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BRIEF COMMUNICATION

Glutamine: a suitable nitrogen source for enhanced shoot multiplication in *Cucumis sativus* L.

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Abstract

Shoot tip explants of cucumber (*Cucumis sativus* L. cv. Poinsett 76) were cultured *in vitro* on Murashige-Skoog medium with L-glutamine, ammonium nitrate, adenine sulphate, asparagine, ammonium succinate, potassium nitrate and sodium nitrate as the nitrogen sources along with optimal concentration of 0.044 mM benzyladenine to study their effects on *in vitro* morphogenesis. The explants grown with 0.068 mM L-glutamine displayed the highest culture response (74.6 %) and greatest shoot number per explant (13.6) at the end of two subcultures. The explants cultured with other nitrogen sources resulted in low culture frequency and low number of shoots per explant accompanied by basal callusing and necrosis.

Additional key words: benzyladenine, cucumber, shoot tip explants.

The importance of the culture medium especially the contents of cytokinins and auxins in callus induction, organogenesis and multiplication has been demonstrated for a large number of plant species. However, the variation in hormone ratios cannot be the sole mechanism controlling *in vitro* developmental processes. Preece (1995) suggested that optimization of the mineral component of the culture medium would reduce the concentrations of plant growth regulators required. Influence of the absolute and relative amounts of nitrate and ammonium on the induction and differentiation of plant cell cultures have been reported for a number of *in vitro* systems (Halperin and Wetherell 1965, Wetherell and Dougall 1976, Chaleff 1983, Grimes and Hodges 1990, Cousson and Tran Thauh Van 1993). Reinert *et al*. (1967) observed that the concentration of nitrogen and the relative amounts of ammonium and nitrate in the culture medium would be critical for growth and morphogenesis of plant cells. The present study was undertaken to investigate the effects of various nitrogen sources in *in vitro* morphogenesis of cucumber cv. Poinsett 76 for the first time.

Seeds of cucumber (*Cucumis sativus* L.) cv. Poinsett 76 (Indo-American hybrid seeds *Pvt Ltd*, Bangalore, India) were soaked in tap water for 15 min disinfected with 70 % alcohol for 1 min and 2.5 % (v/v) commercial bleach '*Teepol*' (5.25 % sodium hypochlorite; *Reckit & Benckiser of India Ltd*, Kolkatta, India) for 15 min followed by three rinses with sterile distilled water. Seeds were further disinfected by soaking in 0.1 % mercuric chloride (m/v) for 3 min and rinsed four times with sterile distilled water. Disinfected seeds germinated in darkness for 48 h in 25 × 150 mm test tubes (*Borosil*, Mumbai, India) containing sterile moist cotton and then cultured at an irradiance of 30 μmol m^{-2} s^{-1} with 16-h photoperiod, and temperature of 25 ± 2 ºC. Shoot tips (5 mm length) from 5-d-old *in vitro* grown seedlings were excised and used as explants.

The explants were inoculated in 25 × 150 mm test tubes containing 10 cm^3 of MS medium (Murashige and

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Abbreviations: BA - 6-benzyladenine; MS medium - Murashige and Skoog medium.
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Skoog 1962), containing 3 % sucrose, 0.8 % agar and 0.044 mM benzyladenine (BA). To this, different concentrations of nitrogen compounds were used individually: 0.034, 0.068, 0.102 mM L-glutamine, 0.062, 0.124, 0.187 mM ammonium nitrate, 0.013, 0.027, 0.040 mM adenine sulphate, 0.033, 0.066, 0.099 mM asparagine, 0.053, 0.106, 0.159 mM ammonium succinate, 0.049, 0.098, 0.148 mM potassium nitrate and 0.058, 0.117, 0.176 mM sodium nitrate (all compounds from *Himedia Laboratories Pvt Ltd*, Mumbai, India) (shoot induction medium). The pH of the medium was adjusted to 5.8 before autoclaving at 1.06 kg cm^{-2} (121 ºC) for 20 min. The cultures were maintained for 4 weeks to shoot initiation under the same conditions as mentioned above. MS medium containing BA (0.044 mM) alone served as control.

Explants initiated shoot buds at the apical region of shoot tip explants after 1 week of initial culture in shoot induction medium were subcultured in MS medium containing the same concentration of BA and nitrogen sources as mentioned above. The explants were transferred to fresh medium of same composition at 10-d intervals. Two subcultures were done at a period of 4 weeks each.

