Indian J.L.Sci. 5 (1) : 33-38, 2015

ISSN : 2277-1743 (Print)
ISSN : 2278-7879 (Online)

IMPACT OF DIFFERENT CONCENTRATIONS AND COMBINATIONS OF GROWTH REGULATORS, SUPPLEMENTED IN MS BASAL MEDIUM ON EPICOTYLS EXPLANTS OF *Cajanus cajan*

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ABSTRACT

***Cajanus cajan* (L) Millsp. (Pigeon pea) is most important pulse crop in North India. The seeds of the plant provide rich protein food to the common people in this area. Seeds of pigeon pea were collected from the local traders of Muzaffarpur and were surface sterilized in the laboratory. Above sterilized seeds were placed on pre-sterilized moist filter paper lined in the Petriplates. Hypocotyls were excised from 7-8 days seedlings and used for culture. Tissue culture study was done with the above hypocotyls for the development of an efficient protocol for callus induction. MS basal medium supplemented with 3% sucrose were also supplemented with various concentrations and combinations of the growth regulators viz., 2,4-D + IBA + BAP or 2,4-D + IBA + KN, and gelled with 0.8% agar. MS + 0.5 mg/l 2,4-D + 0.5 mg/l IBA + 0.5 mg/l KN induced callus in 92.6% of the explants, while at the similar concentration BAP induced callus in 90.3% of the explants. The concentrations and combinations of different growth regulators were found to be critical factors for both the frequency and the type of callus formation as well as the growth rate of the calli. The green coloured calli with compact texture and excellent growth rate were obtained in the same medium, where higher percentage of induction was found. It was further noted that at the similar concentrations, 2,4-D + IBA + BAP the percentage of response was 90.3%. Both the lower and higher concentrations of the growth regulators were not promising.**

KEYWORDS : Epicotyl, *Cajanus cajan*, Pigeon pea, Callus, Protocol, Efficient

Cajanus cajan is an oldest pulse crop. The world production of pigeon pea sums up to 3.25 million tons. India is the largest producer contributing to around 85% of the world total production, followed by Myan mar and Malaur. It is grown in 4.3 million hectares land in the world and India has 85% of the area covered. Maharashtra is top in the production while Uttar Pradesh is second and Karnataka third. Bihar is the last. Again India is largest consumer as well as largest importer of the pulses. It is second most important crop next to pea. Here domestic consumption is estimated about 3.4 million tons. Out of this country imports 4-5 lakh tons annually.

Cajanus cajan belongs to family Papilionoideae. This crop is biennial as it is planted in late June and harvested in late March. Its plants may become woody if left for longer periods. In additions to the pulse, its green branches are used as fodder; dry main stalk is used as fuel wood. The plant is also used as folk medicines.

Tissue Culture Studies

The potential of practical application of plant tissue and cell cultures are many. Induction of haploid through anther culture, pollen culture is of immense significant for crop improvement, because at one hand double haploid can be produced while on the other hand mutant can be easily detected. Through this technique China has produced several commercial varieties of rice and wheat. It is also being used to produce inter specific and intergeneric hybrids. (Chopra & Sharma 1991) The protoplast culture has opended up the possibility of modifying the plants genome by uptake of foreign DNA and by creating hybrids between sexually incompatible species through protoplast fusion. Somatic hybrids are being used for disease resistance, stress tolerance, quality characters, cytoplasmic male sterility, etc.

Tissue culture studies and induction of callus have been reported by different workers. Eapen and Georgi (1993); Patel et al; (1994), Nalini et al; (1996), Sree nivasu K et al; (1998), Anbazhagan and Ganapatti (1999), Mohan and Krishnamurthy (1998, 2002), Singh et al; (2002), Chandra et al; (2003), Krishna et al; (2010), all have reported induction of callus from various explants of pigeon pea (*Cajanus cajan* (L) Millsp) such as leaf disc, hypocotyls, cotyledonary segments etc. They have also reported somatic embryogenesis and morphogenesis in the above calli and induction of multiple shoots. They also noted impact of growth regulators on callusing.

