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Agro - Morphological and Molecular Analysis of Somaclonal Variation Among Regenerated Plants from Tomato (*Lycopersicon esculentum* Mill.) Varieties

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ABSTRACT

Genetic variations in tissue-culture tomato plants has been examined by agro-morphological evaluation and molecular markers analysis. Four tomato varieties were used as donor parents, two types of explants (Cotyledone and hypocotyle) and two combinations of auxin and cytokinin with different concentrations were used for the first time to study the effect of genotype, type of explants and growth regulators on callus induction and plant regeneration in tomato. Significant differences among tomato varieties were observed in all tissue culture studied traits (percentage of callus induction, regeneration rate, number of regenerated plants and number roots per explant). These differences were depending on genotype, explant type, type and concentration of growth regulators. The best regeneration medium from each growth regulator combination distinct used for establishment of regenerated plants. Regenerated plants from each combination of growth regulators which exhibited wide variations compared with its donor parent were selected and subjected to somaclonal variation analysis using molecular markers and agro-morphological traits. Two molecular marker systems, ISSR (inter simple sequence repeat) and SRAP (sequence related amplified polymorphism) were used to analysis of somaclonal variation. These markers revealed polymorphism showing distinct different banding patterns in all somaclones, which were prominent in their differences from the donor parents. To confirm stability of these variation, regenerated plants (R_0) from donor parents were transferred to the greenhouse and the first generation of these somaclones (R_1) as well as their donor parents were evaluated for plant height, number of branches per plant, No. of inflorescences per plant, No. of flowers per inflorescence, No. of flowers per plant, number of fruits per plant and yield per plant. Generally, the results revealed that some somaclones exceeded their donor parents in one or more traits.

Keywords: Somaclonal variation, Molecular markers, Tomato, In vitro culture, Yield

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops belongs to the family Solanaceae. It is a diploid plant with 2n = 24 chromosomes. Fruits of tomato are very rich in vitamins A and C and it contains approximately 20–50 mg of lycopene/100 g (Kalloo 1991).

Genetic variations in crop plants are one of the most important sources to obtain elite genotypes which could be used in hybridization and breeding programs.

Somaclonal variations which occur among regenerated plants from *in vitro* culture has been considered as one of the most important sources of genetic variants in crop plants (Anil *et al.,* 2018). Thus, the establishment of simple and efficient regeneration method is an essential requirement of taking advantage of cell and tissue culture for genetic improvement.

The *in vitro* culture of the tomato has been successfully used in different biotechnological applications including the clonal propagation of high-value commercial cultivars, virus-free plants, and genetic transformation (Hanus-Fajerska 2006; Yarra *et al.,* 2012; Namitha and Negi 2013).

The success of plant regeneration from tomato tissue culture depends on several factors, such as genotype, explant type, growth regulators, physiological state of donor plants and laboratory conditions (Mamidala and Nanna 2011; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). Different types of explants such as cotyledons, hypocotyls, petioles, leaves and anthers were used for plant regeneration from tissue culture in tomato (Yasmeen 2009; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). The type of explant determines not only the frequency of the explants' organogenesis but also determines the number of shoots produced per explant (Bahurpe *et al.,* 2013; Jehan and Hassanein 2013). Mamidala and Nanna (2011) found that cotyledon explants showed callus induction and regeneration superiority over hypocotylexplants.

Plant growth regulators especially auxins and cytokinins play an important role in regulating callus induction, shoots and roots differentiation. Auxins such as IAA (indole acetic acid), 2,4-D (2,4-Dichlorophenoxyacetic acid), NAA (a-Naphthalene acetic acid), and cytokinins such as BA (benzyl amino purine) and KIN (Kinetin) are the growth regulators widely used in *in vitro* ultures of tomato to improve callus induction and plant regeneration (Ishag *et al.,* 2009; Chaudry *et al.,* 2010; Kantor *et al.,* 2010; Mamidala and Nanna 2011; Zhang *et al.,* 2012; Namitha and Negi 2013).

Somaclonal variation is widespread among tissue culture - derived regenerants (Nehra *et al*., 1992). Many researchers reported that variation appeared in tissue culture regenerated plants may be resulted from the pre-existing variation of the explants (Nwauzoma and Jaja 2013), the long period of the culture (Masoud and Hamta 2008), plant growth regulators in the culture medium (Soniya *et al*., 2001; Bairu *et al*., 2011), the number of sub-cultures (Eeuwens *et al*., 2002), the genotype dependence (Zucchi *et al*., 2002), single gene mutations, activation of transposable elements (Bairu *et al*., 2011), and hypo- or hyper-methylation of DNA (Evans and Sharp 1983; Abdellatif *et al*., 2012).

Many types of molecular markers such as Random Amplified Polymorphic DNA (RAPD), Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeat (ISSR), Sequenced Related Amplified Polymorphism (SRAP), and Simple Sequence Repeat (SSR) are widely used to detect and characterize somaclonal Variations at the DNA level (Soniya *et al.,* 2001; Hu *et al.,* 2011; Karácsonyi *et al.,* 2011; Aruingtyas *et al.,* 2012; Ali *et al.,* 2017; Al Hattab *et al.,* 2017; El-Shahed *et al.,* 2017; Martínez-Estrada *et al.,* 2017) .

The objectives of this study were to a) study the effect of genotypes and different combinations of auxins and cytokinins on plant regeneration in tomato, 2)

investigate the influence of genotypes, and growth regulators on somaclonal variation in the tomato varieties and c) detect and characterize somaclonal variations using molecular markers and agro-morphological traits.

MATERIAS AND METHODS

This work was carried out at the Tissue culture and Bio-technology Laboratory, Genetics Department, and Experimental Farm of the Department of Vegetable Crops, Faculty of Agriculture, Assiut University, Assiut Egypt, during 2016-2018.

Plant Materials

Four varieties of tomato (*Lycopersicon essculentum* Mill) were used in the present investigation, Namely; Castle Rock (CR) (Castle Seed, USA), Super Strain B (SB) (Sun seed, Parma, Idaho USA), UC_{97-3} (UC) (Peto Seed, USA) and Super Marmand (SM) (Daehnfeltd, Holland).

The effect of genotype, type of explants and growth regulators on callus formation and plant regeneration was studied in two experiments. Each experiment was designated on the basis of the following factors; four genotypes, two types of explants (hypocotyls and cotyledons) and a combination of different levels of growth hormones. Each experiment was constructed to study one combination of auxins and cytokinin's as shown in Table (1) and designated in randomized complete block design with three replicates.

Preparation of explants

Tomato seeds from each variety were soaked in 0.1% mercuric chloride for three minutes. Then in a solution of 25% of commercial bleach (which contained 5.5% (W.V) NaOCl) for 20 Min, This was followed by rinses three times in sterile distilled water, under sterile conditions of laminar flow hood. The seeds were germinated on a pre-sterilized filter paper in scrow capped glass vials (50x100mm) containing 10 ml of ¼ MS (Murashige and skoog 1962) medium free of growth hormones. The vials were incubated at 25±2°C under continuous light. After10 days, the cotyledonary leaves and the hypocotyl segments (ca.10mm) were excised from the good germinated seedlings (5-8 cm length) and aseptically transferred to the culture media.

Culture media

The basic medium consisted of major and minor salts according to Murashige and Skoog (1962) and supplemented with one combination of growth hormones as shown in Tablee (1). The pH was adjusted to be 5.8 before the addition of 0.8% (W.V) agar and autoclaved (121^{o}C for 15 min) at 1.2 Kg cm^{2}. Cotyledonary leaf and hypocotyl segment explants were cultured at each type of medium. The cultures were incubated at 25±2ºC under continuous fluorescent light. After four weeks' incubation, cultures were evaluated in the basis of the following parameters: Callus formation (C %), calculated as percentage of explants produced callus, Regeneration rate (Reg %), calculated as percentage of explant product shoot, Number of shoots per explant (Shoots/exp) and Percentage of rooting (Root %), calculated as percentage of shoots that formed roots.

Table 1. Combinations of growth hormones used for tissue culture studies.

No	Combination (Kin +IAA)	Combination (BA+NAA)
1	IK-1 (4mg/l Kin + 2mg/l IAA)	NB-1 (0.2mg/l BA+0.2mg/l NAA)
2	IK-2 (4mg/l Kin + 4mg/l IAA)	BN-2 (0.2mg/l BA+0.5mg/l NAA)
3	IK-3 (4mg/l Kin + 8mg/l IAA)	NB-3 (2mg/l BA+0.1mg/l NAA)
5	Ik-4 (8mg/l Kin + 4mg/l IAA)	NB-4 (2mg/l BA+0.5mg/l NAA)

Where: IAA = 3- Indole acetic acid ($C_{10}H_9No_2$, M.W = 175.18), Kin = Kinetin ($C_{10}H_9N_{50}$, M.W = 215.21), NAA= α-Naphthalene acetic acid ($C_{12}H_{10}O_2$, M.W =186.20), BA = benzyle amino purine ($C_{12}H_{11}N_5$, M.W = 225.255)

Establishment of tomato somaclones

The rooted shoots were aseptically transferred to hormone-free medium for further development. The well rooted plantlets were transferred to pots contained a 1:1:1 mixture of peat, sand, and soil. Pots were covered with a transparent plastic cover for two weeks to keep the humidity and transferred to the greenhouse for adaptation and further growth. Healthy plants (R_o) were then transferred to the field (Farm of Department of Vegetable Crops, Faculty of Agriculture, Assiut university).At maturity, tomato fruits were collected and brought to the laboratory for seed collection (R_1 generation).

Somaclonal Variation

In this part of the present investigation, both molecular analysis and morphological evaluation were carried out to study and determine somaclonal variations among *in vitro* tomato plants regenerated from the cultivars Castle Rock (CR), Super Strain B (SB), UC_{97-3} (UC) and Super Marmand (SM).

Molecular studies

Genomic DNA was extracted from frish leaves of the four tomato varieties (donor plants developed in hormone free MS medium) besides to its regenerated plants (five regenerated plants from each IK-2 and NB-4 medium for each tomato varieties) using the CTAB method outlined by Doyle and Doyle (1990).

The quantity and quality of DNA were measured using a UV spectrophotometer at the wave lengths of 260/280 nm and the quality of extraction was observed using 0.8% agarose gel electrophoresis.

