Journal of Spices and Aromatic Crops
Vol. 14 (1) : 51-55 (2005)

Indian Society for Spices

# Callus culture and regeneration from root tip of garlic (*Allium sativum* L.)

Anil Khar, A Asha Devi & K E Lawande

*National Research Centre for Onion and Garlic*
*Rajgurunagar, Maharashtra – 410 505, India.*
*E-mail: kashir@sify.com*

Received 17 August 2004; Revised 03 January 2005; Accepted 21 February 2005

**Abstract**

Callus induction and subsequent plant regeneration was studied in a commercial Indian cultivar of garlic, cv. G-41 (short-day type). 2,4-dicholorophenoxyacetic acid at lower concentration (0.25 mg $l^{-1}$) and picloram at higher concentration (1.0 mg $l^{-1}$) were suitable for efficient callus formation from the root tips. Callus was obtained only from the apical portions of root-tips treated with 2,4-dicholorophenoxyacetic acid whereas picloram led to callus formation throughout the root segment except the non apical (subjacent) portion. Callus obtained from induction medium containing both auxins and cytokinins responded well to regeneration. The average shoot regeneration frequency ranged from 16.7% to 50.0%. The best combination for callus culture and regeneration was callus formation in root tip medium 2 (CRT 2) (2,4-dicholorophenoxyacetic acid 0.25 mg $l^{-1}$ + 6-benzylaminopurine 1.0 mg $l^{-1}$) induction medium followed by garlic regeneration medium 3 (GR 3) (kinetin 1.0 mg $l^{-1}$) as the regeneration medium. In other regeneration media, profuse root formation and appearance of dark green callus was also observed.

**Key words**: *Allium sativum*, callus induction, garlic, shoot regeneration.

**Abbreviations**: BA: 6-Benzylaminopurine, CRT: Callus formation in root tip medium, 2,4-D: 2,4-Dicholorophenoxyacetic acid, GR: Callus regeneration medium, KIN: Kinetin, MS: Murashige and Skoog (1962), Pi: Picloram, RG: Callus regeneration medium.

Garlic (*Allium sativum* L.) is a vegetatively propagated crop that does not set seed under normal growing conditions in India. Due to its sexually sterile nature, garlic breeding has been limited to clonal selection of land races or spontaneous mutants. However, development of new garlic cultivars has become increasingly feasible now by sexual hybridisation (Kamenetsky & Rabinowitch 2001) or by genetic transformation (Kondo *et al.* 2000). Genetic transformation or development of somaclonal variants depends upon the availability of highly efficient callus production and regeneration systems. Regeneration via somatic embryogenesis from calluses of bulb, leaf disc, stem tip, leaf, receptacle and flower bud and direct organogenesis or embryogenesis from shoot tip or stem disc explants in garlic has been reported by various authors and summarised by Khar *et al.* (2003a). Haque *et al.* (1997) obtained high frequency shoot regeneration from root tips of garlic without an intervening callus phase. Garlic root tips are commonly used for development of garlic regeneration system with or without a callus phase (Robledo *et al.*

2000). Most of the work on garlic micropropagation is based entirely on long day, temperate garlic, whereas, work on Indian garlic (short day type) is lacking except for a few reports (Bhojwani 1980; Koul *et al.* 1994; Khar *et al.* 2003b). The present experiment was designed to study the potential of callus culture and regeneration in short day, tropical garlic at National Research Centre for Onion and Garlic, Maharashtra.

