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ADVENTITIOUS SHOOT REGENERATION FROM LEAF EXPLANTS OF KAKROL CULTURED *IN VITRO*

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Abstract

Adventitious shoot bud differentiation occurred from the petiolar cut ends of leaf explants of *Momordica dioica* (Roxb.) Willd.. cultured on Murashige and Skoog medium supplemented with different concentrations and combinations of BA and NAA. The best response of shoot proliferation was obtained in medium supplemented with 2.0 mg/l BA and 0.2 mg/l NAA. Direct and indirect (via callus) shoot bud differentiation were observed. After 3 weeks of culture, calli were transferred onto the MS medium supplemented with aforesaid growth regulator for bud regeneration. Rooting was induced with 1.0 mg/l BA and plantlets were survived successfully to field condition. This is the first description of adventitious bud regeneration from leaf explants of kakrol.

Introduction

Two of the basic strategies used for micropropagation of plants are direct regeneration and indirect regeneration *via* an intermediate callus phase. Indirect regeneration often results in somaclonal variations. Therefore, direct regeneration, which involves morphogenesis without an intermediate callus phase, is a more reliable method for clonal propagation (Rao *et al.*, 1996).

Momordica dioica, commonly known as 'kakrol' (Teasle gourd) is an economically important cucurbitaceous summer vegetable in Bangladesh and its neighboring countries. Slices of unripe fruits are served in different types of curries and fried forms and have good nutritional value. The young twigs and leaves of this crop are also used as vegetable (Fakir *et al.*, 1992). With increase in its popularity, the commercial cultivation of kakrol has been expanded. Micropropagation through cotyledon has been described as an alternative for mass production (Hoque *et al.*, 2000). However, there are no reports of the regeneration of kakrol from leaves or other organs. In this paper, we report a method for the direct and indirect regeneration of kakrol plants from leaves, and we discuss the potential of this approach for the micropropagation of kakrol.

Materials and Methods

The experiments were done with explants of tetraploid *Momordica dioica* (Roxb.) Willd. Young fully expanded leaf explants were excised from micropropagated plants obtained through cotyledon culture as described by Hoque *et al.*, (2000) and maintained *in vitro* on MS medium supplemented with 2.0 mg/l BA and 0.2 mg/l NAA. Leaf explants were cultured on MS medium solidified with 0.7% agar and supplemented with different combinations of BA and NAA. Explants were placed, with abaxial surface

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down, on the regeneration medium. The protocol for plantlets regeneration used a sequence of four step, direct shoot bud initiation, callus development, shoot regeneration *via* callus and rooting. For regeneration, a part of nodular callus (6-7 mm^3) was cultured onto the same media into the culture vessel (data not shown). Rooting of *in vitro* derived shoots was accomplished on MS medium supplemented with 1.0 mg/l BA. After that, process of acclimatization and transfer to field were same as described in our previous report (Hoque *et al.*, 2000).

The explants were directly incubated under light (16-h photoperiod, 45 µmol $m^{-2}s^{-1}$) at 26 ± 1°C. In all these steps the basal medium consisted of MS (Murashige & Skoog, 1962) salts supplemented with vitamins and 30 g/l sucrose, with the pH adjusted to 5.8. Each treatment was randomized completely, with three replications per treatment and 18 explants per replicate. Observations were recorded at every two week intervals for adventitious shoot proliferation, callus induction and rooting. The comparison of means was analyzed using the Duncan (1955) multiple range test at $P<0.05$.

Results and Discussion

Leaf explants of kakrol were inoculated on different combination of BA and NAA. The results showed a variable shoot forming capacity depending on the combination of growth regulators used in the culture medium (Table 1). Of the several combinations, MS medium supplemented with 1.0~2.0 mg/l BA and 0.2 mg/l NAA were the most effective giving high number of shoots per explant. The number of shoots produced increased with the concentration of BA until 3.0 mg/l of the cytokinin. In the presence of 4.0 mg/l of BA a high frequency of explants exhibiting friable green callus was observed. When these calli were excised and maintained on MS medium supplemented with 2.0 mg/l of BA and 0.2 mg/l of NAA, shoot regeneration was obtained within 4 weeks. Explants cultured without growth regulator or with BA alone did not significantly develop callus (data not shown). Callus initiation was quick and started from the petiolar cut ends of the leaf explants with higher concentrations of NAA.

Table 1. Direct and indirect shoot formation in kakrol with BA and NAA combination after 6 weeks of culture onto the MS medium.

