ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2017.155.164

Research Article
Differential *In vitro* Direct Regeneration of Tomato Genotypes on Various Combinations of Growth Regulators

[1]Nadia M El-Shafey, [1]Nada Hassan, [1]Salah El-Din A Khodary and [2]Abdelfattah Badr

[1]Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt
[2]Department of Botany and Microbiology, Faculty of Science, Helwan University, Cairo, Egypt

Abstract

Background and Objective: Tomato (*Lycopersicon esculentum* L.) is one of the most extensively consumed crops. Optimizing the conditions of its *in vitro* regeneration besides extending the technology to a wider range of cultivars will enhance transformation results. The current study aimed at evaluating the effect of genotype and growth regulators in both of pre-culture and regeneration media on direct shoot regeneration from cotyledonary explants. Attention is particularly given to study of the interaction between the genotype, growth regulators and media type. **Materials and Methods:** Cotyledonary leaf explants from five tomato genotypes (National Gene Bank's accession numbers, E1: 13139, E2: 13163, E3: 13145, F4: 12676 and E5: 12702) were cultured on two different pre-culture media (PCM) for 2 days and then sub-cultured on three different shoot induction media (SIM) consisting of Murashig and Skoog (MS) media supplemented with various combinations of growth regulators. **Results:** SIM3 (MS medium supplemented with 1.0 mg L^{-1} 6-benzylaminopurine and 1.0 mg L^{-1} zeatin) was the most effective in scoring high regeneration frequency and shoot number as well as the highest shoot length. Although PCM1 (0.5 mg L^{-1} indol-3-acetic acid and 0.5 mg L^{-1} kinetin) was less effective in direct regeneration from most of the investigated tomato genotypes, it worked effectively with E3 by exhibiting the highest shoot number (13±0.08) directly regenerated/explant, high shoot length (0.87±0.23 cm) and high regeneration frequency (86%) after transferring to SIM3. The genotype F4 came after E3 by exhibiting high shoot number and length as well as the highest regeneration frequency, in addition to scoring neither callus nor adventitious roots, while E1, E2 and E5 were superior in callus induction and adventitious roots. **Conclusion:** The differential response of the investigated genotypes to the types and combinations of growth regulators depends on the endogenous levels of hormones in the tested tomato genotypes.

Key words: *Lycopersicon esculentum*, cotyledonary explant, growth regulators, direct shoot regeneration, pre-culture medium, tomato genotype

Citation: Nadia M El-Shafey, Nada Hassan, Salah El-Din A Khodary and Abdelfattah Badr, 2017. Differential *in vitro* direct regeneration of tomato genotypes on different combinations of growth regulators. Biotechnology, 16: 155-164.

Corresponding Author: Nada Hassan Mohamed, Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt Tel: 00201221509173

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most popular and extensively consumed crops. Tomato is rich in vitamins A and B and the richest source of the anti-oxidant lycopene, the compound that has a protecting effect from many oxidants causing cancer[1]. Tomato is a short duration vegetable crop that grows under different climates, tropical, sub-tropical and temperate areas[2]. Egypt occupies fifth rank all over the world in production of tomato after China, India, USA and Turkey[3]. Being an economically important plant, tomato was a target for many genetic engineering trials for production of cultivars resistant to biotic and abiotic stresses[4,5]. Hence, improving the efficiency of regeneration, besides extending the technology to a wider range of commercial cultivars will have a positive impact on transformation results[6].

Optimizing the conditions for better *in vitro* tomato regeneration is still an empirical process[7]. The frequency of tomato regeneration has been reported to be controlled by some factors such as genotype, nutrient media, concentrations and combinations of growth regulators, explant type, light and temperature. Numerous studies on tomato plant regeneration from different tissues and organs have been conducted, including cotyledons, hypocotyl, apical meristem and leaves[8,9]. However, most researches referred to the superiority of cotyledonary explant in tomato regeneration and *Agrobacterium* mediated transformation over any other explant[4,5,10,11]. In addition, many reports confirmed that pre-culturing of explants prior to transformation gave better regeneration response[12-14].

In vitro regeneration as a prerequisite for plant transformation, it is more desirable to regenerate transgenic plants by direct rather than indirect organogenesis. This not only saves time but also eliminates undesirable somaclonal variations associated with long callus culture period[15]. Various hormonal combinations are used to induce adventitious shoots. The most widely used cytokinins for direct multiple shoot regeneration from tomato cotyledon explants are zeatin, thidiazorun (TDZ), kinetin (Kn) and 6-benzylaminopurine (BAP)[6,16-18]. The concentration of growth regulators employed is dependent on the cultivar being cultured and the particular cytokinin or auxin being employed[8]. Mamidala and Nanna[9] reported that among the factors affecting on tomato regeneration, genotype was the most limiting one and due to the specific plant growth regulator requirements for each genotype, it is necessary to deal with each genotype individually[8].

The current study aims at evaluating the effect of genotype and growth regulators in both of pre-culture and regeneration media on direct shoot regeneration from cotyledon explants of different tomato genotypes. Attention is particularly given to study of the interaction between the genotype, growth regulators and media type in order to choose the best genotype to be directly regenerated on the appropriate medium.

MATERIALS AND METHODS

The present study was carried out in Laboratory of Plant Tissue Culture and Molecular Biology, Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt. In the period from January, 2016-October, 2016.

Plant materials: Seeds of five tomato (*Lycopersicon esculentum* L.) genotypes were provided by National GeneBank (NGB), Agriculture Research Center (ARC), Giza, Egypt. The genotypes included four Egyptian accessions and one from France and have been identified in this study by their accession number in the genebank but also coded as E1, E2, E3, F4 and E5 (Table 1). All the chemicals, culture media and growth regulators were purchased from Duchefa Biochemie bv, Haarlem, the Netherlands, unless stated otherwise.

