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In Vitro Multiplication of Kakrol (*Momordica dioica* Roxb.)

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A study on *in vitro* plant regeneration in kakrol was carried out. The combination of 1 mgl^{-1} BAP and 0.1 mgl^{-1} NAA in MS media was found most suitable in callus induction followed by 0.2 mgl^{-1} BAP. The combination of 1 mgl^{-1} BAP and 0.1 mgl^{-1} NAA produced the highest number of multiple shoots from the callus. Half strength MS medium supplemented with IBA showed better performance than with IAA for number of roots but the later produced tallest shoot. Four types of explants, node, shoot tip, leaf and cotyledon, were used but only the cotyledon exhibited the best performance. Plantlets were successfully established in normal soils.

INTRODUCTION

Kakrol (*Momordica dioica* Roxb.) is a cucurbitaceous vegetable of Indo–Malayan origin (Rashid, 1976; Singh, 1990). It is dioecious in nature and usually propagated vegetatively through tuberous roots. It is rich in carotene, protein, carbohydrate (Rashid, 1976) and vitamin C (Bhuiya *et al.*, 1977). It has been cultivated in India, Bangladesh and neighboring countries for a long time.

Kakrol is an important summer vegetable in Bangladesh. Its cultivation is gaining popularity for its high economic return as compared with that of cereals (Das, 1988; Anonymous, 2001). It has better shelf life and being exported to Middle East, UK and other countries thereby earning foreign currencies. In spite of many advantages, many efforts have not been paid to the crop for its improvement. Recently, homosexual crossing (induction of bisexuality using chemicals) is made possible and sets seed (Hossain *et al.*, 1996; Ali *et al.*, 1991), which may serve as a source of variation for commencing breeding program. But true breeding is seemed to be handicapped due to poor germinability of crossed seeds i.e. F_1, F_2 and subsequent generations (Rasul, 2000, personal communication).

The normal propagation entirely depends on underground tuberous roots that occupy the cultivable lands for a long time i.e. until next growing season. Growers dig out the tuberous roots to free the field for next crop and preserve them in holes at the corner of the field. Sometimes they preserve them in sand under shady place. With the time pass-

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es, the roots become rotten and get shrunk. This hampers the quality of roots to be used as planting propagule next year.

In vitro micropropagation of kakrol will greatly help to overcome many problems related to its successful production, seed germination and creating variability. Adequate information on these aspects is scanty. Therefore, the present study was undertaken to identify suitable explants and to develop a standard protocol for *in vitro* micropropagation in kakrol.

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of Biotechnology Division, Bangladesh Agricultural Research Institute, Gazipur 1701, Bangladesh during November 1999 to April 2000.

Plant parts used as explants

Cotyledons, shoot tips, nodes and leaves of kakrol were used as explants. The true seeds and other plant parts were taken from the field grown mature plant from the research field of the Department of Genetics and Plant Breeding of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

Preparation of Stock solution of MS media and growth regulators

Stock solution for MS media was prepared according to Murashige and Skoog (1962). Four types of growth regulators, 6–benzyl aminopurine (BAP), α–naphthalene acetic acid (NAA), indole acetic acid (IAA) and indole butyric acid (IBA), were used. NAA and BAP were dissolved in 0.1 N NaOH, while IAA and IBA were in 0.1 N KOH.

Preparation of explants

The explants were washed thoroughly under running tap water in plastic pots for 10–15 minutes to reduce the surface organism. They were cut into pieces and washed with detergent powder and Tween 20 for 10 minutes and then sterilized with 0.1% mercuric chloride for 5 minutes. They were washed 3–4 times with sterilized water under laminar airflow cabinet. Finally the explants were prepared under laminar airflow cabinet excising small segments (0.5–1.0 cm) and were cultured on MS medium. In case of seeds, they were decoated, washed thoroughly and then placed on MS medium following the same procedure described earlier. After a week when the cotyledon opened the shoot tip was removed and the remaining cotyledon was allowed to callus formation.

Subculture and shoot and root induction

Subcultures were done at every 30 days intervals. Cultures were kept at 25 ± 1 °C under 16 hr photoperiod at a light intensity of 2000–3000 lux from florescent tubular light. The proliferated shoots were cut at the base and subcultured for shoot induction. The regenerated multiple shoots were cut and individual shoots were placed in half strength MS medium containing different concentration of IBA and IAA for root induction.

