

# IN VITRO STUDY THE EFFECT OF PLANT GROWTH REGULATORS AND RESPONSE OF TWO VARIETIES OF IRAQI MAIZE

SAJA. J. S. BADAY

College of Administration and Economic, University of Baghdad, Iraq  
E-mail: sajajawad3@gmail.com

**Abstract-** In vitro plant tissue culture study the effect of plant growth regulators and response of two varieties of maize (5018 and Baghdad-3) to callus induction and regeneration using the 2,4-D (0.75, 1.5, 2.25 and 3.0) mg.L<sup>-1</sup> to induce callus, the [BAP (1.0, 2.0, 3.0, and 4.0) mg.L<sup>-1</sup> and NAA (0.0, 0.5, 1.0 and 1.5) mg.L<sup>-1</sup>] to regenerate plantlet from induced callus and the IBA (0.0, 1.5, 3.0, 4.5 and 6.0) mg.L<sup>-1</sup> to reform roots. The highest percentage to callus induction was 78% and the lowest was 13%. The highest and lowest average wet and dry weights to callus (245.3 and 102.4) mg (27.4 and 11.2) mg respectively. The highest and lowest average branches shoot number and length (20.0 and 1.8) branches.plant<sup>-1</sup> and (11.3 and 2.3) cm respectively. The highest and lowest average root number and length (25.3 and 3.8) root.plant<sup>-1</sup> and (6.4 and 2.4) cm respectively. The results show the presence of significant differences between the two varieties and growth regulators concentrations where the variety (5018) exhibits higher response.

**Index terms-** Callus induction, Plant growth regulators, Maize.

## I. INTRODUCTION

(Zeamaiz L.) belongs to the gramineae family is the third most important cereal crop in the world after wheat and rice as an important food source for humans, animal feed and other materials used in various industries and source of products such as sweeteners, starch, alcohol, oil and others (1). Maize is characterized by its ability to adapt to a variety of environmental conditions, and maize is preferred to other crops for agriculture in many countries (2). Its production in Iraq was about 3.5 and 4.3 tons/ha in 2015 and 2016 respectively (3). The maize crop is very important in improving the world's agricultural economy (4). To meet the growing needs of maize as an important source for many food industries, crop production and reproduction must be increased. The first attempt to grow the immature embryos of yellow maize was made by Greens and Phillips 1975 (5). Plant growth regulators are essential factors in agriculture, especially in the cultivation of plant tissues in the laboratory, through which the growth of the plant is directed as required. Auxins and cytokinins can be said to be among the most important growth regulators used in plant tissue culture, encouraging cells to divide, elongate and differentiation (6). Previous studies have indicated the importance of auxins in the induction of callus and the role of auxins and cytokinins in the regeneration of callus and the effectiveness of auxins in the reformed of rooting. The researchers are urging the callus to get many numbers of non-specialized cells whose growth and differentiation are then directed to the growth of vegetables, roots or embryos through plant growth regulators. Hosseini et al. (2014) when using different concentrations of Kin, IAA, NAA, and 2,4-D, noted that the highest percentage of regeneration of the

