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TISSUE CULTURE OF INNALA (*SOLENOSTEMON ROTUNDIFOLIUS* (POIR.) J.K. MORTON) HIGH FREQUENCY PLANT REGENERATION FROM LEAF EXPLANTS

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ABSTRACT

Leaf explants of innala (*Solenostemon rotundifolius* (Poir.) J.K. Morton) were cultured on media prepared with MS salts, organic constituents of NN, sucrose (40 g/l w/v) and growth regulators. NAA, 2,4-D, BAP and Kn were used as growth regulators. Callus formation and subsequent shoot regeneration from cultured explants were observed in the presence of 0.8-1.0 mg/l BAP in the medium. When low concentration (0.5 mg/l) of NAA was present in the medium, a higher level (5-6 mg/l) of BAP was required for induction of shoots. The frequency of callus formation or shoot regeneration in such media was as high as 100%. The regenerated shoots could be rooted in hormone-free MS medium and successfully established in soil.

INTRODUCTION

Innala (*Solenostemon rotundifolius* (Poir.) J.K. Morton) is an annual herb possibly of Ethiopian origin. It is presently distributed in south- east Asia (Sri Lanka, Malaysia, Indonesia) and tropical Africa (Tindall, 1983). Both the leaves and tubers of this crop are consumed as cooked vegetables. The tubers may also be baked or fried and sometimes used as a substitute for Irish potato (*Solanum tuberosum* L.). The plant grows well at altitudes of 40-1300 m and prefers a warm climate. It requires a period of six months from planting to harvesting hence an early maturing cultivar is very desirable.

Innala is vegetatively propagated by stem cuttings or tubers. Lack of genetic variability has greatly affected the improvement of this crop. Somaclonal variation has been identified as a potential means of creating genetic variability of crops. So far no reports are available on plnant regeneration from tissue (callus) cultures of innala. Therefore testing of callus-inducing media and optimization of protocols for plant regeneration are necessary prerequisites for generating somaclones.

This paper describes the establishment of a successful method for high frequency plant regeneration from tissue cultures of innala.

MATERIALS AND METHODS

Shoot-tips of innala were obtained from healthy mother plants grown in a glasshouse. They were aseptically cultured in glass jars containing growth regulator-free, MS (Murashige & Skoog, 1962) medium with sucrose (3% w/v) and maintained at 25+1°C under fluorescent light (2000 lux; 8 hours) for 2-3 weeks. Leaf explants (1 cm^2), obtained from these *in vitro*-grown plants were cultured in media which were prepared with basic salts of MS, organic compounds of NN, (Nitsch and Nitsch, 1967) sucrose (4% w/v) and growth regulators. The growth regulators used were NAA,1-naphthaleneacetic acid (0.5-5.0 mg/l), 2,4-D, 2-4-dichlorophenoxyacetic acid (0.5 - 2.0 mg/l), BAP, 6-benzylaminopurine (0.1-6.0 mg/l) and Kn, 6-furfurylaminopurine (0.5,3.0 mg/l). Leaf cultures were also maintained under light (approximately 250 lux, 10 h). All media (pH 5.7) used in the study were solidified with Difco-bacto agar (0.8% w/v), autoclaved for 20 minutes (at 121°C with 1.05 kg/cm^2 of steam pressure) and dispensed (20 ml aliquots) into sterile culture vessels under aseptic conditions. Glass jars (150 ml capacity) were used for culturing shoot-tips, and for leaf tissues Petri dishes (10 cm d.m.) were used.

Shoots regenerated from leaf explants, isolated and cultured on growth regulator-free MS medium, were used for the maintenance of shoot cultures. Plants (5-7 cm) regenerated from leaf explants were finally planted in pots (5 cm) containing a mixture of sand and compost (3:1). After 2-3 weeks of growth at room temperature 26 + 3°C) under diffused, diurnal light, these plants were transferred to larger pots (15 cm) containing garden soil and maintained in the glasshouse till maturity.

