

# High Frequency Induction of Callus from Seedling Explants of Pigeonpea

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<https://doi.org/10.55041/ijstmt.v2i4.459>

**Cite this Article:** Hitesh, J., Nimita, U. & D, P. L. (2026). High Frequency Induction of Callus from Seedling Explants of Pigeonpea. International Journal of Science, Strategic Management and Technology, 02(04). <https://doi.org/10.55041/ijstmt.v2i4.459>

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## ABSTRACT

A simple, efficient, and reproducible protocol for high-frequency callus induction was established using leaf, hypocotyl, and embryo explants excised from 12-day-old seedlings of pigeonpea genotypes GT 101 and UPAS 120. Callus induction was observed at varying frequencies across different combinations of 2,4-D and BAP. Initiation of callus was evident from the cut surfaces of explants after a mean period of 10.4 days of inoculation. The highest callus induction frequency (85%) was recorded on MS (Murashige and Skoog) medium supplemented with 2.0 mg/L 2,4-D and 1.0 mg/L BAP when hypocotyl explants were used.

**Key Words:** Pigeonpea, Callus induction, Regeneration, Tissue Culture

## I. Introduction

Food legumes are of major importance in human diet and animal feed contributing the chief source of vegetarian protein. They are an economic source of not only protein but of carbohydrate, minerals and B-complex vitamins particularly in vegetarian diet [1]. Among the various pulses, Pigeonpea is an important multipurpose grain legume and good source of protein for populations living in the semi-arid tropics and cultivated under rain-fed agricultural condition. It contains about 20-22 per cent protein particularly sulphur containing amino acids, namely methionine and cysteine [2], which is almost three times than cereals. Also contains 45-55% starch, 3-5% soluble sugar, 1-2% fats, 3-4% crude fiber and 3-4% ash [3].

Pigeonpea is one of the important crop plants and have been subjected of efforts to improve desirable traits including their *in vitro* culture response. Since Pigeonpea is 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 [4]. For the successful development of transgenic plant an effective regeneration system is essential. Callus induction from various explants have been attempted, these include leaf [5-9], hypocotyls [10-11], cotyledonary node [4,12-13] and embryo [14-15].

Hence, the present study aims to attempts produce callus from hypocotyl, leaf and embryo explants of pigeonpea (*Cajanus cajan* [L] Millsp.) for further transformation and/or transgenic plants development.

## II. Material and Methods

### A. Plant material and explant preparation

The experimental material of pigeonpea genotypes GT 101 and UPAS 120 were collected from "Pulse Research Station", Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. Seeds were surface sterilized with 70 per cent (v/v) ethanol for 30 seconds, followed by 0.1 per cent (w/v) aqueous mercuric chloride (HgCl<sub>2</sub>) containing 1-2 drops of Tween-20 solution for 7-8 min and then rinsed five times with sterile double distilled water and germinated

aseptically on MS [16] (Murashige and Skoog, 1962) basal medium. Leaf, hypocotyl and embryo were used as explants for callus induction excised from 12 day old seedling.

### B. Culture medium and condition

The Murashige and Skoog (MS) solid medium supplemented with various combinations of auxin (2,4-D) and cytokinin (BAP) was tested for callus induction (Table 1). Leaf margins were cautiously trimmed and inoculated in the test tube and/or bottle in such a way that the lower surface of explant remains in intimate contact with callus induction medium. Explants (leaf, hypocotyl and embryo) were placed in a test tube containing 12-13 ml of callus induction medium and/or Glass bottles containing 30-35 ml of callus induction medium. pH was adjusted to 5.7 prior to autoclaving. Cultures were incubated at 25°C with 16 hr photoperiod. Observation of days to callus initiation and per cent callus induction was recorded.

### III. Results and Discussion

Surface-sterilized seeds cultured on MS basal agar medium devoid of growth regulators exhibited 80–95% germination within 3–5 days. Explants such as hypocotyl, leaf (excised from 12-day-old seedlings), and embryo derived from these seedlings were subsequently used to assess morphogenic responses.

