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Studies on anther culture in tomato (*Lycopersicon esculentum*)

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SUMMARY

Anther or pollen culture have been used in mutation and F1 hybrid breeding programme in many plant species. In order to get haploid plants, three tomato varieties were used in this study. Anther were removed from 2-4 mm, 5-6 mm and 8-10 mm length tomato flowers. Two different nutrient media were investigated to get callus. N6 medium + 2 mg/L NAA + 1 mg/L kinetin was most efficient medium for anther callus growth. Calluses were subcultured but calli did not show any response for further callus growth and haploid plantlets were not obtained.

Key words : Anther culture, Callus formation and tomato

Tomato (*Lycopersicon esculentum* Mill.) which belongs to family *solanaceae* is the most abundantly produced vegetable crop in the world (Anonymous, 2002). With wide range of adaptability of soil and climate. It is most popular because of its high nutritive value and diversified uses.

For improvement of any crop, variability in the basic population is important which can be created through hybridization and induced mutation followed by selection. Tissue culture is one of the technique which can be used to create the genetic variability among basic population within short period of time.

The regeneration of plants from pollen grains of angiosperms has a relatively recent history dating back to the discovery by Guha and Maheshwari (1964) of the production of embryo like structures (embryoids) from anthers of *Datura innoxia* culture in a complex medium. In subsequent studies several investigators established the origin of embryoids from pollen grains and their regeneration into plants (Maheshwari *et al.*, 1982; Bajaj, 1983).

Therefore, present investigation has been undertaken on anther culture using three varieties of tomato, Vaishali, Wild (*Lycopersicon khasianum*) and Pusa ruby.

MATERIALS AND METHODS

Present investigation was carried out at Tissue Culture Centre, Marathwada Agricultural Univrsity, Parbhani during 2005-06. The material used for conducting the experiment and methods employed are described by following data.

Three cultivators of tomato namely Vaishali, Wild (*L. khasianum*) and Pusa Ruby were used in the experiment.

Preparation of media:

Basal media used in present study was N6 medium (Nitsch *et al.*, 1969) and DBM 2 medium (Gresshoff and Doy, 1977). The stock solutions of macro and microelements were pipetted out in required proportion and mixed well. The stock solution of vitamins and hormones were also added in required quantities and the volume was made up using double distilled water. Sucrose 4 per cent was used as gelling agent.

Media for callus induction:

– N6 medium+2.0 mg/lit. NAA + 1.0 mg /lit. Kinetin

– DBM2 medium+2.0mg/lit. NAA+5.0 mg/lit. Kinetin

Media for regeneration:

– MS + 2.0 mg/lit BAP + 1.0 mg/lit. NAA

The pH of medium was adjusted to 5.8 by using dil. NaOH or dil. HCl before addition of noble agar. The media was boiled after addition of noble agar to dissolve it. This was distributed uniformly @ 30 ml of media / Petridish. The dishes were autoclaved at 1.06 kg/cm^2 (15 lbs/inch^2) pressure and 121^{O}C temperature for 20 minutes. The dishes are ready for inoculation after cooling.

Preparation and inoculation of explant:

Unopened flowerbud of different sizes *viz.*, 2-4 mm, 5-6 mm and 8-10 mm of each of three cultivars were selected. Surface sterilization of flowerbud was done in the following steps.

– Transferred to 0.1 % Tween 20 solution and kept for 5 minutes then washed four times with distilled water.

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– Dipped in 70 % ethanol for 15 to 20 sec. Followed by washing four times with distilled water.

– Transferred to sodium hypochlorite 0.5 % solution for 5 minutes in LAF and then washed four times with sterile distilled water.

Anthers were excised from flower buds and inoculated in Petridish containing media. The dishes were wrapped with parafilm after inoculation and exposed to cold treatment at 8^0C for 2 days, 4 days and 10 days. After cold treatment incubation was done in dark at 23 + 1^0C.

RESULTS AND DISCUSSION

Anthers from three different sizes of flower buds *viz.*, 2-4 mm, 5-6 mm and 8-10 mm of three different tomato cultivars *viz.*, Vaishali, Wild (*Lycopersicon khasianum*) and Pusa ruby were inoculated on N6 medium supplemented with 2.0 mg/lit NAA + 1.0 mg/lit kinetin. After incoulation they were subjected to cold treatment 8^0C for 2 days.

