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INFLUENCE OF GROWTH REGULATORS ON CALLUS INDUCTION IN A LOCAL CULTIVAR OF CUCUMBER (*CUCUMIS MELO*) HYBRID THROUGH ANTHERS CULTURE

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ABSTRACT

Anther culture response of a cucumber hybrid on MS culture media with altered concentrations of growth regulators was evaluated. Anther culture is an efficient and convenient technique for rapid production of doubled haploids which are helpful in crop breeding programs.Callus induction is one of the pathways required for haploid plant regeneration through anther culture. The effect of different combination of growth regulators on anthers of cucumber to induce callus formation were herein evaluated. The developmental stage of anthers were fisrt deterimend by acetocarmine test and then anthers with mid to late uni-nucleate stage were cultured on MS media supplemented with different combination of growth regulators. Among the nine treatments highest percent of callus induction (93.33%) was recorded in treatment T7 containing 5 mg/l BAP and 0.5 mg/l NAA. Whereas, anthers cultured on MS media containing combination of 2, 4-D and NAA failed to induce callus. The present study revealed that combination of different growth regulators play significant role in order to achieve high callus induction.

Key words : PGRs, Callus, Haploids, Anther culture, Double haploids, Cucumber.

Introduction

Cucumber is the important vegetable crop belongs to family cucurbitacea. Development of homozygous line is very crucial for exploitation of heterosis. DH technique can advance the breeding cycles for the isolation of inbreeds within a short period. Haploids can be used to develop the homozygous lines. Haploid plants have the gametic chromosomes number that is a single set of chromosomes in sporophyte. Haploid can be induced by several *in vivo* and *in vitro* techniques. In cucumber, haploids can be obtained *via in situ* parthenogenesis (pollination mostly with irradiated pollen), androgenesis (*in vitro* culture of anthers and microspores) and gynogenesis (*in vitro* culture of ovules and ovaries). These methods have been used for over 30 years to develop haploid and DH lines in cucurbits (Sari *et al.*, 2021). But, anther culture is an efficient method for haploid induction compare to other methods (Bajaj, 1990; Ferrie *et al.*, 1995; Dong *et al.*, 2016). Androgenesis is a process of production of haploids through anther or pollen culture and to date, it has been reported in 135 species. The principle involved in the process is to halt the development of pollen cells into a gamete and induce it in a suitable environment to develop into a haploid plant. Anther culture has been utilized as an important tool for production of homozygous lines by developing haploid plants followed by doubling of chromosome number which bypass the inbreeding process (Germana, 2011). It is the fastest method for homozygous line production as it only takes an year whereas conventional methods takes 5-6 generation of inbreeding for development of homozygous lines (Dong *et al.*, 2016). Unfortunately, low percentages of both callus induction and plant regeneration are the main constraints in establishing successful anther culture in cucumber since, these critical culturing responses are

genotype dependent and also influenced by many other factors like physiological state and condition of plants, stage of pollen development, pre-treatment, *in vitro* culture conditions and plant growth hormones (Germana, 2011; Dong *et al*., 2016). The effective culture medium used for some cucumber varieties may not be appropriate for others, and the composition of culture media should be carefully selected when the anthers of particular cucumber variety was subjected to culture.

The effect of plant growth regulators have been widely investigated in anther culture. The composition of media and PGRs and their combination and concentration play pivotal roles in the induction and differentiation of callus from cultured anthers. However, in a few plant species, including *Nicotiana tabacum* (Nitsch and Nitsch, 1969), *Datura innox* (Sopory and Maheshwari, 1976) and *Hyoscyamus niger* (Raghavan, 1975), embryoid cells have been developed from anthers even in the basal medium devoid of growth regulators. However, many reports are available in which either one or other growth regulator has been found effective for the induction of anthers, calluses and embryos in the majority of plant species. The requirement for type and concentration of growth regulators may differ with genotype, species and within varieties. Xie *et al*. (2005) and Shalaby (2006) found that cucumber and squash genotype significantly affects callus induction ability and they reported that there is a strong genotypical difference in callus induction and plantlets regeneration among the genotypes of cucumber and summer squash. Zhan *et al*. (2009) reported genotype as a key factor in embryoid induction on cucumber microspore culture, where embryoids yield varied from 1.5 to 33.4 embryoids per dish among different genotypes.