Callus induction was observed on MS medium supplemented with different concentrations and combinations of 2,4-D and BAP within 10–11 days of incubation in hypocotyl and leaf explants, whereas embryo explants required 15–16 days for initiation. Callus formation was recorded across all media formulations; however, a wide variation in the percentage of callus induction was observed. The detailed results are presented in Tables 1 and 2.

Table 1: Effect of different concentration of 2,4-D and BAP on callus formation from leaf, hypocotyl and embryo explants of pigeonpea genotype GT 101. Means±SE, n=3

PGR Concentration (mg/L)		Days to callus initiation			Per cent callus induction (%)		
2,4-D	BAP	Leaf	Hypocotyl	Embryo	Leaf	Hypocotyl	Embryo
0.5	0.5	22.4±0.11	19.9±0.80	24.5±0.17	38.0±4.50	37.2±2.10	23.5±1.51
1.0	0.5	18.4±0.07	17.7±0.22	20.7±0.08	55.5±5.55	50.0±2.88	33.3±1.73
1.0	1.0	17.3±0.08	16.5±0.24	19.4±0.30	60.0±5.77	60.0±3.46	46.7±3.33
2.0	0.5	13.8±0.14	12.5±0.17	17.8±0.29	62.2±2.22	62.2±2.22	50.0±2.31
2.0	1.0	10.7±0.06	10.4±0.07	16.2±0.24	83.3±1.75	85.0±1.00	62.2±2.22
3.0	0.5	16.8±0.10	15.0±0.12	18.2±0.14	58.3±3.08	52.5±1.62	38.5±1.31

Table 2: Effect of different concentration of 2,4-D and BAP on callus formation from leaf, hypocotyl and embryo explants of pigeonpea genotype UPAS 120. Means±SE, n=3

PGR Concentration (mg/L)		Days to callus initiation			Per cent callus induction (%)		
2,4-D	BAP	Leaf	Hypocotyl	Embryo	Leaf	Hypocotyl	Embryo
0.5	0.5	22.1±0.11	20.2±0.12	25.0±0.12	35.0±2.79	38.3±1.50	25.5±2.47
1.0	0.5	18.3±0.33	17.2±0.08	20.3±0.14	53.3±3.33	50.0±0.58	37.8±2.22
1.0	1.0	17.6±0.10	16.5±0.08	19.9±0.11	63.3±8.81	60.0±1.73	43.3±3.33
2.0	0.5	13.9±0.22	13.3±0.24	17.9±0.11	65.0±5.00	69.5±2.78	50.0±2.00
2.0	1.0	11.3±0.09	10.5±0.17	15.8±0.28	78.3±1.67	76.7±1.67	64.5±2.22
3.0	0.5	16.5±0.17	15.4±0.12	18.8±0.15	62.3±4.80	55.5±2.36	42.5±2.21

The maximum callus induction (85%) was recorded on Murashige and Skoog (MS) medium supplemented with 2.0 mg/L 2,4-D and 1.0 mg/L BAP when hypocotyl explants of genotype GT 101 were used. In comparison, a callus

induction frequency of 78.3% was observed on the same medium when leaf explants of genotype UPAS 120 were cultured. The callus developed from leaf and hypocotyl explants in both genotypes was hard and compact in nature, whereas callus derived from embryo explants was comparatively less friable and exhibited browning (Figure 1).

These findings are in agreement with earlier reports, which documented callus induction within 20–25 days on medium supplemented with 10  $\mu\text{M}$  BA and 0.5  $\mu\text{M}$  IAA using leaf explants of pigeonpea variety ICPL-1615. Furthermore, it has been reported that MS medium containing 2.0 mg/L 2,4-D in combination with 0.5 mg/L kinetin resulted in the shortest duration for callus initiation (9.2 days) in dehusked seed explants and produced the highest callus induction frequency (87.5%). However, under the same conditions, callus induction was not observed in embryo and leaf explants, indicating explant-specific variability in response to growth regulator combinations [17].