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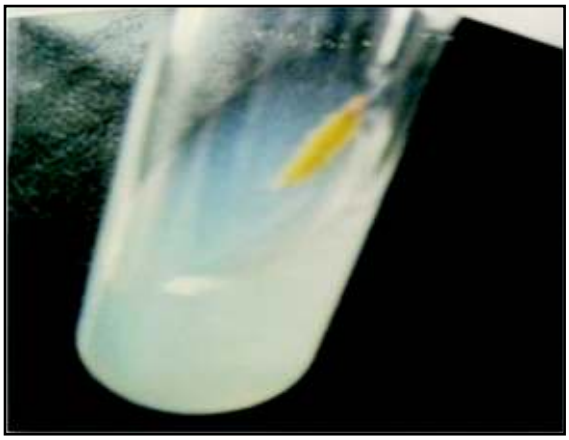

Figure 1 : Induction of Callus in MS medium + 0.2 mg 2,4-D + 0.2 IBA + 0.2 BAP (mg/l)

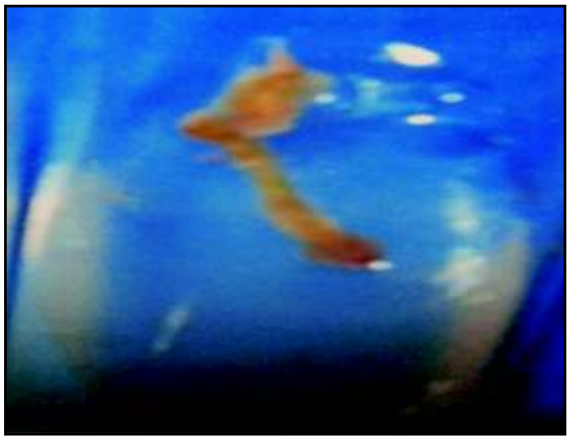

Figure 2 : Subculture 0.4 2,4-D + 0.4 IBA + 0.4 BAP

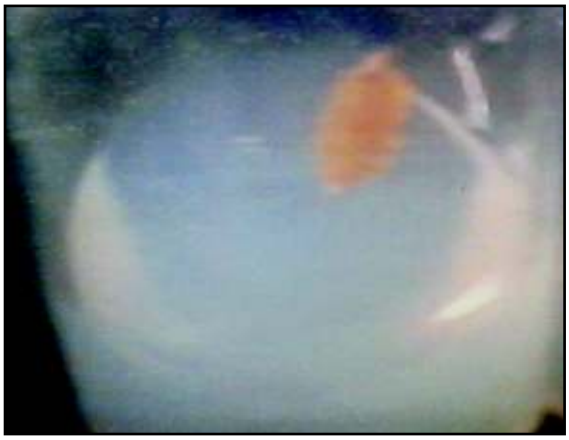

Figure 3 : 0.5 2,4-D + 0.5 IBA + 0.5 BAP

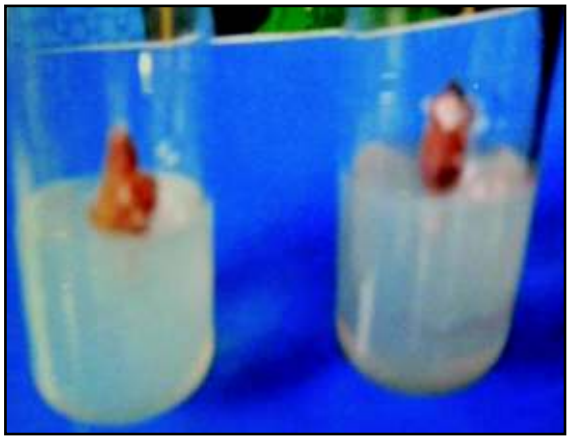

Figure 4 : 0.5 2,4-D + 0.5 IBA + 0.5 KN

Figure 5 : 1.0 2,4-D + 1.0 IBA + 1.0 KN

Figure 6 : 0.4 2,4-D + 0.4 IBA + 0.4 KN

Figure 7 : 0 .5 2,4-D + 0.5 IBA + 0.5 KN (Subculture)

Tissue culture studies of pigeon pea for micropropagation in general and induction of calli in particular is getting more and more importance. The calli has multipurpose utilization. They may be used for genetic transformation either directly or via protoplasts. In the present study attempt has been made to develop a protocol for efficient induction of calli so that they may be utilized either for selection against the pathogens that is the resistant cell lines so that the differentiated plants shall be disease resistant against that pathogen.

MATERIALS AND METHODS

Seeds of *Cajanus cajan* (L) Millsp. Commonly called pigeon peas were purchased from authorized seed traders at Muzaffarpur. Seeds were washed in running tap water, keeping them in 1 L conical flasks whose mouth was covered with muslene clothes for 20 min, and then they were washed thoroughly with pre sterilized distilled water with few drops of liquid detergent (tween 20). Seeds were treated with drops of sodium hypochlorite in 200 ml distilled water. The flask was manually shaken for 10 min. The seeds were again washed thoroughly with distilled water twice. Fresh water was used every time. This was followed with treatment with 0.1% $HgCl_2$ for 2 min and the flask was shaken vigorously for uniform mixing. Seeds were taken out and washed 3-4 times with distilled water for 3-4 min of duration. These seeds were germinated on pre sterilized and moist filter paper lined the Petriplates. Epicotyl was excised from 7 days old seedlings to be used as explants.