Callus initiation:

Callus initiation was observed after 4-5 weeks inoculation of anthers. Mean performance for variety, flower bud size and their interaction effects for callus parameters are presented in Table 1. It is revealed that variety Vaishali was significantly superior over other two varieties. Earlier callus initiation was observed in Vaishali (26 days) followed by Pusa ruby (27 days) and wild (29 days). Flower bud size 2-4 mm was significantly superior over other two treatments for callus initiation. From 2-4 mm bud size callus initiation required (26 days) followed by 5-6 mm size of flower bud (27 days) and 8-10 mm size of flower bud (29 days).

Diploid plants of tomato were regenerated from anthers by Cappodocia and Sreeramulu (1980) and Brasileiro *et al*. (1999). This techniques have not been widely adopted in tomato because until, recently, callus production and plantlet formation rates were < 0.7 % (Zamir *et al*., 1981). The slow adoption of anther culture as a breeding tool is a result of the many difficulties involved in generating useful diploid plants.

The present study was oriented with an aim to obtain callus initiation, proliferation and plantlet regeneration from anthers of tomato cultivars *viz*., Vaishali, Wild (*Lycopersicon khasianum*) and Pusa ruby. Anthers of three different sizes of flower buds *viz.*, 2-4 mm, 5-6 mm and 8-10 mm of three different tomato cultivars *viz.*, Vaishali, Wild (*Lycopersicon khasianum*) and Pusa ruby were inoculated on N6 medium supplemented with 2.0 mg/lit NAA + 1.0 mg/lit kinetin. After inoculation they were subjected to cold treatment at 8^0C for 2 days.

Earlier callus initiation from anthers was observed in Vaishali (26 days) followed by Pusa ruby (27 days) and Wild (29 days). Ozzambak (1994) reported callus formation after 2 weeks in tomato. Jaramilo and Summers (1990) also reported callus induction after 4 to 8 weeks of dark light exposure. Brasileiro *et al*. (1999) reported that the anther and flower bud both are significantly correlated with anther development stage and anthers containing prophase-I produced highest callus frequency. Anthers from flower bud size 2-4 mm was significantly superior over 5-6 mm and 8-10 mm sizes of flower buds for callusing. These observations are in agreement with Summers *et al*. (1992). In the present investigation, the cold treatment was given to the inoculated anthers of different flower buds (8^0 C for a period of 2 days). Ma You Hui *et al*. (1999) also given pretreatment to anthers at 4^0 C for 3 days.

Fresh and dry weight (g):

Fresh and dry weights were measured after second

Table 1 : Mean performance of variety, flower bud size and their interaction effect on days required for callus initiation, fresh and dry weights (g) of tomato

Sr. No.	Source of variation	Mean days required for callus initiation	Mean fresh weight (g)	Mean dry weight (g)
	Variety			
1.	V_1 Vaishali	26.83	0.420	0.032
2.	V_2 Wild (*L. Khasianum*)	29.50	0.413	0.026
3.	V_3 Pusa Ruby	27.66	0.415	0.029
	S.E. ±	0.254	0.0005	0.0004
	C.D. (P=0.05)	0.828	0.001	0.001
	Flower bud size			
4.	S_1 2-4 mm	26.33	0.423	0.033
5.	S_2 5-6 mm	27.83	0.415	0.028
6.	S_3 8-10 mm	29.83	0.410	0.025
	S.E. ±	0.254	0.0005	0.0004
	C.D. (P=0.05)	0.828	0.001	0.001
	Variety x flower bud size			
7.	V_1 S_1	25.50	0.428	0.035
8.	V_1 S_2	26.50	0.420	0.031
9.	V_1 S_3	28.50	0.413	0.029
10.	V_2 S_1	27.50	0.420	0.031
11.	V_2 S_2	29.50	0.411	0.027
12.	V_2 S_3	31.50	0.408	0.027
13.	V_3 S_1	26.00	0.422	0.021
14.	V_3 S_2	27.50	0.415	0.034
15.	V_3 S_3	29.50	0.409	0.027
	S.E. ±	0.440	0.0008	0.0007
	C.D. (P=0.05)	1.435	0.002	0.002

subculturing. Mean performance of variety, flower bud size and their interaction effect on fresh and dry weight (g) are presented in Table 1. It is observed from Table 1 that Vaishali variety was highly significant over other two varieties for fresh and dry weight (0.420 g and 0.032 g, respectively). Anthers from flower bud size 2-4 mm showed higher fresh and dry weights (0.423 g and 0.033 g, respectively) followed by anthers from 5-6 mm (0.415 g and 0.028 g, respectively) and 8-10 mm flower bud size (0.410 g and 0.025 g, respectively).