Growth substances (mg/l)		Direct regeneration		Indirect regeneration	
BA	NAA	% of explant producing shoot	Number of shoots per explant	% of explant producing callus	Number of shoots per callus
1	0.1	15.3 h*	3.3 ± 0.4 e*	10.6 h*	4.2 ± 0.3 d*
	0.2	36.5 de	8.8 ± 0.6 c	25.3 f	6.1 ± 0.8 cd
	0.5	26.2 g	7.4 ± 1.5 d	30.4 e	7.6 ± 0.6 c
2	0.1	45.7 d	12.7 ± 1.1 b	15.1 g	8.4 ± 0.7 bc
	0.2	66.8 a	17.1 ± 1.2 a	30.1 e	10.6 ± 0.9 b
	0.5	62.4 b	11.3 ± 0.7 b	26.7 f	9.2 ± 0.6 bc
3	0.1	35.1 e	10.9 ± 0.8 b	30.9 e	7.3 ± 0.5 c
	0.2	64.9 a	15.7 ± 1.3 a	32.4 de	9.2 ± 0.7 bc
	0.5	56.3 c	9.6 ± 0.5 c	35.1 d	11.6 ± 1.1 b
4	0.1	32.6 f	9.3 ± 0.7 c	45.3 c	8.5 ± 0.6 bc
	0.2	45.2 d	12.7 ± 0.9 b	48.7 b	10.8 ± 0.8 b
	0.5	38.6 de	7.5 ± 0.8 d	55.2 a	14.6 ± 1.2 a

*Comparison of the mean values obtained in treatments were made using Duncan's multiple range test; the values with different letters within a column are significantly different at $p<0.05$.

The shoot regenerated directly from the petiolar cut ends of leaf explants (Fig. 1B) were successfully separated and micropropagated during 6 weeks on MS medium supplemented with 2.0 mg/l of BA and 0.2 mg/l of NAA. Plant regeneration from leaf explants as well as petiolar part are reported in other plants (Kumar *et al.*, 1998; Nyochembeng & Garton, 1998; Dronne *et al.*, 1999; Rey *et al.*, 2000; Ibrahim & Debergh, 2001). After this period, individual shoots were rooted on MS medium supplemented with 1.0 mg/l of IBA (Hoque *et al.*, 2000) within 2 weeks of culture.

After that, cultures were kept for 10 days at room temperature (28°~30°C) for hardening. About 88% of these semi-hardened plantlets survived in non-sterile soil and sand mixture but direct transfer of plantlets resulted in a low survival rate of 60%. To maintain high humidity during the initial 4 or 5 days the pots were covered with polythene bags with perforations and after 30 days the plants were transferred to field where they produced flowers and fruits. The plants were free from growth defects as revealed by visual analysis of morphological traits, based on leaf shape, growth habit, flower shape and flowering period. The occurrence of somaclonal variation also should be studied for its improvement.

This study showed that the adventitious shoot regeneration from leaf explants of kakrol can be a useful method for the multiplication of this important vegetable, allowing for the rapid commercial propagation of kakrol.

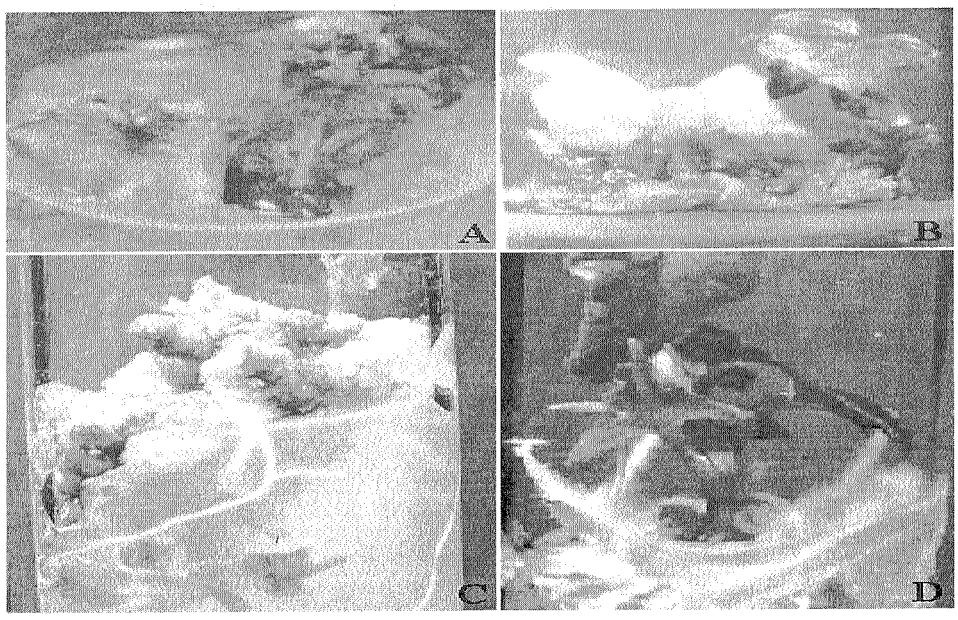

Fig. 1. A-D – Adventitious shoot formation in leaf explant cultures of *Momordica dioica*. A. Formation of callus and protrusions from petiolar cut ends of leaf on MS with 2.0 mg/l BA and 0.2 mg/l NAA, 2 weeks after culture. B. Direct shoot formation from petiolar part of leaf explants on MS with 2.0 mg/l BA and 0.2 mg/l NAA, 4 weeks after culture. C. Shoot regeneration from leaf-derived callus on MS with 2.0 mg/l BA and 0.2 mg/l NAA, 3 weeks after subculture. D. Development of adventitious roots from excised regenerated shoot on MS with 1.0 mg/l BA.

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