Molecular analysis:

ISSR and SRAP analyses

Twelve ISSR primers and fifteen SRAP primer combinations (Table 2), were obtained from Metabion International AG Company (Germany) and used to amplify the template DNA. The reaction conditions were optimized and the mixtures (25 µL total volume) were composed of 11.7 µL dH_2O, 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 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 ISSR amplification conditions were, initial denaturation for 5 min at 94ºC, 45 cycles of 1 min denaturation at 92ºC, 1 min annealing at 38 ºC - 44 ºC (annealing ISSR primer) and 2 min extension at 72ºC, 10 min final extension at 72ºC, then followed by a final hold at 4ºC. The SRAP amplification program was followed as

initial denaturation for 5 min at 94ºC, 10 cycles of 1min denaturation at 92ºC, 1 min annealing at 35ºC and 2 min extension at 72ºC, 35 cycles of 1 min denaturation at 92ºC, 1 min annealing at 50-55ºC and 2 min extension at 72ºC, 10 min final extension at 72ºC, then followed by a final hold at 4ºC. Amplification products were separated on agarose gel 2% and 2.5% for 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 pair wise 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:

$$\text{"PIC"} = 1 - [(p)^2 + (q)^2]$$

(Ghislain *et al.,* 1999), "MI"= PIC x ηβ (Powell *et al.,* 1996) and "Rp"=∑Ib, (Prevost and Wilkinson 1999).

Morphological evolution

Regenerated plants (R_0) from donor parents were transferred to the greenhouse, while only ten somaclones from each variety were established in the field and produced fruits. The progeny of tomato somaclones (R_1) and their donor parents, were cultivated in the field in a randomized complete block design with three replicates. The somaclones and their donor parents were evaluated for the previously mentioned traits.

Table 2. Primer sequences and codes used.

Primer codes		Sequence (5' to 3')
ISSR-1	UBC 807	AGAGAGAGAGAGAGAGT
ISSR-2	UBC 810	GAGAGAGAGAGAGAGAT
ISSR-3	HB09	5'-GTG TGT GTG TGT GG -3'
ISSR-4	HB10	5'-GAG AGA GAG AGA CC -3'
ISSR-5	HB12	5'-CCA CCA CCA GC-3'
ISSR-6	HB15	5'-GTG GTG GTG GC-3'
ISSR-7	UBC 823	TCTCTCTCTCTCTCTCC
ISSR-8	UBC834	AGAGAGAGAGAGAGAGYT
ISSR-9	UBC844	CTCTCTCTCTCTCTCTRC
SRAP-1	Em 1a	5′–GAC TGC GTA CGA ATT AAT–3′
	Me1b	5′–TGA GTC CAA ACC GGA AG–3′
SRAP-2	Em 2	5′–GAC TGC GTA CGA ATT TGC–3′
	Me3	5′–TGA GTC CAA ACC GGA AT–3′
SRAP-3	Em 1c	5′–GAC TGC GTA CGA ATT AAC–3′
	Me4	5′–TGA GTC CAA ACC GGA CC–3′
SRAP-4	Em6	5′–GACTGCGTACGAATTGCA–3′
	Me5	5′–TGAGTCCAAACCGGAAG–3′
SRAP-5	Em 1a	5′–GAC TGC GTA CGA ATT AAT–3′
	Me2	5′–TGA GTC CAA ACC GG AGC–3′
SRAP-6	Em11	5′–GACTGCGTACGAATTCCA–3′
	Me7	5′–TGAGTCCAAACCGGACA–3′
SRAP-7	EM10	5′–GACTGCGTACGAATTCAG–3′
	Me10	5′–TGAGTCCAAACCGGACG–3′

RESULTS AND DISCUSSION

Tissue culture response

Generally, the results revealed that embryogenic callus with globular structures was developed from all tomato varieties and both types of cultured explants (hypocotyle and cotyledon) on MS medium supplemented with different combinations and concentrations of growth regulators as shown in Fig. (1a-m).

Fig. (1a-m). Plant regeneration from tomato tissue culture, (a-g) embryogenic callus having globular structures and early development of shoot regeneration after 3 - 4 weeks of culture, (h and i) Shoot and root regeneration from the embryogenic callus after 4 - 5 weeks of culture, (j-k) The regenerated tomato plants grown in the greenhouse after 4-5 months of tissue culture, (i) The regenerated tomato plants grown in the field, and (m) The regenerated tomato plants grown in the field at flowering and fruiting stages.

Callus formation (C %)

The results revealed that callus was developed from the explants of all genotypes on all tested concentrations of growth hormones in both combinations (Table 3). However, the percentages of callus formation (C %) were varied according to the type of explant as well as the concentration of growth hormones. As average over the genotypes, calli were developed from 53.65 to 73.74 % of the cultured cotyledons and from 56.96 to 66.37 % of the cultured hypocotyls on combination of IAA + Kin, while on the combination of NAA + BA, calli were development from 58.25 to 96.27 % of the cultured cotyledons and from 75.73 to 89.95 % of the cultured hypocotyls (Table 3). These differences were significant (Table 4).

High significant differences in C % were also found between the tested levels of growth regulators in both combinations (Table 4). Overall Genotypes and explants, the results in Table (3) showed that the medium IK-3 in combination with IAA + Kin and the NB-2 medium in

combination with NAA + BA exhibited the highest percentages of callus formation (66.67% and 95.31%, respectively).

The results also revealed significant interaction between the type of explant and genotypes. These results suggested that the explant performed differently from one genotype to another in the same medium (Table 3). In addition, the significant interaction between the type of explant and concentration of the growth hormones also revealed that the explant performed differently from one concentration to another (Table 3).

Table 3. Mean percentages of culls formation, percentages shoot formation, Mean values of number of shoot/exp and Mean percentages of root formation for hypocotyle (H) and cotyledonary (C) explants on culture medium supplemented with different concentration of Kin +IAA and BA + NAA.

			Combination of IAA + Kin						Combination of NAA + BA					
	Parents	**Exp**	**IK-1**	**IK-2**	**IK-3**	**IK-4**	**AVR**	**M**	**NB-1**	**NB-2**	**NB-3**	**NB-4**	**AVR**	**M**
Callus formation (C %)	CR	C	70.75	70.75	80.28	61.35	70.78	69.00	92.68	92.68	78.54	68.73	83.16	82.76
		H	65.99	70.75	70.75	61.35	67.21		88.49	78.68	78.68	83.59	82.36	
	SB	C	61.00	56.36	75.29	56.36	62.25	60.12	78.14	96.90	78.14	68.33	80.38	81.18
		H	61.56	56.80	61.44	52.15	57.99		72.38	96.14	86.90	72.47	81.97	
	UC	C	50.95	65.12	69.95	46.30	58.08	57.46	89.21	98.45	7.83	74.78	67.57	77.16
		H	50.95	60.35	65.12	50.95	56.84		93.83	89.21	89.21	74.78	86.76	
	SM	C	64.65	55.25	69.42	50.60	59.98	61.10	73.38	97.05	68.48	58.95	74.47	78.65
		H	58.64	58.64	68.17	63.40	62.21		86.51	95.75	76.99	72.08	82.83	
	AVR	C	61.84	61.87	73.74	53.65	62.77		83.35	96.27	58.25	67.70	76.39	
		H	59.29	61.64	66.37	56.96	61.06		85.30	89.95	82.95	75.73	83.48	
	Mean		60.56	61.75	70.05	55.31			84.33	93.11	70.60	71.71		
	LSD 0.05		Geno		Explant		Medium		Geno		Explant		Medium	
			0.566		0.4002		0.566		0.1816		0.1283		0.1816	
Regeneration rate (Reg %)	G1	C	33.03	75.52	14.29	61.35	46.05	38.96	17.12	3.80	17.12	17.61	13.91	9.74
		H	18.73	52.06	18.73	37.96	31.87		3.35	3.35	7.79	7.79	5.57	
	G2	C	23.11	75.29	13.74	56.36	42.13	34.07	8.54	8.54	4.10	27.28	12.12	8.96
		H	23.55	42.81	14.18	23.55	26.02		2.35	2.35	2.35	16.16	5.80	
	G3	C	27.56	65.12	3.89	36.96	33.38	23.07	6.34	1.90	6.34	10.78	6.34	6.17
		H	3.89	32.26	1.65	13.26	12.77		2.55	2.55	6.99	11.92	6.00	
	G4	C	26.93	59.89	17.07	50.60	38.62	31.01	6.23	1.79	6.23	10.67	6.23	4.99
		H	6.94	54.00	6.94	25.68	23.39		1.53	5.97	1.53	5.97	3.75	
		C	27.66	68.96	12.25	51.32	40.04		10.83	4.17	9.72	15.64	10.09	
		H	13.28	45.28	10.38	25.11	23.51		2.44	3.55	4.66	10.46	5.28	
			20.47	57.12	11.31	38.22			6.64	3.86	7.19	13.05		
	LSD 0.05		Geno		Explant		Medium		Geno		Explant		Medium	
			0.562		0.3973		0.562		0.0691		0.0489		0.0691	
Number of shoots /explant (Shoots/exp)	G1	C	12.18	29.85	10.18	18.18	17.60	15.39	4.80	3.80	4.47	4.80	4.47	3.99
		H	10.52	18.85	10.18	13.18	13.18		3.35	3.35	3.68	3.68	3.52	
	G2	C	9.53	27.86	5.86	16.19	14.86	11.62	4.43	4.43	4.10	6.43	4.85	3.77
		H	6.63	12.97	5.97	7.97	8.39		2.35	2.35	2.35	3.68	2.68	
	G3	C	4.78	22.78	2.38	11.45	10.35	6.46	3.55	2.55	3.55	3.22	3.22	3.01
		H	2.07	5.45	1.65	1.12	2.57		2.55	2.55	2.88	3.22	2.80	
	G4	C	6.08	18.42	5.08	15.08	11.17	8.96	2.12	1.79	2.46	2.79	2.29	1.77
		H	3.17	14.83	3.17	5.83	6.75		1.00	1.33	1.00	1.67	1.25	
		C	8.14	24.73	5.88	15.23	13.49		3.73	3.14	3.65	4.31	3.71	
		H	5.60	13.03	5.24	7.03	7.72		2.31	2.40	2.48	3.06	2.56	
			6.87	18.88	5.56	11.13			3.02	2.77	3.06	3.69		
	LSD 0.05		Geno		Explant		Medium		Geno		Explant		Medium	
			0.564		0.388		0.563		0.0721		0.051		0.0721	
Percentage of Rooting (R %)	G1	C	14.29	56.70	9.85	18.73	24.89	28.93	8.57	56.31	3.80	27.98	24.17	25.10
		H	18.73	70.75	14.29	28.10	32.97		17.83	17.67	8.12	60.49	26.03	
	G2	C	23.11	42.37	9.30	13.74	22.13	27.02	4.10	66.00	4.10	42.61	29.20	30.75
		H	18.62	75.73	9.74	23.55	31.91		26.53	26.53	2.35	73.78	32.30	
	G3	C	8.33	41.66	6.49	3.89	15.09	18.73	7.32	64.45	7.32	41.06	30.04	27.02
		H	10.28	50.95	10.53	17.70	22.37		2.55	21.80	2.55	69.12	24.01	
	G4	C	8.19	41.26	8.19	12.63	17.57	21.59	1.79	68.60	1.79	25.97	24.54	28.69
		H	20.75	63.40	6.94	11.38	25.62		16.27	49.64	1.79	63.69	32.85	
		C	13.48	45.50	8.46	12.25	19.92		5.45	63.84	4.25	34.41	26.99	
		H	17.10	65.21	10.38	20.18	28.22		15.80	28.91	3.70	66.77	28.79	
			15.29	55.35	9.42	16.22			10.62	46.38	3.98	50.59		
	LSD 0.05		Geno		Explant		Medium		Geno		Explant		Medium	
			0.563		0.398		0.563		0.065		0.0458		0.065	

Regeneration rate (Reg %)

Generally, the results revealed that shoots were regenerated from the explants of all genotypes on all concentrations of hormones (Table 3). However, the rates of regeneration were varied depending upon the genotype, type of explant and concentration of the growth hormones.

