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Effect of 2, 4-D and BAP on *In vitro* Regeneration of Garlic

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Abstract: Vigorously growing root tips, basal discs and leaf discs of three garlic strains (G-101, G- 102, G-103) were used as explants. The explants were collected from cloves germinated in a basal medium. Different combinations and concentrations of growth regulators like 2, 4-D and BAP were used in MS medium to observe the callus induction, proliferation, organogenesis and to produce regenerate plantlets under *in vitro* condition. The highest callusing was recorded at the highest concentration of 2, 4-D (2.0 mg l^{-1}) + BAP (1.0 mg l^{-1}) in MS medium for G-103 (80%). The other two strains (G-101, G-102) showed 70 and 75% callusing ability respectively. Highest performance in the proliferation of calli were found at MS+2,4-D (2.0 mg l^{-1}) +BAP (0.5 mg l^{-1}) in G-103 (90%). Maximum number of regenerated plantlets were obtained at MS + NAA (2.0 mg l^{-1}) + BAP (1.0 mg l^{-1}) in all the strains (35%). Plantlets thus, obtained were successfully transferred to the potted soil and subsequently to the field. The survival rate was 40-60% in all the cases.

Key words: *In vitro* regeneration, 2,4-D, BAP, explant MS medium, callus induction, proliferation, garlic (*Allium sativum*)

Introduction

Garlic (*Allium sativum* L.) is an aromatic spice crop used for various culinary purposes and traditional Unani and Ayurvedic treatments. It is the second most widely cultivated species after onion (Bose and Som, 1990). The origin of the crop is believed to be central Asia (Katyal and Chandha, 1996). Presently the major garlic producing countries are China, South Korea, Spain, India, Egypt, United States, Thailand and Turkey. China leads in garlic cultivation both in world production and acreage, 4.65 million tons and 5.57 million hectare respectively (Anonymous, 1998). Considering the total annual requirement (85000 mt), there exists a great deficit in garlic production (40000 mt). The yield rate of garlic is 5.06 t ha^{-1} in China, whereas in Bangladesh is only 3.08 t ha^{-1} (Anonymous, 1999).

Garlic is mainly propagated through vegetative means. Owing to the reported chromosomal irregularities occurring in this material, sexual reproduction is restricted. Further, during the evolutionary process some genetic modifications occurred, resulting in the flower production in general (Zhang, 1999). Therefore improvement of garlic production can be achieved through chromosomal manipulation and selecting the desirable variants e.g. large bulb size, number of cloves/bulb, storability etc. The alteration of chromosome constitute in a vegetatively propagated plant like garlic can be made possible through *in vitro* techniques. The regeneration of plants from tissue culture is an important and essential component of biotechnological research and sometimes is required for the genetic manipulation of plants. High frequency regeneration of plants from *in vitro* culture is a pre-requisite for successful application of tissue culture technique for crop improvement. More recently many plant breeders are getting interest in tissue culture techniques, especially in crops where sexual reproduction is relatively complicated or difficult. Considering the aspects mentioned above the present research program has been undertaken with the following objectives:

- To determine the optimum concentration of 2, 4-D and BAP for *in vitro* regeneration of garlic.
- To obtain successfully cultured tissues and consequent regenerants from root tip, basal disc and leaf disc explants of garlic.

Materials and Methods

The study was carried out during the period from April, 2000 to September 2001 in the tissue culture laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University.

Vigorously growing root tips, basal disc and leaf discs of the three garlic strains (G-101, G-102, G-103) were used as explants. The explants were collected from cloves germinated in basal medium. (Murashige and Skoog, 1962). A nutrient medium consists of organic and inorganic salts, iron, a carbon source, some vitamins and growth regulators like 2, 4-D (2, 4-dichlorophenoxy acetic acid) and BAP (6-benzyl amino purine) were used.

The culture vessels contained the medium were autoclaved at 121°C for 40 min at 1.16 kg cm^{-2} pressure and allowed to cool under normal condition. The culture room was initially cleaned with 70% ethyl alcohol. Generally, laminar airflow cabinet was sterilized by ultra violet (UV) light and wiping the working surface with 70% alcohol.

