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Full Length Article

Efficient plantlet regeneration from nodal explant culture of Blackgram (*Vigna mungo L.)* Hepper

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ABSTRACT

Nodal explants of Black gram (*Vigna mungo* L.) Hepper Var Vamban-1 was cultured on MS medium containing various concentrations of cytokinins BAP/Kn/TDZ (0.5-3.0 mg/L) alone and also in combination with auxins IAA (0.5 mg/L). Maximum number of shoot bud proliferation was observed at (0.5 mg/L) IAA + (2.0 mg/L).TDZ, compared to all other concentrations of Kn/BAP alone. As the concentration was increased above (2.0mg/L) the shoot bud induction was reduced gradually in both the cytokinins tested. The synergistic effect of both auxin and cytokinin combination was found to be effective in inducing maximum number of shoots. High frequency of shoots was induced at (0.5mg/L IAA + (2.5mg/L) Kn and (0.5mg/L) IAA+ (2.0mg/L) TDZ. The *in vitro* regenerated shoots produced more number of roots on MS medium containing (1.0mg/L) IBA. Thus the plant developed *in vitro* using nodal cultures were established in pots containing garden soil outside under shade in wound temperature and light conditions. These plants flowered after 8 weeks following transfer to pots. The protocol established can be used for rapid multiplication of the specific producing true to type plants.

Key Words: - Black gram, *In vitro* culture, Plant growth regulators, Plant Regeneration, and *Vigna mungo*

INTRODUCTION

Legumes are one of the most important and first cultivated crop plants. They are also useful as animal feed, extracting vegetable oils, for improving the soil nitrogen content etc and their benefits are recognized around the world. Legumes serve as a model plant in plant biotechnological studies providing useful information in crop improvement. The biochemistry of legumes is distinct from that of other plant groups and has many unique molecules. Legume seeds are generally characterized by relatively large content of protein 17% to 40%, an even greater concentration of carbohydrates and small amount of oil (Bressaniand Elias, 1980). Black gram *(Vignamungo L.* Hepper) is an indispensable conventional crop the world over. It is a short duration crop (70-110).

A legume are adapted to tropical and sub tropical condition, require low inputs, yields highly, and serves as a brilliant source of protein as seed or sprout. The area under this crop continues to amplify for the reason that it can be cultivated in fallow lands or as an alternation crop after rice and groundnut in normal soils (Sreenivasulu *et al.,* 2000).

Legumes in general are recalcitrant to tissue culture and are highly genotype specific (Sujatha *et al.,* 2007), so regeneration has been quite difficult among these plants. Forage legumes e.g., clover are more agreeable to in vitro plant regeneration than are seed legumes (Phillips and Collins, 1984).

Several legume species have been regenerated through in vitro culture, but most

cases regeneration is at low frequency (Flick *et al.,* 1983; Kysely *et al.,* 1987and Prakash *et al.,* 1994). The earlier reports focused on the cotyledonary node since the morphogenetic potentiality is confined to that region. Even though plants have been successfully transformed by using cotyledonary nodal explants, the major problem of chimerism still persists (Meurer *et al*., 1998). This is primarily due to pre-existing meristematic shoot buds, which continue to grow effectively on a medium containing a selectable agent like antibiotic or herbicide. Christou *et al.,* (1988) could effectively select transgenic calli after bombarding protoplasts but they failed to produce transgenic plants. Optimization of callus induction in *Lathyrus sativus* L (Swapan *et al.,* (2014), Induction of callus from cowpea (*V. unguiculata* (L.) Walp) through *in vitro* culture (Dadmal and Navhale (2012), Callus Induction and Plant Regeneration of *V. mungo* (L.) Hepper via half seed explants (Harisaranj *et al.,* 2010). Callus Induction and Organogenesis in Soybean (*Glycine max* (L.) Merr. Cv. Pyrami) from Mature Cotyledons and Embryos.(Joyner *etal.,*2010). Effect of thidiazuron (TDZ) on *in vitro* regeneration of blackgram (*Vigna mungo* L.) embryonic axes (Sumita *et al.,* 2012)

The present investigation describes a micropropagation technique using nodal explants culture as the source of direct production of multiple shoot in Black gram (*Vigna mungo* L.) HepperVar Vamban-1