***In vitro* seed germination:** Mature seeds were surface-sterilized using 70% (v/v) ethanol (purchased from Sigma-Aldrich, CAS No. 64-17-5) for 30 sec, followed by 10% (v/v) commercial bleach (Clorox with 5% NaOCl) for 15 min. After decanting the bleach, seeds were rinsed at least three times in sterile distilled water and germinated on half strength Murashige and Skoog[19] (MS) medium supplemented with 30 g L^{-1} sucrose. The medium was adjusted to pH 5.8 and solidified with 8 g L^{-1} agar prior to autoclaving at 121°C and 15 psi for 20 min. The cultured seeds were incubated at

Table 1: Accession number, source and collection date of the five tomato genotypes used in the current study

Codes	Number of accession	Source of accessions	Collection date
E1	13139	El Faiyum-Egypt	2005
E2	13163	El Gharbia-Egypt	2005
E3	13145	El Gharbia-Egypt	2005
F4	12676	France	2005
E5	12702	(Ty 3059 Hy) Egypt	2005

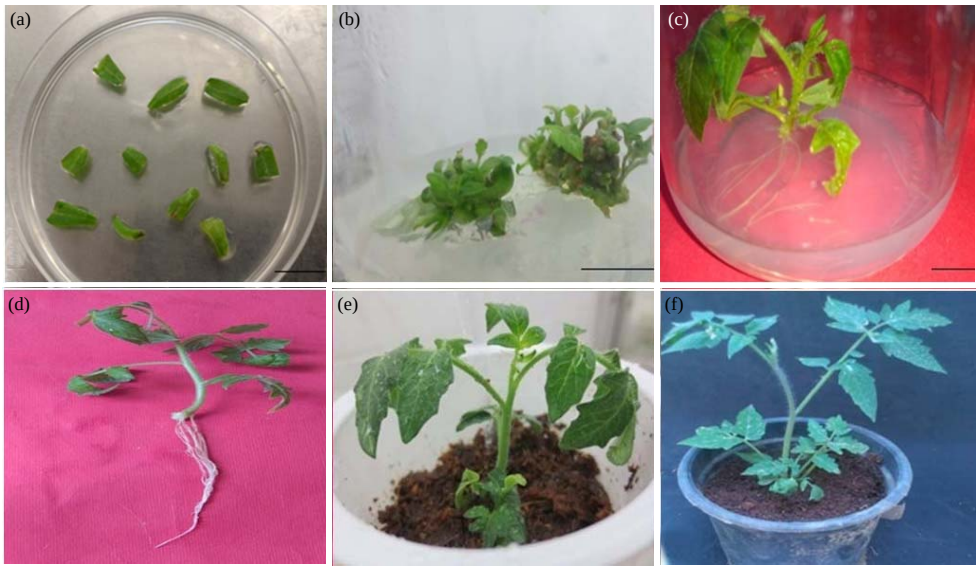

Fig. 1(a-f): Tomato direct regeneration, (a) Cotyledonary explants of E3 genotype cultured on pre-culture medium PCM1, (b) Direct shoot induction from cotyledonary explants on shoot induction medium SIM3, (c) Induction of roots from regenerated shoots on MS supplemented with 1 mg L^{-1} IAA, (d) Rooted shoot, (e) *In vitro* regenerated plantlet transferred to peat moss soil during acclimatization and (f) Regenerated plant transferred to larger plastic pot in green house after survival. Scale bar length is 1 cm

25±2°C for 3 days in dark until germination and then transferred to 16 h light/8 h dark photoperiod.

Direct shoot regeneration: Cotyledonary leaf explants (Fig. 1a) were excised from 12-14 days old seedling and cultured on two different pre-culture media (PCM1 and PCM2). The media contained MS medium supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar and different combinations of IAA, Kn and BAP (Table 2). Cultures were incubated in dark at 25±2°C. After 2 days explants were transferred to three shoot induction media (SIM1, SIM2 and SIM3) containing MS medium supplemented with 30 g L^{-1}sucrose, various combinations of IAA, Kn, BAP, TDZ, zeatin (Table 2) and 7 g L^{-1} agar. Cultures were incubated at 25±2°C under a 16 h light/8 h dark photoperiod. Explants with regenerated shoots (Fig. 1b) were sub-cultured once after 3 weeks.

Root induction and acclimatization: Regenerated shoots with 2 cm long were excised and transferred to root induction medium (RM). Four different RM containing hormone-free ½ MS, hormone-free MS and MS medium supplemented with 0.25 mg L^{-1} indole-3-butyric acid (IBA) or 1 mg L^{-1} IAA. The media also contained 30 g L^{-1} sucrose and solidified with 8 g L^{-1} agar. Cultures were incubated as mentioned above. After rooting, rooted plantlets were removed from jars and the number and length of roots were recorded (Fig. 1c and d). Roots of the plantlets were washed gently under running tap water to remove agar and then treated with solution (1 mg L^{-1}) metalaxyl fungicide (The National Company for Agrochemicals Production, Egypt) and planted in disposable plastic pots containing peat moss soil. Pots with cultured plants were covered with transparent plastic bags allowing light transmission and irrigated with ½ MS basal salts and kept in growth chamber for two weeks. During this period the plants were gradually acclimatized by decreasing the percentage of humidity gradually. After two weeks, the plants were transferred to green house (Fig. 1e and f) and the percentage of survival was recorded.