Bulbs of garlic strains (G-101, G-102, G-103) were used as seed. Initially cloves were separated from healthy and mature bulbs with the removal of membranous scale. It was accomplished manually. The cloves were then washed throughly in running tap water. Special care was taken to avoid all types of injury. The surface sterilization of these cloves were carried out under a laminar air-flow cabinet. The cloves were washed 3 times with distilled water and then immersed into 70% alcohol for one min and then throughly washed. The alcohol treated cloves were immersed into 0.1% $HgC1_2$ solution for 3 min followed by 3-4 rinses in autoclaved distilled water to remove trace of $HgC1_2$ which would be toxic to the explant if kept for longer duration. The cloves were then ready for placement into the media.

Culture methods: Sterilized cloves were placed into sterilized germinating medium in culture vessels, then incubated in dark till the germination of cloves, then transferred to 12 h light period. Within 3-5 days roots were initiated and was ready for use as explants.

Explant culture: Different kinds of explants like root tips and basal discs were used. The seedling raised in the axenic culture provided contamination free shoot and root explant.

Root tip culture: The aseptically grown seeding were rescued and placed on a sterile petridish. Young root tips measuring 5 mm in length were separated and placed in to the sterile culture media for induction. Different concentrations and combinations of growth regulators like 2.4-D and BAP were used for callus induction. One or two root tips were inoculated in each vessel and plugged with cork as well as non-absorbent cotton.

Basal disc culture: Basal disc from each germinated clove was separated by a sterile scalpel. Each disc was divided into 3-4 pieces

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and then placed into the culture vessel.

Leaf disc culture: Leaf disc from each germinated clove was separated by a sterile scalpel. Each disc was divided into 3-4 pieces and then placed into the culture vessel.

Sub culture or transfer: When the calli attained a convenient size they were removed aseptically from the culture vessels and placed on a sterilized petridish inside the laminar airflow cabinet. The calli were cut into small pieces and were placed into freshly prepared sterilized media with appropriate combinations and concentrations of growth regulators.
These were again subcultured to freshly prepared medium containing different hormonal supplements for the maintenance of callus or for root-shoot differentiation. The culture vessels showing signs of contamination were discarded. Repeated subculturing was done at an interval of 15 days for maintenance of calli.

Incubation: Culture vessels with inoculated explants were incubated both in dark and light conditions under controlled temperature ($25 \pm 2°C$). About 16 h photoperiod with a light intensity of 2000-3000 lux was maintained. Observations were carried out daily to note the response.

Preparation of pot: Potting mixture containing ground soil and cowdung in the ratio 1:1 was mixed properly and were placed into a 10 cm plastic pots for growing the plantlets *in vivo* condition after proper autoclaving.

Transfer of plantlets on to soil: When the plantlets became 4-8 cm in length with 3-6 well developed leaves and roots, the plantlets were removed from conical flasks. Medium attached to roots was gently washed out with tap water. Plantlets were then transplanted to pots containing the above mentioned potting mixture. Immediately after transplantation, the plants along with pots were covered with moist polythene bags to prevent evapotranspiration. To reduce sudden shock, the pots were kept in the controlled environment in growth room for 7 to 15 days. The polythene bags were removed when the plantlets appeared to be self-sustainable and then transferred to the field.

Results and Discussion

A detailed investigation was carried out to examine the appropriate growth conditions that may help in callus induction, proliferation, plantlets regeneration in different garlic strains.

Callus induction

Response at different concentration of 2,4-D: Root tips, basal discs and leaf discs of the three garlic strains (G-101, G-102, G-103) were cultured on MS medium supplemented with different concentration of 2,4-D (0.0, 0.5, 1.0, 2.0 mg l^{-1}) and the callusing response was evaluated. The results indicated that the concentration 2.0 mg l^{-1} of 2,4-D showed highest callus induction frequency in G-103 (80%) strain, other two strains showed 70% (G-101) and 75% (G-102) callusing ability (Fig. 1, Table 1). In all cases, these findings showed similarities to the finding of Jang *et al*. (2000) and Kudou *et al*. (1995).

