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In vitro Regeneration of Garlic Through Callus Culture

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Abstract: Garlic (*Allium sativum*) root tips were induced to regenerate shoots through callus culture and somatic embryogenesis to obtain plantlets. The experiment was designed to investigate the regeneration potentiality of two garlic verities and also to develop an efficient protocol for regeneration of garlic via callus culture. Higher percentage of callus was initiated from the combination of 5 mg l^{-1} Kinetin and 1.5 mg l^{-1} 2,4-D. Embryogenic callus produced higher number of shoots in MS medium supplemented with 10 BAP. Rooting of individual shoots was induced after transfer to medium without growth regulator. The plantlets were established in the soil after acclimization. Cultivar differences in regeneration from root tips were observed.

Key words: *In vitro*, garlic, callus

INTRODUCTION

Garlic (*Allium sativum* L.) is an important and widely cultivated plant with both culinary and medical uses stemming from its biological activities, which include antibiotic, anticancer, antithrombotic and lipid lowering cardiovascular. It is cultivated vegetatively and its breeding has been limited to clonal selection of wild varieties, the production of virus free stocks or spontaneous mutants, because this species does not from fertile flowers, i.e. it is sexually sterile. It is exclusively propagated vegetatively[1]. The propagation rate of garlic in the field is very slow and it takes many years to produce a new verity for practical cultivation. *In vitro* grown micro plants are some of the newer avenues through which rapid propagation of garlic is possible. *In vitro* regeneration of garlic achieved through formation of adventitious shoots through callus culture. This investigation was undertaken with the following objectives:

- development and identify the best protocols for *in vitro* regeneration of garlic.
- study the potentiality of callus, shoot and root induction ability of two garlic cultivars.

MATERIALS AND METHODS

Explants were taken from sprouted garlic cloves cultured on ½ MS medium. Two garlic varieties namely local and exotic were taken to investigate their *in vitro* regeneration potentiality. Garlic cloves were surface sterilized with 70% alcohol for 30 sec and then 0.1% $Hgcl_2$ solution with 2 drops Tween–20 per 100 ml for 5 minutes subsequently washed by distilled water for three times. Root tips of spouted cloves about 2-3 mm long were used as explants. Explants were placed on MS[2] medium supplemented with different concentrations (0, 0.5, 1.0, 1.5, 2. mg l^{-1}) of 2.4-D with the combination of BAP (0, 5, 10, 15 and 20 mg l^{-1}) were used. Forty-five days older callus was subcultured in BAP (0, 5, 10,15 and 20 mg l^{-1}) for regeneration. The experiment was laid out in Complete Randomized Block Design with 5 replications. The treatment mean were compared with LSD values. The whole experiment was conducted in Tissue Culture Laboratory, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh.

RESULTS AND DISCUSSION

The ultimate goal of this experiment was plant regeneration via unorganized calli. To achieve this goal root tips of local and exotic varieties were cultured. From the present investigation it was evident that combinations of Kinetin and 2,4-D produces satisfactory performance for callus induction, where 5 mg l^{-1} Kinetin + 1.5 mg l^{-1} 2,4-D showed best result but higher and controlled combination show poor performance. Among the varieties exotic variety show better performance than the local one for callus induction (Table 1 and Fig. 1) Robledo *et al*.[3] also reported that good callus induction was observed using root tip in MS medium supplemented with Kinetin and 2,4-D.

MS medium supplemented with different concentrations of BAP showed wide variation in shoot proliferation for both exotic and local variety. It was

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Table 1: Role of different concentration of 2,4-D and Kinetin for callus induction

	0.0 (mg lG^{1})		0.5 (mg lG^{1})		1.0 (mg lG^{1})		1.5 (mg lG^{1})		2.0 (mg lG^{1})	
2,4-D Kinetin	Days to callusing	%callus induction	Days to callusing	%callus induction	Days to callusing	%callus induction	Days to callusing	%callus induction	Days to callusing	%callus induction
L×5.0 (mg lG^{1})	15	10	8	52	7	65	6	82	14	20
E×5.0 (mg lG^{1})	14	12	6	55	7	68	6	85	12	25
L×10 (mg lG^{1})	12	15	9	60	8	70	7	70	15	25
E×10 (mg lG^{1})	13	15	9	65	8	72	6	75	13	30
L×15 (mg lG^{1})	14	22	10	48	9	53	8	65	15	15
E×15 (mg lG^{1})	16	20	10	50	8	55	8	68	16	15
L×20 (mg lG^{1})	21	25	11	43	9	45	8	55	18	10
E×20 (mg lG^{1})	20	25	11	43	9	50	9	58	18	10

