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BIOCHEMICAL ANALYSIS OF IN VITRO INDUCED CALLUS OF CUCUMIS ANGURIA L.

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ABSTRACT

Among the flowering plants, the members of cucurbitaceae are duly useful for human being that is agood source of vegetables and also a better resource for phytochemical compounds. The present study was aimed to induce the callus from *in vitro* grown seedling explants. The effect of BAP and NAA have been investigated, the combination of BAP and NAA results in the higher callus induction. The callus was further analyzed for biochemical changes at 15 and 30 days of interval. The steep increase in phenol in 30 days callus compared to 15 days old callus, which could be one of the reason for browning of callus.

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INTRODUCTION

Cucurbits are vegetable crops (Cucurbitaceae), comprising of about 130 genera and more than 900 species of which only a few are cultivated (Jeffrey, 1962). In India, a number of cucurbits are cultivated in several commercial cropping systems and also as popular kitchen garden crops. Cucurbits share about 5.6% of the total vegetable production of India and are highly utilized for culinary purposes. The fruits contain rich amount of vitamins, iron and minerals, phosphorous and presents good dietary fiber levels. Recent development in biotechnology has opened up several avenues for cucurbit breeding using genetic transformation, in which heterologous genes can be introduced into existing cultivars (Sorowar et al., 2003). Micro propagation and shoot regeneration protocols for cucumber are required to decrease the cost of hybrid seed production which is usually higher than 30% of total seedling cost (Konstas and Kintzios, 2003). Regeneration of cucumber plants have been reported with limited success either directly or indirectly on various explants (Ziv,1992). Poor development of embryos, low differentiation of callus into shoots and poor survival rate of plants after acclimatization has been reported earlier (Ziv&Gadasi, 1986; Kim *et al*., 1988; Ziv, 1992). Alsop *et al*., (1978) obtained only callus from several organ explants with various concentrations of NAA and BAP, each. Aziz *et al*., (1986) also described bud-like nodules on callus derived from internode pieces of cucumber, but they could induce root formation only. However, callus from cotyledons was characterized by proliferation of fibrous roots whereas callus derived from hypocotyls did not (Novak & Dolezeloa, 1982).

Normally in *Cucumis anguria*, the seed setting and seed germination is low, probably due to the presence of a thin nucellar membrane lending impermeability to water and gases and make them dormant for many days (Devendra*et al.,* 2008).Biotechnological approaches,specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants (Rao&Ravishankar, 2002). The capacity for plant cell, tissue and organ cultures to produce and accumulate many of the same valuable chemicalcompounds as the parent plant in nature, has been recognized almost since the inception of *in vitro* technology.The strong and growing demand in today's marketplace for natural, renewable products has refocused attention on *invitro* plant materials as potential factories for secondaryphytochemical products (Karuppusamy, 2009). In the present investigation, an attempt was made to evaluate the choice of auxin, cytokinin for callus induction and the biochemical analysis (Aminoacids, Protein, Starch and Phenol) of various stages of callus, in order to understand the biochemical changes during the development. We believe that our findings could facilitate the understanding of problems associated with the callus maturity and organogenesis of callus of this commercially important vegetable. It may also helpful to generate reproducible regeneration system in *C. anguria* and other related species.

MATERIALS AND METHODS

Explant sources and sterilization procedures

To raise aseptic seedlings, mature seeds were washed in running tap water for three minutes and then washed repeatedly in double distilled water. Under aseptic conditions the seeds were surface sterilized with 70% ethanol for one minute followed by a twenty minute treatment with 2% sodium hypochloride and washed with sterilized triple distilled water five times followed by 0.1% Mercuric chloride (HgCl2) for five minutes and rinsed five times in sterile distilled water. The sterilized seeds were then placed on MS basal medium (Murashige and Skoog1962) solidified with 0.8% agar for germination in 250 ml culture bottles,20 seeds were cultured per bottle containing 30 ml of medium. This was incubated in dark at 26^{o}C till it germinated and then transferred to cool-white-fluorescent light room and incubated at 24±2^{o}C and allowed to

grow. The plant after reaching a height of 8 – 10 centimeters was taken in an aseptic condition and primary leaves were excised using a sterile scalpel and cut into 6-8 mm sections. The plant parts excised and used as explant for further callus induction experiment.

