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EFFECT OF AUXIN -CYTOKININ BALANCE AND EXPLANT ON CALLUS GROWTH AND REGENERATION IN SUNFLOWER

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ABSTRACT

Various combinations of hormones were used for culturing different explants (immature embryo, hypocotyl, cotyledon and leaf) of two sunflower genotypes (viz. cv. Miak and a synthetic population). The data showed that the highest growth rate across three growth passages of callus derived from immature embryos of cv. Miak (13.4 mg/day). This callus was obtained on MS medium supplemented with 0.5 mg L^{-1} Naphthaline acetic acid (NAA) and 1.0 mg L^{-1} Benzylamino purine (BAP) followed by MS medium supplemented with 2.0 mg L^{-1} NAA and 0.1 mg L^{-1} BAP (12.0 mg/day). In general, average growth rate was highest at the 1st passage (12.5 mg/day), then it was reduced to 6.1mg/day at the 2nd one, and to 2.5 mg/day at the third one. For regeneration percentage from the same calli, using MS medium containing 0.1 mg L^{-1} NAA and 2.0 mg L^{-1} BAP was the best (70.5%) followed by MS medium supplemented with 2.0 mg L^{-1} NAA and 0.1 mg L^{-1} BAP (54%). Either the absence of auxin or cytokinin or the high concentrations of both of them caused loss of regeneration ability. However, regeneration was favored by combinations of low NAA with high BAP or by high NAA with low BAP. It seems that there is no relation between callus growth rate and regeneration ability.

Using the MS medium supplemented with 2.0 mg L^{-1} NAA and 0.1 mg L^{-1} 6-BAP showed superiority over the other tested media for giving the highest growth rate of callus derived from cotyledons and leaves of cv. Miak and the synthetic population. Generally, growth rate of callus derived from cotyledons of the Syn. Pop was lesser than that of Miak, however, no regeneration was observed in the Syn. Pop using cotyledon explant. For hypocotyl explant, MS medium with 0.1 mg L^{-1} NAA and 2.0 mg L^{-1} BAP gave the highest callus growth rate for both genotypes and average callus growth rate was gradually increased from the 1st through the 3rd passage for both genotypes.

In general, hypocotyl was the best explant for obtaining high regeneration response as compared to cotyledon and leaf explants. Only MS medium supplemented with 0.5 mg L^{-1} of both NAA and 6-BAP was able to

regenerate plants from cotyledons of Miak. Regeneration percentage was the highest from Miak hypocotyls, when using MS medium supplemented with 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} BAP, followed by MS medium supplemented with 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} BAP. While, the Syn. Pop showed high regeneration by using MS medium containing 0.1 mg L^{-1} NAA and 0.5 mg L^{-1} BAP, followed by MS medium containing 0.1 mg L^{-1} NAA and 2.0 mg L^{-1} BAP. It could be concluded that the most efficient hormone concentration for regeneration in sunflower depends on genotype and type of explant.

Key words: ***Helianthus annuus, Naphthaline acetic acid, Benzylamino purine, Hypocotyl, Cotyledon, Immature embryo.***

INTRODUCTION

Successful applications of new methods of biotechnology can induce new types of variability (somaclonal variation) for selection of new genotypes, and contribute to overcome the problem of narrow genetic base of cultivated species, and at the same time accelerate the breeding process (Friedt 1996).

The use of tissue culture techniques by plant breeders is very valuable since it offers new tools that conventional methods do not have.

Plant regeneration from tissue culture would be particularly useful in sunflower breeding because regenerable cultures will be the first step toward *in vitro* selection among the somaclonal variations. Callus formation, shoot organogenesis of callus, and direct shoot regeneration were dependent on the explant (shoot tips, stem nodes, hypocotyl segments, leaves, or cotyledons) as well as the medium composition and genotype (Greco *et al* 1984 and Paterson 1984). Sunflower stem nodes appeared to be the most suitable explant in developing shoots directly, whereas middle hypocotyl segments gave the best response to callus induction and consequent shoot regeneration (Greco *et al* 1984). Therefore the present work aims to determine the best medium composition and the best explant for callus growth and regeneration of two genotypes of sunflower.

MATERIALS AND METHODS

Preliminary experiment: Effect of hormone combinations on callus derived from immature embryos

This experiment was carried out in 1996. The material for the experiment was the commercial sunflower cultivar Miak, which was grown in an open field in the Agricultural Experiments and Research station, Faculty of Agriculture, Cairo University, Giza, Egypt.

Immature embryos of globular-to torpedo-stage ranged in size from 3 to 5 mm (12 days after pollination) of the Miak variety were excised, after surface sterilization of their immature seeds (Azzam 2000).

Immature zygotic embryos were dissected and then cultured on MS medium (Murashige and Skoog 1962) supplemented with different concentrations of NAA (naphthalene acetic acid- auxin) and 6-BAP (6-benzylaminopurine-cytokinin) represented fourteen different media (M1 through M14) as shown in Table (1).

