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Effect of Media Supplements on *in Vitro* Response of Sesame (*Sesamum indicum* L) Genotypes

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Abstract: The *invitro* response of sesame varieties CO 1, VRI 1, TMV 3 and AHT 123 was studied on ten media components for callus induction, multiplication, shooting and rooting. CO1 recorded the highest callusing frequency at, 3mg per litre of 2, 4 D with 100 ml of coconut milk followed by 3mg per litre of 2,4 D with 0.1 mg per litre casein hydrosylate. CO 1 and TMV 3 recorded significant shoot multiplication ratio and length in MS media supplemented with 1mg per litre of IAA, 1.0mg per litre of BA and 1.25mg per litre of kinetin and 1mg per litre of IAA, 1.5mg per litre of BA and 1.25mg per litre of kinetin respectively. CO 1 produced profuse rooting in full strength MS media with hormonal concentration of 1.0g/l of IBA and 1.25 mg/l of IAA followed by VRI 1. Least response to rooting was showed by TMV 3 at hormonal concentration of 0.5g/l of IBA and 0.5mg/l of IAA.

Key words: *Sesamum indicum*, callusing, shooting and rooting *in vitro*

INTRODUCTION

Sesame seeds constitute rich quantities of nutrients such as oil, proteins, sugars, fibre, minerals and vitamins. Sesame oil has been evaluated as one of the familiar health foods ancient times. Tissue culture methods involving a callus phase or regeneration via somatic embryogenesis are known to produce stable variants [1]. Successful induction of somatic embryos directly from the surface of the zygotic embryos of sesame in culture was also established. Tissue culture methods can also be used to facilitate successful wide crosses using embryo culture techniques. Although conventional hybrid crosses between cultivated sesame and its wild relatives have been attempted[2], hybrids were difficult to produce. In preliminary studies, zygotic embryos were cultured at various developmental stages and plants were regenerated from embryos obtained 15 days after pollination. Similar methods could be used to regenerate plants from embryos generated from wide hybrid crosses. Regeneration can be accomplished through *in vitro* culture and large number of regenerants could be produced in a short span of time. The present investigation was carried out to determine the influence of various growth regulators upon *in vitro* culture.

MATERIALS AND METHODS

The seeds of four sesame genotypes *viz.*, CO 1, VRI 1, TMV 3 and AHT 123 were used as explants for induction of callus and shoot. The seeds were subjected to series of sterilization that includes; initially the seeds were washed with 0.1 per cent mercuric chloride for two minutes and then rinsed thrice with sterile double distilled water. The embryos (1.0 to 1.5 mm) were aseptically excised from the seeds and placed on semi-solid culture medium. The cultures were incubated at 28°c (± 2°c) with a 16/8-hour photoperiod from cool-white fluorescent lights with an intensity of 2,000 lux. For each treatment, three replication with twenty tubes per replication was maintained. The callus induction in different genotypes was assessed using various growth regulating factors along with basal media usually Murashige and Skoog[3]. The cultures were incubated at 28°c. The cultured embryos were scored for the appearance of embryogenic callus formation and were expressed in percentage. The callus derived from various media compositions were used for shooting. The media for callus induction was supplemented with various growth regulator *viz.*, two levels of IAA, BA and kinetin. The combinations of the same were also maintained. The study was designed to investigate the effect of media supplements upon *in vitro* culture. Plant regeneration was carried out as described

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Table 1: Percentage of callus induction in sesame genotypes.

Treatment	Genotypes			
	CO 1	VRI 1	TMV 3	AHT 123
2,4-D				
2.0mg/l	34.81	38.36	39.42	36.12
2.5mg/l	38.31	43.24	45.18	37.04
3.0mg/l	43.43	51.20	54.18**	41.39
3.5mg/l	48.18	46.80	39.91	39.81
2,4-D + CH				
2.0mg/l+0.1g/l	43.14	47.65	49.18	42.01
2.5mg/l+0.1g/l	62.84	61.94	45.06	43.81
3.0mg/l+0.1g/l	60.18**	59.86**	54.18**	51.61
3.5mg/l+0.1g/l	59.08**	58.18**	58.64**	57.54**
2,4-D+Coconut Milk				
2.0mg/l+0.1g/l	43.24	44.34	47.84	41.85
2.5mg/l+0.1g/l	56.18**	54.10	52.84**	54.60**
3.0mg/l+0.1g/l	68.10**	64.20**	53.86**	52.84**
3.5mg/l+0.1g/l	48.28	58.20**	62.14**	46.84
SEd 1.22				
CD(0.05)	2.45			
CD(0.01)	3.26			