Each treatment consisted of at least 20 explants and each experiment was conducted thrice. A complete randomized design was used in all experiments and analysis of variance and mean separations were carried out using Duncan's Multiple Range Test (DMRT) with significance determined at 5 % level (Gomez and Gomez 1976).

Bud-like protuberances appeared from the apical region of 5-d-old shoot tip explants after a week on MS medium containing 0.044 mM BA and different nitrogen sources. Maximum frequency (74.6 %) of bud-like protuberances was recorded in explants inoculated in MS medium fortified with 0.044 mM BA and 0.068 mM L-glutamine. In this medium, explants responded most favourably to shoot bud induction with minimal necrosis (< 10 %). Shoot bud initiation (64.8 %) was also good in MS medium supplemented with 0.124 mM of ammonium nitrate. However, the induction was delayed beyond three weeks. Moderate shoot bud initiation response (52.8 % and 41.8 %) was recorded in MS medium with adenine sulphate (0.027 mM) and asparagine (0.066 mM), respectively, along with the appearance of basal callusing of explants. Other nitrogen sources evoked low frequency of shoot bud induction (Table 1). Shoot bud initiation did not occur either in MS basal medium alone or in the presence of any nitrogen sources without BA (data not shown).

Shoots regenerated from shoot buds were transferred

Table. 1. Effect of different nitrogen sources on multiple shoot induction from 5-d-old shoot tip explants of cucumber cv. Poinsett 76 on MS + BA (0.044 mM) after initial culture (1 week) and two subcultures of 4 weeks each. Mean ± SE of three replicates with 20 explant per treatment. Means with different letters are significantly different according to Duncan′s Multiple Range Test (DMRT) at 5 % level.

Nitrogen sources	[mM]	Initial culture Culture response [%]	Number of shoot [explant^{-1}]	1st subculture Number of shoot [explant^{-1}]	2nd subculture Number of shoot [explant^{-1}]
L-glutamine	0.034	58.4 ± 0.32c	4.2 ± 0.16c	8.4 ± 0.16b	12.4 ± 0.16c
	0.068	74.6 ± 0.16a	5.6 ± 0.48a	10.4 ± 0.48a	13.6 ± 0.16a
	0.102	43.6 ± 0.32fg	3.4 ± 0.32de	7.2 ± 0.16d	8.6 ± 0.16f
Ammonium nitrate	0.062	48.4 ± 0.20e	3.2 ± 0.16e	6.4 ± 0.16e	9.4 ± 0.16d
	0.124	64.8 ± 0.16b	5.0 ± 0.32a	8.2 ± 0.16b	10.6 ± 0.16b
	0.187	34.6 ± 0.48h	2.6 ± 0.48f	5.2 ± 0.16g	7.2 ± 0.16gh
Adenine sulphate	0.013	43.6 ± 0.43fg	2.0 ± 0.16h	3.8 ± 0.16h	7.2 ± 0.16g
	0.027	52.8 ± 0.58d	3.6 ± 0.16d	5.8 ± 0.16i	8.6 ± 0.48e
	0.040	22.4 ± 0.58lm	1.4 ± 0.09l	2.6 ± 0.48k	5.2 ± 0.16j
Asparagine	0.033	32.4 ± 0.16ij	1.2 ± 0.16jk	1.6 ± 0.48m	4.2 ± 0.43jk
	0.066	41.8 ± 0.48g	2.4 ± 0.16fg	3.2 ± 0.86ij	5.8 ± 0.81i
	0.099	14.8 ± 0.32o	1.0 ± 0.71k	1.2 ± 0.48n	3.4 ± 0.43lm
Ammonium succinate	0.053	24.6 ± 0.32kl	0.8 ± 0.32kl	1.0 ± 0.16l	2.8 ± 0.16mn
	0.106	32.8 ± 0.16hl	1.8 ± 0.16hi	2.4 ± 0.16hi	3.8 ± 0.58k
	0.159	10.6 ± 0.32l	0.6 ± 0.16l	0.6 ± 0.16m	2.0 ± 0.32no
Potassium nitrate	0.049	16.8 ± 0.16n	0.6 ± 0.16m	0.8 ± 0.32o	2.2 ± 0.16n
	0.098	24.4 ± 0.32l	1.2 ± 0.16j	1.6 ± 0.16m	2.8 ± 0.16l
	0.148	8.2 ± 0.16q	0.4 ± 0.16mn	0.4 ± 0.16p	1.6 ± 0.16o
Sodium nitrate	0.058	11.4 ± 0.16p	0.4 ± 0.16l	0.4 ± 0.16p	1.0 ± 0.16o
	0.117	16.6 ± 0.16no	0.6 ± 0.16m	0.8 ± 0.16o	1.6 ± 0.16no
	0.176	6.4 ± 0.32qr	0.2 ± 0.01m	0.2 ± 0.16pq	0.8 ± 0.16p

