Shoot Propagation From Three Sunflower(Helianthus annuus L.)Genotype Callus Induction in Vitro

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ABSTRACT - ***An experiment was conducted in faculty of sciences labs, Baghdad university carried out during 2014-2015 .This study aims to observe the effect of , benzyl adenine and culture conditions on sunflower (Helianthus annuus L.) callus induction and indirect plant regeneration to propagate three genotype of sunflower (Shumoos, Flammy, Akmar) by using plant tissue culture technique. Vegetative propagation program was put on the above mentioned sunflower genotype in vitro included conducting few experiment to determine the best media for each growing period during propagation (culturing initiation) .Randomized complete block design was used with six replication as a factorial experiment with two factors concentration (first factor) six levels and genotype (second factors) three levels. Results showed that the apical meristem, had better response in tissue culture for all cultivars in the media M.S. the best way to sterilized lateral buds in this experiment was sodium hypochloride at 8% for 10 min or mercuric chloride at 0.01% for 2min. while , for the apical meristem thy alcohol 70% for 5 min. gave good result. Shoot multiplication experiment for sunflower genotype that applying (BA) into media gave a signification increase in the number of shoots at its lower concentration compared to control 0.5 mg/ l resulted in a higher number of shoots (74.61 shoot/plant) with length of 1.51 cm .***

Key words :sunflower , tissue culture , callus , plantlets, regeneration , propagation, benzyl adenine ,Apical meristem .

I.INTRODUCTION

Plant tissue culture is the sterilized culture of cells, tissues, organs and their components under defined physical and chemical conditions in vitro. Tissue culture techniques have been applied to the plant species in an attempt to produce new clones and cultivars with improved character. Mainly in the early 1960s plant tissue culture[1-3] was developed and have been use into a standard procedure for modern biotechnology, and today one can recognize five major areas where in vitro cell cultures are presently applied: large-scale propagation of identified materials, generation of genetic modified productive individuals, as a model system for fundamental plant cell physiology aspects, protection of rare species, and metabolic engineering of fine chemicals (Vargas and Flota, 2006). Calls is an unorganized mass of plant cell and its formation is controlled by growth regulating substance present in the medium.(auxins and cytokinins) (shah, et al., 2003) . The specific concentration of plant regulators needed to induce callus formation , varies from species to species and can even depend on the source of explant (Charrier, et al., 1999). Furthermore , genotype is of the most important factors for callus induction like shoot regeneration in tissues culture studies (sharrafi et al., 1996) [4-9] . The first trying started to use this kind of technology through German scientist haberlandt (1902) when isolation cells from pith and epidermis of paranchyma cells. Sunflower (Helianthus annuus L.) is a member of thistle family Asteraceae . Sunflower plants are herbaceous, annuals that grow from 4' to 20" (1-6m)tall, usually with only a single, hair covered stem that may be more than an inch in diameter. Leaves are as long as 12 inches (30 cm) and are borne on Petioles arranged alternately on the stem Sunflower has a typical head type of Inflorescence[10- 15] . The ray florets are located around the margin of the head 'are sterile. The corollas of the flower are the petals of the sunflower. The disc florets (flowers) located in the central part of the head are fertile but incomplete. The ovary of the disc flower develops into an achene, the same type of fruit as that of safflower The Genus Helianthus, is named from Greek where 'helios' means 'Sun', 'anthus' means 'flower'. Helianthus annuus was so named by C. Linnaeus, to when the only sunflower known, lived in a single season (annual). Sunflower probably originated in South-West United States and its seed was very early used for food by Indians. The plant tissues culture technique in vitro the modern abiotic techniques used in agricultural industrial and medical fields as technology used in genetics research and plants physiology and breeding of plants (Mariam,et al., 2014). Sunflower is an annual herbaceous crop (Asteraceae) that is propagated by seed only[16-20] . The cultivars grown were open- pollinated and cross-pollinated mostly by insect self-incompatibility cab be seen in sunflowers which is why they rely heavily on pollen movement between plants by insect and bee The ray florets. Poor seed germination is one of major problem in sunflower cultivation and also stratification is require to induce seed germination. The integration

