IV- RESULTS AND DISCUSSION

IV- Stage A

IV.A.1- Phenolic compounds in the explant and medium :

IV.A.1.a- Phenolic compounds :

Table (1) & Fig. (1) show the effect of antioxidant pretreatment on the level of the phenolic compounds in the different explants. Dealing with the content of phenolic compounds in the different explants before pretreatment, it is clear that leaf disc explants contained the highest significant amount of phenolic compounds as compared with all other tested explants. However, internode segments contained the lowest amount of phenolic compounds without significancy in relation to apical meristem or single node cutting explants. In the meantime, significant difference was lacking between apical and single node cuttings in their phenolic compounds content.

Considering the phenolic compounds content in the different explants after pretreatment with an antioxidant solution, it appears that internode segments have significantly the lowest content of phenolic compounds as compared with all other explants. However, the reverse was true concerning leaf disc explants. On the other hand, single node cuttings showed a slight decrease in relation to phenolic compounds of apical meristem.The rate of reduction in phenolic compounds occurred as a result of antioxidant pretreatment was the same as that in internode segments, apical meristem and single node cuttings.

Table (1): Effect of antioxidant pretreatment on phenolic compounds accumulation in different coffee explants.

Explant	No. pretreatment (control) (ppm)	After antioxidant pretreatment (ppm)	Average (ppm)
Leaf disc	3.1	2.0	2.55
Internode segments	1.7	1.0	1.35
Apical meristem	2.2	1.5	1.85
Single node cuttings	2.1	1.4	1.75
L.S.D. at 0.05	0.55	0.10	
L.S.D. at 0.01	0.83	0.17	

Table (2): Phenolic compounds accumulation as affected by time in both explant and medium of the cultured coffee explants.

Explant / No. of weeks	Leaf disc (p.p.m)		Internode segment (p.p.m)		Apical meristem (p.p.m)		Single node cutting (p.p.m)	
	Explant	Medium	Explant	Medium	Explant	Medium	Explant	Medium
1	1.7	0.6	0.8	0.3	1.20	0.5	1.40	0.5
2	1.1	0.6	0.7	0.3	0.9	0.5	1.00	0.5
3	0.9	0.7	0.6	0.4	0.7	0.6	0.70	0.6
4	0.8	0.7	0.6	0.5	0.7	0.7	0.70	0.7
L.S.D. at 0.05	0.74	0.13	0.74	0.10	0.08	0.10	0.42	0.10
L.S.D. at 0.01	1.10	0.20	1.10	0.17	0.18	0.17	0.64	0.17

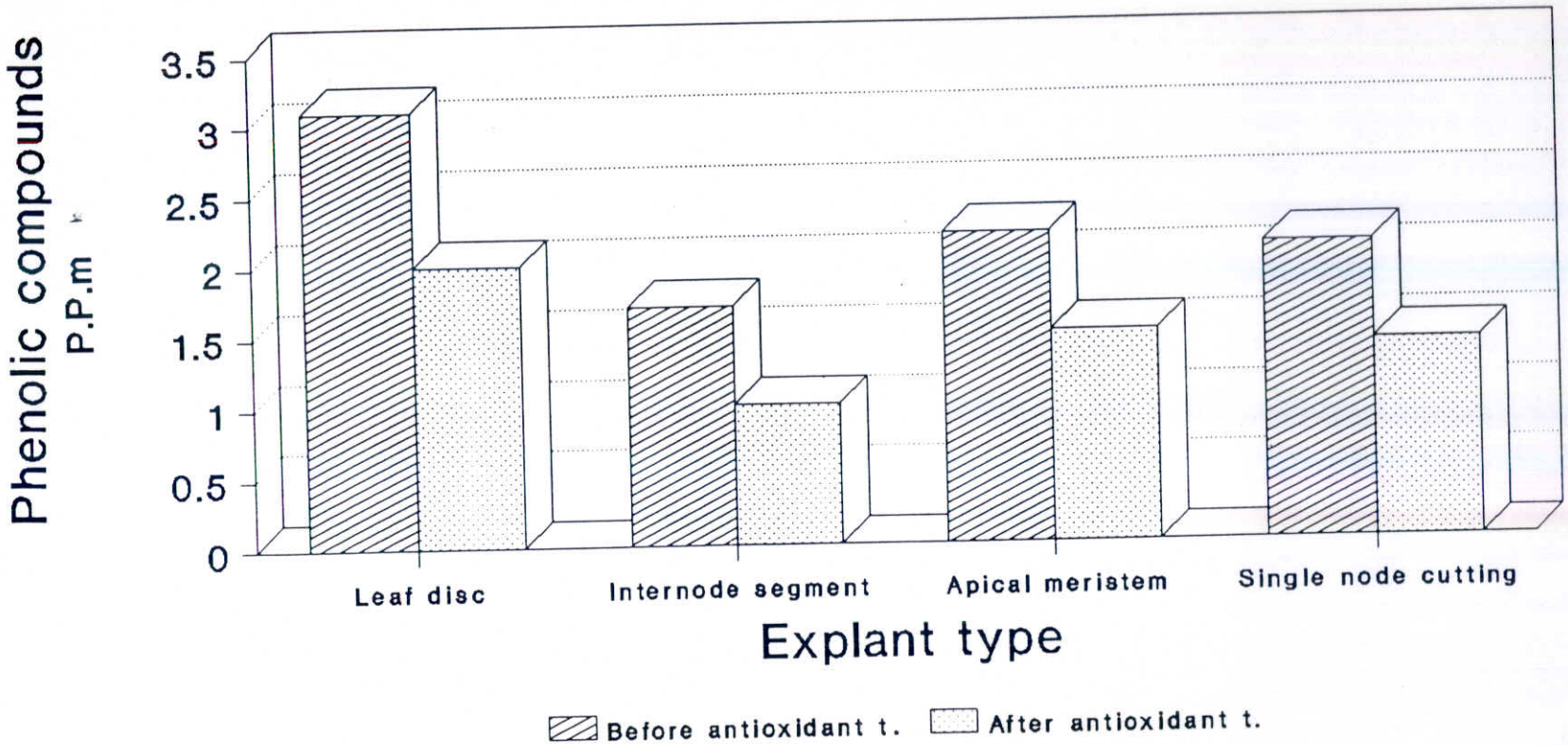


Fig.(1):Phenolic compounds level before and after antioxidant pretreatment in different coffee explants.

Table (2) & Fig. (2) refer to the gradual accumulation of phenolic compounds in the cultured explant and medium through the period of establishing time. It is quite evident that phenolic compounds were significantly reduced at the end of establishing period (after 4 weeks) as compared to the level after one week from culturing. However, the cultured medium of the leaf disc showed no significant increase in accumulation of phenolic compounds during the whole period of establishing medium. On the other hand, establishing medium of the internode segments, apical meristem and single node cuttings showed highly significant increase of phenolic compounds accumulation at the end of establishing period as compared to starting medium. Furthermore, apical meristem and single node cuttings showed highly significant reduction in phenolic compounds after 4 weeks as compared to that after one week from culturing time. Moreover, the final content of phenolic compounds in all explants and the cultured media were more or less similar at the end of the establishing period except internode segment and its medium. Furthermore, data of Table (3) showed that internode segments had the lowest level of phenolic compounds followed by apical meristem and single node cuttings. However, leaf disc explant had the highest content of phenolic compounds. Regardless of internode segments medium, all other media had approximately similar content of phenolic compounds.

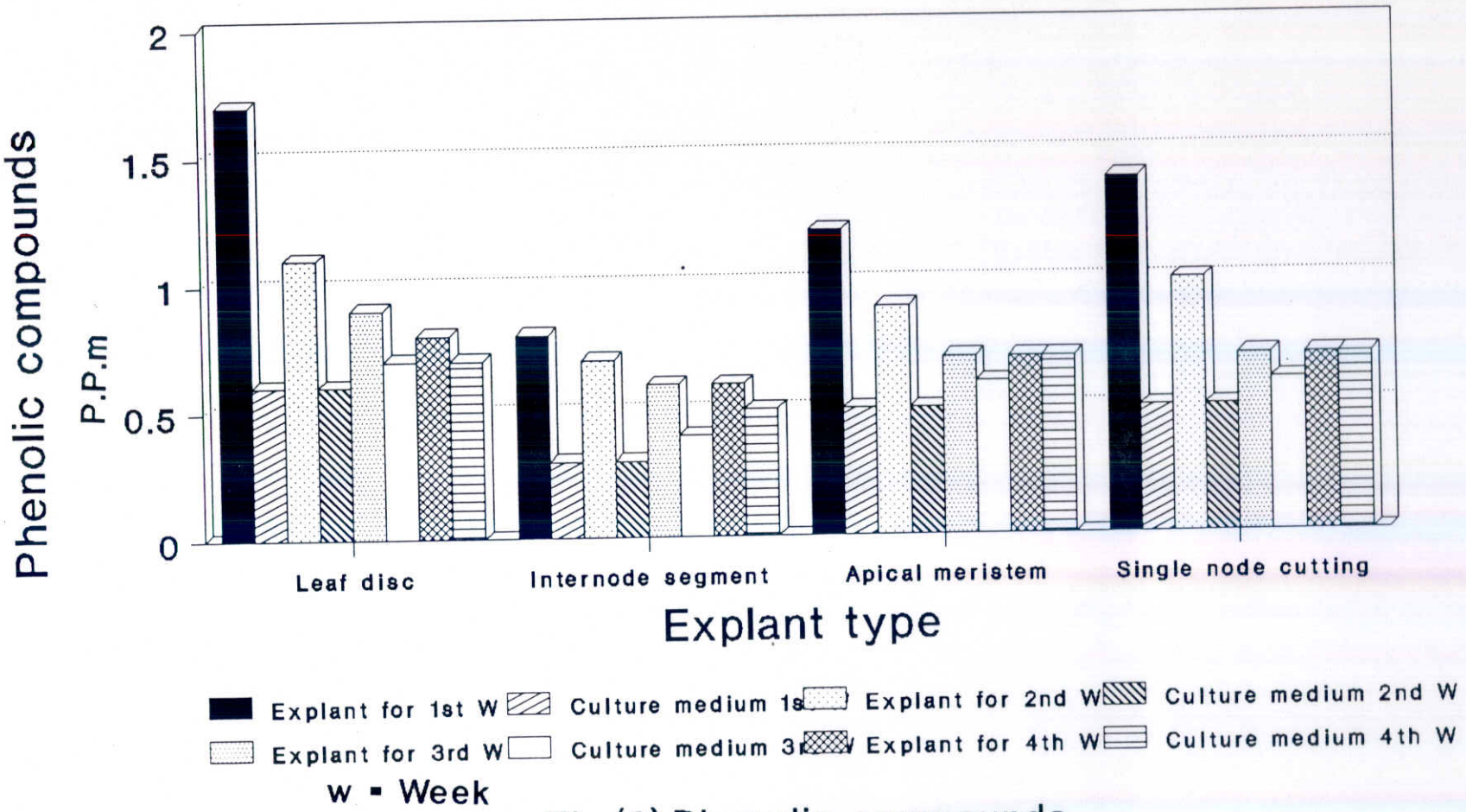


Fig.(2):Phenolic compounds accumulation as affected by time in both explant & medium in the cultured explant

Table (3): Effect of the explant type on the overall average of the phenolic compounds accumulation in both cultured explants and medium of coffee plants.

