Pak.J.Bot., 31(1): 31-35, 1999.

PLANT REGENERATION FROM HYPOCOTYL EXPLANTS OF *CITRULLUS LANATUS* THUNB.

R. ISLAM, A. HOQUE, M.H. RAHMAN AND A. AHAD

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

Abstract

Adventitious shoot regeneration from hypocotyl explants was examined in *Citrullus lanatus* Thunb. Hypocotyl explants of 2,3,4,5 and 6 week old seedlings when cultured on LS medium containing BA, Kn, 2ip with or without 0.5-1.0 mg/l IAA showed that 1.0 mg/l BA and 1.0 mg/l IAA proved ideal for shoot differentiation from 2 week old hypocotyl explants. Shoots elongated on the medium with 0.5 mg/l GA_3 and 0.1 mg/l BA and later rooted on half-strength MS medium containing 1.0 mg/l IBA.

Introduction

Watermelon (*Citrullus lanatus*) of family Cucurbitaceae, is an economically important fruit crop in Bangladesh. Application of *in vitro* technique is of great value as an alternative method to achieve advances in watermelon breeding programme. Cotyledon, hypocotyl, leaf, stem, shoot tip, nodal cutting, embryo, anther, protoplast explants have been used for *in vitro* studies of different species of cucurbits (Jelaska, 1986; Melepszy, 1988; Moreno & Roig 1990; Debeaujon & Branchard, 1992; Misra & Bhatnagar, 1995). However, less attention has been given to tissue culture of watermelon. The only successful *in vitro* regeneration system available for the watermelon is propagation by cotyledons and embryo axis (Dong & Jia, 1992; Ahad *et al.*, 1994). The present report describes an *in vitro* procedure for the regeneration of plantlets from hypocotyl explants of *C. lanatus*.

Materials and Methods

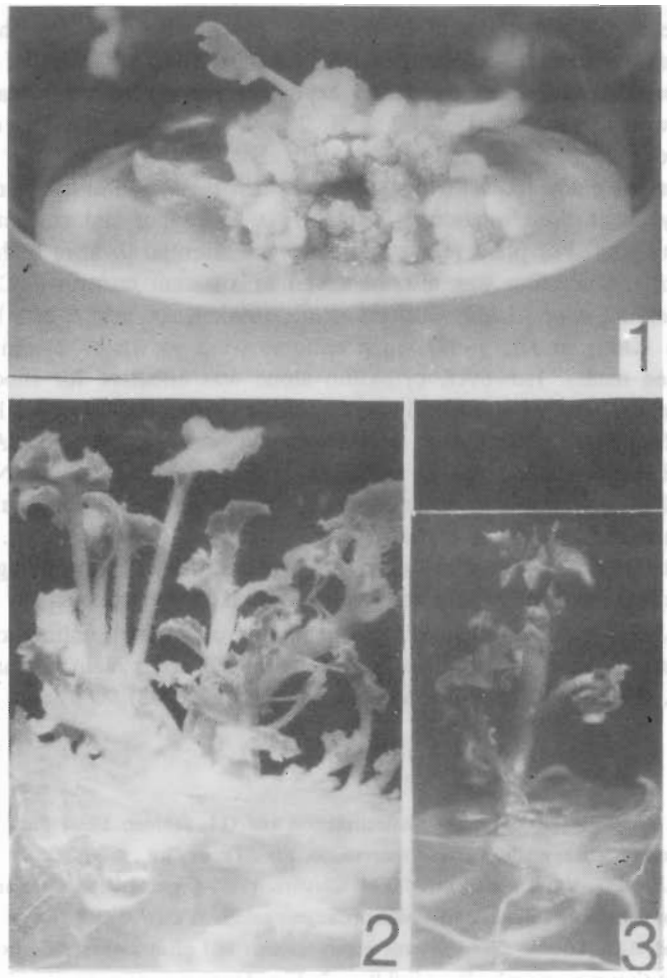
Seeds of *C. lanatus* were obtained from Natore Horticultural Station. The decoated seeds after surface sterilization in 0.1% $HgCl_2$ solution for 2 min., and subsequent washing 3 times in sterilized distilled water were transferred aseptically in culture tubes containing LS medium with 2% sucrose and 0.7% agar. About 5-6 mm long hypocotyl explants were excised from 2-6 week old aseptic seedlings. Excised hypocotyl explants were planted on LS medium with 100 mg/l casein hydrolysate (CH), 3% sucrose and 0.7% agar. This medium was supplemented with various concentrations of BA, Kn and 2ip with or without IAA. For elongation, the shoots were transferred to 0.5 mg/l GA_3 and 0.1 mg/l BA. Elongated shoots were rooted on half-strength MS medium containing 1.0 mg/l BA. The pH of all the media were adjusted to 5.7 ± 0.1 prior to autoclaving. Cultures were maintained in a growth chamber under a 16-h photoperiod provided by warm white fluorescent light (approximately 60 μ Mol $m^{-2}s^{-2}$) at $26 \pm 1^{\circ}C$.