MATERIALS AND METHODS

Nodal segments (1.0 cm- 2.5 cm) of Black gram (*Vigna mungo* L.) HepperVar Vamban-1 bearing and axillary buds were collected from healthy, young branches of one year old plant growing in the research field Department of Botany Kakatiya University Dist Warangal (T.G.). The explants were washed under running tap water and treating with 5% teepol for 5 minutes. There were washed thoroughly under running tap water and then surface sterilized with 0.1% w/v Mercuric chloride ($HgCl_2$) for 4-5 minutes and later rinsed at least thrice with sterile distilled water. Sterilized nodal segments were dried on sterile filter paper before inoculation

Culture media and culture conditions:

The explants were inoculated on MS medium containing (30gm/L) Sucrose fortified with different concentrations of Cytokinin BAP/KN/TDZ and in combination with IAA (Table 1,2 and 3) and solidified with 0.8% agar (Difcobacto) All media were adjusted to pH 5.8 before addition of 0.8% agar and autoclaved at 121^0 C under 15 psi for 15-20 minuts.

Culture tubes were maintained at $25+2^0$ C with 16 hrs photo period under white fluorescent light (40-50) for subculture of differentiating explants MS+ BAP/KN/TDZ and MS+IAA+BAP /KN/TDZ media was used. The proliferated axillary shoots were transferred to rooting medium after 6 weeks of culture.

RESULTS AND DISCUSSION

The results of the axillary /Nodal bud cultures on the development of multiple shoots and roots are shown in Table (1-3).The Nodal buds of Black gram (*Vigna mungo* L.) cultured on different hormonal combinations showed varied results. The axilary buds became active with in weak after inoculation and new shoots became distinct by the seconds and third weak with leaves and internodes. The explants survival from nodal segments of nature plant of Black gram (*Vigna mungo* L.) varied with season. According to the present observations, the explants were collected from field grown plants thought out the year to determine the ideal season for culture established. Explants collected in August to October period showed less time for sprouting and quick shoot bud proliferation.

Effect of BAP

The results on nodal bud culture of Black gram (*V. mungo* L.) on MS medium + BAP (0.5-3.0mg/L) alone are presented in (Table-1) and shown in (Fig-A). The medium containing (2.0 mg/L) BAP induced maximum number of shoots (3.0± 0.29 cm) and also showed high percentage (80%) of responding cultures. As the concentration of BAP was increased up to (2.0 mg/L) gradually the shoot bud proliferation was found to be decreased and when BAP concentration was increased above (2.0 mg/L) the rate of shoot multiplication was reduced.

Effect of Kn

The result on nodal bud culture of Black gram (*V. mungo* L.) on MS medium + Kn (0.5 – 3.0 mg/L) was observed. High percentage (70) of responding cultures was found at (2.0 mg/L Kn compared to all other concentrations tested. Whereas more number of shoots were regenerated from Nodal explants at (2.0 mg/L) Kn (2.5±0.36 shoots/explant) followed by (1.8 mg/L) Kn at

(0.5, 1.0 and 1.5 mg/L) Kn produced (1.8 ± 0.02), (2.0 ± 0.37) and (2.2 ± 0.24) shoots/explant. With 50, 60 and 65 cultures response was recorded (Table-1).

Effect of TDZ

The result on nodal explants culture of Black gram (*V. mungo* L.) on MS medium supplemented with TDZ (0.5–3.0 mg/L) was observed. High percentage (60) of responding cultures were found at (2.0 mg/L) TDZ compared to all other concentrations tested. Whereas more number of shoots were regenerated from nodal explants at (2.0 mg/L) TDZ (4.2± 032 shoots/explant) followed by (2.5 mg/L) TDZ. At (0.5, 1.0 and 1.5 mg/L) TDZ (3.2 ± 0.22), (4.0 ± 0.32) and 4.2 ± 0.32) shoots/explant. With 48, 50 and 58 percentage of cultures response was recorded (Table-1, Fig -B).

