Greener Journal of Biological Sciences
ISSN: 2276-7762 ICV 2012: 5.99

Effect of Different Auxin Concentration on Rooting in Pigeonpea (*Cajanus cajan* [L.] Millsp.) *cv.* Manak (H77216) *Via* Cotyledonary Node Explants

By

Jharna Srivastava
Pramod K. Raghav

Research Article

Effect of Different Auxin Concentration on Rooting in Pigeonpea (*Cajanus cajan* [L.] Millsp.) *cv*. Manak (H77216) *Via* Cotyledonary Node Explants

Jharna Srivastava*, Pramod K. Raghav

Department of Biotechnology and Allied Sciences, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan (India) – 303122.

*Corresponding Author's Email: srivastava.jharna16@gmail.com

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) fortified with 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 + 1.0 mg/l GA_3. Elongated shoots were transferred to root induction media (RIM) using different concentration of IAA and IBA. Highest percentage (93) of rooting was observed on modified MS media + 1.0 mg/l IBA.

Keywords: *Cajanus cajan;* cotyledonary node explants; IAA; IBA; MS media.

ABBREVIATIONS

BAP, 6-Benzylaminopurine; GA_3, Gibberellic acid; IAA, Indole-3-acetic acid; IBA, 3-Indolebutyric acid; MS, Murashige and Skoog.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] with chromosome number of 2n=22, is an important grain legume crop of rainfed agriculture in the semi-arid tropics of the Indian sub-continent and is also widely grown in eastern and southern Africa, Latin America and the Caribbean (Nene and Sheila, 1990). Pigeonpea is cultivated in more than 25 tropical and subtropical countries, either as a sole crop or intermixed with cereals, such as sorghum (*Sorghum bicolor*), pearl millet (*Pennisetium glaucum*), or maize (*Zea mays*), or with other legumes, such as peanuts (*Arachis hypogaea*). Being a legume, the pigeonpea enriches soil through symbiotic nitrogen fixation.

Ripe seeds are eaten fried or boiled, often after being soaked first, or boiled into porridge. In the Indian subcontinent pigeonpea is mainly used as a pulse, in the form of 'dhal' or 'dal' (soaked, dried, hulled and split seeds), and this use is carried on by Indian communities in Africa. Pigeonpea is useful in hedges and windbreaks on dry soils and in agroforestry (e.g. in alley cropping systems, where it is pruned to supply green manure). It is also grown as a shade crop, cover crop, or as support for vanilla.

Shoots developed in presence of higher cytokinin to auxin ratio generally lack roots that prevent the generation of complete plantlets. In general, the rooting medium has low salt e.g. ½ or even ¼ salts of the MS medium, and reduced sugar levels (usually 1.0 g/L). Generally 0.1-1.0 mg/L NAA or IBA is required for rooting. Shoots are usually rooted in an agar medium, but the recent trend is to root them directly in vermiculite or potting mix. The cut ends of shoots are treated with a suitable auxin solution or powder mix, transplanted in pots and kept under high relative humidity and low light intensity. This saves cost as rooting and soil transfer stages are combined and rooting medium is eliminated.

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 (Eapen and George, 1993; Eapen *et al*., 1998; Geetha *et al*., 1998; Kumari *et al*., 2001; Singh *et al*., 2002; Dayal *et al*., 2003), cotyledonary node (Shivprakash *et al*., 1994; Franklin *et al*., 1998; Geetha *et al*., 1998; Mohan and Krishnamurthy, 1998; Singh *et al*., 2002), epicotyls (George and Eapen, 1994) and shoot apices (Geetha *et al*., 1999; Singh *et al*., 2004). A variable frequency (20-80%) of regeneration was reported by the workers implicating genotype-dependency on regeneration process.

In 1957, Skoog and Miller described the essentiality of plant growth regulators concentration in culture media. 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 (Mohan and Krishnamurthy, 1998; Yadav and Padmaja, 2003; Dayal *et al*., 2003), IBA (Shivprakash *et al*., 1994; Geetha *et al*., 1998), NAA (George and Eapen, 1994) and 2,4-D (Anbazhagan and Ganapathi, 1999) were used in various combinations with cytokinins like kinetin (Mohan and Krishnamurthy, 1998; Geetha *et al*., 1998; Dayal *et al*., 2003; Villers *et al*., 2008), BAP (Shivaprakash *et al*., 1994; Geetha *et al*., 1998; Pudukottai, 1998; Mohan and Krishnamurthy, 1998), TDZ (Eapen *et al*., 1998) to promote cell division, regeneration of shoots and to enhance proliferation and growth of auxiliary buds.