Media composition and sterilization

Murashige&Skoog (1962) basal medium was usedfor seed germination and callus induction of C. anguria. MS medium with different concentrations of (0.5,1, 1.5 and 2 mgL^{-1}), BAP and NAA separately (0.5,1, 1.5 and 2 mgL^{-1}), and incombinations of BAP and NAA (0.5+0.5, 1.0 + 1.0 mgL-1, respectively). Media were sterilized properly in an autoclave at 15 psi pressure for 20 minutes.

Culture conditions

After inoculation, the cultures were maintained in the growth room at 25 ± 2°C with 2500 lux of light intensity.

Data collection

For each treatment, a minimum of 24 cultures were raised and each experiment was repeated at least thrice. The cultures were examined periodically and the morphological changes were noted on the basis of visual observations. The results have been expressed as percentage of responding cultures.

RESULTS AND DISCUSSION

***In vitro* seed germination and CALLUS induction**

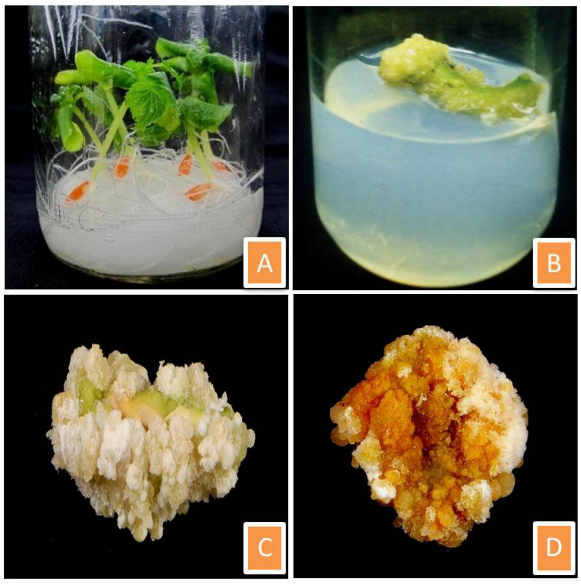
Seeds of *Cucumis anguria* responded positively for germination *in vitro* when cultured on MS medium. *C.anguria* seeds (58%)were germinated in12-15 days of culture. The leaves derived from *in vitro* raised 15 to 20 days old seedlings were used as explants for callus induction (Fig.1). On MS medium containing BAP(80.00%)increased callus induction percentage in all the concentrations tested compared to NAA(76%), however, the Combinations of BAP (1.0 mgL^{-1}) and NAA (1.0 mgL^{-1}) had significantly increased the callusing percentage at maximum of 92.00% (Table 1). Increased concentrations of PGRs showed increased in callus induction percentage, but it resulted in the browning of callus. Over all among different concentrations tested for callus induction, the highest callus induction was observed in MS medium containing NAA and BAP. Our results are in accordance with the findings of Kim *et al*.,(1988) and Ali *et al*., (1991) who induced callus from cotyledon of Burpless hybrid cucumber. The findings of Garcia-Sogo (1990), Lou &Kako (1994) and Ladyzynski*et al*., (2001) who developed an efficient method for obtaining callus from cotyledon, hypocotyls and first leaves in cucumber are in agreement as significantly higher calli induced on higher levels of 2,4-D.However, our results are in conformity with the findings of Alsop *et al*., (1978) and Punja *et al*., (1990) who observed callus induction from cotyledon explant. Our findings are further supported by the observations of Gambley& Dodd (1990) who obtained callus from cv. Crystal Salad hybrid cucumber. Punja *et al*., (1990) and Gambley& Dodd (1990) who obtained genotypic differences for callus induction from cv. Crystal Salad hybrid cucumber and this may be attributed to the use of different genotypes for callus induction.