Table 2: Tomato shoots regeneration media with various combinations of growth regulators

	Growth regulators (mg L^{-1})				
Media	IAA	Kn	BAP	Zeatin	TDZ
PCM1	0.5	0.5	-	-	-
PCM2	0.2	-	0.5	-	-
SIM1	0.2	2	-	-	-
SIM2	-	-	1	-	1
SIM3	-	-	1	1	-

Statistical analysis: Experiments were repeated twice and data were analyzed using SPSS Software (V16) program for

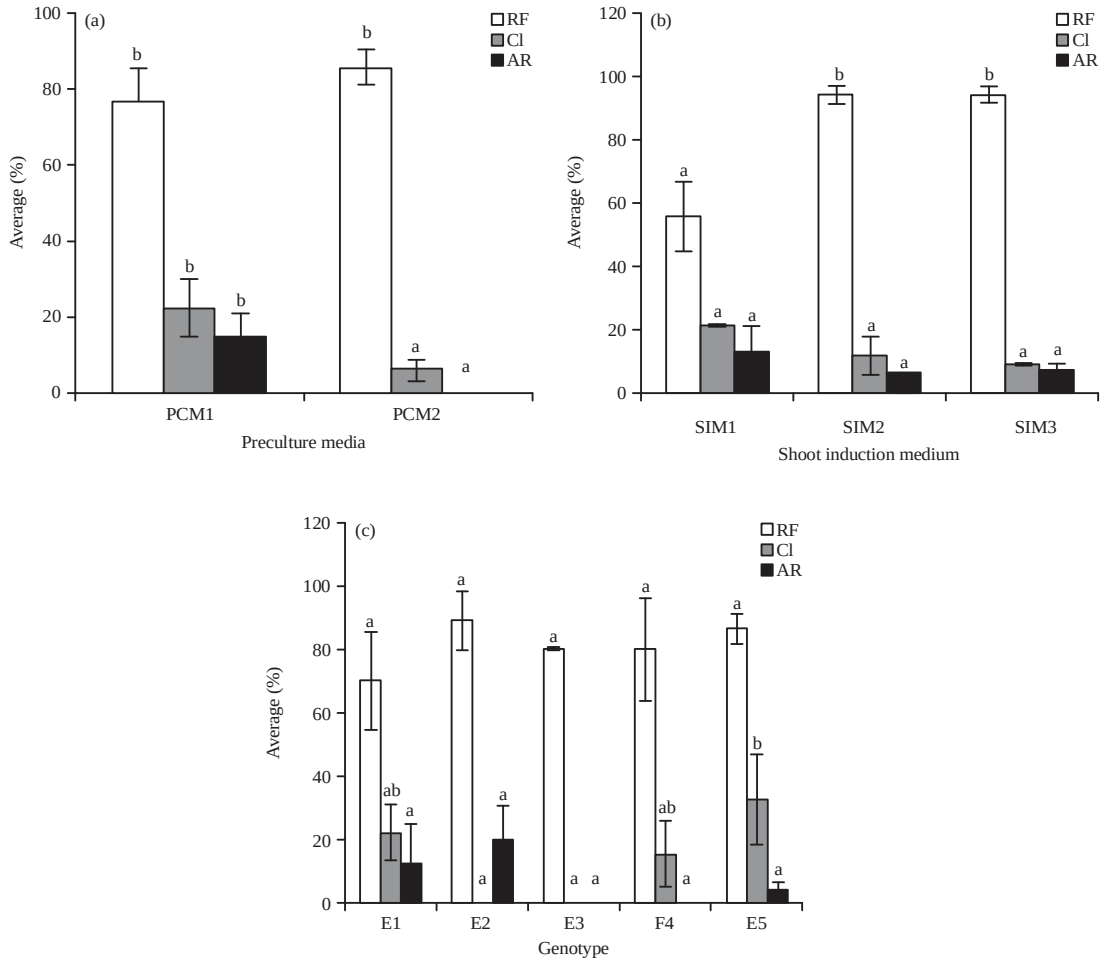

Fig. 2(a-c): Effect of (a) Pre-culture media, (b) Shoot induction media and (c) Tomato genotypes on mean percentage of regeneration (RF), callus induction (CI) and adventitious root (AR).Values are Mean±SE, Values with the same letter are not significantly different at $p \leq 0.05$

Windows (SPSS Inc. Headquarters, 2335 Wacker Drive, Chicago, IL 60606, USA). One-way analysis of variance (ANOVA) was applied and the significant differences among values were analyzed with Duncan's new multiple range tests at 5% level of significance. To illustrate how the differences across and within the genotypes, pre-culture media and shoot induction media return to the interaction among the above mentioned factors, three-way ANOVA analysis was conducted. Data presented in the study were means of five replicates (n = 5) ±SE.

RESULTS

Direct shoot regeneration: The effects of pre-culture media, shoot induction media and tomato genotypes on mean percentages of regeneration (RF), callus induction (CI) and adventitious root formation (AR) are illustrated in Fig. 2. Means comparison showed that pre-culture medium (PCM1) supplemented with 0.5 mg L^{-1} IAA and 0.5 mg L^{-1} Kn exhibited high mean percentage of callus induction and adventitious roots, whereas all explants pre-cultured on PCM2 that supplemented with 0.2 mg L^{-1} IAA and 0.5 mg L^{-1} BA (Fig. 2a) did not record any induction of adventitious root and only recorded a negligible percentage of callus induction. On the other hand, there was no significant difference between PCM1 and PCM2 concerning their effect on mean regeneration frequency. It seems that both SIM2 and SIM3 (Fig. 2b) non-significantly varied in scoring the highest mean regeneration frequency, while SIM1 significantly scored the lowest one. The differences in the induction of callus and adventitious roots were non-significant in response to the various investigated shoot induction media (SIM1, SIM2 and SIM3). All the investigated genotypes varied non-significantly in their regeneration frequency on the various tested media.