Various genotypes were used for development of regeneration protocol like cv. ICEAP 00557, ICEAP 00020, ICPL 88039, ICPL 86012, ICEAP 00040, ICPL 87091, ICEAP 00554, and ICEAP 00053 (Villers *et al*., 2008), AL 15 & Hyderabad C (Cheema and Bawa, 1991), ICPL 87119 (Ugandhar *et al*., 2012), LRG-41(Raghavendra *et al*., 2012), AL 201(Kaur *et al*., 2012), JKR105 (Krishna *et al*., 2011), LGG-29 (Guru *et al*., 2011), Bahar & UPAS120 (Yadav and Chand, 2001), ICPL 93086, Tanzania-7 & F1 Hybrid (Tyagi *et al*., 2001), ICP 26 &ICP 28 (Srinivasan *et al*., 2004) but till date no regeneration protocol for cv. Manak (H77216) has been developed using cotyledonary node explants.

Hence, the present study aims to attempt produce of cotyledonary node explants of pigeonpea cv. Manak (H77216) from suitable auxin combination for *in vitro* regeneration study.

MATERIALS AND METHODS

Plant materials and culture conditions

Seeds of pigeonpea variety Manak (H77216) were used for all the experiments in the present studies. Unless mentioned otherwise, all media contained MS salts and organic constituents (Murashige and Skoog, 1962), 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. For shoot elongation and rooting, 250ml conical flask were used closed with plugs made with non-absorbent cotton. All the growth regulators including BAP, GA_3, IAA and IBA were added after filter sterilization with nylon filter (Millipore) of 0.22µm pore size. The cultures were incubated at 25±2℃ temperature, with a light regime of 45-60µE/m^2/s for 16 h and 8 h at dark.

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.

Explant preparation and shoot regeneration

Seed coats from the pre-soaked seeds were removed under aseptic conditions and seeds were inoculated in media comprising of MS, B_5 vitamins, 2mg/l BAP, 3% (w/v) sucrose and 0.7% agar. Embryonic axes obtained from overnight soaked sterilized seeds. Embryonic axes were further excised by removing radicle apex and plumule apex along the axis resulting in two explants per seed.

Elongation of shoots

In the present study, BAP alone was found to be suitable for both multiple shoot bud induction and proliferation. However, the multiple shoots obtained on various concentrations of 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 1mg/l GA_3 for elongation.

Rooting of shoots

Elongated and well-developed shoots (> 3 cm long) were excised from the shoot clumps and transferred to MS medium augmented with various concentrations of IAA and IBA alone for root initiation. The hormone combinations used were [1mg/l, 2mg/l & 3mg/l] & IBA [1mg/l, 2mg/l & 3mg/l] IAA in RIM1, RIM2, RIM3, RIM4, RIM5 and RIM6 media respectively. The rooting frequency was further calculated. (Table I).

RESULTS AND DISCUSSION

Seeds inoculated in SIM media containing MS, B_5 vitamins, 2mg/l BAP, 3% (w/v) sucrose and 0.7% agar produced explants after 15 days. Each explant was then transferred to SIM for 7 days and then to SEM fortified with 1.0 mg/l GA_3 for 15 days. Each elongated shoot was then transferred to rooting media with different auxin at different concentration. Elongated shoots transferred to RIM1 media fortified with 1mg/l IBA showed maximum rooting. RIM1 media gave thickest and the most developed rooting.

Fig.1: RIM1 media (a) Shoot in RIM1 media, (b) Shoot in RIM1 media after 7 days showing thick rooting, (c) Most dense rooting in RIM1 media after 15 days.

Fig.2: RIM2 media (a) Shoot in RIM2 media, (b) Shoot in RIM2 media after 7 days showing least rooting, (c) Least rooting in RIM2 media after 15 days.