Explant	Leaf disc		Internode segment		Apical meristem		Single node cutting	
	Explant	Medium	Explant	Medium	Explant	Medium	Explant	Medium
Phenolic compound content (p.p.m)	1.13	0.65	0.70	0.38	0.88	0.58	0.95	0.58

Table (4): Phenolic compounds accumulation in callus and cultured medium at various ages of coffee callus.

Callus age weeks from culturing	Callus (p.p.m)	Medium (p.p.m)
4 weeks	0.7	0.4
6 weeks	0.5	0.4
8 weeks	0.4	0.5
L.S.D. at 0.05	0.23	0.09
L.S.D. at 0.01	0.38	0.15

Meanwhile, Table (4) reflects the level of phenolic compounds accumulation in the callus and the culture medium at various ages of callus. It is quite evident that phenolic compounds decreased significantly after 8 weeks as compared to 4 weeks old callus, in both callus and culture medium.

Generally, the mentioned results showed that leaf disc explants contained the highest level of phenolic compounds and this is in agreement with the findings of **Lopes et al. (1974)**. They declared that coffee chlorogenic acid occurs with high concentrations in seeds (2.7-10.3%) and leaves (0.3-1.7%). Also phenolic compounds decreased in the cultured explant to the lowest level at the end of establishing time. However,the cultured media took the other way around in accumulation of phenolic compounds during the period of establishing medium. In the same time, the cultured medium always contained very low phenolic compounds content as compared to the explants. These results are partially confirmed with the findings of **Frischknecht et al. (1977)**. They stated that average caffeine content at 49 days old culture contained 25 times as much as the primary culture. The tissue always contained more caffeine than the culture medium. Furthermore, the results of **Frischknecht and Baumann (1980)** indicated that the rate of methylation and the biotransformation potency of theobromine to caffeine were highest in the lag phase.

IV.A.I.b- Antioxidant treatments :

Table (5) and Fig. (3) involved the effect of antioxidants either as a pretreatment or in the medium on necrosis and callus production of both leaf discs and internode segments explants. It is clear that necrosis was significantly decreased in both leaf disc and internode segment explants when antioxidant solution + activated charcoal + L-cysteine were followed by antioxidant solution + activated charcoal combination treatment as compared to either any of the control, antioxidant solution, activated charcoal or L-cysteine solely. However, necrosis dominate the explant completely when no antioxidant treatment was applied(control).
Also, the addition of activated charcoal to the medium either alone or in combination with any antioxidant encouraged appreciably the reduction in necrosis. On the other hand, callus production took the other way around in this respect.

Furthermore, Table (6) and Fig. (4) deal with the effect of different antioxidant treatments on necrosis and plantlets regeneration from both apical meristem and single node cuttings of coffee plants. It appears that necrosis decreased significantly with the combination of antioxidant solution, activated charcoal and L-cysteine treatment in both apical meristem and single node cutting explants as compared to either control, antioxidant solution, activated charcoal or L-cysteine alone. However, an indirect relationship was obtained between necrosis and the number of regenerated plantlets as affected by different antioxidant treatments.

Table (5): Effect of different antioxidant treatments on necrosis and callus production of coffee leaf disc and internode segment explants.

Treatment	Leaf discs		Internode segments	
	Necrosis (scores)*	Callus production (scores)*	Necrosis (scores)*	Callus production (scores)*
Control	5.00	1.00	5.00	1.00
Antioxidant solution	2.67	3.00	3.00	3.00
Activated charcoal	2.67	3.33	2.67	3.00
L-cysteine	3.00	2.67	3.33	2.67
Antioxidant sol. + Activated charcoal	1.33	4.33	2.00	3.67
Antioxidant sol. + L-cysteine	1.67	4.00	2.67	3.33
Activated charcoal + L-cysteine	2.00	3.33	2.67	3.00
Antioxidant sol. + activated charcoal + L-cysteine	1.00	4.67	1.33	4.33
L.S.D. at 5%	0.84	1.27	0.86	1.22
L.S.D. at 1%	1.14	1.81	1.13	1.78

* Scores :
No. callus and no necrosis = 1, below average =2, average = 3, above average = 4, excellent callus volume and complete necrosis = 5.

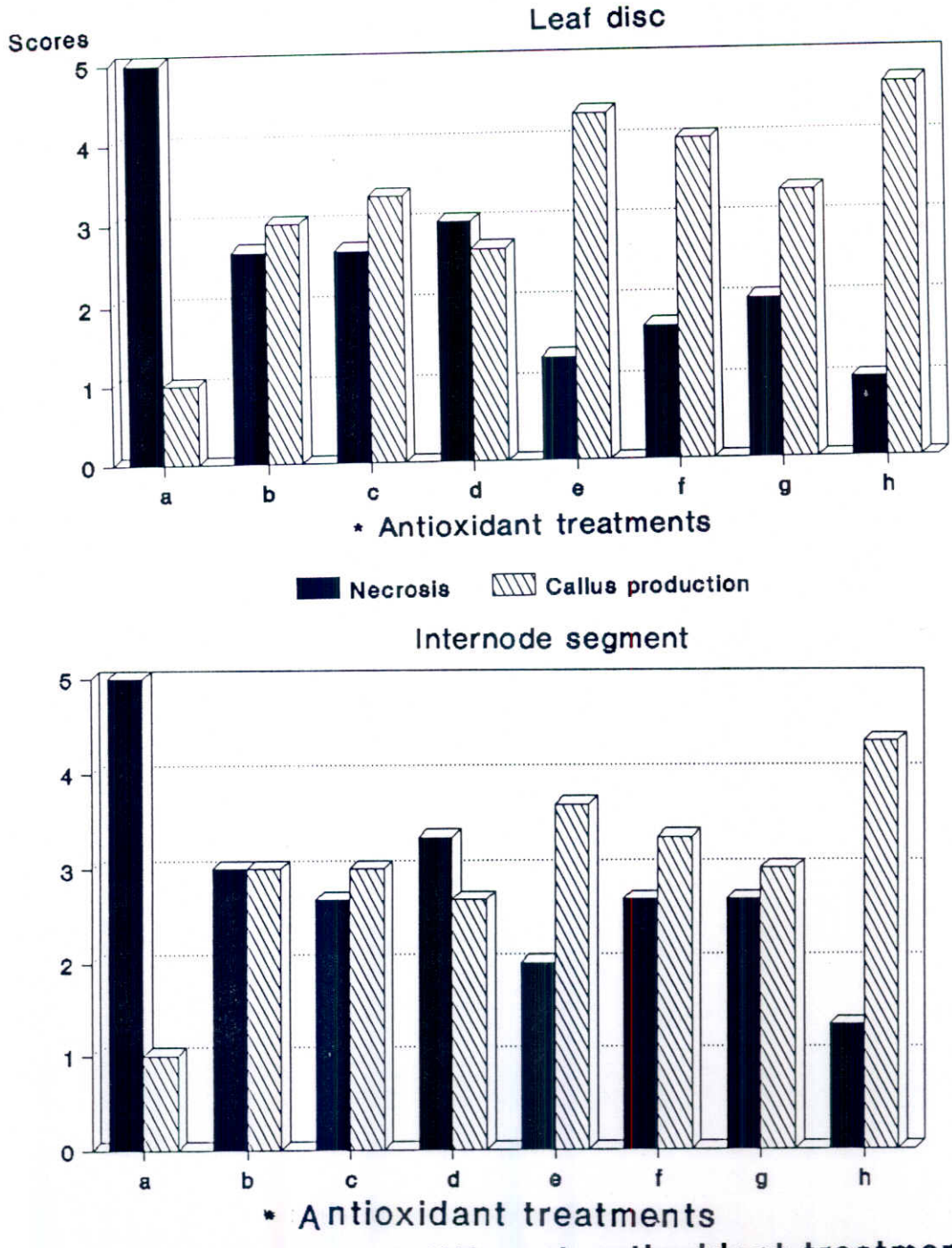


Fig.(3): Effect of different antioxidant treatments on necrosis and callus production of coffee leaf disc and internode segment explants.

* Antioxidant treatments

a- Control.
b- Antioxidant solution.
c- Activated charcoal.
d- L-Cysteine.
e- Antioxidant sol. + activated charcoal.
f- Antioxidant sol. + L-cysteine.
g- Activated charcoal + L-cysteine.
h- Antioxidant sol. + activated charcoal + L-cysteine.

** Necrosis & Callus scores

No callus & No necrosis = 1, below average = 2, average = 3, above average = 4, excellent = 5.

Table (6): Effect of different antioxidant treatments on necrosis and plantlets regeneration of both apical meristem and single node cuttings of coffee plants.

Treatment	Apical meristem		Single node cuttings	
	Necrosis (scores)*	No. of regenerated plantlets	Necrosis (scores)*	No. of regenerated Plantlets
Control (water)	5.00	1.00	5.00	1.00
Antioxidant solution	4.00	2.67	4.00	2.33
Activated charcoal	3.00	2.67	3.67	2.33
L-cysteine	4.00	2.33	4.00	2.00
Antioxidant sol. + Activated charcoal	2.67	2.67	3.00	2.67
Antioxidant sol. + L-cysteine	3.67	2.33	3.33	2.67
Activated charcoal + L-cysteine	2.67	3.00	3.00	2.67
Antioxidant sol. + activated charcoal + L-cysteine	2.33	3.33	2.67	3.00
L.S.D. at 0.05	0.81	0.73	0.91	0.77
L.S.D. at 0.05	1.11	1.01	1.26	1.07

* Scores : No necrosis = 1, below average =2, average = 3, above average = 4, high necrosis = 5.

