A novel combination of plant growth regulators for in vitro regeneration of complete plantlets of guar [Cyamopsis tetragonoloba (L.) Taub.]

S Verma1, K S Gill2, V Pruthi1, K S Dhugga3 & G S Randhawa1 *

1Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee 247 667, India
2Department of Crop and Soil Science, Washington State University, P O Box: 646420, Johnson Hall, 277, Pullman, WA 99164-6420, USA
3DuPont Agricultural Biotechnology, DuPont Pioneer, 7300 NW 62nd Avenue, Johnston, IA 50131, USA

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A novel combination of plant growth regulators comprising indole-3-butyric acid (IBA), 6-benzylaminopurine (BA) and gibberellic acid (GA$_3$) in Murashige and Skoog basal medium has been formulated for in vitro induction of both shoot and root in one culture using cotyledonary node explants of guar, (Cyamopsis tetragonoloba). Highest percentages of shoot (92%) and root (80%) induction were obtained in the medium containing (mg/L) 2 IBA, 3 BA and 1 GA$_3$. Shoot regeneration from the cotyledonary node explants was observed after 10-15 days. Regeneration of roots from these shoots occurred after 20 to 25 days. The regenerated plantlets showed successful acclimatization on transfer to soil. This protocol is expected to be helpful in carrying out various in vitro manipulations in this economically and industrially important legume.

Keywords: Cotyledonary node, Guar, In vitro, Regeneration

Cluster bean [Cyamopsis tetragonoloba (L.) Taub.], popularly known as guar, is a drought-hardy, annual and subtropical legume. It is mainly grown in the semi-arid regions of north-west India and to a limited extent in Pakistan, Brazil, Australia, South Africa and the USA. The galactomannan gum, a polysaccharide derived from guar seed endosperm, is used in many industries like pharmaceutical, textile, food, cosmetic, coal mining and paper. A rise in the demand of guar gum, particularly for fracking, which involves deep horizontal drilling to release gas from the rocks, brought an agricultural boom in guar cultivation in the year 2012.

Traditional plant breeding has led to the release of a number of commercial varieties but their development is constrained by the cleistogamous floral morphology of guar. Now, as the molecular components that make galactomannan, the economically important polysaccharide in the endosperm, have been isolated, it has become possible to alter the composition of this polysaccharide for improved industrial uses through a transgenic approach. To meet this objective, in vitro regeneration of guar is required. Although in vitro regeneration of plantlets in guar has been reported, in all these reports the regeneration of complete plants required several transfers from one combination of hormones to another. The present work has been undertaken to develop an efficient system for in vitro regeneration of both root and shoot in a single culture using a suitable explant of guar.

Materials and Methods

Plant material—The seeds of three guar varieties used, viz., RGC 936, RGC 1002 and RGC 1066 were obtained from Central Arid Zone Research Institute (CAZRI) Jodhpur, India.

Plant growth medium—The Murashige and Skoog (MS) medium used for plant growth contained (w/v) 0.01% myo-inositol, 3% sucrose and 0.8% agar-agar. The pH of the medium was adjusted to 5.8. MS medium was supplemented with different combinations of plant growth regulators, viz., indole-3-acetic acid (IAA), 1-naphthlenacetic acid (NAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), thidiazuron (TDZ), 6-benzylaminopurine (BA), kinetin (Kn) and gibberellic acid (GA$_3$) for shoot and root regeneration.

Germination of seeds—All the manipulations were carried out under aseptic conditions. Seeds were kept in 3% (v/v) teepol (Qualigens, Mumbai, India) solution for 30 s, rinsed with sterilized distilled water.
4 times and then placed in sterilized distilled water for 1 h. For surface sterilization, the soaked seeds were kept in 0.1% (w/v) HgCl₂ solution for 1-2 min and subsequently washed 4 times with sterilized distilled water. These seeds were placed on half-strength MS basal medium for germination.

Culturing of explants—Hypocotyl and cotyledon explants were excised from 10 days old in vitro germinated seedlings. The cotyledonary nodes were taken from 15 days old in vitro germinated seedlings. Leaf explants were excised from 40-50 days old guar plants growing in green house. The leaves were washed with 70% (v/v) ethanol for 2 min, surface-sterilized by keeping in 0.1% (w/v) HgCl₂ for 3 min and rinsed 4-5 times with sterilized distilled water. Each of the four explants, viz., hypocotyl, cotyledon, cotyledonary node and leaf, was cut into small pieces of 1-1.5 cm size and cultured on MS medium containing various combinations and concentrations of plant growth regulators. All cultures were kept under the conditions of 16 h of light/8 h of dark period at 25±2 °C for growth.