Fig.3: RIM3 media (a) Shoot in RIM3 media, (b) Shoot in RIM3 media after 7 days showing less thick rooting, (c) Less dense rooting in RIM3 media after 15 days.

Fig.4: RIM4 media (a) Shoot in RIM4 media, (b) Shoot in RIM4 media after 15 days showing no rooting.

Fig.5: RIM5 media (a) Shoot in RIM5 media, (b) Shoot in RIM5 media after 15 days showing no rooting.

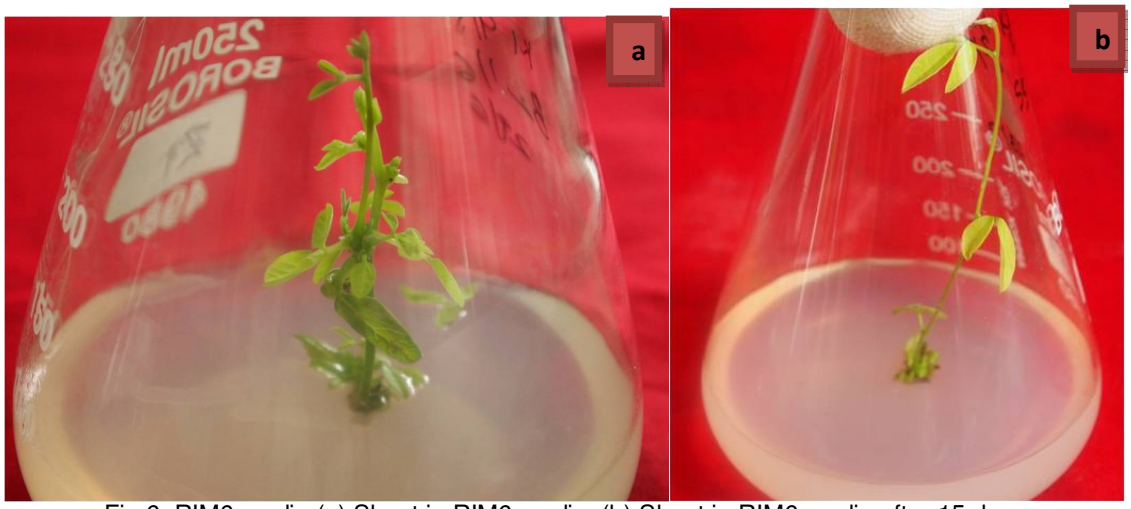

Fig.6: RIM6 media (a) Shoot in RIM6 media, (b) Shoot in RIM6 media after 15 days showing no rooting.

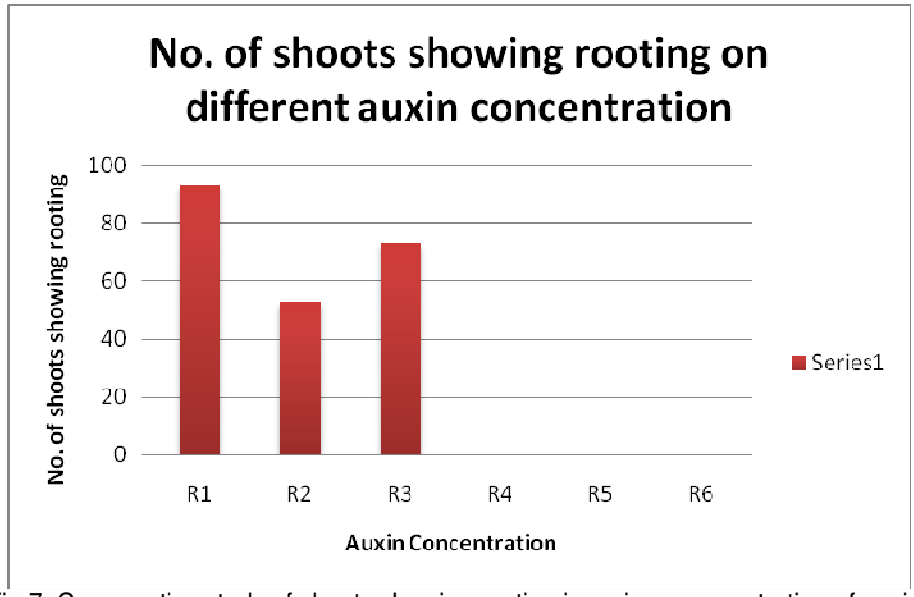

Fig.7: Comparative study of shoots showing rooting in various concentration of auxin.

