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In vitro Regeneration of Cabbage (*Brassica oleracea* L. var. Capitata) through Hypocotyl and Cotyledon Culture

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Abstract

The best response toward direct regeneration of multiple shoots from seven-day-old seedling was observed on half-strength MS supplemented with 0.5 mg/l BA. Hypocotyl and cotyledon explants produced highest percentage (73 and 66, respectively) of shoots. The maximum number of shoots (12) and the highest shoot length of 5.9 cm were also observed in this medium. On the other hand, indirect regeneration via callus was observed in cotyledonary explants. Maximum percentage of callus formation was observed on MS containing 1.0 mg/l 2,4-D and 0.5 mg/l NAA. Highest frequency of shoot regeneration was achieved on MS fortified with 2.0 mg/l BA and 0.1 mg/l NAA in cotyledon derived callus. Shoot regeneration was not obtained in hypocotyl-derived callus. Shoots rooted well when they were excised individually and implanted in half-strength MS with 0.5 mg/l IBA in which 98% rooting was achieved within 10 - 12 days. The well rooted *in vitro* raised plantlets were successfully transferred to soil and their survival rate under natural environment was 86%.

Introduction

Cabbage is one of the most important vegetable crops in Asia including Bangladesh. It is a good cash crop as well as valuable source of calcium, crude fibre and vitamin-C in daily diet (Telekar and Griggs 1981). It is mainly used for curries, pickle etc. and also for feeding stocks of chicken. It is a temperate crop, but nowadays there is a strong demand of this crop in South East Asian countries (Shinohara 1980). It is known that high temperature is one of the major factors for its profitable cultivation in the tropical areas. The productivity and quality of this crop also suffer due to its susceptibility to a number of diseases and insect/pests. The crop is extensively damaged by infestation of a fungal disease caused by *Alternaria brassicae* and *Pythium* sp. and also by cut worm and cabbage butterfly insects. The traditional breeding method for the improvement of cabbage has its limitation. It requires 10 to 15 years to complete a selection cycle. As an alternative, genetic transformation can be employed for development of disease and pest resistant as well as heat tolerant cultivars of this crop.

There are a good number of reports regarding *in vitro* regeneration of cabbage from different explants via organogenesis (Zhang et al. 1998, Deng et al. 1991, Bajaj and Nietsch 1975, Xiaoou et al. 2007), somatic embryogenesis and microspore culture (Kuginuki et al. 1999). The present study was undertaken to establish a reproducible protocol for *in vitro* regeneration of cabbage using hypocotyls and cotyledons as explants.

Materials and Methods

Cotyledon and hypocotyl of cabbage (*Brassica oleracea* L. var. Capitata) excised from aseptically germinated seven-day-old seedlings were used as explants for multiple shoot formation. Freshly mature and dried seeds were collected from the market and washed thoroughly under running tap water to eliminate the dust and surface contaminants. The seeds were then washed with distilled water for 3 - 4 min followed by a wash in 70% ethanol for 1 min. Then they were transferred to sterile conical flask and surface sterilized with a 0.1% $HgCl_2$ for 12 min under laminar flow. These were washed with sterile distilled water for three times. Finally they were cultured in germinating medium (containing 2% sucrose and 1% agar). Cotyledones and hypocotyls were separated from axenic seedlings and cultured on MS and half strength MS containing different concentrations of hormones. The media contained 3% sucrose, 0.7% Difco-bacto agar and the pH was adjusted to 5.8 prior to autoclaving. All the cultures were maintained under a 16 h photoperiod (3000 lux, approx.) regime at 26 ± 2^0C. Subcultures were maintained at a regular interval of four weeks. Data on different parameters were recorded after four weeks of culture.

