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In vitro regeneration in cole crops

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Abstract

The experiment was conducted to optimize a suitable protocol for *in vitro* regeneration in cole crops. Callus initiation was excellent in the variety Early Tropical. Highest percentage of callus proliferation was observed in Early Tropical (75.0%) followed by Tangail Special Pauslali (55.0%) and the lowest in Tara (40.0%). Maximum callus proliferation (68.5%) was observed in MS + 3.0 mgL^{-1} BAP + 0.1 mgL^{-1} 2,4-D + 2.0 mgL^{-1} $AgNO_3$. Callus proliferation was lowest (40.0%) in MS + 2.5 mgL^{-1} BAP + 0.1 mgL^{-1} 2,4-D + 2.0 mgL^{-1} $AgNO_3$. MS medium supplemented with 3.0 mgL^{-1} BAP + 1.0 mgL^{-1} 2,4-D + 2.0 mgL^{-1} $AgNO_3$ was the best for shoot initiation & plantlet regeneration. The highest number of shoots per vial was 7.20 and the lowest number of shoots per vial was 4.40. Among the concentration MS + 3.0 mgL^{-1} BAP + 0.1 mgL^{-1} 2,4-D + 2.0 mgL^{-1} $AgNO_3$ showed the highest performance of shoots per vial. The variety Tangail Special Pauslali was the best for root initiation.

Keywords: *Brassica*, Cole crops, Callus, *In vitro,* Regeneration

Introduction

The genus *Brassica* belongs to the family Brassicaceae under the order Rhoedales (Benson, 1957) has three categories namely the Rape seed, the Mustard and the Cole. (Yarnell, 1956). Cabbage (*Brassica oleracea* var. *capitata*) and cauliflower (*Brassica oleracea* var. *botrytis*) are the most important members of cole crops in the tropic and temperate regions of the world (Siddiqui, 2004).In Bangladesh, out of 260.32 thousand hectares of vegetables growing area, cabbage and cauliflower covers 9.64% of the total land and contributes 13.97% of total production (BBS, 2004). The curd (white head of cauliflower) is an early stage of inflorescence development as its formation invariably precedes floral initiation (Bose and Som, 1986). Conventional breeding methods are time consuming, extending over seven to eight years involving crossing and wise selection of desirable traits. In addition, genetic incompatibilities in some cases restrict many important gene transfer by interspecies hybridization. More recently, genetic engineering (gene technology) has advanced and opened a new avenue for crop improvement. An efficient tissue culture system is thought to be essential to the success of plant genetic engineering. This technique can be used to add desirable traits from wild to existing cultivars. Therefore, it is essential to develop an efficient transformation system (Henzi *et al.*, 2000) which offers the potential for the introduction of specific genes from any source (related or unrelated plant species or even from animal) into the existing elite plant lines. And to do, a well adapted regeneration protocol for cabbage and cauliflower should be developed using the locally cultivated varieties.

Keeping the above facts in mind, the present study was taken to standardize a suitable protocol for *in vitro* regeneration of different genotypes of Cole crops.

Materials and Methods

The seed materials of two cabbage varieties like Tara, Summer Star and two cauliflower varieties like Early Tropical, Tangail Special Pauslali were collected from the local market of Mymensingh town and the experiment was conducted in the Tissue Culture Laboratory of the Department of Genetics & Plant Breeding, Bangladesh Agricultural University, Mymensingh. Healthy and uniform seeds were surface sterilized with 70% ethyl alcohol and 0.1% $HgCl_2$. Half MS used for *in vitro* germination. Six days old seedlings were used as source of explants and cultured on MS medium supplemented with different concentrations of BAP (2.0, 2.5, 3.0 mgL^{-1}) with a constant concentrations of 2,4-D (0.1 mgL^{-1}) and $AgNO_3$ (2.0 mgL^{-1}) for callus induction and subsequent plantlet regeneration. The regenerated plants were transferred to sterile culture vessels containing rooting media (half strength MS medium supplemented with 0.5 and 1.0 mgL^{-1} NAA). When the plantlets became 5-6 cm in length with sufficient root system, they were transferred in plastic pots containing a 3:1 mixture of autoclaved soil and sand for hardening and incubated at 25±1 °C in the culture room for a week. The plants were finally transferred to the pots to the field condition.