The quality of the callus was assessed after 3 weeks of culture by assessing its morphology. The combination of NAA and BAP produced brownish compact callus (Figure 1.D) with high callusing response (92.00%). Nabi et al. (2002), Devendra et al. (2009) and Selvaraj et al. (2006) found that the combination of BAP with NAA or 2,4-D produced organogenic callus in *Momordicadioica* and *Cucumis sativus*. The combination of 7.7 µM NAA with 2.2 µM TDZ produced greenish compact callus from leaf explants of *M. charantia*. Handley and Chambliss (1979) reported that NAA and Kn combination in MS medium produced nodular compact callus in cucumber. Selvaraj et al. (2006) obtained nodular, greenish compact and organogenic callus in the presence of 2,4-D and BAP for hypocotyl explants of cucumber. Punja et al. (1990), Seo et al. (2000) and Selvaraj et al. (2007) reported callus formation in cucumber cultivars in the combination of NAA and BAP for petiole, leaf and cotyledon explants, respectively.

As per earlier reports in cucurbits, cotyledons were foundto be more regenerative than other parts in *Cucumis sativus*(Custers et al. 1980, Kim et al.1988) and *Cucumis melo*(Trulson and Shahin 1986, Bouabdallah and Branchard 1986). *Cucurbita* spp. are one of the most widely studied groups of the family Cucurbitaceae and several workers have reported high frequency regeneration from cotyledon explants of several varieties (Ananthakrishnan et al. 2003, Kathiravan et al. 2006) but no work has been done as yet on Indian varieties of Cucurbita.

An important factor affecting morphogenic response in the Cucurbitaceae is the type of explant used for callus induction. To understand the problem associated with the browning of the callus, we intended to understand the biochemical changes like aminoacid, total protein, starch and phenol. The callus samples (15 & 30 days) induced by the combination of BAP and NAA were taken for the analysis. The results were clearly shown that the amount phenol was drastically increased in 30 days old callus than the 15 days old callus(Fig. 2). The increased phenol has direct relation to the intensity of browning callus. This browning of callus is reduce the further regeneration capacity of cells.

Fig. 1 : Callusing of *Cucumis anguria*



1.A: *In vitro* Explant source of *Cucumis anguria*
1.B: BAP induced callus (15 days)
1.C: NAA induced callus (15 days)
1.D: BAP + NAA induced callus (15 days)

Table - 1
Effect of NAA and BAP on callus induction of Cucumis anguria

PlantGrowth Regulator	Concentration (mgl^{-1})	No. of Explants Callusing/inoculated	% of callusing
NAA	0.5	9/25	36
	1.0	13/25	52
	1.5	16/25	64
	2.0	19/25	76
BAP	0.5	11/25	44
	1.0	14/25	56
	1.5	18/25	72
	2.0	20/25	80
NAA+BAP	0.5+0.5	18/25	72
	1.0+1.0	23/25	92

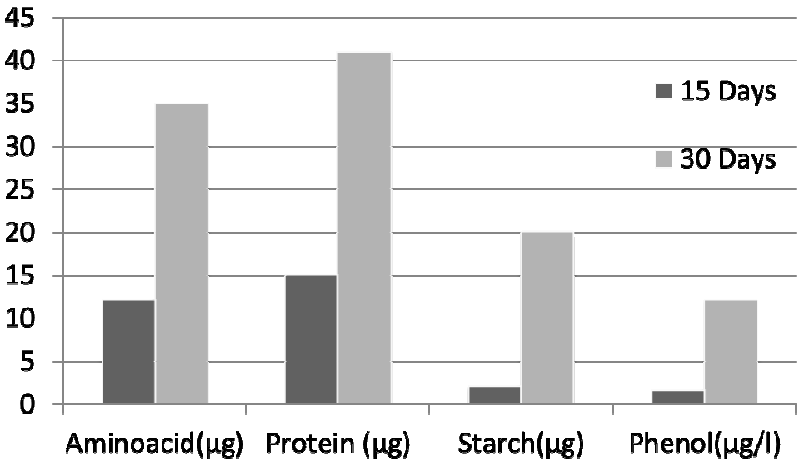


Fig. 2: Biochemical changes of callus at 15 and 30 days of interval

CONCLUSION

Plant tissue culture is an important frontier area in plant biotechnology and to support the production of phytochemical compounds in laboratory conditions. The vitro derived phytochemicals has many advantages than the synthetic production. The present study may reveal the problems associated with organogenesis in *Cucumisanguria*.

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