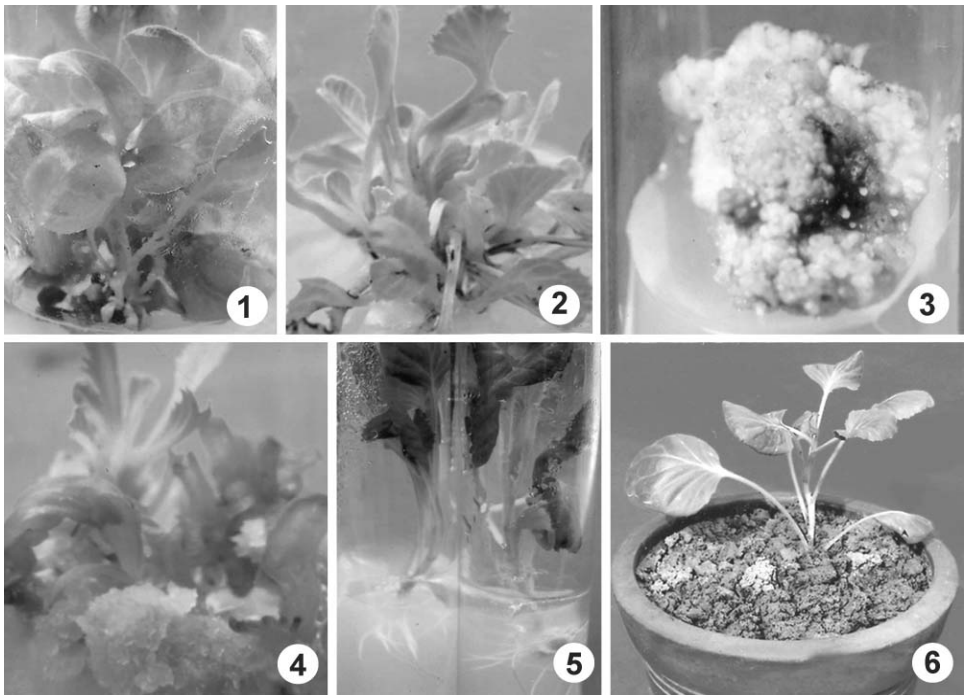
Results and Discussion

Hypocotyl and cotyledon explants excised from aseptically grown seven-day-old seedlings of cabbage were cultured on MS and half strength MS supplemented with various hormonal concentrations for production and development of multiple shoots. BA, Kn and 2ip at different concentrations were added to MS and half-strength MS to determine their effect for shoot development and multiplication. Shoot development and shoot multiplication were achieved at all media composition containing hypocotyl explants (Table 1). Morphogenic potential of explants differed between the two types of explants. Hypocotyl proved to be the most potential for multiple shoot regeneration directly compared to cotyledons. However, Chowdhury and Prakash (1992) believed that plant multiplication by *in vitro* using nodal explants is simple, efficient and also economical in *Dianthus caryophyllus*. But the present study showed that hypocotyl explants are also efficient for *in vitro* shoot regeneration and shoot multiplication. The effect of different hormones showed the production of maximum percentage (73) of shoots on half-strength MS fortified with 0.5 mg/l

Table 1. Production of multiple shoots on MS and half strength MS medium containing various concentrations of cytokinins from hypocotyl and cotyledon explants of *Brassica oleracea*.

Supplements (mg/l)	Hypocotyls			Cotyledons		
	% of responsive explants	No. of shoots/ explants (± SE)	Mean length of shoots (cm)	% of responsive explants	No. of shoots/ explant (± SE)	Mean length of shoots (cm.)
MS + 0.0	46	4 ± 0.54	4.4	-	-	-
BA						
0.5	48	2 ± 0.15	4.6	40	2 ± 0.18	4.8
1.0	52	4 ± 0.48	5.3	45	3 ± 0.52	4.8
2.0	50	3 ± 0.72	4.6	40	3 ± 0.48	4.5
MS + Kn						
0.5	30	3 ± 0.85	4.4	24	2 ± 0.32	4.6
1.0	44	2 ± 0.18	4.2	40	2 ± 0.35	4.7
2.0	30	2 ± 0.24	4.3	30	2 ± 0.28	4.6
MS + BA						
0.5	48	2 ± 0.15	4.6	40	2 ± 0.18	4.8
1.0	52	4 ± 0.48	5.3	45	3 ± 0.52	4.8
2.0	50	3 ± 0.72	4.6	40	3 ± 0.48	4.5
MS + Kn						
0.5	30	3 ± 0.85	4.4	24	2 ± 0.32	4.6
1.0	44	2 ± 0.18	4.2	40	2 ± 0.35	4.7
2.0	30	2 ± 0.24	4.3	30	2 ± 0.28	4.6
MS +2iP						
0.5	46	5 ± 0.65	5.2	38	4 ± 0.62	5.3
1.0	38	4 ± 0.38	4.9	35	4 ± 0.58	5.4
2.0	35	3 ± 0.25	4.6	26	2 ± 0.33	4.7
½ MS + 00	62	7 ± 0.82	4.8	-	-	-
½ MS + BA						
0.5	**73**	**12 ± 1.25**	**5.9**	**66**	**6 ± 0.57**	**5.6**
1.0	65	8 ± 1.12	5.4	62	5 ± 0.62	5.5
2.0	60	5 ± 0.88	5.3	48	3 ± 0.35	5.2
½ MS + Kn						
0.5	62	5 ± 0.68	5.1	58	4 ± 0.42	5.5
1.0	58	3 ± 0.35	4.6	42	3 ± 0.37	4.8
2.0	52	3 ± 0.28	4.4	38	3 ± 0.34	4.4

BA in hypocotyl explants. The highest number of shoots (12) per explant was also recorded in these media from hypocotyl (Fig. 1). On the other hand, cotyledon produced maximum number (66) of shoots at the same medium (Fig. 2). The highest length of shoots derived from hypocotyl in the same medium was 5.9 cm. With repeated subcultures at an interval of four weeks the number of regenerated shoots per culture gradually increased.