Results and Discussion

Initiation and maintenance of callus: Calli raised from hypocotyls of each of two varieties of cabbage and cauliflower were successfully maintained by subculturing at an interval of 15 days on MS medium supplemented with different concentrations of BAP (2.0, 2.5 and 3.0 mg L^{-1}) with constant concentration of 2,4-D (0.1 mg L^{-1}) and $AgNO_3$ (2.0 mg L^{-1}).

Among the concentration the best callus proliferation (68.5%) was observed in MS + 3.0 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$ (T_3) (Fig. 1). Callus proliferating was lowest (40.0%) in MS + 2.5 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$ (T_2). The varieties showed wide range of variations in callus proliferation (Table 1). Callus proliferation was the highest (75.0%) in Early tropical (Plate 1) followed by Tangail special pauslali (55.0%) (Plate 2) and the lowest in Tara (40.0%) (Fig. 2). Callus proliferating was lowest (40.0%) in MS + 2.5 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$ (T_2).

After two to three subcultures, the calli exhibited variation in color and texture i.e. calli became friable in texture and greenish in color. The subcultured calli then started regeneration by shoot bud initiation.

From Table 1, it was evident that both the higher and lower concentration of cytokinin was not suitable for callus maintenance. A medium concentration of cytokinin (2.5 mg L^{-1} BAP and 2.0 mg L^{-1} $AgNO_3$) with lower concentration of auxin (0.1 mg L^{-1} 2,4-D) was suitable for callus maintenance. Similar results were reported by Hasan (2006) who found that the best callus proliferation (69.5%) was observed in Snow Queen using the concentration MS + 3.0 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$.

Table 1. Maintenance of calli derived from hypocotyls of cabbage and cauliflower varieties in different concentrations and combinations of phytohormone

BAP conc.	Varieties	No. of explants incubated	No. of explants showing callus proliferation	% callus proliferation	Days of callus subculture
2.0 mgL^{-1}	Tara	20	6	30	15
	Summer star	20	11	55	15
	Early tropical	20	17	85	15
	Tangail special pauslali	20	10	50	15
2.5 mgL^{-1}	Tara	20	10	50	15
	Summer star	20	6	30	15
	Early tropical	20	12	60	15
	Tangail special pauslali	20	12	60	15
3.0 mgL^{-1}	Tara	20	8	40	15
	Summer star	20	10	50	15
	Early tropical	20	16	80	15
	Tangail special pauslali	20	11	55	15

Note: BAP was added to MS medium supplemented with 0.1 mgL^{-1} 2,4-D and 2.0 mgL^{-1} $AgNO_3$.

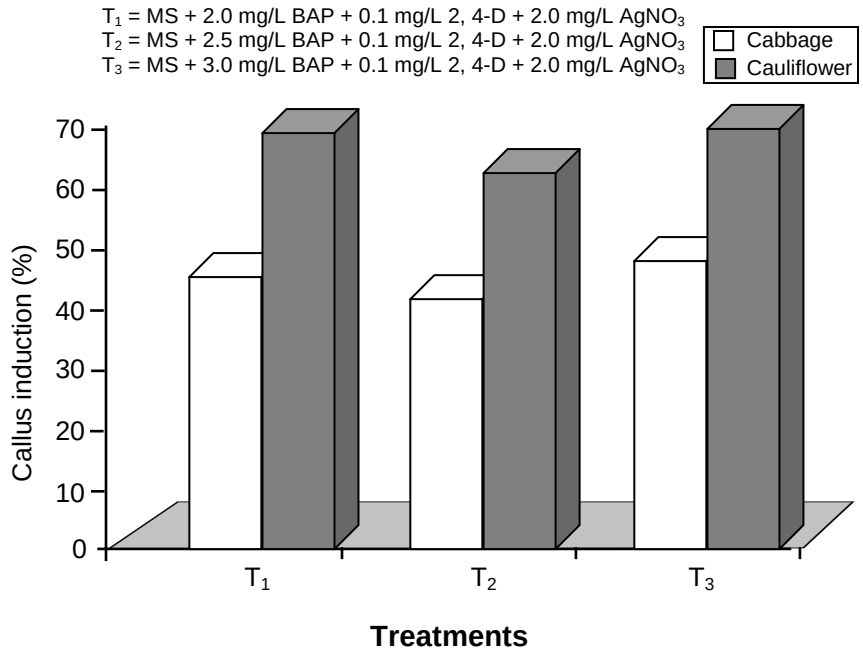

Fig. 3. Response of different treatments on cabbage and cauliflower varieties towards callus proliferation

