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EFFECT OF DIFFERENT GIBBERELLIC ACID CONCENTRATION ON PIGEONPEA (*CAJANUS CAJAN* [L.] MILLSP.) *CV*. MANAK (H77216) *VIA* COTYLEDONARY NODE EXPLANTS

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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, these sterilized seeds were germinated aseptically in shoot induction media (SIM) MS media supplemented with 2mg/l BAP. Cotyledonary node explants (excised from 15 day old seedlings) were transferred to shoot elongation media (SEM) for elongation fortified with MS media with different concentration of GA_3. Highest percentage (93.33) of shoot elongation was observed on modified MS media + 1.0 mg/l GA_3.

Key Words: *Cajanus cajan,* cotyledonary node explant, MS media, BAP, elongation.

Abbreviations

BAP, 6-Benzylaminopurine; MS, Murashige and Skoog, GA_3, Gibberellic acid 3.

[I] INTRODUCTION

Pigeonpea (*Cajanus cajan* [L.] Millispaugh*)* is an important grain legume of family *Fabaceae*. It is an out-crossed, diploid (2n=2x=22) crop with genome size of 800 Mbp. Pigeonpea is mainly cultivated in tropical and subtropical regions of the world. Globally pigeonpea is cultivated on 4.6 m h with annual production of 3.25 mt. India accounts for 78% of the global output with current production of 2.60 mt from 3.5 mh [1]. In India, pigeonpea is mainly grown in states of Madhya Pradesh, Uttar Pradesh, Rajasthan, Karnataka and Andhra Pradesh. Pigeonpea is rich in protein (20-22%) particularly sulphur containing amino acids, namely methionine and cysteine [2]. Besides, seeds also contain about 57.3-58.7% carbohydrates, 1.2-8.1% crude fibres and 0.6-3.8% lipids [3]. The seed and pod husk make a quality feed, whereas dry branches and stem serve as domestic fuel. Fallen leaves from the plant provide vital nutrients to the soil and also enrich soil through symbiotic nitrogen fixation. The pigeonpea varieties are broadly categorized into three classes based on duration of maturity *viz. early duration* (140-150 days), *medium duration* (160-200 days) and *late duration* varieties (more than 200 days).

In pigeonpea, attempts to regenerate plants from various explants had been done and direct shoot induction has been obtained from various explants. These include leaves [4,5,6,7,8,9], cotyledonary node [6,8, 10,11, 12], epicotyls [13] and shoot apices [14,15]. A variable frequency (20-80%) of regeneration was reported by the workers implicating genotype-dependency on regeneration process.

The essentiality of plant growth regulators concentration in culture media is reported in [16]. The relative concentration is very critical for growth and morphogenesis. The ratio of cytokinin to auxin depicts the occurrence of changes in plants. The higher cytokinin to auxin ratio is found to be suitable for shoot regeneration. Usually, the following growth regulators were used in pigeonpea regeneration: auxins like IAA [9, 12, 17], IBA [6, 10], NAA [13] and 2,4-D [18] were used in various combinations with cytokinins like kinetin [6, 9, 12, 19], BAP [6, 10, 12, 20], TDZ [5] to promote cell division, regeneration of shoots and to enhance proliferation and growth of auxiliary buds. The gibberellins are commonly used for shoot elongation and somatic embryo germination. The ranges of hormones used were as follows: auxins (0.1-3.0 mg/L), cytokinins (0.1-3.0 mg/L) and GA_3 (0.1-1.0 mg/L).

Hence, the present study attempts to produce elongated shoots developed from cotyledonary node explants of pigeonpea *cv.* Manak (H77216) from suitable gibberellic acid combination for *in vitro* regeneration study.

[II] MATERIALS AND METHODS

2.1 Plant materials and culture conditions

Seeds of pigeonpea varieties Manak (H77216) were used for all the experiments in the present studies. Unless mentioned otherwise, all media contained MS salts and organic constituents [21], 3% sucrose, 0.7% (w/v) agar, and the pH was adjusted to 5.8 before autoclaving. For explant culture and shoot bud development, 50ml conical flask were used for seed inoculation closed with plugs made with non-absorbent cotton. All the growth regulators including BAP and GA_3 were added after filter sterilization with nylon filter (Millipore) of 0.22µm pore size. The cultures were incubated at 25±2°C temperature, with a light regime of 45-60µE/m^2/s for 16 h and 8 h at dark.