of modern biotechnology like plant tissues culture into breeding programs may provide powerful tools to overcome these limitation. The first report of successful plant regeneration from sunflower callus was observed by sadhu (1974).by growing stem pith on a modified white's medium with 1Mg/L IAA, callus was induced after 10 weeks, one piece of callus. differentiated into several plant lets. (Greco et al ., 1984) showed that callus capable of regeneration can be obtained from every part of the seedlings (except root). Peterson and Everett (1985) reported embryogenesis of sunflower inbreed from 12 days old . seedlings hypocptyl explant on a modified Ms medium (supplemented with 6.9 gm/L total kn 03, 40 Mg/L adenine phosphate 500 Mg/L casamino acid , 1 Mg/L IBA, 1.0 Mg/L NAA, and 1 Mg/L GA3 , cava lini and lupi (1987) studied cytological condition of in vitro shoot apical meristem derived calli and regeneration shoots.Kenittel al., 1991)obtained high frequency plant regenerated from mature sunflower apical meristem.(pugliesi, et al., 1991) showed plant regeneration and genetic variability in tissue cultures of sunflower cotyledons.(Jamil et al.,2014) refers to, the effect of cotyledon and hypocotyl explants of five sunflower genotypes (Azargol, Farokh, Maste, CIRENA and ESBIBA) were studied on different hormonal combinations IAA, BA, NAA, BAP, KIN and 2,4-D on regeneration of Sunflower. Variance analysis results showed that evaluated cultivars had significant differences in response to different hormonal combinations[21-24] .The present study efforts was have been made to determination bettered explant to response for tissue culture and the best chemical material to sterilized of explant of the genotype of sunflower and determine the best media adequate for vegetative propagation in vitro.

II.EXPERIMENTAL & MATERIALS

An experiment was conducted in faculty of sciences labs, Baghdad university carried out during 2014-2015 .The presents study include the following :

1- Genotype used in this study: using in this research[14] three sunflower genotype (Shumoos , Flammy and Akmar)

2-Explant used in tissues cultural : Shoot tips which contain on internal apical meristem in length (0.7– 1cm) and mind (1-2cm) in length.

3- Sterilized materials : Using in this study two types from sterilized materials $NaOCl_2$ (sodium hypo chloride at 10% for 10 min and mercuric chloride at 0.01% for 2 min, for the apical meristem $NaOCl_2$ concentration (1 ,4 ,8)% by 250 ml For all concentration , added tween-20 . preparation $HgCl_2$ 1.0% concentration which using to sterilized sunflower explant by immerse in this solution for (1,2,4)min. And carried out all operations within the air laminar air flow –hood.

4- MS components : used food medium known as MS[15] (Murashige and Skoog, 1962) table(1)

Table (1): Composition of MS medium (Murashige and Skoog, 1962).

Ingredients	Concentration mg/L
NH_4NO_3	1650
KNO_3	1900
$CaCl_2.2\ H_2O$	440
$MnSO_4.H_2O$	370
$KH_2.PO_4$	170
B- Micronutrients	
H_3BoO_3	6.2
KI	0.83
$MnSO_4.\ 7H_2O$	16.9
$ZnSO_4.\ 7H_2O$	8.6
$Na_2MoO_4.5H_2O$	0.025
$CuSO_4.5H_2O$	0.025
$CoCl_2.\ 6H_2O$	0.025
C- Chelated Iron components	
Na_2 EDTA	37.25
$FeSO_4.\ 7H_2O$	27.84

Table (2) nutrients media components used in culturing initiation stage .

	Ingredients	Concentration mg/L
1-	MS salts	Stock solution
2-	Thiamin – HCl	0.4
3-	Benzyl Adenine (BA)	0.5
4-	Kinetin	0.5
5-	Inositol	100
6-	Sucrose	30000
7-	Agar	8000
8-	PH	5.70

Shoot multiplication : Used Benzyl adenine (BA) in the shoot multiplication.

Benzyl adenine (BA) prepared by dissolving 50 mg of BA in 1 ml HCl to ensure the full dissolving and complete volume to 50 ml with distilled water to become our fundamental solution concentration of 1 mg/ml and Study the differentiation concentration effected from (BA) (0 , 0.5 , 1, 1.5 , 2, 2.5) mg/L where he distribution nutrient media in a rate of 30 ml / tubes and transplanted into each tubes five plantlets by six replication for each concentration of (BA) and each genotype .Incubated in the same pervious incubation condition and took notes after 10 days after of agriculture included number of shoot , lengths , Fresh and dry weights of Shoot .

