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Effect of glutamine and polyamines in micropropagation of strawberry plants

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Abstract: *This research was conducted in the plant tissue culture Lab. College of Agriculture / University of Baghdad – Al-Jadriya. In vitro shoot clusters of (FragariaXAnanassaDuch cv. Festival) were used as experimental materials. Explants were collected from 4weeks old virus free aseptic proliferated meristems. Shoot clusters of strawberry explants without roots were cultured on MS media which were supplemented with (1 mg.L^{-1} BA and 0.1 mg.L^{-1} IBA) and amino acid (Glutamine) which were add separately at various concentrations(0, 25, 50 and 100 mg.L^{-1}),and add two selected polyamines (Putrescine, spermidine) at concentration (0, 0.07, 0.14 and 0.28 mg.L^{-1}). In rooting stage, shoots obtained from the shooting stage were transferred for culturing on MS nutrient medium supplemented 0.1 mg.L^{-1} IBA with Glutamine and polyamines (Putrescine, spermidine) at the same previously concentrations. The results indicate that the treatment of glutamine at concentration of 100 mg.L^{-1} has given the highest increase in the number and length of shoots of 7.60 and 1.88 cm respectively.The interaction between concentration of polyamines and their type significant results where the addition of putrescine at (0.07 mg.L^{-1}) to the culture nutrient medium was the highest significant result in stimulating the number of shoots/explant and their length of (6.6) and2.67 cm, respectively, while the addition of spermidine at (0.07 mg.L^{-1}) to the culture nutrient medium was the highest significant result in stimulating the number of roots/explant and their length of (9.8) and 8.35 cm, respectively*.

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I. Introduction

Strawberry (*Fragaria X Ananassa*Duch)are members of the Rosaceae family, is one of the most flavored fruit in the world. It is a delicious small fruit widely appreciated mainly for its characteristic aroma,rich in vitamin B, vitaminC, fiber, folic acid and potassium (Kessel, 2003; Hasan et al., 2010). The acreage of strawberry in the world reached about 401862hectare, with production of 9118336 tons, the main producing countries are China then USA, Mexico, Egypt, Turkey (Fao, 2016).The propagation of strawberries isachieved either by runners or by in vitromicropropagation. Conventional propagation methods are slow, laborious, and expensive with many limitations and maynot be recommended for effective andcommercial multiplication,so we go to tissue culture (Ashrafuzzaman et al., 2013).Micropropagation of strawberries fromrunners was initially reported for achieving efficient generationandlarge numbersof disease free plants (Moradi et al.,2011). Root development is under the control of hormonal, metabolic, and environmental cues,these processes involvenot only the five 'classical' plant hormones, but also other growth regulators, such as polyamines. Polyamines are lightweight molecular that arepresent in all living organisms. Polyamines are necessary for thegrowth of prokaryotes and eukaryotes. Putrescine,spermidine and spermine are the major Polyamines in plants (Fazilati and Forghani, 2015). Muthu et al. (2012) spermidine has a significant role in cell division, growth and differentiation and has improved the number of leaves and chlorophyll content in it when added to the nutrient solution of the troysterange plants.Al-Juboury and Hamad(2015) also studied the effect of adding levels of spermidineon *in vitro* micropropagation of *Citrusvolkameriana* rootstock,and they foundIn multiplication stage, MS medium supplemented with 0.07 mg /l spermidine was the highest for shoot proliferation (6.5 shoot/explant). Whereas, those obtained from MS medium supplemented with 0.14mg/l spermidine revealed best results for shoot terminals (5.5 shoots / explant).In another studywas investigating the influence ofcombinations of Indole butyric acid (IBA), Naphthalene acetic acid (NAA) and three concentration of polyamine Spermidine (0, 0.5 and 1 mg.L^{-1})on rooting of shoots of *citrus volkameriana* rootstock, Al-Khazali and Hamad (2016) found the MS mediumsupplemented with 0.5 mg.L^{-1}spermidine gave the highest percentage of rooting and root number that was notsignificantly different than other concentrations of spermidine.

Amino acid mixtures such as casein hydrolysate, L-glutamine,Lasparagine and adenine are frequentlyused as sourcesof organic nitrogen in culture media. Gerdakaneh et al. (2012)demonstrated that the type and concentration ofamino acid have imperative effectson the somatic embryogenesis process andembryo developmentof the strawberrycultivars.El-Sharabasyet al (2015) conducted an experimentto investigate the

effect of threeamino acid compounds (Tyrosine, Arginine, or Glutamine) at different concentrations on the shooting and rooting stages aswell as the residual effects of the examined amino acid compounds on the harvested rooting plantlets for the acclimatization stage,As they foundhighest significant result ofshoot number/explant and root number of cultured strawberrywas obtained at (25 mg/L) foreach studied amino acid compound, while that there was no significant differenceamong the tested concentrations (25, 50and 100 mg/L) of each amino acid typein shoot length/explant after twosubcultures during multiplication stage. This study aimed to conduct theoptimum type and concentrations of thetwo selected polyamines (Putrescine, spermidine) and two amino acid (Arginine and glutamine) for an effort of enhancing in vitro regeneration of (*FragariaX Ananassa*Duch cv. Festival) during shooting and rooting stagessince there were no studies investigated for strawberrymicropropagation in this approach.