callus maize was 4% at the concentration of 2.0 mg.L<sup>-1</sup> from Kin and IAA (7). Cilar et al. (2006) utilized mature form of the embryo among 5 different species of the wheat that give rise to two mutually different media of MS like Murashige and Skoog (MS) media that is NAA or 2, 4-D in the results of callus induction (8). Use Jianget al. (2015) in tissue culture characteristics of maize haploid coleoptile sections to induction of callus 0.5 mg.L<sup>-1</sup> 2,4-D, 2.0 mg.L<sup>-1</sup> 2,4-D to subculture and 0.5 mg.L<sup>-1</sup> NAA to rooting (9). Show Mahato (2016) when study in regeneration of plantlets through anther culture of maize some swelling in the anther was observed in MS medium after 40 days of inoculations with 2.0 mg.L<sup>-1</sup> 2,4-D and 4.0 mg.L<sup>-1</sup> 2,4-D and 30 days of inoculations for N6 medium with 1.0 mg.L<sup>-1</sup> 2,4-D, 2.0 mg.L<sup>-1</sup> 2,4-D, 3.0 mg.L<sup>-1</sup> 2,4-D and 2.0 mg.L<sup>-1</sup> 2,4-D + 1.0 mg.L<sup>-1</sup> Kin (10). Use Gonzalez et al. (2012) when study somatic embryogenesis and plant regeneration capacity in maize inbred lines 2.0 mg.L<sup>-1</sup> 2,4-D to callus induction and 0.25 mg.L<sup>-1</sup> NAA to somatic embryo development and regeneration (11). Show Manivannan et al. (2010) when studying induction of callus and regeneration of the maize inbreds, the best concentration of the callus induction 2.0 mg.L<sup>-1</sup> 2,4-D, when using concentrates of 2,4-D (1.0, 2.0 and 3.0) mg.L<sup>-1</sup>, and IAA and BAP at (0.5 and 1.0) mg.L<sup>-1</sup> were used respectively for regeneration (12). Use Saputro et al. (2017) 2,4-D 3.0 mg.L<sup>-1</sup> when callus induction through in vitro culture for study dynamics expression of Osr40c1 gene and growth of maize calluses in responding to salt stress (13). Morshed et al. (2014) use (0.5, 1.0, 1.5 and 2.0) mg.L<sup>-1</sup> 2,4-D to callus induction, IAA (0.0, 0.5, 1.0 and 1.5) mg.L<sup>-1</sup> and BAP (0.5, 1.0, 1.5 and 2.0) mg.L<sup>-1</sup> to regeneration, when the study efficient plant regeneration using mature and immature embryos of maize, show highest percentage of callus induction 1.5 mg.L<sup>-1</sup> 2,4-D and percentage

highest to regeneration (77.44%) 1.0 mg.L<sup>-1</sup> and 0.5 mg.L<sup>-1</sup>BAP and IAA respectively(14). In this work the aim is to in vitro study the effect of different concentration of growth regulators (2,4-D, BAP, NAA and IBA) of two varieties of maize to induce callus to for regenerate and root.

## II. EXPERIMENTALS AND METHOD

Two Iraqivarieties ofmaize(5018 and Baghdad-3) were used to stimulate the callus. The mature shoot meristem (30-35) mmwere surfactant sterilized with 70% (v/v) ethanol/water for two minutes, then treated with sodium hypochlorite (NaOCl) for three minutes and rinsed with sterile distilled water for (3-5) times and subsequently sterilized with 0.1% (v/v) mercuric chloride (HgCl<sub>2</sub>). Theapical meristem (10-15) mmwere planted after sterilization on the media(MS). Sucrose 30g L<sup>-1</sup> , agar 7.0 g L<sup>-1</sup> and 2,4-D(0.75,1.5,2.25 and 3.0) mg.L<sup>-1</sup>were added to induce callusby 10 replicates per treatment at pH 5.7, thenincubated in the dark at temperature 25±2C° for five weeks,percentage of the callus induction and the wet and dry weight of the callus was calculated as follows:

$$\text{Callus induction \%} = (\text{Number of induced callus} \times 100) / \text{Number of inoculated explant}$$

The highest percentage of callus induction and the highest wet weight wereat2.25 mg.L<sup>-1</sup>from 2,4-D for callus subculture.The regeneration of the callus and cultivatedon media containing BAP (1.0, 2.0, 3.0, and 4.0) mg.L<sup>-1</sup> and NAA (0.0, 0.5, 1.0 and 1.5) mg.L<sup>-1</sup>. The plantlets were incubated under lighting heavily 1000 lux 16 hours light and 8 hours darkness and temperature of 25±2C° of eight weeks.The average of (number and length)of branches incubated were calculated. The plantlets were planted in media containsIBA (0.0, 1.5, 3.0, 4.5 and 6.0) mg.L<sup>-1</sup> to reform roots andincubated under lighting 16 hours light and 8 hours darkness of four weeks. The average of (number and length) of roots incubated were calculated.

## III. STATISTICAL ANALYSIS

All trials were performed using (CRD) and global experiments. The results were analyzed using the genestate program, and the mean was measured with the (LSD) and 5% probability (15).