Auxin and cytokinin have been widely used for *in vitro* anther culture. The type and concentration of growth regulators in the induction medium determine the specific development pathway of the anther. The induction media containing 2, 4-D promotes callus induction, whereas induction media supplemented with NAA induces direct embryogenesis (Dong *et al*., 2016). Hamidvand *et al.* (2013) studied the effect of plant growth regulators on callus induction in cucumber anther culture and reported that types and concentrations of PGRs show significant impact on callus induction. There is a contrasting response of callus induction with growth regulators and chemical supplements among cucumber varieties. Hence, there is further need to study the influence of factors such as auxin, cytokinin in the media that favor the formation of high quality callus in a shorter time. The objective of this study was to determine the effect of Growth regulators on callogenesis and embryogenesis from cultured anthers of cucumber hybrid.

Materials and Methods

Plant material

The landraces are cultivated throughout in many regions due to their qualitative features and taste of the fruit. Therefore, the production of DH plants in these landraces helps to accelerate the breeding programs for cultivar development.

Local cultivars of cucumber (*Cucumis melo*) were used to develop F1hybrid. This F_1 of cucumber was used as donor plant. The donor plants were grown in a glass house of Department of Biotechnology and crop Improvement, University of Horticultural Sciences, Bagalkot in 2022. Staggered sowing of seeds was followed to make sure that the flower bud would be available for a longer period of time and managed using standard agronomic practices.

Determination of anther developmental stage

For anther culture unopened male flower buds at different stages were collected (Fig. 3A) and buds were measured for length of the flower buds. Then anthers were squashed in 1% acetocarmine solution and stage of the anther was observed under the microscope. Based on the observations, suitable flower buds (1.2 cm-1.4 cm) *i.e* the flower buds with mid to late uninucleate stage of microspore were selected. As Uninucelate stages response is high responsive and the pollen grains are with single nucleus pre mitotic stage.

Pre-treatment and Sterilization,

Male flower buds containing microspores at the mid- to late uninucleate stages (Fig. 3B and 3C) were collected and placed on sterile Petri dishes containing sterile wet filter paper and kept in a refrigerator at 4! for cold pre-treatment. After 2 days, flower buds were surface sterilized using 70 % ethanol for 1 minute, followed by rinsing with sterilized distilled water for 1 minute. Next, the flower buds were shaken gently with 2% sodium hypochlorite for 15-20 minutes and washed with sterilized distilled water for 3 to 4 times to remove the residue of chemical.

Culture conditions and callus induction

Anthers were isolated and inoculated into gamma-ray pre-sterilized Petri dishes of 60 X 15 MM and 90 X 14 MM containing callus induction media supplemented with 2,4-D, BAP, NAA and Kn, then pre incubated at 32! for 24 hours and then transferred to dark room with 25 ± 1^0C till callus induction.

Design and statistical analysis

The experiment was arranged in a completely randomized design with three replications. The observations like *per cent* of callus induction, days to callus induction and weight of the callus were recorded. ANOVA for statistical analysis were computed using the WASP and IBM SPSS software program. *Per cent* values with 0 or 100 were transferred to arcsine and other data's with a zero value were transformed using the square root transformation before they were used in the statistical analysis. The means were compared by Duncan's multiple range test ($p \leq 0.01$).

Results and Discussion

Effect of growth regulators on percent of callus induction

Auxins are essential growth regulators for the induction of callus from anthers, but previous studies have reported that the combination of auxins and cytokinins were more effective for callus induction than auxins alone (Kumar *et al*., 2003). Among the auxins, 2, 4-D and NAA and among cytokinins, BAP and Kn, are the most usually used growth regulators for the callus induction (Dong *et al*., 2016). Hence, in the present study, different combinations of auxins and cytokinins have been used for the induction of callus from the anthers. In the present study, percent of callus induction varied among the treatments from 0% to 91.33%. The per cent of response was recorded after 30 days of inoculation of anthers. MS Media containing 5 mg/l BAP and 0.5 mg/l NAA has produced highest percentage of callus (91.33%) followed by MS medium supplemented with 4 mg/l BAP and 0.5 mg/l NAA (90%) (Table 2). The anthers inoculated on MS media without growth regulators (controls) and also on MS media supplemented with 2, 4-D and NAA combination didn't show any response. The induced the callus was light yellow in colour, watery and friable in nature (Fig. 3D). The highly significant difference was observed among the treatments for per cent of callus induction ($P < 0.01$) (Table 1). The findings are in concord with Thaneshwari *et al*. (2018), who reported the 80.33% of callus induction in marigold when anthers were cultured on MS media containing BAP and NAA combination. Among different combinations of growth regulators, combination of BAP and NAA found more effective for callus induction followed by combination of Kn and NAA. The present finding is in agreement with Usman *et al*. (2015), who concluded that combination of BAP and NAA is more effective for induction of callus in bitter gourd. It probably due to ability of cytokinins to induce cell division provided the auxin was present in the media. BAP and kinetin are often used in plant tissue culture for inducing