OBSERVATIONS AND RESULTS

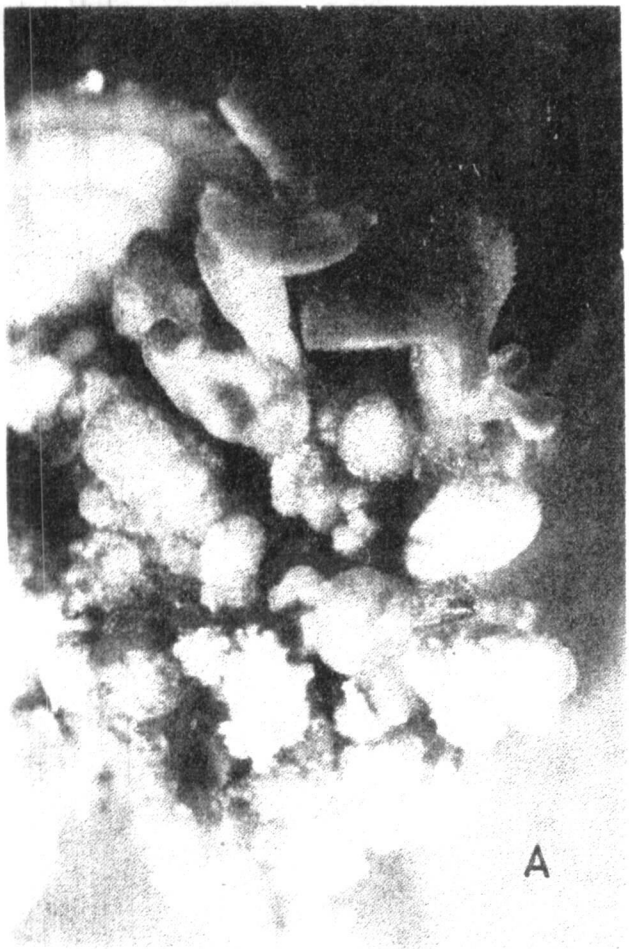
Shoot tips obtained from glasshouse-grown mother plants grew into healthy plantlets within 3 weeks of culture. Cell proliferation and growth of cultured leaf explants occurred in 2-3 weeks of culture. Cell proliferation and callus formation, at first visible on cut surfaces, subsequently spread over upper and lower surfaces of the explants. Calli developing in media containing only cytokinin (BAP or Kn) were white with green patches and those forming in the presence of both BAP and NAA were with dark necrotic regions. Calli forming in the presence of a combination of 2,4-D and Kn were pale green and friable.

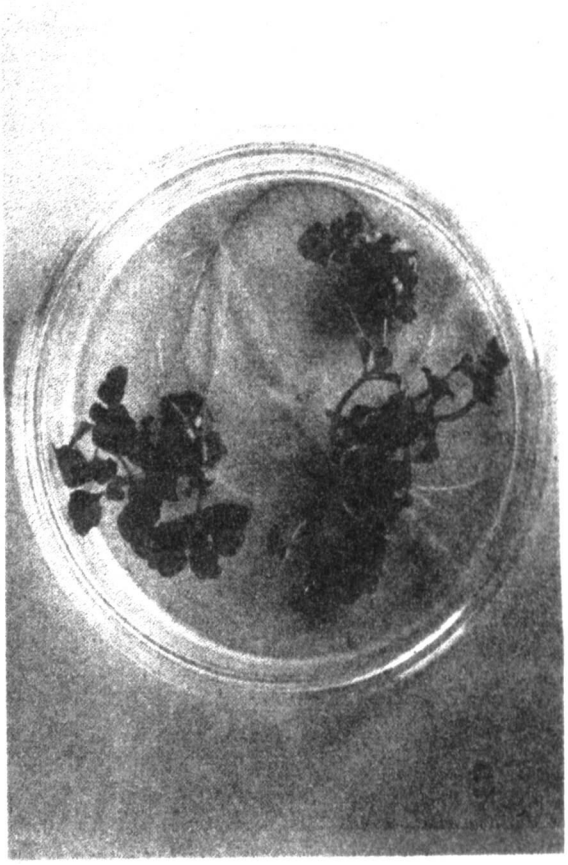
Growth responses of cultured leaf explants to different growth regulators are summarized in table 1. Shoot regeneration from explants occurred within 4-5 weeks of culture (Fig.A) in the presence of 0.8 - 1.0 mg/l BAP. When low concentration of NAA was present in the medium BAP was required at a higher concentration (5.0 - 6.0 mg/l) to regenerate normal and healthy shoots (Fig.B). The frequency of callus formation or shoot regeneration of cultured explants in growth inducing media was observed to be 100%.

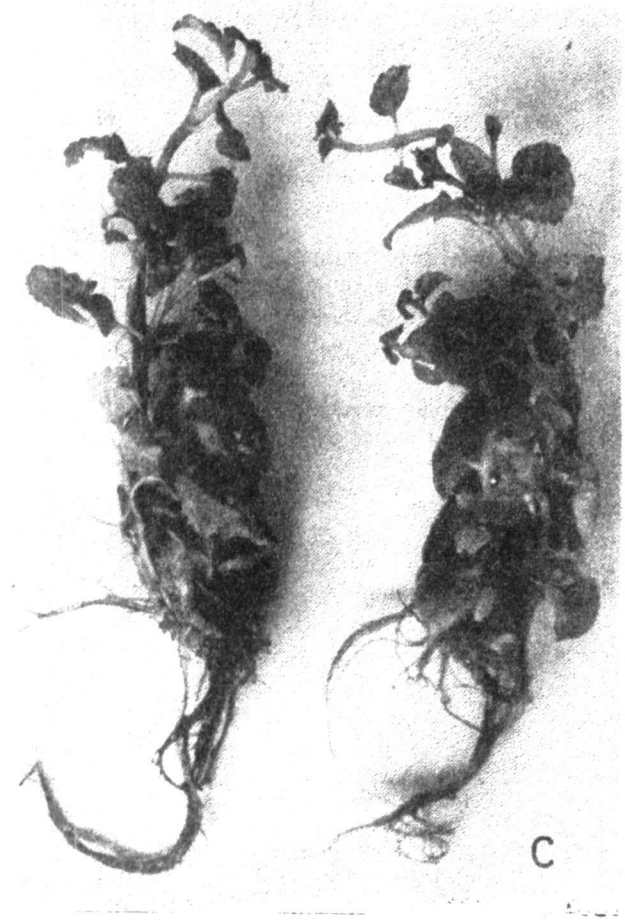
Explant-derived shoots continued to grow when cultured in the hormone-free medium and produced healthy roots within 2-3 weeks (Fig.C). These plants grew well and produced tubers upon transfer to soil (Fig.D).

