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SOMATIC EMBRYOGENESIS FROM IMMATURE COTYLEDONS OF CHICKPEA (*CICER ARIETINUM L.*)

R. ISLAM

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

Regeneration of plants by means of somatic embryogenesis is of utmost importance for improvement through somatic cell genetics. This is preferred over normal regeneration via organogenesis due to various advantages when used for transformation. High number of regenerants can be obtained originating from few or single cells which increases the likelihood of achieving transformed plants. Little progress has been made towards developing an in *vitro* regeneration system for chickpea, an important food legume crop. To date, chickpea has been regenerated only from preexisting shoot meristems (Rao & Chopra, 1989'; Rao & Reddy, 1992). This study describes the development of chickpea somatiic embryos on immature cotyledon explants.

Green pods containing immature seeds collected from field grown plants of chickpea were surface sterilized with 0.1% $HgCl_2$ for 10 min., immediately rinsed in sterilized distilled water and the seeds removed. The cotyledons without embryo axis were separated aseptically from decoated seeds. The immature cotyledons approximately 5 mm in diameter were cultured on B5 medium (Gamborg *et al.*, 1968) supplemented with various concentrations and combintions of 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA), kinetin, 6-benzyladenine (BA) and zeatin. The explants were always placed with adaxial surface facing the medium. All media were solidified with 0.7% agar (BDH) and pH was adjusted to 5.7 prior to autoclaving. The cultures were maintained at 26 ± 2°C with a 16 h photoperiod.

The cotyledon explants when cultured on B5 medium supplemented with 1-3 mg/l zeatin gave rise to embryogenic cultures without undergoing intermediate callus stage (Fig.1A) within 3-4 weeks of culture initiation. BA and kinetin supplemented media failed to induce somatic embryogenesis. Maximum percentage (54%) of explants produced somatic embryos and highest number (19.7) of somatic embryos per explant were obtained on medium containing 2 mg/l zeatin. The percentage of embryogenic cultures and number of somatic embryos decreased considerably when 0.1 mg/1 2,4-D or NAA was added together with zeatin. In these cases some callus formation was also observed. The medium containing only 2,4-D or NAA (1 mg/l) had no effect on somatic embryo induction and induced the explants to produce callus only. The callus was crystalline and transparent in appearance, white, tan, brown or yellow in colour and appeared as loosely packed, friable regions of vacuolated cells which produced more callus, frequently roots but never embryoids.

The induced somatic embryos were green, globular and loosely attached to the parental tissue. By 5-6 weeks after culture initiation the embryoids acquired shapes ranging from globular to torpedo stage and well formed bipolar structures bearing cotyledons were observed (Fig.1B). Occurrence of various aberration structures, such as root like structures, were also observed. In some cases cotyledons tended to fuse

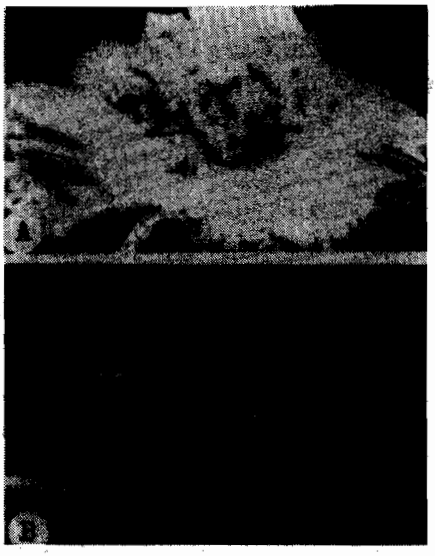

Fig.1. Somatic embryogenesis in chickpea. A. Development of somatic embryos on immature cotyledons after 3 weeks of culture on 2 mg/l zeatin. B. Cotyledonary stage of somatic embryos.

into one or two irregular masses. They resembled abnormal embryoids as seen in soybean (Lazzarie *et al.*, 1985) and groundnut (Ozias-Akins, 1989).

The present investigation demonstrates the feasibility of somatic embryogenesis on immature cotyledons of chickpea using zeatin and in the absence of auxin. Subsequent studies to optimize factors for embryo conversion to plantlets make the system highly adaptable for biotechnology and transformation studies.

Table 1. Effect of growth regulators on somatic embryogenesis on immature chickpea cotyledons.

Growth regulators (mg/l)	Embryogenesis %	No. of somatic embryos per explant
Kinetin 1-3	---	---
BA 1-3	---	---
Zeatin 1	30	14.3
Zeatin 2	54	19.7
Zeatin 3	50	11.2
2, 4-D 1	---	---
NAA 1	---	---
Zeatin 2+2,4-D 0.1	18	3.2
Zeatin 2+NAA 0.1	34	3.2

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