Ten immature embryos were placed on the surface of each of the 14 different solidified media in plastic petri dishes (15x100 mm) containing 25 ml of the medium and were repeated 4 times. The petri dishes were incubated in the dark at a temperature of 25°C ±1°C for 3 weeks. The calli were transferred to fresh media, and kept under cool-white fluorescent light (35 μ Em^{-2} s^{-1}) 16/8h light (1500 lux)/dark cycle at a temperature of 25°C ±1°C. Every 3 weeks the calli were transferred to fresh media. Adventitious shoot formations occurred from callused cotyledonary tissue after about 4 passages, particularly along the cut edges. The initiated shoots were transferred to shooting-medium [MS hormone free medium] for shoot development, the shoots were developed after about 2-3 weeks. To get the rooted shoots, shoots were then transferred to (R-medium) rooting-medium (half-strength MS medium supplemented only with 0.5 mg L^{-1} NAA). This stage needed about 10-15 days.

Table 1. Combinations of auxin and cytokinin concentrations in MS media used in this study

Medium designation	Auxin (NAA mg L^{-1})	Cytokinin (6-BAP mg L^{-1})
M1	-	0.5
M2	-	2.0
M3	0.1	0.1
M4	0.1	0.2
M5	0.1	0.5
M6	0.1	1.0
M7	0.1	2.0
M8	0.5	0.1
M9	0.5	0.5
M10	0.5	1.0
M11	2.0	-
M12	2.0	0.1
M13	2.0	0.5
M14	2.0	1.0

pH = 5.8

The plantlets with well-developed roots were transferred to pots containing standard soil compost for sunflower and kept for 10 days, under

colorless transparent plastic sheets to prevent desiccation, in a greenhouse with 16h photoperiod at 25°C (6000 lux). Plantlets were initially covered and gradually exposed to ambient humidity. After 10 days, plants were transferred to ordinary greenhouse conditions and were successfully grown to maturity. Recovered fertile plants after 6 weeks were self-pollinated by hand. Seeds have been obtained from regenerated plants.

Data on callus growth rate and regeneration response percentage were recorded as follows:

$$\text{Callus growth rate (mg/day)} = \frac{\text{Final weight of callus - initial weight of callus}}{\text{No. of days}}$$

$$\text{Regeneration response of calli \%} = \frac{\text{No. of the responded calli to regeneration}}{\text{Total no. of induced calli}} \times 100$$

Testing different explants, auxin-cytokinin combinations and genotypes

Two genotypes, cv. Miak and a Synthetic population (Syn. Pop.), the latter is an open pollinated population developed by the Agronomy Dept., Faculty of Agriculture, Cairo University (Shabana 1990), were used as experimental material in this experiment. Three explants were used i.e., hypocotyl, cotyledon and leaf.

The tested culture media were MS medium supplemented with different combinations of NAA and 6-BAP.

The MS medium supplemented with different concentrations of NAA in combination with 6-BAP were used, making six different media (M4, M5, M7, M9, M10 and M12), the composition of these media is shown in Table (1).

After 5 days from sowing seeds on MS hormone free medium, seedlings with one pair of cotyledonary leaves were used as source of cotyledon explants. Cotyledons were excised and cut into 2 parts (longitudinally). The two half-cotyledon explants were placed on the surface of different 6 solidified media (MS medium with different hormone combinations) in 10 cm plastic petri dishes containing 25 ml agar medium (10 parts/petri dish).

Ten-day-old seedlings, with the third pair of non-cotyledonary leaves were used as source of leaf explants. The non-primary leaves were

excised and cut into 2 parts (longitudinally). The two half-leaves were cultured on the different 6 solidified media.

Eleven-day-old seedlings were used as a source of hypocotyl explants. Hypocotyls were excised, cut into sections (each section was about 20-30 mm long) and placed (10 parts/ petri dish) on the surface of the different 6 solidified media.

The conditions of the callus induction and plant regeneration were similar to those mentioned before. The recorded data are: callus growth rate (mg/day), unfriable calli %, friable calli % and the regeneration response percentage. Percentages of friable and unfriable calli were estimated as follows:

$$\text{Friable calli \%} = \frac{\text{No. of friable induced calli}}{\text{No. of used explants}} \times 100$$

$$\text{Unfriable calli \%} = \frac{\text{No. of unfriable induced calli}}{\text{No. of used explants}} \times 100$$

RESULTS AND DISCUSSION

The preliminary experiment:

The growth rates of sunflower calli derived from immature embryos of Miak cultivar through three passages (each passage =21 days) on MS medium supplemented with the different combinations of auxin (NAA) and cytokinin (6-BAP) are presented in Table (2).