Table 2: Effect of genotype on percent shoot induction from callus

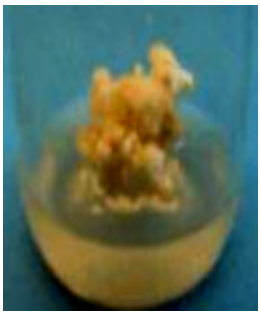
Genotype	% Shoot regeneration	Days required for shoot regeneration (ns)	Number of shoots/callus
Local	33.44a	123.124	8.4a
Exotic	31.84b	124.120	7.96b

Table 3: Effect of different concentration of BAP on percent shoot induction from callus

Hormone (BAP)	% Shoot regeneration	Days required for shoot regeneration	Number of shoots/callus
0	11.20c	130.1a	2.8c
5	44.8b	123.6b	11.20b
10	56.80a	115.1d	14.20a
15	45.2b	118.8c	11.30b
20	5.20d	132.4a	1.40d
LSD	3.27	2.99	0.804
SE	0.85	0.77	0.209

Table 4: Combined effect of genotype and different concentration of BAP on percent shoot induction from callus

Effect	% Shoot regeneration	Days required for shoot regeneration	Number of shoots/callus
Local×0	13.6c	128.6ab	3.4c
Exotic×0	8.8d	132.4a	2.2d
Local×5	44.8b	125.8b	11.2b
Exotic×5	44.8b	131.6a	11.2b
Local×10	57.6a	113.8c	14.4a
Exotic×10	56.3a	121.4cd	14.0a
Loca×5	45.6b	118.8cd	11.4b
Exotic×15	44.0b	116.4de	11.2b
Local×20	5.6d	132.4a	1.6de
Exotic×20	4.0d	118.8a	1.2e
SD	3.45	4.23	0.87
SE	1.23	1.10	0.30



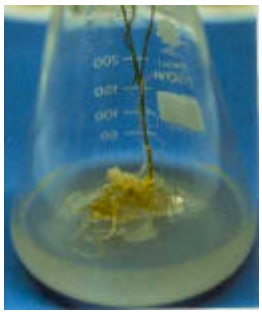
(Local)

(Exotic)

Fig. 1: Callus induction from root tips of local and exotic variety in MS+5 mg lG^{1} Kinetin+1.5 mg lG^{1} 2,4-D

evident from the experiment that per cent of shoot regeneration and number of shoots per callus increased with the increasing concentrations of BAP up to 10 mg lG^{1}. Highest regeneration and highest number of shoots/callus were found in 10 mg lG^{1} BAP (Table 3). It was worth noting that plant regeneration depended on diverse factors including the genotypes, hormone supplements and so on. Statistical analysis revealed that significant variation was presented among varieties, hormonal concentrations and their interaction for percent shoot regeneration, days required for shoot regeneration and number of shoots per callus (Table 2, 3 and 4 and

(Local)



(Exotic)

Fig. 2: Shoot regeneration through callus, derived from leaf base of local and exotic variety in MS+10 mg l^{-1} BAP

(Local)

(Exotic)

Fig. 3: Plantlet in plastic pot

Fig. 2). In the present study, we observed that use of BAP helped in shoot regeneration, this result corroborated with the findings of Kudou *et al*.[4] and Chi *et al*.[5] who reported that BAP was the most effective stimulator for shoot formation and increased percentage of shoot regeneration.

Regenerated shoots from different explants needed roots to establish them in soil. From the present study it was evident that MS medium with out any growth regulator is best for root induction in garlic. This finding showed similarity with that of Shoto *et al*.[6] reported that garlic callus initiate roots on hormone-free MS medium.

After satisfactory growth of root system, the plantlets were removed from vessels and transferred into soil in small pots and the pots were transferred into greenhouse for proper hardening. About 75% of plantlets of both varieties survive (Fig. 3).

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