2.2 Seed sterilization

Seeds of pigeonpea were collected and washed thoroughly under running tap water for 10 min and washed with autoclaved double distilled water 2-3 times. Then rinse in 1-2 drops of Tween-20 (liquid detergent) for 20 min and wash with tap and distilled water until all foam is removed. Then wash seeds with 70% alcohol for 5 min, followed by treatment with a solution of 0.1% (w/v) NaOCl (bleach) for 5 min and finally with autoclaved distilled water in laminar air flow. The seeds were soaked for overnight in autoclaved distilled water.

2.3 Explant preparation and shoot regeneration

Seed coats from the pre-soaked seeds were removed under aseptic conditions and seeds were inoculated in shoot induction media (SIM) comprising of MS, B_5 vitamins, 3% (w/v) sucrose, 2.0 mg/l BAP and 0.7% agar. After 15 days, embryonic axes were excised by removing root apex and shoot meristem was dissected along the axis resulting in two pieces.

Each piece was used as cotyledonary node explants. Each explants was further inoculated in SIM for shoot proliferation for 7 days.

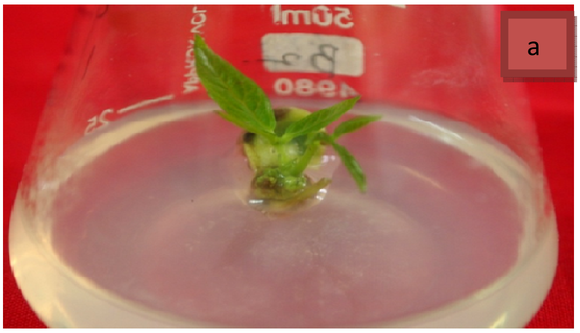
2.4 Elongation of shoots

Clumps of multiple shoots were transferred to 250 ml conical flask containing shoot elongation media (SEM) comprising of MS, B_5 vitamins, 3%

(w/v) sucrose, 0.7% agar and various concentration of GA_3 for further proliferation and elongation of shoots. The hormone concentration used were 1mg/l GA_3 in SEM1, 2mg/l GA_3 in SEM2, 3mg/l GA_3 in SEM3 & 4mg/l GA_3 in SEM4. The cultures were maintained as mentioned previously and subcultured onto the same fresh media after 2 weeks. Shoots longer than 1.0 cm were counted and harvested after 4 weeks and planted on fresh medium. Hence, shoot elongation frequency was further calculated.

[III] RESULTS AND DISCUSSION

In the present study, BAP alone was found to be suitable for both multiple shoot bud induction and proliferation. However, the multiple shoots obtained from BAP failed to elongate on the same medium resulting in rosettes of shoots. Hence it was necessary to develop suitable media for proliferation and elongation of shoot buds. Clumps of multiple shoots were transferred to conical flask containing various concentration of GA_3 for elongation. Addition of GA_3 (1.0–4.0 mg/l) enhanced the number of multiple shoots and shoot elongation (Table I). The optimum level of GA_3 that promoted the highest number of multiple shoot was 1.0 mg/l. In the present study the problem of shoot elongation was overcome by transferring shoot cultures to a shoot elongation medium containing GA_3 (Fig. 1, 2, 3, 4). The highest number of multiple shoots was scored GA_3 (1.0 mg/l). The results were recorded in Table 1. and Fig. 1, 2,3,4,5.



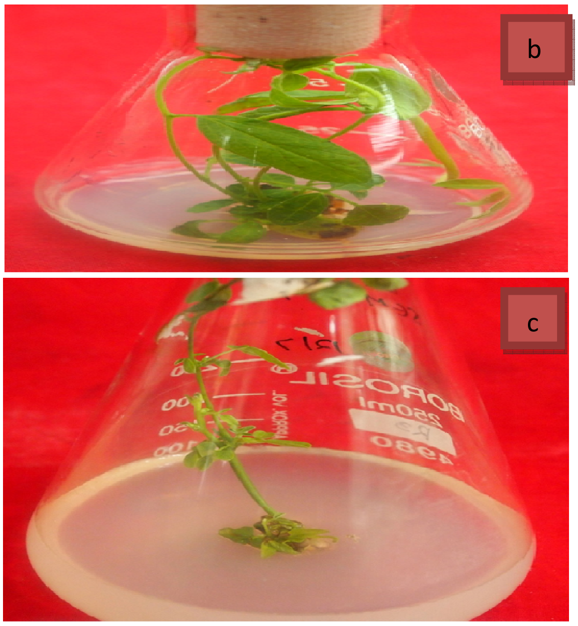


Fig.1.SEM1 elongation (a) Explant in SIM media, (b) Explant in SEM1 media after 7 days showing elongation (c) Very good shoot elongation in SEM1 media after 15 days.