Hardening—The developed plantlets were taken out of the culture vessels, washed to remove the adhering medium and planted in Cocopeat mixture contained in plastic pots. The pots were watered with 0.5% bavistin (BASF India Limited) solution. For next 8-10 days, high humidity was maintained around the plantlets by covering them with polybags. After 8-10 days the plantlets were transferred to pots containing a mixture of soil, sand and farm yard manure. On successful acclimatization, the plantlets were transplanted to soil.

Statistical analysis—Shoot and root regeneration percentages were calculated for different combinations of growth hormones. The data were analyzed by one-way ANOVA (analysis of variance) and the treatment mean values were compared by Tukey’s Post HOC test ($P \leq 0.05$) using SPSS software [Statistical Package for the Social Sciences; version 17; International Business Machinery (IBM) Corporation New York, USA].

Results and Discussion

To begin with four different types of explants, viz., cotyledon, hypocotyl, leaf and cotyledonary node were tried for in vitro shoot induction. Only the cotyledonary node explants regenerated shoots. The variety RGC-936 was found to be most responsive among the three different varieties (i.e. RGC 936, RGC 1002 and RGC 1066) tested for in vitro shoot regeneration. As the cotyledonary node explants of this variety underwent expansion during 4-5 initial days of culture, there was a change in colour from green to pale green and callus initiation at the cut ends appeared after about 7-8 days. Shoot regeneration occurred after another 3-7 days. Hence, from day one of culture, it took 10-15 days for shoot induction (Fig. 1a). An increase in BA concentration resulted in a rise in percentage shoot regeneration (Table 1). The maximum success (92% in vitro shoot induction) was observed on MS medium containing 2 mg/L IBA, 3 mg/L BA and 1 mg/L GA₃ in this variety. The ratio of hormones with respect to each other as well their concentrations affected the frequency of shoot induction. The shoots underwent adequate elongation and formation of rosettes or ill-defined shoots was not observed (Fig. 1b).

In the variety RGC-1002 shoot induction occurred after 10-15 days but the highest percentage of shoot induction was much lower (61%) as compared to variety RGC-936 (Table 1). Delay of about one week was observed in shoot regeneration in variety RGC-1066. Regeneration of chlorophyll deficient shoots was more frequent in the MS medium supplemented with 2,4-D and kinetin. On increasing the IBA concentration to 2.5 mg/L, the rate of callus proliferation increased which hindered the growth of shoots (Table 1).

Initiation of roots from the in vitro regenerated shoots occurred in the same culture after another 10 days in the variety RGC-936 (Fig. 1c). Highest percentage (80%) of in vitro root induction was observed from regenerated shoots in MS medium containing 2 mg/L IBA, 3 mg/L BA and 1 mg/L GA₃ in this variety (Table 2). Regeneration of roots from shoots did not result in any other combination of plant growth regulators except in MS medium supplemented with IBA, BA and GA₃. Joersbo et al.¹³ achieved multiple shoot induction without root induction on B5 medium containing TDZ, BA and GA₃. In contrast root regeneration along with shoot regeneration was observed in the present study when TDZ was replaced with IBA. From these results IBA appeared to be a potent root inducer.

It took nearly 35 days for in vitro formation of a complete plantlet with well developed shoot and root system (Fig. 1d). The plantlets thus obtained showed successful acclimatization on transfer from culture medium to Cocopeat mixture (Fig. 1e) and on
Fig. 1—In vitro plant regeneration in variety RGC 936 of guar [C. tetragonoloba (L.) Taub.]. (a) Cotyledonary node explants showing callus proliferation and initiation of shoots after 10 days in MS medium containing 2 mg/L IBA, 3 mg/L BA and 1 mg/L GA3, (b) in vitro regenerated shoots showing elongation along with callus proliferation at the base, (c) in vitro regeneration of roots from shoots in the same culture, (d) complete in vitro regenerated plantlet with well developed root and shoot system taken out of culture medium after 35 days, (e) in vitro regenerated plantlet transplanted to Cocopeat mixture for hardening.
subsequent transfer to soil. The percentage survival of the transplanted plantlets obtained by culturing cotyledonary node explants was much higher (90%) than reported by Prem et al. The plants showed proper growth till maturity.

In the earlier reports on in vitro regeneration in guar, regeneration of complete plantlets required multiple transfers from one combination of hormones to another. The present communication reports a novel combination of plant growth regulators for induction of both shoots and roots in guar. To the best of our knowledge this is the first report of regeneration of complete plantlet of this legume in one culture. These reports a novel combination of 11-16 hormones to another combination of growth regulators for induction of both shoots and roots in guar. To the best of our knowledge this is the first report of regeneration of complete plantlet of this legume in one culture. These

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<th>BA</th>
<th>GA&lt;sub&gt;3&lt;/sub&gt;</th>
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F-value: 62.761 81.42 17.215

The F-value is significantly different when P≤0.05.

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References


