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EFFECT OF DIFFERENT CYTOKININS ON SHOOT INDUCTION IN PIGEONPEA (*CAJANUS CAJAN* L.) MANAK (H77216) VARIETY

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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 and soaked overnight. This sterilized seeds were decoated and inoculated in MS media with various combination of cytokinin for cotyledonary node explants. Seeds inoculated were used for morphogenic response and various frequencies were observed using (0mg/l,1mg/l,2mg/l,3mg/l and 4mg/l) concentration of BAP, KIN and ZEA simultaneously. Highest percentage (88.35) of cotyledonary node explant formation was observed on MS media + 2.0 mg/l BAP, 3% sucrose, 0.7% (w/v) agar.

KEY WORDS: ***Cajanus cajan,*** **cotyledonary node explant, MS media, BAP, Kinetin, Zeatin.**

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INTRODUCTION

Pigeonpea (*Cajanus cajan* [L.] Millispaugh*)* is an important grain legume of family *Fabaceae*. Pigeonpea is rich in protein (20-22%) particularly sulphur containing amino acids, namely methionine and cysteine (Singh *et al*., 1990). 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. Due to its deep root system, pigeonpea offers less competition to associated crops than some other legumes, and it is often used in intercropping systems with cereals such as millet, sorghum, and maize or with short duration legumes such as cowpea. 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), cotyledon (Ugandhar *et al*., 2012), 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 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 axillary 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 HK & Bawa J,1991), ICPL 87119 (Ugandhar , *et al*.,2012), LRG-41(Raghavendra, *et al*.,2012), AL 201(Kaur A *et al*.,2012), JKR105 (Krishna G, *et al*.,2011), LGG-29 (Guru Prasad M *et al*.,2011), Bahar & UPAS120 (Yadav V & Chand L ,2001), ICPL 93086, Tanzania-7 & F1 Hybrid (Tyagi AP *et al*.,2001), ICP 26 &ICP 28 (Srinivasan T *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 attempts produce cotyledonary node explants of pigeonpea *cv*. Manak (H77216) from suitable cytokinin 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. All the growth regulators including BAP, KIN and ZEA 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.

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 different media comprising of MS, B_5 vitamins, 3% (w/v) sucrose and 0.7% agar. The hormone combinations used were [0mg/l, 1mg/l, 2mg/l, 3mg/l & 4mg/l] BAP, [0mg/l, 1mg/l, 2mg/l, 3mg/l & 4mg/l] KIN & [0mg/l, 1mg/l, 2mg/l, 3mg/l & 4mg/l] ZEA. The explants frequency was further calculated.

RESULTS AND DISCUSSION

The seeds were germinated on MS basal medium with various concentrations of BAP, KIN and ZEA to suppress the growth of the primary shoot. Culturing the seedlings on SIM containing 2mg/l BAP suppressed the axillary bud growth; it instead resulted in multiple shoot formation from the suppressed axillary bud region. Bulging at the cotyledonary node region was observed and by 12 d the differentiation of adventitious shoot buds occurred at the bulged portion surrounding the axillary bud. These shoot buds became more defined by 15 d, while growth of the axillary bud was further suppressed. Fully differentiated shoots developed in tissue surrounding the suppressed axillary bud resulting in multiple shoots. If allowed to grow further, multiple shoots were formed in the axillary regions of the seedlings. If the pre-soaked and de-coated seeds are germinated on hormone-free MS medium normal germination occurs, resulting in the development of two axillary buds. However, when these seeds are germinated on SIM containing 2mg/l BAP, it results in complete suppression of the axillary and apical buds, and differentiation of adventitious shoot buds occurs in the tissue surrounding the axillary bud (Fig. 1C). While it was observed that 1mg/l BAP media provided less no. of shoots as compared to 2mg/l BAP media whereas 3mg/l BAP and 4mg/l BAP concentration proved to be high and unfavourable for development of multiple shoots. Kinetin and Zeatin were also used in different concentrations but these do not supported manak variety to develop multiple shoots. 0 mg/l KIN provided no adventitious shoot, 1 mg/l KIN media gave 6-7 shoots, 2 mg/l KIN media gave 5-6 shoots, 3mg/l gave 3-4 shoots and 4 mg/l gave 0 adventitious shoots. Zeatin hormone was the least supporter of multiple shoots. 0 mg/l ZEA provided no adventitious shoot, 1 mg/l ZEA media gave 2-3 shoots, 2 mg/l ZEA media gave 4-5 shoots, 3mg/l gave 1-2 shoots and 4 mg/l gave 0 adventitious shoots.