Culture Medium

MS basal medium was prepared from the stock solutions prepared earlier. Requisite volume of each stock solution was kept in one L conical flask. Then the vol was made 500 ml. 3% Sucrose and required growth hormones were added. The pH was adjusted to 5.8. 0.8% agar was dissolved by heating in 400 ml distilled water. This was mixed with the above solution and the volume was made 1000 ml. by adding extra glass distilled water.

Above medium was dispensed in culture tubes (20 ml) and culture flasks of 250 ml (40 ml). The mouth was covered with cotton plugs covered with muslene cloth. They were wrapped with Aluminium foil & autoclaved at 15 lb pressure & 121^{0}C temp for 20 min. These cultures were cooled at room temp and stored in freeze. They were used for inoculation after two days.

Inoculation

Inoculation was done in the aseptic conditions under the Laminar flow chamber.

Incubation

After inoculation the tubes and flasks were incubated in culture rooms at 26±2^{0}C and 12 h photoperiod at 3000 lux generated through the white fluorescent tubes (Phillips). Observation was made on an alternate day. All the contaminated tubes were discarded. Final data were collected after three weeks of incubation. Average of 3 replica, each containing 20 cultures have been taken.

RESULTS AND DISCUSSION

Pre sterilized seeds germinated efficiently on moist filter paper lined in the Petriplates or in culture flasks, containing MS basal medium without any growth regulators. On seventh day 95-98% germination was observed. Hypocotyl explants were prepared from these plantlets which was excised with the help of a sterilized

Table 1 : Impact of Various Concentrations and Combinations of Growth Regulators Supplemented in MS Basal Medium on Epicotyl Explants of *Cajanus cajan* L.

Growth Regulators	% response	Status of Callus		
2,4 - D+IBA+BAP (mg/l)		**Colour**	**Texture**	**Growth**
0.1+0.1+0.1	23.6	W	L	+
0.2+0.2+0.2	29.3	W	L	+
0.3+0.3+0.3	42.8	W	L	+ +
0.4+0.4+0.4	72.4	G	C	+ + +
0.5+0.5+0.5	90.3	G	C	+ + + + +
1.0+1.0+1.0	64.7	G	C	+ + +
1.0+ ----- +1.0	70.4	G	C	+ + + +
----- 1.0+1.0	63.6	G	C	+ + +
0.5+0.5+1.0	61.5	W	L	+ + +
0.5+ ----- +1.0	62.4	W	L	+ + +
------ +0.5+1.0	54.6	W	L	+ + +
2,4 - D+IBA+KN				
0.1+0.1+0.1	24.8	W	L	+
0.2+0.2+0.2	30.6	W	L	+ +
0.3+0.3+0.3	43.2	G	L	+ + +
0.4+0.4+0.4	74.6	G	C	+ + + + +
0.5+0.5+0.5	93.2	G	C	+ + + +
1.0+1.0+1.0	83.4	G	C	+ + + +
1.0+ ----- +1.0	72.5	G	C	+ + +
------ +1.0+1.0	65.7	W	C	+ + +
0.5+ ------ +1.0	62.2	W	L	+ + +
------- +0.5+1.0	60.8	W	L	+ + +
0.5+ ------- +1.0	56.6	W	L	+ +

20 explants for each treatment.

Final data were collected after 4 weeks.

+ Average, + + Good, + + + Better, + + + + Best, + + + + +Excellent

W= White, G= Green, C= Compact, L= Loose

sharp blade.

Induction of callus was observed in MS basal medium fortified with different concentrations of 2,4-D, IBA with either BAP or KN, after 18th day of incubation. Virtually calli were induced in all the combinations and concentrations of the growth regulators, however, difference in percentage of response was evident. Highest percentage of callus induction was observed in MS + 0.5 mg/l 2,4-D + 0.5 mg/l IBA + 0.5 mg/l BAP, which was 90.3. This was followed by 0.4 mg/l of each of the growth regulators. It was further noted that at both the lowest and the higher concentration of the above growth regulators the response was lower than 0.5 mg/l of each of the growth regulators.

From the table 1 it is clear that growth rate of calli, their colour and texture were, excellent, green and compact in the medium where the highest percentage of response was observed. Similarly average growth, white colour and loose texture were noted on the lowest concentration where the response was also poor.