During the first passage, the highest growth rate of calli was noticed with the combination of 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} 6-BAP (M10), where the growth rate was 27.3 mg/day, followed by M12 (2.0 mg L^{-1} NAA and 0.1 mg L^{-1} 6-BAP) and M5 (0.1 mg L^{-1} NAA and 0.5 mg L^{-1} 6-BAP) media which exhibited 24.6 and 21.6 mg/day, respectively. During the second passage, the combination of 0.1mg L^{-1} NAA and 2.0mg L^{-1} 6-BAP (M7) produced the highest callus growth rate (9.3 mg/day) followed by M1 medium which had only 0.5 mg L^{-1} 6-BAP (8.8 mg/day), as shown in Table (2). During the third passage, the callus growth rate was high for the media rich in Auxin such as M13, M10, and M12 (7.3, 5.9 and 5.2 mg/day, respectively).

Table 2. Mean growth rate (mg/day) ± standard error for sunflower calli derived from immature embryos of cv. Miak.

Passage	Auxin/cytokinin balance (mg L^{-1})							
	NAA \ BAP	0.0	0.1	0.2	0.5	1.0	2.0	X`
1st Passage	0.0	-	-	-	10.9 ± 1.3	-	10.7 ± 1.1	10.8
	0.1	-	8.9 ± 3.7	11.6 ± 1.4	21.6 ± 10.1	4.5 ± 2.8	11.1 ± 1.3	11.5
	0.5	-	7.2 ± 2.0	-	9.8 ± 1.8	27.3±10.3	-	14.8
	2.0	12.5 ± 1.6	24.6 ± 8.0	-	13.2 ± 12.3	1.3 ± 0.6	-	12.9
	X`	12.5	13.6	11.6	13.9	11.0	10.9	
2nd Passage	0.0	-	-	-	8.8 ± 4.1	-	6.5 ± 3.9	7.7
	0.1	-	2.5 ± 0.6	6.2 ± 3.9	4.7 ± 1.7	3.1 ± 0.8	9.3 ± 4.3	5.2
	0.5	-	3.6 ± 1.0	-	5.5 ± 2.7	7.1 ± 1.3	-	5.4
	2.0	8.2 ± 4.0	6.0 ± 3.8	-	8.1 ± 8.3	1.4 ± 0.6	-	6.0
	X`	8.2	4.0	6.2	6.8	3.9	7.9	
3rd Passage	0.0	-	-	-	0.3 ± 0.1	-	0.9 ± 0.6	0.6
	0.1	-	1.9 ± 0.5	1.1 ± 0.7	3.7 ± 1.7	1.7 ± 0.4	0.5 ± 0.3	1.8
	0.5	-	2.3 ± 0.9	-	4.2 ± 2.6	5.9 ± 1.2	-	4.1
	2.0	0.4 ± 0.2	5.2 ± 3.4	-	7.3 ± 8.4	0.7 ± 0.5	-	3.4
	X`	0.4	3.1	1.1	3.9	2.8	0.7	
X``		7.0	6.9	6.3	8.2	5.9	6.5	

The means, over the three passages, indicated that the highest callus growth rate (13.4 mg/day) was produced on M10 medium, followed by M12 and then M13 (Table 3).

The general average of callus growth rate, over all used media, was highest at the first passage (12.5 mg/day) then it was reduced to 6.1 mg/day at the second one, and to 2.5 mg/day at the third passage. Therefore, the ratio of callus growth rate was about 5: 2.4: 1 for the first, second and third passages, respectively.

Finally, it is suggested that both NAA and 6-BAP should be avaliable in the medium to induce callus from zygotic immature embryos. However, the optimal growth regulator combination depended on the specific interaction between the two growth regulators. These results are in harmony with those obtained by Espinasse and Lay (1989) and Ahmad *et al* (1994) who reported that all sunflower cultivars used by them showed the best callus growth on medium containing both NAA and BA.

The effects of previously used media on the percentage of regeneration response from calli derived from immature embryos of Miak cultivar are illustrated in Fig. (1).

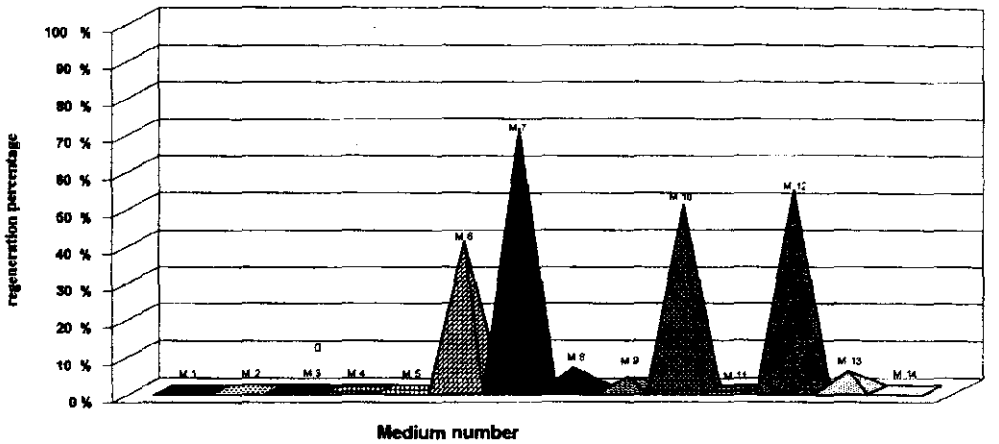


Fig 1. Plant regeneration from immature embryos of Miak cultivar as affected by different combinations of NAA and 6-BAP in MS media

Table 3. Mean growth rate (mg/day) over passages and media for sunflower calli derived from immature embryos of cv. Miak.