Mature garlic cloves of cv. G-41 were used in this study. Cloves were peeled off and immersed in Bavistin 0.5% solution along with two drops of Tween-20 per 100 ml for 15 min and then washed 8–10 times with double distilled water. After that, the cloves were immersed in 70% alcohol for 2 min, rinsed thoroughly 3–4 times with sterile water and surface sterilised with 2% (v/v) aqueous sodium hypochlorite for 15 min and then washed with sterile water 5–6 times. After cutting the upper half portion of the cloves and trimming sideways and removing the basal necrotic portions, the cloves were cultured on MS basal medium for root initiation. After 10–12 days, the aseptically grown roots were used for further experiments. Apical root tips of 1 cm length were cut from the axenic seedlings and transferred to the callus induction medium. This medium consisted of MS basal salts and vitamins with sucrose (3% w/v) and phytagel (0.3%) with different concentrations of auxins and cytokinins (Table 1). The medium was adjusted to pH 5.8 before autoclaving. The petridishes containing explants were placed at a temperature of 25 ± 1°C with a 16 h photoperiod (2500–3000 Lux). The dishes were sealed with parafilm and explants were sub-cultured every 4 weeks while the frequency of callus induction (percentage of root segments with callus formation) was recorded after 8 weeks. After 8 weeks, all the callus forming explants were transferred to different regeneration media (RG2, RG7, RG8, GR1, GR3 and GR4) and shoot regeneration (percentage of callus with at least one shoot) was determined after 1 month.

Use of auxin i.e., 2,4-D alone was found to initiate callus formation at lower concentration (0.25 mg $l^{-1}$) only and increase in con-

**Table 1.** Effect of various auxin and cytokinin combinations on callus formation from root tip of garlic

| Establishment or induction medium | Supplement (mg $l^{-1}$) | | | | No. of explants used | No. of explants forming callus | % response |
|---|---|---|---|---|---|---|---|
| CRT 1 | 2,4-D | 0.25 | | | 48 | 39 | 81.3 |
| CRT 1.1 | | 0.50 | | | 12 | 1 | 8.3 |
| CRT 1.2 | | 1.00 | | | 48 | 0 | 0.0 |
| CRT 2 | 2,4-D | 0.25 | BA | 1.00 | 49 | 46 | 93.9 |
| CRT 2.1 | | 0.50 | | 1.00 | 48 | 47 | 97.9 |
| CRT 2.2 | | 1.00 | | 1.00 | 36 | 31 | 86.1 |
| CRT 3 | | 0.25 | | 2.25 | 68 | 56 | 82.4 |
| CRT 3.1 | | 0.50 | | 2.25 | 91 | 76 | 83.5 |
| CRT 3.2 | | 1.00 | | 2.25 | 37 | 30 | 81.1 |
| CRT 4 | 2,4-D | 0.25 | KIN | 1.00 | 69 | 63 | 91.3 |
| CRT 4.1 | | 0.50 | | 1.00 | 79 | 68 | 86.1 |
| CRT 4.2 | | 1.00 | | 1.00 | 37 | 30 | 81.1 |
| CRT 5 | | 0.25 | | 2.25 | 37 | 32 | 86.5 |
| CRT 5.1 | | 0.50 | | 2.25 | 57 | 53 | 92.9 |
| CRT A.1 | Pi | 1.00 | | | 12 | 9 | 75.0 |
| CRT B | | 0.25 | BA | 1.00 | 37 | 37 | 100.0 |
| CRT C | | 0.25 | | 2.25 | 13 | 12 | 92.3 |
| CRT D | Pi | 0.25 | KIN | 1.00 | 12 | 11 | 91.7 |
| CRT E | | 0.25 | | 2.25 | 12 | 12 | 100.0 |

CRT=Callus formation in root tip medium

centration led to sharp decline in callus formation whereas Pi at higher concentration (1.0 mg $l^{-1}$) was able to initiate callus formation in garlic root tip. This is in agreement with previous studies on other *Allium* species which indicated Pi as the most suitable auxin (Phillips & Luteyn 1983). Addition of cytokinins (BA and KIN) led to increase of callus formation from apical portion of the roots with CRT 2.1 (2,4-D 0.5 mg $l^{-1}$ + BA 1.0 mg $l^{-1}$) showing maximum callus formation (97.9%). Increase in BA concentration from 1.00 to 2.25 mg $l^{-1}$ did not increase callus formation efficiency. Similarly, in the case of KIN (1.00 and 2.25 mg $l^{-1}$), addition of KIN 1.0 mg $l^{-1}$ along with 2,4-D 0.25 mg $l^{-1}$ (CRT 4) registered maximum callus formation efficiency (91.3%); increase in 2,4-D concentration from 0.25 to 0.50 mg $l^{-1}$ and KIN from 1.00 to 2.25 mg $l^{-1}$ resulted in 92.9% callus formation efficiency from root tips only. When Pi alone was used at higher concentration of 1 mg $l^{-1}$, callus formation was observed from the apical root tip. The results are in conformity with Haque *et al.* (1998), and Robledo *et al.* (2000) who also reported callus formation from root tip of garlic but are contrary to the findings of Zheng *et al.* (2003) where callus formation from both apical and non apical portion of roots has been reported. In our studies, callus formation was observed throughout the various zones of roots in callus induction medium supplemented with Pi or Pi + cytokinin combination at various concentrations. Callus formation on non-apical portions was not observed. But in auxin i.e., 2,4-D and 2,4-D + cytokinin combinations, callus formation from root tip only was observed.

