations recorded in this work were 105-106 del TC in TSW12 gene that created a missense mutation, and consequently, the translation phenylalanine changed to proline. This led to the creation of a stop codon after 17 amino acids to produce a truncated protein with 52 amino acids instead of a complete polypeptide chain (P>L36Fs*17). In addition, 130-132 del ATG at 43 positions in TAS14 protein caused the deletion of the amino acid methionine. The 3D structure predicted by protein stability prediction tools, as well as MutPred2, revealed that there is a great change in the polypeptide chain which might affect the function of the gene and these changes might be responsible for delays in the development of the callus compared to the untreated one.

TSW12 is responsible for non-specific lipid transfer proteins (nsLTP) and is thought to have a role in the intermembrane transfer of molecules [18], yet the results obtained in our previous work, did not show any significant difference in membrane properties between the control and SVC cells [11]. This confirms that this mutation has no significant detrimental effect on cell functions of SVC.

Finally, under environmental conditions generating reduced water potential, tomato produce stress-responsive proteins as a part of a global stress response to protect the cell metabolism. The synthesis of TSW12 and TAS14 proteins is a major part of the response to water-deficit conditions. Mutations generated to the response of 250mM mannitol-induced drought stress are benign with a possible damaging consequence without reducing the stress tolerance of the SVC.

5. Conclusions

The exposure of SVC cells to a sublethal concentration of mannitol resulted in minor genetic modifications on the two investigated responsive genes (TSW12 and TAS14), and therefore, neglected changes of corresponding proteins. Since SVC cells survived a relatively high concentration of mannitol, other genes may be responsible for these results which is planned to be a subject of a future study.

6. Abbreviations

ABA: abscisic acid; **CIM:** callus induction medium; **CTAB:** Cetyltrimethylammoniumbromide; **NCBI:** National Center for Biotechnology Information; **nsLTP:** non-specific lipid transfer proteins; **SVC:** Somaclonal variant cells; **SNPs:** Single nucleotide polymorphisms.

7. References

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