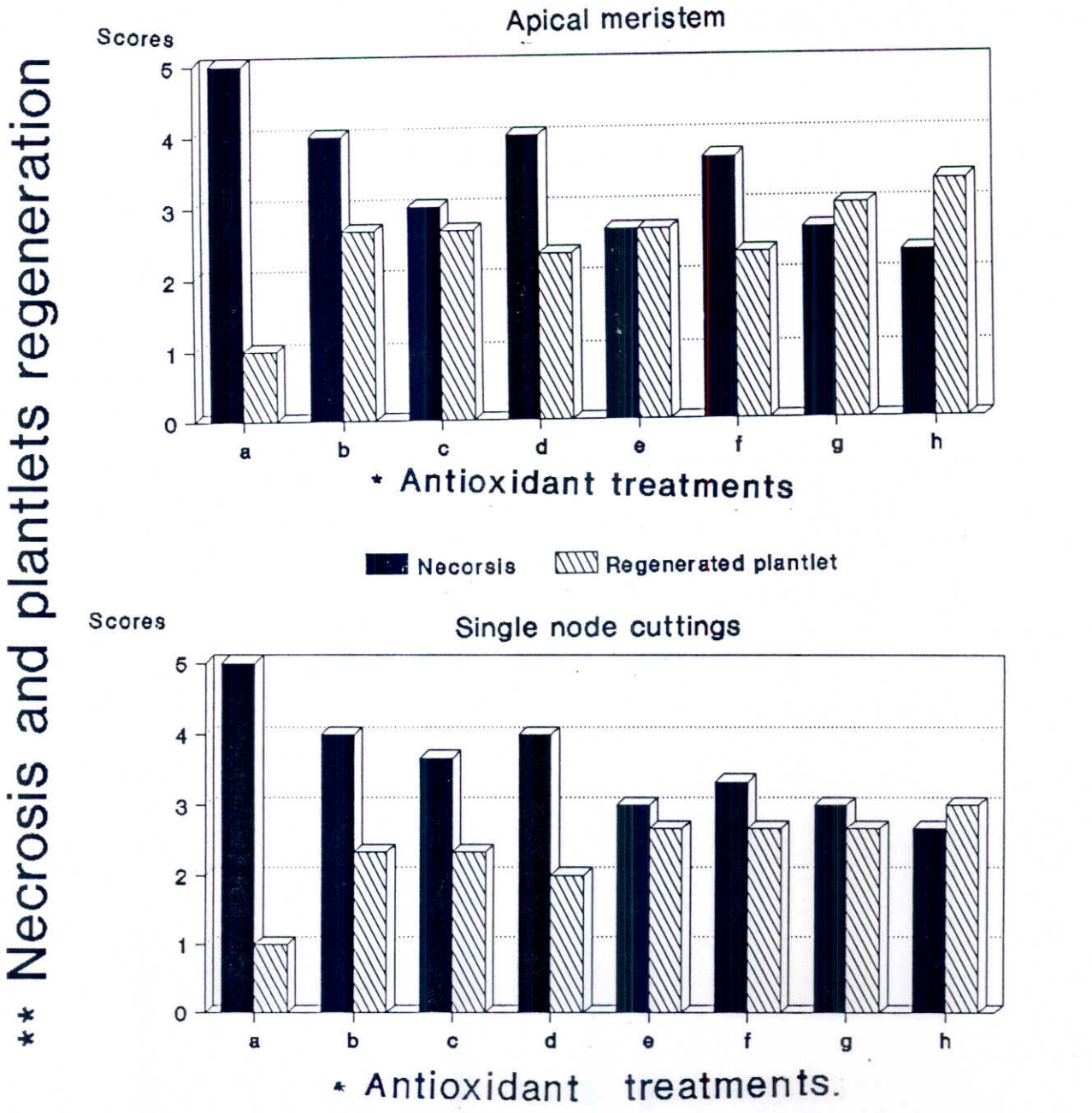


Fig.(.A.): Effect of different antioxidant treatments on necrosis and plantlets regeneration of both Apical meristem and single node cutting explants.

* Antioxidant treatments

a- Control.
b- Antioxidant solution.
c- Activated charcoal.
d- L-Cysteine.
e- Antioxidant sol. + activated charcoal.
f- Antioxidant sol. + L-cysteine.
g- Activated charcoal + L-cysteine.
h- Antioxidant sol. + activated charcoal + L-cysteine.

** Necrosis & plantlets regeneration scores : No necrosis & no plantlets regeneration = 1, below average = 2, average = 3, above average = 4, excellent = 5.

Meanwhile, number of regenerated plantlets behaved negatively in this sphere.

The above mentioned data show that charcoal treatment either alone or combined with any other antioxidant reduced necrosis. These results are in general agreement with the findings of **Anagnostakis (1974) and Bajaj and Nietsch (1975).** They reported that the addition of activated charcoal at optimal concentration in the cultured medium induced accumulation in inhibitory substances. However, data of activated charcoal effect in encouraging callus production or plantlets regeneration disagree with the findings of **Sondahl (1977).** He declared that activated charcoal has no detectable effect upon the growth or development of cultured coffee leaf explant.

IV.A.2- Somatic embryogenesis :

IV.A. 2.1- Callus production :

Table (7) explain the effect of different concentrations of 2,4-D combined with different concentrations of kinetin on callus production of coffee leaf disc explants. It is clear that increasing 2,4-D concentrations up to 1 and 2 mg/L resulted in highly significant increase in callus production from leaf disc explants as compared to 0 and 0.5 mg/L 2,4-D levels. However, increasing kinetin concentration from 0.01 mg/L to 1 mg/L induced a slight increase in callus production while both above mentioned concentrations of kinetin showed

Table (7): Effect of different concentrations of 2,4-D combined with different concentrations of kinetin on callus production of coffee leaf disc explants.

Kinetin (mg/L)	2,4-D (mg/L)				
	0.0	0.5	1.0	2.0	Average
0.0	1.0	1.3	2.7	3.0	2.00
0.01	1.0	1.7	3.7	3.7	2.53
0.1	1.0	1.7	3.7	4.0	2.60
1.0	1.3	2.7	4.7	4.7	3.35
Average	1.08	1.85	3.70	3.85	2.62
LSD at 5%	2,4-D 0.35		kinetin 0.35		2,4-D X kinetin 0.68
LSD at 1%	0.48		0.48		0.91

Table (8): Effect of different concentrations of 2,4-D combined with different concentrations of kinetin on callus production of coffee internode segment explants.

Kinetin (mg/L)	2,4-D (mg/L)				
	0.0	0.5	1.0	2.0	Average
0.0	1.0	1.3	2.30	3.0	1.90
0.01	1.0	1.7	3.00	3.3	2.25
0.1	1.0	2.0	3.67	3.7	2.59
1.0	1.0	2.7	3.67	4.3	2.92
Average	1.00	1.93	3.16	3.58	2.42
LSD at 5%	2,4-D 0.46		kinetin 0.46		2,4-D X kinetin 0.93
LSD at 1%	0.61		0.61		1.30

highly significant increase as compared to the control treatment. Meanwhile, 1 mg/L kinetin produced the highest significant increase in callus production in relation to all other used kinetin concentrations. On the other hand, 1 and 2 mg/L levels of 2,4-D combined with 1 mg/L kinetin treatments showed highly significant increase in callus production with respect to other treatments except 2 mg/L 2,4-D combined with 0.1 mg/L kinetin treatment.

Regarding internode explants, Table (8) reflect the effect of different concentrations of 2,4-D combined with different concentrations of kinetin on callus production of coffee internode segment explants. It is obvious that the addition of 2,4-D with 1 and 2 mg/L concentrations resulted in highly significant increment of callus production as compared to other 2,4-D concentrations. However, increasing kinetin concentration up to 0.1 and 1 mg/L induced highly significant increase in callus production in relation to the control treatment. In contrast, 2 mg/L 2,4-D combined with 1 mg/L kinetin treatment resulted in highly significant increase in callus production as compared to all other treatments of 0 and 0.5 mg/L 2,4-D combined with 0, 0.01, 0.1 and 1 mg/L levels of kinetin. Furthermore, Table (9) clearly show the effect of different IBA concentrations combined with different levels of kinetin on callus production of coffee leaf disc explant. It indicates that the addition of 2 or 4 mg/L IBA caused highly significant increase in callus production as compared to all other IBA concentrations. However, increasing kinetin

Table (9): Effect of different concentrations of IBA combined with different kinetin levels on callus production of coffee leaf disc explants.

Kinetin (mg/L)	IBA (mg/L)				
	0.0	1.0	2.0	4.0	Average
0.00	1.0	1.0	1.5	2.0	1.38
0.01	1.0	1.0	2.0	2.0	1.50
0.10	1.0	1.0	2.0	2.7	1.68
1.00	1.0	1.7	2.7	4.0	2.35
Average	1.00	1.18	2.05	2.68	1.73
LSD at 5%	IBA 0.23		kinetin 0.23		IBA X kinetin 0.47
LSD at 1%	0.31		0.31		0.63

Table (10): Effect of different concentrations of IBA combined with different concentrations of kinetin on callus production of coffee internode segments explants.

Kinetin (mg/L)	IBA (mg/L)				
	0.0	1.0	2.0	4.0	Average
0.00	1.0	1.0	1.3	2.0	1.33
0.01	1.0	1.0	1.7	2.0	1.43
0.10	1.0	1.0	1.7	2.3	1.50
1.00	1.0	1.3	2.3	2.3	1.73
Average	1.00	1.08	1.75	2.15	1.49
LSD at 5%	IBA 0.36		kinetin 0.36		IBA X kinetin 0.73
LSD at 1%	0.49		0.49		0.98

concentrations up to 1 mg/L resulted in highly significant increase in callus production with respect to all other kinetin concentrations. Besides, 4 mg/L IBA combined with 1 mg/L kinetin produced the highest significant increment in this respect as compared to all other treatments.

Considering the effect of different concentrations of IBA combined with different levels of kinetin on callus production of coffee internode segment explants as presented in Table (10),it is quite evident that increasing IBA concentrations up to 2 and 4 mg/L resulted in highly significant increase in callus production from internode segment explants as compared to lower levels (0 and 1 mg/L). However, a slight increase in callus production occurred by increasing kinetin concentrations up to 0.1 mg/L.In contrast, 1 mg/L kinetin induced significant increase in callus production with respect to other kinetin concentrations. In the meantime, 2 and 4 mg/L levels of 2,4-D with 1 mg/L kinetin treatments resulted in the highest significant increase as compared to the lower concentrations of IBA (0 and 0.5 mg/L) combined with different concentrations of kinetin.

Moreover,Table (11 & 12) refer to the effect of different concentrations of IAA combined with different levels of kinetin on callus production of leaf disc and internode segment explants. It is clear that 1 and 2 mg/L IAA induced a slight increase in callus production in relation to the control when either leaf discs or internode segment were used. However,

Table (11): Effect of different concentrations of IAA combined with different kinetin levels on callus production of coffee leaf disc explants.

