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Regeneration of Eggplant Through Anther Culture

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Abstract: The study was conducted to develop a protocol for anther culture of eggplant. Six eggplant cultivars viz., Dohazari, IPM 31, Laffa S, Ishurdi L, ISD-006 and Jessore L were implanted on MS medium with different hormone supplements viz., 2 mg L^{-1} NAA + 2 mg L^{-1} BAP, 1 mg L^{-1} IAA + 2 mg L^{-1} BAP and 2 mg L^{-1} 2, 4-D + 2 mg L^{-1} BAP for callus induction. The highest survivability of anthers (35 %) and the highest percentage of callus induction (30%) were recorded in cv. ISD-006 with 2 mg L^{-1} NAA + 2 mg L^{-1} BAP supplemented MS medium. The minimum days (27.25) required for callus initiation in the cultivar ISD-006 on MS medium with hormone supplement 2 mg L^{-1} 2-4,D + 2 mg L^{-1} BAP. Anther derived calli of eggplant cultivars were transferred to MS medium with various combinations and concentrations of auxin and cytokinin for shoot regeneration but the calli did not produce any shoot. Root formation was observed from the cultured calli. For different parameters in root formation, the cultivar ISD-006 showed the best performance followed by Jessore L. Among the media, MS medium with 2 mg L^{-1} NAA +2 mg L^{-1} BAP and MS medium with 2.5 mg L^{-1} NAA +2 mg L^{-1} BAP showed better performance with insignificant variation.

Key words: Eggplant, anther culture, shoot regeneration, rooting

INTRODUCTION

Eggplant (*Solanum melongena* L.) belongs to the family Solanaceae and is one of the most popular, palatable and nutritious vegetable crops in Bangladesh. It is the second most important vegetable crop next to potato in Bangladesh in respect of total areas in production[1]. It is cultivated in 15% of total vegetable cultivated land and 80 lakh farm families are related with eggplant cultivation. About 40 million people earn their livelihood on eggplant cultivation[2]. But infectious diseases like *Phomopsis* fruit rot and foot rot and the insect shoot and fruit borer limit harvest target of eggplant. Thus contribution of eggplant growers to national economy is jeopardized. As a result, eggplant improvement has taken a considerable interest of researchers in different areas of biotechnology. One very popular approach for this purpose is anther culture. It is one of the most promising parts of tissue culture to obtain haploid and doubled haploid plants. Haploid was first produced by Guha and Maheshwari[3]. By doubling the chromosomes in haploid plants, it is possible to obtain completely homozygous lines in a short time thereby providing a method for speeding up and increasing the selection efficiency[4]. Anther culture has since been successfully applied throughout the world with many different species and genera. With intensive application anther culture to species of economic importance, progress has been made on the cereals, such as barley[5], wheat[6], linseed[7] rice[8] and Solanaceous crops, namely pepper[9] and potatoes[10]. Very few researches conducted on anther culture of eggplant. Raina and Iyer[11] were the first to start anther culture in eggplant. In 2003, Prabhavathi *et al.*[12] were able to regenerate some plants from eggplant anthers. However, the anther culture protocol for eggplant needs to be developed allowing more economic production of eggplant in Bangladesh. Considering the above facts, this research program aims to optimize the efficiency of the anther culture technique for the production of haploid plants and to incorporate this method in the eggplant breeding programs of Bangladesh.

MATERIALS AND METHODS

The study was carried out during the period from December, 2003 to November, 2004 at the Integrated Pest Management Laboratory, Department of Plant Pathology and Biotechnology Laboratory, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh.

Plant material: Six eggplant cultivars viz., Dohazari, IPM 31, Laffa S, Ishurdi L, ISD-006 and Jessore L were used. Donor plants were grown in field condition. Before sowing, seeds were treated with hot water by Vegetable Seed Treating Plant to ensure disease free healthy seedling production. Seedlings were raised in plastic trays

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in the net house. Forty eight days old healthy seedlings were transplanted in the field followed by watering. Fifteen healthy seedlings of each cultivar were planted in each sub plot by maintaining plant to plant distance 75 cm and line to line 1 m. Each plot was fertilized with Cow dung (10000 kg ha^{-1}), Oil cake (500 kg ha^{-1}), Urea (130 kg ha^{-1}), T.S.P. (125 kg ha^{-1}), M.P. (100 kg ha^{-1}).

