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TISSUE CULTURE STUDIES IN *VIGNA UNGICULATA L*

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ABSTRACT

Cowpea is a dicotyledonous plant belonging to the family Fabaceae and sub-family, Fabiodeae. Cowpea is often called as "black-eyed pea" due to its black- or brown- ringed hylum. *Vigna* is a nutritious source of food, its grains contain about 25% Protein, especially rich in folate, potassium, iron, magnesium and the essential amino acids lysine and tryptophan. It is also used as animal fodder, cover crop and green manure. Cowpea is often called as "black-eyed pea" due to its black- or brown-ringed hylum. *Vigna* originated in West Africa India, Nigeria accounts for 70% of the world's production of cowpea beans. The plant tissue culture is also proving to be rich and novel sources of variability with a great potential in crop improvement without restoring to mutation or hybridization. The plant with long seed dormancy can be raised faster by *in vitro* clonal propagation. In the present study shooting was initiated from nodal and shoot tip explants on MS medium containing BAP alone used as phytoharmone source. Rooting in the form of organogenesis was obtained from leaf explants in the presence of NAA alone as phytoharmone.

KEYWORDS: Phytoharmone, Clonal propagation, Organogenesis, Mutation, Hybridization.

INTRODUCTION

Cowpea is a dicotyledonous plant belonging to the family Fabaceae and sub-family, Fabiodeae (Agbogidi *et al*., 2010). *Vigna* contains good protein quality and high nutritional value and often referred to as the "poorman's meat". A survey of the literature, especially on cowpea morphology and taxonomy (Padulosi and Ng, 1997) and genetics (Fery, 1985; Fery and Singh, 1997, showed that it is an edible legume of the family Fabaceae with high protein contents. India, Nigeria accounts for 70% of the world's production of cowpea beans.

Cowpeas are one of the most important food legume crops in the semiarid tropics covering Asia, Africa, southern Europe, and Central and South America (Singh *et al*., 1997). Of all the grain-legumes, it is the most extensively cultivated distributed and traded food crop in India (Ogbo, 2009; Agbogidi *et al*., 2011; Philips and McWalters, 1991) and one of the best hopes for combating shortage in food supply. Cowpea is called the "hungry-season crop" because it is the first crop to be harvested before the cereal crops (Gomez, 2004). Cowpea has great flexibility in use, farmers can choose to harvest them for grains or to harvest forage for their livestock, depending on economical or climatological constraints (Gomez, 2004). *Vigna unguiculata* is an important legume that can be easily cultivated in areas of low rainfall and poor soil. The seed is diuretic and is used to strengthen the stomach. When boiled and eaten as a food it is considered to destroy worms in the stomach (Chopra *et al*., 1986). Traditional of cowpea are known as “Vegetable meat” due to high amount of quality protein (23.4%), carbohydrate (60.3%), fat (1.8%), iron (57 mg/100g) and vitamins such as riboflavin (0.18 mg/100 g), nicotinic acid (1.9 mg/100 g) and thiamine (Singh *et al*., 1997, Nielsen *et al*., 1997).

MATERIALS AND METHODS

Five domesticated cultivars of cowpea Gangothri, Pusakomal, Navarathna, Gowathmi and Gomchi were collected from National Seeds Corporation, in Moulali, Hyderabad, Telangana. The young disease free leaves and nodal portions are used as explants for the production of Shoots and Roots on the MS medium supplied with various hormones like, 2-4,D, NAA, Kinetin and BAP individually or in combination.

The excised plant part called explant are at first washed with liquid detergent (5% v/v ‘teepol’) then the explants are surface sterilized by 0.1% w/v mercuric chloride (Hgcl2) for a limited time (generally 10-15 minutes). The sterile explants are properly washed with sterile distilled water in the laminar air flow chamber before inoculation. The explants are incubated on the nutrient medium supplied with hormones and incubated under controlled physical condition. The suitable temperature for *in vitro* growth is usually 25 ± 2ºC. The cultures are and Roots.

RESULTS

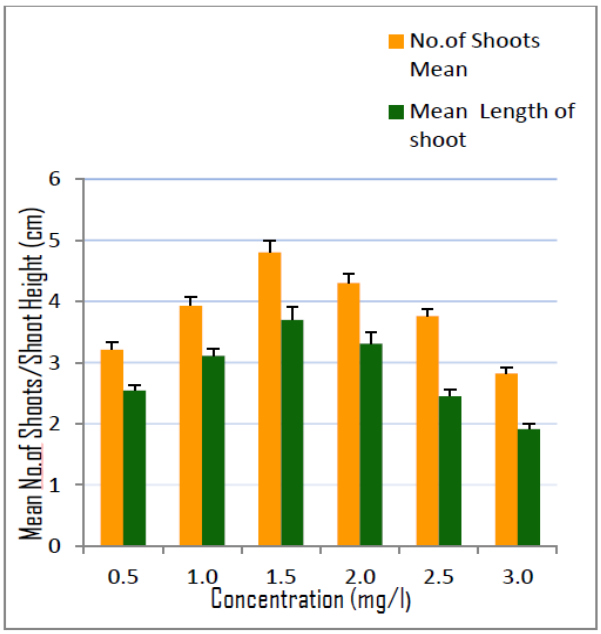
Shooting was initiated from nodal and shoot tip explants on MS medium containing BAP alone used as phytoharmone source (fig 32). When BAP alone as a phytohormone with

concentrations ranging from 0.5 to 3.0mg/l is used to induce callus from nodal explants in MS medium responded as multiple shoots without callus formation. The suitable concentration for the shoot formation was found to be BAP with 1.5mg/l (Table 1).