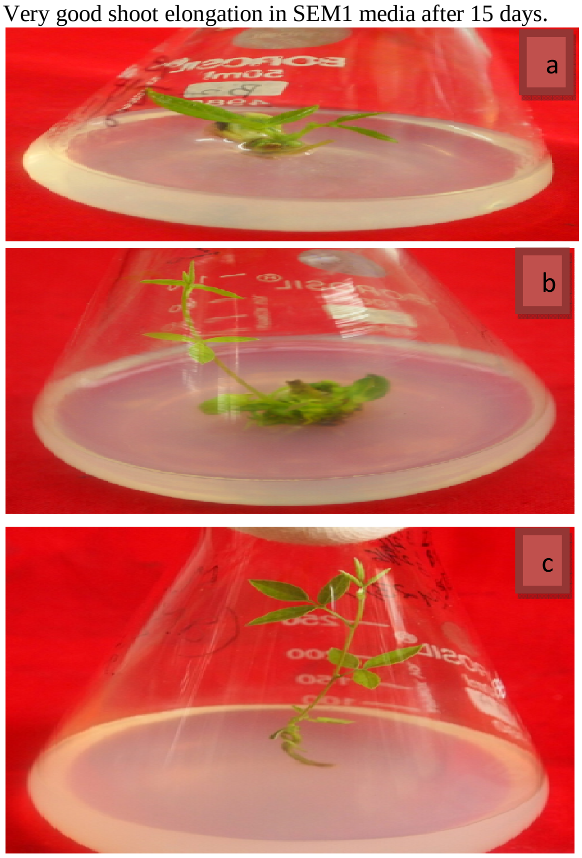


Fig.2. SEM2 elongation (a) Explant in SIM media, (b) Explant in SEM2 media after 7 days showing less elongation (c) Comparatively less shoot elongation in SEM2 media after 15 days.

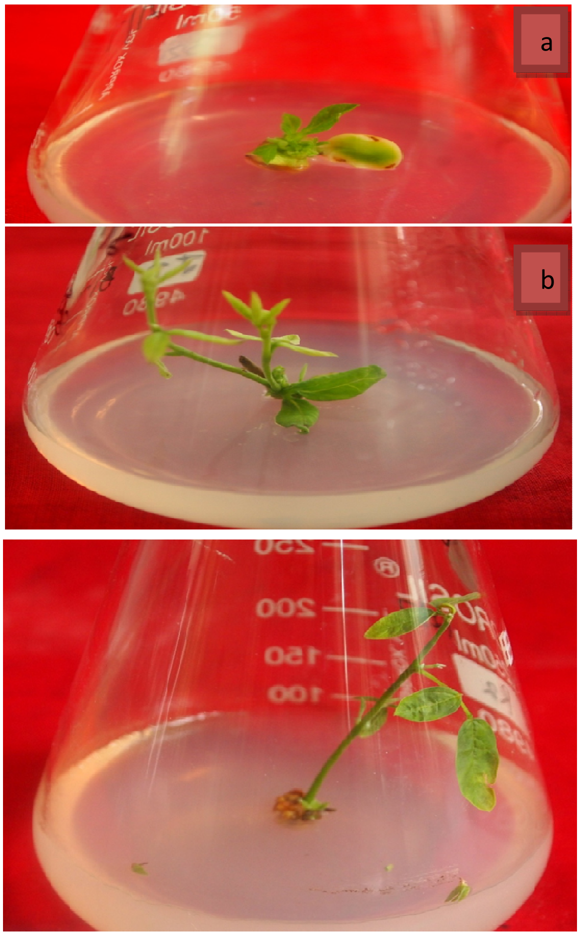
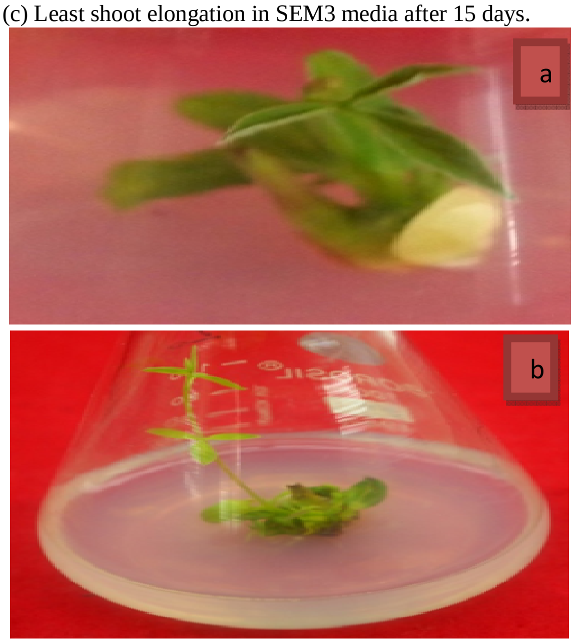


