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In Vitro Study the Callus Induction of Two Varieties of Wheat Seeds by Plant Growth Regulators

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Abstract: Two Iraqi varieties of wheat seeds (Alfares and Tamoz-2) were induced to form callus using growth regulators for the regeneration of plantlet from callus induction. The growth regulators used are 2,4-D 0.0, 1.5, 2.0, 2.5 and 3.0 mgL^{-1} and Kinetin 0.0, 0.3, 0.6, 9.0 and 1.2 mgL^{-1} to the induction of callus. IAA 0.0, 0.5, 1.0, 1.5 and 2.0 mgL^{-1} and BAP 0.0, 0.1, 0.2, 0.3 and 0.4 mgL^{-1} to regeneration of plantlet from callus. IBA 0.0, 2.0, 4.0, 6.0 and 8.0 mgL^{-1} to reform root. Results showed significant differences between wheat varieties as recorded the highest average of wet and dry weights of callus was reached 113.2 and 9.9 mg, respectively at 2.5 and 0.9 mg of 2,4-D and Kinetin, respectively for the variety (Alfares) while the lowest average of wet and dry weights of callus was reached 100.5 and 9.3 mg respectively at 2.5 and 0.9 mg of 2, 4-D and Kinetin respectively for the variety Tamoz-2. Results showed that the highest percentage of plantlet regenerationn from callus 13.8 % at 1.5 and 0.3 mg of IAA and BAP, respectively for the variety (Alfares) while the lowest percentage of plantlet regeneration from callus 0.0% at 0.0and 0.0 mg of IAA and BAP, respectively for the varieties (Alfares and Tamoz-2). Results showed that the highest and lowest percentage of roots reformation were 6.8 and 0.0% of the variety (Alfares and Tamoz-2), respectively at 6.0 and 0.0 mg, respectively from IBA.

Key words: 2,4-D, Kinetin, IAA, BAP, IBA, wheat, callus

INTRODUCTION

The importance of wheat is due to its production average, about 230 mln. ha with production 343 mln.tons in the world. The cultivated area in Iraq is estimated at 920096 ha and the production is estimated at 3.66 ton.ha for the year 2016 (FAO., 2013). The various sciences of progress and prosperity are dealt with the technologies (plant tissue culture) that mostly use experiments in laboratory with the conditions of industrial nutrition under complete sterilization (Razdan, 2003). Plant tissue culture technology was used to induce the plant to form callus from their plant parts (root, stalk, leaves or seeds). General it is possible to make multicellular plants to produce callus when planted on suitable food media (Yeoman and Macleod, 1977). Growth regulators are important compounds in the cultivation of plant tissues such as auxins and cytokines by providing growth to the tissue or organ planted. Auxins is important in in the development and maintenance of callus. Auxins hormones take important part in the elongation of the cells and legs (Satyavathi *et al*., 2004). 2,4-D is effective hormone in the growth of callus and embryonic (Malik *et al*., 2004). Cytokines play a large role in the cultivation of plant tissues as they promote plant cell division and differentiation pointed out (Ioio *et al*., 2007). Hakam *et al*. (2015) found that the highest percentage of plantlets regeneration was 60.44 and 52.55% using 2.0 and 2.5 mgL^{-1} of 2, 4-D, respectively when studying efficient callus induction and plantlets regeneration in bread wheat. Rashid *et al*. (2009) showed that the maximum callus induction 97.18% was observed in Tatara when 2.0 mgL^{-1} of 2, 4-D and Tatara maximum regeneration of 12.25% was obtained on 0.1 mgL^{-1} IAA and 2.0 mgL^{-1} of BAP. (Upadhyaya *et al*., 2015) all the three varieties exhibit highest frequency of callus induction at 2.0 mgL^{-1} 2, 4-D when study callus induction and plant regeneration of rice varieties Sita, Rupali and Swarna Masuri. Beck (2013) the best results were achieved with a combination of concentration 1.0 mgL^{-1} of 2, 4-D. Shah *et al*. (2003) found when using 2, 4-D and BAP in different concentrations increasing in the percentage of callus induction in wheat. Rahman *et al*. (2008) studied the response of mature embryos in spring wheat when using different concentrations 2ip and 2, 4-D. Dagustu, (2008) found that the highest average of dry weight of callus when using different concentration of auxins and cytokines. Fahmy *et al*. (2012) found that the highest average of callus wet weight is 3.2 mg when using 2, 4-D 2 mgL^{-1} and BAP 1.5 mgL^{-1}. Abdallah *et al*. (2012) calculated the highest percentage of regeneration of wheat callus which was 14% at NAA 1 mgL^{-1} and BAP 0.2 mgL^{-1}. Yu *et al*. (2008) found that the percentage of regeneration increased when using different