From the table 1 it is further evident that 0.5 mg/l 2,4-D + 0.5 mg/l IBA + 0.5mg/l KN induced callusing in 93.2% of the explants. Here 2,4-D 1.0 mg/l , IBA 1.0 mg/l and KN 1.0 mg/l gave the next higher percentage i.e., 83.4%. Here also at both the lowest and higher concentrations the response was poor. Similarly, either 2,4-D + BAP or IBA+BAP or 2,4-D + KN or IBA + KN had also lower response.

It was noted that 2,4-D + IBA + BAP gave lower response in percentage induction or growth rate than 2,4-D + IBA + KN. Induction of callus in pigeon pea is of immense importance. Aubazhagan and Ganpathi (1999) reported that

sucrose was the best source of carbon for the induction of callus in pigeon pea. They reported higher concentrations of the growth regulators, but in contrast in the present study higher concentrations were not promising for the induction of callus. Different concentrations of 2,4-D, IBA with either BAP or KN, supplemented to MS medium displayed the formation of white/green coloured calli, which is in conformity with the findings of Baken et al; (1995); Nalini et al; (1996), Sreenivasu et al; (1998), Singh et al; (2002), Krishna et al; (2010). MS basal medium is suitable for the induciton of calli has been also confirmed by Mohan and Krishnamurthy (2002); Chandra et al; (2003); Singh et al; (2002).

In pigeon pea, *Fusarium* wilt is most prevalent and causes heavy loss to the farmer. Selection of disease resistant cell lines is possible. For that callus induction is essential. Selection of resistant cell lines had been reported by Behnke (1979) in potato against Phytophthora infestans; Daub (1986); Arcioni et al; (1987) in alfalfa against *Fusarium oxysporum* F. Sp. medicagivis, Chawla and Wenzel (1987) in barley & wheat against Helminthesporium sativum; Vidayasekaran et al; (1990) in rice against Helminthosporium toxin. Gayatri et al; (2005) in turmeric against *Pythium graminicolum*. But we get scanty report of such works in pigeon pea. Similarly, protoplasts obtained from the calli, may be used for somatic hybridization or somatic embryogenesis. Such works have been reported by Dutis et al; (1980); Austin et al; (1985), Barwale and Kerns (1986); Krishna et al; (1986), Pental et al; (1986), Leelavathi et al; (1987), Chatterjee et al; (1988), Komatsu & Ohyama (1988), Parrot and Hoffman (1989); Bailey et al; (1993), Georgi & Eapen (1993); Baken et al; (1995), Anbazhan and Ganapathi (1999); Helgeson et al; (1986, 1993); Krishna et al; (2010); Prabhakaran (2011) and Pawar et al; 2014.

ACKNOWLEDGEMENTS

Authors are obliged to The Head, University Department of Botany, B. R. Ambedkar Bihar University, for kind permission to utilize the Library and Laboratory of the Department.

REFERENCES

Arcioni S., Pezzotti M, Damiani F., 1987. "In *vitro* selection of alfalfa plants resistant to *Fusarium oxysporum* F. Sp. medicaginis". Theor. Appl. Genet. 74: 700-705.

Aubazhagan V. R., Ganapathi A., 1999. Somatic embryogenesis in cell suspension cultures of Pigeon pea (*Cajanus cajan* L.) Plant Cell Tiss. Org. Cult. **56**: 179-184.

Austin S, Bacr MA, Helgeson J.P., 1985. Transfer of resistance to potato leaf roll virus from *Solanum brevidens* into *Solanum tuberosum* by somatic fusion. Plant Sci. **39**: 75-82.

Bailey MA, Parrott WA, Boerma HR, 1993. Genotype effects on proliferative embryogenesis and plant regeneration of Soybean. In *Vitro* Cell Dev. Biol. Plant. **29**: 102-108.

Baker C.M., Durham RE, Burns JA, Parrott WA, Wetzstein HY, 1995. High frequency of somatic embryogenesis in peanut (*Arachis hypogaea* L.) using mature dry seed. Plant Cell Rep., **15**: 38-42.

Barwale U B, Kerns HR, Widholm J.M., 1986. Plant regeneration from callus cultures of soybean genotypes via embryogenesis and organogenesis. Planta, **167**: 473-481.

Behnke M., 1970. "Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of plants." Theor. Appl. Genet., **55**: 69-71.

Chandra A., Gupta V., Burma P., Pental D., 2003. Patterns of morphogenesis from cotyledon explants of pigeon pea. In *Vitro* Cell. Dev. Biol. Plant. 39: 514-519.