The regeneration rate ranged from 23.07% in UC_{97-3} to 38.96% in Castle Rock and from 4.99 in Super Marmand to 9.74% in Castle Rock on culture medium supplemented with IAA + Kin and NAA + BA, respectively. These differences between the genotypes were highly significant (Table 4). The results revealed that the cotyledone explants exceeded the hypocotyle explants in shoot formation (Table 3). These differences were highly significant in both combinations (Table 4).

Overall explants and genotypes, the IK-2 medium revealed the highest % of shoot formation (57.12%), while the IK-3 was the lowest one (10.38%) in combination of IAA + Kin. However, the NB-4 medium exhibited highest % of shoot formation (13.05%), while the NB-2 was the lowest one (3.86%) in combination of NAA + BA. These differences between the medium were highly significant (Table 4. This indicating that the regeneration rate depends on the genotype, explant and growth hormones combinations.

Number of shoots per explant (Shoots/exp)

The mean values of Shoots/exp ranged from 6.46 in UC_{97-3} to 15.39 in Castle Rock and from 1.77 in Super Marmand to 3.99 in Castle Rockin both combinations of IAA + Kin and NAA + BA, respectively (Table 3). These differences between the genotypes were significant (Table 4).

Table 4. Analysis of variance for the percentage of explant produced callus (C%), regeneration rate (Reg%), number of shoots per explant (No. shoots/exp) and percentage of explant produced root (Rot%)

Source	df	Combination of IAA + Kin				Combination of NAA + BA			
		C%	Reg %	No. Sh/exp	Rot %	C%	Reg %	No. Sh/exp	Rot %
REP	2	12.57	12.95	12.76	12.80	0.37	1.37	1.32	1.46
G	3	591.7**	1066.2**	352.0**	537.9**	151.0**	107.3**	23.7**	139.3**
E	1	72.4**	6576.7**	805.9**	1639.5**	1203.7**	552.7**	30.9**	79.2**
C	3	898.6**	9836.9**	864.4**	10662.7**	2780.6**	358.7**	3.8**	13796.4**
G*E	3	49.80**	47.67**	15.81**	6.80**	480.80**	55.30**	3.25**	211.53**
G*C	9	77.3**	47.9**	3.0**	61.8**	641.9**	94.1**	0.8**	277.6**
E*c	3	117.9**	731.8**	156.8**	386.2**	1034.5**	61.1**	0.5**	4723.5**
G*E*C	9	34.9**	148.7**	16.2**	103.8**	645.8**	30.1**	0.2**	68.3**
Error	62	0.96	0.95	0.95	1.00	0.10	0.01	0.02	0.01

As average over the genotypes, the results in Table (3) revealed that the mean numbers of shoots per explant ranged from 5.88 to 24.73 of the cultured cotyledons and from 5.24 to 13.03 of the cultured hypocotyls on the combination with IAA + Kin and from 3.14 to 4.31 of the cultured cotyledons and ranged from 2.31 to 3.06 of the cultured hypocotyls on the combination with NAA + BA. These differences between the type of explants were highly significant and suggested that the cotyledon explants exceeded the hypocotyle explants in shoot regeneration.

Overall explants and genotypes, the IK-2 and NB4 media revealed the highest number of shoots/exp (18.88 and 3.69 shoots/exp, respectively) while the IK-3 and NB-2 showed the lowest mean values (5.56 and 2.77, respectively) (Table 3). These differences between the medium were highly significant (Table 4). This indicating that the regeneration rate depends on the genotype, explant and concentration of the growth hormones. The different types of interactions were also significant suggesting that number of shoots per explant depends upon the genotype, the explant, the concentration of growth hormones as well as the interaction between them.

Percentage of Rooting (R%)

The results in Table (3) revealed that the percentage of rooting varied according to the genotypes, the type of explant and concentration of the growth hormones in both combinations. The results in Table (4) revealed that the differences between each of the concentrations of the growth hormones, genotypes, and explants as well as the interaction between them were significant. Table (3) showed that the IK-2 and NB-4 media exhibited the highest percentage of root formation in both combinations (55.35 and 50.59%, respectively). The genotype Castle Rock possessed the higher percentage of rooting (28.93 %) in combination of IAA + Kin, while this genotype revealed the lowest percentage of root formation (25.1 %) in combination of NAA + BA.

Both IK-2 and NB-4 medium possessed high numbers of shoots/exp (18.88 and 3.69, respectively) and high % of rooting (55.35 and 50.59, respectively) which suggesting that the IK-2 and NB-4 medium were more suitable for regeneration of tomato plants than the other medium in both combinations.

Genetic diversity among tomato varieties

ISSR and SRAP marker systems being employed to assess the genetic diversity among the four tomato varieties were quite informative and were able to generate adequate polymorphism and unique DNA fingerprints for identification of these varieties.

In total, nine ISSR primer, and seven SRAP primer combinations gave reproducible results that were further considered for data analysis. Table 5 shows the total number of bands and the percentage of polymorphisms for each primer or primer combinations.

Of the 75 bands amplified by nine ISSR primers, 45 were polymorphic, with an average of five polymorphic fragments per primer. The percentage of polymorphism ranged from 50.0 % (ISSR-2, ISSR-3 and ISSR-5) to a maximum of 77.8 % (ISSR-9), with an average of 60.0 % (Table 5).

Seven SRAP primer combinations generated a total of 97 bands across the four tomato varieties where out of them, 53 bands were polymorphic with an average of 6.63 per primer. Maximum percentage of polymorphism was observed using primer SRAP-7 (63.6 %), while minimum percentage was observed using primer SRAP-2 (40.0 %), with an average of 54.6 % (Table 5).

Table 5. Number of amplified DNA-bands and polymorphic bands in four tomato varieties investigated with ISSR and SRAP primers.

	CR	SB	UC	SM	TB	MB	PB	%PB
ISSR-1	7	7	7	8	10	3	7	70.0
ISSR-2	6	7	5	6	8	4	4	50.0
ISSR-3	6	7	7	8	10	5	5	50.0
ISSR-4	6	5	4	5	7	2	5	71.4
ISSR-5	6	6	6	5	8	4	4	50.0
ISSR-6	6	7	6	7	9	4	5	55.6
ISSR-7	8	7	6	7	9	4	5	55.6
ISSR-8	3	3	5	3	5	2	3	60.0
ISSR-9	6	5	5	7	9	2	7	77.8
Total	54.00	54.00	51.00	56.00	75.00	30.00	45.00	--
AVR	6.00	6.00	5.67	6.22	8.33	3.33	5.00	60.04
SRAP-1	13	9	11	14	16	7	9	56.3
SRAP-2	11	12	13	11	15	9	6	40.0
SRAP-3	12	12	10	15	16	8	8	50.0
SRAP-4	9	9	9	12	16	6	10	62.5
SRAP-5	8	11	8	10	12	5	7	58.3
SRAP-6	8	6	7	9	11	5	6	54.5
SRAP-7	9	7	7	8	11	4	7	63.6
Total	70.00	66.00	65.00	79.00	97.00	44.00	53.00	--
AVR	10.00	9.43	9.29	11.29	13.86	6.29	7.57	55.04
Total	124.00	120.00	116.00	135.00	172.00	74.00	98.00	--
AVR	7.75	7.50	7.25	8.44	10.75	4.63	6.13	57.85

CR (Castel Rock), SB (Super Strain B), UC (UC_{97-3}), SM (Super Marmand), TB (Total bands), MB (Monomorphic bands), PB (Polymorphic bands), %PB (% Polymorphic bands)

Combined analysis of molecular data

Combined analysis was carried out using ISSR and SRAP marker systems data to obtain more accurate genetic estimates. The two molecular marker systems produced a total of 172 DNA fragments, with an average of 10.12 bands per primer, and the average of their polymorphism was 65.98% (Table 5). The genotype Super Marmand displayed the highest number of DNA fragments (135 bands) followed by Castle Rock (124 bands) and Super Strain B (120 bands), while the genotype UC_{97-3} revealed the least number of bands (116 bands). Both marker systems were successful in characterizing the four tomato varieties by unique positive and/or negative markers (Table 6).

Table 6. Tomato varieties identified by unique presence or absence of specific ISSR and/or SRAP markers.

Markers	CR		SB		UC		SM	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
ISSR-1	245 bp	403 bp, 380 bp		492 bp		437 bp		320 bp, 180 bp
ISSR-2		564 bp	227 bp					
ISSR-3			760 bp		372 bp	674 bp	349 bp	
ISSR-4	412 bp	446 bp		243 bp				
ISSR-5	339 bp		231 bp					318 bp
ISSR-6		877 bp			344 bp	502 bp	756 bp	
ISSR-7	260 bp			515 bp		689 bp		402 bp
ISSR-8					180 bp			404 bp
ISSR-9	616 bp	430 bp	696 bp	240 bp		369 bp		
SRAP-1	541 bp			321 bp, 144 bp	374 bp	798 bp, 176 bp	276 bp	
SRAP-2	1056 bp	290 bp			964 bp			
SRAP-3	317 bp	145 bp, 91 bp				461 bp, 253 bp	629 bp, 228 bp	
SRAP-4	380 bp	328 bp	395 bp		751 bp 520 bp	987 bp	335 bp, 286 bp, 146 bp	
SRAP-5		446 bp				703 bp, 480 bp, 306 bp	244 bp	
SRAP-6	204 bp		477 bp	173 bp			369 bp	
SRAP-7	447 bp		265 bp	712 bp		395 bp		624 bp

Somaclonal variation

In this part of the present investigation, both molecular analysis and morphological evaluation were used to study and determine somaclonal variations in tomato plants regenerated from the four tomato varieties (donor parents).