Kinetin (mg/L)	IAA (mg/L)				
	0.0	1.0	2.0	4.0	Average
0.00	1.0	1.0	1.0	2.0	1.25
0.01	1.0	1.0	1.0	2.0	1.25
0.10	1.0	1.0	1.7	2.3	1.50
1.00	1.0	1.7	2.0	3.3	2.00
Average	1.00	1.2	1.4	2.4	1.50
	IAA		kinetin	IAA X kinetin	
LSD at 5%	0.23		0.23	0.46	
LSD at 1%	0.31		0.31	0.61	

Table (12): Effect of different concentrations of IAA combined with different concentrations of kinetin on callus production of coffee internode segment explants.

Kinetin (mg/L)	IAA (mg/L)				
	0.0	1.0	2.0	4.0	Average
0.00	1.0	1.0	1.0	1.3	1.08
0.01	1.0	1.0	1.3	2.0	1.33
0.10	1.0	1.0	1.3	2.0	1.33
1.00	1.0	1.7	2.0	2.8	1.85
Average	1.00	1.18	1.40	2.00	1.39
	IAA		kinetin	IAA X kinetin	
LSD at 5%	0.27		0.27	0.53	
LSD at 1%	0.36		0.36	0.71	

4 mg/L IAA resulted in highly significant increment in callus production for both leaf disc and internode segment explants as compared to other IAA concentrations. Meanwhile, 1 mg/L kinetin enhanced callus production with high significancy as compared to other kinetin concentrations in both explants. Furthermore, 4 mg/L IAA combined with 1 mg/L kinetin treatment resulted in highly significant increase in callus production in relation to lower IAA concentrations (0 and 1 mg/L) combined with all kinetin concentrations treatments.

Considering the effect of overall average of auxins (2,4-D, IBA and IAA) on both leaf discs and internode segment explants, Table (13) indicate that 2,4-D was superior in callus formation followed by IBA and IAA in a descending order. Also, leaf disc explants surpassed internode segments in this respect.

Briefly, 2,4-D was the most promising auxin and the highest concentrations of both auxins and kinetin were the most effective treatments in callus production. Also, leaf disc explants were most superior in callus production than internode segments. These results are in partial agreement with the findings of **Sharp et al. (1973)**. They stated that callus production was best on the medium provided with various levels of auxins and kinetin with high levels of 2,4-D. However, results of the best explant coincided with the finding of **Staritsky (1970)** who reported that internode segments of orthotropic shoot of coffee are most suitable for both callus initiation and production.

Table (13): Effect of different auxins (2,4-D IBA, and IAA) combined with kinetin on callus production of coffee leaf disc and internode segment explant.

Auxin \ Explant	Leaf discs (scores)*	Internode segments (scores)*	Average
2,4-D	2.62	2.42	2.52
IBA	1.73	1.49	1.61
IAA	1.50	1.39	1.45
Average	1.95	1.77	-

* Scores : No callus production = 1 below average = 2, averages 3, above average = 4, excellent callus production = 5.

Table (14): Effect of different organic additives (casein hydrolysate as well as malt and yeast extracts) on induction of somatic embryogenesis of coffee (C. arabica) plants.

Organic additives (mg/L)	Kinetin concentrations (mg/L)			
	0.0	0.1	0.5	Average
Control	1	3	4	2.7
200 mg/L casein hydrolysate	3	3	5	3.7
300 mg/L malt extract	2	2	4	2.7
300 mg/L yeast extract	1	3	4	2.7
Average	1.8	2.8	4.3	
LSD at 5%	Additives 0.64	Kinetin 1.10	Additives X kinetin 2.43	
1%	0.98	1.69	3.68	

Moreover, Fig. (5) illustrate the initiation and development of callus produced from leaf disc explants. It is clear that callus was initiated at the cut edge of the leaf disc and increased in volume toward the center of the explant to dominate the whole explant. Also, the quantity of callus produced from leaf disc explants seemed to be abundant. However, Fig. (6) explains the steps of callus formation from internode segment explants. Callus formation starts at the cut edge of the explant and continued to dominate the whole explant. On the other hand, Fig. (7) reflects the type of callus produced either friable or globular. Globular callus is the only type of callus that is able to develop and form lobes where the somatic embryogenesis is formed.

IV.A.2.2- Induction and development of somatic embryogenesis:

Table (14) involves the effect of different organic additives (casein hydrolysate as well as malt and yeast extracts) on induction of somatic embryogenesis of coffee plants. It is quite clear that the addition of 200 mg/L casein hydrolysate enhanced the highest significant induction of somatic embryogenesis as compared to the control or other organic additives

However, other organic additives failed completely to show any signs of encouraging somatic embryogenesis in relation to the control. In addition, increasing kinetin concentration to 0.5 mg/L induced significant increase in somatic embryogenesis as compared to 0.1 mg/L kinetin while a highly significant increase in somatic embryos was noticed in

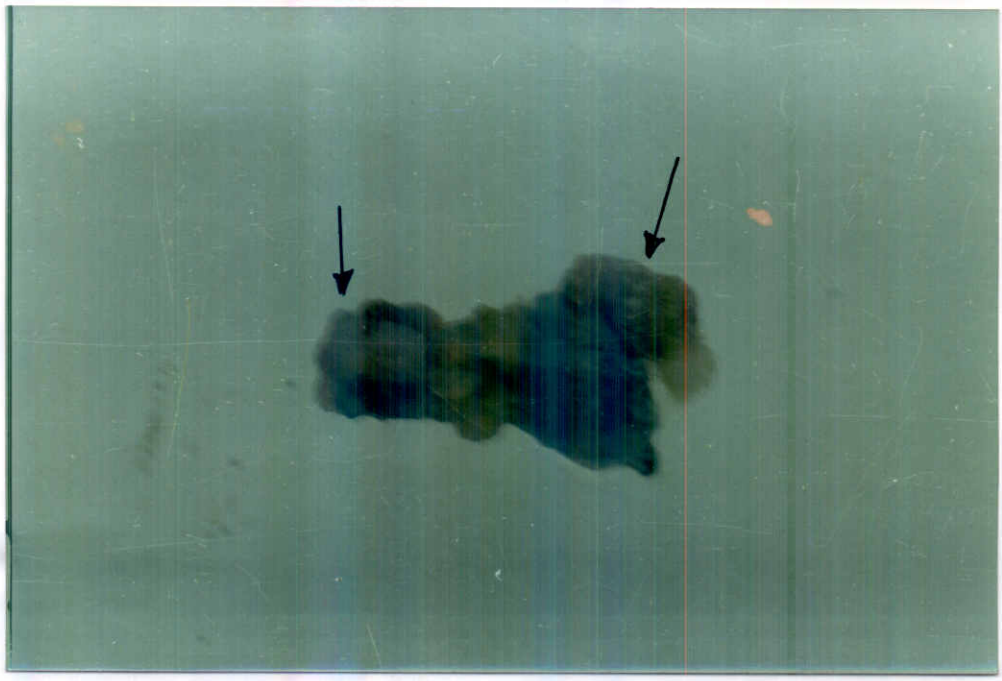
Fig. (5):Callus production steps of coffee leaf disc explants

A
Leaf disc explant at planting time.

B
Leaf disc after 7 days from planting. Callus production started at the edge of the leaf disc.

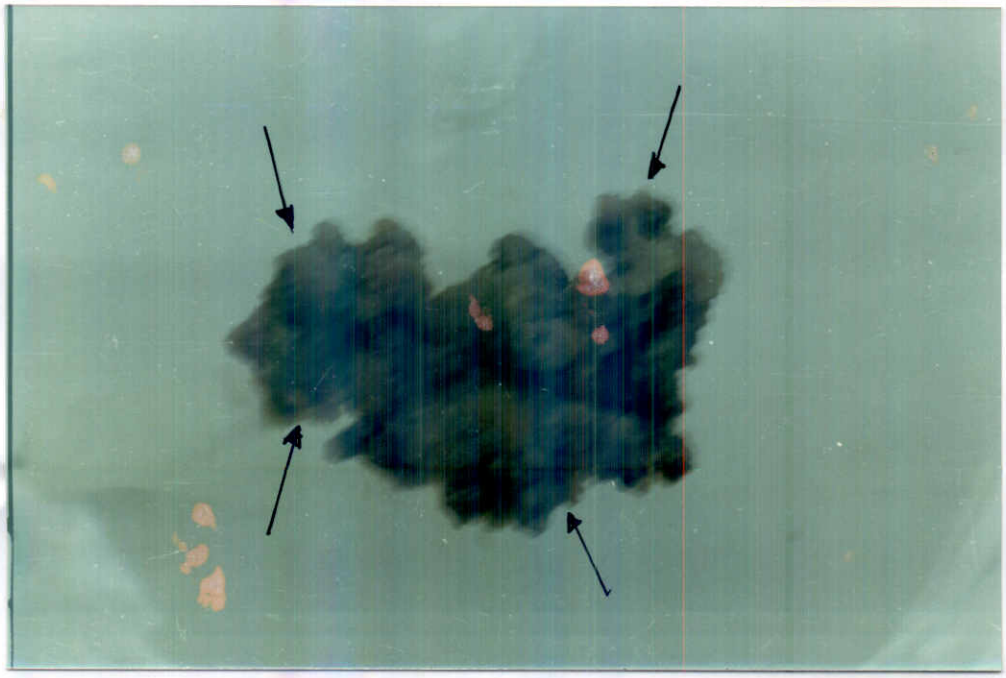
C
The volume of callus increased after 2 weeks from culturing time of the leaf disc.

Fig. (5): Continued.



D

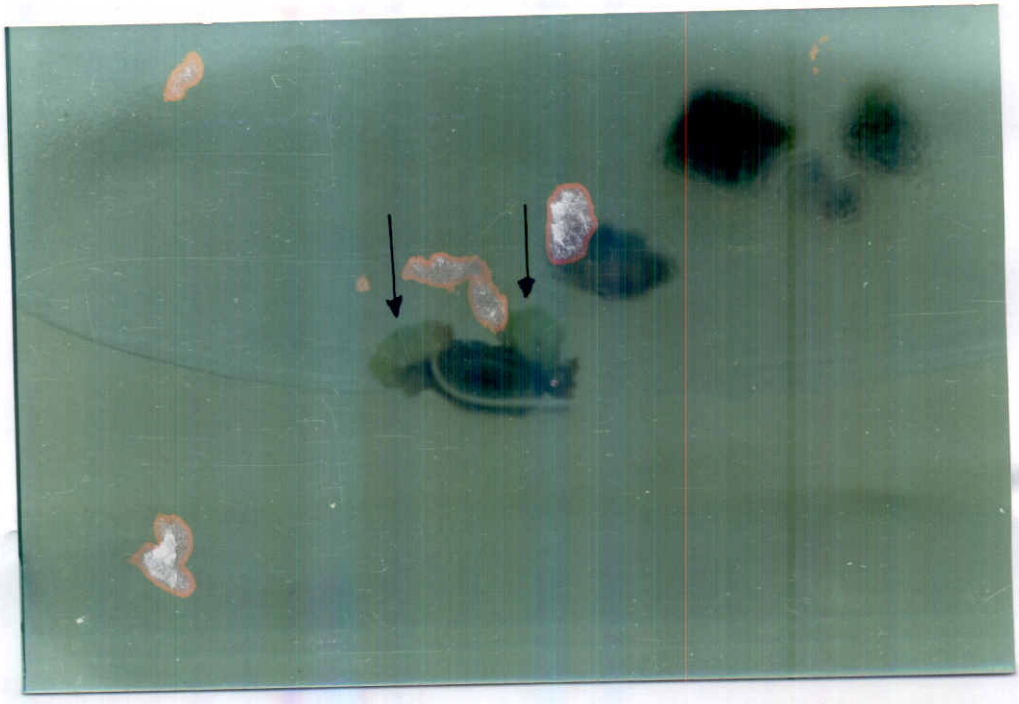
Callus formation dominate most of the leaf disc explant after 4 weeks from culturing time.