Media	Hormone mg L^{-1}		The Callus growth rate (mg/day)			Average growth rate over passages
	NAA	BAP	1st passage	2nd Passage	3rd Passage	
M1	-	0.5	10.9 ± 1.3	8.8 ± 4.1	0.3 ± 0.1	6.7
M2	-	2.0	10.7 ± 1.1	6.5 ± 3.9	0.9 ± 0.6	6.0
M3	0.1	0.1	8.9 ± 3.7	2.5 ± 0.6	1.9 ± 0.5	4.4
M4	0.1	0.2	11.6 ± 1.4	6.2 ± 3.9	1.1 ± 0.7	6.3
M5	0.1	0.5	21.6 ± 10.1	4.7 ± 1.7	3.7 ± 1.7	10.0
M6	0.1	1.0	4.5 ± 2.8	3.1 ± 0.8	1.7 ± 0.4	3.1
M7	0.1	2.0	11.1 ± 1.3	9.3 ± 4.3	0.5 ± 0.3	7.0
M8	0.5	0.1	7.2 ± 2.0	3.6 ± 1.0	2.3 ± 0.9	4.4
M9	0.5	0.5	9.8 ± 1.8	5.5 ± 2.7	4.2 ± 2.6	6.5
M10	0.5	1.0	27.3 ± 10.3	7.1 ± 1.3	5.9 ± 1.2	13.4
M11	2.0	-	12.5 ± 1.6	8.2 ± 4.0	0.4 ± 0.2	7.0
M12	2.0	0.1	24.6 ± 8.0	6.0 ± 3.8	5.2 ± 3.4	12.0
M13	2.0	0.5	13.2 ± 12.3	8.1 ± 8.3	7.3 ± 8.4	9.5
M14	2.0	1.0	1.3 ± 0.6	1.4 ± 0.6	0.7 ± 0.5	1.1
Average of growth rate over			12.5	5.8	2.6	7.0

Medium number 7 showed the highest response to promote shoots and regenerate plants of Miak cultivar; the regeneration response percentage was 70.5% followed by M12 medium (54%) and M10 medium (50%) as shown in Fig. (1).

Combinations of low NAA (0.1 mg L^{-1}) with high 6-BAP (2.0 mg L^{-1}) (M7) , high NAA (2.0 mg L^{-1}) with low 6-BAP (0.1 mg L^{-1}) (M12) had favored regeneration. Medium concentration of 6-BAP (1.0 mg L^{-1}) combined with low concentration of NAA (0.5 or 0.1 mg L^{-1}) exhibited high response to regeneration. Moreover, the absence of cytokinin or the high concentration of both auxin and cytokinin inhibited regeneration ability. It is suggested that both NAA and BAP should be present in medium to induce callus and regenerated plants from immature embryos whereas, the optimum combination depends on specific interaction between the two hormones. The results are in agreement with those reported by Espinasse *et al* (1989), Jeannin and Hahne (1991), Kraeuter and Friedt (1990) who mentioned that combination of low auxin with high cytokinin favoured shoot organogenesis in several sunflower genotypes. With several sunflower genotypes examined by Witrzens *et al* (1988), 3.0 mg L^{-1} 6-BAP was the optimum.

Testing different explants, auxin-cytokinin combinations and genotypes

The growth rate of calli produced from cotyledon, hypocotyl and leaf explants of Miak and Syn. Pop as affected by different combinations of NAA and 6-BAP concentrations through three passages are presented in Table (4).

Cotyledon explant

The maximum callus growth rate during the first passage of cv. Miak was exhibited by M12 medium (106 mg/day), followed by M9 (100 mg/day). The lowest callus growth rate was 65 mg/day which was produced by the M7 medium (Table 4).

Medium M10 was the best for giving the highest growth rate (47 mg/day) for callus derived from cotyledons of the Syn. Pop in the first passage.

During the second passage, the callus growth rate of Miak was generally increased as compared to the first one, while it was decreased for Syn. Pop. (Table 4). Maximum callus growth rate in the second passage was obtained by using M12 medium in both genotypes (188 and 27 mg/day for Miak and the Syn. Pop, respectively).