Collection of flower buds: Flower buds were collected before anthesis period when the microspores were at the early to mid uninucleate stage. The developmental stages of the microspores were determined in using microscopic slides prepared according to the acetocarmine squash method[13].

Culture media: The MS medium was used for callus induction with different concentration and combination of auxin (NAA, IAA, 2, 4-D) and cytokinin (BAP). For plant regeneration, MS medium was used with different concentration and combination of auxin (NAA) and cytokinin (BAP, Kinetin) (Table 2).

Culture technique: Flower buds were surface sterilized by immersion in 70% ethanol for one minute and subsequently rinsed three times with sterilized distilled water. Thereafter, the anthers were isolated and plated on the callus induction media (1 anther/test-tube) and incubated at 25°C in dark condition[12]. Six to seven weeks after inoculation, the calli attained convenient size and were transferred onto regeneration medium where they were cultured at 25°C under 16 h photoperiod.

Data recorded: To investigate the effect of different treatments and response of different cultivars, data were recorded on different parameters of callus induction and root formation[14].

Statistical analysis: The data for the parameters recorded in the present study were statistically analyzed by the statistical package MSTATC and Microsoft Excel wherever applicable. The experiment was conducted in growth room (IPM Laboratory and Biotechnology Laboratory) and arranged in Completely Randomized Design. The analysis of variances for different parameters was performed and means were compared by the Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Callus induction: Result of Table 1 showed that among the six cultivars, ISD-006 showed the highest percentage (32.66) of survivability followed by Jessore L (25.66) and Ishurdi L (24.66) and showed significant difference. The least survivability (19.58%) was recorded in IPM 31. Dohazari and Laffa S ranked third and were not statistically different.

Callus initiation from the anthers of eggplant cultivars started approximately after 28 days of culture. The cultivar Jessore L took minimum time (28.25 days) for callus initiation followed by ISD-006, Ishurdi L and Dohazari. Requirement of time of these cultivars for callus initiation was significantly different. Time required for cultivar IPM 31 and Laffa S was not significantly different and they required maximum time (38.41) for callus initiation. The cultivar ISD-006 performed significantly better as compared to other cultivars for callus induction followed by Jessore L and Ishurdi L with significant difference. Performance of the cultivar IPM 31 was the least among all the cultivars.

Among the three callus induction media, MS media containing 2 mg L^{-1} NAA + 2 mg L^{-1} BAP showed the best result for per cent survivability followed by MS+ 1 mg L^{-1} IAA+2 mg L^{-1} BAP and MS+ 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP which were significantly different (Table 2).

MS media containing 2 mg L^{-1} 2, 4-D + 2 mg L^{-1} BAP took minimum time (32.54) for callus initiation followed by MS media containing 1 mg L^{-1} IAA + 2 mg L^{-1} BAP which was not significantly different. This result is in line with the findings of Prabhavathi *et al.*[12]. MS media containing 1 mg L^{-1} NAA + 2 mg L^{-1} BAP took maximum time and significantly different than other two indicating those days to callus initiation was found to be influenced by different hormone supplementation for anther culture of eggplant.

Table 1: Effect of cultivars on callus induction

Cultivar	Survivability of anther (%)	Days to callus initiation	Callus induction (%)
Dohazari	21.66 c	35.33 b	18.33 d
ISD-006	32.66 a	28.41d	26.66 a
Laffa S	21.66 c	38.41 a	17.58 d
Ishurdi L	24.66 b	31.66 c	20.00 c
IPM 31	19.58 d	37.66 a	15.33 e
Jessore L	25.66 b	28.25 d	22.33 b
$LSD_{(0.05)}$	1.06	1.31	1.31
CV (%)	8.90	5.01	8.00

Table 2: Effect of media with different hormone supplementation on callus induction

Media with different hormone supplementation	Survivability of anther (%)	Days to callus initiation	Callus Induction (%)
MS+ 2 mg L^{-1} NAA + 2 mg L^{-1} BAP	27.33 a	34.50 a	22.79 a
MS+1 mg L^{-1} IAA + 2 mg L^{-1} BAP	23.95 b	32.83 b	20.00 b
MS+ 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP	21.66 c	32.54 b	17.33 c
$LSD_{(0.05)}$	0.75	0.92	0.92
CV (%)	8.90	5.01	8.00