concentration of auxin and cytokines. The aim of the study is to determine the ideal concentration of plant growth regulators in the induction of callus, regeneration and root reformation of two varieties of Iraqi wheat (Alfares and Tamoz-2).

MATERIALS AND METHODS

Two Iraqi wheat seeds varieties (Alfares and Tamoz-2) were washed with water and sterilized by immersing them in the ethanol 70% for 30 sec; seeds again were sterilized with sodium hypochlorite (NaOCl) solution (0.0, 1.0, 2.0, 3.0 and 4.0%) with the addition of two drops of (Tween-20) with continuous shaking for 10 min and then washed with sterilized distilled water three times to remove traces of sterile material. The sterilized seeds were transferred to sterile petri dishes containing filter paper with a quantity of distilled water to ensure that the seeds were well soaked with Para film. The seeds were incubated in total darkness at 25±2°C for 36 h to stimulate embryos to grow. The mature embryos of the sterile seeds were then removed by a small pressure operation on the back of the embryo using surgical blades. The mature embryos were implanted at the average of one embryo and inverted in each. (MS) media prepared containing 30 g/L sucrose with the addition of 2, 4-D 0.0,1.5, 2.0, 2.5 and 3.0 mgL^{-1} and interact with Kinetin 0.0, 0.3, 0.6, 0.9 and 1.2 mgL^{-1} to determine the appropriate focus for the development of callus. The seeds were grown after their eradication and planted on the food circles per 10 replicates. The cultivated callus then incubated in the dark at a temperature 25±2°C for 5 weeks. The wet and dry weights were measured using a sensitive balance and the callus was dried by oven at 40°C. The newly developed callus was transferred into a nutritional media to induce regeneration which contains IAA 0.0, 0.5, 1.0, 1.5 and 2.0 mgL^{-1} and BAP 0.0, 0.1, 0.2, 0.3 and 0.4 mgL^{-1}. The resulting plants were regeneaveraged of the newly reformed callus induced into the media of the rooting which was contained IBA 0.0, 2.0, 4.0, 6.0 and 8.0 mgL^{-1}.

Statistical analysis: The trials were done using Complete Random Design (CRD) and global experiments. The results were analyzed using the genestate program and the mean was measured with the (LSD) and 5% probability (Sahuki and Wahib, 1990).

RESULTS AND DISCUSSION

The results Table 1 show that the lowest percentage of contamination was 50.0% to the variety (Alfares) which

Table 1: The percentage of contamination to the varieties (Alfares and Tamoz-2) by NaOCl after seven days

	Sodium hypochlorite (%)					
Varity	0.0	1.0	2.0	3.0	4.0	Average
Alfares	100	83	45	12	10	50.0
Tamoz-2	100	85	48	16	10	51.8
LSD 0.05	2.1	NS				
Average	100.0	84.0	46.5	14.0	10.0	
LSD 0.05	1.5					

Table 2: The average of wet weight to callus of the variety Alfares by 2,4-D and Kinetin after five weeks