Table 1: Effect of different concentrations of 2, 4-D in MS medium on callus induction from root tips basal discs and leaf discs of three garlic strains

Supplements		No. of explants	No. of explants showing callus			% callus induction			
2, 4-D (mg l^{-1})	Strains	Inoculated	RT	BD	LD	RT	BD	LD	Days to callus induction
0			3	2	4	15	10	20	
0.5			4	5	5	20	25	25	
1.0	G-101	20	5	7	6	25	35	30	14-21
1.5			11	13	11	55	65	55	
2.0			14	14	14	70	70	70	
0			2	-	-	10	-	-	
0.5	G-102	20	5	3	-	25	15	-	
1.0			6	5	2	12	15	10	14-21
1.5			12	11	10	60	55	50	
2.0			14	15	15	70	75	75	
0			-	-	-	-	-	-	
0.5			3	4	5	15	20	25	
1.0	G-103	20	7	5	6	35	25	30	14-21
1.5			12	12	13	60	60	65	
2.0			16	16	16	80	80	80	

Table 2: Effect of different concentrations of 2, 4-D and BAP in MS medium on callus induction from root tips, basal discs. and leaf explants of three garlic strains

Supplements (mg l^{-1})			No. of explants	No. of explants showing callus induction			% callus induction			
2, 4-D	BAP	Strains	Inoculated	RT	BD	LD	RT	BD	LD	Days to callus induction
0				1	1	2	10	10	20	
0.5				3	4	3	30	40	30	
1.0	1.0	G-101	10	4	5	4	40	50	40	7-14
1.5				6	7	5	60	70	60	
2.0				8	8	8	80	80	80	
0				0	2	1	0	20	10	
0.5				2	3	2	20	30	20	
1.0	1.0	G-102	10	3	6	3	30	60	30	7-14
1.5				7	8	4	70	80	40	
2.0				8	8	8	80	80	80	
0				2	-	-	20	-	0	
0.5				3	3	2	30	30	20	
1.0	1.0	G-103	10	4	6	3	40	60	30	7-14
1.5				5	7	5	50	70	50	
2.0				8	8	8	80	80	80	

RT, BD & LD means root tip, basal disc & leaf disc respectively.

Table 3: Effect of different combinations of 2, 4-D and BAP in MS medium on callus proliferation derived from root tips basal discs and leaf discs of three garlic strains

Supplements (mg l^{-1})	Strains	No. of vessels in which callus inoculated	No. of vessels in which callus proliferated			% callus proliferated			Days to callus proliferation
			RT	BD	LD	RT	BD	LD	
1.0 2,4 - D + 0.5 BAP	G101		5	6	6	50	50	60	
	G102	10	6	6	5	60	60	50	15
	G103		7	7	5	70	70	50	
1.5 2,4 - D + 0.5 BAP	G101		5	7	6	50	70	60	
	G102	10	6	8	8	60	80	80	15
	G103		5	7	5	50	70	50	
2.0 2,4 - D + 0.5 BAP	G101		8	8	8	80	80	80	
	G102	10	8	8	8	80	80	80	15
	G103		9	9	9	90	90	90	

Table 4: Effect of different combinations of NAA and BAP in MS medium on plant regeneration of three garlic strains using root tip, basal disc and leaf disc explant

Supplements (mg l^{-1})				No. of plants showing regeneration			% Regeneration		
BAP	NAA	Strains	No. of explants inoculated	RT	BD	LD	RT	BD	LD
1.0+0.5		G101		3	5	4	15	25	20
		G102	20	5	5	4	25	25	20
		G103		4	5	4	20	25	20
1.0+1.0		G101		5	5	6	25	25	30
		G102	20	5	5	6	25	25	30
		G103		5	5	6	25	25	30
1.0+1.5		G101		4	7	4	20	35	20
		G102	20	5	6	5	25	30	25
		G103		6	7	6	30	35	30
1.0+2.0		G101		7	7	7	35	35	35
		G102	20	7	7	7	35	35	35
		G103		7	7	7	35	35	35

* RT, BD & LD means root tip, basal disc and leaf disc respectively

Table 5: Survival rate of regenerants in the three garlic strains after transfer in soil