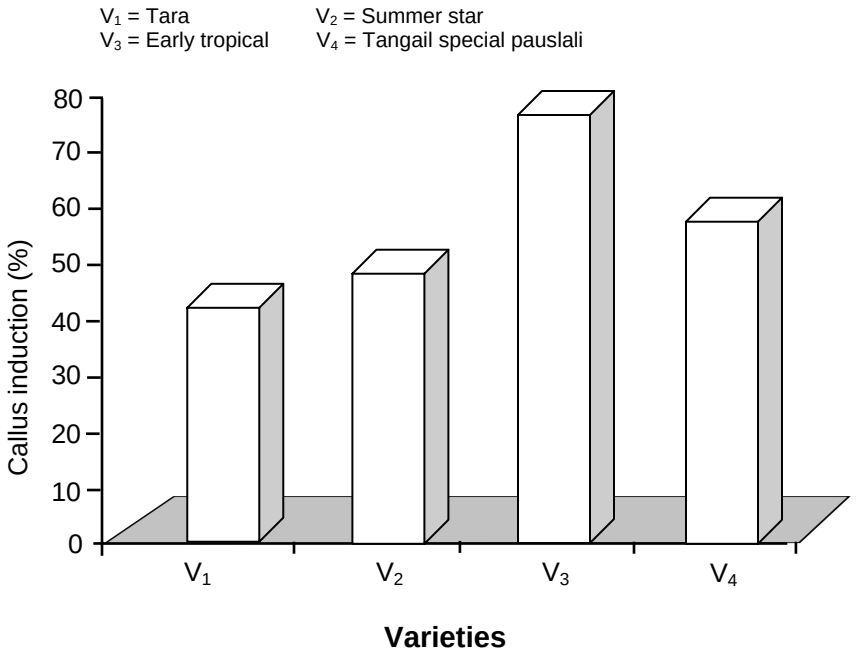

Fig. 4. Response of two cabbage and two cauliflower varieties towards callus proliferation

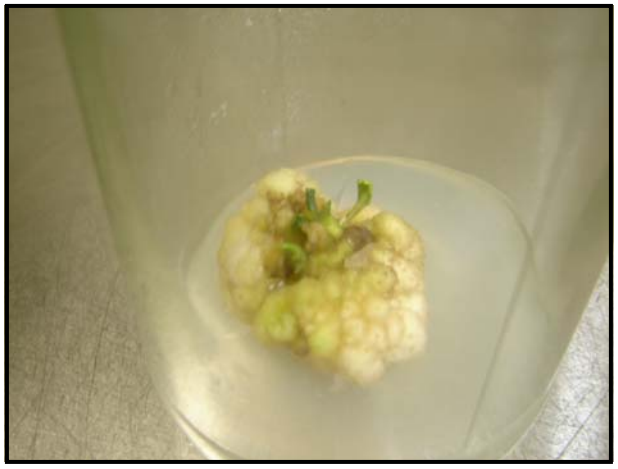

Plate 1. Photograph of proliferated calli of the variety Early Tropical in 3.0 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$

Plate 2. Photograph of proliferated calli of the variety Tangail Special Pauslali in 3.0 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$

Plate 3. Regenerated plantlet of Tangail Special Pauslail in plastic pot kept in net house for hardening

Effect of phytohormones: Among the phytohormone combinations, MS + 3.0 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$ (T_3) showed superior performance in respect of number of shoots per explant (1.75), number of shoots per callus (2.55) and number of shoots per vial (6.10). Whereas MS + 2.5 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$ (T_2) showed the lowest performance. So, it can be concluded that MS medium supplemented with MS + 3.0 mg L^{-1} BAP+ 0.1 mg L^{-1} 2,4-D + 2.0 mg L^{-1} $AgNO_3$ (T_3) was the best for shoot initiation and plantlet (Plate 3) regeneration (Table 2). These results are similar to the observation of Hasan (2006), Du *et al.* (2000).