Figs. 1-6. *In vitro* propagation of cabbage (*Brassica oleracea* L.). 1. Multiple shoot regeneration in half MS + 0.5 mg/l BA from hypocotyl. 2. Shoot proliferation in half MS + 0.5 mg/l BA from cotyledon. 3. Callus induction in MS fortified with 1.0 mg/l 2,4-D and 0.5 mg/l NAA. 4. Shoot regeneration via callus in MS+ 2.0 mg/l BA + 0.5 mg/l NAA. 5. Rooting of *in vitro* regenerated shoot in half MS + 0.5 mg/l IBA. 6. Potted plant after three weeks of transfer.

Meanwhile, MS supplemented with 0.5 - 2.0 mg/l BA, Kn, 2ip showed comparatively less response toward shoot multiplication from both hypocotyl and cotyledon (Table 1). A considerable improvement of multiple shoot induction from hypocotyl and their subsequent growth were also observed on MS supplemented with 1.0 mg/l BA. However, cotyledons produced comparatively lower number of shoot/culture at all concentrations of hormones. Interestingly, hypocotyl responded best in both MS and half strength MS even without any hormonal supplements. Gupta et al. (1980) obtained only two shoots/culture when they used hypocotyl of *Tectona grandis* in MS supplemented with 0.1 - 2.0 mg/l BA. They also observed that in the media supplemented with Kn and BA there were only two to three adventitious shoots/culture in shoot apices, nodal segments and hypocotyls. In turmeric and ginger, Balachandran et al. (1990) reported that BA alone was adequate for shoot multiplication and their finding is partially supported by the present investigation. Lazzeri and Dunwell (1984) also observed *in vitro* plant regeneration of cabbage in MS fortified with BA and Kn. These results are also in agreement with the present study in which Kn was found to be less effective.

Table 2. Effect of different concentrations of IAA, IBA and NAA in half-strength MS on root formation of *in vitro* grown shoots.

Conc. (mg/l)	% of shoots rooted	Average No. of roots/shoot mean ± SE	Average length of roots (cm) mean ± SE	Days to rooting
IAA				
0.5	-	-	-	-
1.0	28	4.1± 0.3	4.7± 0.2	12 - 14
1.5	35	5.7± 0.1	4.5± 0.4	12 - 14
2.0	26	3.8± 0.1	4.2± 0.1	12 - 14
IBA				
0.5	**98**	**8.4 ± 0.1**	**6.5 ± 0.3**	**10 - 12**
1.0	95	7.8 ± 0.3	6.2 ± 0.1	10 - 12
1.5	81	7.5 ± 0.2	6.0 ± 0.3	10 - 12
2.0	78	6.4 ± 0.3	5.7 ± 0.1	10 - 12
NAA				
0.5	77	7.6 ± 0.2	5.4 ± 0.2	12 – 15
1.0	70	6.5 ± 0.2	4.5 ± 0.3	12– 15
1.5	64	4.1 ± 0.1	4.2 ± 0.2	12 – 15
2.0	61	3.8 ± 0.3	4.1 ± 0.3	12 – 15

Callus induction was observed after two - three weeks of culture in cotyledons grown in different hormonal concentrations and combinations. Best callus induction was obtained in MS containing 1.0 mg/l 2,4-D and 0.5 mg/l NAA (Fig. 3). Moderate callus induction was observed at the same hormonal combination in hypocotyl. Shoot regeneration via callus was obtained on MS fortified with 2.0 mg/l BA and 0.1 mg/l NAA (Fig. 4). The maximum number of shoots regenerated per callus mass was six.

In vitro raised shoots were transferred on to half-strength MS supplemented with four concentrations (0.5, 1.0, 1.5 and 2.0 mg/l) of IAA, IBA and NAA. It was observed that shoots rooted at all the media except the one containing 0.5 mg/l IAA. Among the concentrations, 0.5 mg/l IBA was found most suitable for percentage, number and length of roots. These results are in agreement with the findings of Hossain et al. (1995) in *Aegle marmelos*. However, in NAA supplemented medium, root induction was low and shoots in the medium had a tendency to callus at their base. Caboni and Tonalli (1999) reported that IBA is the most effective auxin for root induction in a wide range of plant species. It was also found that IBA is superior to IAA or NAA for its more stable nature (Hutchinson 1995, Litz and Jaiswal 1990). The present study also proved that IBA is the best over IAA and NAA for root induction. After thorough washing of the well-developed roots with tap water, *in vitro* raised plantlets were transferred to earthen pots containing a mixture of soil and compost (2 : 1) and gradually acclimated (Fig. 6). About 86 % plantlets were successfully established in the experimental field after acclimation.

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