Data collection and experimental design

Completely Randomized Design (CRD) with three replications was followed. Each treatment had 24 test tubes per replication. Data on response of explants, success percentage of tubes containing callus, morphogenic response of callus, number and length of multiple shoots, number and length of roots were recorded. The success of callus growth was calculated by the following formula:

Success (%)=(Number of test tubes showed callus induction per treatment/Total number of test tubes set for callus induction per treatment)×100.

Shoots developed from explants were removed and those from callus were allowed to grow/multiplication. Number of shoots was the average number of shoots developed in 15 conical flasks per treatment (taking 5 flasks from each replication).

Duncan's Multiple Range Test (DMRT) was used during mean separation using MSTAT software.

RESULTS AND DISCUSSION

Morphogenic responses

Cotyledon showed high performance in callus induction than shoot tip, leaf and node (Table 1). Swelling of leaf occurred in treatments with 2 mgl^{-1} BAP+0.2 mgl^{-1} NAA and 3 mgl^{-1} BAP+0.2 mgl^{-1} NAA, but there were no further response. Direct shoots developed from node and shoot tip without callusing in treatments with 3 mgl^{-1} BAP+0.2 mgl^{-1} NAA and 1 mgl^{-1} BAP+0.3 mgl^{-1} NAA. The cotyledon showed the maximum shoot induction in 1 mgl^{-1} BAP+0.1 mgl^{-1} NAA. Hoque *et al.* (1995) had more or less similar observation in kakrol. They found that the combination of 1.5 mgl^{-1} BA+0.1 mgl^{-1} NAA was the most

Table 1. Effects of different explant and plant growth regulators on morphogenic response of kakrol.

Concentration (mgl^{-1})		Morphogenic response of explants[z]			
BAP	NAA	Shoot tip	Node	Leaf	Cotyledon
0	0	–	–	–	NS
1.0	0	–	–	–	NS
2.0	0	–	–	–	NS
3.0	0	–	–	–	–
0	0.1	–	–	–	C+S, S
1.0	0.1	–	–	–	C+S, S
2.0	0.1	–	–	–	C+S, S
3.0	0.1	–	–	–	C+S, S
0	0.2	–	–	–	C+S, S
1.0	0.2	–	–	–	C+S, S, R
2.0	0.2	–	–	Sw	NS
3.0	0.2	S	S	Sw	–
0	0.3	–	–	–	–
1.0	0.3	S	S	–	–
2.0	0.3	–	–	–	S
3.0	0.3	–	–	–	S

[z]–: No growth (response), S: Direct Shoot, Sw: Swelling, NS: No shoot but initial callus, C+S: Shoot from callus, R: Root.

Table 2. Effects of BAP and NAA on callus induction of kakrol from cotyledon.

Concentration (mgl^{-1})		Production of callus		Morphogenic response of plantlet differentiation
BAP	NAA	Amount of callus[z]	Success (%)[y]	
0	0	+	8.3	Callus dried up
1.0	0	+	20.8	Callus dried up
2.0	0	++	20.8	Good callus growth with shoot initiation
3.0	0	0	0	No callus growth
0	0.1	+	16.6	No callus growth but shoot initiation
1.0	0.1	+++	79.1	Good callus growth with shoot initiation
2.0	0.1	++	16.6	Good callus growth with shoot initiation
3.0	0.1	++	20.8	Good callus growth with shoot initiation
0	0.2	++	50.0	Slow callus growth
1.0	0.2	+++	29.1	Good callus growth with shoot and root
2.0	0.2	+++	41.6	Good callus growth with shoot and root
3.0	0.2	0	0	No callus growth
0	0.3	0	0	No callus growth
1.0	0.3	0	0	No callus growth
2.0	0.3	+	16.6	No callus growth but shoot initiation
3.0	0.3	+	20.8	Slow callus growth but shoot initiation

[z]Profuseness of callus based on eye estimation. 0: No callus, +: Low callus, ++: Medium callus, +++: High callus.
[y]Success (%)=(Number of test tubes showed callus induction per treatment)/(Total number of test tubes set for callus induction per treatment)×100.

suitable for multiple shooting.

One mgl^{-1} BAP+0.1 mgl^{-1} NAA resulted the maximum success of callus growth (79.1%) after 12 days of culture followed by 0.2 mgl^{-1} NAA which was 50% (Table 2).