Table 3: Effect of cultivars on root formation

Cultivars	Days to root initiation	No. of calli showing root	No. of root/callus at different week after explanting					
			2nd	3rd	4th	5th	6th	7th
Dohazari	15.33a	1.25bc	0.33c	0.58c	1.19b	2.33bc	2.83b	3.50b
ISD-006	14.08b	2.25a	1.16a	2.00a	4.33a	4.66a	5.33a	5.66a
Laffa S	12.16c	0.66c	0.58bc	0.83bc	1.50bc	1.83c	2.58b	3.00b
Ishurdi L	13.00bc	1.16bc	0.66bc	1.16b	1.83bc	2.33bc	2.91b	3.58b
IPM 31	12.83bc	1.00bc	0.83ab	1.08b	1.83bc	2.41c	2.75b	3.25b
Jessore L	13.16bc	1.58b	0.58bc	1.16b	1.33c	2.16bc	2.75b	3.08b
$LSD_{(0.05)}$	1.17	0.64	0.37	0.46	0.52	0.51	0.59	0.54
CV (%)	10.64	9.92	6.45	9.99	10.21	13.90	8.74	10.02

Table 4: Effect of media with different hormone supplementation on root formation

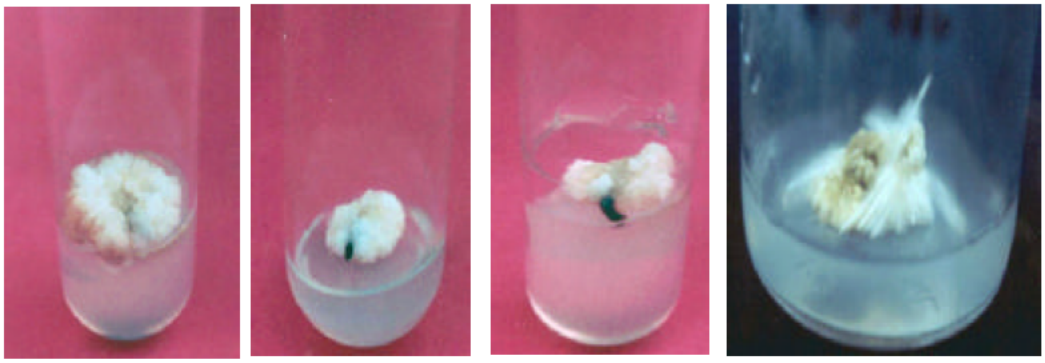
Media with different hormones	Days to root initiation	No. of calli showing root	No. of root/callus at different week after explanting					
			2nd	3rd	4th	5th	6th	7th
MS+ 1 mg L^{-1} NAA + 2 mg L^{-1} BAP	15.00a	0.87b	0.41b	0.87b	1.91b	2.29b	2.95a	3.45b
MS+ 2 mg L^{-1} NAA+ 2 mg L^{-1} BAP	13.04b	1.41a	0.75a	1.20a	2.04b	2.66a	3.25a	3.66ab
MS+ 2.5 mg L^{-1} NAA + 2 mg L^{-1}BAP	12.25b	1.66a	0.91a	1.33a	2.41a	2.91a	3.37a	3.91a
$LSD_{(0.05)}$	0.82	0.45	0.26	0.32	0.31	0.36	0.42	0.38
CV (%)	10.64	9.92	6.45	9.99	10.21	13.90	8.74	10.02

Performance for per cent callus induction was influenced by the media composition. MS media containing 2 mg L^{-1} NAA + 2 mg L^{-1} BAP showed the best result for per cent callus induction followed by MS + 1 mg L^{-1} IAA + 2 mg L^{-1} BAP and MS + 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP which were significantly different. The callus from anther of cultivar ISD-006 proliferated better in MS medium with 2 mg L^{-1} NAA + 2 mg L^{-1} BAP than the anther from the same cultivar or other cultivars on different media (Fig. 1a-c). In case of interaction it was observed that the cultivar ISD-006 showed the highest percentage of survivability on the media MS+ 2 mg L^{-1} NAA + 2 mg L^{-1} BAP followed by ISD-006 X MS with 1 mg L^{-1} IAA + 2 mg L^{-1} BAP showing significant variation.