Molecular analysis of tomato somaclones

Nine ISSR primers and seven SRAP primer combinations which gave reproducible and polymorphic

bands among the four tomato varieties were used to examine the genetic variation between the regenerated plants (somaclones) and their donor parents as well as among somaclones.

Both marker systems generated a total of 191 bands across 40 somaclones and four donor parents out of them, 173 bands (90.58%) were polymorphic and 18 bands (9.42) were common between all genotypes (Table 7). The number of polymorphic bands ranged from 6 (ISSR-8) to 18 (SRAP-1) with an average of 10.81 polymorphic bands per primer. The percentage of polymorphism across all primers ranged from maximum 100% (ISSR-4, ISSR-6, ISSR-8, SRAP-5and SRAP-6) to minimum 76.47% (SRAP-2). The PIC values ranged from 0.24 (ISSR-3) to 0.34 (SRAP-3) with an average 0.29. The MI The values ranged from 1.85 (ISSR-8) to 4.91 (SRAP-1). The highest RP value 8.91 was recorded for SRAP-3 while the minimum value 2.64 was recorded for ISSR-8 (Table 7). Figure 2, for example shows, the genetic variations between the donor parents and its regenerated plants using ISSR and SRAP primers.

Table 7. Genetic marker information generated from tomato donor parents and its somalones using ISSR AND SRAP marker systems

Markers	TB	PB	%PB	PIC	MI	RP
ISSR-1	13	12	92.31	0.32	3.88	6.23
ISSR-2	9	8	88.89	0.30	2.38	3.86
ISSR-3	10	8	80	0.24	1.9	3.14
ISSR-4	7	7	100	0.33	2.29	3.36
ISSR-5	9	8	88.89	0.26	2.06	3.27
ISSR-6	10	10	100	0.29	2.89	4.18
ISSR-7	10	8	80	0.27	2.14	4.14
ISSR-8	6	6	100	0.31	1.85	2.64
ISSR-9	10	9	90	0.29	2.58	4.05
SRAP-1	19	18	94.74	0.27	4.91	7.5
SRAP-2	17	13	76.47	0.25	3.27	6.5
SRAP-3	16	13	81.25	0.34	4.43	8.91
SRAP-4	16	15	93.75	0.27	4.01	6.27
SRAP-5	15	15	100	0.31	4.67	7
SRAP-6	12	12	100	0.29	3.48	4.82
SRAP-7	12	11	91.67	0.26	2.83	4.09
Total	191	173	--	--	--	--
AVR	11.94	10.81	90.58	0.29	3.1	5

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

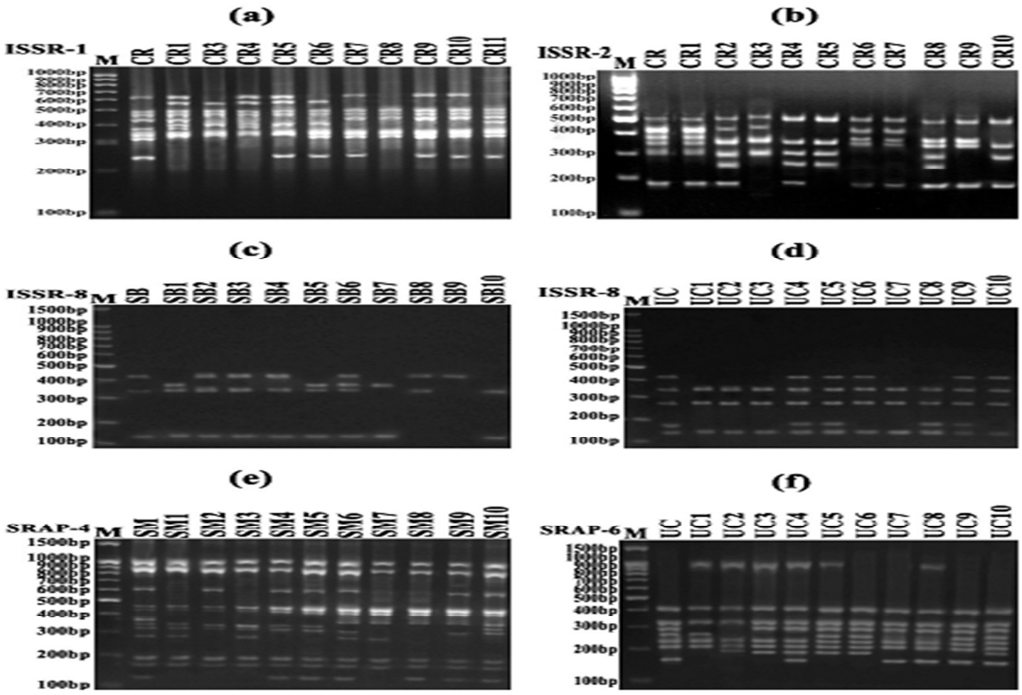

Fig . 2. ISSR and SRAP profile of donor parents plant and its somaclonals (a and b) ISSR-1 and ISSR-2 profiles for CR donor parent and its somaclones (c) ISSR-8 profiles for SB donor parent and its somaclones (d) ISSR-8 profiles for UC donor parent and its somaclones (e) SRAP-6 profiles for SM donor parent and its somaclones (f) SRAP-6 profiles for UC donor parent and its somaclones.

Genetic variations between each donor parent and its regenerated plants (somaclones)

For donor parent Castle Rock (CR) and its regenerated plants, both marker systems generated a total of 141 DNA fragments, 89 bands of them (63.12%) were polymorphic. Out of the 89 polymorphic bands, 72 were parental bands which were absence in some somaclones and 17 were novel non-parental bands which were appeared only in some somaclones.

Evidently, CR3 somaclone had the maximum genetic change as represented by the presence of 7 non-parental and the absence of 29 parental bands, while CR8 had undergone minimum genetic change containing 9 non-parental bands and absence of 11 parental bands.

The percentage of polymorphism (54.74%) between the donor parent Castle Rock and its somaclones regenerated from IK-2 medium was higher than the polymorphism between the donor parent and its somaclones regenerated from NB-4 medium (42.34%). As well as the percentage of polymorphism among somaclones regenerated from IK-2 medium was higher compared with the polymorphism among the somaclones regenerated from NB-4 medium (55.47% and 38.93%, respectively).

For donor parent Super Strain B (SB) and its regenerated plants, both marker systems generated a total of 140 DNA fragments, 99 bands (70.71%) of them were polymorphic. Out of 99 polymorphic bands, 79 parental bands were absence in some somaclonales and 20 new DNA fragments which were appeared only in some somaclones.

Somaclones SB8 and SB9 had the maximum genetic change as represented by the presence of 5 and 7 non-parental bands, respectively and the absence of 36 and 34 parental bands, respectively, while SB5 somaclone had the minimum genetic change containing 9 new DNA fragments which not appeared in donor parent and missing 4 parental bands.

The percentage of polymorphism (61.48%) between the donor parent Super Strain B and its somaclones regenerated from NB-4 medium was higher than the polymorphism between the donor parent and its somaclones regenerated from IK-2 medium (50.0%). As well as the percentage of polymorphism among somaclones regenerated from NB-4 medium was higher compared with the polymorphism among somaclones regenerated from IK-2 medium (57.14% and 48.15%, respectively).

For donor parent UC_{97-3} (UC) and its regenerated plants, both marker systems generated a total of 135 DNA Fragments out of them, 70 bands (51.85%) were polymorphic. Among polymorphic bands, 51 parental bands were not detected in some somaclones and 19 non-parental bands were generated only in some somaclones. The somaclone UC3 had the highest genetic changes as represented by the presence of 12 new DNA bands and the missing of 16 parental bands, while UC6 somaclone had the lowest genetic changes containing 9 non-parental bands and absence of 4 parental bands.

The percentage of polymorphism (47.37%) between the donor parent UC97-3 and its somaclones regenerated from IK-2 medium was higher than the polymorphism between the donor parent and its somaclones regenerated from NB-4 medium (44.36%). While the percentage of polymorphism among somaclones regenerated from NB-4 medium was higher compared with polymorphism among somaclones regenerated from IK-2 medium (42.11% and 40.61%, respectively)

For donor parent Super Marmand (SM) and its regenerated plants, both marker systems generated a total of 137 DNA fragments, included 61 polymorphic bands (44.53%). Among polymorphic bands, 59 were parental bands which were not detected in some somaclones and only two bands were non-parental which were found only in five somaclones regenerated from NB-4 medium. The SM4 somaclone had the maximum genetic change as represented by the absence of 29 parental bands, while SM9 had the minimum genetic change containing 2 non-parental bands and absence of 18 parental bands.

The percentage of polymorphism (37.23%) between the donor parent Super Marmand and its somaclones regenerated from NB-4 medium was higher than the polymorphism between the donor parent and its somaclones regenerated from IK-2 medium (33.33%).

Similarly, the percentage of polymorphism among somaclones regenerated from NB-4 medium was higher compared with the polymorphism among somaclones regenerated from IK-2 medium (33.83% and 29.13%, respectively

Genetic similarity

The genetic similarity among all somaclones and their donor plants ranged from minimum 0.585 between somaclones SB9 and CR2 to maximum 1.0 between SM1 and SM3 with an average of 0.698. The dendrogram generated based on a combined ISSR and SRAP data set revealed a better representation of the relationship between the tested tomato varieties than individual markers (Fig. 3). The dendrogram grouped all somaclones and their donor plants into two major groups with genetic similarity 0.698.

The first group comprised of the donor parent Super Strain B (SB) and its regenerated plants (ten somaclones) with genetic similarity 0.744. This group divided into two main clusters, the first one contained the donor parent (SB) and the five somaclones regenerated from IK-2 medium with genetic similarity 0.852. Cluster II included the five somaclones regenerated from NB-4 medium with genetic similarity 0.784. Within this group, similarity index between the SB donor parent and its somaclones ranged between 0.767 and 0.907. The highest genetic similarity was found between donor parent and SB5 somaclone with value of 0.907, whereas SB9 somaclone had undergone the highest degree of genetic change from the parent which was evidenced by the lowest value for genetic similarity (0.767) between them. Similarity index among the somaclones also varied from 0.653 to 0.878. SB4 and SB8 displayed the lowest similarity 0.653, while the highest genetic similarity was observed between SB3 and SB4 (0.878). Overall somaclones and medium combinations, the similarity index among somaclones regenerated from IK-2 medium was higher than those regenerated from NB-4 medium.