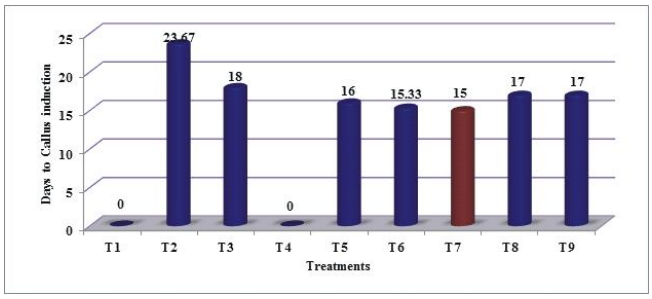

Fig. 1 : Effect of plant growth regulators on days to callus induction from anther culture of cucumber. **Note:** (T_1) MS0 (T_2) MS0+ 1 mg/l 2,4-D, (T_3) MS0+ 1 mg/l 2,4-D + 1 mg/l BAP, (T_4) MS0+ 1 mg/l 2,4-D +1 mg/l NAA, (T_5) MS0+ 3 mg/l BAP +0.5 mg/l NAA, (T_6) MS0+ 4 mg/l BAP + 0.5 mg/l NAA, (T_7) MS0+ 5 mg/l BAP + 0.5 mg/l NAA (T_8) MS0+ 2.5 mg/l Kn + 0.2 mg/l NAA, (T_9) MS0+ 5 mg/l Kn + 0.2 NAA.

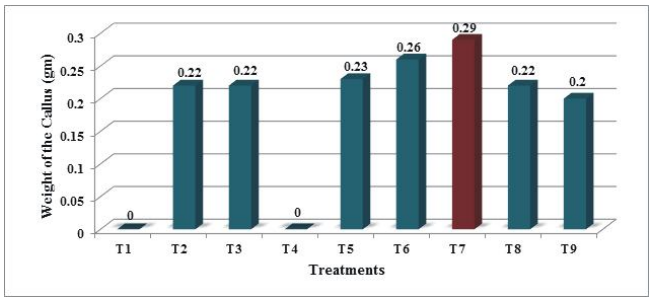

Fig. 2 : Effect of plant growth regulators on weight of the callus from anther culture of cucumber. **Note:** (T_1) MS0 (T_2) MS0+ 1 mg/l 2,4-D, (T_3) MS0+ 1 mg/l 2,4-D + 1 mg/l BAP, (T_4) MS0+ 1 mg/l 2,4-D +1 mg/l NAA, (T_5) MS0+ 3 mg/l BAP +0.5 mg/l NAA, (T_6) MS0+ 4 mg/l BAP + 0.5 mg/l NAA, (T_7) MS0+ 5 mg/l BAP + 0.5 mg/l NAA (T_8) MS0+ 2.5 mg/l Kn + 0.2 mg/l NAA, (T_9) MS0+ 5 mg/l Kn + 0.2 NAA.

Table 1 : ANOVA for effect of growth regulators on callus induction from anther culture of cucumber.

Source	*Df*	MSS		
		% of callus induction	Days to callus induction	Weight of the callus
Treatments	8	2352.158	6.697	0.014
Error	18	1.559	0.005	0.00

formation of callus in conjunction with auxin (Usman *et al*., 2015). In many plant species, including tomato (Brasileiro *et al*., 1999), purple coneflower (Zhao *et al*., 2006), flax (Burbulis *et al*., 2009), marigold (Li *et al*., 2007; Yingchun *et al*., 2011; Ravindra Kumar *et al*., 2019; Taneshwari, 2018), chrysanthemum (Gao *et al*., 2011), pumpkin (Kurtar *et al*., 2016) combination of BAP and NAA was reported as a effective growth regulators for induction of callus from anther culture.

