EFFECT OF COCONUT WATER ON CALLUS GROWTH OF CYAMOPSIS TETRAGONOLOBUST

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Abstract

The main aim of this study is to minimize the time for callus induction and shoot generation with easily available low cost natural materials. Coconut water is a rich source of carbohydrate and other nutrients which enhance callus formation. Cyamopsis tetragonolobust has hypoglycemic activity. This study shows higher viability and rapid callus generation through micropropogation technique of Cyamopsis tetragonolobust seeds.

Key Words: Cyamopsis tetragonolobust, micropropogation, seeds, shoot generation

Introduction

Guar is a native to the Indian subcontinent. Guar is grown mainly in India, Pakistan, United States and also in some part of Africa and Australia. The important source of nutrition to human and animals is the legume, it regenerates soil nitrogen and the endosperm of guar seed is an important hydrocolloid widely used across a broad spectrum of industries. The mature seeds of guar contain a huge reserve of galactomannan and to a lesser extent other galacto-oligosaccharides [1], it has high hypoglycemic activity [2].

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods [3]. It is not a new technology and application of innovative methods have served to overcome barriers to progress in the efficient multiplication of elite plant species and further improvements in the technique and methodology are anticipated. Growth and development In vitro are considerably influenced by several factors including genotype, the age of plant, the age of the tissue or organ (explant), the physiological state and many more. As a mean of securing pathogen free plants, culture of shoot apical meristem is ideal.

Materials and Methods

Isolation of coconut water: The coconut water is simply drained from immature coconuts by drilling holes through two of the micropyles. Extract of water from each fruit separately was checked that it is not fermented before addition to the bulk. Collected water from all the fruits was heated at 60°C for 10 minutes with continuous stirring to precipitate out the proteins, fats and other materials.

The precipitates were separated by filtration and the filtrate is stored at -20 °C for future use [4].

Harvesting, sterilization and germination of seeds: The seeds of Cyamopsis tetragonolobust were collected and washed under tap water for 10 minutes. The seeds were sterilized [5]. The seeds

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of Cyamopsis tetragonolobust were inoculated on simple MS medium and incubated at 25 ± 2 °C with 14 hours photoperiod for 4 weeks.

Shoot multiplication: After 4 weeks of incubation, all the germinated seeds were transferred to the experimental media which is MS basal salts [6] supplemented with various levels of 2,4-D (1.5, 2.0 and 2.5 mg/L) along with coconut water (15, 20 and 25 % v/v) as shown in Table 1. The pH of the media were adjusted to 6.77 prior to autoclaving by adding 1N NaOH and/or 1N HCl.

The media were solidified with 2.5 gm/L Agar added after adjusting the pH and before autoclaving. All the cultures were kept at 22 ± 2°C with 14 hours photoperiod. Data regarding the length and number of shoots along with the number of nodes were recorded for 5 weeks and mean with standard deviation were calculated (Table 1).

Rooting and acclimatization: After 5 weeks, the multiplied shoots were transferred on root initiation media comprised of half MS medium containing IBA 0.02 mg/L. Data were recorded after every week for four weeks. The rooted plantlets were transferred to the green house for acclimatization. The potting mix used for acclimatization contained 80% sand and 20% farmyard manure (v/v).

Results and Discussion

The seeds of *Cyamopsis tetragonolobust* started germination after 4 weeks and were fully germinated within 8 weeks. At the height of 1.0 - 1.5 cm, these germinated plantlets were transferred to the experimental media (Table 1).

It was observed that both the 2,4-D and coconut water had a synergistic effect and either of them was not able to produce the same result when used separately. It was noted that MS media containing 20% coconut water (v/v) with 2 mg/L of 2,4-D (A10) resulted in the maximum increase in number (11.5 ± 1.1) and length of shoots (7.2 ± 0.16). The highest number of nodes (4.2 ± 0.12) was also recorded when the same A10 medium was used (Table 1).

Table: 1 Effect of coconut water on shoot generation

Media	2,4-D Level (mg/L)	Coconut water %	Mean shoot length*(cm)
A0	0.00	0	2.1 ± 0.14
A1	0.00	5	2.4 ± 0.12
A2	0.00	10	3.1 ± 0.18
A3	0.00	15	3.2 ± 0.24
A4	1.50	0	3.3 ± 0.21
A5	1.50	5	3.9 ± 0.18
A6	1.50	10	3.8 ± 0.27
A7	1.50	15	4.2 ± 0.09
A8	2.00	0	3.8 ± 0.17
A9	2.00	5	5.2 ± 0.11
A10	2.00	10	7.3 ± 0.13
A11	2.00	15	4.6 ± 0.05
A12	2.50	0	5.1 ± 0.12
A13	2.50	5	4.4 ± 0.19
A14	2.50	10	4.6 ± 0.11
A15	2.50	15	4.4 ± 0.38

It was also noted that all the growth parameters were highly influenced by the addition of coconut water as the cultures grown on the media without coconut water (A0, A4, A8, A12) showed much lesser growth as compared to the cultures grown with the coconut water (Table 1). It was also observed that the cultures, grown without coconut water, turned brown after four weeks and need to be transferred to the fresh media whereas the addition of coconut water to the media had made cultures to survive for 8 weeks. After 8 weeks of time, the multiplied shoots of good length (7.2 ± 0.16) were transferred on rooting media where the root initiation started within 10 days and prolonged enough in 4 weeks time.IBA (0.02 mg/L) induced sufficient shooting in Cyamopsis tetragonolobust. The plants were then shifted to the green house and were well acclimatized using 90% soil and 10% farmyard

manure as a potting mix. More than 95% of the plants, transferred to green house, survived under semi-controlled environment.

The effect of coconut water for the enhanced In vitro propagation of *Cyamopsis tetragonolobust* was evaluated. During the course of study, apart from the enhanced shoot multiplication, two major effects of coconut water were observed. First the addition of coconut water to the media resulted in about 95% increment of overall phosphorus (P) content of the media [7]. This effect of coconut water ultimately resulted in the doubling of sub-culturing time from four to 8 weeks. This elimination of transferring the plantlets to the fresh medium resulted in the reduction of overall cost of labor and chemicals as the number of plants produced was same. The second important effect of coconut water is that it proved as a very useful pre-conditioner to achieve bigger and more robust plants.

The addition of coconut water to the culture media resulted in the plants with a greater nutritional and carbohydrates contents as coconut water itself contained 21.8 gm/L sugars in total [4]. This high robustness and survival rate (>95%) of the In vitro grown plants might be due to their high carbohydrates contents which could be used to meet respiratory demands while surviving the physiological shocks of ex vitro procedures associated with the shifting of plant from controlled to semi controlled environment [8].

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