Chatterjee G., S.R. Sikdar, S. Das, S.K. Sen., 1988. Intergeneric somatic hybrid production through protoplast fusion between *Brassica juncea* and Diplotaxis muralis. Theoritical & Applied Genetics. **76**(6): 915-922.

Chawla, HS. Wenzel, G., 1987. "In vitro selection of barley and wheat for resistance against *Helminthosporium sativum*". Theor. Appl. Genet. **74**: 841-845.

Daub, 1986. "Tissue culture and the selection of resistance to pathogens." Annu. Rev. Phytopathol. **24**: 159-186.

Eapen S., George L., 1993. Plant regeneration from leaf discs of peanut and pigeon pea- Influence of benzyladenine, IAA and IAA- amino acid conjugates. Plant Cell Tiss Org. Cult. **35**: 223-227.

Gayatri M C., V. Roopa darshini, R. Kavyashree, 2005. Selection of turmeric callus tolerant to culture of *Pythium graminicolum* and regeneration of plants. Plant Cell. , Tissue and Organ Culture, **83**(1): 33-40.

George L., Eapen S, 1993. Influence of genotype and explant source on somatic embryogenesis in peanut. Oleagineux. **48**: 361-363.

Helgeson JP; Heent GJ; Haberlach GT and Austing S. 1986. "Somatic hybrids between Solanum brevidens and *Solanum tuberosum*: expression of a late blight resistance gene and potato leaf roll resistance". Plant Cell Rep., **5**(3): 212-214.

Kinsara A, Patnaik S.N., Cocking EC, Power J.B., 1986. Somatic hybrid plants of *Lycopersicon esculentum* Mill and *Lycopersicon peruvianum* Mill. J. Plant Physiol., **125**: 225-234.

Komatsuda T., Ohyama K., 1988. Genotypes of high competence for somatic embryogenesis and plant regeneration in soybean. Glycine max. Theor. App. Gen., **75**: 695-700.

Krishna G., Reddy P. S., Ramtake PW, Bhattachary PS., 2010. Progress of tissue culture and genetic transformation research in pigeon pea (*Cajanus cajan* (L) Millsp.) Plant Cell Rep. 29: 1079-1095.

Leelavathi S, Reddy VS, Sen SK., 1987. A scheme for alien chromosomal transfer in *Brassica* through anther culture. Cauciferae Newslett. pp-12.

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

Mohan ML. Krishnamurthy K.V., 2002. Somatic embryogenesis and plant regeneration in pigeon pea. Biol. Planta, **45**: 19-25.

Nalini M, Reena MJT, Sastri DC, Moss JP., 1996. Somatic embryogenesis in pigeon pea (*Cajanus cajan* L. (Millsp.)) Indian J. Exp. Biol. 34: 282-284.

Parrott WA, Hoffman LM, Hildebrandt DF, Williams EG, Collin GB., 1989. Recovery of Primary transformants of Soybean. Plant Cell Rep., **7**: 615-617.

Patel D. B., Barve DM, Nagar N, Mehta AR., 1994. Regeneration of pigeon pea (*Cajanus cajan*) somatic embryogenesis. Ind. J. Exp. Biol., **32**: 740-744.

Pawar B. D., A. S. Jadhav, Sharad V. Pawar, V. P. Chinote and Anil A. Kale, 2014. An efficient regeneration system for pigeon pea (*Cajanus cajan* L.) Journal of Crop. Improvement., **28**(6): 825-833.

Pental D., Hamill J.D., Pirrie A, Cocking EC, 1986. Somatic hybridization of *Nicotiana tabacum* and *Petunia hybrida*. Mol. Gen. Genet., **191**: 244-252.

Prabhakaran M., Ehemalai S., Prakasam S Rav., 2011. High frequency Induction of callus from seedling explants of Pigeon pea (*Cajanus cajan* (L) Millsp.) for genetic transformation. Curr. Bot. 2(6): 15-17.

Singh ND, Sahoo L, Sonia JPK, 2002. In *vitro* shoot organogenesis and plant regeneration from cotyledonary node and leaf explants of pigeon pea. (*Cajanus cajan* L. Millsp.) Physiol. Mol. Biol. Plants, **8**: 133-140.

Sreenivaseu K, Mlik SK, Ananda KP, Sharma RP, 1998. Plant regeneration via somatic embryogenesis in pigeon pea (*Cajanus cajan* (L.) Millsp.). Plant Cell. Rep., **17**: 194-297.

Vidayasekaran, P. Ling, DH Borrowmeo, ES, Zapata, F.J., Mew TW, 1990. Selection of Brown spot-resistant rice plants from *Helminthosporium oryzae* toxin resistant calluses. Ann. Appl. Biol., **117**: 515-523.