II. Material and Methods

This research was conducted in the plant tissue culture Lab. College of Agriculture / University ofBaghdad – Al-Jadriya.In vitro shoot clusters of (*FragariaXAnanassa*Duch cv.Festival) wereused as experimental materials. Explantswere collected from 4weeks old virusfree aseptic proliferated meristems; the invitro propagation was carried out according to the protocol ofKhierallah and Ahmad(2014)to investigate the effect ofamino acid (Glutamine) at different concentrations on the shooting and rooting stages and two selected polyamines (Putrescine, spermidine) at different concentrations. Sterilize with a 3% NaOCl solution for 15 minutes with Tween20 drops, then wash with distilled water three times.Planted in the MSmedia with 0.5 mg. L^{-1} BA and 0.1 mg. L^{-1} of IBA and then incubating the plants at 25 ° C under 1000 lumens and 16/8 light / dark for four weeks. The branches obtained from the shooting stage are transferredfor culturing on MS nutrient medium supplemented with 1 mg. L^{-1} BA and 0.1 mg. L^{-1} from IBA. We then study the effect of polyamines and amino acids.

Shooting stage: Shoot clusters of strawberry explants without roots were cultured on MSmedia (Murashige and Skoog, 1962)which were supplemented with (1 mg.L^{-1} BA and 0.1 mg.L^{-1} IBA) and amino acid (Glutamine) which wereadd separately at variousconcentrations(0, 25, 50 and 100 mg.L^{-1}). And add two selected polyamines (Putrescine, spermidine) at concentration (0, 0.07, 0.14 and 0.28 mg.L^{-1}). Datarecorded inthis stage included the number and length of shoots.

Rooting stage: Shoots obtained from theshooting stage were transferred for culturing on MS nutrient medium supplemented0.1 mg.L^{-1} IBAwithGlutamine and polyamines (Putrescine, spermidine)at the same previously concentrations. In this stage data recordedwas regarding the root numbers andlengths (cm) after (4-6) weeks of culturing. After inoculation, the culture jarswere maintained at a temperature of at 25 ° Cwith a 16 hours/day photoperiod.Lighting was supplied using fluorescentlamps with 1000 lux for the multiplication and rooting stage.

Statistical analysis:

The experiments were carried outusing completely randomized design.Each treatment consisted of ten replicates, each of this replicate consisted ofone explant.The results were analyzedusing the analysis of variance and themeans were compared using the LeastSignificant Difference (LSD) at the 5%levelaccording to (Elsahookie and Wuhaib, 1990).

III. Results and Discussion

Influence of the Glutamine concentration on shoot number and lengths, root number and length:

The results of Table (1) indicate that the treatment of glutamine at concentration of 100 mg.L^{-1} has given the highest increase in the number and length of shoots of 7.60 and 1.88 cm respectively. While the control treatment gave the highest number of roots and lengths, which reached at 9.70 and 6.80 cm respectively.This investigation clearly established that amino acids play a vital role inthe induction and development of themaximum number of multiple shoots.The results demonstrated that factors such asthis type of amino acid and the amountsemployed in the culturing process provided significant effects on the induction ofmultiple shoots(Karlidag et al., 2009).

Table (1) Influence of theGlutamine concentration on shoot number and lengths, root number and lengths of Festival strawberry cultivar

Glutamine concentration mg.L^{-1}	Shoot number	Shoot lengths (cm)	root number	root lengths (cm)
0	6.40	1.13	9.70	6.80
25	1.40	1.22	4.60	4.00
50	2.00	1.58	2.30	3.58
100	7.60	1.88	1.40	3.96
L.S.D 5%	0.73	0.20	2.16	1.25

Shoot number:

From data in Table (2)the supplementation of two typesof polyamines separately at the studied concentrations to the MS nutrient mediumenhanced significantly the shoot number of the culturedexplant of (*Fragaria X Ananassa* cv. Festival).The highest significant result ofshoot number/explant of cultured strawberrywas obtained at (0.07 mg.L^{-1}) foreach studied polyamines compound (6.5) followed significantly by the addition ofconcentration of (0.14 mg.L^{-1}) its gave 5.8 shoot number/explant. Control culture mediums without the addition of polyaminesrecorded the lowestsignificant result (2.4) of increasing inshoot number/ explant. Data indicated that when spermidine was added to culture nutrient medium, thehighest significant result was obtained inincreasing shoot number/explant (4.8). The interaction betweenconcentration of polyamines and their typesignificant results where the addition of putrescine at (0.07 mg.L^{-1}) to the culture nutrient medium wasthe highest significant result in stimulatingthe number of shoots/explant (6.6).