Effect of IAA + BAP

Influence of Auxin - cytokinin combination such as IAA (0.5 mg/L) + BAP (0.5- 3.0 mg/L) in Nodal explants showed variable response (Table-2). Auxin (0.5 mg/L) IAA was taken in combination with cytokinin BAP (2.0 mg/L) showed maximum percentage (82%) responding cultures and high frequency of shoot induction (6.2 ± 0.23 shoots /explant) (Plate XIX-Fig-a) followed by (2.5 mg/L BAP + 0.5 mg/L IAA). At (0.5 mg/L) IAA + (3.0 mg/L) BAP (4.5 ± 0.36) shoots /explant with 60% cultures response was recorded (Table -17).

Effect of IAA + Kn

Similarly Nodal explants were cultured on MS medium supplemented with IAA (0.5 mg/L) in combination with various concentrations of BAP (0.5 - 3.0 mg/L) (Table -17). The results showed the direct shoot regeneration in all the concentrations and combinations tested. Nodal explants cultured on (0.5 mg/L) IAA in combination with (2.0 mg/L) Kn showed highest number of shoots (6.8 ± 0.32) with 72 percentage of responding cultures. As the concentration of Kn was increased up to (0.5 mg/L) gradually the shoot bud proliferation was also found to be increased and when BAP concentration was increased above 2.0 mg/L the rate of shoot multiplication and elongation was reduced (Table-2, Fig-D).

Table – 1 Direct shoots Proliferation from Nodal explants of Black gram (*Vignomungo* L.) HepperVar Vamban-1 on MS medium supplemented with various concentrations of BAP, Kn and TDZ

Hormone concentration (mg/L)	% of cultures response	Mean number of shoots /explants ±(S.E.)*
BAP		
0.5	60	2.3 ± 0.17
1.0	70	2.4 ± 0.34
1.5	75	2.5 ± 0.31
2.0	80	3.0 ± 0.29
2.5	72	1.5 ± 0.32
3.0	58	1.3 ± 0.23
Kn		
0.5	50	1.8 ± 0.02
1.0	60	2.0 ± 0.37
1.5	65	2.2 ± 0.24
2.0	70	2.5 ± 0.36
2.5	68	1.8 ± 0.34
3.0	52	1.6 ± 0.32
TDZ		
0.5	48	3.2 ± 0.32
1.0	50	4.0 ± 0.23
1.5	58	4.6 ± 0.32
2.0	60	5.0 ± 0.32
2.5	52	3.2 ± 0.36
3.0	40	3.0 ± 0.34

***SE Standard Error**

Table – 2 Direct shoots Proliferation from Nodal explants of Black gram (*Vignomungo* L.) HepperVar Vamban-1 on MS medium supplemented with IAA in combination with BAP, Kn and TDZ

Hormone concentration (mg/L)	% of cultures response	Mean number of shoots /explants ±(S.E.)*
IAA+BAP		
0.5+0.5	65	5.0 ± 0.32
0.5+1.0	72	5.5 ± 0.34
0.5+1.5	76	5.6 ± 0.32
0.5+2.0	82	6.2 ± 0.23
0.5+2.5	70	4.8 ± 0.32
0.5+3.0	60	4.5 ± 0.36
IAA+Kn		
0.5+0.5	58	5.0 ± 0.36
0.5+1.0	62	5.2 ± 0.32
0.5+1.5	68	5.4 ± 0.32
0.5+2.0	72	5.8 ± 0.32
0.5+2.5	70	7.0 ± 0.36
0.5+3.0	60	4.8 ± 0.32
IAA+TDZ		
0.5+0.5	50	5.4 ± 0.34
0.5+1.0	56	5.6 ± 0.32
0.5+1.5	60	5.8 ± 0.32
0.5+2.0	62	7.4 ± 0.36
0.5+2.5	56	5.6 ± 0.32
0.5+3.0	50	5.4 ± 0.36

***SE Standard Error**

Table -3: Rooting ability of regenerated shoots from Nodal explants culture of Black gram (*V. mungo* L.) Hepper Var Vamban-1cultured on MS medium supplemented with IAA and IBA.