Fig. 3 : Callus induction response *in vitro* in cucumber anthers: (A) Different stage of flower buds collected to determine the developmental stage of microspore (a) 0.6 cm (b) 0.8 cm (c) 1 cm (d) 1.2 cm (e) 1.4 cm (f) opened flower (B) Unopened flower buds of 1.2 – 1.4 cm with mid to late uni nucleate stage of microspore (C) Mid uni-nucleate stage of microspore (D) Induction of callus from anthers cultured on MS0 + 5 mg/l BAP + 0.5 mg/l NAA (T_7) and MS0 + 2.5 mg/l Kn + 0.2 mg/l NAA.

Table 2 : Effect of growth regulators on callus induction from anther culture of cucumber.

Treatment Code	Induction media	% of callus induction
T_1	MS0 (Control)	0 (1.65)^e
T_2	MS0 + 1 mg/l 2, 4-D	17.00 (24.34)^d
T_3	MS0 + 1 mg/l 2, 4-D + 1 mg/l BAP	64.33 (53.33)^c
T_4	MS0 + 1 mg/l 2, 4-D +1 mg/l NAA	0.00 (1.65)^e
T_5	MS0 + 3 mg/l BAP +0.5 mg/l NAA	76.00 (60.71)^b
T_6	MS0 + 4 mg/l BAP + 0.5 mg/l NAA	90.00 (71.62)^a
T_7	MS0 + 5 mg/l BAP + 0.5 mg/l NAA	91.33 (72.92)^a
T_8	MS0 + 2.5 mg/l Kn + 0.2 mg/l NAA	68.67 (54.94)^c
T_9	MS0 + 5 mg/l Kn + 0.2 NAA	72.67 (58.69)^b

Days to callus induction and weight of the callus

The days to callus induction was recorded when the 50% of cultured anthers showed response. There is significant difference among the treatments at 1% level of significance for days to callus induction (Table 1). Minimum days for callus induction (15 days) was reported in MS media supplemented with 5 mg/l BAP and 0.5 mg/l NAA (T_7) followed by (15.33 days) MS medium containing 4 mg/l BAP and 0.5 mg/l NAA (T_6) (Fig. 1). The potentiality of BAP in combination with auxin results in increasing rate of cell division may result in early callus initiation. This finding was in close agreement with the finding of Thaneshwari *et al*. (2018), who reported that MS Medium supplemented with 4.44 µM BAP and 1.07 µM NAA took minimum days for callus induction (16.67 days) in marigold. Significantly maximum weight of the callus (0.29 gm) was observed in MS medium supplemented with 5 mg/l BAP and 0.5 mg/l NAA (Fig. 2). This may be due to higher cell dedifferentiation and protein synthesis resulting in maximum callus weight (Prabhuling *et al*., 2018).

Conclusion

In conclusion, the effectiveness of callus induction depends on concentration and combination of growth regulators. The results revealed that *in vitro* cultured anthers of cucumber responded differently according to the growth regulators added to the culture medium. A combination of BAP and NAA proved to be an effective PGR combination. Maximum callus induction (99.33%) was obtained on MS medium supplemented with 5mg/l BAP and 0.2 mg/l NAA with significantly higher percent of callus induction, min days to callus induction and max weight of the callus. These findings will be immense value in the application of *in vitro* androgenesis for cucumber crop improvement. In future induced callus can be used for the regeneration of haploids and double haploids in cucumber by further standardizing the regeneration media.

Declaration

The authors should declare that they do not have any conflict of interest.

Authors contribution

Prajwala P: Conduct of experiment, Phenotyping and data nalysis

Rekha Chiitapur: Planning and Execution, Manuscript preparation and Data analysis

Prabhuling G: Guidance for Invitro culturing of anthers Analysis of data

Namita Raut : Data interpretation

Mallikarjun Awati : Manuscript preparation

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References

Bajaj, Y.P.S. (1990). *In vitro* production of haploids and their use in cell genetics and plant breeding. In : Bajaj, Y.P.S. (ed) *Haploids in crop improvement I*. Springer, Berlin, p. 3–44.

Burbulis, N., Blinstrubiene A., Kupriene R. and Zilenaite L. (2009). Effect of genotype and medium composition on flax (*Linum usitatissimum* L.) anther culture. *Agron. Res*., **7**, 204-209.

Dong, Y.Q., Zhao W.X., Li X.H., Liu X.C., Gao N.N., Huang J.H., Wang W.Y., Xu X.L. and Tang Z.H. (2016). Androgenesis, gynogenesis and parthenogenesis haploids in cucurbit species. *Plant Cell Reports*, **35**, 1991-2019.

Ferrie, A.M.R. and Caswell K.L. (2010). Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production. *Plant Cell, Tissue and Organ Culture*, **104**, 301–309.