TABLE I: Influence of auxin on rooting of *in vitro* derived shoots of pigeonpea after 2 weeks of culture

Type of media	Plant Growth Regulator	Concentration	Explant Response (mean ± SD)	Percentage Response
R1	IBA	1mg/l	9.33±1.15	93
R2	IBA	2mg/l	5.33±1.15	53
R3	IBA	3mg/l	7.33±1.15	73
R4	IAA	1mg/l	NR	NR
R5	IAA	2mg/l	NR	NR
R6	IAA	3mg/l	NR	NR

NR: Not Responded
10 plantlets were transferred to each media

While elongated shoots transferred to RIM2 media fortified with 2mg/l IBA gave minimum rooting. Roots in RIM2 media were very weak and underdeveloped. But elongated shoots transferred to RIM3 media fortified with 3mg/l IBA gave comparatively more thick rooting but less dense as in RIM1 media as shown in Fig 1, 2, 3.

Likewise, elongated shoots transferred to RIM4 media fortified with 1mg/l IAA showed no rooting. Similarly, elongated shoots transferred to RIM5 media fortified with 2mg/l IAA and RIM6 media fortified with 3mg/l IAA showed no rooting as shown in Fig. 4, 5, 6.

It is observed that elongated shoots did not respond to IAA while shoots only showed rooting in media containing IBA. On lower concentration of IBA i.e., 1mg/l 93.0 % rooting was obtained whereas 2mg/l IBA produced minimum of 53.0 % of rooting was obtained. Similarly, at 3mg/l, 73.0 % rooting was obtained as shown in Fig. 7.

It was observed that Manak variety showed indeterminate results i.e., in 1mg/l IBA highest rooting frequency is obtained while at 2mg/l IBA lowest rooting frequency is obtained and at 3mg/l IBA medium rooting frequency is obtained. So, we can definitely assign Manak as indeterminate variety.

ACKNOWLEDGEMENT

Pigeonpea seeds variety Manak (H77216) provided by Indian Institute of Pulses Research (IIPR), Kanpur and Financial support provided by Department of Science and Technology (DST), Rajasthan is greatly acknowledged.

REFERENCES

Anbazhagan V.R. and Ganapathi A., 1999. Somatic embrogenesis in cell suspension culture of pigeonpea (*Cajanus cajan* L.). Plant Cell, Tissue Organ Cult, 56: 179-184.

Cheema H.K. and Bawa J., 1991. Clonal multiplication via multiple shoots in some legumes (*Vigna unguiculata* and *Cajanus cajan*). Acta Horti, 289:93–94.

Dayal S., Lavanya M., Devi P. and Sharma K.K., 2003. An efficient protocol for shoot regeneration and genetic transformation of pigeonpea (*Cajanus cajan* (L.) Millsp.) by using leaf explants. Plant Cell Rep, 21:1072-1079.

Eapen S. and George L., 1993. Plant regeneration from leaf discs of peanut and pigeonpea: influence of benzyladenine, indole acetic acid and indole acetic acidamino acid conjugants. Plant Cell Tissue Organ Cult, 35:223-227.

Eapen S., Tivarkar S. and George L., 1998. Thidiazuron induced shoot regeneration in pigeonpea (*Cajanus cajan* L.). Plant Cell Tissue Organ Cult, 53:217-220.

Franklin G., Jeyachandran R., Melchias G. and Ignacimuthu S., 1998. Multiple shoot induction and regeneration of pigeonpea (*Cajanus cajan* (L) Millsp.) cv. Vamban 1 from apical and axillary meristem. Curr Sci, 74:936-937.

Geetha N., Venkatachalam P. and Lakshmisita G., 1999. *Agrobacterium*-mediated genetic transformation of pigeonpea (*Cajanus cajan* L.) and development of transgenic plants via direct organogenesis. Plant Biol, 16:213-218.