Table (2) Influence of polyamines type and their concentration on shoot number of Festival strawberry cultivar

polyamines concentration mg.L^{-1}	Polyamines type		mean
	Putrescine	spermidine	
0.00	1.3	3.5	2.4
0.07	6.6	6.4	6.5
0.14	3.7	7.9	5.8
0.28	5.9	1.3	3.6
Mean	4.4	4.8	
L.S.D 5%	Concentration	type	interaction
	0.44	0.31	0.62

Shoot length:

Data in Table (3) clearly revealedthat there was no significant differenceamong the tested polyamines added to culture nutrient medium an increase in shoot length/explant.However, polyamines concentration was significant effect on shoot length , The highest significant result of shoot length/explant of cultured strawberry was obtained at (0.07 mg.L^{-1}) foreach studied polyamines compound (1.88)cm . Regarding the interaction of thedifferent concentrations of polyamines and their type, Putrescine gave themost significant difference at (0.07 mg.L^{-1})in increasing in shoot lengths of the cultured explants of 2.67 cm. It is consistent with a study by Anjum (2011) on the effect of Spermidine added to the nutrition medium of theTroyer Citrange, which improved the leaves number and leaves chlorophyll content, Polyamine has an effect on cell division, growth and development and has the ability to protect plants from non-life stresses.

Table (3) Influence of polyamines type and their concentration on shoot length (cm) of Festival strawberry cultivar

polyamines concentration mg.L^{-1}	Polyamines type		mean
	Putrescine	spermidine	
0.00	1.24	1.38	1.31
0.07	2.67	1.08	1.88
0.14	1.00	0.85	0.93
0.28	1.00	2.33	1.67
Mean	1.48	1.41	
L.S.D 5%	Concentration	type	interaction
	0.19	N.S	0.27

Root number:

From data in Table (4)the supplementation of two typesof polyamines separately at the studied concentrations to the MS nutrient mediumenhanced significantly the root number of the culturedexplant of (*Fragaria X Ananassa* cv. Festival).The highest significant result ofroot number/explant of cultured strawberrywas obtained at (0.07 mg.L^{-1}) foreach studied polyamines compound (9.7) followed significantly by the control treatmentit's gave 5.7root number/explant. Data indicated that when spermidine was added to culture nutrient medium, thehighest significant result was obtained inincreasing root number/explant (9.7). The interaction betweenconcentration of polyamines and their typesignificant results where the addition ofspermidine at (0.07 mg.L^{-1}) to the culture nutrient medium wasthe highest significant result in stimulatingthe number of shoots/explant (9.8).

Table (4) Influence of polyamines type and their concentration on root number of Festival strawberry cultivar

polyamines concentration mg.L^{-1}	Polyamines type		mean
	Putrescine	spermidine	
0.00	4.8	6.6	5.7
0.07	9.6	9.8	9.7
0.14	3.7	3.9	3.8

0.28	1.2	2.8	2.0
Mean	4.8	5.8	
L.S.D 5%	Concentration	type	interaction
	1.23	0.87	1.74

Root length:

Data in Table (5) demonstrated that after six weeks during rooting stages the addition of polyamines concentration significantly effect on root length. The highest significant result of root length/explant of cultured strawberry was obtained at (0.07 mg.L^{-1}) for each studied polyamines compound (8.15 cm) followed significantly by control treatmentit's gave 5.46 cmroot length/explant. Data indicated that when spermidine was added to culture nutrient medium, the highest significant result was obtained in increasing root length/explant (5.46 cm). The interaction between concentration of polyamines and their type significant results where the addition of spermidine at (0.07 mg.L^{-1}) to the culture nutrient medium was the highest significant result in stimulating the length of roots/explant (8.35 cm).These effectsmay be related to the involvement of polyamines in the control of cell division and differentiation, which playsan important role in the root apex and during lateral and adventitious root formation (Couee et al, 2004).

Table (5) Influence of polyamines type and their concentration on root length (cm) of Festival strawberry cultivar

polyamines concentration mg.L^{-1}	Polyamines type		mean
	Putrescine	spermidine	
0.00	4.80	6.12	5.46
0.07	7.95	8.35	8.15
0.14	3.20	4.13	3.67
0.28	2.00	3.22	2.61
Mean	4.49	5.46	
L.S.D 5%	Concentration	type	interaction
	1.17	0.82	1.65

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