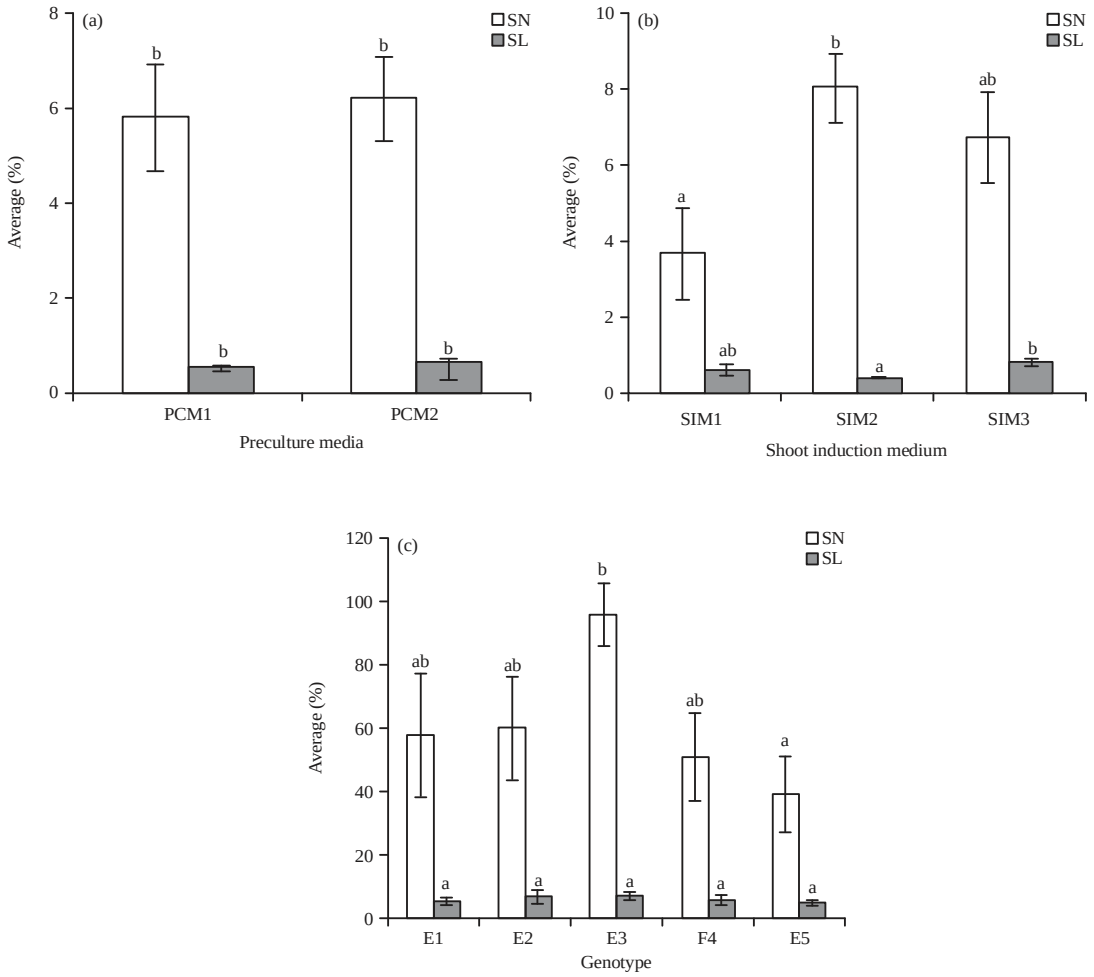

Fig. 3(a-c): Effect of (a) Pre-culture media, (b) Shoot induction media and (c) Tomato genotypes on mean shoot number/explant (SN) and shoot length (SL). Values are Mean±SE, Values with the same letter are not significantly different at $p \leq 0.05$

Nevertheless, E3 was the best in achieving neither callus induction nor adventitious roots, followed by E2 and F4 for showing only one of them. In contrast, E1 and E5 were superior in exhibiting both criteria.

The effect of two pre-culture media, three shoot induction media and tomato genotypes on mean shoot number/explant (SN) and shoot length (SL) are illustrated in Fig. 3a, b and c, respectively. It seems that culturing tomato cotyledon explants on two different pre-culture media showed no significant difference in mean shoot number/explant and shoot length (Fig. 3a). On the contrary, the variation in composition of shoot induction media significantly affected mean shoot number as well as shoot length (Fig. 3b). Over the evaluated shoot induction media, SIM2 induced the highest number of shoots, although it induced the lowest shoot length. Interestingly, SIM3 not only exhibited a number of shoots that was non-significantly different from that of SIM2 but also displayed the highest shoot length compared with the other shoot induction media (SIM1 and SIM2). Showing the least effectiveness, SIM1 induced the lowest number of shoots/explant. Among the assessed genotypes, E3 revealed the best quality being the one that exhibited the highest shoot number (although non-significant to E1, 2 and F4) while E5 induced the lowest one. At the same time, no significant variation was recorded among the shoot length of the five genotypes (Fig. 3c).