Treatments with 2 mgl^{-1} BAP+0.2 mgl^{-1} NAA, 1 mgl^{-1} BAP, 2 mgl^{-1} BAP and 3 mgl^{-1} BAP+0.1 mgl^{-1} NAA showed good response to callus induction from the cotyledons. The treatment with 1 or 2 mgl^{-1} BAP+0.2 mgl^{-1} NAA only induced roots in cotyledon explants.

Shoot proliferation

Shoots emerged both from callus and explants. The shoots emerged directly from explants are true to type and it is desirable when exact copy of parental multiplication are expected. But the numbers were very poor (data were not recorded). Shoots derived from callus were, therefore, recorded. One mgl^{-1} BAP+0.1 mgl^{-1} NAA produced the highest number of multiple shoots (25.3) closely followed by 2 mgl^{-1} BAP (21.3) that differed significantly from other treatments (Table 3). The regenerated shoots were comparatively weak in the treatment with 1 mgl^{-1} BAP+0.1 mgl^{-1} NAA. Hoque *et al.* (1995) found maximum 8.8 shoots per explant, and it was close to the minimum value of our study.

Shoot growth

Shoot elongation showed the more or less similar trend as observed in multiple shoot proliferation. Treatment with 1 mgl^{-1} BAP+0.1 mgl^{-1} NAA produced the longest shoots (4.5 cm) closely followed by 2 mgl^{-1} BAP+0.2 mgl^{-1} NAA (4.1 cm) and 1 mgl^{-1} BAP+

Table 3. Effects of BAP and NAA on shoot proliferation in kakrol using cotyledon as explants.

Concentration (mgl^{-1})		Number of shoots[z]	Length of shoots (cm)
BAP	NAA		
0	0	–[y]	–
1.0	0	–	–
2.0	0	21.3 b[x]	2.2 e
3.0	0	–	–
0	0.1	–	–
1.0	0.1	25.3 a	4.5 a
2.0	0.1	10.6 d	2.6 d
3.0	0.1	10.0 d	2.3 de
0	0.2	–	–
1.0	0.2	8.6 d	3.4 c
2.0	0.2	15.6 c	4.1 b
3.0	0.2	–	–
0	0.3	–	–
1.0	0.3	–	–
2.0	0.3	–	–
3.0	0.3	–	–

[z]Number of shoots=Number of shoots developed in 15 conical flasks per treatment during subculture (taking five flasks from each replication)/15.
[y]No shoots.
[x]Mean separation within columns by DMRT at 5% level.

0.2 mgl^{-1} NAA (3.4 cm) that differed significantly from other treatments (Table 3). Though longer shoots are weak but it yield more explants which is desirable for further rapid *in vitro* multiplication. There was no citation on the growth and elongation of shoots of kakrol earlier. Hoque *et al.* (1995) failed to elongate the shoots in kakrol on the medium when BA and kinetin were alone used.

Root induction and growth

In vitro grown multiple shoots were excised and cultured on a half strength MS medium supplemented with IBA and IAA. Most of the cultured shoots produced roots in

Table 4. Effects of IAA and IBA in half strength MS medium for root induction and growth of kakrol using cotyledon as explants.

Treatment	Concentration (mgl^{-1})	Number of roots per plantlet	Length of roots (cm)
Control	0	1.9 b[z]	3.5 ab
IAA	0.2	1.1 c	4.1 a
	0.3	2.6 a	3.5 ab
IBA	0.2	2.6 a	2.8 b
	0.3	2.8 a	1.9 c

[z]Mean separation within columns by DMRT at 5% level.