Geetha N., Venkatachalam P., Prakash V. and Lakshmisita G., 1998. High frequency induction of multiple shoots and plant regeneration from seedling explants of pigeonpea (*Cajanus cajan* L.). Curr Sci, 17:1036-1041.

George L. and Eapen S., 1994. Organogenesis and embryogenesis from diverse explants in pigeonpea. Plant Cell Rep, 13:417-420.

Guru Prasad M., Prasad N.V.K.V.T. and Sudhakar P., 2011. In vitro proliferation of shoot regeneration from embryo of *Cajanus cajan* L (var.LGG-29). J of Develop Tissue Eng, 3:62-65.

Kaur A., Devi R. and Dev A., 2012. Efficient in vitro regeneration in pigeonpea from cotyledonary node explants. J Cell Tissue Res, 12:3075-3080.

Krishna G., Reddy S.P., Ramteke W.P., Rambabu P., Sohrab S.S. *et al*., 2011. *In vitro* regeneration through organogenesis and somatic embryogenesis in pigeonpea [*Cajanus cajan* (L.) Millsp.] cv. JKR105. Physio Mol Biol Plants, 17:375-385.

Kumari P.V., Kishor P.B.K. and Bhalla J.K., 2001. *In vitro* plant regeneration in pigeonpea [*Cajanus cajan* L.] via organogenesis. Plant Cell Bio Mol Bio, 2:49-56.

Mohan M.L. and Krishnamurthy K.V., 1998. Plant regeneration in pigeonpea (*Cajanus cajan* (L) Millsp.) by organogenesis. Plant Cell Rep, 17:705-710.

Murashige T. and Skoog F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physio Plant, 15:473–497.

Nene Y.L. and Sheila V.K., 1990. Pigeonpea geography and importance In: Y L Nene. S D Hall, V K Sheila (eds), The Pigeonpea, CAB, Wallingford, UK, pp 1-14

Pudukottai V., 1998. Multiple shoot induction and regeneration of pigeonpea (*Cajanus cajan* Millsp.) cv.Vamban 1 from apical and axillary meristem. Curr Sci, 74(11):936-937.

Raghavendra T., Sudhakar P., Reddy K.B.H., Venkaiah K., Latha P. *et al*., 2012. *In vitro* shoot regeneration from cotyledon of redgram (*Cajanus cajan* [L] Var. LRG-41.

Shiva Prakash N., Pental D. and Bhalla-Sarin N., 1994. Regeneration of pigeonpea (*Cajanus cajan*) from cotyledonary node *via* multiple shoots formation. Plant Cell Rep, 13:623-627.

Singh N.D., Sahoo L., Saini R. and Jaiwal P.K., 2002. *In vitro* shoot organogenesis and plant regeneration from cotyledonary node and leaf explants of pigeonpea (*Cajanus cajan* L. Millsp.). Physio Mol Plants, 8:133- 140.

Singh N.D., Sahoo L., Saini R., Sarin N.B. and Jaiwal P.K., 2004. In vitro regeneration and recovery of primary transformation from shoot apics of pigeonpea using *Agrobacterium tumefaciens*. Physiology Mol Bio Plants, 10:65-74.

Srinivasan T., Verma V.K. and Kirti P.B., 2004. Efficient shoot regeneration in pigeonpea [*Cajanus cajan* (L) Millisp.] using seedling petioles. Curr Sci, 86:30–32.

Tyagi A.P., Comai L. and Byers B., 2011. Comparison of plant regeneration from root shoot and leaf explants in pigeonpea (*Cajanus cajan*) cultivars. Sabrao J, 33:59–71.

Ugandhar T., Shekhar G.P.V., Venkateshwarlu M., Srilatha T. and Jagan Mohan Reddy K., 2012. Plantlet regeneration from mature zygotic embryo culture of pigeonpea [*Cajanus cajan* (L.) Millsp] using thidiazuron. Life Science Feed, 1:1-4.

Yadav P.B.S. and Padmaja V., 2003. Shoot organogenesis and plantlet regeneration from leaf segments of pigeonpea (*Cajanus cajan* L.). Plant Cell, Tissue Organ Cult, 73:197-200.

Yadav V. and Chand L., 2001. Plantlet regeneration from decapitated embryonic axes of pigeonpea varieties. Indian J Plant Physio, 6:208- 211.