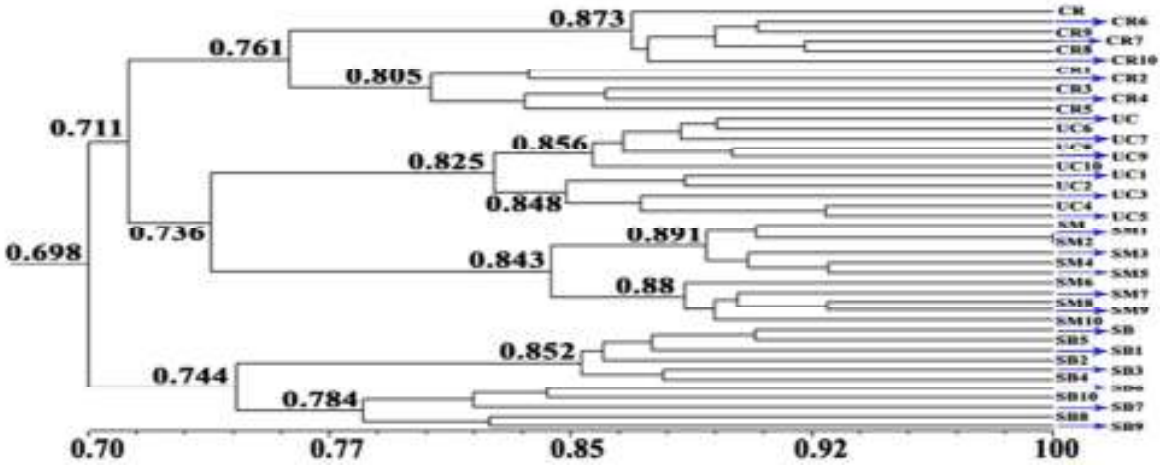

Fig. 3. Dendrogram of tomato donor parents and its somaclones developed from ISSR and SRAP data using UPGMA analysis. The scale is based on Dice coefficients of similarity.

The second group comprised of 30 somaclones and three donor parents with genetic similarity 0.711which was divided into three sup-groups.

Sup-group I comprised of the donor parent Castle Rock (CR) and its somaclones with genetic similarity 0.761. This sup-group I further divided into two clusters. Cluster I contained the donor parent and all somaclones regenerated from NB-4 medium with genetic similarity 0.873. Cluster II included all somaclones regenerated from IK-4 medium with genetic similarity 0.805. Within sup-group I, similarity index between the donor parent and its somaclones was ranged between 0.804 and 0.879. The highest genetic similarity value 0.879 was found between the donor parent and CR6 while the lowest value 0.804 was recorded between the donor parent and somaclone CR3. Similarity index among the somaclones also varied from 0.676 to 0.922. The lowest degree of genetic similarity among the 10 somaclones was found between CR2 and CR9 (0.676), while the highest degree of genetic similarity was observed between CR7 and CR8 (0.922). Overall somaclones and medium combinations, the similarity index among somaclones regenerated from NB-4 medium was higher compared with similarity index among somaclones regenerated from IK-2 medium.

Sup-group II comprised of the donor parent $UC_{97\text{-}3}$ (UC) and its somaclones with genetic similarity 0.825. Sup-group II divided into two main clusters. Cluster I contained the donor parent and all somaclones regenerated from NB-4 medium with genetic similarity 0.8596. Cluster II included all somaclones regenerated from IK-4 medium with genetic similarity 0.848. Similarity index between the parent UC and its somaclones ranged between 0.815 and 0.895. Maximum genetic similarity was between the donor parent and UC6 somaclone (0.895), whereas UC3 had the highest degree of genetic change from the donor parent which was evidenced by the lowest value of genetic similarity (0.815) between them. Genetic similarity among the somaclones regenerated from UC donor parent also varied from 0.771 to 0.929. The highest degree of genetic similarity among the 10 somaclones was found between the between UC4 and UC5 (0.929), while the lowest similarity value of 0.771 was found between the UC2 and UC9.

Overall somaclones and medium combinations, the similarity index among somaclones regenerated from IK-2 medium was higher compared with similarity index among somaclones regenerated from NB-4 medium.

Sup-group III comprised of the donor parent Super Marmand (SM) and the ten somaclones regenerated from it with genetic similarity 0.843. Sup-group III further divided into two clusters. Cluster I contained the donor parent and all somaclones regenerated from IK-2 medium with genetic similarity 0.891. Cluster II include all somaclones regenerated from NB-4 medium with genetic similarity 0.88.

The genetic similarity between the SM donor parent and its somaclones was ranged between 0.877 and 0.913. The highest genetic similarity was recorded between the donor parent and SM9 somaclone (0.913), while the lowest value (0.877) was found between the donor parent and each of somaclones SM7, SM8 and SM10 which had the highest degree of genetic changes from the donor parent. Similarity index among the somaclones also ranged from 0.8 to 1.00. Close genetic similarity was found between the SM1 and SM3 (1.00), while the lowest similarity was recorded between SM4 and SM7 (0.8). Overall somaclones and medium combinations, the similarity index among somaclones regenerated from IK-2 medium was higher than that among somaclones regenerated from NB-4 medium.

Overall donor parents and medium combinations, the highest polymorphism was recorded for the somaclones regenerated from Super Strain B variety (70.71%) followed by somaclones regenerated from Castle Rock and $UC_{97\text{-}3}$ varieties (63.12 and 51.85%, respectively) while the minimum polymorphism was recorded for somaclones regenerated from Super Marmand variety (44.53%). Somaclones regenerated from NB-4 medium exhibited higher polymorphism compared with plants regenerated from IK-2 medium for all tomato varieties except $UC_{97\text{-}3}$ variety.

Morphological evaluation of tomato somaclones

Regenerated plants (R_0) from Castle Rock (CR), Super Strain B (SB), UC_{97-3} (UC) and Super Marmand (SM) were transferred to the greenhouse. Somaclones which exhibited wide variations with its donor parent were established in the field and produced fruits. The first generation of these somaclones (R_1) as well as their donor parents were evaluated for different plant characters, the mean values of plant height, number of branches per plant, no. of inflorescences per plant, no. of flowers per inflorescence, no. of flowers per plant and number of fruits per plants and yield per plant are summarized in Tables (8). The analyses of variance for these characters are shown in table (9).

Generally, the results revealed that some somaclones exceeded their donor parents in one or more traits, while significant decrease in some traits were also observed (Table 8). Significant variations between the tested genotypes were observed for all studied traits, except no. of inflorescences per plant and no. of flowers per inflorescence for somaclones regenerated from the donor parent UC_{97-3}, and number of flowers per plants and yield per plant for somacloncs regenerated from the donor parent Super Marmand (Table 9).

Table 8. The mean values of plant height (PH), number of branches/plant (n.Brh), number of fruits/plants (n. Frt/P), yield/plant (Yi/P), number of inflorescences/plant (n. Inf/P), number of flowers/inflorescence (n.Fl/Inf), and number of flowers/plant (n. Fl/P) in tomato somaclones in comparison with their donor parent.

Parents	PH	n. Brh	n. Frt	Yi/P(g)	n. Inf/P	n. Fl/Inf	n. Fl/P
CR	64.64	12.83	35.58	3050.33	12.19	5.23	47.14
CR1	65.04	12.35	35.09	3061.33	11.58	4.63	45.12
CR2	64.01	11.24	33.47	2805.99	10.16	5.38	43.60
CR3	61.24	11.20	32.70	2933.43	11.91	5.82	46.34
CR4	66.34	14.23	33.67	2963.36	9.64	4.58	48.33
CR5	67.62	14.09	36.35	3077.82	13.84	4.33	44.28
CR6	70.82	13.65	38.27	3315.19	14.28	7.39	50.09
CR7	68.39	14.94	37.51	3092.01	14.95	6.59	48.91
CR8	73.47	15.92	40.62	3352.89	13.59	5.98	49.84
CR9	69.54	14.58	35.84	3073.04	13.84	5.83	46.94
CR10	71.15	13.67	41.37	3287.84	13.60	6.71	51.98
LSD. 0.05	3.928	1.763	3.955	190.5	0.5885	0.4711	3.103
SB	68.32	10.35	33.16	2781.43	9.25	4.42	37.68
SB1	68.47	13.32	34.82	2892.14	12.13	6.03	41.28
SB2	72.21	12.31	35.59	2983.34	12.97	5.38	40.67
SB3	70.97	10.55	35.81	2854.21	10.93	5.62	41.43
SB4	70.16	11.53	33.26	2813.37	10.09	4.59	38.41
SB5	72.42	10.57	35.94	3065.52	10.91	5.79	39.97
SB6	65.19	10.49	29.51	2570.75	8.34	3.81	37.53
SB7	67.15	8.27	28.16	2566.30	8.94	4.75	38.21
SB8	67.53	9.08	33.31	2901.21	7.44	4.12	38.28
SB9	68.46	9.41	32.04	2815.21	8.74	4.79	38.95
SB10	63.18	10.33	34.31	3046.68	9.88	4.36	39.81
LSD. 0.05	1.59	1.72	1.133	153.3	0.496	0.8442	0.4053
UC	75.58	15.08	40.37	2646.90	13.41	4.14	44.67
UC1	78.52	13.89	39.49	2699.23	14.21	4.93	46.64
UC2	75.00	12.13	39.02	2484.66	12.94	3.69	41.84
UC3	79.12	13.70	42.26	2737.92	13.90	5.24	46.26
UC4	78.64	15.34	41.54	2672.33	13.08	4.74	46.08
UC5	76.31	14.61	42.89	2762.81	14.80	5.39	47.40
UC6	82.73	18.04	45.04	2863.27	14.82	5.19	46.82
UC7	73.48	16.68	42.83	2860.39	15.36	5.38	47.76
UC8	74.46	15.72	38.07	2581.21	13.85	5.43	39.39
UC9	72.68	14.31	37.94	2516.82	11.86	3.58	44.17
UC10	74.89	16.66	40.91	2793.02	13.00	4.23	44.77
LSD. 0.05	3.622	2.402	3.525	199.7	2.692	1.266	3.461
SM	85.95	17.54	36.22	3572.06	10.04	5.96	57.34
SM1	87.08	19.15	37.56	3661.23	11.22	6.28	62.37
SM2	82.63	17.20	31.26	3456.26	9.64	5.08	55.43
SM3	84.57	20.46	38.70	3725.31	13.93	6.58	62.59
SM4	81.47	15.87	32.30	3380.41	13.34	5.60	57.64
SM5	88.51	15.05	38.44	3866.15	10.07	6.81	57.88
SM6	88.45	19.74	41.56	3814.79	13.82	6.47	61.97
SM7	88.36	19.44	38.37	3888.88	12.10	7.92	63.57
SM8	88.67	21.98	38.09	3687.84	10.26	6.22	59.63
SM9	90.19	21.23	39.56	3748.60	13.60	6.45	64.56
SM10	89.48	18.60	36.27	3598.08	10.32	5.88	55.70
LSD. 0.05	3.863	2.450	4.028	334.0	2.370	1.293	5.586

Table 9. The analysis of variance for mean values of plant height (PH), number of branches/plant (n.Brh), number of fruits/plants (n. Frt/P), yield/plant (Yi/P), number of inflorescences/plant (n. Inf/P), number of flowers/inflorescence (n.Fl/Inf), and number of flowers/plant (n. Fl/P) in tomato somaclones and their donor parent.