The cultivar IPM 31 displayed the poorest performance for per cent survivability on MS medium with 2 mg L^{-1} 2, 4-D + 2 mg L^{-1}BAP. These results indicated that interaction between cultivar and media composition played a vital role for per cent survivability. Days required for callus initiation, was significantly affected by the interaction between the cultivars and media composition. The cultivar Jessore L took minimum time (26 days) on MS medium with 2 mg L^{-1} 2,4-D+ 2 mg L^{-1}BAP followed by ISD-006 X MS medium with 2 mg L^{-1} 2,4-D + 2 mg L^{-1} BAP and ISD-006 x MS medium with 1 mg L^{-1} IAA+ 2 mg L^{-1} BAP and displayed insignificant variation. The cultivar IPM 31 and Laffa S took maximum time (40.00 days) on MS medium with 2 mg L^{-1} NAA + 2 mg L^{-1} BAP. From the Fig 2. it can be concluded that the cultivar ISD-006 performed the best for percentage of callus induction on MS medium with 2 mg L^{-1} NAA + 2 mg L^{-1} BAP.

Root formation: Callus derived from cultured anther of six cultivars of eggplant was implanted on MS medium supplemented with NAA and BAP in three combinations. Root was directly initiated from cultured calli instead of shoot on regeneration medium. Assessment on root regeneration studied through the parameters, days to root initiation, number of calli showing root and number of roots per callus at different week after explanting. The cultivar Laffa S performed the best for days to root initiation. It took minimum days (12.16) compared to other cultivars. Next to Laffa S, except Dohazari, other cultivars varied insignificantly for the parameters. Number of calli showing root was maximum in the cultivar ISD-006 and showed significant variations. Other cultivars varied insignificantly for this parameter. Maximum number of roots produced by the cultivar ISD-006. Root number increased gradually at different weeks after explanting which was faster during the 4th week (Table 3).

Performance of the media composition for root formation is presented in the Table 4. Minimum days (12.25) required on MS medium + 2.5 mg L^{-1} NAA + 2 mg L^{-1} BAP to initiate root which was statistically similar to MS medium + 2 mg L^{-1} NAA + 2 mg L^{-1} BAP. Significantly more time (15.0 days) required on MS medium with 1 mg L^{-1} NAA + 2 mg L^{-1} BAP. Number of roots was the maximum on MS medium containing 2.5 mg L^{-1} NAA+ 2 mg L^{-1} BAP and MS medium containing 2 mg L^{-1} NAA+ 2 mg L^{-1} BAP. These two media showed insignificant variation for the parameters. This result corresponds with that obtained by Chen[15] where he found sturdy growth of root in NAA rich medium. In all the three root inducing media, number of roots per callus increased with time. The increase



1a 1b 1c 1d

Fig. 1a: Callus from anther of cv. ISD-006 incubation in MS + 2 mg L^{-1} NAA + 2 mg L^{-1} BAP after 45 days of culture,
b: Callus from anther of cv. ISD-006 incubation in MS + 1 mg L^{-1} IAA + 2 mg L^{-1} BAP after 45 days of culture
c: Callus from anther of cv. Dohazari after incubation in MS+2 mg L^{-1} 2,4-D+2 mg L^{-1} BAP after 45 days of culture
d: Root development in cv. ISD-006 on MS medium with 2.5 mg L^{-1} NAA + 2 mg L^{-1} BAP after 35 days of culture

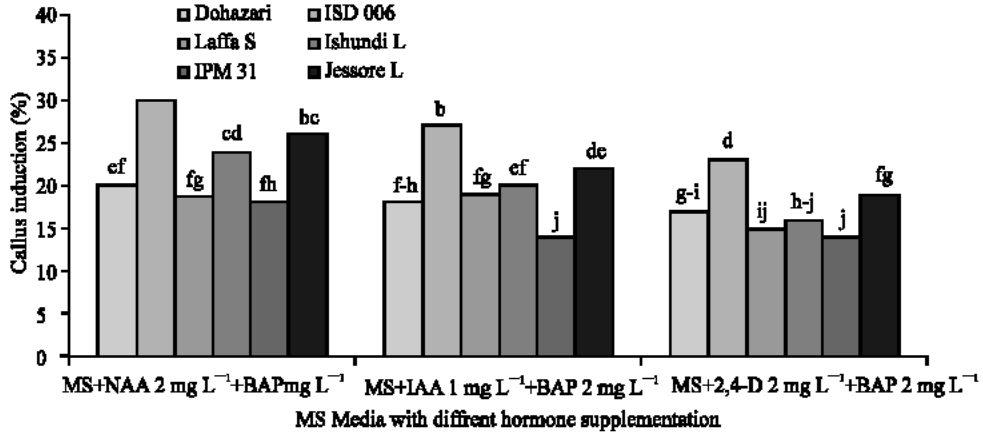


Fig. 2: Combined effect of cultivars and media composition for percent callus induction after 45 days of culture