Table 2. Performance of different concentrations and combinations of phytohormones on different characters of shoot regeneration

Phytohormone combinations	No. of shoots per explant	No. of shoots per callus	No. of shoots per vial
2.0 mgL^{-1} BAP	1.550ab	2.050b	5.500b
2.5 mgL^{-1} BAP	1.300b	1.700b	5.250b
3.0 mgL^{-1} BAP	1.750a	2.550a	6.100a

BAP was added to MS medium supplemented with 0.1 mgL^{-1} 2,4-D and 2.0 mgL^{-1} $AgNO_3$.
Note: Mean values having common letter(s) are statistically identical and those having different letter(s) are statistically different

Effect of hormone × variety interaction: The results related to hormone × variety interaction for shoot regeneration characters like, number of shoots per explant, number of shoots per callus and number of shoots per vial is presented in Table 3. The interaction between hormone × variety for the concentration and combination of 2.0, 2.5 and 3.0 mg L^{-1} BAP were found to be non significant. Among the characters the higher number of shoots per vial was 7.20 and the lower number of shoots per vial was 4.40. Among the concentrations, MS + 3.0 mg L^{-1} BAP + 0.1 mg L^{-1} 2, 4-D + 2.0 mg L^{-1} $AgNO_3$ (T_3) produced the higher number of shoots per vial.

Table 3. Effect of hormone × variety on different characters of shoot regeneration of cabbage and cauliflower

Hormone × Variety		No. of shoots per explant	No. of shoots per callus	No. of shoots per vial
2.0 mgL^{-1} BAP	Tara	1.200a	1.600a	5.400a
	Summer star	1.400a	2.200a	5.800a
	Early tropical	1.000a	1.400a	5.200a
	Tangail special pauslali	1.600a	2.400a	5.600a
2.5 mgL^{-1} BAP	Tara	1.800a	2.200a	6.000a
	Summer star	1.200a	1.600a	4.600a
	Early tropical	1.400a	2.200a	5.400a
	Tangail special pauslali	1.000a	1.600a	4.400a
3.0 mgL^{-1} BAP	Tara	1.800a	2.600a	6.000a
	Summer star	1.400a	1.600a	5.400a
	Early tropical	2.200a	2.600a	6.400a
	Tangail special pauslali	2.400a	3.200a	7.200a

BAP was added to MS medium supplemented with 0.1 mgL^{-1} 2,4-D and 2.0 mgL^{-1} $AgNO_3$.

References

BBS. 2004. Monthly Statistical Bulletin, December, 2004. Bangladesh Bureau of Statistics. Statistics Division, Ministry of Planning, Govt. of the People's Republic of Bangladesh. pp. 50-57.

Benson, L. 1957. Plant classification, Raytheon Education company, Boston, Massachusetts, USA.

Bose, T.K. and Som, M.G. 1986. Vegetable Crops in India. Naya Prokakash, Calcutta, India. pp. 165-166, 190-196.

Du, H., Zhuang, D.H. and Hunang, W.H. 2000. Stimulation effect of silver nitrate on shoot regeneration in cotyledon tissue culture of *Brassica oleracea*. J. Trop. Subtrop. Bot., 8(2): 109-112.

Hasan, M.M. 2006. *In vitro* regeneration in cauliflower (*Brassica oleracea* var *botrytis*)

An unpublished Thesis of Master of Science. Department of Biotechnology. Bangladesh Agril. Univ., Mymensingh. pp. 1-3.

Henzi, M.X., Christey, M.C. and Mc Neil, D.L. 2000. Factors that influence Agrobacterium rhizogenes-mediated transformation of broccoli (*Brassica oleracea* L. var. *italica).* Plant Cell Rep., 19: 994-999.

Siddiqui, M.A.H. 2004. Effect of zinc, boron and molybdenum on seed production of cauliflower. An unpublished Thesis of Master of Science. Department of Horticulture, Bangladesh Agril. Univ., Mymensingh. pp. 1-3.

Yarnell, S.H. 1956. Cytogenetics of vegetable crops. Crucifers. Bot. Rev. 22(2): 81-166.