Growth Hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IAA	IBA		
00	00	23	1.0 ± 0.12
0.5	-	60	5.3 ± 0.37
1.0	-	70	7.2 ± 0.38
2.0	-	73	5.6 ± 0.38
-	0.5	54	4.3 ± 0.36
-	1.0	73	8.3 ± 0.87
-	2.0	70	6.3 ± 0.36

*** Mean ± Standard Error**

Effect of IAA + TDZ

Nodal explants responded to all the concentrations of TDZ (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) in combination of auxin IAA (0.5mg/L) of tried. MS medium fortified with (2.0 mg/L) TDZ supported better results as compared to other concentrations of BAP and Kn in terms of period required for bud break, per cent bud break, number of shoots regenerated and shoot length in Nodal explants showed variable response. Auxin (0.5 mg/L) IAA was taken in combination with cytokinin TDZ (2.0 mg/L) showed maximum percentage (62%) responding cultures and high frequency of shoot induction (7.0 ± 0.36 shoots /explant) (Plate XIX-Fig-c) followed by (2.5 and 3.0 mg/L) TDZ + (0.5 mg/L) IAA. At (0.5 mg/L) IAA in combination with TDZ (0.5, 1.0 and 1.5 mg/L) produce (5.4 ± 0.34), (5.6 ± 0.32) and (5.8 ± 0.32) shoots/explant. With 50, 56 and 60 percentage of cultures response was recorded (Table- 2, Fig-C).

***In vitro* rooting**

Fully elongated healthy shoots were transferred on MS medium fortified with different concentration of IAA (0.5 – 2.0 mg/L) and IBA (0.5 – 2.0 mg/L).Profuse rhizogenesis was observed on 1.5 mg/L IAA, compared to 0.5 -2.0 mg/L) IAA/ IBA on MS medium containing 1.5 mg/L IBA whereas 96% of plants produced roots with (14.3 ± 0.27 roots/ explants) (Table -3), (Fig-E).

Acclimatization

Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to polypots containing pre- soaked vermiculite and maintained inside a growth chamber set at 28 ^{o}C and 70 – 80 % relative humidity. After three weeks they were transplanted to poly bags containing mixture of soil + sand + manure in 1: 1: 1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland'ssoulation every 3 days for a period of 3 weeks (Fig-F).

We were successful in shoots regenerating plants from nodal bud cultures on MS medium fortified with different concentrations of cytokinining i.e. BAP, Kn and TDZ individually and also in combination with (0.5 mg/L) IAA + BAP/Kn/TDZ (0.5 – 3.0 mg/L). Maximum numbers of shoots were induced at (0.5 mg/L) IAA + (2.0 mg/L) TDZ in comparison to (0.5 mg/L) IAA +BAP/Kn as a role growth regulators. However the shoot bud proliferation was found be more on (2.0 mg/L) TDZ in combination with compared to (0.5 mg/L) IAA + BAP (2.0mg/L). BAP might have triggered the action of TDZ in proper way for inducing more number of plant let regeneration among all hormonal combinations and concentrations used.

The effectiveness of BAP on the induction of bud break and shoot proliferation has been reported in *Rotula aquatic* (Sebastian *etal.,* 2002), BAP found to enhance the regeneration frequency as reported by Gulati and Jaiwal (1930) and Chandra, and Pal (1995). Nodal explants were also used to get higher rates of shoot multiplicationof several plants (Shekawat and Galston1983). Immature cotyledonary node explants produced a high frequency of plant regeneration in several species (Tivarekar, and Eapen 2001). Multiple shoot formation from cotyledonary node was obtained on MS medium supplemented with 4.44µM BA, (Sujatha *etal.,* 2007).In the present investigation multiple shoot induction was observed in the presence of BA (1.0mg/L). In black gram there was no results were observed the culture supplemented with IAA and BAP (Murgananthame*t.al.*2005) in our study results were observed