** Significant at 1 per cent level

Table 2: Effect of Media on shoot multiplication in sesame genotypes

Treatment No.	Growth regulator (mg/l)			Genotypes			
	IAA	BA	Kinetin	CO 1	VRI 1	TMV 3	AHT 123
1	0.5	1.0	0.50	2.31	2.46	1.81	1.41
2	0.5	1.5	0.50	2.41	2.81	2.81	1.84
3	0.5	1.0	1.25	1.91	3.81	2.82	3.21
4	0.5	1.5	1.25	4.21*	4.81**	4.18*	4.45**
5	0.5	1.0	0.50	3.61	3.16	2.81	3.28
6	0.5	1.5	0.50	3.81	2.51	3.83	5.18
7	0.5	1.0	1.25	6.18	4.98	5.81	4.12
8	0.5	1.5	1.25	4.95	5.16	6.12	5.98
9	Control			2.16	2.91	1.91	2.25
Sed	0.23						
CD (0.05)	0.47						
CD(0.01)	0.63						

*Significant at 5 percent level ** Significant at 1 percent level

Table 3: Effect of Growth hormones on root induction in sesame genotypes

Hormone combination	Concentration (mg/l)	Number of roots after three weeks			
		CO 1	VRI 1	TMV 3	AHT 123
IBA + IAA	0.5+0.50	4.85	3.91	2.18	2.36
	0.5+1.00	8.31	7.85**	5.81	6.12
	0.5+1.25	9.91**	10.81**	8.21	6.81
	0.5+1.50	9.21**	8.17**	7.91	6.21
IBA + IAA	1.0+0.50	9.23**	5.39	4.82	4.35
	1.0+1.00	10.22**	8.41	6.38	6.21
	1.0+1.25	13.81**	11.21**	10.12**	9.15**
	1.0+1.50	11.60**	10.21**	8.01	7.41
SEd	0.48				
CD(0.05)	0.98				
CD(0.01)	1.32				

*Significant at 5 percent level **Significant at 1 percent level

by Shanthi[4]. The regenerated plantlets were transplanted to a soil mixture and grown to maturity in the green house.

RESULTS AND DISCUSSION

Callus induction: Callus induction found to increase with increase in the concentration of 2,4-D upto 3.5mg/l. 2,4-D along with casein hydrosylate found to increase callus induction upto 3.5mg/l. Jeya Mary and Jeyabalan[5] reported that among the different auxins tested, 2,4-dichlorophenoxy acetic acid was the most effective and resulted in the highest frequency of responding cultures and highest average number of somatic embryo per responding cultures. While CO 1 (59.08) recorded higher

per centage of callus induction at 3.5mg/l of 2,4-D and 0.1g/l of Casein hydrosylate, AHT 123 (42.01) registered lower per centage of callus induction at 2.0mg/l of 2,4-D and 0.1g/l of Casein hydrosylate. Among sesame genotypes, CO 1 recorded good response and high per centage of callus induction (68.10) at 3.0mg/l of 2,4-D in addition with 0.1g/l of coconut milk. But, AHT 123 recorded lower value (52.84) at the same concentration of constituents. Sehrawat *et al.*[6] observed that the coconut milk had a similar effect as that of auxin.

Shoot multiplication: All the genotypes responded positively to the different levels of IAA, BA and Kinetin during shooting. The highest significant shoot multiplication ratio was observed in treatment combination of 1.0mg/l of IAA+1.0mg/l of BA+1.25mg/l of Kinetin followed by 1.0mg/l of IAA+1.50mg/l of BA+1.25mg/l of Kinetin. While CO 1 recorded highly significant shoot multiplication ratio (1:6.18), the lowest was observed in TMV 3 at control (1:1.08). The treatment combination 0.5 mg/l of IAA+1.0mg/l of BA+0.5mg/l of Kinetin was found to be less effective. The results were supported Sontakey *et al.* [7].

Root multiplication: Among varieties, CO 1 produced profuse rooting in full strength MS media with hormonal concentration of 1.0g/l of IBA and 1.25 mg/l of IAA followed by VRI 1 at the hormonal concentration. Least response to rooting was showed by TMV 3 at hormonal concentration of 0.5g/l of IBA and 0.5mg/l of IAA. Similar report also quoted by Morris [8].

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