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High Frequency Induction of Callus from Seedling Explants of Pigeonpea (*Cajanus cajan* [L] Millsp.) for Genetic Transformation

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Abstract

Pigeonpea (*Cajanus cajan* [L] Millsp.) is an important grain legume of the semi-arid tropics. It provides protein rich food. Seeds of Pigeonpea were collected and surface sterilized, this sterilized seeds were germinated aseptically. Various explants epicotyls (excised from 7 day old seedlings) cotyledons were used for morphogenic response. Callus induction was achieved from epicotyl and cotyledon explants of Pigeonpea. Callus induction at various frequencies were observed using different concentration and combination of IAA, kinetin and 2,4-D. Highest percentage (95) of callus formation was observed on modified MS (Murashige and Skooge) media + 1.0 mg l^{-1} IAA (Indole Acetic Acid) + 0.9 mg l^{-1} kinetin and 90 percentage of callus formation was observed on modified MS (Murashige and Skooge) media + 1.0 mg l^{-1} 2,4-D.

Key Words: *Cajanus cajan,* Epicotyl, cotyledon, MS (Murashige and Skooge) media, IAA, Kinetin, 2,4-D, Callus

Introduction

Pigeonpea (*Cajanus cajan* [L] Millsp.) is an important grain legume of the semi-arid tropics [10]. Many attributes have contributed to the widespread use of pigeonpea in the semi-arid tropics. The crop is grown mainly for its grain, which contains between 17 and 28% protein and is an important diet supplement for resource-poor farmers, who eat mainly low-protein cereal and root crops. The deep root system of more than 1.5 m permits the crop to extract water at depth and thus confers great ability to withstand drought stress [13].

Pigeonpea is an important multipurpose pulse legume in the tropics and subtropics. It is grown for its wide range of products. The seed, pod, and the leaf are used for human and livestock nutrition and the crop generally enhances soil fertility through leaf litter and biological nitrogen fixation [17].

It is cultivated in more than 25 tropical and sub-tropical countries either as sole crop or intermixed with some cereals and other legumes [12]. Rainfall is a limitation to pigeonpea production in semi-arid regions where it is mostly produced [8].

Legumes are one of the most important groups of crop plants and have been subjected of efforts to improve desirable traits including their *in vitro* culture response. Since legumes are notoriously recalcitrant to regenerate from tissue culture, much effort has been devoted to developing and optimizing efficient in vitro regeneration system to facilitate a variety of technologies [11].

In Pigeonpea attempts to regenerate plants from various explants have been attempted, these include leabes [2, 3, 5, 16, 1] cotyledonary node [14, 4, 5, 9, 16], epicotyls [7], and shoot apics [16, 15].

Hence, the present study aims to attempts produce callus from epicotyls and cotyledon explants of Pigeonpea (*Cajanus cajan* [L] Millsp.) for further transformation or transgenic plants production.

Materials and methods

Plant material and explant preparation

Seeds of Pigeonpea(*Cajanus cajan* [L] Millsp.) were collected and washed thoroughly under running tap water for 10 min and washed with a autoclaved filtered double distilled water, add 3 drpos of tween 20 (liquid detergent) and add 3 drops of sodium hypochlorite (bleach) swirl it for 8 ½ min then decant or discard the water and washed it thoroughly with distilled water and add 0.1% $HgCl_2$ and swirl it for 2 ½ min, then immediately add 70% alcohol. Then wash it 3 to 4 times with distilled water and 2 times washed with autoclaved filtered double distilled water and germinated aseptically. Various explants epicotyl (excised from 7 day old seedlings), cotyledons were used for morphogenic response.

Culture medium and condition

The explants were transferred in culture jar with MS medium contained 3% sucrose and 0.7% agar, supplemented with different hormone (IAA, kinetin and 2,4-D) concentration for callus induction. pH was adjusted to 5.7 prior to autoclaving. Cultures were incubated at 25˚C with 16 hr photoperiod.