Table 1. Response of hypocotyl explants excised from 2 week old seedlings of *Citrullus lanatus* to BA and IAA in LS medium.

Growth regulators	% of explants formed shoots ± SE	No. of shoot buds per explant ± SE
BA 0.5 + IAA 0.0	0	0
BA 0.5 + IAA 0.5	48.2 ± 4.13	19.8 ± 2.15
BA 0.5 + IAA 1.0	38.2 ± 3.89	11.4 ± 1.99
BA 1.0 ± IAA 0.0	0	0
BA 1.0 + IAA 0.5	45.3 ± 3.92	8.2 ± 1.76
BA 1.0 + IAA 1.0	54.3 ± 4.11	16.3 ± 2.32
BA 2.0 + IAA 0.0	0	0
BA 2.0 + IAA 0.5	30.2 ± 3.33	7.5 ± 1.54
BA 2.0 + IAA 1.0	28.7 ± 2.47	8.9 ± 2.00
Kn 1.0 + IAA 0.0	0	0
Kn 1.0 + IAA 1.0	29.3 ± 4.30	6.7 ± 2.19
2ip 1.0 + IAA 0.0	0	0
2ip 1.0 + IAA 1.0	0	0

Results and Discussion

Hypocotyl explants enlarged and thickend within 10-12 days of culture. Callus initiation was observed initially at the cut ends which later covered the entire surface of the explants. Regeneration of shoot buds occurred within 3 weeks of culture (Fig.1). In addition to shoot regeneration, profuse callus growth was observed when LS medium was supplemented with 2.0 mg/l of BA and 0.5 or 1.0 mg/l of IAA. The callus was white and friable in nature. Shoot regeneration was maximum (54.3%) on LS + 1.0 mg/l BA and 1.0 mg/l IAA (Table 1). Highest number of shoots was obtained on LS + 0.5 mg/l BA and 0.5 mg/l IAA. If the explants were not subcultured onto the elongation medium within 6 weeks, shoot elongation did not occur. Callus growth was moderate on the medium containing 1.0 mg/l BA + 1.0 mg/l IAA and 0.5 mg/l BA + 0.5 mg/l IAA and the callus was hard and white. On the medium supplemented with 1.0 mg/l BA + 0.5 mg/l IAA and 0.5 mg/l BA + 1.0 mg/l IAA callus growth was poor and the callus was hard and light green. Hypocotyl explants cultured on the medium with BA alone showed intitial stages of shoot development but such shoots never grew further. Kinetin alone did not produce any shoot buds and when combined with IAA, it was less effective than BA for shoot induction. The cytokinin, 2ip, alone



Figs.1-3. Plant regeneration from hypocotyl explants of *Citrullus lanatus*. 1. Development of shoot buds on LS + 2.0 mg/l BA and 0.5 mg/l IAA after 3 weeks of culture. 2. Elongation of shoots on LS + 01 mg/l BA + 0.5 mg/l GA_3 after 4 weeks of culture. 3. Induction of adventitious roots on half-strength MS + 1.0 mg/l BA after 3 weeks of culture.

or in combination with IAA failed to induce any shoot bud. Further development of shoot buds occurred on the medium when level of BA was reduced to 0.1 mg/l. Further increase in shoot length was achieved when 0.5 mg/l GA_3 was combined with BA (Fig.2). Root induction and development was 80% on half-strength MS medium containing 1.0 mg/l of BA (Fig.3).

Hypocotyl segments derived from seedlings of 5 different ages (2,3,4,5 and 6 weeks) were cultured on 1.0 mg/l BA and 1.0 mg/l IAA. The percentage of shoot buds from hypocotyl explants rapidly declined when explants from cultures older than 3

weeks were used. The 6 week old seedlings did not produce any shoot buds. The number of shoots per explant also depended considerably on the age of the leaf. Shoot differentiation frequencies of hypocotyl explants obtained from 2,3,4 and 5 week-old seedlings were 51.4, 45.0, 30.2 and 20.6% and the number of shoots per culture were 17.5, 14.5, 8.2 and 6.5, respectively.