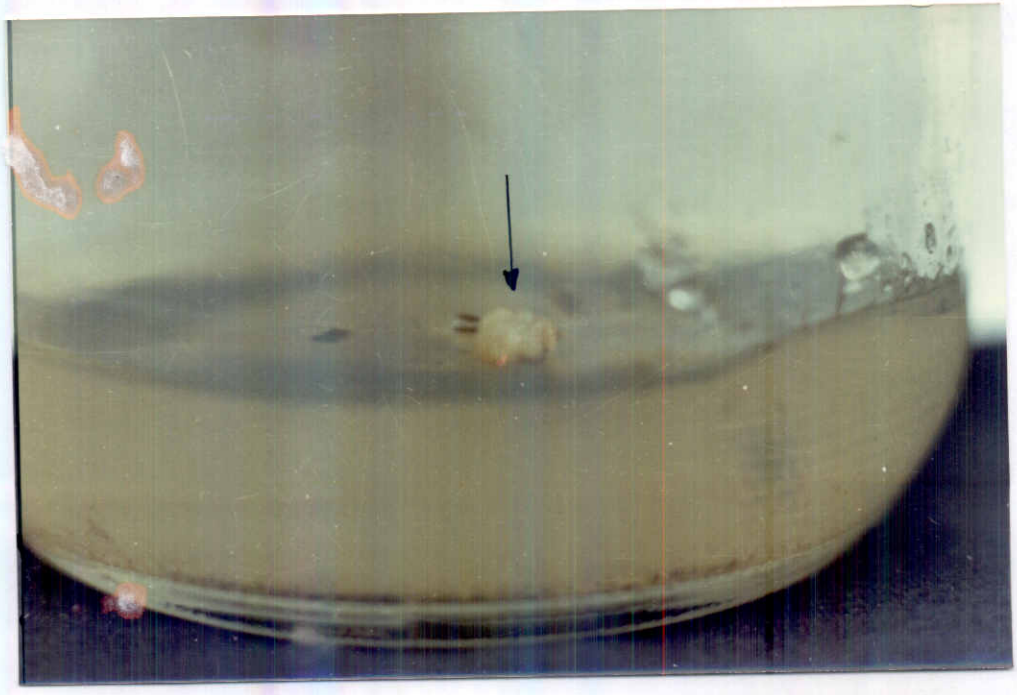
E

All leaf disc explant developed completely into callus after 6 weeks from culturing time.

Fig. (6): Callus production steps of coffee internode segments explants.



A. Starting of callus formation at the edge of the internode explant.



B. Internode explant converted completely with callus of white colour.

Fig. (7): Callus type and development.

A. Friable callus which failed to form somatic embryo.

B. Globular callus which formed somatic embryos.

relation to the medium free of kinetin. Also, 200 mg/L casein hydrolysate treatment combined with 0.5 mg/L kinetin induced significant increase in somatic embryogenesis as compared to other additives at 0.0 kinetin discarding casein hydrolysate treatment.

IV.A.2.3- Regeneration :

Table (15) & Fig. (8), show that the combination of 0.1 mg/L GA3+ 0.1 mg/L kinetin in addition to 1 mg/L NAA enhanced the largest number of regenerated plantlets as compared with the other concentrations of NAA. However, increasing the concentration of NAA to 2 mg/L reduced the number of regenerated plantlets.

Besides, Table (16) show that the addition of 20 or 40 ml/L of coconut milk to the medium supplemented with GA3 +0.1 mg/L kinetin + 1 mg/L NAA increased the number of regenerated plantlets as compared with other treatments.

IV.A.3- Histological studies :

Table (17) & Fig. (9) explain the induction and development of somatic embryos and colouration in relation to somatic embryogenesis developmental stages at different callus ages. They indicate that there was a direct relationship between callus colour, age, somatic embryo stage, average number of lobes per callus and average number of somatic embryo per lobe.Moreover, callus colouration, globular embryo and lobes started after 3-4 weeks from culturing time.Somatic embryos developed into heart shape after 6 weeks and finally

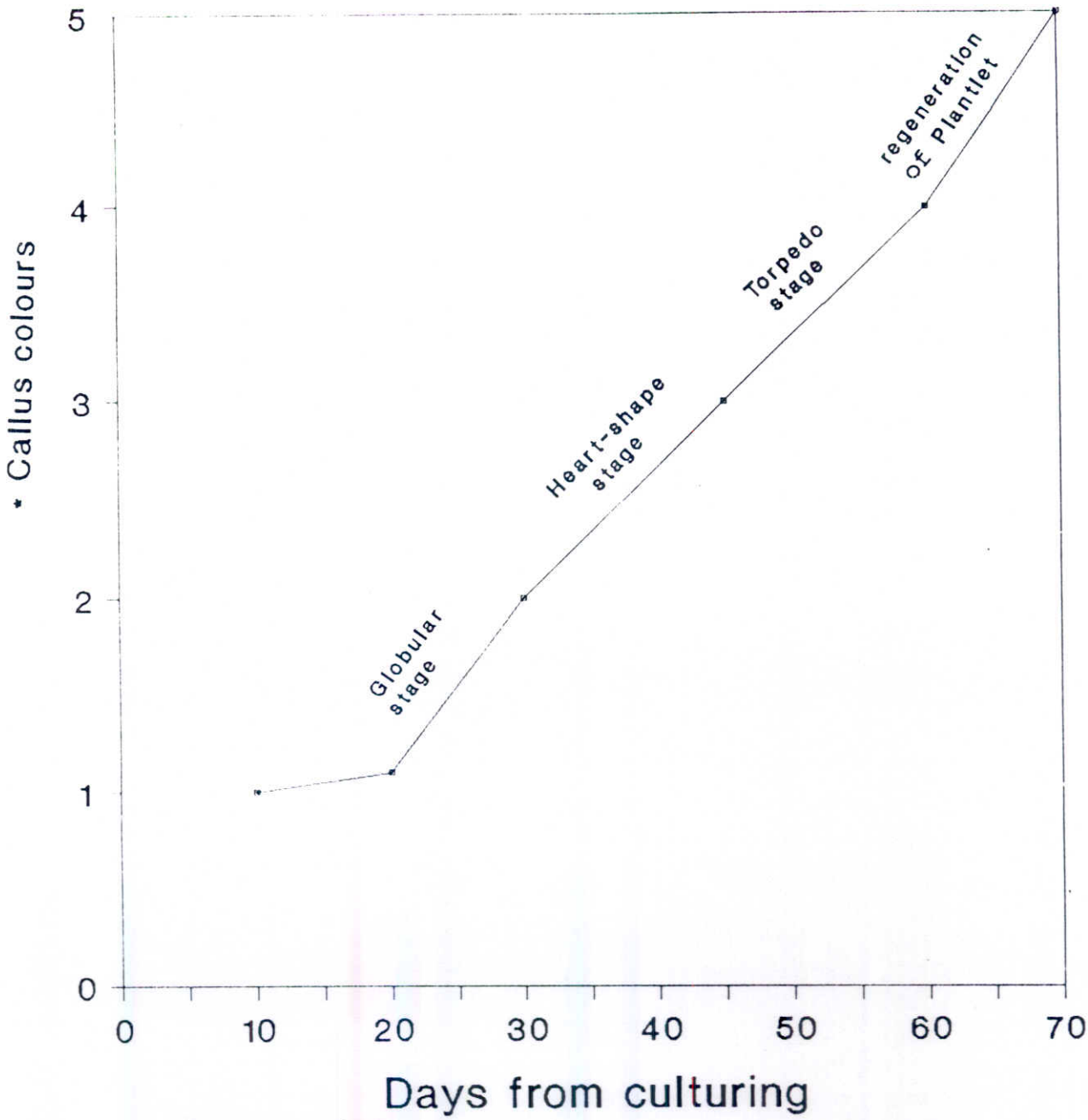
Table (15): Effect of different concentrations of NAA on plantlets regeneration of coffe/plants.

NAA (mg/L)	0	0.5	1.0	2.0
* No. of reg. plantlets	0	1	3	1

Table (16): Effect of different concentrations of coconut milk on plantlets regeneration of coffee plants.

Coconut milk (ml/L)	0	10	20	40	80
* No. of reg. plantlets	1	1	3	3	0

* Number of regenerated plantlets from full developed somatic embryos.



* Callus colours

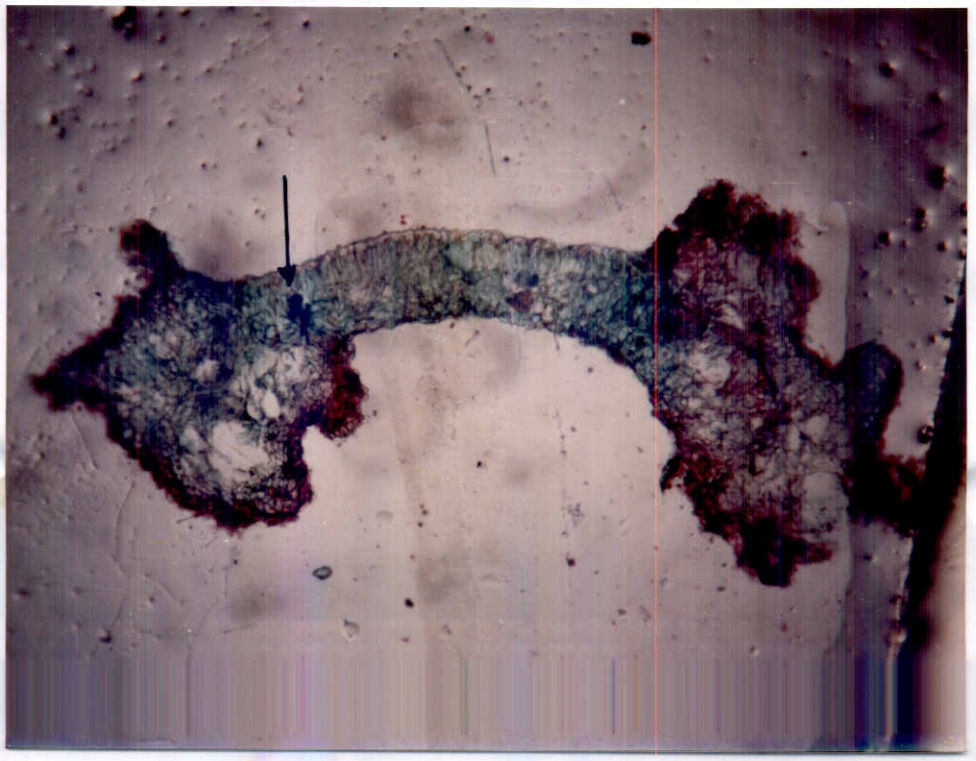
1- White
2- White-green
3- Green-brown
4- Brown
5- Dark brown

Fig.(3): Callus development into somatic embryos and colouration in relation to somatic embryogenesis stages of coffee plants.