Table 4. **Mean growth rate (mg/day) ± standard error of sunflower calli derived from different explants of Miak and Syn. Pop through three passages (each passage = 21 days) using MS medium supplemented with different concentrations of NAA and 6-BAP.**

Explants	Media			Genotypes							
				Miak				Syn. Pop.			
	Medium designation	NAA mg L^{-1}	BAP mg L^{-1}	Callus growth rate (mg/day)			X`	Callus growth rate (mg/day)			X`
				1st passage	2nd passage	3rd passage		1st passage	2nd passage	3rd passage	
Cotyledon	M4	0.1	0.2	75 + 27	100 + 17	223+ 117	**132.5**	38 + 6	15 + 7	10+ 2	**21.0**
	M5	0.1	0.5	75 + 11	27 + 25	144 + 58	**82.1**	29 + 4	9 + 3	47 + 24	**28.4**
	M7	0.1	2.0	65 + 7	58 + 24	120 + 85	**80.9**	25 + 1	12 + 9	32 + 19	**22.9**
	M9	0.5	0.5	100 + 18	109 + 20	244 + 55	**150.8**	27 + 4	12 + 11	22 + 7	**20.2**
	M10	0.5	1.0	69 + 6	105+ 54	190 + 14	**121.2**	47 + 15	9 + 6	53 + 6	**36.3**
	M12	2.0	0.1	106 + 13	188+ 51	255 + 87	**182.9**	27 + 7	27 + 25	88 + 17	**47.3**
		X`		**81.5**	**97.9**	**195.9**	**125.0**	**32.3**	**14.1**	**42.0**	**29.4**
Hypocotyl	M4	0.1	0.2	84 + 35	53 + 15	176 + 16	**104.4**	38 + 11	97 + 50	159+ 96	**97.7**
	M5	0.1	0.5	49 + 8	57 +20	114 + 94	**73.5**	45 + 11	88 + 20	167 + 41	**100.0**
	M7	0.1	2.0	41 + 15	87 + 27	336+ 165	**154.8**	27 + 3	92 + 16	242+ 10	**120.3**
	M9	0.5	0.5	43 + 24	43 + 30	97 + 29	**60.9**	29 + 7	94 + 7	196+ 88	**106.5**
	M10	0.5	1.0	41 + 8	57 + 21	74 + 36	**57.1**	50 + 18	75 + 80	155+ 49	**93.5**
	M12	2.0	0.1	33 + 12	44 + 8	109 + 17	**62.2**	47 + 5	81 + 57	90 + 28	**72.7**
		X`		**48.6**	**57.0**	**151.0**	**85.5**	**39.3**	**87.8**	**168.2**	**98.5**
Leaf	M4	0.1	0.2	35 + 7	66 + 16	298 + 43	**132.9**	36 + 3	27 + 41	92 + 40	**84.6**
	M5	0.1	0.5	19 + 3	31 + 11	74 + 12	**41.4**	31 + 4	43 + 12	87 + 66	**53.6**
	M7	0.1	2.0	25 + 9	63 + 18	87 + 36	**58.4**	26 + 7	46 + 6	115 + 19	**62.9**
	M9	0.5	0.5	43 + 17	93 + 32	252 + 19	**129.4**	33 + 3	43 + 12	231 + 85	**101.9**
	M10	0.5	1.0	30 + 8	99 + 21	208 + 17	**112.4**	49 + 24	47 + 12	262 + 156	**119.1**
	M12	2.0	0.1	54 + 16	141 + 24	264+ 122	**153.3**	51 + 12	135 + 5	305 + 49	**98.5**
		X`		**34.2**	**82.3**	**197.2**	**104.6**	**37.8**	**56.9**	**198.2**	**97.7**

During the third passage, the average callus growth rate of both genotypes for cotyledon explant was further increased as compared to first and second passages (196 and 42 mg/day for Miak and Syn. Pop., respectively). The maximum growth rate in the third passage for callus derived from cotyledon explants was exhibited by using M12 medium (255 and 88 mg/day for Miak and Syn. Pop., respectively).

As an average of the three passages, the medium containing high NAA with low BAP concentration (M12) showed superiority in growth rate over all other tested media which was approximately 183 and 47 mg/day from callus derived from cotyledons of Miak and the Syn. Pop, respectively. Generally, growth rate of the callus derived from cotyledons of the Syn. Pop was less than one fourth of that of the Miak cultivar.

Many investigators e.g., Lupi *et al* (1987), Robinson *et al* (1987), Mohammad and Hassan (1988), Pugliesi *et al* (1991), Ahmad and Punia

(1994) and Sison and Godwin (1994) studied the effects of auxin/cytokinin balance in media for cotyledon culture on callus induction and maintenance. They concluded that hormonal balance depends upon the endogenous hormone level and/or different sensitivity to hormones.