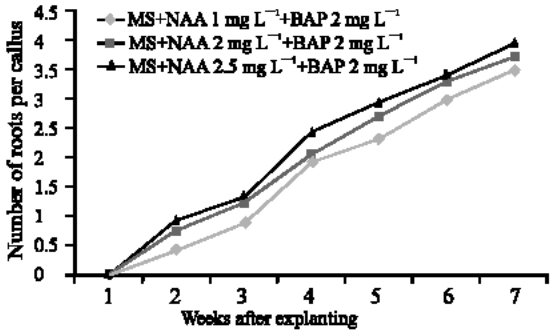


Fig. 3: Effect of different hormone supplementation on number of roots per callus of cv. IS D-006 at different weeks after explanting

in number of roots was steady and sharp which was more pronounced in MS medium containing 2.5 mg L^{-1} NAA+ 2 mg L^{-1} BAP and less in MS medium containing 1 mg L^{-1} NAA+ 2 mg L^{-1} BAP. The difference for root development in the three media was significant from the beginning up to 5th week which diminished towards 6th and 7th week (Fig. 3). In case of interaction, the cultivar IPM 31 took minimum time on MS medium with 2.5 mg L^{-1} NAA+ 2 mg L^{-1} BAP for root initiation followed by the other cultivars on the same medium and they displayed insignificant variation. The cultivar ISD-006 took maximum time on MS medium with 1 mg L^{-1} NAA+ 2 mg L^{-1} followed by the cultivar Dohazari on the same medium. Number of roots per callus was maximum in the cultivar ISD-006 on MS medium with 2.5 mg L^{-1} NAA+ 2 mg L^{-1} BAP followed by the same cultivar on MS medium with 2 mg L^{-1} NAA+ 2 mg L^{-1} BAP. From the above discussion, it can be concluded that both cultivar and media composition affected root formation from anther derived calli of eggplant cultivars.

Shoot regeneration: The anther derived callus was transferred to MS medium with different concentrations

of auxin (NAA) and cytokinin (BAP and Kinetin) and for shoot regeneration. Observations continued for 45 days but initiation of shoot couldn't be traced. Probhavathi *et al.*[12] observed shoot initiation in anther derived callus of eggplant using the same medium. But, in the present experiment, after two weeks of implanting the callus, roots started to initiate. This result is somewhat similar to the findings of Wang and Zapata[16] who used higher concentration of auxin and lower concentration of cytokinin for shoot regeneration from callus. With passage of time, they observed that callus showed root induction tendency instead of shoots.

Low yield of regenerated shoots has often been a problem in production of haploids in many crops[7]. The previous efforts in anther culture had also encounted this problem. Nichterlein *et al.*[17] did not obtain any shoot from anther culture in linseed but were able to improve on this in later work[18]. They observed that shoot and plant regeneration from anthers could be induced from linseed genotypes grown under controlled environmental conditions in a phytotron which helped to avoid the application of chemicals, since the plants remain healthy until the bud harvest was finished. They also observed that shoot regeneration from anther callus strongly increased by using a modified N_6 medium containing zeatin (1 mg L^{-1}) with lower growing temperature (20°C) in the growth chamber. Their findings were in line with Hebrle-Bors and Reinert[19] in *Nicotiana tabacum* and with Keller and Stringham[20]; Thurling and Chay[21] in *Brassica napus*. Addition of $AgNO_3$ (2 mg L^{-1}) to the regeneration medium could greatly increase the frequency of shoot regeneration[22]. Koh and Loh[23] reported that continued secondary embryogenesis was possible on hormone free medium, thereby excluding the complication of exogenous plant growth regulators.

In summary, the result of the study indicate that the anther culture protocol of eggplant could be improved by allowing measures like a controlled environmental condition for the production of donor plants, a modified N_6 medium with zeatin for culture of anther callus and addition of $AgNO_3$ to the regeneration medium.