The detailed results on the effects of different pre-culture media, shoot induction media and tomato genotypes on shoot number/explant, shoot length, percentage of regeneration, callus induction and adventitious roots are given in Table 3. It is clear that the response of explants that excised from different five genotypes varied across and within the various hormonal combinations of the pre-culture and shoot induction media. The genotype E3 scored the highest shoot

Table 3: Effect of genotypes, pre-culture media (PCM) and shoot induction media (SIM) on tomato regeneration

Genotypes	PCM	SIM	SN	SL (cm)	RF (%)	CI (%)	AR (%)
E1	1	1	0.0±0.00	0.00±0.00	0	0	75
		2	11.7±1.40^{c}	0.50±0.00^{ab}	100	40	0
		3	5.0±1.40^{b}	0.80±0.12^{b}	83	50	0
	2	1	3.2±1.50^{ab}	0.80±0.37^{b}	60	33	0
		2	11.6±2.10^{c}	0.45±0.14^{ab}	78	10	0
		3	3.6±1.02^{ab}	0.60±0.10^{ab}	100	0	0
E2	1	1	5.7±0.74^{bc}	1.03±0.22^{ab}	100	0	44
		2	5.5±0.76^{bc}	0.29±0.04^{a}	100	0	16
		3	13.0±1.30^{d}	0.71±0.10^{ab}	91	0	60
	2	1	1.2±0.83^{a}	0.14±0.09^{a}	44	0	0
		2	7.6±1.30^{c}	0.33±0.05^{a}	100	0	0
		3	3.4±0.67^{ab}	1.55±0.88^{b}	100	0	0
E3	1	1	8.5±0.50^{a}	0.75±0.25^{ab}	40	0	0
		2	10.7±2.50^{a}	0.43±0.06^{a}	100	0	0
		3	13.0±0.08^{a}	0.87±0.23^{ab}	86	0	0
	2	1	11.5±3.50^{a}	1.25±0.25^{b}	75	0	0
		2	6.7±1.80^{a}	0.31±0.06^{a}	80	0	0
		3	7.7±0.85^{a}	0.62±0.12^{a}	100	0	0
F4	1	1	0.0±0.00	0.00±0.00	0	62	0
		2	5.7±1.18^{b}	0.39±0.05^{bc}	100	0	0
		3	4.6±0.33^{b}	0.66±0.16^{c}	100	0	0
	2	1	3.2±0.48^{b}	1.00±0.15^{d}	80	30	0
		2	8.6±1.16^{c}	0.35±0.06^{b}	100	0	0
		3	9.0±0.95^{c}	1.00±0.10^{d}	100	0	0
E5	1	1	2.0±0.43^{a}	0.71±0.17^{b}	86	88	11
		2	2.9±0.82^{ab}	0.25±0.05^{a}	82	54	0
		3	2.6±0.60^{ab}	0.55±0.16^{ab}	80	40	13
	2	1	1.5±0.67^{ab}	0.45±0.181^{b}	71	0	0
		2	9.4±2.10^{c}	0.32±0.04^{ab}	100	14	0
		3	5.4±0.83^{b}	0.65±0.08^{ab}	100	0	0

For each genotype, a single factor analysis of variance was performed between the different pre-culture and shoot induction media. Values followed by the same letter are not significantly different at $p \leq 0.05$. Values are Mean±SE. Shoot number/explant (SN), shoot length (SL), regeneration frequency (RF), callus induction (CI) and adventitious roots (AR)

number (13±0.87 shoots/explant) as well as high shoot length and regeneration frequency (0.87±0.23 cm and 86%, respectively), when pre-cultured on PCM1 and then transferred to SIM3 (Table 3, Fig. 1a, b). It also induced high shoot number and shoot length (11.5±3.5 shoots and 1.25±0.25 cm, respectively) and low regeneration frequency (75%), when cultured on PCM2/SIM1 media. Although E2 exhibited the same number of shoots in addition to high regeneration frequency in response to PCM1 followed by SIM3, it showed a high percentage of adventitious roots. A high number of shoot/explant (11.7±1.4) was recorded in explants of E1 pre-cultured on PCM1 and then transferred to SIM2, however a remarkable percentage (40%) of callus was induced on the same media. The genotypes E1 and E5 significantly revealed high numbers of shoots/explant when pre-cultured on PCM2 and then transferred to SIM2 but the average shoot length (0.45±0.14 and 0.32±0.04 cm, respectively) was low. It is noticeable that mixing between PCM1 and SIM1 in the regeneration protocol resulted in very high percentage of callus induction (88 and 62% in E5 and F4, respectively) or adventitious roots (75% in E1), besides the least effectiveness in shoot induction/explant. Interestingly, the genotype F4 not only scored a significant high number of shoots (9±0.95) for explant but also showed the highest regeneration frequency (100%) and neither callus nor adventitious roots were induced from the explants that cultured on PCM2 and then transferred to SIM3 for direct shoot induction. Three-way ANOVA (Table 4) showed that the interaction effect of genotype, pre-culture media combination and shoot induction media combination was highly significant ($p \leq 0.001$) with respect to shoot number/explant and shoot length.

Rooting and acclimatization: Four root induction media consisting of two hormone-free media (½ MS or MS) and two MS media supplemented with IAA (1 mg L^{-1}) or IBA (0.25 mg L^{-1}) have been tested. The rooted shoots were hardened and acclimatized gradually and plantlets survival was recorded after transferring to *ex vitro* conditions (Table 5, Fig. 1c, d, e and f). Above all the media, MS supplemented with IAA (1 mg L^{-1}) was the most effective one

Table 4: Interaction effect of genotype, pre-culture and shoot induction media on average shoot number and shoot length

	Shoot number		Shoot length	
Sources	df	F	df	F
PCM	1	0.15^{NS}	1	2.40^{NS}
SIM	2	27.01***	2	11.22***
Genotype	4	13.46***	4	1.20^{NS}
SIM*PCM	2	4.89**	2	0.89^{NS}
Genotype*PCM	4	10.71***	4	1.29^{NS}
Genotype*SIM	8	5.63***	8	1.32^{NS}
Genotype*SIM*PCM	8	4.11***	8	4.25***

PCM: Pre-culture medium, SIM: Shoot induction medium, NS: Non-significant, significant at **$p \leq 0.01$ and***$p \leq 0.001$

Table 5: Effect of various rooting media on root induction and development and plant survival

Media	IAA (mg L^{-1})	IBA (mg L^{-1})	Rooting (%)	Root length (cm)	Root number	Survival (%)
½ MS	-	-	55.5	7.6±0.61^{ab}	4.8±0.69^{a}	60.0
MS	-	-	57.1	6.8±0.48^{a}	5.8±0.58^{a}	77.7
MS	1	-	81.1	8.5±0.63^{ab}	6.6±0.77^{a}	81.9
MS	-	0.25	72.2	9.1±0.59^{b}	5.0±0.53^{a}	80.7

Values followed by the same letter are not significantly different at $p \leq 0.05$. Values are Mean±SE

by exhibiting the highest percentage of rooting (81.1%), root number (6.6) and percentage of surviving (81.9%). Media supplemented with IBA (0.25 mg L^{-1}) came after that of IAA and recorded high percentage of rooting (72.2%) and surviving (80.7%) as well as the highest root length (9.1 cm). The two hormone-free media (½ MS or MS) showed less effectiveness in rooting and survival of the regenerated tomato.

