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ROLE OF GROWTH HORMONES ON THE CALLUS PRODUCTION FROM ANTHERS OF *LYCOPERSICON ESCULENTUM* MILL.

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Attempts were made to obtain the maximal production of callus in the cultured anthers of *L.esculentum* CV PKM. For the induction of the callus, both MS Medium and DBM 1 were found to be suitable; while for sub-culturing MS medium alone showed response. Among the different hormonal combinations 2,4-D with BAP showed a good callus production. The maximum response of the cultures (89%) were noted at the concentration level of 0.5/0.3 mg l^{-1} 2,4-D/BAP; showed fragile, green coloured calli. The anthers at the mononucleate stage, pretreated with 2,4-D (10mg/1) for 6 hrs enhanced the callus induction.Post-treatment of the cultured anthers in the dark for one week did not influence the induction of callus, instead many degenerated pollen grains were noted.

Keywords: Anther; Callus & hormones; Culture;*Lycopersicon esculentum.*

Introduction

Tomato (*Lycopersicon esculentum Mill.*) is an important vegetable crop grown all over the world. The traditional tomato improvement and its multiplication has been performed through conventional breeding techniques. Crop improvement through *in vitro* anther culture is the one, among the recent techniques and it also requires further study. In an attempt to optimize the production of tomato anther derived callus, significant improvement was noted when the concentration and type of growth hormones were modified.The present study in undertaken to standardize the technique for the production of anther callus, especially to determine the effects of growth hormones on the induction of callus.

Materials and Methods

Seeds of *L.esculentum* CV PKM, obtained from Tamil Nadu Agricultural University, Coimbatore, were used for the present study. The seeds were sown in the seed bed and transplanted on 30th day in the experimental field. The flower buds collected from these plants were used as the source material for anther culture.

Flower buds of about 5-11 mm length containing microspores at various stages of cell division were removed from plants of the cultivar. Prior to culture on synthetic medium, the intact flower buds were soaked in 2.4-D(10mg/l) for 6 hrs at 4° C in dark. Thereafter, they were sterilized in 70% ethanol for 10 seconds followed by immersion in a 7% calcium hypochlorite for 5 minutes. The five anthers of each bud were excised and cultured together on MS medium[1] and DBM 1 Medium of Gresshoff and Doy[2] supplemented with various growth adjuvants (Table 1). The media were solidified by using 0.7% agar after adjusting the pH to 5.8. For control the seeds were soaked in distilled water for 6 hrs.

The cultures were kept in the dark for 7 days and were then transferred to

alternating 16 hrs 6000 lux light and 8 hrs dark at 28° ± 2° C. For control the cultures were directly placed under light condition. Observations were recorded 8 weeks after culture and they were tabulated.

Results and Discussion

The results obtained by using different hormonal combination in anther culture are given in Table1. Among these various combinations, IAA with KN was totally ineffective for the induction of callus and in the regenerating anthers, only swollen pollen grains could be seen (0.5/1.5mg/1) (Fig.1). NAA + KN showed limited response i.e. 1mg/1 NAA and 2mg/1 KN showed very little response (8%) whereas the other concentration (2 + 1 mg/l) showed comparatively fair response (17%), the combination of 2, 4-D and BAP enhanced the callus induction and were comparably highest to those obtained with NAA + KN. The calli obtained from these were fragile and green in colour (Fig. 2 & 3). Among this 0.3 3mg/1 2,4-D with 0.1mg/1 BAP showed moderate callus growth whereas, 0.5mg/1 and 0.3mg/1 (2, 4-D and BAP respectively) showed a good callus production and found most suitable for the induction of the callus (Table-1, Fig.2). The two chosen medium MS, DBM 1 showed successful response in the induction and proliferation of the callus. But on subculture, the MS medium was found most suitable then DBM 1, irrespective of their initiation medium.

Further observations made on the anther culture revealed that when the five undivided anthers of a flower bud were placed joined together in the same test tube respond positively for the successful production of callus. Whereas, the anthers placed separately showed only the swollen pollen grains rather than callus (Fig.1) Moreover, succusful callus productions were obtained from the pollen grains at the mononuclecte stage. The mature antheres

Table 1. Comparative responses of isolated anthers of tomato to various growth hormones.

Medium	Hormones	Concentr-ation mg/1	Response	% of cultures showing response
MS/DBM 1	IAA + KN	0.5 + 1.0	No response	—
MS/DBM 1	IAA + KN	0.5 + 1.5	No response (Swollen pollen grains)	—
MS/DBM 1	NAA + KN	1 + 2	Very little response.	08
MS/DBM 1	NAA + KN	2 + 1	Limited callus growth	17
MS/DBM 1	2, 4-D+BAP	0.3 + 0.1	Moderate Callus growth	57
MS/DBM 1	2,4-D+BAP	0.5 + 0.3	Good callus growth	89