DISCUSSION

Leaf explants have been used for establishing successful tissue culture systems of plants such as strawberry (Toyoda *et al.* 1990). Lisianthus (Griesbach and Semeniuk, 1987), cucumber (Malepszy and Nadolska-Orczyka, 1983), pea (Mroginski and Kartha, 1981; Rubluo *et al.* 1984) and several *Solanum* pecies (Zenkteler, 1972; Roest and Bokelmann, 1976; Gleddie *et al.* 1983; Hendrix *et al.* 1987). A major factor which determines successful plant regeneration from callus is the auxin/cytokinin ratio of the culture medium in which tissues are grown. It confirms earlier observations with some species that shoot regeneration is dependent on a low auxin/cytokinin ratio (Davies and Dale, 1979; Kowalezyk *et al.* 1983). Shoot regeneration can also occur with lower frequency in the absence of auxin (Bhatt *et al.*, 1979). In the present study, leaf explants of innala showed that a low concentration of BAP alone was adequate to induce callus which regenerates shoots in the same medium.







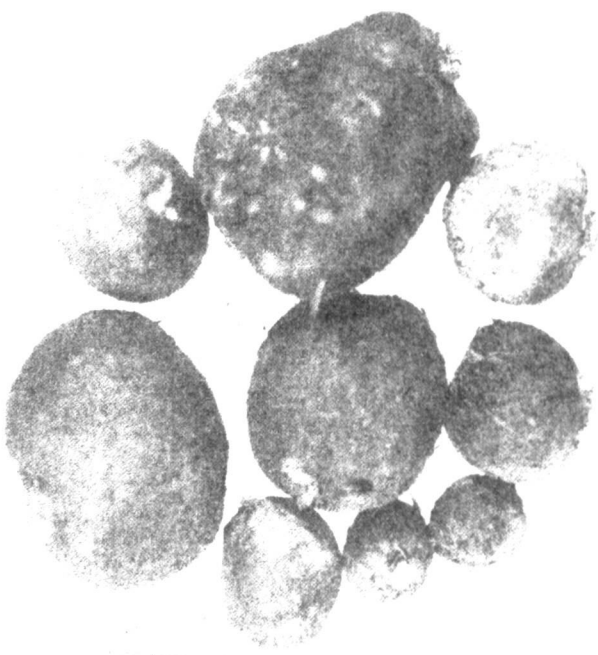


Fig.A - Shoot induction from leaf callus (x12); **Fig.B** - Multiple shoots regenerated from callus (x12); **Fig.C** - Plantlets obtained from regenerated shoots (x1); **Fig.D** - Tubers produced from tissue cultured plants (x0.8)

Table 1. Growth response of cultured leaf explants of innala with respect to different levels of auxins and cytokinins in the medium

Growth regulator (mg/l)					Growth response
Auxin		Cytokinin		Callus growth	Shoot regeneration (No of shoots/explant)
2,4-D	DNA	BAP	KN		
-	-	0.1	-	No callus	-
-	-	0.5	-	+	-
-	-	0.8	-	++	3-4
-	-	1.0	-	++	1-2
-	-	1.5	-	++	-
-	-	3.0	-	+++	-
-	0.5	1.0	-	++	-
-	0.5	2.0	-	++	-
-	0.5	3.0	-	++	-
-	0.5	4.0	-	++	-
-	0.5	5.0	-	++	4-7
-	0.5	6.0	-	++	2-4
-	1.0	5.0	-	++	-
-	2.0	-	-	++	-
-	2.0	0.8	-	+++	-
-	2.0	5.0	-	+++	-
-	5.0	-	-	No Callus	-
-	5.0	3.0	-	+++	-
-	5.0	-	3.0	+++	-
0.5	-	-	0.5	++	-
1.0	-	-	0.5	+++	-
1.5	-	-	0.5	++	-
2.0	-	-	0.5	++	-
-	-	-	0.5	No Callus	-
-	-	-	-	No Callus	-
-	-	-	3.0	+++	-

+ Slow ++ Moderate +++ Rapid

Auxins such as 2,4-D and NAA favour callus induction of most plant tissues (Yamada, 1977). A combination of auxin and cytokinin favours callus formation and shoot regeneration from leaf segments of pomegranate (Omura *et al.* 1987) and strawberry (Toboda *et al.* 1990). In the present study with innala, BAP in combination with NAA induced callus which regenerated shoots. Complete plants could be formed from the regenerated shoots.

The leaf explants of innala exhibited a higher frequency of plant regeneration. Therefore, the protocol developed for tissue culture in the present study can be effectively utilized for generating somaclonal variants of innala.

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