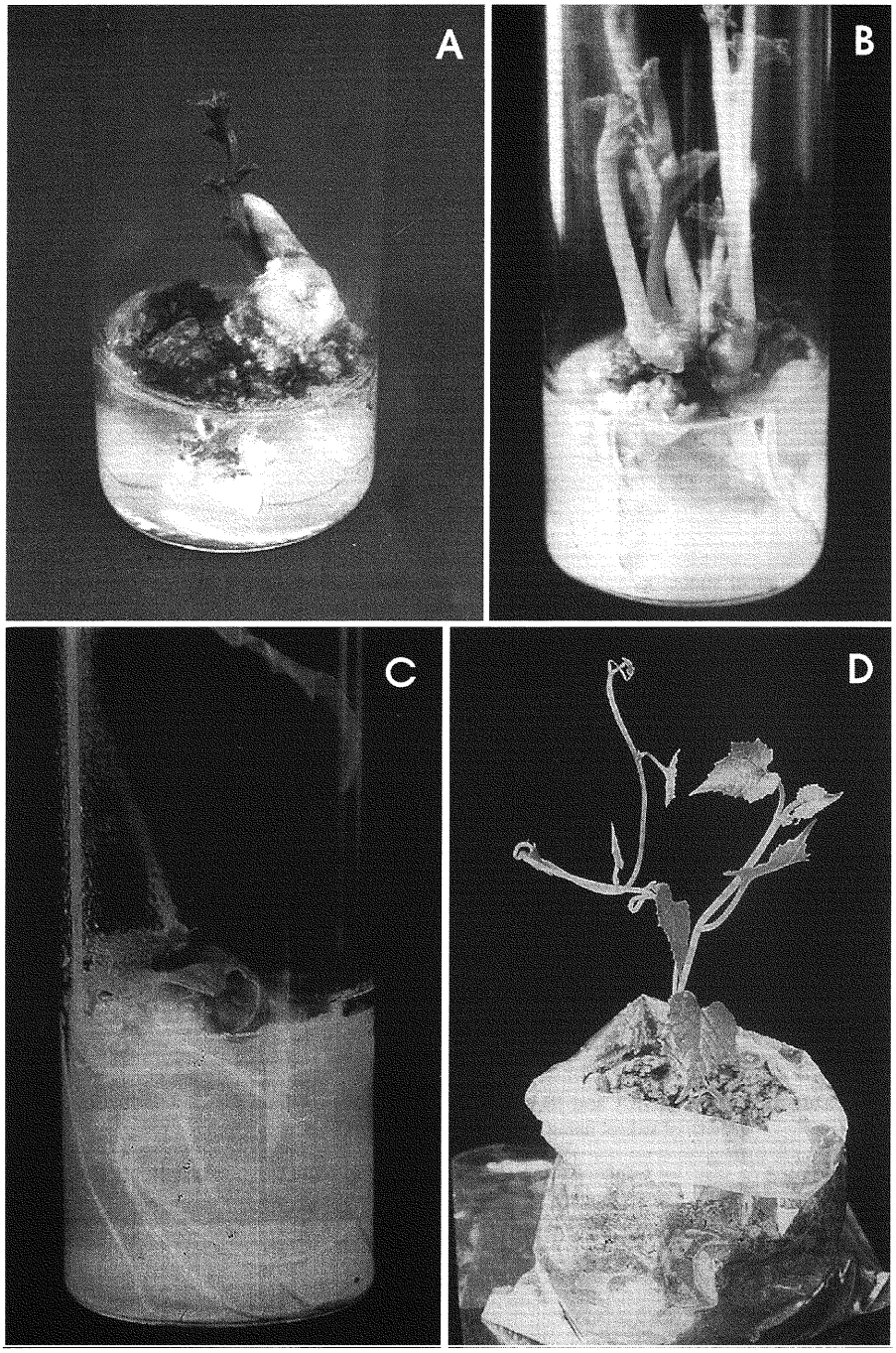

Fig. 1. Sequential steps of micropropagation of kakrol. A; callus induction, B; multiple shooting in subculture, C; rooting and D; ex vitro establishment

12–20 days of subculture. The highest number of roots (2.8) was produced by the treatment with 0.3 mgl^{-1} IBA followed by 0.2 mgl^{-1} IBA (2.6) and 0.3 mgl^{-1} IAA (2.6) (Table 4). IBA performed better than IAA for rooting ability. In our study the root number is lower than that of Hoque *et al.* (1995). It may be due to genotypic variation of the explants along with cultural and environmental conditions. The longest root (4.1 cm) was found in 0.2 mgl^{-1} IAA.

Ex vitro establishment

For the establishment of plant, regenerated healthy rooted plantlets were placed at room temperature for 10–14 days. Then the plantlets removed from the culture bottles were carefully cleaned to remove the adhering agar. The plantlets were sprayed with fungicides and planted to normal and sterilized soils in polyethylene bags in a net house. After 25 days of hardening, the seedlings were transplanted in the field and were successfully acclimatized in the soil. The overall sequential steps of micropropagation are shown in Fig. 1.

Conclusion

Although genetic variation might occur in micropropagation through callus, the advisable procedure for *in vitro* multiplication of kakrol is first to induce callus from cotyledons on MS medium supplemented with 1.0 mgl^{-1} BA and 0.1 mgl^{-1} NAA, then to subculture the callus for shoot initiation on the same composition of plant hormones as for callus induction on MS medium, and finally to transplant on a half strength MS medium with 0.2–0.3 mgl^{-1} IAA or IBA for rooting.

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