	2,4-D (mgL^{-1})					
Kinetin (mgL^{-1})	0.0	1.5	2.0	2.5	3.0	Average
0.0	0.0	51.3	77.8	97.2	80.2	61.3
0.3	54.2	62.4	79.4	98.5	84.3	75.8
0.6	72.3	75.3	81.6	100.4	86.8	83.3
0.9	77.4	78.5	95.3	113.2	97.3	92.3
1.2	79.3	68.3	87.2	95.2	92.5	84.5
LSD 0.05	1.3					0.8
Average	56.6	67.2	84.3	100.9	88.2	
LSD 0.05	0.5					

does no significantly different with the second variety (Tamoz-2) where the percentage of contamination was 51.8%. At 3.0% from NaOCl exhibit lowest percentage contamination about 14% while 0.0% from NaOCl exhibit highest percentage of contamination about 100%. The interaction was with lowest percentage of contamination about 12% in the variety (Alfares) at 3.0% from NaOCl and the highest was 100% to both varieties at 0.0% from NaOCl. The percentage of contamination was 10% at 4.0% from NaOCl to both varieties but wilt and yellowing in the explant as a result to the using sterilizer and exposure to oxidation.

The results of Table 2 related to variety (Alfares) show that the highest average wet weight of induced callus was 92.3 mg at 0.9 mgL^{-1} Kinetin this is because of the increase in the concentration of cytokines because it has a significant role in increasing the division of parenchyma cells that have lost their specialization and turned into meristematic cells, leading to an increase in the size of different tissues (Arteca, 1996) while the lowest was 61.3 mg at 0.0 mgL^{-1} Kinetin. At 2.5 mgL^{-1} from 2, 4-D exhibits highest average of wet weight about 100.9 mg this may be due to the role of 2, 4-D in stimulating the elongation of plant cells through its effective role in stimulating the softness of the cellular wall, causing the breakage of the wall and its return to new sites under the influence of inflationary pressure which contributes to the size and expansion of the cell (Nickell, 1979) and the lowest was 56.6 mg at 0.0 mgL^{-1} 2, 4-D. The effect of interaction between the varieties and the concentrations shows the highest average of wet weight was 113.2 mg at the 2.5 and 0.9 mgL^{-1} from 2, 4-D and Kinetin, respectively it is important to create a delicat balance between the

Table 3: The average of wet weight to callus of the variety Tamoz-2 by 2, 4-D and Kinetin after five weeks

	2, 4-D (mgL^{-1})					
Kinetin (mgL^{-1})	0.0	1.5	2.0	2.5	3.0	Average
0.0	0.0	50.2	73.2	92.4	78.4	58.8
0.3	53.2	61.3	76.3	93.6	79.3	72.7
0.6	71.2	72.4	78.0	96.4	80.1	79.6
0.9	75.3	73.8	91.2	100.5	95.2	87.2
1.2	77.2	62.2	83.4	92.0	91.3	81.2
LSD 0.05	0.8					0.3
Average	55.4	64.0	80.4	95.0	84.9	
LSD 0.05	0.4					

Table 4: The average of dry weight to callus of the variety Alfares by 2, 4-D and Kinetin after five weeks

	2, 4-D (mgL^{-1})					
Kinetin (mgL^{-1})	0.0	1.5	2.0	2.5	3.0	Average
0.0	0.0	6.3	7.6	8.6	8.3	6.9
0.3	6.0	7.2	7.9	8.8	8.4	7.7
0.6	7.5	7.7	8.0	9.6	8.9	8.3
0.9	7.8	8.5	8.7	9.9	9.5	8.9
1.2	7.9	7.3	8.2	8.3	9.0	8.0
LSD 0.05	0.19					0.05
Average	5.8	7.4	8.1	9.0	8.8	
LSD 0.05	0.08					

concentrations of auxins and cytokines in the media because the increase in cytokines relative to auxins stimulates the formation of vegetative branches while high ratios of auxins to cytokines promote the formation of transverse roots and their balanced levels lead to continued callus growth and this is agreed with (Shah *et al*., 2003, Rahman *et al*., 2008; Tomar and Punia, 2003) and the lowest was 0.0 mg at 0.0 mgL^{-1} 2, 4-D and Kinetin.

