

## 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

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### Abstract

Callus induction and subsequent plant regeneration was studied in a commercial Indian cultivar of garlic, cv. G-41 (short-day type). 2,4-dichlorophenoxyacetic acid at lower concentration ( $0.25 \text{ mg l}^{-1}$ ) and picloram at higher concentration ( $1.0 \text{ mg l}^{-1}$ ) 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-dichlorophenoxyacetic 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-dichlorophenoxyacetic acid  $0.25 \text{ mg l}^{-1}$  + 6-benzylaminopurine  $1.0 \text{ mg l}^{-1}$ ) induction medium followed by garlic regeneration medium 3 (GR 3) (kinetin  $1.0 \text{ mg l}^{-1}$ ) 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-Dichlorophenoxyacetic 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 pro-

duction 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 phytigel (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  $25 \pm 1^\circ\text{C}$  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 ( $0.25 \text{ mg l}^{-1}$ ) 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 ( $\text{mg l}^{-1}$ )				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 ( $1.0 \text{ mg l}^{-1}$ ) 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  $0.5 \text{ mg l}^{-1}$  + BA  $1.0 \text{ mg l}^{-1}$ ) showing maximum callus formation (97.9%). Increase in BA concentration from  $1.00$  to  $2.25 \text{ mg l}^{-1}$  did not increase callus formation efficiency. Similarly, in the case of KIN ( $1.00$  and  $2.25 \text{ mg l}^{-1}$ ), addition of KIN  $1.0 \text{ mg l}^{-1}$  along with 2,4-D  $0.25 \text{ mg l}^{-1}$  (CRT 4) registered maximum callus formation efficiency (91.3%); increase in 2,4-D concentration from  $0.25$  to  $0.50 \text{ mg l}^{-1}$  and KIN from  $1.00$  to  $2.25 \text{ mg l}^{-1}$  resulted in 92.9% callus formation efficiency from root tips only. When Pi alone was used at higher concentration of  $1 \text{ mg l}^{-1}$ , 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 con-

centrations. 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 @  $30 \text{ g l}^{-1}$  and cytokinins (BA  $1.00$ ,  $2.25$ ,  $2.50$ ,  $3.00 \text{ mg l}^{-1}$  and KIN  $1.0$ ,  $2.0 \text{ mg l}^{-1}$ ) (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 ( $\text{mg l}^{-1}$ )				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<sup>-1</sup> + BA 1.0 mg l<sup>-1</sup>) medium (Fig. 1) but was later transferred to regeneration medium GR 3 (KIN 1.0 mg l<sup>-1</sup>) (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<sup>-1</sup> and KIN at a lower concentration of 1 mg l<sup>-1</sup> 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<sup>-1</sup> + BA 1.0 mg l<sup>-1</sup>) for callus formation and subsequent transfer to regeneration medium with only cytokinin (KIN 1.0 mg l<sup>-1</sup>) is found suitable for

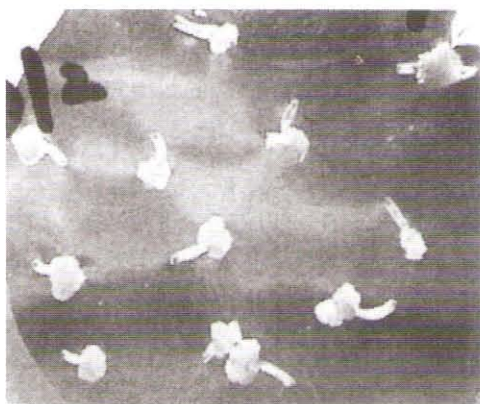


Fig. 1. Callus culture in garlic from root tip at 4 weeks of culture on medium CRT 2 (2,4-D 0.25 + BA 1.0 mg l<sup>-1</sup>)

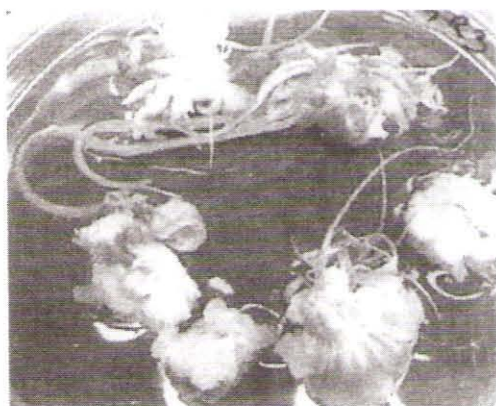


Fig. 2. Callus regeneration in garlic at 1 month of culture on medium GR 3 (KIN 1 mg l<sup>-1</sup>)

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.

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