Table 1

Effect of growth regulators on induction of adventitious shoot buds from explants of pigeonpea. MS basal medium was used for all combinations

Plant growth regulators (mg/l)					
BAP	KIN	ZEA	No. of explants with shoots	No. of explants with elongated shoots	Explant with shoots (%)
0	-	-	NR	NR	-
1	-	-	12.33	37	61.65
2	-	-	17.67	53	88.35
3	-	-	8.33	24	41.65
4	-	-	1.33	4	6.65
-	0	-	NR	NR	-
-	1	-	6.33	19	31.65
-	2	-	5.67	17	28.65
-	3	-	3.67	11	18.35
-	4	-	NR	NR	-
-	-	0	NR	NR	-
-	-	1	2	6	10
-	-	2	4.33	13	21.65
-	-	3	1.33	4	6.65
-	-	4	NR	NR	-

**** Results are averages of three replications where each replicate included 10 explants.***
Values are expressed as mean ^ SE. The experiment was repeated three times.

Figure. 1

(A) No explant in B0 media, (B) explant in B1 media, (C) explants with multiple shoot in B2 media, (D)explants with less shoot in B3 media (E) explants with least shoot in B4 media.

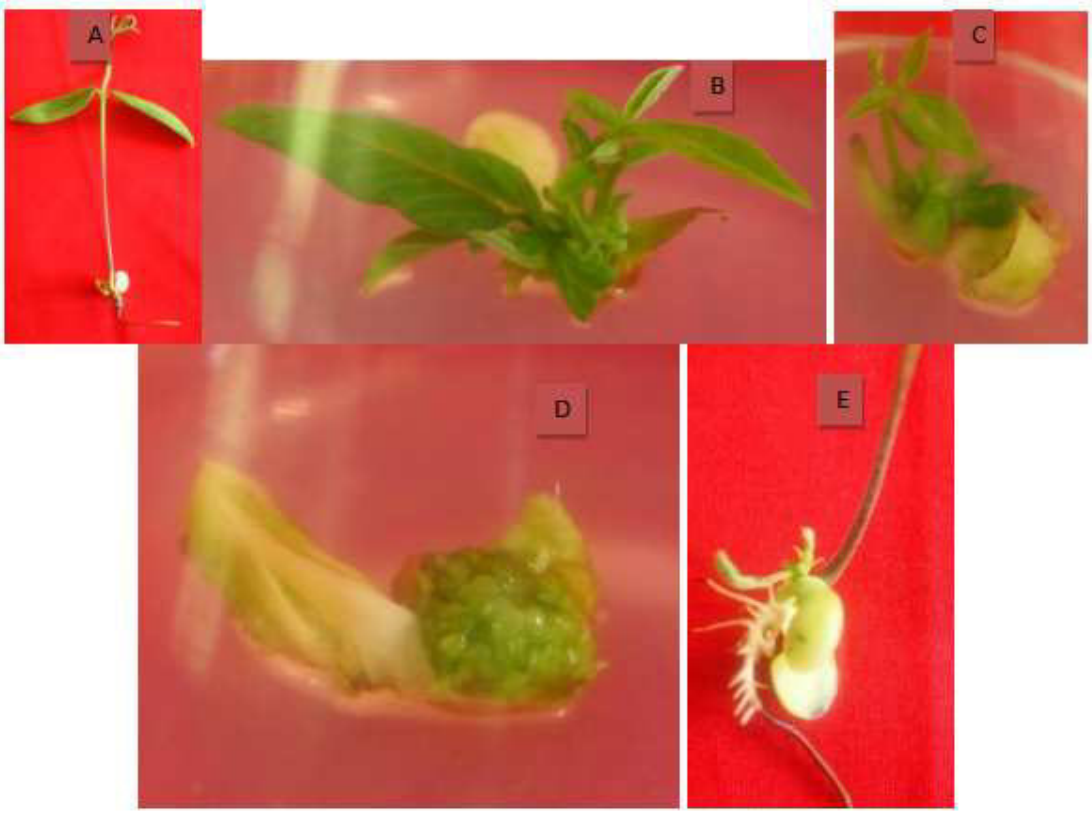

Figure.2
(A) No explant in K0 media, (B) explant with less shoots in K1 media, (C) explants with less shoots in K2 media, (D)explants with less shoots in K3 media (E) No explant in K4 media.