DISCUSSION

Direct regeneration of tomato has been reported to vary with concentrations and combinations of hormones, light incubation, genotype and explants used[8]. In this study comparison of the mean data illustrated that there was no significant variation between the different combinations of pre-culture media (PCM1 and PCM2) concerning regeneration efficiency expressed as regeneration frequency, shoot number and shoot length. However, a significant variation in the ability of the two media to induce callus and adventitious roots from cotyledonary explants was recorded, since the pre-culture medium that supplemented with 0.5 mg L^{-1} IAA and 0.5 mg L^{-1} Kn (PCM1) was superior when compared with PCM2 that supplemented with 0.2 mg L^{-1} IAA and 0.5 mg L^{-1} BAP in induction of callus and adventitious roots, which means negative impact on direct regeneration. The difference in this ability may be due to the difference in the concentration of auxin (IAA) or the type of cytokinin (Kn or BAP) added to the media. Yasmeen[14] has found that pre-culturing tomato explants on 1mg L^{-1} BAP and 0.1 mg L^{-1} NAA enhanced regeneration as well as transformation compared with plain MS. Ahsan *et al.*[20], found no significant difference in regeneration frequency between pre-culturing tomato cotyledons on 4 mg L^{-1} IAA+4 mg L^{-1} Kn and on 0.1 mg L^{-1} NAA+1 mg L^{-1} BAP. However, they observed a statistical significant difference between the pre-cultured and fresh perforated cotyledonary explants as the fresh ones exhibited more regeneration frequency. The data also confirmed that the composition of pre-culture medium has a great influence on the regeneration pathway (direct or indirect) and organogenesis rather than the efficiency expressed as shoot number, shoot length and regeneration frequency in tomato.

The three SIM non-significantly differed in their influence on callus induction and adventitious roots. Among the tested media, SIM1 significantly showed the least efficiency of regeneration expressed as regeneration frequency and shoot number/explant indicating that the concentrations or the types of hormones (IAA and Kn) in this medium were not suitable for tomato direct regeneration. Similarly, Kn was found to be an inferior hormone for tomato regeneration compared with other cytokinins[21]. Although, SIM2 and SIM3 non-significantly differed in scoring high regeneration frequency and high shoot number, the shoots produced by the SIM2 were too short. These results revealed that SIM3 is the most effective medium for direct regeneration from cotyledonary leaf explant of most of the investigated tomato genotypes. Since both media contained the same concentration of BAP (1.0 mg L^{-1}), it seems that the difference in synergism between BAP and TDZ or zeatin is the reason of variation in regeneration efficiency. It was suggested that TDZ showed better response when combined with BAP[22]. However, the present study showed that supplementing the shoot induction media with TDZ resulted in shorter regenerated shoots which in turn leads to unsuccessful

rooting and acclimatization, while using the same concentration of zeatin combined with BAP resulted in high regeneration frequency and shoot number as well as the highest shoot length leading to more successful rooting. These results agree with those reported by Ichimura and Oda[23], who concluded that zeatin is the most efficient phytohormone for *in vitro* regeneration of tomato. It was also found that zeatin and BAP were superior to Kn for shoot formation from tomato leaf explants[24]. The three-way ANOVA analysis revealed that the factor that significantly ($p \leq 0.001$) affected shoot length was the hormonal combination of shoot induction medium.

In addition to the pre-culture media, the genetic factor also crucially controlled the frequency of callus and adventitious roots as well as the number of shoots regenerated per explant. It was previously reported that callus induction is strongly genotype-dependent[25]. The genotypes E1, E2 and E5 induced callus and/or adventitious roots on most of the investigated media combinations. This response was controlled by the interaction between the hormonal combination and the genotype. In another words, pre-culturing the genotype E1 on both PCM1 and PCM2 exhibited high ratio of adventitious roots and callus induction on all the investigated SIMs (Table 3). At the same time, E2 genotype markedly showed adventitious roots on all the tested SIMs, after pre-culturing on PCM1. A similar situation has been shown with E5. In contrast, E3 never induced any of those criteria when pre-cultured on PCM1. This obviously points out to the integration of the physiological mechanisms that are controlled by hormonal combination and the genotype. Means comparison showed that E3 significantly scored the highest number of shoots, whereas E5 scored the lowest one. Although, PCM1 was shown to be less effective in direct regeneration from tomato explant, it worked effectively with E3 by inducing the highest shoot number directly regenerated/explant, high shoot length and high regeneration frequency after transferring to SIM3 indicating the appropriation of pre-culturing the explants of this genotype on this combination. Interaction between genotype and PCM also significantly ($p \leq 0.001$) affected shoot number/explant. Nogueira *et al.*[26], also reported that lower concentrations of IAA with cytokinins enhanced the shoot development in tomato. Differently from E3, F4 showed the highest direct regeneration frequency and high shoot number as well as shoot length on PCM2 and SIM3, while regenerated less efficiently when pre-cultured on PCM1 or transferred to SIM1. Moreover, the same genotype never regenerated when pre-cultured on PCM1 and then transferred to SIM1 indicating that a combination of IAA and Kn is not appropriate for regeneration of this genotype. It also indicates that the differential response of the investigated genotypes to the various types and combinations of growth regulators is depending on the endogenous levels of hormones.

