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Micropropagation of Tomato Red Rock using BA and Kinetin

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ABSTRACT

This investigation was carried out in Plant Tissue Culture Laboratory. Department of Horticulture, College of Agriculture and Forestry, University of Mosul the objective of this study was to investigate the influence of different concentrations of benzyl adenine (BA) or Kinetin (kin) on multiplication of shoot tips of Tomato Red Rock and the effect of Indole-3 butyric acid (IBA) on rooting shoots propagation *in vitro*, the results can be summarized as follows: the highest shoots number 3.40 shoots per explant achieved from cultured shoot tips on medium supplemented with 1.5 mg/l BA, while highest shoots number 2.0 shoot/explant achieved from cultured shoot tips on medium supplemented with 6.0 mg/L Kin. highest rooting percentage, roots number, root length were100% rooting, 4.4 roots/explant, 4.34 cm were achieved respectively from cultured shoots on MS medium supplemented with 2.0 mg/L IBA.

Key words: *Tomato, Shoot tips, BA, Tissues culture.*

Introduction

Tomato is one of the most important vegetable crop grown throughout the world (Shihab and Abood, 2019; Kumar *et al.,* 2019). It is recognized a highly valuable and nutritious food. Different applications of in vitro culture is used in tomato production such as; virus free plant production (Moghaieb *et al.,* 2004), Now a days tomato is one of the major vegetable throughout the world. Its is planted in almost 4 million hectares worldwide (Chaudhry *et. al.*, 2007) Several In vitro investigations have been conducted on tomato based on its relationship with tobacco, and in account of its consequently expected good workability Devlin and Witham (1983). The tissue culture technique has played Important role in its ability of propagate plant and to obtain a large number of plants in a short time as compared with traditional propagation method (Kadhim *et al.,* 2019).

Phytohormones have a major role in plant development and plant responses to the environment, and their action is associated with low and very low concentrations (AL-Taey and Saadoon, 2012; AL-Taey, 2017). Auxins and cytokines play an important role in the process of plant development and evolution and in stimulating cell division, elongation and plant cell differentiation (Al- Duraid *et al.,* 2019; Hamza and Al-Taey, 2020)

Plant tissue culture is important for modern plant

improvement program to produce virus free plants (Moghaleb *et al.*, 1999) to introduce new traits into selected plant, to multiply elite selection and to develop suitable cultivars in short time (Taiz *et al*., 2002). In vitro plant regeneration has been found to depend on several factors, of which must important are genotype, explant, composition of basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels and vessel covers (Reed 1999 and Al-Shareefi *et al*., 2020). In vitro regeneration ability of tomato remains the main limiting factor for efficient genetic transformation (Lima *et al*., 2004). Ishag *et al.* (2009) achieved the highest number of shoots 2 shoot/explant from cultiver shoot tip explants in MS medium supplemented with 4 mg/L Kin and achieved the highest number of roots 22 root/explant when cultured explant in 1/2MS medium with 0.5 mg/LNAA after six weeks. Osman *et al*. (2010) achieved the highest number of shoots 2.0 shoot/explant from cultured shoot tips of *Lycopersicon esculentum* in MS medium supplemented with 4mg/L KIN and achieved the highest length of shoot 4.6 cm. Vinoth *et al.* (2012) recorded the highest number of roots 17.4 root/explant where cultured shoot tips of *Lycopersicon esculentum* in MS medium supplemented with 0.4 mg/L IBA after five weeks. Fedaa and Hassan (2015) achieved Maximum significant shoot average number of shoot (1.5, 3.66) were cultured shoot tips in MS medium obtained BA and Kinetin were combined together for 593 and Baladi cultivar respectively.

The study aims to Induce the shoots from shoot tips of tomato to find out the best concentration of cytokinin and then rooting the shoots to obtain plantlets.

Materials and Methods

This investigation was carried out at plant tissue culture laboratories/ College of Agriculture and forestry/ University of Mosul during the period 1/8/2020 to 15/1/2021. Seeds were used in this study, surface sterilized using 10% of Chlorox (sodium hypochlorite) for 10 minutes containing 1% (v/v) tween 20 as wetting agent, then were rinsed three time with sterile distilled water for three minutes every time. Culture Media Murashige and Skoog (1962) The medium was supplemented with 30 mg/l of sucrose and 0.1 mg/L of myo-inistol, in addition of 7 mg/L of Agar. The pH was adjusted to 5.8, and then the medium was sterilized in the autoclave at 121 ^{o}C for 21 minutes. Tomato seeds were cultured in vessels cultured containing 20 ml of MS basal media, ten seed were planted in each vessels. They were transferred to incubator and kept for 10-15 days at 23 ^{o}C under 16 h day light of and 8 h dark period. *Multiplication Experiment* In this experiment, shoots tips of about 1 cm length were cut from the establishment seedlings after 15-20 days of germination, tips were transferred into MS basal media supplemented with BA (0.0, 0.5, 1.0, 1.5, 2.0) mg/l or Kinetin (2.0, 4.0, 6.0, 8.0) mg/l for multiplication. For rooting, the regenerated shoots transferred to MS media with different concentration (0.0, 0.25, 0.5, 0.75) mg/L IBA for rooting.