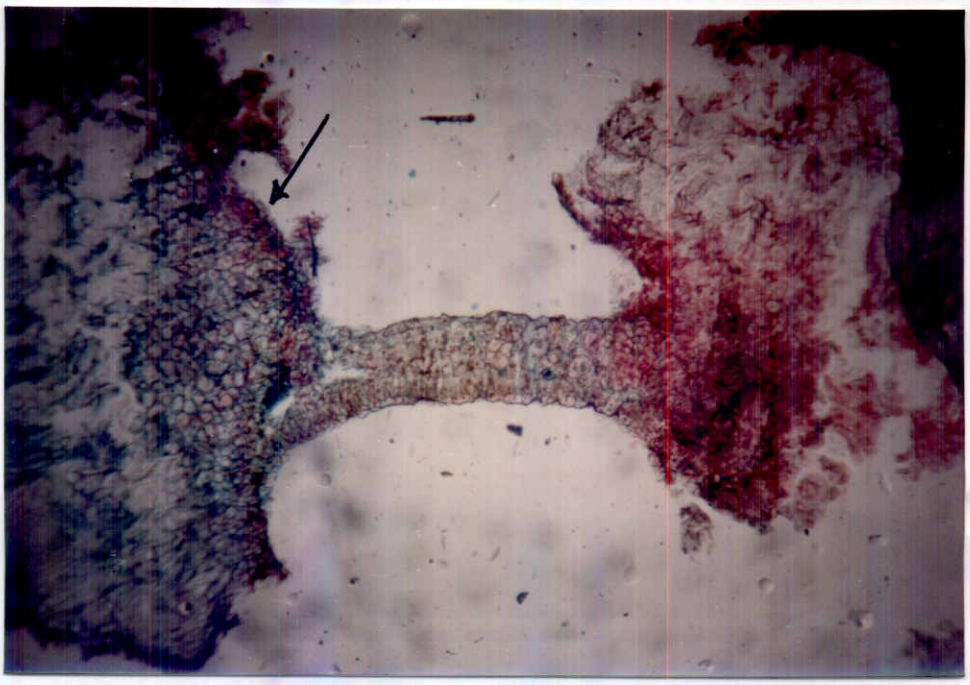
into turpedo shape after 8-10 weeks from culturing time. Also, number of lobes multiplied every 2 weeks up to 67 days then increased slightly after that However, number of somatic embryos per lobe are more or less stable. The best number of somatic embryos are obtained from 8-10 callus age with dark brown colour.

Regarding Fig. (10) it is clear from diagram (A) that

Fig. (10): Steps of formation and development of callus into somatic embryos.

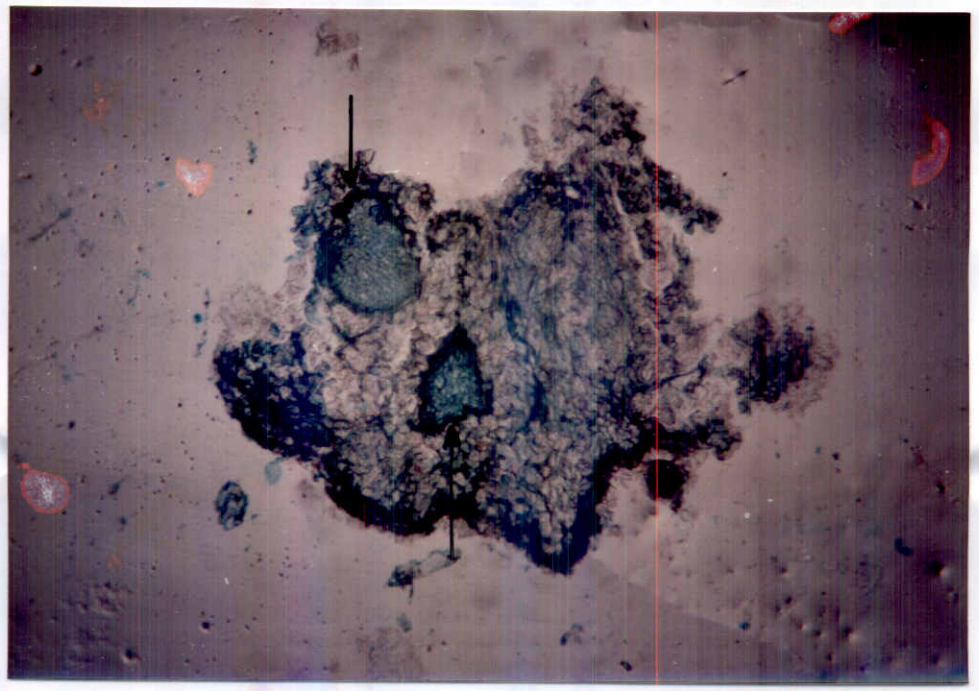


A. Number of cells start to form somatic embryos after 2 weeks from culturing time.

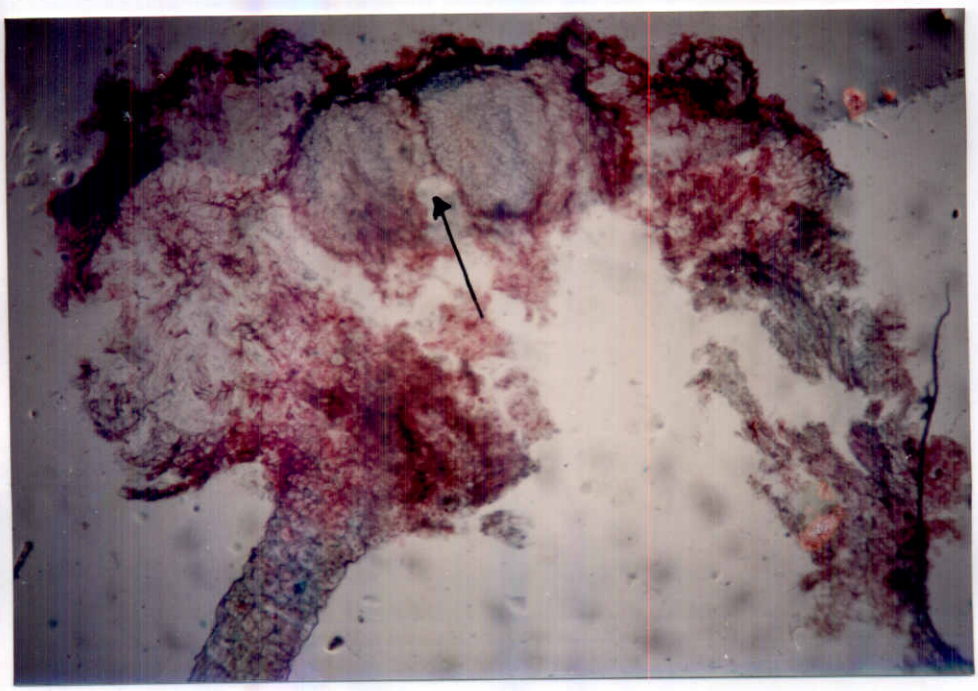


B. The cells aggregated and large number of cells are formed developing a globular embryo and this took place after 4 weeks from culturing time.

Fig. (10): Continued.

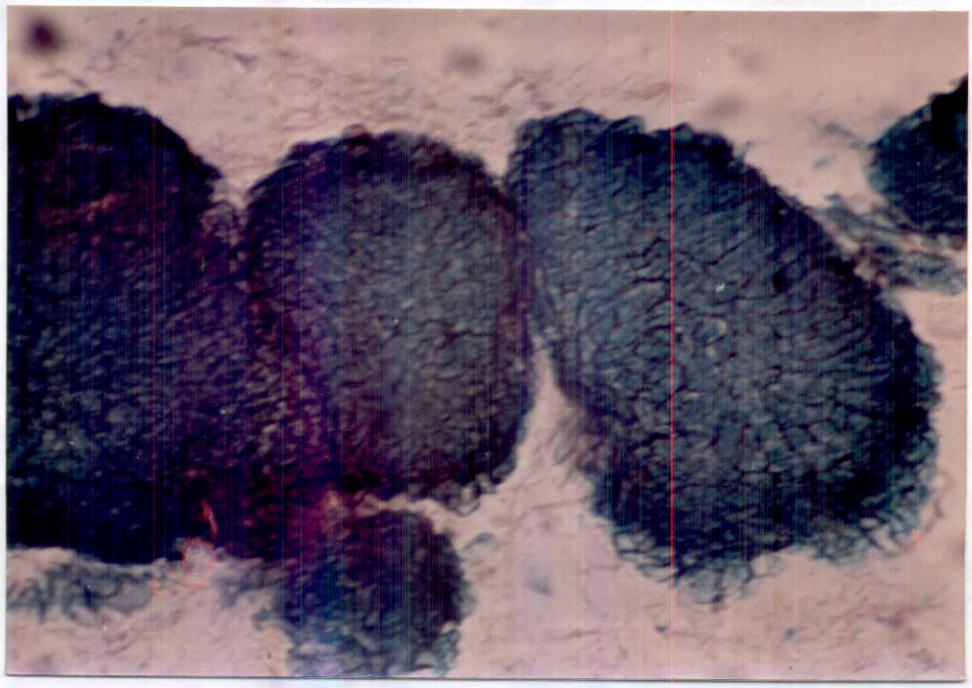


C. Two embryoid stages, the upper one is globular while the lower one is at heart shaped embryo.



D. Proliferative globular embryo as the number of somatic embryos increased.

Fig. (10): Continued.