Statistical analysis :

All experiments carried out using a randomized complete block design (R.C.B.D) and factorial experiment were analysis and compared the results statistically by using duncans test and the level of probability of at 0.01(Al-rawi and Khalaf Allaha, 2000).

Results and Discussion

Culturing initiation Experiment :

1- Explant sterilized

A-sodium hypo chloride using in sterilized

Table (3) shows that lower of sodium hypo chloride concentration were not effective in sterilizing the lateral buds obtained from sunflower the results appearance that use $Naocl_2$ in (1,4)% concentration for 10 minutes given the higher pollution ratio to culturing buds (% 73.3 for 0.1 concentration from $Naocl_2$ and %43 for %4 concentration), and the pollution rate in Akmar variety was higher than other cultivar. Sterilized by 8% from Naocl2 concentration given lower pollution ratio for culturing explant (20, 16.6 , 25)% for genotypes (Shumoos , Flammy , Akmar) respectively. The growth for this plantlets it best and not appearance any toxic indicators these results agree with a number of researchers who used $NaOCl_3$ in sterilization of explant to be grown in vitro of the sunflower genotype (Weber et al., 2000) .

Table (3) : Sodium hypo chloride concentration effected on pollution ratio of lateral shoots of sunflower genotype .

Concentration of $NaOCl_2$ %	Percentage Pollution of Lateral Buds %			
	Shumoos	Flammy	Akmar	Average
1	70.0	66.6	83.3	73.3
4	40.2	33.3	55.5	43.0
8	20.0	16.6	25.0	20.5

The results in table (4) refer to the lateral buds sterilized of the time period for the genotype of sunflower by $HgCl_2$ by concentration 1.0% a higher proportion of the pollution in the a biotic and growth culturing bud. It was found the immersion of explant for one minute had given the higher pollution proportion , especially the genotype Akmar which reaching 50% while the sterilized for a two minute have given best result in reduction in the rate of pollution and the best growth of cultivated buds has pollution ratio (15.6,14.0,19)% for variety (Shumoos , Flammy , Akmar) respectively. While the sterilized of lateral buds by $Hgcl_2$ for 4 minute Its toxic effects on the cultivated buds These results are consistence with what referred to (Ozyigit et al., 2007).

Table (4) :Effected of sterilized period by mercuric chloride (1.0 %)on pollution ratio of laterals shoots of sunflower genotype .

Period of sterilized Min	Pollution ratio		
	Shumoos	Flammy	Akmar
1	32.3	24.5	49
2	15.6	14.0	19
4	-	-	9.5

Source of explant : Results indicated shown in table (5) was increased significantly in the success ratio of the explant of cultivated of sunflower genotypes, where he found that apical meristem gave a best response for multiplication and growth after (4 weeks) of agriculture genotype to respond 100% ratio while the lower respond for lateral buds compared to the apical meristem and amounted to 80% of the two genotypes (Shumoos and Flammy) and the 70% for the genotype of (Akmar). The high respond to the apical meristem tissues planting activity due to cell of this part and the speed of division and growth, as well as contain auxines that stimulated cells division and growth (Ozyigit et al.,2006;). These results are consistent with those reached by many researchers about the source explant a depended in this research on the of the apical meristem in the propagation of sunflower genotype study restriction (Pandurang et al .,2012).

Table (5) : Effected of benzyl adenine in vegetative indicators of sunflower genotypes.

Genotype	Explant	Replicate number	Growth replicate number	Succeed Ratio
Shumoos	Apical meristems	10	10	100.0
	Lateral shoots	10	8	80.0
Flammy	Apical meristems	10	10	100.0
	Lateral shoots	10	8	80.0
Akmar	Apical meristems	10	10	100.0
	Lateral shoots	10	7	70.0

Vegetative propagation experiment

1- Effected of Benzyl adenine (BA) different levels in the vegetative indicators.