Parents	S.O.V	df	MS						
			PH	n. Brh	n. Frt	Yi/P	n. Inf/P	n. Fl/Inf	n. Fl/P
CR	Rep	2	2.997	23.013	3.15	99014.7	34.924	6.011	10.344
	Geno	10	39.534**	6.668**	23.991**	84887.8**	8.921**	2.811**	20.373**
	Error	20	7.093	1.429	7.19	16673.7	0.1592	0.102	4.427
SB	Rep	2	35.85	19.21	16.001	54826.8	37.713	3.49	13.804
	Geno	10	23.972**	6.155**	19.277**	81816.6**	8.299**	1.577**	5.979**
	Error	20	1.162	1.36	0.5897	10796.5	0.1131	0.3276	0.0755
UC	Rep	2	54.834	32.951	8.989	14249	26.402	1.5678	5.382
	Geno	10	26.386**	8.14*	14.884*	49030.6*	3.129	1.458	19.299**
	Error	20	6.029	2.651	5.711	18328.4	3.332	0.7363	5.505
SM	Rep	2	10.959	30.058	7.788	129080	11.112	3.118	8.409
	Geno	10	24.511**	14.091**	27.552**	78363	8.928**	1.593	31.861*
	Error	20	6.859	2.759	7.456	51288	2.582	0.7686	14.341

For somaclones regenerated from the donor parent Castle Rock, all somaclones regenerated from NB-4 medium were superior over their parent in all studied traits except number of flowers/plants for somaclone CR9. While the somaclones regenerated from IK-2 medium exhibited significant decrease in most traits compared with the donor parent (Table 8). Overall somaclones and medium combinations, the highest mean values for plant height (73.47 cm), number of branches (15.92), yield/plant (3352.89 g), number of flowers per inflorescence (5.98) were recorded for the somaclone CR-8. The highest mean values for number of fruits/plant (71.37), and number of flowers/plant (50.09) were recorded for CR10 while the highest mean value of number of inflorescences per plant (14.95) was recorded for CR7 (Table 8). On the other hand, the lowest mean values for plant height (61.24 cm), number of branches per plant (11.20) and number of fruits/plant (32.70) were recorded for the somaclone CR3. The lowest mean values for yield/plant (2805.99) and number of flowers/plant (43.60) were recorded for the somaclone CR2 while the lowest mean values for number of inflorescences/plant (9.64) and number of flowers/inflorescence (4.33) were recorded for the somaclones CR4 and CR5, respectively (Table 8).

For somaclones regenerated from the donor parent Super Strain B, all somaclones regenerated from IK-2 medium were superior over their donor parent in all studied traits. While the somaclones regenerated from NB-4 medium exhibited significant decrease in most traits compared with their donor parent (Table 8). Overall somaclones and medium combinations, somaclone SB-5 exhibited the highest mean values for plant height (72.42 cm), number of fruits/plant (35.94) and yield/plant (3065.52). The highest mean values for number of branches/plant (13.32) and number of flowers/inflorescence (6.03) were recorded for SB1, while the highest mean values for number of inflorescences/plant (12.97) and number of flower/plant (41.43) were recorded for somaclones SB2 and SB3, respectively (Table 8).On the other hand, the lowest mean value for plant height was recorded for somaclone SB10 (63.18 cm), somaclone SB7 showed the lowest mean values for number of branches/plant (8.27), number of fruits/plant (28.16) and yield/plant (2566.60 g), lowest number of inflorescences/ plant was recorded for SB8, while the lowest mean values for number of flowers/ inflorescence (3.81) and number of flowers/plant (37.53) were recorded for somaclone SB6 (Table 8)

For somaclones regenerated from the donor parent $UC_{97\text{-}3}$, most somaclones regenerated from both medium combinations were superior over their parent in one or more studied traits. While some somaclones exhibited significant decrease in most traits as compared with the donor parent (Table 8). In this respect, the somaclone UC6 significantly exceeded their donor parent in all studied traits. Meanwhile, the somaclones UC2 and UC9 revealed significant decrease in all studied traits compared with donor parent. Overall somaclones and medium combinations, the somaclone UC6 possessed the highest mean values for plant height (82.73 cm), number of branches/plant (18.04), number of fruit/plant (45.04) and yield/plant (2863.27 g), somaclone UC5 exhibited the highest mean value for number of flowers/inflorescence (5.39). The highest mean values for number of inflorescences/plant (15.36) and number of flower/plant (47.76) were recorded for somaclone UC7 (Table 8). Meanwhile, the lowest mean values for plant height (73.48 cm), number of branches/plant (12.13) and number of flower/plant (39.39) were recorded for somaclones UC7, UC2 and UC8, respectively, while the somaclone UC9 exhibited the lowest mean values for number of fruit/plant (37.94), yield/plant (2516.82 g), number of inflorescences/plant (11.86) and number of flowers/inflorescence (3.58) (Table 8).

For somaclones regenerated from the donor parent Super Marmand, all somaclones regenerated from NB-4 medium were superior than their parent in all studied traits except number of flowers/inflorescence and number of flowers/plants for somaclone SM10. Some somaclones regenerated from IK-2 medium exhibited significant decrease in most traits compared with the donor parent while the other somaclones were significantly exceeded their parent in all or most studied traits (Table 8). Overall somaclones and medium combinations, the highest mean values for plant height (90.19 cm) and number of flower/plant (64.56) were recorded for somaclone SM9, while the highest mean values for yield/plant (3888.88 gm) and number of flowers/inflorescence (7.92). The three somaclones SM8, SM6 and SM3 exhibited the highest mean values for number of branches/plant (21.98), number

of fruit/plant (41.56) and number of inflorescences/plant (13.93), respectively. The somaclone MS2 exhibited the lowest mean values for all studied traits except plant height and number of branches/plant. The lowest mean value for plant height (81.47 cm) and number of branches/plant (15.05) were recorded for somaclones SM4 and SM5, respectively (Table 8).

Disscussion

The present investigation was carried out to analyze the somaclonal variations among plants regenerated from *in vitro* culture of tomato. Four tomato varieties, two types of explants and two combinations of auxin and cytokinine with different concentrations were used to establish plant regeneration. Several previous studies on tomato *in vitro* culture referred that, callus induction and plant regeneration are affected by many factors such as genotype, explant type, physiological state of donor parent, plant hormones such as (auxins and cytokinins) and environmental conditions (Mamidala and Nanna 2011; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). Our results revealed that the differences among tomato varieties were significant for all tissue culture studied traits. These differences in response to tissue culture from one genotype to another were affected by type of explants and medium composition as shown Table (3). The effect of genotype on response to tissue culture in tomato were also observed by Ishag *et al.* (2009); Chaudhry *et al.* (2007) and Jabeen *et al.* (2005) who reported that the *in vitro* reactions of the genotypes to regeneration ability were differed significantly and dependent on the culture medium. In the present investigation two types of explants, cotyledon and hypocotyl were used and revealed that shoot regeneration can easily be obtained from them. However, the cotyledonary leaves exceeded the hypocotyls in shoot regeneration in both combinations of growth hormones under different concentrations. These results are inagreement with (Ali *et al.,* 2012; Sharma and Srivastava 2014; Gerszberg *et al.,* 2016) who found that the cotyledonary explants produced the highest number of shoots. Also Madhulatha *et al.* (2006) who observed that among three explants, cotyledon was found most effective in callus induction and shoot regeneration ability. In contrast, Namitha and Negi (2013) observed that hypocotyls explant was superior on cotyledons and leaf explants. The two types of plant hormones, auxin and cytokinin alone or in different combinations has been widely used in tomato tissue culture media. These hormones play an important role in regulating callus formation, shoots and roots initiation (Ishag *et al.,* 2009; Chaudry *et al.,* 2010; Mamidala and Nanna 2011; Zhang *et al.,* 2012; Namitha and Negi 2013). The results of this study revealed that all tissue culture traits were varied from one growth regulator combination to another as well as from one concentration to another on the same combination of hormones (Table 3), these differences were significant (Table 4). Our results also revealed that the combinations of IAA + Kin were superior over NAA + BA for all studied traits except for percentage of callus formation. These results are in line with Sharma and Srivastava (2013) and Sharma and Srivastava (2014) who found that the maximum shoot regeneration was obtained on combination of Kin and IAA. These results revealed that the interactions among three factors genotypes, type of explant and concentration of growth were also significant suggesting that plants regeneration depends upon the genotype, the explant, the concentration of growth regulators as well as the interaction between them. Overall varieties, type of explants and growth regulators, results indicated that cotyledon were the best explant for regeneration and the IK-2 medium (4mg/l Kin + 4mg/l IAA) and NB-4 medium (2mg/l BA + 0.5mg/l NAA) were the best for shoot regeneration in both combination of Kin + IAA and BA + NAA, respectively.

Regenerated plants from cotyledonary cultured on both IK-2 and NB-4 medium for each tomato varieties were subjected to analysis of somaclonal variation through molecular markers analysis and agro-morphological traits evaluation. The two molecular marker systems ISSR and SRAP were used to detect the genetic variation among the donor parents. Both markers differ in technical principle, type of inheritance, reproducibility, distribution in the plant genome and amount of polymorphism (Zietkiewicz *et al.,* 1994; Provan *et al.,* 1999; Li and Quiros 2001). Both marker systems were quite informative and were able to generate adequate polymorphism and unique DNA fingerprints for identification of these varieties. All ISSR and SRAP primers generated a total of 172 bands through the four donor parents. The number of generated DNA fragments (97 bands) using SRAP primers was more than the DNA fragments (75 bands) amplified by ISSR primers while the ISSR markers exhibited polymorphism among donor parents higher than polymorphism with SRAP markers. The two marker systems were successful in characterizing the four tomato varieties by unique positive and/or negative markers (Table 6). The present finding is consistent with the earlier report of Comlekcioglu *et al.* (2010); Aguilera *et al.* (2011); Mane *et al.* (2013), Henareh *et al.* (2016); Al Shaye *et al.* (2018) and Kiani and Siahchehreh (2018).