Regeneration of shoots in response to Kn has been observed in *Kaempferia galangal* (Chirangini *etal.,* 2005) and *Phyllanthus niruri* (Karthikeyan *etal.,* 2007). Shoot regeneration was noticed in nodal segments inoculated on the medium supplemented with various concentrations of BAP/Kn/TDZ. A combined effect of BAP + IAA and Kn + IAA and TDZ + IAA was also studied on the nodal explants. The per cent bud break increased with increase in the concentration of BAP/Kn/TDZ from (0.5-2.0 mg/L). Among the various media fortified with different concentrations of TDZ and Kn, (2.0 mg/L) TDZ+ (0.5 mg/L) IAA was proved better in terms of per cent bud break and number of shoots differentiated per explants. The explants cultured on MS medium fortified with TDZ + IAA gave better results as compared to Kn + IAA and BAP+ IAA. Among the six concentrations of TDZ (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) + IAA (0.5 mg/L) applied, most favorable results were obtained on the MS medium supplemented with (2.0 mg/L) TDZ + (0.5 mg/L) IAA. Similar observations have been made in *Albizzia lebbeck* (Vargeese and Kaur, 1988), *Tagecte serecta* (Vanegas *etal*., 2002), *Spilanthe acmella* (Saritha *etal*., 2003). High cytokinins to auxins ratio has been shown to promote shoot formation in *Rhodiola rosea* (Kirichanko *etal.,* 1993). The development of axillary shoots from nodal explants was accompanied by basal callusing of the explants. However this remained undifferentiated. Same type of observation have been made by Singh and Lal (2007) and Nandwani and Ramawat (1991) working with *Prosopis juliflora and Leucaenal eucocephala* respectively.

During the present investigations multiple shoots were induced on MS Medium supplemented with various concentrations of cytokinins such as BAP, Kn and TDZ alone and also in combination with auxins IAA except at the concentration of (0.5 mg/L) BAP. Similarly Sudharshan *etal*., (2000) have observed the multiple shoot bud induction from nodal segments of *Ziziphus mauritiana*on MS medium supplemented with BAP alone.

Fig. a) Direct multiple shoots developed on MS + 2.0 mg/L BAP from nodal explant culture.

Fig. b) Direct multiple shoots developed on MS +2.0 mg/L TDZ from Nodal explant culture.

Fig. c) Proliferation of multiple shoots developed on MS + 0.5 mg/L IAA + 2.0 mg/L TDZ. From Nodal explant culture.

Fig. d) Formation of Elongated shoots on MS+ 0.5 mg/L IAA + 2.0 mg/L TDZ. From Nodal explant culture after six weeks

Fig e) Rooting of individual micro shoots on MS+IBA (3.0mg/L)

Fig. f) hardening of plantlet

It was also recorded the same results in *Vanilla planifolia* on MS + BAP alone (Geetha and Shetty, 2000). When BAP and Kn concentration was increased (above 2.0 mg/L) the rate of shoot multiplication and elongation was reduced in the present investigation. Similar results were obtained in *Canavali anirosa* (Kathiravan and Ignacimuthu, 1999); *Vigna radiate* (Gulati and Jaiswal, 1994) and *Pisonia alba* (Jagadishchandra *et al*., 1999). Shoot tip and nodal were found to be the best explants for multiple shoot formation.

Direct regeneration of multiple shoots form nodal explants as observed on IBA + BAP / Kn supports the finding of Shahzad *et al*., (2000) on *Ocimum sanctum*, Gulati and Jaiwal (1992) on *Vigna radiat a*and Varisaimohamed *etal*., (1998) on *Macrotylom auniforum*, Bais *et al*., (2000) have also observed the maximum number of shoots on MS medium supplemented with auxin + cytokinin combination in nodal culture of *Decalepish amiltonii*as it was found in *Mentha arvensis* (L.) The same synergistic effect was also recorded in *Plumbago indica* inducing maximum number of (17) of shoots / nodal explants on MS + IAA (0.1 mg/L) + BAP (3.0 mg/L). Gill *et al*., (1996) have also obtained multiple shoots of *Azadirachta indica* on MS + IBA + BAP. Similarly, it was reported in *Morus indica* (Jagadishchandra and Suryanarayana, 1997). Similarly this stimulatory effect of a single supplement of cytokinin was reported earlier in other medicinal species including *Curcuma* spp and *Zingiber officinale* (Balachandran *etal*.. 1990) *Chlorophytum borinilianum* (Pattnaik and Chand 1996; Sahoo *et al*., 1997) and *Tridax procumbens* (Sahoo and Chand, 1998).

Thus, direct multiple shoot production was observed from nodal segments of the species studied. This type of clonal propagation has advantage, by producing true to type plants from a single individual in a relatively short time.

Our result on Micro propagation using meristem / Nodal bud culture shows the considerable importance for large – scale propagation of Black gram (*Vignomungo* L.) an import ant commercial and medicinally plant.

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