E. Well developed somatic embryos.

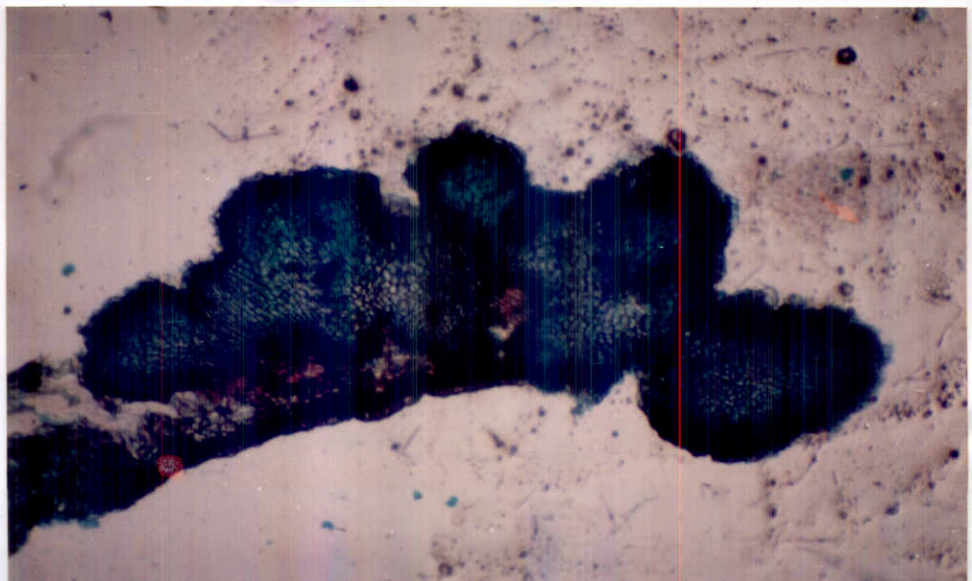
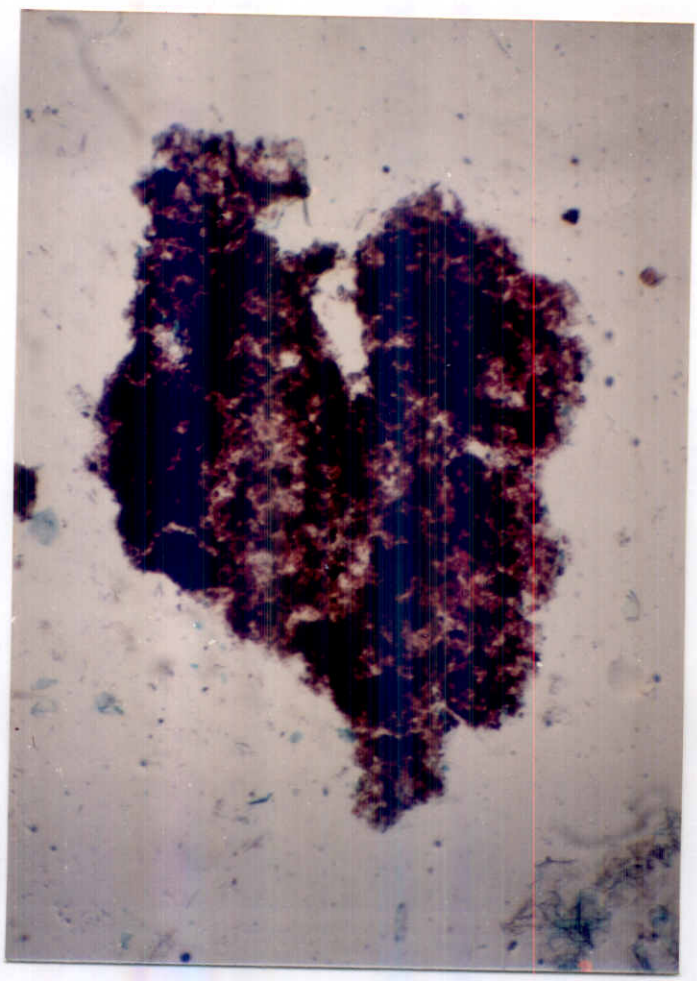


Fig. (11): The final shape of somatic embryo (Turpedo-shape).



However, **Sondahl et al. (1979a)** stated that globular embryo developed into turpedo shaped somatic embryo.

IV.A.4- Microcuttings propagation :

Table (18) involved the effect of different concentrations of IBA combined with different levels of 6-benzylaminopurine on plantlets regeneration of apical meristem of coffee plants. It is clear that the average number of regenerated plantlets from apical meristem was significantly increased with increasing the concentration of IBA up to 2 mg/L. However, increasing BAP concentration in the medium up to 1 mg/L resulted in a highly significant increase of regenerated plantlets as compared with the control and other BAP concentrations. In the same time, 2 mg/L IBA combined with 0.1,0.5 and 1 mg/L levels of BAP as well as 1 mg/L IBA combined with 1 mg/L BAP treatments resulted in a significant increase of regenerated plantlets with respect to other treatments.

Furthermore, Table (19) included the effect of IBA combined with different levels of 6-benzylaminopurine on plantlets regeneration of apical meristem of coffee plants.It is found that increasing IBA up to 1 mg/L resulted in highly significant increase in regenerated plantlets as compared to the control and 0.5 mg/L IBA treatments. However, increasing IBA concentrations up to 2 mg/L caused a slight decline in regenerated plantlets of single node cuttings. Meanwhile, 0.5 and 1 mg/L BAP concentrations caused a highly significant increase in plantlets regeneration with respect to the

Table (18): Effect of different concentrations of IBA combined with different levels of 6-benzylaminopurine on plantlets regeneration from apical meristem of coffee plants.

IBA (mg/L)	6-benzylaminopurine (mg/L)				
	0.0	0.1	0.5	1.0	Average
0.0	1.0	1.3	1.6	2.0	1.48
0.5	1.7	2.0	2.7	3.0	2.35
1.0	3.0	3.0	3.3	4.0	3.33
2.0	2.7	4.0	4.3	5.0	4.00
Average	2.10	2.58	2.98	3.50	2.79
LSD at 5%	IBA 0.30		BAP 0.30	IBA X BAP 0.61	
LSD at 1%	0.41		0.41	0.82	

Table (19): Effect of IBA concentrations combined with different levels of 6-benzylaminopurine on plantlets regeneration from single node cutting of coffee plants.

IBA (mg/L)	6-benzylaminopurine (mg/L)				
	0.0	0.1	0.5	1.0	Average
0.0	1.0	1.0	1.3	2.7	1.50
0.5	1.0	1.3	2.7	3.7	2.18
1.0	3.0	3.7	3.7	4.3	3.68
2.0	2.7	3.0	3.7	4.3	3.43
Average	1.93	2.25	2.85	3.75	2.70
LSD at 5%	IBA 0.34		BAP 0.34	IBA X BAP 0.67	
LSD at 1%	0.45		0.45	0.91	

control and 0.1 mg/L of BAP treatments. Anyhow, 1 and 2 mg/L IBA combined with 1 mg/L BAP induced highly significant increase in regenerated plantlets as compared to different concentrations of IBA combined with 0 and 0.1 mg/L BAP except 1 mg/L IBA combined with 0.1 mg/L BAP treatment.

Regarding the effect of different concentrations of IAA combined with different levels of 6-benzylaminopurine on plantlets rgeneration from apical meristem of coffee plants were tabulated in table (20). It is obvious that continuous increase in IAA concentrations from 0 up to 2 mg/L increased significantly the number of regenerated plantlets. Also, increasing 6-benzylaminopurine level up to 1 mg/L resulted in highly significant increase in number of regenerated plantlets as compared with other levels of BAP. Meanwhile, 2 mg/L IAA combined with all concentrations of 6-benzylaminopurine and 1 mg/L IAA together with 0.5 & 1 mg/L 6-benzylaminopurine induced highly significant increase in regenerated plantlets as compared to all other treatments except 1 mg/L IAA combined with 0.1 mg/L 6-benzylaminopurine treatment.

Considering the effect of different IAA concentrations combined with different 6-benzylaminopurine levels on single node cuttings as shown in table (21), it is clear that 1 and 2 mg/L IAA caused the highest significant increase in regenerated plantlets as compared to 0.0 and 0.5 mg/L levels of IAA. Also, increasing 6-benzylaminopurine concentrations up to 1.0 mg/L resulted in highly significant increase in

Table (20): Effect of different concentrations of IAA combined with different concentrations of 6-benzylaminopurine on plantlets regeneration from apical meristem of coffee plants.

IAA (mg/L)	6-benzylaminopurine (mg/L)				
	0.0	0.1	0.5	1.0	Average
0.0	1.0	1.0	1.0	1.7	1.2
0.5	1.0	1.3	3.0	2.7	2.0
1.0	3.0	3.3	4.0	4.7	3.8
2.0	4.3	4.3	4.0	4.7	4.3
Average	2.3	2.5	3.0	3.5	2.8
LSD at 5%	IAA 0.35		BAP 0.35	IAA X BAP 0.69	
LSD at 1%	0.47		0.47	0.93	

Table (21): Effect of different concentrations of IAA combined with different concentrations of 6-benzylaminopurine on plantlets regenerated from single node cuttings of coffee plants.

IAA (mg/L)	6-benzylaminopurine (mg/L)				
	0.0	0.1	0.5	1.0	Average
0.0	1.0	1.0	1.0	1.3	1.1
0.5	2.0	2.3	2.7	3.7	2.7
1.0	4.0	4.0	4.3	5.0	4.3
2.0	4.0	4.0	4.7	4.7	4.4
Average	2.8	2.8	3.2	3.7	3.1
LSD at 5%	IAA 0.27		BAP 0.27	IAA X BAP 0.55	
LSD at 1%	0.37		0.37	0.74	

Table (22): Effect of auxins (IBA & IAA) combined with 6-benzylaminopurine (BAP) on necrosis and plantlets regeneration of coffee plants.

Explant / Auxin	Apical meristem (scores)*	Single node cuttings (scores)*	Average
IBA	2.8	2.7	2.8
IAA	2.8	3.1	3.0
Average	2.8	2.9	-

regenerated plantlets in relation to other 6-benzylaminopurine concentrations. Meanwhile, 1.0 and 2.0 mg/L levels of IAA combined with all 6-benzylaminopurine concentrations increased regenerated plantlets with high significancy as compared to all other treatments except 0.5 mg/L IAA combined with 1 mg/L 6-benzylaminopurine treatment. In addition, Table (22) indicates that IAA is the most effective auxin in inducing large number of regenerated plantlets.

Single node cuttings, on the other hand, surpassed apical meristem in regenerated plantlets. Also, BAP concentration is important for enhancing large plantlet regeneration. This result is in partial agreement with the findings of **Sondahl et al. (1981)**. They reported that number of developed coffee buds are controlled by the level of cytokinin. Higher cytokinin levels induced higher number of developed buds.