Two months after callus induction, the callus lines were transferred to regeneration medium consisting of MS basal salts and vitamins supplemented with sucrose @ 30 g $l^{-1}$ and cytokinins (BA 1.00, 2.25, 2.50, 3.00 mg $l^{-1}$and KIN 1.0, 2.0 mg $l^{-1}$) (Table 2). It was observed that the callus derived on induction medium containing only auxins led to profuse root formation (RF) and no shoot formation, whereas, in callus obtained from induction medium supplemented with both auxins and cytokinins, led to shoot regeneration in some combinations and root formation was also observed in some cases. This is in agreement with the findings of Haque *et al.* (1997) and Zheng *et al.* (2003), who observed that only auxins in combination with cytokinins induced shoot regeneration. In some combinations, formation of dark green callus was observed when transferred to the regeneration medium (RG7) but no shoot formation was observed after 1 month of culture. Khar *et al.* (2003b) also reported formation of dark green callus but the regeneration was observed only after 55–60 days of culture on the regeneration medium supplemented with thidiazuron (TDZ), a highly potent urea derivative. Higher efficiency of

**Table 2.** Regeneration efficiency (in per cent) of garlic in different media at 4 weeks of culture

| Establishment or induction medium | Callus induction medium (mg $l^{-1}$) | Type of response on regeneration medium (% response) | | | | | |
|---|---|---|---|---|---|---|---|
| | | RG2 | GR1 | RG 7 | RG 8 | GR 3 | GR 4 |
| | | BA 1.0 | BA 2.25 | BA 2.5 | BA 3.0 | KIN 1.0 | KIN 2.0 |
| CRT 2 | 2,4-D 0.25 BA 1.00 | RF | RF | DG | 20.0 | 50.0 | - |
| CRT 2.1 | 2,4-D 0.50 BA 1.00 | - | - | 16.7 | 16.7 | - | - |
| CRT 3 | 2,4-D 0.25 BA 2.25 | 16.7 | 16.7 | DG | 25.0 | - | - |
| CRT 3.1 | 2,4-D 0.50 BA 2.25 | - | RF | - | 25.0 | - | - |
| CRT 3.2 | 2,4-D 1.00 BA 2.25 | - | 20.0 | - | 20.0 | - | - |
| CRT 4 | 2,4-D 0.25 KIN 1.00 | 16.7 | RF | - | - | 25.0 | - |
| CRT 5.1 | 2,4-D 0.50 KIN 2.25 | - | 16.7 | - | 16.7 | 16.7 | - |
| CRT C | Pi 0.25 BA 2.25 | - | - | - | - | 16.7 | 16.7 |

RF=Root formation; DG=Dark green callus; CRT=Callus formation in root tip medium; RG=Callus regeneration medium; GR=Callus regeneration medium

regeneration (50%) was observed from callus which was induced on CRT 2 (2,4-D 0.25 mg $l^{-1}$ + BA 1.0 mg $l^{-1}$) medium (Fig. 1) but was later transferred to regeneration medium GR 3 (KIN 1.0 mg $l^{-1}$) (Fig. 2). Similarly, callus obtained on other combinations of auxins and cytokinins, when transferred to the regeneration medium, gave regeneration ranging from 16.7% to 25.0%. It was observed that the use of BA at a higher concentration of 3 mg $l^{-1}$ and KIN at a lower concentration of 1 mg $l^{-1}$ gave good results.