to MS medium supplemented with 0.044 mM BA with different nitrogen sources. Maximum number of shoots developed from shoot buds two weeks after transfer to MS medium with 0.068 mM L-glutamine, while moderate number of shoots formed at the same period in MS medium with 0.124 mM ammonium nitrate. Shoot multiplication occurred at lower frequency when adenine sulphate, asparagine and ammonium succinate were used separately in MS medium. Addition of potassium nitrate and sodium nitrate in MS medium was not suitable for shoot bud differentiation (Table 1). Further, the explants developed basal callusing, exhibited necrosis and turned brown in cultures containing higher concentrations of these nitrogen sources. In the present study, MS medium containing BA (0.044 mM) alone (control) evoked shoot regeneration but the frequency of culture response and number of multiple shoots/explant were enhanced only in its combination with L-glutamine.

The shoot tip explants with young shoots were transferred to MS medium containing BA (0.044 mM) with any one of nitrogen sources after 4 weeks of first transfer. Shoot regeneration frequency (74.6 %) and average number of regenerated plants per explant (13.6 per explant) increased marginally during the second transfer with the maximum response scored in MS medium supplemented with BA (0.044 mM) and L-glutamine (0.068 mM) (Table 1). A total of 13 plants was produced per shoot tip explant after the end of second transfer. Further sub-culture was stopped as there was no increase in the number of shoots.

In the present study, maximum shoot regeneration response with highest number of shoots per explant occurred in MS medium fortified with optimal BA concentration (0.044 mM) (Vasudevan *et al.* 2001) and 0.068 mM L-glutamine. Selvaraj *et al*. (2002) also reported the influence of L-glutamine on high frequency shoot multiplication from single node explants of field grown cucumber plants of cv. Poinsett 76. The enhancement of growth rate by L-glutamine could be explained on the basis that L-glutamine provided a readily available source of nitrogen, the implication being that the formation of necessary carbon skeleton or the reduction of nitrate to ammonia is a limiting factor in the cells (Gamborg 1970). Addition of L-glutamine, which is relatively non-toxic (Gamborg *et al.* 1968) would enable the cells to maintain a high growth rate for a longer period. In tissue culture, this can be accomplished by adding organic nitrogen into the medium as observed in the optimization of the polyembryogenesis using oak callus tissue (Nuutila *et al*. 2000).

Locy and Wehner (1982) reported that the MS nitrogen source was not the best one to use for tissue culture experiments in cucumber in which shoot growth, but not the callus growth, was desired. They suggested that the best nitrogen source for the growth of cucumber shoots in culture was asparagine. In their investigation, the nitrate and ammonium nitrogen sources produced only moderate shoot frequency. In our investigation, it was glutamine and not asparagine which induced maximum shoot regeneration and inorganic nitrate or ammonium source produced less number of shoots per explant. In addition, the explants developed necrosis which suppressed shoot multiplication. This is in agreement with Khanna and Raina (1997) that nitrate or ammonium as sole nitrogen source is not sufficient for efficient shoot regeneration in rice. While at high nitrate concentration, accumulation of nitrite has been attributed as the cause for necrosis and poor regeneration, with ammonium the problem appears to be one of the acidification of medium that accompanies NH_4^+ uptake.

Tazawa and Reinert (1969) demonstrated that plant regeneration ability in carrot cell cultures was strongly influenced by the quality as well as the form of nitrogen provided. For morphogenesis of shoot tip explants in cucumber, glutamine appears to be the ideal source of nitrogen for highest morphogenetic response. Species-specific nitrogen requirements have already been described for a wide range of different culture systems (Selby and Harvey 1990). Similar requirements are to be investigated for different cucumber cultivars.

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