REFERENCES

1. Anonymous, 2002. Statistical Yearbook of Bangladesh. Bangladesh Bureau of Statistics. Ministry of Planning, Government of the People's Republic of Bangladesh. Dhaka, Bangladesh, pp: 150.
2. Anonymous, 1995. Training Manual. Winter vegetables and spices production. Developed by Horticulture Research and Development Project (FAO/UNDP/ ASDB Project: BGD/87/025), pp: 384.
3. Guha, S. and S.C. Maheshwari, 1966. Cell division and differentiation of embryo in the pollen grains of *Datura in vitro*. Nature, 212: 97.
4. Snape, J.W., 1989. Doubled haploid breeding: theoretical basis and practical applications. Second International Symposium on Genetic Manipulation in Crops, Mujeeb-Kazi, A., Sitch, L.A., pp: 57-68.
5. Savaskan, C., I. Szarejko and M.C. Toker, 1999. Callus production and plant regeneration from anther culture of some Turkish barley cultivars. Turk. J. Bot., 23: 359-365.
6. Hassawi, D.S. and G.H. Liang, 1990. Effect of cultivar, microspore development of anther culture of wheat and *Triticale*. Plant Breed., 105: 332-336.
7. Orhan, K. and M.E. Gareth, 1998. Anther culture potential of linseed (*Linum usitatissimum* L.): Effects of genotypes and pretreatment on callus formation and differentiation. Turk. J. Agric. For., 22: 553-560.
8. Rout, J.R. and N.P. Sharma, 2001. High frequency plantlet regeneration in rice anther callus cultures. Rice Gene. Newslett., 29: 175-182.
9. Ciner, O.D. and R. Tripirdamaz, 2002. The effects of cold treatment and charcoal on the *in vitro* androgenesis of pepper (*Capsicum annuum* L.). Turk. J. Bot., 26: 131-139.
10. Tatiana, B.M. and E.V. Richard, 2002. Phenotypic characterization and bulk segregant analysis of anther culture response in two backcross families of diploid potato. Plant Cell Tiss. Organ Cult., 28: 277-286.
11. Raina, S.K. and R.D. Iyer, 1973. Differentiation of diploid plantlets from pollen callus in anther culture of *Solanum melongena* L.Z. Pflanzenzucht, 70: 275-280.
12. Prabhavathi, K., K. Kumar, M. Singh and A. Mathews, 2003. *In vitro* induction of haploid in eggplant (*Solanum melongena* L.). Capsicum and Eggplant Newsletter, 22: 147-150.
13. Anagnostakis, S.L., 1974. Haploid plants from anthers of tobacco - enhancement with charcoal. Planta, 115: 281-283.
14. Khatun, F., 2004. Development of a protocol for anther culture of eggplant. MS Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh, pp: 71.
15. Chen, Y., 1986. Anther (Pollen) Culture, Haploids of Higher Plants *in vitro*, Part A, Chapter 1, Eds. H. Han, Y. Hongyuan, China Academic Publishers, Beijing.
16. Wang, M.S. and F.J. Zapata, 1987. Somatic embryogenesis in rice (*Oryza sativa*) cultivar IR- 40. Intl. Rice Res. Newslett., 12: 23-24.

17. Nichterlein, K., H. Umbach and W. Friedt, 1989. Investigation on androgenesis in breeding of linseed (*Linum usitatissimum* L). XII. Eucarpia Congress, pp: 13-25.
18. Nichterlein, K., H. Umbach and W. Friedt, 1991. Genotypic and exogenous factor affecting shoot regeneration from anther callus of linseed (*Linum usitatissimum* L). Euphytica, 58: 157-164.
19. Heberle-Bors, E. and J. Reinert, 1981. Environmental control and evidence for predetermination of pollen embryogenesis in *Nicotiana tabacum* pollen. Protoplasma, 109: 249-255.
20. Keller, W.A. and G.R. Stringham, 1978. Production and utilization of microspore derived haploid plants. In: T. Thrope (Ed.). Frontiers of Plant Tissue Culture 1978. University of Calgary Press, Canada, pp: 113-122.
21. Thurling, N. and P.M. Chay, 1984. The influence of donor plant genotype and environment on production of multicellular microspores in cultured anthers of *Brassica napus*. Ann. Bot., 54: 681-693.
22. Du, H., D.H. Zhuang and W.H. Hunang, 2000. Stimulation effect of silver nitrate on shoot regeneration in cotyledon tissue culture of *Brassica campestris*. J. Trop. Subtrop. Bot., 8: 109-112.
23. Koh, W.L. and C.S. Loh, 2000. Direct somatic embryogenesis, Plant regeneration and *in vitro* flowering in rapid cycling *Brassica napus*. Plant Cell Rep., 19: 1177-1183.