It describes the results in table (6) the existence of significant differences between the genotypes in the average number of branched and the total length after 8 weeks of agriculture where he significant increase Shumoos genotypes compared to the Flammy and Akmar in this two of indicator. Average number of branches for this genotypes (62.83 cm) branch and the length (2.06 cm). While given Akmar genotypes lower shoots where the average 36.8 shoots and lower average lengths reaching (1. 61) compared to the other genotypes. The fresh and dry weight the results in the table (6) show not differences significant between the two genotypes Shumoos and Flammy but was increased significantly from the Akmar genotypes. The was given Shumoos genotype the highest rate of the weight of the fresh and dry (2.79 gm) and (147.3 mg) respectively. While the Akmar was given lowest rates for these indicators and reached (1.20gm , 50.23 mg). The differences in number branch and lengths, weights fresh and dry genotypes of sunflower may be due to genetic differences between these genotypes and this agreed with(Ozyigit et al.,2002) .when his studies for his response of six genotypes of sunflower tissue agriculture where he found differences between genotypes in response may be because the genetic differences between genotypes because the enzyme reflection to action of the gene. Also, these results are consistence with ((Paterson and Everett, 1985)where reported that the differences genotypes of sunflower respond to agriculture of tissues from apical meristem.

Table (6): Response of three sunflower genotypes for treatment by different levels from benzyl adenine (BA) in the vegetative indicators.

Studying indicators	Genotypes		
	Shumoos	Flammy	Akmar
Number of shoots	62.83 a	52.07 b	36.8 c
Length of shoots(cm)	2.06 a	1.41 c	1.61 b
Fresh weight (g)	2.79 a	2.44 a	1.20 b
Dry weight(mg)	147.3 a	130.47 a	50.23 b

As for the effect of the (BA) vegetative propagation results in the table (7) shows that the concentration of a benzyl adenine significant effect in culturing initiation has increased the number of branched an increase of a benzyl adenine concentration a compared to control but given concentration of 0.5 mg/L of (BA) higher of branched and reached (74.6) compared to control (40.9) branches. The results show that the number of branched may be a significantly decreased with the higher concentration from (BA) where give the concentration (2.0, 2.5) mg/L a significantly decrease in the number of branched compared to lower concentration from (BA)as well as the treatment of compared to control the number of branched (38.2 and 34.1) branches respectively. As of the branches long it is observed from the table (7) that adding (BA) to the medium has caused a significant decrease in the branched number a compared to control, and that this decline may be increased significantly by increasing the concentration of benzyl adenine. In the medium where the (2.5) mg/L gave the average length of the branches of the amount of (0.81) cm a compared to treatment of control which stood a long of the branches where 3.55 cm. As for the fresh and dry weight for the branched, the concentration of 0.5 mg liter of the (BA) may cause significantly increased in the rate of fresh weight, reaching to (2.81 gm) and increasing significantly in dry weight (155mg) average due to the increasing in number of branched compared to control. Either increasing concentration of (BA) in the medium has led to a decrease in the rate of fresh and dry weight was the average of fresh and dry weight concentration 2.5 mg/L (1.27g , 59.47 mg) respectively, while the rate of weight fresh and dry in control (2.6 gm and 129.73 mg) respectively. The decrease in the fresh and dry weight for the branches because the harmful effects high concentrations from (BA).

Table (7) : Effected of differences levels from benzyl adenine in vegetative indicators.

Studying indicators	Concentration of BA (mg/L)					
	0	0.5	1.0	1.5	2.0	2.5
Number of shoots	40.9 d	74.6 a	70.4 b	45.3 c	38.2 e	34.1 f
Length of shoots (cm)	3.55 a	1.51 b	1.30 bc	1.12 cd	0.97 de	0.81 e
Fresh weight (g)	2.60 a	2.81 a	2.57 a	1.91 b	1.71 b	0.28 c
Dry weight(mg)	129.73 b	155 a	125.3 b	99.93 c	86.5 c	59.47 d

Horizontal rates at which similar not significantly differences a cording to Duncan test at the probability 1%.

In the number and in total length as result of plant uptake of (BA) , which in turn leads to reduce the role of auxin which are responsible for the elongation of the cells when then reduce the length of the branch .That the results consist with the result of Wilcox et al. (1988).when the propagation of the assets of apple trees where he found that adding 2 mg/L from (BA) has given the best results in the multiplication of the number of plants by using tissues, culture. These results show sunflower genotype differ in their propagation response according to different concentrations of the BA added to the MS medium .As for the interference between genotypes and level of (BA) shows the result in the (figure 1) explain were significant in indicator number of shoot which gave the shumoos genotype which planted in medium they provide by 0.5 mg/L BA, highest the total (90.2)buds differed significantly from all other interventions, with the exception of the medium that contains 1 mg/L (BA) for the plants the same genotypes. The lowest rates of the number of the shoots was in the plants Akmar which cultivated in the MS medium contains on (1.5 , 2.0 , 2.5) mg/L it gave (30.3 , 26.8 , 23.7) shoots respectively. These rates of shoots for the Akmar genotype referred to significantly differed from all other interference (fig 1) .