El-Bakry[6] found that both genotypes and growth regulator levels showed significant differences but their interaction was not significantly different. This is in contrast to the differential response of the evaluated genotypes in our experiment which supports the earlier results of Kurtz and Lineberger[27] that most genotypes of tomato uniquely respond to plant growth regulators during regeneration. The MS medium containing zeatin (1 mg L^{-1}) and IAA (0.5 mg L^{-1}) was found best, producing the highest percentage of regeneration and transformation[14]. Also, Zeatin (0.5-2.0 mg) has been recommended to be used for tomato direct and indirect regeneration from cotyledonary explant[4,28]. Plastira *et al.*[29], investigated the effect of various concentrations (0.1-10 mg L^{-1}) of BAP or zeatin on regeneration frequency from hypocotyl, cotyledon and leaf explants of six cultivars. Most cultivars responded to the tested concentrations. Regeneration frequency ranged from 95-100% with the mean number of shoots per explant ranging from 5.3-7.9. Our results are in accordance with those of Kauser *et al.*[30], who concluded that the synergetic effect of cytokinins BAP and zeatin is more effective for direct organogenesis from all of the tested tomato explants (cotyledon, leaf and hypocotyls) as compared to the single one.

Rooting of plantlets is a crucial step in establishment of a regeneration system for any species. The regenerated shoots induced roots on hormone-free MS basal medium coinciding with those of El-Bakry[6]. The tested two media (½ MS and full strength MS) with "Both ½ MS and full strength MS hormone-free media" non-significantly differed in scoring percentage of rooting and root number. Previous reports stated that tomato usually does not require any plant growth regulators for rooting[10,31]. However, these findings showed that transferring the regenerated shoots to MS media supplemented with auxins (IAA or IBA) resulted in higher percentage of rooting and survival confirming the promoting effect of exogenously supplemented auxins on root induction in many tomato genotypes[32,33]. The regenerated shoots favorably responded to IAA when compared to IBA, whereas IAA exhibited higher percentage of rooting than IBA.

CONCLUSION

The shoot induction media supplemented with 1.0 mg L^{-1} BAP and 1.0 mg L^{-1} zeatin (SIM3) was the most effective one in scoring high regeneration frequency and shoot number as

well as the highest shoot length. Although PCM1 (0.5 mg L^{-1} IAA and 0.5 mg L^{-1} Kn) was shown to be less effective in direct regeneration from most of the investigated tomato genotypes, it worked effectively with E3 by exhibiting the highest shoot number, high shoot length and high regeneration frequency after transferring to SIM3. The genotype F4 came after E3 by exhibiting high shoot number and length as well as the highest regeneration frequency on PCM2 and SIM3. The study signified the differential *in vitro* direct regeneration of the investigated tomato genotypes on various combination of growth regulators.

SIGNIFICANCE STATEMENTS

This study confirms that various tomato genotypes exhibit differential direct shoot regeneration on different combinations of growth regulators. The study spots more light on how the difference in pre-culture medium composition controls the pathway of the tomato regeneration. It also provides understanding of the integrated effect of genotype, pre-culture medium and shoot induction medium on not only the number of the regenerated shoots but their length as well. These all will facilitate more initiating successful protocols for tomato direct regeneration and further genetic transformation.

ACKNOWLEDGMENT

Nada Hassan was supported by the grant of Scientists for Next Generation (ARST/SNG/FA/2014-5 no/5), from the Academy of Scientific Research and Technology, Ministry of Scientific Research, Egypt.