## IV. RESULTS AND DISCUSSION

The results table (1) show that the highest percentage of callus induction was 54% to variety (5018) while the lowest was 44% to variety (Baghdad-3). 2,4-D 2.25 mg.L<sup>-1</sup>exhibits highest induction to callus at about 70% and the lowest was 14% using 0.75 mg.L<sup>-1</sup> 2,4-D. The effect of interaction between the varieties

and the concentrations showsthe highest percentage of callus induction were 78% to the variety (5018)using2.25 mg.L<sup>-1</sup>2,4-D and the lowest 13% using 0.75 mg.L<sup>-1</sup>2,4-D to the variety (Baghdad-3). The increase in the percentage of response to the role of auxins may be due to stimulate and induce cells to divide, and this is agreed with (9 and 10).

**Table (1): The percentage of callus induction to the varieties (5018 and Baghdad-3) by 2,4-D after five weeks.**

Variety	2,4-D mg.L <sup>-1</sup>				Average
	0.75	1.5	2.25	3.0	
5018	15	56	78	67	54.0
Baghdad-3	13	43	62	58	44.0
L.sd	42				6
Average	14.0	49.5	70.0	62.5	
L.sd	27				

The results table (2) show that the highest average of wet weight was 203.4 mg to variety (5018) while the lowest was 188.1 mg in variety (Baghdad-3). 2,4-D 2.25 mg.L<sup>-1</sup> exhibits highest average of wet weight about 234.4 mg and the lowest was 110.5 mg using 0.75 mg.L<sup>-1</sup> 2,4-D. The effect of interaction between the varieties and the concentrations shows the highest and the lowest average of wet weight were 245.3 mg to the variety (5018) at 2.25 mg.L<sup>-1</sup>2,4-D and 102.4 mg to the variety (Baghdad-3) at 0.75 mg.L<sup>-1</sup>2,4-D respectively.

**Table (2): The average of wet weight to callus of the varieties (5018 and Baghdad-3) by 2,4-D after five weeks.**

Variety	2,4-D mg.L <sup>-1</sup>				Average
	0.75	1.5	2.25	3.0	
5018	118.5	211.3	245.3	238.6	203.4
Baghdad-3	102.4	200.6	223.5	225.8	188.1
L.sd	43.2				8.5
Average	110.5	206.0	234.4	232.2	
L.sd	19.4				

The results table (3) show that the highest average of dry weight was 20.8 mg in variety (5018) while the lowest was 18.7 mg in variety (Baghdad-3). 2,4-D 2.25 mg.L<sup>-1</sup> exhibits highest average of wet weight about 25.7 mg and the lowest was 12.3 mg at 0.75 mg.L<sup>-1</sup>2,4-D. The effect of interaction between the varieties and the concentrations shows the highest and the lowest average of wet weight were 27.4 mg to the variety (5018) at 2.25 mg.L<sup>-1</sup>2,4-D and 11.2 mg to the variety (Baghdad-3) at 0.75 mg.L<sup>-1</sup>2,4-D respectively.

**Table (3): The average of dry weight to callus of the varieties (5018 and Baghdad-3) by 2,4-D after five weeks.**

Variety	2,4-D mg.L <sup>-1</sup>				Average
	0.75	1.5	2.25	3.0	
5018	13.4	20.6	27.4	21.8	20.8
Baghdad-3	11.2	19.2	24.0	20.3	18.7
L.sd	8.4				1.8
Average	12.3	19.9	25.7	21.1	
L.sd	5.6				

The results table (4) the variety (5018) show that the highest average of branches shoot number regeneration to callus was 15.2 branch.plant<sup>-1</sup> at 1.0 mg.L<sup>-1</sup> NAA, while the lowest is 11.0 branch.plant<sup>-1</sup> at 0.0 mg.L<sup>-1</sup> NAA. BAP 3.0 mg.L<sup>-1</sup> exhibits highest average of branches shoot number regeneration to callus was about 17.7 branch.plant<sup>-1</sup> and the lowest was 4.5 branch.plant<sup>-1</sup> using 1.0 mg.L<sup>-1</sup> BAP. The effect of interaction between the concentrations shows the highest average of branches shoot number regeneration to callus was 20.0 branch.plant<sup>-1</sup> using BAP 3.0 mg.L<sup>-1</sup> and NAA 1.0 mg.L<sup>-1</sup>. The lowest average of branches shoot number regeneration to callus was 2.4 branch.plant<sup>-1</sup> using BAP 1.0 mg.L<sup>-1</sup> and 0.0 mg.L<sup>-1</sup> NAA, and this is agreed with (11, 12 and 14).

