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Induction of Somatic Embryogenesis in the Tissue Cultures of Chick Pea (*Cicer arietinum* L.)

SUMAN SHANKER and H Y MOHAN RAM
Department of Botany, University of Delhi, Delhi 110 007

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2,4-dichlorophenoxyacetic acid (2,4-D) and 4,- amino 3,5,6-trichloropicolinic acid (picloram) (0.1-1.0 mg l^{-1}) either singly or in combination with kinetin (Kn) (0.5-1.0 mg l^{-1}) added to MS medium (semi-solid) induced callusing in the explants of root, leaf, epicotyl, cotyledon and cotyledonary node, obtained from 7 days old seedlings of *Cicer arietinum*. On the same medium, 15 days old callus masses started differentiating globular structures. The latter developed into somatic embryos up to the torpedo stage in the next 15 days. However, they failed to grow into plantlets.

Key Words: Callus, Food legume, Globular structures, Seedling explants, Somatic embryogenesis

Introduction

Chickpea or gram is the most important grain legume of India, accounting for nearly 50% of the area and total production of pulses (Jain 1988). The production of chickpea per unit area has remained more or less steady during the past few years. Some of the major constraints identified in the production of this crop are : (i) low adaptability and low stability of yield (ii) tendency for excessive vegetative growth under irrigation and other inputs (iii) susceptibility to stress, salinity, diseases (*Fusarium* and *Ascochyta* blight) and insect pests (Baldev et al. 1988).

Agricultural scientists have recently stressed the need for speeding up conventional breeding, creating variability through tissue culture and evolving novel genotypes through recombinant DNA (r-DNA) technology (Chopra & Sharma 1991). A major prerequisite for the utilization of biotechnological approaches to the genetic improvement of chickpea is a consistent and high degree of *in vitro* regeneration.

Callus formation and somatic embryogenesis from leaflet explants (Rao & Chopra 1989), callus and shoot bud formation from hypocotyl and cotyledon (Anil et al. 1986, Rao & Reddy 1987), whole plantlet regeneration from shoot tip meristem explants (Kartha et al. 1981, Singh et al. 1982) and shoot bud formation from cotyledonary node of young seedlings (Khan & Ghosh 1984) in chickpea have been reported. In a recent publication, Tawakley et al. (1992) have reported chromosomal instability in the callus cultures raised from explants such as cotyledons embryonal axis, epicotyls and anthers of 6 genotypes of chick pea [*C. arietinum* (cultivated)]. *C. bijugum* and *C. judaicum* (wild spp). In this note we report

induction of somatic embryogenesis in the cultures raised from various explants of *C. arietinum*.

Material and Methods

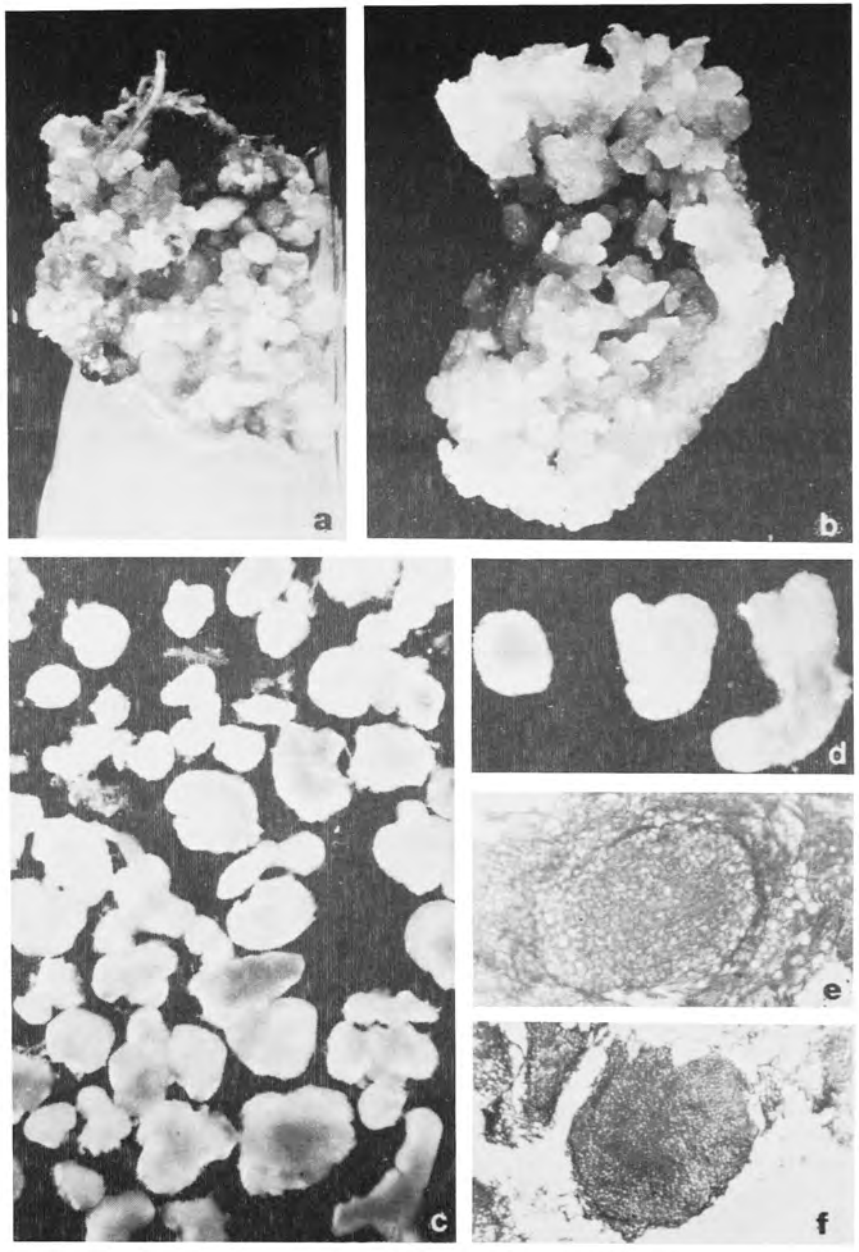
Seeds of *Cicer arietinum* var. Phule G5 obtained from the Directorate of Pulse Research, Kanpur, U.P., India were used in this study. Seeds were washed with the detergent Teepol and then in running tap water. Seeds were surface-sterilized with 70% ethanol followed by 0.2% mercuric chloride ($HgCl_2$) (10 min. each). After thorough washing (4 times) with sterilized distilled water, they were germinated on agar (0.8%) + sucrose (2%). Explants such as root, cotyledonary node, cotyledon, epicotyl, node, internode and whole leaf were obtained from 7 days old seedlings and were cultured on MS medium (Murashige & Skoog 1962) containing.