In our study, the ISSR and SRAP primers were used to analysis somaclonal among regenerated plants and study the effect of genotypes and growth regulator combinations on ability of induced somaclonal variation in tomato tissue culture. The results revealed that both marker systems were sufficient to detect polymorphism among each donor parent and its somaclones, as well as among somaclones. The percentage of polymorphism among each donor parent and its somaclones was different from one parent to another on the same combination of growth regulators or between the two combinations of growth regulators. Also the variations among the somaclones differed from one somaclone to another on the same combination and/or between the two combinations of growth regulators. Our results revealed that the highest polymorphism was found between the donor parent Super Strain B and its somaclones as well as among somaclones themselves while the lowest polymorphism was found between the donor parent Super Marmand and its somaclones. These results indicate that the molecular changes which occurred in tomato tissue culture depending on the genotype (Soniya *et al.,* 2001). The results also revealed that the combination of naphtaline acetic acid and benzyl adenine induced relatively high genetic changes

among regenerated plants in tomato tissue culture compared with the growth regulators combination of kinetin and indole acetic acid. These results indicated that the somaclonal variations among regenerated plants form tomato tissue culture are dependent on the genotypes and growth regulators combination. All polymorphic bands among parent donor and its somaclones were occurred in parental bands which were absence in some somaclonales and/or new non-parental bands which were generated only in some somaclones. This result is in line with determination of genetic polymorphism of somaclones in tomato by isozymes technique Mahmoud *et al.* (2004), RAPD (Soniya *et al.,* 2001; Mansour *et al.,* 2005; Ali *et al.,* 2017), AFLP (Ana *et al.,* 2014) and other plants also such as, potato (Karácsonyi *et al.,* 2011), sugarcane (Martínez-Estrada *et al.,* 2017).

Variations observed in total number of ISSR and SRAP bands as well as the number of bands among the donor parents and regenerated plants indicate genetic differences of the genotypes due to tissue culture and somaclonal variation induced. The presence of specific DNA fragment in the donor parents and absence of it in some regenerated plants referees the loss of certain loci during tissue culture due to somaclonal variation, while the appearance of specific fragments in some regenerated plants and their absence in donor parents may indicate the occurrence of genetic changes leading to formation of new binding sites in these somaclones. Such specific loci are of high importance in the genetic identification of the genotypes or somaclones from each other. Since, even single base change at the primer annealing site is manifested as presence or absence of ISSR and/or SRAP bands, it could be suggested that tissue culture conditions have induced varied amount of genetic changes in different regenerated plants. Some of these changes appeared identical in different plants as represented by appearance of non-parental bands. The reason for such commonness of genetic variation in these plants could be because they were all derived from the same callus (Soniya *et al.,* 2001; Ali *et al.,* 2017). The variations observed in the ISSR or SRAP patterns may be due to different causes including loss or gain of a primer annealing, due to point mutations or by the insertion or deletion of sequence or transposable elements (Peschke *et al.,* 1991).

To confirm stability of genetic variations in progenies of regenerated somaclones. The R_1 somaclone progenies were grown in the field to combared with donor parents on the basis of various agro-morphological characters. Overall screening of the data recorded on somaclones has indicated a wide range of variations for all agro-morphological traits in relation to the donor parent.

Results revealed that some somaclones exceeded their donor parents in one or more traits, while significant decrease in some traits was also observed. Significant agro-morphological differences obtained among the regenerated plants may indicate that the molecular or genetic variation obtained is partly responsible for agro-morphological variations, and also show the possible use of tissue culture in inducing new morphological (possibly new agronomic) traits in the tomato which may be used for breeding purposes. These results coincide with Nayak *et al.,* 2003; Li *et al.,* 2010; Cao *et al.,* 2016 who studied the genetic variation among regenerated plants from tissue culture of different crops and observed that some somaclones were exceeded their donor parents in one or more qualitative and/or quantitative traits.

CONCLUSIONS

This study has shown that in *in vitro* culture of tomato, cotyledonary explants was better responsive in terms of number of shoots per explant than hypocotyl explants and showed that the MS basal medium supplemented with Kin in conjunction with IAA was better for shoots induction compared with MS basal supplemented with BA in conjunction with NAA from cotyledonary explants. These results suggest that the genotypes and plant growth regulators are important factors affected induction of somaclonal variants in tomato tissue culture. Thus, the results suggest that testing a large number of different genotypes and different combinations of plant hormones giving a good chance of obtaining a high percentage of genetic variation among regenerated plants that can be exploited in different breeding programs. The results also refer to the importance of using different molecular marker systems in early detection of the extent of molecular changes occurring in plants regenerated from tissue cultures, which can be directly reflected on agro-morphological characteristics, saving time and effort to obtain new varieties that superior their parents. The study allows selecting somaclonal variants with higher plant height, number of branches/plant, no. of inflorescences/plant, no. of flowers/inflorescence, no. of flowers/plant, number of fruits/plants and yield/plant.

REFERENCES

Abdellatif, K. F.; Hegazy, A. E.; Aboshama, H. M.; Emara, H. A. and ElShahed, A. A. (2012). Morphological and molecular characterization of somaclonal variations in tissue culture-derived banana plants. J Genet Eng Biotechnol, 10: 47–53.

Aguilera, J. G.; Pessoni, L.A.; Rodrigues, G. B.; Elsayed, A. Y.; Silva, D. J. H. and Barros, E. G. (2011). Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill.). Rev. Bras. Ciênc. Agrár.; Recife, 6 (2): 243-252.

Al Hattab, Z. N.; El Kaaby, E. A. and Al Ajeel, S. A. (2017). Molecular analysis of somaclonal variations in chili pepper (*Capsicum annuum* L). Bioscience Research, 14(4): 831-838.

Al Shaye, N.; Migdadi, H.; Charbaji, A.; Alsayegh, S.; Daoud, S.; AL-Anaz, W. and Alghamdi, S. (2018). Genetic variation among Saudi tomato (*Solanum lycopersicum* L.) landraces studied using SDS-PAGE and SRAP markers. Saudi Journal of Biological Sciences, 25: 1007–1015.

Ali, A. A.; El-Denary, M. E.; El-Gendy, A.; Galal, O. A.; Ahmad, M. E. and El-Sayed, T. R. (2017). Detection of somaclonal variations in tomato using RAPD markers. Egyptian J of Genetics and Cytology, 46 (1): 89-99.

Ali, A. A.; Yossef, T. R. and El-Banna, A. (2012). Cytokinin cytokinin interaction ameliorates the callus induction and plant regeneration of tomato (*Solanum lycopersicum* Mill.). Acta Agronomica Hungarica 60: 47-55.

Ana, C.; Dana, Ş.; Ioan, B.; Constantin, D.; Sergiu, V. and Adela, H. (2014). Genetic stability monitoring in micropropagated tomato cultivars. Muzeul Olteniei Craiova. Oltenia. Studii şi comunicări. Ştiinţele Naturii. Tom. 30(2): 38-43.

Anil, V. S.; Bennur, S. and Lobo, S. (2018). Somaclonal variations for crop improvement: Selection for disease resistant variants in vitro. Plant Science Today, 5(2): 44-54.

Aruingtyas, E. L.; Widoretno, W. and Idriyani, S. (2012). Somaclonal variation of soybean (*Glysin max*. L. Merr) stimulated by draught stress based on Random Amplified Polymorphic DNA (RAPDs). American Journal of Molecular Biology, 2: 85–91.

Bahurpe, J. V.; Patil, S. C.; Pawar, B. D.; Chimote, V. P. and Kale, A. A. (2013). Callus induction and plantlet regeneration in tomato (*Solanum lycopersicum* L.). J Cell Tissue Res., 2: 3765–3768.

Bairu, M. W.; Aremu, A. O. and Van Staden, J. (2011). Somaclonal variation in plants: causes and detection methods. Plant Growth Regulation, 63: 147–173.

Cao, Z.; Sui, S.; Cai, X.; Yang, Q. and Deng, Z. (2016). Somaclonal variation in 'Red Flash' caladium: morphological, cytological and molecular characterization. Plant Cell Tissue Organ Cult., 126: 269–279.

Chaudhry, Z.; Afroz, A. and Rashid, H. (2007). Effect of variety and plant growth regulators on callus proliferation and regeneration response of three tomato cultivars (*Lycopersicon esculentum*) Pak. J. Bot., 39(3): 857-869.

Chaudry, A.; Abbas, S.; Yasmin, A.; Rashid, H.; Ahmed, H. and Anjum, M. A. (2010). Tissue culture studies in tomato (*Lycopesricon esculentum*) var. Moneymaker. Pak J Bot., 1: 155–163.

Comlekcioglu, N.; Simsek, O.; Boncuk, M. and Aka-Kacar, Y. (2010). Genetic characterization of heat tolerant tomato (*Solanum lycopersicon*) genotypes by SRAP and RAPD markers. Genet. Mol. Res., 9: 2263–2274.

Dice, L. R. (1945). Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

Doyle, J. J. and Doyle, J. L. (1990). A RAPD-DNA isolation procedure for small quantity of fresh leaf tissues. Phytochemical Bulletin, 19: 11-15.

El-Shahed, A. A.; Abdellatif, K. F.; Ibrahim, A. I.; Mohamed, A. M.; Abdelsalam, I. Z. and Elsehrawy, O. A. (2017). Efficiency of sequence related amplified polymorphism (SRAP) and target region amplified polymorphism (TRAP) markers in detecting banana somaclonal variants. African Journal of Biotechnology, 16(16): 879-888.

Evans, D.A. and Sharp, W. R. (1983). Single gene mutations in tomato plants regenerated from tissue culture. Science, 221: 949–951.

Gerszberg, A.; Hnatuszko-Konka, K.; Kowalczyk, T. and Kononowicz, A. K. (2016). Efficient In Vitro Callus Induction and Plant Regeneration Protocol for Different Polish Tomato Cultivars. Not Bot Horti Agrobo, 44(2): 452-458. DOI : 10.15835 / nbha44210530

Ghislain, M. D.; Fazardo, Z. D.; Huaman, Z. and Hismans, R. H. (1999). Marker assisted ampling of the cultivated Andean Potato (*Solanum fureja*) collection using RAPD markers. Genet Resour Crop Evo., 46: 547-555.

Hanus-Fajerska, E. (2006). Variation in tomato plants regenerated from Cucumber Mosaic Viruse infected tissue. ISHS Acta Hort789: XV Meeting of the EUCARPIA Tomato Working Group. http:// www.actahort.org/books/789/789_40.htm

Henareh, M.; Dursun, A.; Mandoulak̄ani, B. A. and Haliloğlu, K. (2016). Assessment of genetic diversity in tomato landraces using ISSR markers. Genetika, 48 (1): 25-35.