Table 1: Multiple Shoots form Nodal explants with BAP

Hormone conc.	No.of Shoots Mean	No.of Shoots ±SD	Mean Length of shoot	Length of shoot ±SD
0.5	3.21	0.12	2.54	0.09
1.0	3.93	0.15	3.11	0.12
1.5	**4.80**	**0.20**	**3.70**	**0.21**
2.0	4.30	0.15	3.31	0.18
2.5	3.76	0.11	2.45	0.11
3.0	2.82	0.09	1.91	0.08

Graph 1: Multiple shoots form Nodal explants with BAP



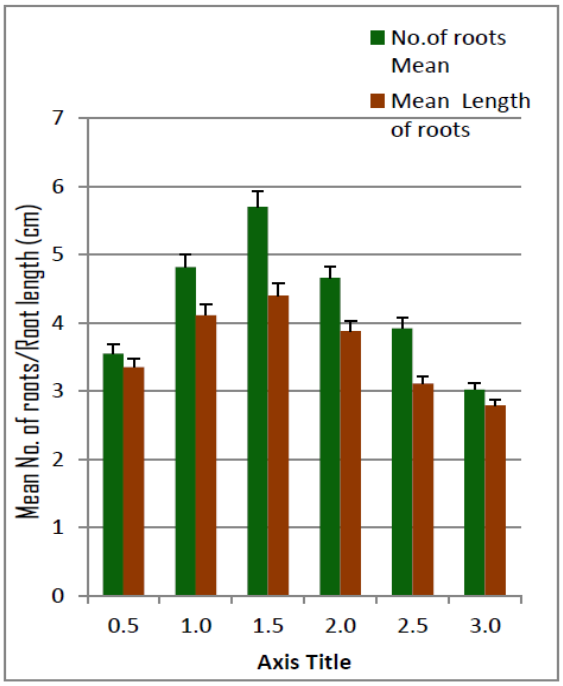
Shooting in the form of organogenesis was obtained from leaf explants in the presence of NAA alone as phytoharmone (fig-1).

When NAA is used as a source of phytohormone with concentration ranging from 0.5 to 3.0 mg/l MS medium, the leaf explants instead of forming callus produced roots as phenomena of direct organogenesis. The rhizogenesis was prominent with NAA+MS medium with a concentration of 3.0mg/l, with mean number of roots as (3.9 ±0.21) and mean length of roots as (3.20 ±0.18) (Table 2).

Table 2: Root formatiom from leaf explants with NAA

Hormone conc.	No.of roots Mean	No.of roots ±SD	Mean Length of roots	Length of roots ±SD
0.5	3.55	0.14	3.35	0.13
1.0	4.82	0.18	4.11	0.16
1.5	**5.70**	**0.23**	**4.40**	**0.18**
2.0	4.66	0.17	3.88	0.14
2.5	3.92	0.15	3.11	0.11
3.0	3.02	0.10	2.79	0.09

Graph 2 : Root formatiom from leaf explants with NAA



Rooting in the form of organogenesis was obtained from leaf explants in the presence of NAA alone as phytoharmone (fig-2).

DISCUSSIONS

In the present study shooting was initiated from nodal and shoot tip explants on MS medium containing BAP alone used as phytoharmone source. Rooting in the form of organogenesis was obtained from leaf explants in the presence of NAA alone as phytoharmone. The present finding is in concurrence with findings of Asim *et al*., 2009a, Diallo *et al*., 2008 and Mao *et al*., 2008, Kartha *et al*., 1981, Manoharmin *et al*., 2008, who have reported the importance of BAP, and auxins in organogenesis and multiple shoot formation. Different explants like primary leaves (Muthukumar *et al*., 1995 and Bao *et al*., 2006), stem (Mao *et*

al., 2006 and Bao *et al*., 2006), shoot tip (Sebastian 1983 and Aasim 2009), cotyledon (Brar *et al*., 1999, Chaudhary *et al*., 2007, Mao *et al*., 2006, and Pellegrineschi., 1997) and shoot meristem (Kartha *et al*., 1981 and Manoharrain 2008) have been employed for plant organogenesis in cowpea. To know the influence of culture medium, Diallo *et al*., (2008) employed two types of basal media (B5 and MS) to regenerate the cotyledonary node explants. They have reported that MS basal medium is efficient for shoot elongation and MS medium for multiple shoot formation. The most common cytokinins were 6-benzylaminopurine (BAP), Kinetin (KI) and Thidiazuron (TDZ). The effect of different concentrations of BAP 2.5-10µm on the regeneration of cotyledonary node was reported by Chaudhury *et al*., (2007).

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