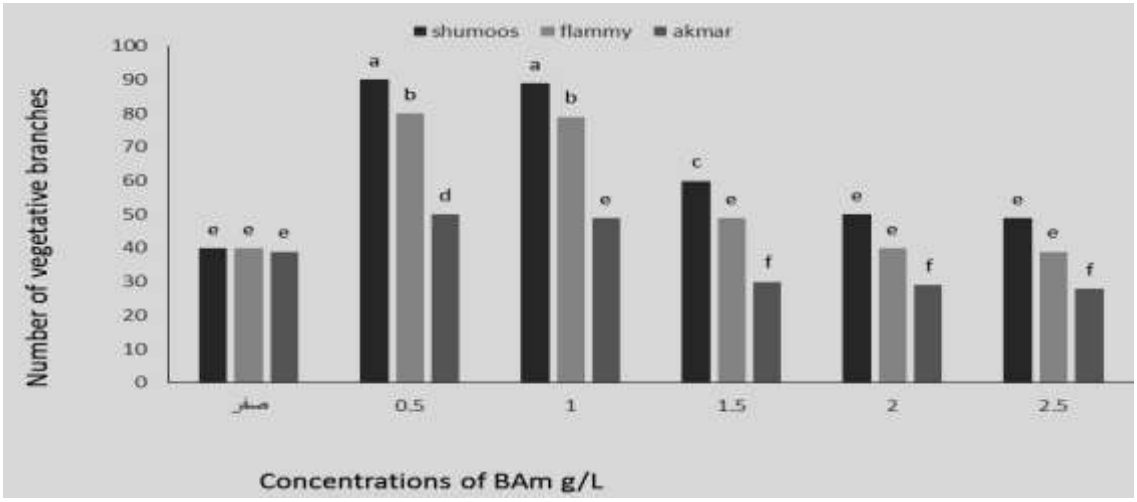

Figure .1:The effect of interaction between genotypes and BA concentrations (mg / L) in the number of branches plants sunflower genotypes according to a Duncan test at the level of probability of 0.01 .

As the interaction between the genotypes of sunflower and level (BA) added to the media in the average length of vegetative branches caused interaction interference between the genotypes and level of (BA) figure(2). Has reached the highest rate for the length of the shoots in the control and significantly differed with the rest of the levels as well as the significantly differences between the genotypes of sunflower from each other in this indicator where the significantly increased vegetative branches in length of shumoos genotype for all concentrations the average length of the shoots in the media without (BA) (5) cm followed by Flammy genotype where the average length of shoots total(3cm) either Akmar genotype average length of shoots it has reached (1.98 cm) and decreased plant lengths and all genotypes with increase (BA) concentration in the media. Was the lowest rate for the length of the shoots of the plants Akmar which planted in media which contain 2.5 mg/L (BA), which reaching (0.66 cm) and significantly differed from for all interaction between genotypes and concentration of (BA).

