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IN VITRO PLANT REGENERATION FROM ZYGOTIC EMBRYOS OF *CITRULLUS LANATUS* THUNB

R. Islam, A. Ahad, M. A. Reza, A. N. K. Mamun and O. I. Joarder

Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

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A procedure has been developed which allows high frequency plant regeneration from mature zygotic embryos of *Citrullus lanatus*. Prolific shoot regeneration occurred on Linsmaire and Skoog (LS) medium containing BA-IAA combinations. Embryo axes were more responsive explants than cotyledon explants. Regenerated shoots were rooted on Murashige and Skoog (MS) medium supplemented with 1 mg/l NAA.

Key Words: Plant regeneration, Zygotic embryos, *C. lanatus*.

Introduction

Watermelon (*Citrullus lanatus* Thunb.) is widely grown in the tropics and subtropics, most part of the south-east Asia, Africa, the Carribean and the southern parts of United States. It is not only an economically important crop but also a valuable alternative source of water in desert. The introduction of new genes conferring characters into the watermelon by means of genetic manipulation is of greater potential value, especially traits that would confer resistance to pathogens and pests. The usefulness of cell and tissue culture technique often depend upon the development of an efficient *In vitro* plant regeneration system. Howevr, less attention has been given to tissue culture of watermelon than the closely related cucumber, *Cucumis melo* [1], *Cucurbita pepo* [2] and squash [3, 4]. The only *In vitro* system presently available for the watermelon is micropropagation by shoot tip, cotyledon and hypocotyl cultures [5]. This paper describes a method for regeneration from mature zygotic embryos of watermelon.

Materials and Methods

Mature seeds of watermelon were collected from Natore Horticultural Centre. The seeds were washed thoroughly and surface sterilized in 0.1% $HgCl_2$ for 10 min and finally washed three times with autoclaved distilled water. Seeds were decoated, embryo axes were excised and cotyledons were separated.The embryonic axes and cotyledons were cultured separately on LS medium [6] containing 3% sucrose and 0.7% Difco Bacto-agar. The medium was supplemented with different concentrations of benzyladenine (BA) and indoleacetic acid (IAA). For adventitious rooting MS [7] with 2% sucrose, 0.7% agar and 0.1-1.0 mg/l napthaleneacetic acid (NAA) was used. Explants were initially cultured in test tubes (150 x 25 mm) containing 20 ml of medium and transferred after 5 weeks to 250 ml flasks containing 50 ml of medium. The pH of all media was adjusted to 5.7 prior to autoclaving at 120°C, 1.4kg/cm^2 for 15 min and the cultures were maintained at 27 ± 1°C with 16 h photoperiod (3000 lux). All experiments were repeated twice and statistical analysis was conducted [8].

Results and Discussion

A number of preliminary experiments revealed that phytohormones were essential for induction of adventitious shoots from mature zygotic embryo explants and no shoot bud was induced on basal LS medium. Therefore, attempts were made to induce adventitious shoot formation using individual cytokinins and auxins. It was noticed that both BA and kinetin alone could induced adventitious shoots. However, addition of a low concentration (0.1-0.5 mg/1) of IAA or napthaleneacetic acid (NAA) showed beneficial effects on shoot organogenesis. Of the two cytokinins, BA was more efficient than kinetin, and of the two auxins, IAA was better than NAA. Therefore, only BA-IAA combinations were used in the present investigation for induction of adventitious shoots from mature zygotic embryo explants.

The embryo axis when cultured on media containing BA-IAA swelled within 1-2 weeks and multiple shoot buds differentiated directly within 3-4 weeks of culture initiation. Initially adventitious buds were nodule like structures which subsequently developed into shoots within 1-2 weeks of additional culture. Cotyledon explants were green and swollen within 3-4 weeks of culture. Though some adventitious buds developed directly on cotyledon surface, most did so on certain swollen, whitish areas formed at the proximal end from which embryo axes were removed. It was observed that two third distal part of cotyledon produced less shoots than one third proximal part of cotyledon where prolific shoot regeneration occurred.

The type of explants influenced the overall shoot organogenesis and embryo axis was found to be more responsive than cotyledon explants (Table 1). In case of embryo axis, the highest organogenesis was afforded on medium with 1-2

mg/l BA and 0.2 mg/l IAA (Fig.1). On the other hand the highest rate being obtained by medium with 2 mg/l BA and 0.2 mg/l IAA in case of cotyledon explants. The mean number of shoots per regenerating explant also varied greatly. Embryo axis explants formed greater number of shoots than did cotyledon explants regardless of the concentrations of growth regulators used. Among the different treatment combinations 2 mg/l BA and 0.2 mg/l NAA was found to be the best formulation where maximum number of shoots per explant were produced from both the explants.