The results of Table 3 related to variety (Tamoz-2) show that the highest average of wet weight of induced callus was 87.2 mg at 0.9 mgL^{-1} Kinetin while the lowest was 58.8 mg at 0.0 mgL^{-1} Kinetin. At 2.5 mgL^{-1} from 2,4-D exhibits highest average of wet weight about 95.0 mg and the lowest was 55.4 mg at 0.0 mgL^{-1} 2, 4-D. The effect of interaction between the varieties and the concentrations shows the highest average of wet weight was 100.5 mg at the 2.5 and 0.9 mgL^{-1} from 2,4-D and Kinetin, respectively and the lowest was 0.0 mg at 0.0 mgL^{-1} 2, 4-D and Kinetin. The results show that the increasing in the average of wet weight to the induced callus increased by the increasing in the concentration growth regulators while the average of wet weight decreased at higher concentrations of growth regulators with significant differences between the varieties used.

The results Table 4 related to variety (Alfares) show that the highest average of dry weight of induced callus was 8.9 mg at 0.9 mgL^{-1} Kinetin while the lowest was 6.9 mg at 0.0 mgL^{-1} Kinetin. At 2.5 mgL^{-1} from 2,4-D exhibits highest average of wet weight about 9.0 mg and the

Table 5: The average of dry weight to callus of the variety Tamoz-2 by 2, 4-D and Kinetin after five weeks

	2, 4-D (mgL^{-1})					
Kinetin (mgL^{-1})	0.0	1.5	2.0	2.5	3.0	Average
0.0	0.0	6.1	7.2	8.4	8.0	6.0
0.3	5.7	6.8	7.3	8.6	8.1	7.3
0.6	7.0	7.5	7.7	9.0	8.2	7.9
0.9	7.3	8.0	8.5	9.3	9.0	8.4
1.2	7.4	7.2	8.0	8.2	8.3	7.8
LSD 0.05	0.14					0.04
Average	5.5	7.0	7.7	8.7	8.3	
LSD 0.05	0.06					

Table 6: The percentage of callus regeneration to the variety Alfares by IAA and BAP after eight weeks

	IAA (mgL^{-1})					
BAP (mgL^{-1})	0.0	0.5	1.0	1.5	2.0	Average
0.0	0.0	3.2	4.6	7.2	6.5	4.3
0.1	3.8	4.2	6.4	8.2	8.0	6.1
0.2	5.3	5.8	6.8	8.9	8.2	7.0
0.3	8.7	9.3	10.1	13.8	10.2	10.4
0.4	5.7	6.3	7.8	9.4	8.3	7.5
LSD 0.05	0.34					0.21
Average	4.7	5.8	7.1	9.5	8.2	
LSD 0.05	0.26					

lowest was 5.8 mg at 0.0 mgL^{-1} 2, 4-D. The effect of interaction between the varieties and the concentrations shows the highest average of dry weight was 9.9 mg at the 2.5 and 0.9 mgL^{-1} from 2, 4-D and Kinetin, respectively it should be noted that the same treatment achieved the highest average of wet weight of the callus tissue induced and surpassed here may be an extension of that superiority (Table 2 and 3) and the lowest was 0.0 mg at 0.0 mgL^{-1} 2, 4-D and Kinetin.

The results Table 5 related to variety (Tamoz-2) show that the highest average of dry weight of induced callus was 8.4 mg at 0.9 mgL^{-1} Kinetin while the lowest was 6.0 mg at 0.0 mgL^{-1} Kinetin. At 2.5 mgL^{-1} from 2, 4-D exhibits highest average of wet weight about 8.7 mg and the lowest was 5.5 mg at 0.0 mgL^{-1} 2, 4-D. The effect of interaction between the varieties and theconcentrations shows the highest average of dry weight was 9.3 mg at the 2.5 and 0.9 mgL^{-1} from 2, 4-D and Kinetin, respectively, this is agreed with (Dagustu, 2008; Fahmy *et al*., 2012; Nasircilar *et al*., 2006) and the lowest was 0.0 mg at 0.0 mgL^{-1} 2, 4-D and Kinetin. The variety (Alfares) significantly different where gave the highest dry weight 9.9 mg while variety (Tamoz-2) gave 9.3 mg.