The present study indicates that *in vitro* plant regeneration of watermelon can be obtained by proper combination of explant age and types and concentrtion of plant growth regulators. For plant regeneration, it was essential to have both cytokinin and auxin. Plant regeneration was also observed in different cucurbits (Chee & Tricoli, 1982; Bergervoet *et al.*, 1989; Clusters *et al.*, 1990; Kageyama *et al.*, 1990) including watermelon (Dong & Jia, 1992) when cultures were grown on cytokinin and auxin-supplemented media. However, cytokinin alone was effective for shoot induction in *Cucumis melo* (Kathal *et al.*, 1988) and *C. sativus* (Misra & Bhatnagar, 1995).

High frequency adventitious root formation was obtained in BA-supplemented medium. However, Dong & Jia (1992) obtained rooted plants in NAA-containing medium. This could possibly be due to genotypic differences. The results showed that young tissues of watermelon are more responsive than the older ones. This could be attributed to high plasticity of cells at younger stage. The younger tissues are physiologically more active and are easily affected by environmental factors such as exogenous hormones. A similar effect on the age of donor seedlings on regeneration from leaf or cotyledon explants has been noted in *Cucumis melo* (Kathal *et al.*, 1988; Niedz *et al.*, 1989) and watermelon (Compton & Gray 1993).

References

Ahad, A., R. Islam, M. Hossain, M. Khalequzzaman and O.I. Joarder. 1994. Plant regeneration from immature and mature embryo axis of watermelon. *Plant Tissue Cult.*, 4: 39-44.

Bergervoet, J.H.W., F. Van der Mark and J.B.M. Clusters. 1989. Organogenesis verus embryogenesis from long term suspension cultures of cucumber (*Cucumis sativus* L.). *Plant Cell Rep.*, 8: 116-119.

Chee, P.P. and D.M. Tricoli. 1988. Somatic embryogenesis and plant regeneration from cell suspension culture of *Cucumis sativus* L. *Plant Cell Rep.*, 7: 274-277.

Clusters, J.B.M., S. Zijlstra and J. Jansen. 1990. Somaclonal variations in cucumber (*Cucumis sativus* L.) plants regenerated via somatic embyogenesis. *Acta Bot. Neerl.*, 39: 153-161.

Compton, M.E. and D.J. Gray. 1993. Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid and tetraploid watermelon. *J. Am. Soc. Hort. Sci.*, 118: 151-157.

Debeaujon, I and M. Branchard. 1992. Induction of somatic embryogenesis and callogenesis from cotyledon and leaf protoplast-derived colonies of melon (*Cucumis melo* L.). *Plant Cell Rep.*, 12: 37-40.

Dong. J. and S. Jia. 1992. High frequency plant regeneration from cotyledons of watermelon (*Citrullus vulgaris* Schrad.). *Plant Cell Rep.*, 9: 559-562.

Jelaska, S. 1986. *Cucurbits* In: YPS Bajaj (Ed.). *Biotechnology in Agriculture and Forestry,* Vol. 2., Crops I. pp. 371-386, Springer-Verlag, Berlin, Heidelberg.

Kageyama, K.K. Yabe and Miyajima. 1990. Somatic embryogenesis and plant regeneration from stem, leaf and shoot apex of melon (*Cucumis melo* L.). *Plant Tissue Cult. Lett.*, 7: 193-198.

Kathal, R.S., P. Bhatnagar and S.S. Bhojwani. 1988. Regeneration of plants from leaf explants of *Cucumis melo* cv. Pusa Sharbati. *Plant Cell Rep.*, 7: 449-451.

Melepszy, S. 1988. Cucumber (*Cucumis sativus* L.) In: Y.P.S. Bajaj (Ed.). *Biotechnology in Agriculture and Forestry*; Vol.6, Crops, II, pp. 277-293, Springer-Verlag, Berlin, Heidelberg.

Misra, A.K. and S.P. Bhatnagar. 1995. Direct shoot regeneration from the leaf explant of Cucumber (*Cucumis sativus* L.). *Phytomorph.*, 45: 47-55.

Moreno, V. and L.A. Roig. 1990. Somaclonal variations in Cucurbits. In: Y.P.S. Bajaj (Ed.), *Biotechnology in Agriculture and Forestry*, Vol. II, Somaclonal Variation in Crop Improvement I, pp. 435-464, Springer-Verlag, Berlin, Heidelberg.

Niedz, R.P., S.S. Smith, K.B. Dunbar, C.T. Stephens and H.H. Murakishi. 1989. Factors influencing shoot regeneration from cotyledonary explants of *Cucumis melo*. *Plant Cell Tiss. Org. Cult.*, 18: 313-319.

(Received for Publication 24 November, 1997.)