Only a few of the shoots could elongate if the explants with multiple shoots were maintained on regeneration medium for longer than six weeks. However, these shoots could elongate normally when subcultured onto LS medium containing 0.1 mg/l BA (elongation medium). After excision of the elongated shoots the remaining explants with small shoots attached to it could be recultured on elongation medium for further elongation. Following this process, more than 10 shoots from a cotyledon explant and more than 20 shoots for an embryo axis explant could be recovered.

TABLE 1. SHOOT REGENERATION FROM EMBRYO AXIS AND COTYLEDON EXPLANTS OF WATERMELON IN LS MEDIUM CONTAINING VARIOUS CONCENTRATIONS OF BA AND IAA. DATA WERE RECORDED AFTER 5 WEEKS OF CULTURE. EACH TREATMENT CONSISTED OF 15-18 EXPLANTS AND THE DATA ARE THE MEANS OF TWO EXPERIMENTS.

Growth regulators mg/l		Embryo axis		Cotyledon	
BA	IAA	% regeneration	No.of shoots per explant	% regeneration	No. of shoots per explant
1.0	0.1	63e	8.4e	52ef	5.3c
2.0	0.1	69cd	11.0d	50f	6.0bc
5.0	0.1	65dc	11.5d	54e	5.0c
1.0	0.2	88a	16.2b	74a	7.2b
2.0	0.2	84a	18.8a	70b	10.0a
5.0	0.2	74bc	13.2c	62c	7.4b
1.0	0.5	77b	11.4d	52ef	5.4c
2.0	0.5	73bc	11.0d	53ef	6.6b
5.0	0.5	74bc	8.2e	58d	6.0bc

In each column means followed by the same letter are not significantly different at p=0.05 level by Duncan's Multiple Range test.

TABLE 2. ADVENTITIOUS ROOTS FORMATION IN *IN VIRTRO* OF *LANATUS* MICROSHOOTS ON MS MEDIUM WITH NAA. DATA WERE RECORDED AFTER 4 WEEKS OF CULTURE. EACH TREATMENT CONSISTED OF 12-15 EXPLANTS AND THE DATA ARE THE MEANS OF TWO EXPERIMENTS.

NAA Concentration mg/l	% Shoots rooted	No. of roots per shoot	Average length (cm) of roots
0.1	70b	2.1d	1.2c
0.2	81a	5.4a	3.2a
0.5	64c	4.2c	2.5b
1.0	62c	3.8b	2.3b

In each column means followed by the same letter are not significantly different at p=0.05 level by Duncan's Multiple Range test.

Fig.1&2. Plant regeneration from zygotic embryo of watermelon. (1). Multiple shoot formation on embryo axis in LS medium supplemented with 1 mg/l BA and 0.2 mg/l IAA, after 5 weeks of culture. (2). Adventitious root formation on micropropagated shoot on MS medium with 1 mg/l NAA, after 4 weeks in culture.

Elongated shoots (>2 cm long) were excised and cultured on MS medium supplemented with 0.1-1.0 mg/l NAA. Rooting was observed within 2 weeks of culture. Formation of callus at cut bases, malformation and slow growth of roots, and smaller number of roots per shoot were observed at high concentrations (0.5-1.0 mg/l) of NAA (Table 2). Low concentrations (0.1-0.2 mg/l) of NAA, on the other hand, promoted root elongation and proliferation and inhibited base callusing. Maximum rooting (81%) with 3-5 roots per shoot was recorded on the medium containing 0.2 mg/l NAA (Table 2, Fig. 2). Regenerated plantlets were transferred to pots containing soil mixed with compost. During the initial one week, the potted plantlets were covered with glass beakers to provide high humidity. Transplantation survival was 74%.

The results presented here indicated that *In vitro* regeneration of complete plantlets is possible from mature zygotic embryo of watermelon. Both embryo axis and cotyledon explants were capable of producing adventitious shoots. These adventitious shoots differentiated directly with no intermediate callus stage. However, while the regeneration capacity of the embryo axis was 63-88%, the morphogenetic capacity of the cotyledons was lower (50-74%). The embryo axis explants are known to be good source for regenerative capacity, as has been shown in *Hordium* [9], *Camellia* [10] and *Calotropis* [11]. The technique of zygotic embryo culture may be useful for rescuing embryos from incompatibe crosses. Beacuse of their juvenile nature, embryos have a high potential for regeneration and hence may be used for *In vitro* clonal propagation. The system presented here will facilitate rapid propagation of watermelon, and is potentially useful in the transformation via *Agrobacterium*.

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