2, 4-D (2, 4-dichlorophenoxyacetic acid) and picloram (4, -amino-3, 5, 6-trichloropicolinic acid) singly and in combination with Kn (kinetin). A total of 48 cultures per treatment were prepared and the experiments were repeated twice. The cultures were stored in a sterile room at $26 \pm 2°C$ under 16/8hr light/dark cycle. Light was supplied from fluorescent tubes at an intensity of 200 $\mu E\ m^{-2}s^{-1}$.

Results and Discussion

Callus was formed 15 days after culture in all the explants in response to the following treatments: (i) 2,4-D (0.1-1.0 mg l^{-1}), (ii) picloram (0.1-1.0 mg l^{-1}), (iii) Kn (0.5, 1.0 mg l^{-1}) + picloram (0.1, 0.5 mg l^{-1}), (iv) Kn (0.5, 1.0 mg l^{-1}) + 2,4-D (0.1, 0.5 mg l^{-1}). The callus was friable and creamish white. In another 15 days time it showed differentiation of organized globular masses over the surface (figure 1a, b). A portion of the callus observed under the microscope showed somatic embryos (mostly globular and a few heart- and rarely torpedo-shaped structures (figure 1c-f). The maximum potentiality to form somatic embryos was shown by root explants (followed by nodal explants > cotyledonary node > leaf > internode > cotyledon. The number of embryos formed per culture varied from 30 to 60.

Our findings are similar to an earlier work by Rao and Chopra (1989), wherein they have reported induction of embryogenic callus, consisting of globular structures, from the leaf explants of chickpea on MS medium supplemented with 0.5 mg/l 2,4-D and BAP (benzylaminopurine) each. Factors such as a dark incubation phase and withdrawal of 2,4-D during further subculturing were found to be important for producing high frequency of embryogenesis in their study. A certain percentage of embryos develop into plantlets (Pers. communication Dr V L Chopra). Induction of somatic embryos by 2,4-D and picloram has been reported earlier in the tissue cultures of grain legumes such as *Phaseolus vulgaris* (Martins & Sondahl 1984, Saunders et al. 1987), *Pisum sativum* (Jacobsen & Kysley 1984, Kysley et al. 1987), *Glycine max* (Lippman & Lippman 1984, Barwale et al. 1986) and *Lens culinaris* (Saxena & King 1987). In this investigation all explants showed early stages of somatic embryo differentiation in response to 2,4-D and picloram, used singly or in combination with Kn. However, when the cultures were retained on the same medium for 55-60 days, roots were formed only in treatments containing 2,4-D. Callusing followed by root differentiation occurred from different explants of *C. arietinum* on B_5 medium supplemented with either 0.5, 0.75 mg/l BAP or NAA + BAP (0.4 mg/l) (Tawakley et al. 1992). Callus cells taken from these cultures when analysed for chromosomal counts showed ploidy levels of 2n, 3n, 4n, 5n, 6n, 8n and even higher number of chromosomes (more than 100-150) (Tawakley et al. 1992).



Figures 1a-f **a,** Culture raised from cotyledonary nodal segment on MS + picloram (0.5 mg l^{-1}) showing differentiation of globular, organised masses of cells; **b,** An enlarged view of a culture raised from an epicotyl segment on Kn (0.5 mg l^{-1}) + picloram (0.1 mg l^{-1}); **c,** Somatic embryos of various sizes and at several stages of development; **d,** A few embryos enlarged from the callus mass; **e,f,** Globular (e) and heart-shaped (f) embryos in longitudinal section

Thirty days old callus cultures bearing somatic embryos, raised on medium containing picloram or 2,4-D were transferred to MS medium. However, the embryos failed to develop into plantlets. Our study confirms the earlier statement by Rao and Chopra (1989) that in the case of chick pea, concentrations and combinations of hormones in the medium on which callus is raised strongly influence the embryogenic response and the subsequent development of somatic embryos. A consistently high rate of regeneration of plants from somatic embryos is yet to be reported in this plant.

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