The highest percentage of callus formation was reported from leaf explants of pigeonpea genotypes ICPL 93086, Tanzania-7, and their hybrid when cultured on medium supplemented with 125  $\mu\text{L}$  2,4-D in combination with 50  $\mu\text{L}$  kinetin and 50  $\mu\text{L}$  NAA, indicating the effectiveness of this hormonal combination for inducing callogenesis in these genotypes [7]. Callus induction was observed within 15 days, with a maximum callus formation frequency of 90% on medium supplemented with 1.0 mg/L 2,4-D and 0.9 mg/L kinetin when epicotyl and cotyledon explants were used [4]. Embryogenic callus induction ranging from 50–60% was observed from leaf explants after 40 days of culture on medium supplemented with 0.5 mg/L 2,4-D and 0.5 mg/L IAA [9].

The present investigation demonstrates that callus induction in pigeonpea is strongly influenced by the interaction of explant type, genotype, and the concentration of plant growth regulators (PGRs), particularly 2,4-D and BAP. The observed increase in callus formation with increasing concentrations of 2,4-D in combination with BAP up to an optimum level, followed by a decline at higher concentrations, is consistent with the classical concept of auxin–cytokinin balance regulating cellular dedifferentiation and proliferation.

Auxins such as 2,4-D are known to play a crucial role in initiating cell division and promoting dedifferentiation, whereas cytokinins like BAP enhance cell proliferation and callus growth. The synergistic effect of these hormones at optimal concentrations (2 mg/L 2,4-D + 1 mg/L BAP) observed in this study aligns with earlier reports indicating that a balanced ratio of auxin and cytokinin is essential for efficient callus induction [18-19]. The decline in callus formation at higher concentrations may be attributed to hormonal toxicity or disruption of endogenous hormonal balance, which can inhibit cell division and lead to tissue necrosis.

Among the explants tested, hypocotyl explants exhibited the highest callus induction frequency, followed by embryo and leaf explants. This variation can be explained by differences in physiological and developmental status of the tissues. Hypocotyl tissues are relatively less differentiated and possess higher meristematic activity, making them more competent for *in vitro* dedifferentiation. In contrast, leaf tissues are highly differentiated and may have lower regenerative rate, resulting in reduced callus formation. Similar findings have been reported in leguminous crops, where hypocotyl explants showed superior response compared to other tissues [11,18].

Genotype-dependent variation observed between GT 101 and UPAS 120 further highlights the role of genetic factors in determining tissue culture response. GT 101 consistently exhibited higher callus induction across all explants, suggesting a greater inherent rate for *in vitro* growth and regeneration. Such variability among genotypes has been widely documented and is often attributed to differences in endogenous hormone levels, metabolic activity, and gene expression patterns controlling morphogenesis [20].

The overall pattern observed in this study supports the concept that successful callus induction requires optimization of multiple factors, including explant source, genotype, and precise hormonal combinations. The superior performance of hypocotyl explants of GT 101 at optimal PGR concentration suggests their potential utility in large-scale tissue culture, genetic transformation, and crop improvement programs in pigeonpea.

#### IV. Conclusion

The present study demonstrates that callus induction in pigeonpea is markedly influenced by explant type, genotype, and the concentration of plant growth regulators. The combination of 2 mg/L 2,4-D and 1 mg/L BAP was found to be optimal for maximum callus formation in both genotypes. Hypocotyl explants proved to be the most responsive, indicating their suitability for in vitro culture studies. Furthermore, genotype GT 101 exhibited superior performance compared to UPAS 120, suggesting its greater potential for tissue culture applications. The observed decline in callus formation at higher hormone concentrations emphasizes the importance of maintaining an optimal hormonal balance for efficient callus induction.

#### V. Acknowledgements

All the authors would like to thank Department of Plant Molecular Biology and Biotechnology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar-385 506, Gujarat, India, for providing us funds and facilities to successfully complete our research.

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