IV- Stage B

IV.B- Proliferation :

Table (23) involved the effect of 6-benzylaminopurine concentrations combined with different levels of IAA on proliferation of coffee plants.It is quite clear that proliferation of coffee plants responded significantly to increased BAP concentrations up to 8 mg/L. However,0.5 mg/L IAA increased proliferation with highly significant levels as compared to other IAA concentrations used. Meanwhile, increasing concentrations of IAA up to 1 and 2 mg/L resulted in highly significant decrease in proliferation as compared to 0.5 mg/L IAA. Furthermore, 6 and 8 mg/L BAP combined with 0.5 mg/L IAA

Table (23): Effect of 6-benzylaminopurine concentrations combined with different levels of indole-3-acetic acid on proliferation of coffee plants.

IAA (mg/L)	6-benzylaminopurine (mg/L)					
	0.0	2.0	4.0	6.0	8.0	Average
0.0	1.0	2.0	3.3	3.7	4.3	2.9
0.5	1.0	2.7	4.0	4.7	5.0	3.5
1.0	1.7	2.0	3.3	3.3	4.3	2.9
2.0	1.0	1.7	1.7	2.0	3.0	1.9
Average	1.2	2.1	3.1	3.4	4.2	2.8
LSD at 5%		IAA 0.22		BAP 0.29		IAA X BAP 0.50
LSD at 1%		0.30		0.39		0.68

Table (24): Effect of different concentrations of kinetin combined with different concentrations of indole-3-acetic acid on proliferation of coffee plants.

IAA (mg/L)	Kinetin (mg/L)					
	0.0	2.0	4.0	6.0	8.0	Average
0.0	1.0	1.0	2.0	2.7	4.3	2.2
0.5	1.0	1.0	2.7	3.3	4.7	2.5
1.0	1.0	1.3	2.3	2.7	4.0	2.3
2.0	1.7	1.0	1.7	2.0	2.7	1.8
Average	1.2	1.1	2.2	2.7	3.9	2.0
LSD at 5%		IAA 0.25		kinetin 0.32		IAA X kinetin 0.55
LSD at 1%		0.34		0.43		0.75

induced highly significant increase in proliferation as compared with other treatments of 0,2 and 4 mg/L 6-benzylaminopurine combined with different levels of IAA.

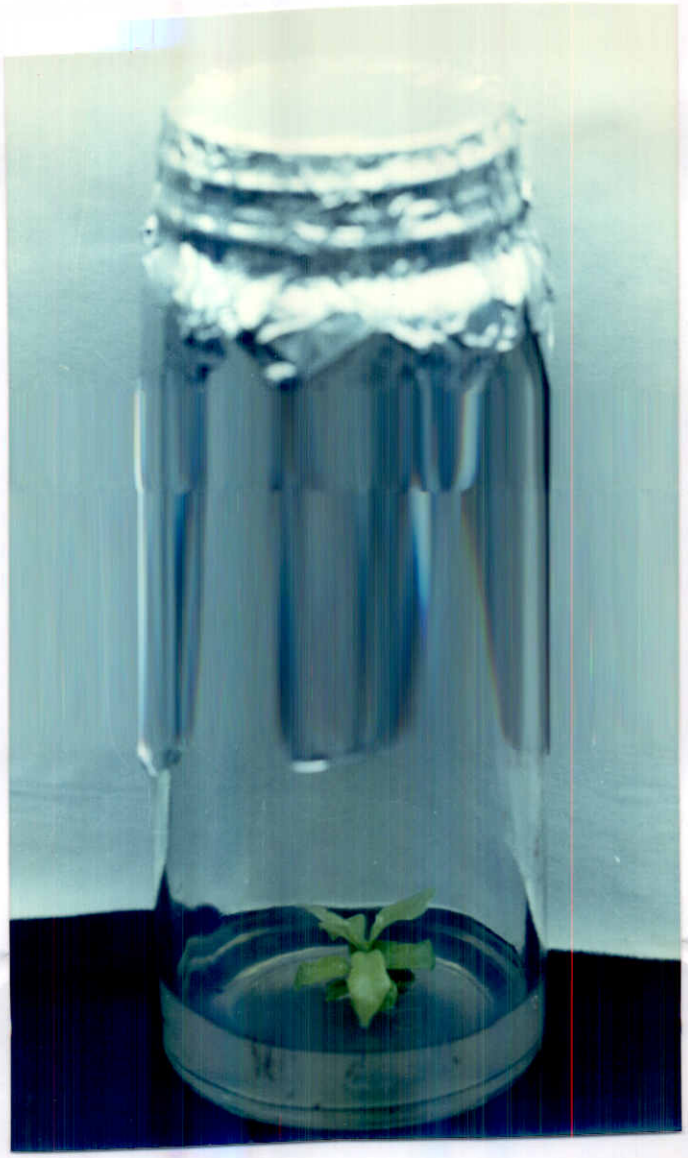
Furthermore, table (24) show the effect of different concentrations of kinetin combined with different levels of IAA on proliferation of coffee plants. It is clear that increasing concentrations of kinetin from 2 to 8 mg/L resulted in highly significant increase in proliferation. However, increasing IAA concentrations to 0.5 or 1 mg/L slightly increased proliferation while increasing the concentration up to 2 mg/L reduced significantly proliferation as compared with other treatments. In addition, 8 mg/L kinetin combined with 0,0.5 and 1 mg/L IAA levels resulted in a significant increase in proliferation with respect to all other treatments.

At all events, the above results showed that BAP with higher concentrations combined with 0.5 mg/L IAA produced the best results of proliferation. These results are in partial agreement with the findings of **Zok (1986)** who found that multiplication rate was increased 4-5 times in C. arabica CV. Bourbon when MS medium was used and supplemented with either 10 BA or 15 mg/L kinetin.

Moreover, Fig. (12) illustrate that plantlets regeneration from the apical meristem (diagram a). However, diagram (b) shows plantlets resulted from apical meristem and single node cutting.

On the other hand, Fig. (13) represent complete plants resulted from apical meristem and single node cuttings.

Fig. (12): Plantlets regeneration from apical meristem and single node cuttings.



a- Regenerated plantlets from apical meristem.

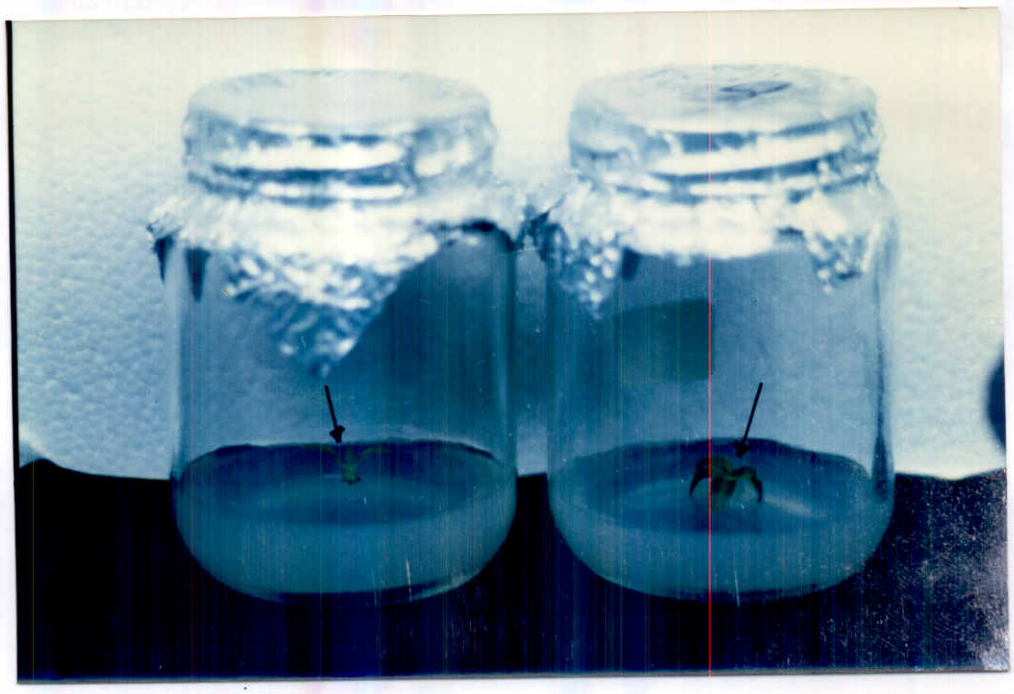


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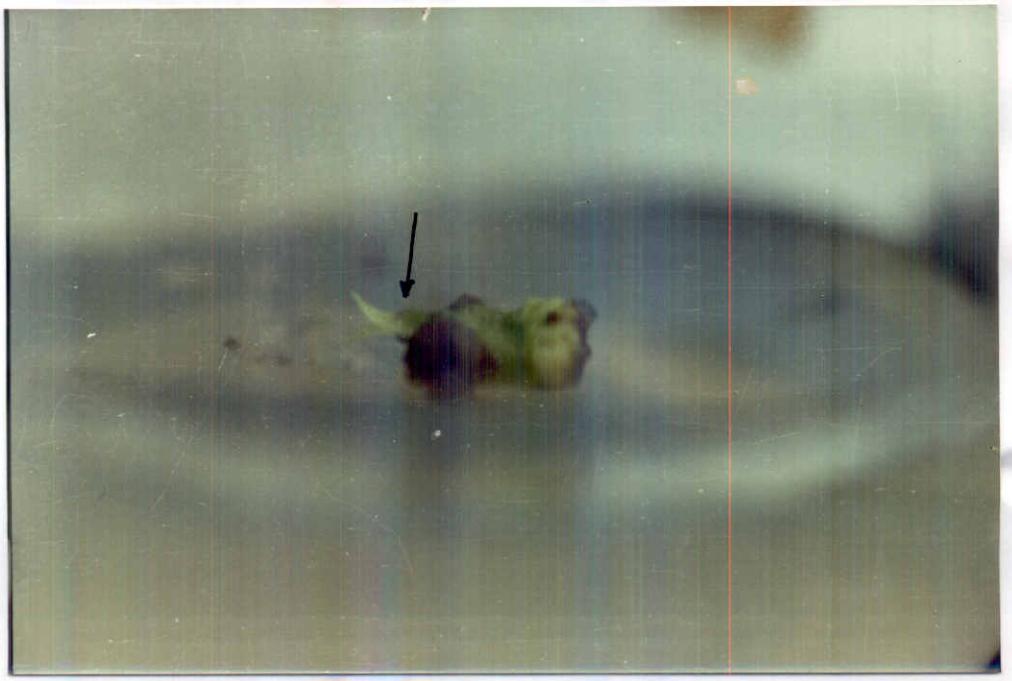
Fig. (13): Micropropagated coffee plants.



a- Regenerated plantlets from apical meristem.

b- Regenerated plantlets from single node cutting.