Amongst the various basal media tested for the germination of seeds, MS containing 2mg/l BAP was found to be superior to other media with respect to the number of seedlings showing the induction of adventitious shoot buds. To optimize the cytokinin for adventitious shoot induction, BAP, KIN and ZEA were tested in different concentrations and the experiment was repeated three times. Comparative study of development of explant in various media (Fig 4).

Figure. 3
(A) No explant in Z0 media, (B) explants with less shoots in Z1 media, (C) explants with less shoots in Z2 media, (D)Explant with least no. of shoot in Z3 media (E) No explant in Z4 media.

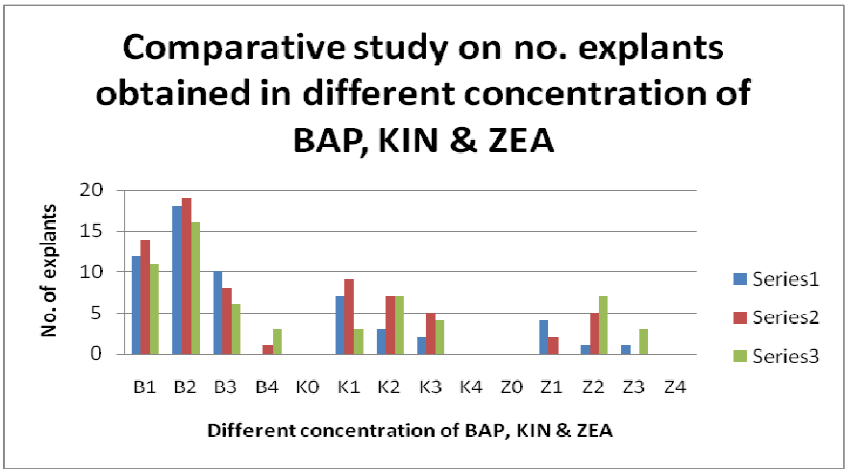

Figure.4t
Comparative study of development of explant in various media

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. Although similar type of regeneration has been observed earlier from cotyledonary node explants of pigeonpea (Shiva Prakash *et al*., 1994), the shoot meristems were thought to differentiate directly without the formation of the intervening primary shoot-like structures, where the axillary shoots and possibly the adventitious shoots were not separated and were mixed. Shiva Prakash *et al*., 1994 did a comparative study of different cytokinins namely BAP, kinetin, zeatin and adenine at different concentrations for different genotypes and BAP 2mg/l produced highest no. of shoots per explants. The best response in terms of no. of shoots per cotyledonary node explants was (43±8.9) obtained from CC11295 cultivar. Similarly Geetha *et al*., 1998 reported maximum frequency of shoot formation from cotyledonary node explants was 93.2% on regeneration medium with BAP and 75.4% with kinetin while Franklin *et al.,* 1998 obtained a maximum of 49 shoots on 13.31 □□M (3.0 mg/l) BAP with seedling explant which is a combined cotyledonary node and shoot tip and only five shoots with cotyledonary node. 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 different cytokinin and in different composition it was observed that 2mg/l BAP proved to be the most suitable for development of multiple shoots in manak variety. On lower concentration of BAP i.e., 1mg/l 61.65 % explants with shoots were obtained but on increasing the BAP concentration to 3mg/l 41.65% and at 4mg/l only 6.65% explants with shoots were obtained. While 2mg/l BAP produced maximum of 88.35% explants with shoots. It was also observed that manak variety did not much responded to kinetin and zeatin media. Kinetin hormone produced a maximum of 31.65% explants with shoots at 1mg/l but on increasing the concentration of kinetin hormone the no. of explants also gradually fall. Likewise with zeatin hormone a maximum of 21.65% explants with shoots were obtained at 2mg/l and the no. shoots also gradually fall on increasing the concentration of growth hormone. So, we can also derive to conclusion that very high concentration of growth hormone proved to be toxic for plant growth. Therefore, 2 mg/l BAP provides maximum no. of multiple adventitious shoots in manak variety and plant growth hormone play a pivotal role in development and growth of plant.

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ABBREVIATIONS

BAP, 6-Benzylaminopurine; KIN, Kinetin; MS, Murashige and Skoog; ZEA, Zeatin.

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