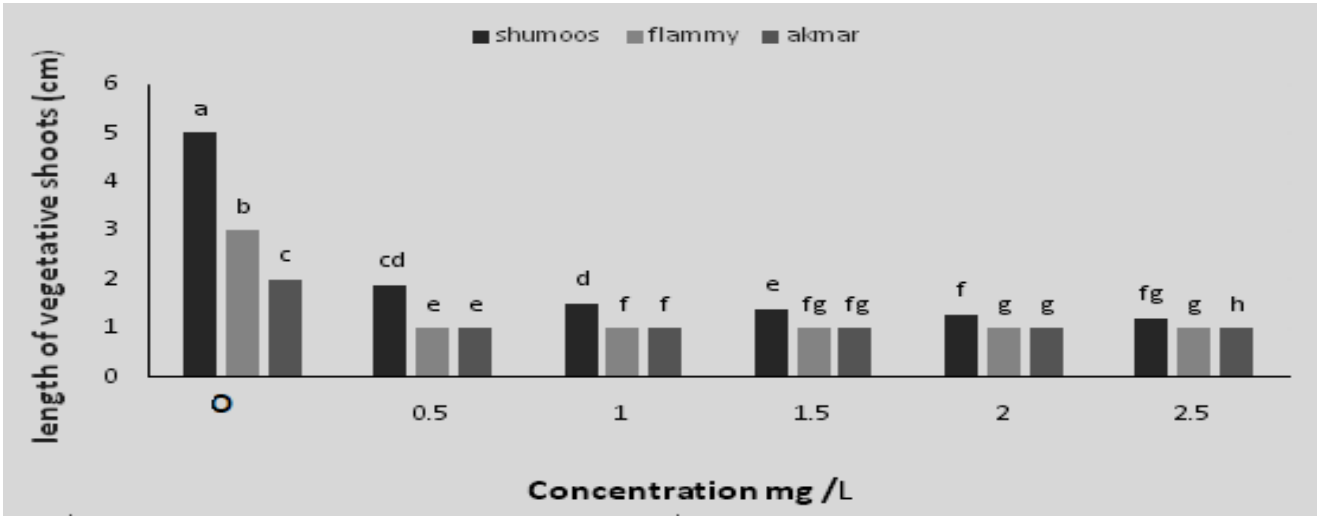

Figure.2:The effect of interaction between genotypes and BA concentrations(mg/ L)in length of vegetative branches (mm) of sunflower genotypes according to a Duncan test on the level of probability of 0.01 .

While for interaction s between concentration of (BA) and genotypes in the rate of fresh weight of the vegetative shoots the results in the (figure 3) show that week significantly interaction between each of the two Shumoos and Flammy genotype and concentrations of (BA) in this characteristic may be greater than genotypes (Shumoos and Flammy).Than the rate of fresh weight in the media which contain 0.5 mg/L (BA) the average fresh weight of the two genotypes (3.45 , 3.4)g respectively and will not differed significantly from the control treatment but they differed significantly of the media containing concentration (1.5 mg/L) (BA) .either lower the weight of the shoots have check in the Akmar genotypes and MS media which contains (2.5 mg/L) and differed significantly from the media and genotypes.

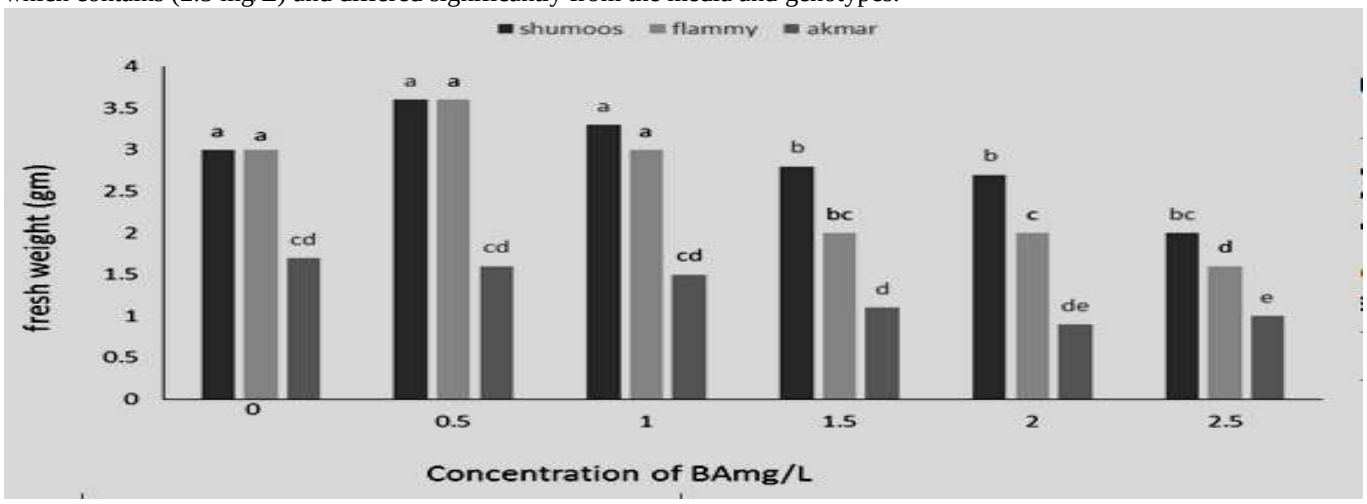

Figure (3): The effect of interaction between genotypes and BA concentrations(mg / L) in the fresh weight of the vegetative branches (gm) of sunflower genotypes according to a test Duncan on the level of probability of 0.01.