Fig.3. SEM3 elongation (a) Explant in SIM media, (b) Explant in SEM3 media after 7 days showing less elongation (c) Least shoot elongation in SEM3 media after 15 days.



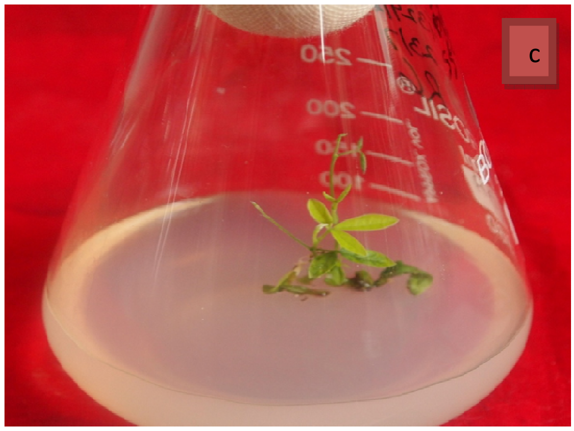


Fig.4 SEM4 elongation (a) Explant in SIM media, (b) Explant in SEM4 media after 7 days showing least elongation (c) Shoot eventually dies in SEM4 media after 15 days.

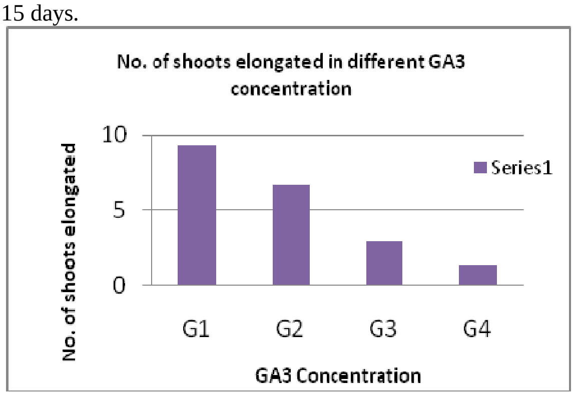


Fig.5 Comparative study of shoot elongation in various concentration of gibberellic acid

Type of media	Plant Growth Regulator	Concen tration	Explant Response* (mean ± SD)	Percentage
SEM1	GA_3	1mg/l	9.33±0.577	93.3%
SEM2	GA_3	2mg/l	6.66±1.52	66.6%
SEM3	GA_3	3mg/l	3±2	30%
SEM4	GA_3	4mg/l	1.33±0.577	13.3%

TABLE I. Effect of GA_3 on elongation of multiple shoots of pigeonpea after 3 weeks of culture

*10 shoots were transferred to each media for elongation

For the successful development of an effective regeneration protocol, selection of most suitable growth hormone composition is imperative. In the present study, we report an efficient protocol by using the axillary bud region of the seedlings, which can be induced to differentiate into adventitious shoots. The method involves suppressing growth of the axillary bud and the primary shoot bud while inducing multiple adventitious shoot buds in the axillary regions of the seedlings.

In this study, we were able to isolate the tissue surrounding the axillary bud, which could be isolated and induced to differentiate into multiple adventitious shoot buds. After inoculation of seeds in SIM, it was observed that 2mg/l BAP proved to be the most suitable for development of multiple shoots in Manak variety but it was unable to elongate the shoots. On lower concentration of GA_3 i.e., 1mg/l 93.30 % shoot elongation were obtained whereas 2mg/l GA_3 produced maximum of 66.6 % of shoot elongation was obtained. Similarly, at 3mg/l, 30% shoot elongation was obtained while at 4 mg/l, 13.33% of shoot elongation was obtained. It was also observed that manak variety did not much responded to increasing the concentration of GA_3 hormone and the no. of shoots elongated also gradually fall. So, we can also derive to conclusion that very high concentration of growth hormone proved to be toxic for plant growth.

This leads to the conclusion that media with lower concentrations of Plant Growth Regulators (PGRs) favour the elongation of shoot buds.

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