Regeneration response (Table 5) from callus derived from cotyledon explants was observed for Miak genotype only by using M9 medium (18.7%). In contrast, no regeneration was observed in Syn. Pop. using cotyledon explant (Table 5). Shoot regeneration from cotyledon explant and number of shoots per explant was dependent on genotypes and media (Knittel *et al* 1991). It is worthy to note that medium containing equal amounts of NAA and BA (M9) was also able to induce somatic embryos from cotyledon capable to regenerate plants

Hypocotyl explant

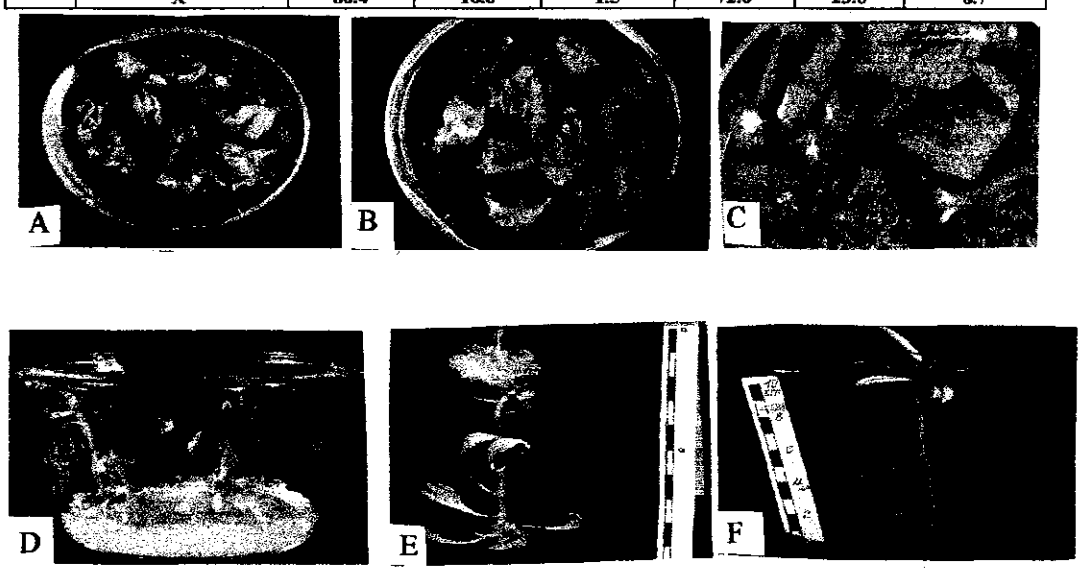
Results showed the highest callus growth rate over the three passages of Miak (154.8 mg/day) was obtained from M7 medium, followed by M4 (104.4 mg/day) and M5 (73.5 mg/day). Also M7 exhibited the same trend in calli produced from the other tested genotype (Syn. Pop), with an average callus growthrate of 120.3 mg / day followed by M9 (106.5 mg/day) and M5 (100.0 mg/day). The M7 medium showed its superiority over the other tested media for enhancing growth rate of callus derived from hypocotyl explant of both genotypes (Miak and Syn. Pop.), as shown in Table (4).

Over all 6 studied media, the growth rate of callus derived from hypocotyl gradually increased from the first through the third passage (the callus growth rate was 48.6, 56.9 and 151.0 mg/day for Miak and 39.3, 87.8 and 168.2 mg/day for Syn. Pop. for first, second and third passage, respectively).

These data exhibited that growth of callus derived from hypocotyls is accelerated in the first passage by a combination of 0.1mg L^{-1} NAA and 0.2mg L^{-1} 6-BAP for Miak and by concentration of 0.5mg L^{-1} NAA and 1.0 mg L^{-1} 6-BAP for the Syn. Pop. During the second and third passages, the hypocotyl callus growth could be accelerated with the addition of low concentration of auxin (0.1 mg L^{-1}) and high concentration of cytokinin (2.0 mg L^{-1}) for both genotypes. Lupi *et al* (1987), Robinson *et al* (1987), Sison and Godwin (1994) and Heupel and Kutschera (1996) also used NAA and BA to induce and propagate calli from sunflower hypocotyl. They have succeeded in callus induction and maintenance, but the effect of each growth regulator varied with the genotype.

Table 5. Behaviour of different sunflower explants of cv. Miak and the Syn. Pop as affected by different concentrations of auxin (NAA) and cytokinin (6-BAP).