The results Table 6 related to variety (Alfares) show that the highest percentage of callus regeneration was 10.4% at 0.3 mgL^{-1} BAP while the lowest is 4.3% at 0.0 mgL^{-1} BAP. At 1.5 mgL^{-1} IAA exhibits highest regeneration to callus at about 9.5% and the lowest was 4.7% at 0.0 mgL^{-1} IAA. The effect of interaction between

Table 7: The percentage of callus regeneration to the variety Tamoz-2 by IAA and BAP after eight weeks

	IAA (mgL^{-1})					
BAP (mgL^{-1})	0.0	0.5	1.0	1.5	2.0	Average
0.0	0.0	3.0	4.3	7.0	6.2	4.1
0.1	3.2	4.1	6.2	8.1	7.8	5.9
0.2	5.2	5.5	6.6	8.4	8.0	6.7
0.3	8.3	9.2	9.3	10.9	9.8	9.5
0.4	5.4	6.0	7.2	9.3	8.2	7.2
LSD 0.05	0.62					0.25
Average	4.4	5.6	6.7	8.7	8.0	
LSD 0.05	0.27					

Table 8: The percentage of reformed roots to the varieties (Alfares and Tamoz-2) by IBA after four weeks

	IBA (mgL^{-1})					
Varity	0.0	2.0	4.0	6.0	8.0	Average
Alfares	0.0	2.2	4.6	6.8	4.2	3.6
Tamoz-2	0.0	2.0	4.3	5.7	4.1	3.2
LSD 0.05	0.23					0.12
Average	0.0	2.1	4.5	6.3	4.2	
LSD 0.05	0.16					

the varieties and the concentrations shows the highest and the lowest percentage of callus regeneration was 13.8% at 1.5 and 0.3 mgL^{-1} from IAA and BAP respectively, the studies pointed out by confirming the need for a balance between cytokines and auxin to maintain the permanence of the growth and development of the plant part, cytokines works by containing adenine as a key to initiating the process of cellular division, growth regulator 2, 4-D affects plant metabolism by producing proteins, thus influencing enzyme efficiency, breathing and cell division (Gamborg, 2002). The lowest percentage of callus regeneration was 0.0% at 0.0 mgL^{-1} from IAA and BAP.

The results in Table 7 related to variety (Tamoz-2) show that the highest percentage of callus regeneration was 9.5% at 0.3 mgL^{-1} BAP while the lowest is 4.1% at 0.0 mgL^{-1} BAP. At 1.5 mgL^{-1} IAA exhibits highest regeneration to callus at about 8.7% and the lowest was 4.4% at 0.0 mgL^{-1} IAA. The effect of interaction between the varieties and the concentrations shows the highest, this is agreed with Abdallah *et al*. (2012), Zale *et al*. (2004) and Yu *et al*., 2008) and the lowest percentage of callus regeneration was 10.9% at (1.5 and 0.3) mgL^{-1} from IAA and BAP, respectively. The lowest percentage of callus regeneration was 0.0% at 0.0 mgL^{-1} from IAA and BAP. The variety (Alfares) significantly different where gave the highest percentage of callus regeneration 13.8% while variety (Tamoz-2) gave 10.9%.

The results in Table 8 show that the highest percentage of reformed roots was 3.6% to variety (Alfares) while the lowest was 3.2% to variety (Tamoz-2). At 6.0 mgL^{-1} IBA exhibits highest percentage of reformed roots at about 6.3% and the lowest was 0.0% at 0.0 mgL^{-1} IBA. The effect of interaction between the varieties and the concentrations shows the highest percentage of reformed roots was 6.8% at 6.0 mgL^{-1} from IBA to the variety (Alfares). The lowest percentage of reformed roots was 0.0% at 0.0 mgL^{-1} from IBA to variety (Tamoz-2).

CONCLUSION

The study showed the efficacy of plant growth regulators 2, 4-D and Kinetin in stimulating the production of callus at concentrations 2.5 and 0.9 mgL^{-1} and the efficacy of IAA and BAP in regeneration of callus at concentrations 1.5 and 0.3 mgL^{-1} and the role of IBA in root reformation at focus 6.0 mgL^{-1}. There was a significant difference between the two varieties with the superiority of the variety Alfares on the variety Tamoz-2.

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