In variety x flower bud size interaction, Vaishali x 2-4 mm flower bud size interaction was significantly superior over other treatments. Maximum fresh and dry weight was observed in Vaishali x 2-4 mm flower bud size interaction (0.428 and 0.035 g, respectively). Maximum fresh and dry weight was obtained in Vaishali. Vaishali variety was significantly superior for fresh and dry weight than Pusa ruby and Wild. 2-4 mm size of flower bud was significant for fresh and dry weights than other treatments. These results are also in agreement with Jaramillo and Summers (1990) and Brasileiro *et al*. (1999).

Subculture:

Calli were transferred to same media for further callus proliferation. In this experiment 2-4 mm, 5-6 mm and 8-10 mm sizes of flower buds of each cultivar did not respond to callus proliferation as well as organogenesis. They became degenerate by changing calli colour on N6 medium supplemented with 2.0 mg/lit NAA and 1.0 mg/lit kinetin.

Several factors like physiological condition of the donor plants, developmental stage of the pollen, pretreatment of anthers, effect of light during culture and density or position of anthers might affected the callus induction and plant regeneration from anther. These observations are in agreement with Ozzambak (1994) who reported the absence of organogenesis from anther callus on N6 medium + 2.0 mg/lit NAA + 1.0 mg/lit kinetin. Present findings are also in agreement with other studies, which suggest that differentiation can not occur from callus (Gu, 1979; Gulshan *et al*., 1982; Karakulluku and Abak, 1992).

REFERENCES

Anonymous (2002). FAO Website http://apps.fao.org/cgi-bin/nphdb.pl?subset=agriculture

Bajaj, Y.P.S. (1983). *In vitro* production of haploids in D.A. Evans, W.R. Sharp, P.V. Ammirato and Y. Yamada (eds.) Handbook of Plant Cell Culture Vol.1. Techniques for propagation by Ethrel. *Nature*, 240:566- 568.

Brasileiro, A.C.R., Willadina, L., Carvalheira, G.G. and Guerra, M. (1999). Callus induction and plant regeneration of tomato (*Lycopersicon esculentum* cv. IPA) *Via anther Culture*, **29** (4) : 619-623.

Cappadocia, M. and Sree Ramulu, K. (1980). Plant regeneration from *in vitro* cultures of anthers and stem internodes in an interspecific hybrid, *Lycopersicon esculentum* L. x L. *peruvianum Mill* and cytogenetic analysis of the regenerated plants. *Plant Sci. Lett.*, **20** : 157-166.

Gresshoff, P.M. and Doy, C.H. (1972). Development and differentiation of haploid *Lycopersicon esculentum* (tomato). *Planta*, **107** : 161-170.

Gu, S.R. (1979). Plantlets from isolated eggplant pollen cultures. *Hort. Abs.*, **49** (8) : 5891.

Guha, S. and Maheshwari, S.C. (1964). *In vitro* production of embryos form anthers of *Datura Nature*, 204-497.

Gulshan, T.M., Varghese and Sharma, D.R. (1982). Studies on anther cultures of tomato *Lycopersicon esculentum* Mill. *Hort. Abs.*, **52** (6): 3921.

Jaramillo, J. and Summers, W. (1990). Tomato anther callus production : Solidifying agent and concentration influence induction of callus. *J. American Soc. Hort. Sci.*, **115** : 1047-1050.

Karakulluku, S. and Abak, K. (1992). Farkli Sicaklik Saklarinin partlicanda embriyo olusumu uzerine etkisi. *Turkive I. Bahce Bit. Kong. Cilt II*., 237-240.

Ma YouHui, Kato, K., Masuda, M. (1999). Efficient callus induction and shoot regeneration by anther culture in male sterile mutants of tomato (*Lycopersicon esculentum* Mill cv. First) *J. Japanese Soc. Hort. Sci*., **68** (4): 768-773.

Maheshwari, S.C., Rashid, A. and Tyagi, A.K. (1982). Haploids from pollen grains-retrospects and prospect. *Amererican J. Bot*., 6**9** : 865-879.

Nitsch, J.P. (1969). Experimental androgenesis in *Nicotiana*, *Phytomorphology*, **19** : 389- 404.

Ozzambak, E. (1994). Anther culture of tomatoes and egg plants. *Acta Hort.*, **366** : 229-231.

Summers, W.I., Jaramillo, J., and Bailey, T. (1992). Microspore developmental stage and anther length influence the induction of tomato anther callus. *Hort. Sci*., **27**(7) : 838-840.

Zamir, D., Tanksley, S.D. and Jones, R.A. (1981). Genetic analysis of the origin of plants regenerated from anther tissue of *Lycopersicon esculentum* Mill. *Plant Sci. Lett*., **21** : 223-227.