**Table (4): The average of branches shoot number to the variety (5018) by BAP and NAA after eight weeks.**

NAA mg.L <sup>-1</sup>	BAP mg.L <sup>-1</sup>				Average
	1.0	2.0	3.0	4.0	
0.0	2.4	13.6	15.3	12.8	11.0
0.5	4.5	14.8	17.4	16.5	13.3
1.0	5.8	16.6	20.0	18.4	15.2
1.5	5.2	16.4	18.2	17.3	14.3
L.sd	2.5				0.8
Average	4.5	15.4	17.7	16.3	
L.sd	1.2				

The results table (5) the variety (Baghdad-3) show that the highest average of branches shoot number regeneration to callus was 13.6 branch.plant<sup>-1</sup> at 1.0 mg.L<sup>-1</sup> using NAA, while the lowest is 8.2 branch.plant<sup>-1</sup> at 0.0 mg.L<sup>-1</sup> NAA. BAP 3.0 mg.L<sup>-1</sup> exhibits highest average of branches shoot number regeneration to callus was about 15.3 branch.plant<sup>-1</sup> and the lowest was 3.7 branch.plant<sup>-1</sup> at 1.0 mg.L<sup>-1</sup> BAP. The effect of interaction between the concentrations shows the highest average of branches shoot number regeneration to callus was 18.3 branch.plant<sup>-1</sup> using BAP 3.0 mg.L<sup>-1</sup> and NAA 1.0 mg.L<sup>-1</sup>. The lowest average of branches shoot number regeneration to callus was 1.8 branch.plant<sup>-1</sup> using BAP 1.0 mg.L<sup>-1</sup> and NAA 0.0 mg.L<sup>-1</sup>. The results above show significant differences between the two varieties and the concentrations. Variation between varieties is due to genetic differences among them and differences in the content of the internal hormones of the varieties.

**Table (5): The average of branches shoot number to the variety (Baghdad-3) by BAP and NAA after eight weeks.**

NAA mg.L <sup>-1</sup>	BAP mg.L <sup>-1</sup>				Average
	1.0	2.0	3.0	4.0	
0.0	3.5	4.3	5.7	4.6	4.5
0.5	4.8	5.6	8.5	7.4	6.6
1.0	6.4	8.7	11.3	8.6	8.8
1.5	5.8	7.0	10.2	6.2	7.3
L.sd	0.34				0.03
Average	5.1	6.4	8.9	6.7	
L.sd	0.24				

The results table (6) the variety (5018) show that the highest average of branches shoot length was 8.8 cm at 1.0 mg.L<sup>-1</sup> using NAA while the lowest was 4.5 cm at 0.0 mg.L<sup>-1</sup> NAA. BAP 3.0 mg.L<sup>-1</sup> exhibits highest average of branches shoot length was about 8.9 cm and the lowest was 5.1 cm at 1.0 mg.L<sup>-1</sup> BAP. The effect of interaction between the concentrations shows the highest average of branches shoot length was 11.3 cm using BAP 3.0 mg.L<sup>-1</sup> and NAA 1.0 mg.L<sup>-1</sup>. The lowest average of branches shoot length was 3.5 cm using BAP 1.0 mg.L<sup>-1</sup> and NAA 0.0 mg.L<sup>-1</sup>.

**Table (6): The average of branches shoot length to the variety (5018) by BAP and NAA after eight weeks.**

NAA mg.L <sup>-1</sup>	BAP mg.L <sup>-1</sup>				Average
	1.0	2.0	3.0	4.0	
0.0	1.8	8.3	12.2	10.3	8.2
0.5	3.2	12.7	14.3	13.7	11.0
1.0	5.0	14.5	18.3	16.6	13.6
1.5	4.6	13.4	16.5	16.2	12.7
L.sd	2.4				0.7
Average	3.7	12.2	15.3	14.2	
L.sd	0.9				