Results and Discussion

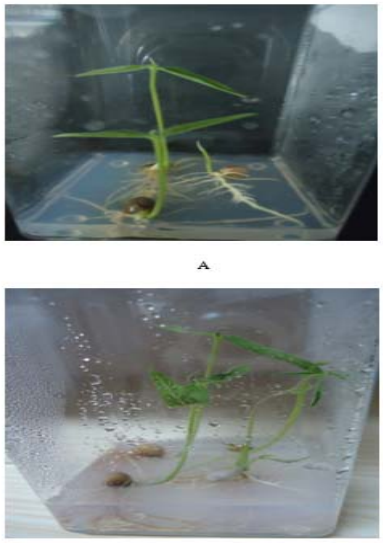
Surface sterilized seeds cultured directly on MS basal agar medium without any growth regulators in the phyta jars showed 40-50% germination in 4 days; 95-100 % germination was observed after 3 days (Fig 1). This seedling plant explants like epicotyl (excised from 7 day old seedlings), cotyledons were used for morphogenic response.

Callus induction was observed onto MS containing different concentrations and combinations of IAA, kinetin and 2,4-D within 15 days incubation of epicotyls and cotyledon explants depending upon the concentration and combination of

hormones. Callus induction was noticed in all media formulations. But there was a wide range of variation in percentage of callus formation and average fresh weight of callus. The results were recorded in Table 1. and Fig. 1, 2,3.

Table 1. Effect of different concentration of IAA + Kinetin and 2,4-D on callus formation from epicotyls and cotyledon explants response (%) of pigeonpea (*Cajanus cajan* (L).) Millsp.) after 15 days.

PGR Concentration (mg / l)		Explant response (%) Epicotyl	Cotyledon
IAA + Kinetin	1 + 0.5	65	70
	1 + 0.6	70	75
	1 + 0.7	80	85
	1 + 0.8	80	85
	1 + 0.9	95	95
2,4-D	0.6	65	75
	0.7	70	80
	0.8	75	80
	0.9	80	85
	1.0	90	90



A

B

Fig. 1. A. 4 days old seedling plant.
B. 7 days old seedling plant

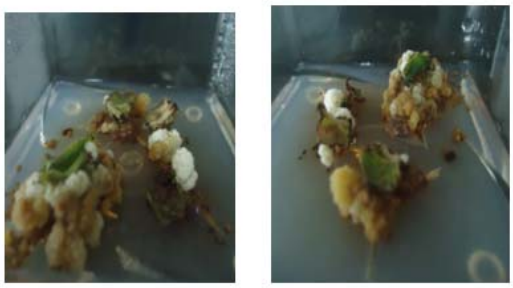


Fig. 2. Callus developing from epicotyl and cotyledon explant on MS + 1.0 mg l^{-1} IAA + 0.9 mg l^{-1} kinetin

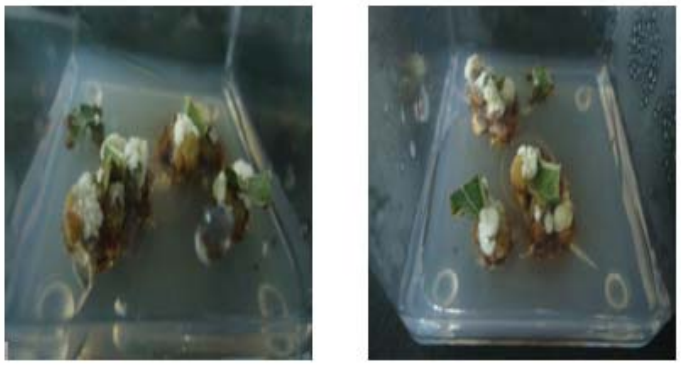


Fig. 3. Callus developing from epicotyl and cotyledon explant on MS + 1.0 mg l^{-1} 2,4-D

The highest percentage of callus induction (95%) was observed on MS medium containing 1.0 mg l^{-1} IAA (Indole Acetic Acid) + 0.9 mg l^{-1} kinetin (Table 1; Fig 2). and 90 percentage of callus formation observed on MS media containing 1.0 mg l^{-1} 2,4-D (Table. 1; Fig 3.).

In Pigeonpea diverse tissue that have been used to obtain regeneration include leaves [2, 3, 16, 1], cotyledonary node [14, 4, 5, 9, 16], epicotyls [7].

In the present study, we report an efficient protocol by using epicotyls and cotyledon region of seedling, which can be induced to differentiate into adventious callus that can be used for efficient production of transgenic Pigeonpea.

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