Explants	Media			Genotypes					
				Miak			Syn. Pop.		
	Medium designation	NAA mg L^{-1}	BAP mg L^{-1}	Unfriable callus %	Friable callus %	Regeneration response %	Unfriable callus %	Friable callus %	Regeneration response %
Cotyledon	M4	0.1	0.2	96 ± 2	2 ± 7	0.0 ± 0.0	50 ± 23	15 ± 7	0.0 ± 0.0
	M5	0.1	0.5	71 ± 15	26 ± 18	0.0 ± 0.0	40 ± 19	4 ± 1	0.0 ± 0.0
	M7	0.1	2.0	31 ± 10	65 ± 6	0.0 ± 0.0	75 ± 16	0 ± 0	0.0 ± 0.0
	M9	0.5	0.5	90 ± 18	0 ± 0	18.7 ± 6.0	62 ± 18	0 ± 0	0.0 ± 0.0
	M10	0.5	1.0	93 ± 27	7 ± 1	0.0 ± 0.0	69 ± 18	31 ± 11	0.0 ± 0.0
	M12	2.0	0.1	89 ± 11	8 ± 3	0.0 ± 0.0	57 ± 15	33 ± 11	0.0 ± 0.0
		X`		78.4	17.8	3.0	59.0	13.7	0.0
Hypocotyl	M4	0.1	0.2	55 ± 11	45 ± 12	0.0 ± 0.0	56 ± 23	42 ± 20	27.1 ± 3.5
	M5	0.1	0.5	66 ± 17	34 ± 12	15.6 ± 5.0	100 ± 0	0 ± 0	60.2 ± 12.4
	M7	0.1	2.0	81 ± 19	19 ± 4	20.8 ± 6.3	92 ± 49	7 ± 3	27.9 ± 40.0
	M9	0.5	0.5	60 ± 24	40 ± 7	22.7 ± 4.9	87 ± 24	14 ± 8	27.1 ± 11.4
	M10	0.5	1.0	56 ± 11	44 ± 24	27.7 ± 15.2	67 ± 20	33 ± 12	14.3 ± 6.3
	M12	2.0	0.1	62 ± 15	38 ± 11	21.1 ± 13.1	49 ± 14	51 ± 15	0.0 ± 0.0
		X`		63.2	36.8	18.0	75.0	24.32	26.1
Leaf	M4	0.1	0.2	100 ± 0	0 ± 0	0.0 ± 0.0	89 ± 58	7 ± 2	19.7 ± 9.0
	M5	0.1	0.5	91 ± 3	7 ± 1	0.0 ± 0.0	88 ± 34	3 ± 1	8.0 ± 4.3
	M7	0.1	2.0	56 ± 10	37 ± 12	0.0 ± 0.0	90 ± 25	9 ± 3	25.0 ± 15.1
	M9	0.5	0.5	64 ± 23	37 ± 11	0.0 ± 0.0	80 ± 21	9 ± 4	0.0 ± 0.0
	M10	0.5	1.0	90 ± 35	2 ± 1	0.0 ± 0.0	40 ± 11	60 ± 44	0.0 ± 0.0
	M12	2.0	0.1	82 ± 14	18 ± 7	8.9 ± 3.7	50 ± 6	50 ± 29	0.0 ± 0.0
		X`		80.4	16.6	1.5	72.6	23.0	8.7



A: Initiated somatic embryos. B and C: Shoot proliferation. D: Root formation
E: Regenerated Plantlet. F:Mature regenerated plant.

Fig. 2. Main steps of plant regeneration process from leaf explants.

Moreover, the M7 medium which contained 0.1 mg L^{-1} NAA + 2.0 mg L^{-1} 6-BAP and M5 medium which contained 0.1mg L^{-1} NAA + 0.5mg L^{-1} 6-BAP induced the highest percentage of unfriable calli for the two genotypes (Table 5).

As indicated in Table (5) all tested media were able to generate shoots from callus derived from hypocotyl explants of the two genotypes, except M4 medium for Miak and M12 for Syn. Pop. which were unable to generate plants. The regeneration response percentage was highest for Miak when using M10 medium and for the Syn. Pop when M5 medium was used. Also, Greco *et al* (1984) and Schettler *et al* (1989) were able to regenerate plants from sunflower hypocotyls using NAA and 6-BAP balance.

Leaf explant

Average callus growth rate over all the tested media using leaf explant was gradually increased from the first through the third passage for both genotypes. For Miak cultivar, the callus growth rate was 34.2 mg/day, 82.3 mg/day and 197.2 mg/day for the first, second and third passage, respectively. However for the Syn. Pop, it was 37.8 mg/day, 56.9 mg/day and 198.2 mg/day for the same passages, respectively (Table 4).

The concentration of 2.0mg L^{-1} NAA and 0.1mg L^{-1} 6-BAP i.e., M12 was the best combination for increasing callus growth rate of the two genotypes through the three passages, as shown in Table (4). This result is in agreement with those obtained by Punia and Bohorova (1992) and Fambrini *et al* (1996).

The highest percentage of unfriable callus produced from leaf explant was 100% (Miak cultivar) and 89.7 % (Syn. Pop.) by using M4 and M7, respectively. These media were supplemented by 0.1 mg L^{-1} NAA and 0.2 mg L^{-1} 6-BAP (M4), or 2.0 mg L^{-1} 6-BAP (M7), indicating indicated that high concentration of auxin may increase the friable calli as shown in Table (5).

Regeneration from callus derived from Miak leaves occurred only when using medium containing 20 NAA: 1 6-BAP (i.e., M12 medium). While for the Syn. Pop, regeneration plants from callus produced from leaf explants by using three media, i.e., M4, M5 and M7, with highest response from M7 medium (25%) are shown in Table (5). The later medium contained NAA and 6-BAP in the ratio 1:20. Moreover, this medium resulted somatic embryos on callus derived from leaf explant of the Syn. Pop, as shown in Fig. (2). Greco *et al* (1984), Bohorova *et al* (1990) and

Punia and Bohorova (1992) concluded that the presence of both auxin and cytokinin in MS media were important factors for plant regeneration from sunflower leaves.