Pot mixture prepared	Strains	No. of plantlets transplanted	No. of plant survived	% survived
50% loamy soil + 50% cowdung	G101	5	2	40
	G102	5	2	40
	G103	5	2	40
Only loamy soil	G101	5	3	60
	G102	5	3	60
	G103	5	3	60

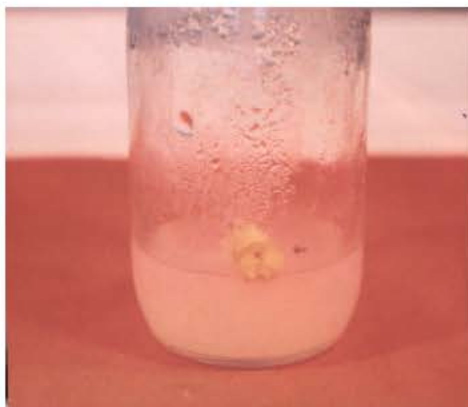

Fig. 1: Callus initiation from root tips of the garlic strain G102 (representative) in MS medium supplemented with 2, 4-D (2.0 mg l^{-1})

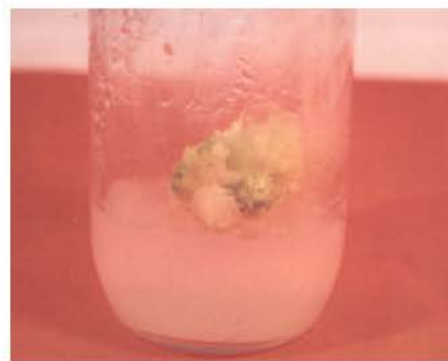

Fig. 2: Callus initiation from root tips of the garlic strain G102 (representative) in MS medium supplemented with 2, 4-D (2.0 mg l^{-1}) and fixed concentration of BAP (1.0 mg l^{-1})

Response at different concentration of 2,4-D and BAP: Different concentration of 2,4-D (0.0, 0.5, 1.0, 2.0 mg l^{-1}) in combination with a constant concentration of BAP (1.0 mg l^{-1}) were used in MS
medium to observe the callus induction ability using all three explants. It was observed that MS medium supplemented with 2.0 mg l^{-1}, 2,4-D and 1.0 BAP exhibited highest percentage of callusing ability 80% in all the three strains indicating the individuality of BAP on the other two strains (Fig. 2, Table 2). Similar results were obtained by Tapia (1996).

Maintenance of calli: The highest performance in the of calli were obtained at MS + 2.0 mg l^{-1}, 2,4-D + 0.5 mg l^{-1} BAP in G-103 (90%). Other two strains showed 80% proliferation ability of the

Fig. 3: Proliferated callus derived from the garlic strain G102 (representative) in MS medium supplemented with 2,4-D (2.0 mg l^{-1}) and fixed concentration of BAP (0.5 mg l^{-1})

Fig. 4: Multiple shoot regeneration of the garlic strain G102 (representative) from proliferated calli derived from root tips in MS medium supplemented with BAP (1.0 mg l^{-1}) and NAA (2.0 mg l^{-1})

Fig. 5: Plantlets of garlic strain G102 established in pot

callus indicating moderate degree of friability. (Fig. 3, Table 3). The statement was supported by Ma *et al.* (1994) and Conci *et al.* (1987).

Regeneration of plantlets: Different concentrations of NAA (0.0, 0.5, 1.0, 2.0 mg l^{-1}) and constant concentration (1.0 mg l^{-1}) of BAP were used to observe the regenerating capacity of proliferated calli

in MS medium. MS medium supplemented with 2.0 mg l^{-1} NAA and 1.0 mg l^{-1} BAP exhibited with the highest regenerating ability (35%) in all the strains. (Fig.4, Table 4). These findings were in agreement with that of Seabrook (1994) and Suh and Park (1995).

Establishment of plantlets: The survival rate of the plantlets ranged from 40-60%. When the plants were transferred on to the soil and cowdung in a ratio 1:1 the rate was lower (40%) than loamy soil (60%) (Fig.5, Table 5).

Many factors are responsible for *in vitro* regeneration as well as establishment of plantlets in garlic. Phytohormones combinations and concentrations play a vita role for *in vitro* regeneration of 2,4-D and BAP are major ones that lead a better performance in garlic regeneration.

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