Ishag, S.; Osman, M. G. and KhalafallA, M. (2009). Effects of growth regulators, explant and genotype on shoot regeneration in tomato (*Lycopersicon esculentum* c.v. Omdurman). Int. J. Sustain. Crop Prod., 4(6): 7-13.

Ishag, S.; Osman, M. G. and Khalafalla, M. M. (2009). Effects of growth regulators and genotype on shoot regeneration in tomato (*Lycopresicon esculentum* c.v. Omdurman). Int J Sustain Crop Prod., 6: 7–13.

Jabeen, N.; Chaudhry, Z.; Rashid, H. and Mirza, B. (2005). Effect of genotype and explant type on in vitro shoot regeneration of tomato (*Lycopersicon esculentum* Mill.). Pak. J. Bot., 37(4): 899-903.

Jehan, S. and Hassanein, A. M. (2013). Hormonal requirements trigger different organogenic pathways on tomato nodal explants. Am J Plant Sci., 4: 2118–2125. doi:10.4236/ajps.2013.411263

Kalloo, G. (1991). Genetic improvement of tomato. Kalloo G (eds) Monographs of Theoretical and Applied Genetics Springer-verlag, Berlin, Heidelberg, New York, 1-9.

Kantor, M.; Sestras, R. and Chowudhury, K. (2010). Identification of the most organogenic-responsive variety of tomato using the variety X medium interaction. Rom Biotechnol Lett., 5: 5640–5645.

Karácsonyi, D. E.; Chiru, N. and Nistor, A. (2011). Microsatellite analysis of somaclonal variation in potato (*Solanum tuberosum* Ssp. Tuberosum) plantlets regenerated from callus. Romanian Biotechnological Letters, 16(1): 81-83.

Kiani, G. and Siahchehreh, M. (2018). Genetic Diversity in Tomato Varieties Assessed by ISSR Markers. International Journal of Vegetable Science, 24(4): 353–360.

Li, G. and Quiros, C. F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor. Appl. Genet., 103: 455-461.

Li, R.; Bruneau, A. H. and Qu, R. (2010). Tissue culture-induced morphological somaclonal variation in st.augustinegrass [*Stenotaphrum secundatum* (walt.) kuntze]. Plant Breed., 129: 96-99. https://doi.org/10.1111/j.1439-0523.2009.01647.

Madhulatha, P.; Pandey, R.; Hazarika, P. and Razam, M. V. (2006). Polymines and maltose significantly enhance shoot regeneration in tomato. Physiology and Molecular Biology of Plants, 12: 295-301.

Mahmoud, M. A. H.; Dakhly, O. F.; Abdel-Rahem, A. T. and Anwar, G. M. (2004). In vitro regeneration and somaclonal variation among regenerated plants of tomato (*Lycopersicon esculentum* Mill.). The 4th Scientific Conference of Agriculture Science, Assiut, December, 2004 885-900.

Mamidala, P. and Nanna, R. S. (2011). Effect of genotype, explants source and medium on in vitro regeneration of tomato. Int J Genet Mol Biol., 3: 45–50.

Mane, R.; Sridevi, O.; Nishani, S.; Salimath, P. M. (2013). Evaluation of genetic diversity and relationships among tomato genotypes using morphological parameters and SRAP markers. Indian J. Hortic., 70: 357–363.

Mansour, A.; Zaki, M.; Fayed, A. H. and Shawky, A. (2005). Biochemical and molecular markers associated with callus induction in some tomato cultivars. Egypt. J. Exp. Biol., (Bot.), 1: 21 – 28.

Mantel, N. A. (1967). The detection of disease clustering and a generalized regression approach. Cancer Res., 27(2): 209-220.

Martínez-Estrada, E.; Caamal-Velázquez, J. H.; Salinas-Ruíz, J. and Bello-Bello J. J. (2017). Assessment of somaclonal variation during sugarcane micropropagation in temporary immersion bioreactors by intersimple sequence repeat (ISSR) markers. In Vitro Cell. Dev. Biol.—Plant, 53:553–560.

Masoud, S. and Hamta, A. (2008). Cytogenetic analysis of somaclonal variation in regenerated plants of Berseem clover (*Trifolium alexandrium* L.). Caryologia, 61(4): 392-396.

Murashige, T. and Skoog, F. A. (1962). Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15: 473–497.

Namitha, K. K. and Negi, P. (2013). Morphogenetic potential of tomato (*Lycopersicon esculentum*) cv. Arka Ahuti to plant growth regulators. Notulae Scientia Biologicae, 2: 220–225.

Nayak, S.; Debata, B. K.; Srivastava, V. K. and Sangwan, N. S. (2003). Evaluation of agronomically useful somaclonal variants in jamrosa (a hybrid cymbopogon) and detection of genetic changes through RAPD. Plant Sci., 164:1029–1035. https://doi.org/10.1016/S0168-9452(03)00090-6

Nehra, N. S.; Kartha, K. K.; Stusnoff, C. and Giles, K. L. (1992). The influence of plant growth regulator concentrations and callus age on somaclonal variation in callus culture regenerants of strawberry; *Plant Cell Tissue Org. Cult.,* 29: 257-268.

Nwauzoma, A. B. and Jaja, E.T. (2013). A review of somaclonal variation in plantain (Musa spp): mechanisms and applications. Journal of Applied Biosciences, 67: 5252 – 5260.

Powell, W.; Morgante, M.; Andre, C.; Hanafey, M.; Vogel, J.; Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225-238.

Prevost, A. and Wilkinson, M. J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics, 98: 107-112.

Provan, J.; Russell, J. R.; Booth, A. and Powell, W. (1999). Polymorphic chloroplast simple sequence repeat primers for systematic and population studies in the genus Hordeum. Mol.; Ecol., 8: 505-511.

Rohlf, F. J. (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, New York, 83 pp

Sharma, C. and Srivastava, D. K. (2014). In vitro Plant Regeneration from Seedlings-derived explants of tomato (*Lycopersicon esculentum* Mill.). International Journal of Agriculture, Environment & Biotechnology, 7(3):421-432.

Sharma, P. and Srivastava, D. K. (2013). In vitro plant regeneration from leaf and petiole tissues of tomato (*Solanum lycopersicon* cv. Solan vajr.). Journal of Cell and Tissue Research, 13(3): 3913-3920.

Sherkar, H. D. and Chavan, A. M. (2014). Studies on callus induction and shoot regeneration in tomato. Sci Res Rep., 1: 89–93.

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

Wagner, D.; Furnier, G.; Saghay-Maroof, M.; Williams, S.; Dancik, B. and Allard, R. (1987). Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proceedings of the National Academy of Science USA, 1987; pp 2097– 2100.

Wayase, U. R. and Shitole, M. G. (2014). Effect of plant growth regulators on organogenesis in tomato (*Lycopersicon esculentum* Mill.) cv. Dhanashri. Int J Pure Appl Sci Technol., 2: 65–71.

Yarra, R.; He, S. J.; Abbagani, S.; Ma, B.; Bulle, M. and Zhang, W. K. (2012). Overexpression of wheat Na^+/H^+ antiporter gene (TaNHX2) enhances tolerance to salt stress in transgenic tomato plants (*Solanum lycopersicum* L.). Plant Cell Tissue Organ Cult., 111: 49–57. doi:10.1007/s11240-012-0169-y

Yasmeen, A. (2009). An improved protocol for the regeneration and transformation of tomato (cv. Rio Grande). Acta Physiol Plant, 31:1271–1277.

Zhang, W.; Hou, L.; Zhao, H. and Li, M. (2012). Factors affecting regeneration of tomato cotyledons. Biosci Methods, 4:27–33.

Zietkiewicz, E.; Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20:176-183.

Zucchi, M. I.; Arizono, H.; Morais, V. A.; Fungaro, M. H. P.; Vieira, M. L. C. (2002). Genetic instability of sugarcane plants derived from meristem cultures. Gen Mol Biol., 25(1): 91-6.

التحليل المورفولوجي والجزيئي للاختلافات الوراثية الناتجة في مزارع الأنسجة لأصناف الطماطم

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تم اختبار الاختلافات الوراثية الناتجة عن طريق التقييم المورفولوجي والتحليل الجزيئي. وذلك باستخدام أربعة أصناف من الطماطم، ونوعين مختلفين من الأجزاء النباتية هما الأوراق الفلقية والسويقة تحت الفلقية وأيضا تركيبات مختلفة من الأكسينات والسيتوكاينينات بتركيزات مختلفة لدراسة تأثير التركيب الوراثي، ونوع الأجزاء النباتية ومنظمات النمو على تكوين الكالس وتكشف النبات في الطماطم. أظهرت النتائج وجود فروق معنوية بين أصناف الطماطم في جميع الصفات المدروسة في زراعة الأنسجة (النسبة المئوية لتكون الكالس، ومعدل التكشف، وعدد النباتات المتكشفة لكل جزء نباتي والنسبة المئوية لتكون الجذور). كما أظهرت النتائج أن هذه الاختلافات كانت تعتمد على التركيب الوراثي ونوع الأجزاء النباتية والتركيزات المختلفة من منظمات النمو. وقد تم اختيار أفضل بيئة أعطت تكشفا للنباتات من كل توليفة من منظمات النمو لتوطيد طريقة لتكشف النباتات. حيث تم انتخاب النباتات المتكشفة والتي أظهرت اختلافات واسعة مع الأب المعطي لتحليل الاختلافات الوراثية الناشئة في مزارع الأنسجة بواسطة الواسمات الجزيئية والصفات المورفولوجية والمحصولية. حيث تم تطبيق نظامين مختلفين من الواسمات الجزيئية هما الـ ISSR والـ SRAP. أظهرت الواسمات الجزيئية تعددا للأشكال في حزم الـ DNA في جميع النباتات المتكشفة بالنسبة لآبائها المعطية. وللتأكد من ثبات هذه الاختلافات تم زراعة النباتات المتكشفة من مزارع الأنسجة (R0) للحصول على بذور الجيل الأول والتي تم زراعتها لإنتاج نباتات الجيل الأول (R1). حيث زرع نباتات الجيل الأول في الحقل لتقييمها مع الآباء المعطية للصفات المورفولوجية والمحصولية الآتية، طول النبات (سم)، عدد الأفرع لكل نبات، عدد النورات لكل نبات، عدد الأزهار لكل نورة عدد الأزهار لكل نبات وعدد الثمار لكل نبات ومحصول الثمار للنبات الواحد بالجرام. وقد أظهرت النتائج أن بعض النباتات المتكشفة من مزارع الأنسجة في الطماطم كانت متفوقة عن الأب المعطي في واحدة أو أكثر من هذه الصفات.