In conclusion, we have reported a regeneration protocol wherein combined use of auxin and cytokinin (2,4-D 0.25 mg $l^{-1}$ + BA 1.0 mg $l^{-1}$) for callus formation and subsequent transfer to regeneration medium with only cytokinin (KIN 1.0 mg $l^{-1}$) is found suitable for efficient regeneration of the short day type tropical garlic cultivar G-41. Further studies on callus formation and regeneration in different tropical garlic cultivars will give a better insight for developing an efficient regeneration protocol which will be useful for genetic transformation studies and for development of somaclonal variants in garlic.

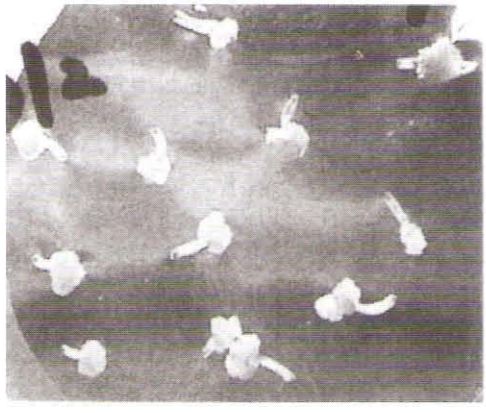

**Fig. 1.** Callus culture in garlic from root tip at 4 weeks of culture on medium CRT 2 (2,4-D 0.25 + BA 1.0 mg $l^{-1}$)

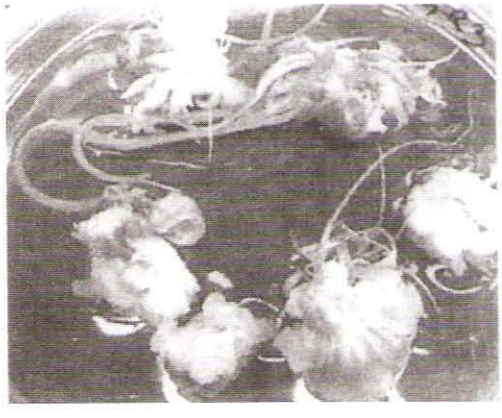

**Fig. 2.** Callus regeneration in garlic at 1 month of culture on medium GR 3 (KIN 1 mg $l^{-1}$)

## References


Bhojwani S S 1980 *In vitro* propagation of garlic by shoot proliferation. Scientia Hort. 13: 47–52.

Haque M S, Wada T & Hattori K 1997 High frequency shoot regeneration and plantlet formation from root tip of garlic. Plant Cell Tiss. Organ Cult. 50 : 83–89.

Haque M S, Wada T & Hattori K 1998 Efficient plant regeneration in garlic through somatic embryogenesis from root tip explants. Plant Prod. Sci. 1 : 216–222.

Kamenetsky R & Rabinowitch R D 2001 Floral development in bolting garlic. Sex. Plant Reprod. 13 : 235–241.

Khar A, Lawande K E & Asha Devi A 2003â Biotechnological approaches in garlic (*Allium sativum* L.)-Past, present and future. Botanica 53 : 155–168.

Khar A, Bhutani R D & Yadav N R 2003b *In vitro* regeneration studies on effect of genotype, explant and media in garlic (*Allium sativum* L.). Veg. Sci. 30 : 138–144.

Kondo T, Hasegawa H & Suzuki M 2000 Transformation and regeneration of garlic (*Allium sativum* L.) by *Agrobacterium* mediated gene transfer. Plant Cell Rep. 19 : 989–993.

Koul S, Sambyal M & Grewal S 1994 Embryogenesis and plantlet formation in garlic (*Allium sativum* L.). J. Spices Aromatic Crops 3 : 43–47.

Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.

Robledo P A, Villalobos Arambula V M & Jofre Garfias A E 2000 Efficient plant regen-

eration of garlic (*Allium sativum* L.) by root tip culture. *In Vitro* Cell Dev. Biol. Plant. 36 : 416–419.

Zheng S J, Henken B, Krens F A & Kik C 2003 The development of an efficient cultivar independent plant regeneration system from callus derived from both apical and non apical root segments of garlic (*Allium sativum* L.). *In Vitro* Cell Dev. Biol. Plant. 39 : 288–292.