In general, percentage of unfriable calli (favorable for regeneration) induced from cotyledons, hypocotyls and leaves was much high than that of friable calli. The best media for inducing unfriable callus were M4 and M10 for Miak and M7 and M10 for Syn. Pop.

For both genotypes, hypocotyl explant was the best source for obtaining high regeneration response as compared to the other tested explants (cotyledons and leaves). Media requirement for regeneration in sunflower varied with explant source (Paterson and Everett 1985; Knittel *et al* 1991).

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تأثير توازن السيتوكينين – الأوكسين و أجزاء النبات على نمو الكالوس و الاستيلاد فى زهرة الشمس

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استخدمت تواليف مختلفة من تركيزات هرمون (حمض النفثالين و بنزيل أمينو بيورين) في بيئة MS لزراعة أنسجة من أجزاء مختلفة من نبات زهرة الشمس (الجنين غير الناضج – السويقة الجنينية السفلي – الفلقتين – الأوراق) للصنف التجارى (مياك) وعشيرة تركيبية من كلية الزراعة جامعة القاهرة بهدف الوصول الى أفضل التواليف لزراعة الأجزاء المختلفة ومدى اختلافها مع اختلاف التركيب الوراثي، وكان المعيار هو معدل نمو الكالوس و القدرة على استيلاد النباتات وقد أظهرت النتائج ما يلي:

١- أعلى معدل نمو للكالوس الناتج من الجنين غير الناضج كمتوسط لثلاث نقلات (١٣,٤ ملليجرام / يوم) كان مع تركيز نصف ملليجرام/ لتر حامض النفثالين + ٠,١ ملليجرام / لتر BAP و تلي ذلك تركيز ٢ ملليجرام NAA + ٠,١ ملليجرام BAP.

٢- كان معدل نمو الكالوس أثناء النقلة الأولى أعلى وتدرج نقص النمو فى النقلات التالية الثانية والثالثة وكان المعدل ٥ : ٢,٤: ١ للنقلات الثلاثة على التوالي.

٣- وجد أن أعلى معدل للاستيلاد من الأجنة غير الناضجة (٧٠,٥%) كان في حالة إضافة ٠,١ ملليجرام / لتر NAA +٢ ملليجرام / لتر BAP أثناء نمو الكالوس و استحداثه و تلتها التوليفة (٢ ملليجرام / لتر NAA + ٠,١ ملليجرام / لتر BAP) التى أعطت حوالي ٥٤ %, و كان لغياب الاوكسين او السيتوكينين و التوليفات التى تحتوى على تركيزات مرتفعة او منخفضة من الاثنان تأثير سلبى على معدل الاستيلاد.

٤- كان من الواضح إنه لا توجود علاقة وثيقة بين زيادة معدل نمو الكالوس و معدل استيلاد النباتات.

٥-وجد أن أفضل نمو للكالوس الناتج من الفلقتين و الأوراق في الصنف مياك على بيئة MS المضاف إليها ٢ ملليجرام / لتر NAA + ٠,١ ملليجرام BAP.

٦- كان معدل نمو الكالوس الناتج من فلقتى العشيرة التركيبية أقل من الصنف مياك بصفة عامة.

٧- وجد أن معدل نمو الكالوس الناتج من السويقة الجنينية السفلي للتركيبين الوراثيين أعلى مع التوليفة (٠,١ ملليجم / لتر NAA + ٢ ملليجرام / لتر BAP) كما لوحظ زيادة معدل نمو الكالوس بتقدم النقلات من الأولى الى الثالثة.

٨-لوحظ ان معدل الاستيلاد من السويقة الجنينية السفلي كان أعلى من معدل الاستيلاد من الفلقتين والأوراق فى التركيبين الوراثيين و كانت البيئة التي تحتوى على ٠,٥ ملليجرام/لتر لكلا من NAA , BAP هي فقط التي أعطت نباتات من فلقتي الصنف مياك.

٩- أمكن الحصول على أعلى معدل للاستيلاد من السويقة الجنينية السفلي للصنف مياك مع إضافة (٠,٥ملليجرام / لتر لكلا من BAP, NAA) و لكن مع العشيرة التركيبية وجد أن أعلى استيلاد كان مع إضافة (٠,١ ملليجم / لتر NAA + ٠,٥ ملليجرام / لتر BAP) وهذا يدل على أن التركيب الوراثي للمصدر له علاقة بالتواليف المضافة من الهرمون للحصول على أعلى معدل استيلاد للنباتات. وقد أمكن الحصول على أعلى معدلات للاستيلاد باستخدام مزارع السويقة الجنينية السفلي بصفة عامة.

١٠- يمكن استنتاج أن تركيز الهرمون في بيئة زراعة الأنسجة لنبات زهرة الشمس يتوقف على التركيب الوراثى والجزء النباتي المستخدم.