The experiments were carried out using Complete Randomized Design (C.R.D) and factor experiments using the statistical program Genstat 12th, The results were analyzed and the arithmetic averages were compared according to the test of the Least Significant Difference (L.S.D.) and the level of propabilty of 0.05 (Al-rawy and Khalaf Allah, 2012).

Results and Discussion

Seedling growth after 4 weeks, all seeds germinated successfully onto the basal medium (Fig. 1), they continued to grow at this media for (3-4) weeks. All of seedlings were clean (no contamination was observed).

Fig. 1. Germinate tomato seeds on medium MS Free after 4week in culture.

Table 1 concludes that the treatment of 1.5 mg/L BA gave the highest response rate 80% and the highest with the number of branches 2.80, 3.40 shoots per explant with length 2.40, 3.42cm and number of leaves 3.70, 4.90 leaf per explant for establishment and multiplication stages (Fig. 3), re-

spectively. Table 2 infers that the addition of Kin was effective on establishment and multiplication stages in terms of response rate and number of shoots the concentration of 6.0 mg/Lproduced the highest response rate 70% and the highest with the number of branches 1.0, 2. 0 shoots per explant with length 1.43, 2.40 cm and number of leaves 1.6, 2.8 leaf per explant respectively. Table 3 shown that the concentration of (1.5, 2.0) mg/L IBA produced the highest rooting percentage 100% the treatment 2.0 mg/L yielded the highest number of roots and length 4.4 root per explant and 4.34 cm respectively.

Fig. 2. Shoot tip of Tomato Red Rock cultivation on MS medium, control at 0.0 mg/L of BA and Kin for each shoot tip.

Multiple shoots regeneration were initiated from the shoot tip explants after (3-4) weeks of culture. The frequency of shoot number was influenced by both the type and concentration of regulater used. Micro-propagation has become a reliable and routine approach for large-scale rapid plant multiplication (Akbas *et al*., 2009). BA is the most important growth regulator for shoot multiplication and are lationship exists between BA levels and shoot number Messeguer *et al*. (1993). The dose of cytokinin is known to be critical in multiple shoots induction (Abdellatef and Khalafallah, 2007). BA was more effective than Kin for shoot production, this can be due to internal structure of BA and the number of double bonds. The side chain of BA contains three double bonds where a Kin has only two. Further more, the activity of cytokinins got increased with

Table 1. Effect of BA on initiation and multiplication of shoot tips cultured on MS medium.

BA (mg/L)	Establishment stage after 4 weeks				Multiplication stage after 8 weeks			
	Response rate(%)	Shoots Numbers	Shoots Length (cm)	Leaves Numbers	Response rate (%)	Shoots Length (cm)	Shoots Numbers	Leaves Numbers
0.0	50	0.50	0.50	0.90	60	0.60	1.06	2.20
0.5	60	0.80	0.80	1.30	60	1.50	1.11	2.20
1.0	70	1.10	1.20	2.40	70	1.70	1.60	2.50
1.5	80	2.80	2.40	3.70	80	3.40	3.42	4.90
2.0	50	1.20	1.10	1.80	50	1.50	1.34	1.95
L.S.D 0.05	0.22	0.75	0.75	1.10	0.22	0.75	0.75	1.10
Average	62	1.28	1.20	2.02	64	1.74	1.71	2.75
L.S.D 0.05	0.09	0.33	0.33	0.49	0.09	0.33	0.33	0.49

Table 2. Effect of Kin on initiation and multiplication of shoot tips cultured on MS medium.

Kin (mg/L)	Establishment stage after 8 weeks				Establishment stage after 4 weeks			
	Response rate (%)	Shoots Numbers	Shoots Length (cm)	Leaves Numbers	Response rate(%)	Shoots Numbers	Shoots Length (cm)	Leaves Numbers
0.0	30	0.3	0.36	0.6	30	0.5	0.40	0.7
2.0	40	0.6	0.58	0.8	40	0.8	0.85	1.5
4.0	60	1.3	1.05	1.4	60	1.5	1.33	2.3
6.0	70	1.0	1.43	1.6	70	2.0	2.40	2.8
8.0	20	0.4	0.25	0.4	20	0.5	0.50	1.0
L.S.D 0.05	0.26	0.63	0.64	0.88	0.26	0.63	0.64	0.88
Average	44	0.72	0.73	0.96	44	1.06	1.096	1.66
L.S.D 0.05	0.12	0.28	0.29	0.39	0.12	0.28	0.29	0.39

Fig. 3. Shoot tip of Tomato Red Rock cultivation on MS medium, control at 1.5 mg/L of BA for each shoot tip.

Table 3. Effect of different of IBA on rooting culture in full MS medium

IBA (mg/L)	Root%	Root Number	Root Length (cm)
0.0	40	0.7	0.4
0.5	80	1.6	1.65
1.0	90	2.2	2.28
1.5	100	4.0	3.48
2.0	100	4.4	4.34
L.S.D 0.05	0.27	0.80	0.80

the rise in the number of double bonds in their side chain (Krishnamoorthy, 1985). Further, the presence of benzene ring on BA structure increases its efficiency and makes it the most effective cytokinine (Wasfy, 1995). Gubis *et al*. (2004) reported that the frequency of adventitious shoot regeneration depends on the type of explant and both the type and concentration of growth regulators. One of the major physiological effects of auxins is stimulation of adventitious roots formation in both *in vitro* or *in vivo* cuttings (Hartmann *et al*., 2002).

Refereance

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