Gao, Y., Chen B. and Zhang J. (2010). Anther culture of garden Chrysanthemum. *XXVIII International Horticultural Congress on Science and Horticulture for People*. p. 103-110.

Germana, M.A. (2011). Gametic embryogenesis and haploid technology as valuable support to plant breeding. *Plant Cell Reports*, **30**, 839–857.

Hamidvand, Y., Abdollahi M.R., Chaichi M. and Moosavi S.S. (2013). The effect of plant growth regulators on callogenesis and gametic embryogenesis from anther culture of cucumber (*Cucumis sativus* L.). *Int. J. Agricult. Crop Sci*., **5**, 1089–1095.

Kumar, H.G.A., Murthy H.N. and Paek K.Y. (2003). Embryogenesis and plant regeneration from anther cultures of *Cucumis sativus* L. *Scientia Horticulturae*, **98**, 213–222.

Kumar, K.R., Singh K.P., Bhatia R., Raju D.V.S. and Panwar S. (2019). Optimising protocol for successful development of haploids in marigold (*Tagetes* spp.) through *in vitro* androgenesis. *Plant Cell, Tissue and Organ Culture*, **138**, 11-28.

Kurtar, E.S., Balkaya A. and Kandemir D. (2016). Evaluation of haploidization efficiency in winter squash (*Cucurbita maxima* Duch.) and pumpkin (*Cucurbita moschata* Duch.) through anther culture. *Plant Cell, Tissue and Organ Culture* (*PCTOC*), **127**, 497-511.

Li, Fu (2007). Identification of the microspore development stages and study of anther vitro culture on *Tagetes erecta*. *Thesis* submitted to Qinghai University, China (Abstract).

Nitsch, J.P. and Nitsch C. (1969). Haploid plants from pollen grains. *Science*, **163**, 85-87.

Prabhuling, G. and Huchesh H. (2018). *In vitro* regeneration in pomegranate (*Punica granatum* L.) cv. Bhagwa using double nodal segments. *Res. J. Biotechnol.*, **13**, 8.

Raghavan, V. (1975). Induction of haploid plants from anther cultures of henbane. *Zeitschrift für Pflanzenphysiologie*, **76**, 89-92.

Sari, N. and Solmaz I. (2021). Doubled Haploid Production in Watermelon. In : Segui-Simarro, J.M. (eds) *Doubled Haploid Technology*. Humana, New York, p. 97-110.

Shalaby, T.A. (2006). Embryogenesis and plantlets regeneration from anther culture of squash plants (*Cucurbita pepo* L.) as affected by different genotypes. *J. Agricult. Res. Tanta Univer.*, **32**, 173-183.

Sopory, S.K. and Maheshwari S.C. (1976). Development of pollen embryoids in anther cultures of *Datura innoxia*: II. Effects of growth hormones. *J. Exp. Botany*, **27**, 58-68.

Thaneshwari, Aswath C., Rao T.M., Rajiv K., Tejaswini P. and Leela S. (2018). Induction of embryogeny and plant regeneration through induced androgenesis/ gynogenesis in marigold (*Tagetes* spp. L.) *Ph.D (Horti.) Thesis*, Indian Agricultural Research Institute, New Delhi.

Usman, M., Bakhsh K., Fatima B., Zaman Q. and Shah M.H. (2015). Exploring embryogenic competence in anthers of Bitter gourd (*Momordica charantia* L.) cultivar Faisalabad long. *The J. Anim. Plant Sci*., **25**, 181-188.

Xie, M., Qin L.Y., Pan J.S., He H.L., Wu A.Z. and Cai R. (2005). Flower morphogenesis and microspore development versus anther culture of cucumber. *Acta Botanica Boreali-Occidentalia Sinica*, **25**, 1096–1100.

Yingchun, Q., Yaomei Y. and Manzh B. (2011). Establishment of plant regeneration system from anther culture of *Tagetes patula*. *Afr. J. Biotechnol.*, **10**, 17332-17338.

Zhan, Y., Chen J.F. and Malik A.A. (2009). Embryoid induction and plant regeneration of cucumber (*Cucumis sativus*L.) through microspore culture. *Acta Horticulturae Sinica,* **36**, 221–226.

Zhao, F.C., Nilanthi D., Yang Y.S. and Wu H. (2006). Anther culture and haploid plant regeneration in purple coneflower (*Echinacea purpurea* L.). *Plant Cell, Tissue and Organ Culture*, **86**, 55-62.