The average dry weight of studied sunflower genotypes the results in (figure 4) show there is a significantly interaction between the genotypes and the concentration of the (BA) in the MS media. The Shumoos genotypes gave was a higher rate of weight dry (224.2) in MS media which contain 0.5 mg/ between the genotypes and level of the MS media. The lowest dry weights was Akmar genotype in the concentration of the (2.5 mg/L)of (BA), where the rate was (17.2 mg/L) dry weight was not significantly differed from the two mediums which contain (0.5 , 0 , mg/L) (BA) but the genotype significantly differed for all interaction between two genotypes of (Shumoos and Flammy).L and differed significantly from the other interactions

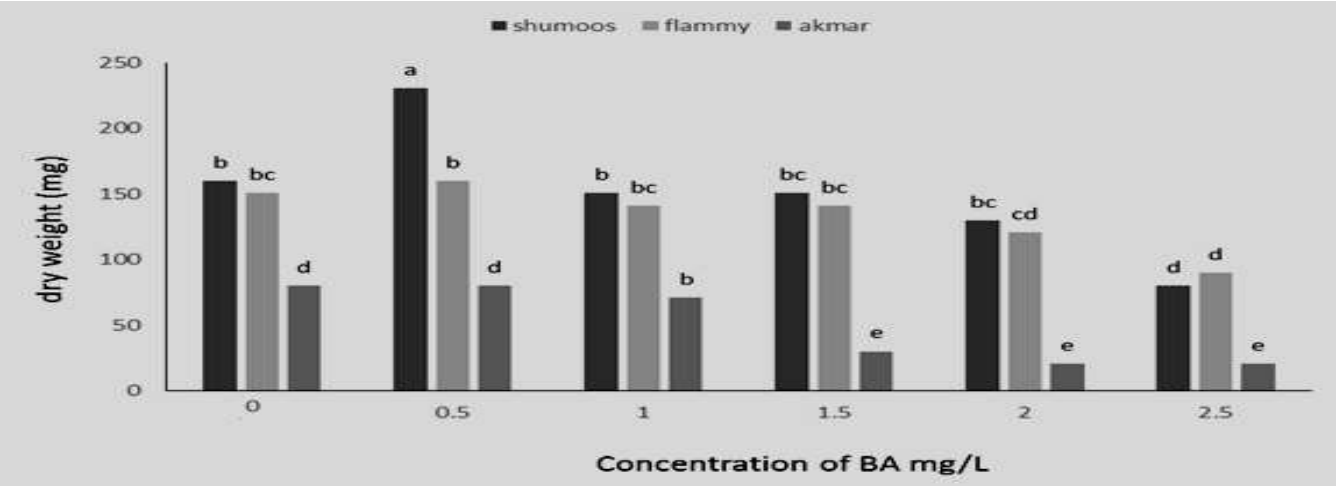

Figure (4):The effect of interaction between genotypes and BA concentrations (mg/ L) in the dry weight of vegetative branches (mg) of sunflower genotypes according to a Duncan test at the level of probability of 0.0

The Elicitation is one of the strategies to stimulate secondary metabolite production in plant cell cultures. Various abiotic and biotic elicitors can be used to induce the biosynthesis of secondary metabolites (Kadhim,2016).significantly differences in the interactions between the genotypes and concentrations of the (BA) in the MS media in the vegetative indicators consistent with what the found (Dagustu et al., 2010;Hayati, 2004;Paterson and Everett, 1985;Gorel, 1994).Result of this study revealed the explants from apical meristem which highest response for shoot production observed from apical meristem explants in Helianthus annuus L. Callus induction from apical meristem was faster than lateral buds (Ozyigit et al, 2006). The best explant recommend the use of vegetative propagation of the plant sunflower is apical meristem compared to lateral buds. Preferably use sodium hypochlorite at (8%) concentration for 10 minutes in the sterilized lateral buds or use of mercuric chloride in concentration (0.1%) for 2 minutes. But the apical meristem which sterilized by methyl alchohol (70%) for (5 min) gave encouraging results. Can be used (BA) in concentration 0.5 mg/L in MS media in vegetative propagation process gave a best results compared with control treatment .

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