REFERENCES

1. Pohar, K.S., M.C. Gong, R. Bahnson, E.C. Miller and S.K. Clinton, 2003. Tomatoes, lycopene and prostate cancer: A clinician's guide for counseling those at risk for prostate cancer. World J. Urol., 21: 9-14.
2. Kimura, S. and N. Sinha, 2008. Tomato (*Solanum lycopersicum*): A model fruit-bearing crop. CSH Protoc, Vol. 105. 10.1101/pdb.emo105.
3. FAO., 2014. Food and Agriculture Organization of the United Nations: Statics 2014.. Food and Agriculture Organization, Rome, Italy.
4. Hamza, S. and Y. Chupeau, 1993. Re-evaluation of conditions for plant regeneration and *Agrobacterium*-mediated transformation from tomato (*Lycopersicon esculentum*). J. Exp. Bot., 44: 1837-1845.
5. Sharma, M.K., A.U. Solanke, D. Jani, Y. Singh and A.K. Sharma, 2009. A simple and efficient Agrobacterium-mediated procedure for transformation of tomato. J. Biosci., 34: 423-433.
6. El-Bakry, A.A., 2002. Effect of genotype, growth regulators, carbon source and pH on shoot induction and plant regeneration in tomoto. *In vitro* Cell. Dev. Biol. Plant, 38: 501-507.
7. Devi, R., M.S. Dhaliwal, A. Kaur and S.S. Gosal, 2008. Effect of growth regulators on *In vitro* morphogenic response of tomato. Indian J. Biotechnol., 7: 526-530.
8. Bhatia, P., N. Ashwath, T. Senaratna and D. Midmore, 2004. Tissue culture studies of tomato (*Lycopersicon esculentum*). Plant Cell. Tiss. Organ Cult., 78: 1-21.
9. Mamidala, P. and R.S. Nanna, 2011. Effect of genotype, explant source and medium on *in vitro* regeneration of tomato. Int. J. Genet. Mol. Biol., 3: 45-50.
10. Velcheva, M., Z. Faltin, M. Flaishman, Y. Eshdat and A. Perl, 2005. A liquid culture system for *Agrobacterium*-mediated transformation of tomato (*Lycopersicon esculentum* L. Mill.). Plant Sci., 168: 121-130.
11. Bhushan, A. and R.K. Gupta, 2010. Adventitious shoot regeneration in different explants of six genotypes of tomato. Indian J. Hortic., 67: 224-227.
12. Wu, Y.F., Y. Chen, X.M. Liang and X.Z. Wang, 2006. An experimental assessment of the factors influencing *Agrobacterium*-mediated transformation in tomato. Russian J. Plant Physiol., 53: 252-256.
13. Gao, N., W. Shen, Y. Cao, Y. Su and W. Shi, 2009. Influence of bacterial density during preculture on *Agrobacterium*-mediated transformation of tomato. Plant Cell Tiss. Organ Cult., 98: 321-330.
14. Yasmeen, A., 2009. An improved protocol for the regeneration and transformation of tomato (cv Rio Grande). Acta Physiologiae Plant., Vol. 31. 10.1007/s11738-009-0364-6.
15. Su, W.W., 2002. Cell Culture And Regeneration Of Plant Tissues. In: Transgenic Plants and Crops, Khachatourians, G.G., A. McHughen, R. Scorza, W. Nip and Y.H. Hui(Eds.)., Taylor and Francis Publishers, New York, pp: 151-176.
16. Moghaieb, R.E.A., H. Saneoka and K. Fujita, 1999. Plant regeneration from hypocotyl and cotyledon explant of tomato (*Lycopersicon esculentum* Mill.). Soil Sci. Plant Nutr., 45: 639-546.
17. Mohamed, A.A., M.R. Ismail and M.H. Rahman, 2010. *In vitro* response from cotyledon and hypocotyls explants in tomato by inducing 6-benzylaminopurine. African J. Biotechnol., 9: 4802-4807.
18. Kalyani, B.G. and S. Rao, 2014. Zeatin induced direct plant regeneration from cotyledon explants of cultivated tomato (*Lycopersicon esculentum* Mill.). World J. Pharm. Pharm. Sci., 3: 1034-1040.

19. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Planta., 15: 473-497.
20. Ahsan, N., S.H. Lee, D.G. Lee, M. Anisuzzaman and M.F. Alam *et al*., 2007. The effects of wounding type, preculture, infection method and cocultivation temperature on the *Agrobacterium*-mediated gene transfer in tomatoes. Ann. Applied Biol., 151: 363-372.
21. Venkatachalam, P., N. Geetha, P. Priya, G. Rajaseger and N. Jayabalan, 2000. High frequency plantlet regeneration from hypocotyl explants of tomato (*Lycopersicon esculentum* Mill.) via organogenesis. Plant Cell Biotech. Mol. Biol., 1: 95-100.
22. Osman, M.G., E.A. Elhadi and M.M. Khalafalla, 2010. Callus formation and organogenesis of tomato (*Lycopersicon esculentum* Mill, CV Omdurman) induced by thidiazuron. Afr. J. Biotechnol., 9: 4407-4413.
23. Ichimura, K. and M. Oda, 1998. Stimulation of phenotypically normal shoot regeneration of tomato (*Lycopersicon esculentum* Mill.) by commercial filter paper extract. Japanese Soc. Hortic. Sci., 67: 378-380.
24. Dhruva, B., T.V. Ramakrishnan and C.S. Vaidyanathan, 1978. Regeneration of hybrid tomato plants from leaf callus. Curr. Sci., 47: 458-460.
25. Xinrun, Z. and A. Conner, 1992. Genotypic effects on tissue culture response of lettuce cotyledons. J. Genetics Breed, Vol. 46.
26. Nogueira, F.T.S., M.G. Costa, M.L. Figueira, W.C. Otoni and F.L. Finger, 2001. *In vitro* regeneration of santa clara tomato plantlets and its natural mutant firme. Sci. Agrotec. Lavras, 25: 36-71.
27. Kurtz, S.M. and R.D. Lineberger, 1983. Genotypic differences in morphogenic capacity of cultured leaf explants of tomato. J. Am. Soc. Hortic. Sci., 108: 710-714.
28. Park, S.H., J.L. Morris, J.E. Park, K.D. Hirschi and R.H. Smith, 2003. Efficient and genotype-independent *Agrobacterium*-mediated tomato transformation. J. Plant Physiol., 160: 1253-1257.
29. Plastira, V.A. and A.K. Perdikaris, 1997. Effect of genotype and explant type in regeneration frequency of tomato *in vitro*. Acta Hortic., 447: 231-234.
30. Kauser, N., S. Khan, S.A. Mohammadi, B. Ghareyazie, E.D. Uliaie and B. Darvishrohani, 2016. Agrobacterium-mediated transformation and direct shoot regeneration in Iranian tomato (*Solanum lycopersicum* L.) cultivar falat-ch. Pak. J. Bot., 48: 2489-2498.
31. Mensuali-Sodi, A., M. Panizza and F. Tognoni, 1995. Endogenous ethylene requirement for adventitious root induction and growth in tomato cotyledons and lavandin microcuttings *in vitro*. Plant Growth Regul., 17: 205-212.
32. Selvi, D.T. and M.A. Khader, 1993. *In vitro* morphogenetic capacity of tomato (*Lycopersicon esculentum* Mill.) var. PKM.1. South Indian Hort., 41: 251-258.
33. Sundararajan, M., 2015. Induction of organogenesis in tomato callus using plant growth promoters including triacontanol and antioxidants. Indian J. Fundamental Appl. Life Sci., 5: 81-87.