The results table (7) the variety (Baghdad-3) show that the highest average of branches shoot length was 6.5 cm at 1.0 mg.L<sup>-1</sup> using NAA, while the lowest is 3.7 cm at 0.0 mg.L<sup>-1</sup> NAA. BAP 3.0 mg.L<sup>-1</sup> exhibits highest average of branches shoot length was about 6.4 cm and the lowest was 3.6 cm at 1.0 mg.L<sup>-1</sup> BAP. The effect of interaction between the concentrations shows the highest average of branches shoot length was 8.7 cm using BAP 3.0 mg.L<sup>-1</sup> and NAA 1.0 mg.L<sup>-1</sup>. The lowest average of branches shoot length was 2.3 cm using BAP 1.0 mg.L<sup>-1</sup> and NAA 0.0 mg.L<sup>-1</sup>. BAP and NAA may be superior to the number of branches and their length to the catalytic function BAP and NAA in stimulating cells to divide, differentiate and increase growth. Cytokinins create attractors that stimulate nutrient transfer, which stimulates cell division, growth of buds, auxins to stimulate cell division and elongation (16).

**Table (7): The average of branches shoot length to the variety (Baghdad-3) by BAP and NAA after eight weeks.**

NAA mg.L <sup>-1</sup>	BAP mg.L <sup>-1</sup>				Average
	1.0	2.0	3.0	4.0	
0.0	2.3	3.5	4.6	4.3	3.7
0.5	3.4	4.8	5.8	5.6	4.9
1.0	4.5	5.3	8.7	7.3	6.5
1.5	4.2	5.0	6.3	5.1	5.2
L.sd	0.25				0.02
Average	3.6	4.7	6.4	5.6	
L.sd	0.12				

The results table (8) show that the highest average of root number was 15.8 root.plant<sup>-1</sup> in variety (5018) while the lowest is 14.5 root.plant<sup>-1</sup> in variety (Baghdad-3). IBA 4.5 mg.L<sup>-1</sup> exhibits highest average of root number was about 24.2 root.plant<sup>-1</sup> and the lowest was 4.2 root.plant<sup>-1</sup> at 0.0 mg.L<sup>-1</sup> IBA. The effect of interaction between the varieties and the concentrations shows the highest average of root number was 25.3 root.plant<sup>-1</sup> to the variety (5018) at 4.5 mg.L<sup>-1</sup> IBA. The lowest average of root number was 3.8 root.plant<sup>-1</sup> to the variety (Baghdad-3) at 0.0 mg.L<sup>-1</sup> IBA. This may be due to the role of auxins in stimulating roots and increasing their number (17).

**Table (8): The average of root number to the varieties (5018 and Baghdad-3) by IBA after four weeks.**

Variety	IBA mg.L <sup>-1</sup>					Average
	0.0	1.5	3.0	4.5	6.0	
5018	4.6	15.9	16.4	25.3	16.8	15.8
Baghdad-3	3.8	14.7	15.3	23.0	15.7	14.5
L.sd	1.3					0.4
Average	4.2	15.3	15.9	24.2	16.3	
L.sd	0.8					

The results table (9) show that the highest average of root length was 4.9cm in variety (5018) while the lowest is 4.5 cm in variety (Baghdad-3). IBA 4.5 mg.L<sup>-1</sup> exhibits highest average of root length was about 6.3cm and the lowest was 2.5cm at 0.0 mg.L<sup>-1</sup> IBA. The effect of interaction between the varieties and the concentrations shows the highest average of root length was 6.4 cm to the variety (5018) at 4.5 mg.L<sup>-1</sup> IBA. The lowest average of root number was 2.4 cm to the variety (Baghdad-3) at 0.0 mg.L<sup>-1</sup> IBA. This may be because high concentration of plant growth regulators may lead to hormonal imbalance and inhibition of growth.

**Table (9): The average of root length to the varieties (5018 and Baghdad-3) by IBA after four weeks.**

Variety	IBA mg.L <sup>-1</sup>					Average
	0.0	1.5	3.0	4.5	6.0	
5018	2.6	3.7	5.8	6.4	5.9	4.9
Baghdad-3	2.4	3.5	5.0	6.2	5.6	4.5
L.sd	0.3					0.1
Average	2.5	3.6	5.4	6.3	5.8	
L.sd	0.2					

## CONCLUSION

The best concentration to give highest callus induction, which in turn exhibits higher wet and dry weight was 2.25 g.L<sup>-1</sup> 2,4-D, and for the regeneration of higher average of plantlet was by using BAP 3 mg.L<sup>-1</sup> and NAA 1 mg.L<sup>-1</sup> and the highest average for the root formation was by IBA 4.5 mg.L<sup>-1</sup>.

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