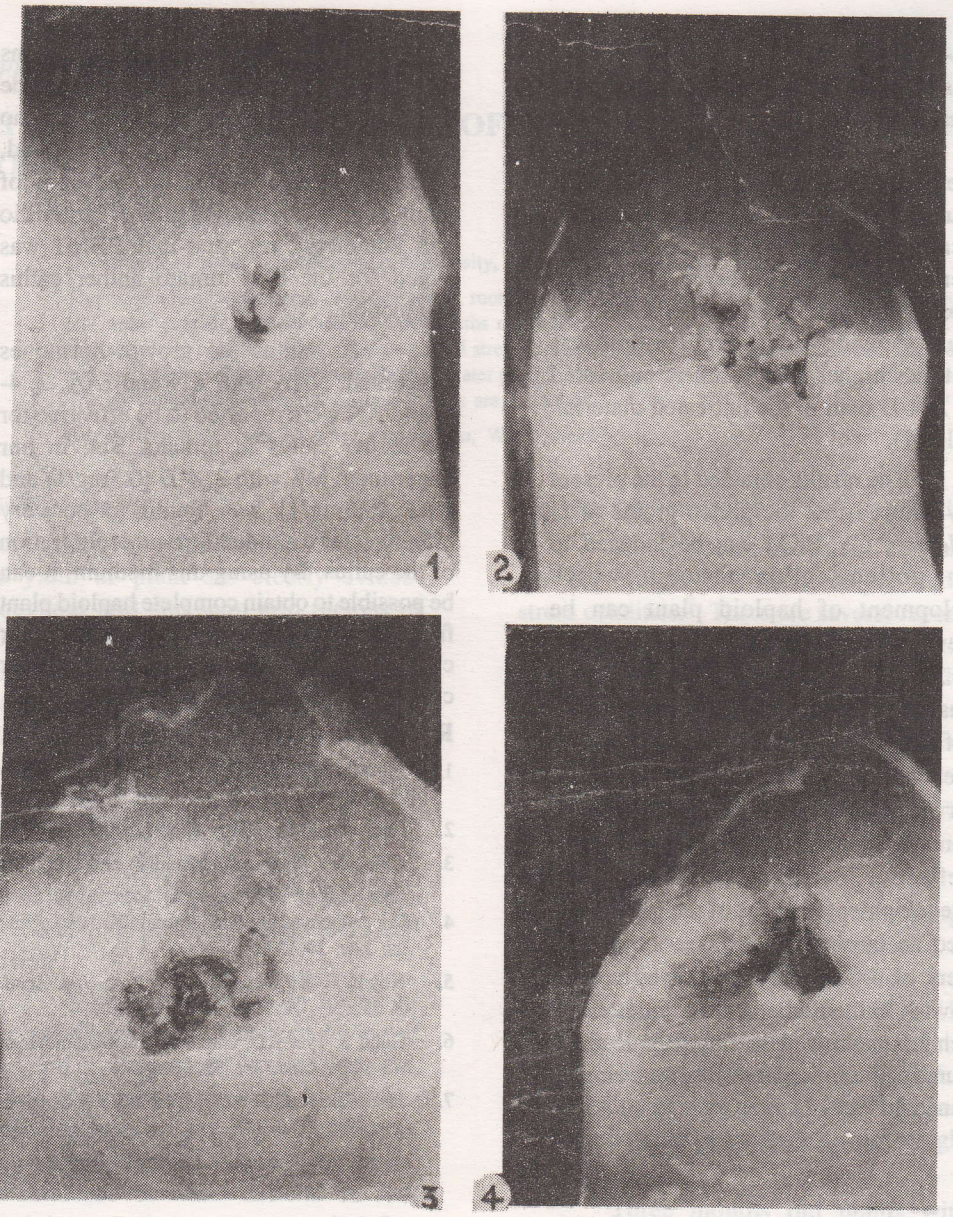

Fig. 1-4 1. Separated anthers showing swollen pollen grains (MS with IAA + KN); 2. Ms with 2, 4-D + BAP. Good callus production showing pale green colour; 3. MS with NAA + KN showing moderate callus growth; 4. Mature anthers showing cream — yellowish callus.

of 4-8 weeks showed cream-yellowish callus and the uninucleate pollen grains produced fragile, pale green coloured callus (Fig. 2 & 4). It was also noted that, the anthers kept in the dark for one week after inoculation did not influence the induction of callus, instead many degenerated pollen grains were observed. It is also important to know that the percentage of anthers showing callus proliferation was four times higher in the anthers pretreated with 2, 4-D than in the untreated material. (Table 1).

The results obtained in the present study showed that the pollen grains of *L. esculentum* CV PKM can be induced to form pollen callus through which development of haploid plant can be achieved[3]. Pretreatment of the flower buds with 2, 4-D had marked influence on the callus proliferation from anthers. The beneficial effect of the pretreatment of flower buds in 2,4-D has been already observed by Cappadocia and Sree Ramulu[4]. In tomato, soaking of the seeds alone was not effective. For the successful cultures, the developmental stages of the pollen also played an important role, i.e., the mature anthers of 4-8 weeks old showed cream-yellowish callus, whereas the uninucleate pollen grains showed the fragile, pale green coloured calli. Smimilar results were already obtained in rice[5,6].

As reported earlier, observations made on tomato anthers, kept under the dark condition for one week showed no response in the callus induction[4]. Instead, further analysis revealed the presence of many degenerated pollen grains. Jaramillio and Summers[7], reported that DBM1 was suited for the best tomato anther callus production.

As far as the growth hormones concerned, NAA with KN and IAA, 2, 4-D and KN were reported to be effective for the anther callus in tomato. But, in our experiment MS with 2, 4-D (0.5mg/1) and BAP (0.3mg/1) was found to be very effective, for the induction and proliferation of the callus. By using this medium, it can be possible to obtain complete haploid plant from this anther callus by using other cultivars also. Further studies on the anther culture is under the investigation.

References

1. Murashige T and Skoog F 1962, *Physiol. Plant,* **15** 473
2. Gresshoff PM and Doy Ch 1972 *Planta* **107** 161
3. Gulshan Y, Varghese TM and Sharma DR 1981, *Biol. Plant.* **23** 414
4. Cappadocia M and Sree Ramulu K. 1980, *Plant Sci. Lett.* **20** 157
5. Niijeki H and Oona K 1961, *Proc. Jpn. Acad,* **44** 554
6. Guha S, Iyer RD, Gupta N and Swaminathan MS 1970, *Curr. Sci.* **39** 174
7. Jaramillio J and Summers W 1990, *J. Amer. Soc. Hort. Sci.* **115** 1047