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EFFICIENT MULTIPLE PLANTLET REGENERATION VIA MICROPROPAGATION IN (*VIGNA RADIATA* L.) WILCZEK.

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ABSTRACT:
Efficient protocol was established for multiple plantlet regeneration from cotyledonary nodal segments of cultivar K851 through micropropagation. Multiple shoots were obtained from cotyledonary nodal segment of 5 days old plantlet of *Vigna radiata* (L.) Wilczek. The decisive role of Naphthaleneacetic acid (NAA) and Benzyl adenine (BA) was reputable on shoot organogenesis. The maximum shoot regeneration was observed in MS medium (Murashige and skoog 1962) fortified with 3.00mg/l of BA. Proliferated shoots were sub cultured under the same concentration. Additional multiple shoots were obtained. Rooting of the *in vitro* propagated shoot was achieved on half strength MS medium supplemented with 3.00mg/l Indole-3-butric acid (IBA). Regenerated plantlets were acclimatized and successfully transplanted to soil with 80%survival.

Key words: (Vigna radiata *L.) Wilczek.*, in-vitro *culture*, *mature explant, Nodal explant, Rooting, Mungbean.*

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 [10]. 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 [3]. Green gram *(Vigna radiata L. Wilczek*) is an indispensable conventional crop the world over. It is a short duration crop (70-110). Legumes are adopted to tropical and sub tropical condition, requires 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 [20]. A number of biotic and abiotic stresses severely affect the full yield potential of green gram [1] In addition to Abiotic limitations associated with drought and heat stress and soil adaptation, mungbean has anumber of specific productivity constraints, caused by foliar diseases and insect pests on-form and postharvest [12]. Therefore, there is need to increase the productivity and also enhance nutritional value of these pulse crops. Green gram has narrow genetic base, therefore to increase its genetic base and incorporate desirable characters, extensive genetic transformation studies are necessary. Before transgenesis, in vitro regeneration of plant is a prerequisite. It is also useful tool for the selection and development of new cultivars. *In vitro* legume cultures were first established in the 1950's by [14]. Legumes in general are recalcitrant to tissue culture and are highly genotype specific [21], so regeneration has been quite difficult among this plants. Forage legumes e.g., clover are more

agreeable to in vitro plant regeneration than are seed legumes [15]. Several legume species have been regenerated through in vitro culture, but most cases regeneration is at low frequency [5,11,16]. Hence, we have developed a practical method for the micropropagation through multiple shoot proliferation from auxiliary buds of 5 days old seedlings. The aim of the present study is development of micropropagation techniques as a means to produce healthy and superior stock plants.

MATERIALS AND METHODS:

Explant preparation:

Freshly collected seeds (C.V-K851) were excised and washed thoroughly in running water and the seeds were treated with teepol (detergent). The seeds were initially disinfected by rinsing it in 80% ethanol for 15 seconds followed by surface sterilization in an aqueous solution of 0.1 % (w/v) $HgCl_2$ for 2-3 minutes. Seeds were again rinsed 3 times with distilled water, and the seeds were inoculated in to sterilized water agar medium. The inoculated seeds were then incubated at 25±2*C under a light intensity of 3000 lux and under 16 hr light and 8 hr dark conditions. The germinated seedlings were used as the initial explant for shoot and root formation.

Shoot formation:

Nodal explants were taken from the germinated 5 days old seedlings and implanted in MS medium supplemented with various concentrations of NAA and BA in combination to find out the suitable hormone concentration for multiple shoot proliferation (Table-1). Cultures were exposed under the conditions mentioned above. Multiple shoot initiation was observed on the 14^{th} day. The obtained multiple shoots were then subcultured in to fresh media every 5^{th} week for exponential shoot production.

Root formation

For the regeneration of whole plant the obtained shoots were subcultured in to rooting media for root induction. Regenerated shoots were excised (2cm length) and inoculated. MS Medium supplemented with IAA or IBA at various concentration (0.5 mg/l, 1.00 mg/l, 2.00 mg/l, 3.00 mg/l, 4.00 mg/l, and 5.00 mg/l) separately. After the root formation the plantlets were gently removed and washed thoroughly to remove trace of agar attached to root and planted in to plastic cups (5cm-dm) filled with mixture of sterilized garden soil and coco peat (3:1). The potted plants were kept in the green house to avoid the desiccation. and after two weeks of acclimatization they were transferred to the botanical garden. The results were statistically analyzed using ANOVA to determine whether the given treatments were significant.

RESULTS AND DISCUSSION

Cotyledonary nodal explants of *Vigna radiata* (L.) Wilczek. were cultured on MS medium supplemented with auxins namely α – Naphthaleneacetic acid (NAA) and Cytokinine – Benzyl adenine (BA) in varying concentration and combinations. Maximum shoots were formed from the cotyledonary nodal explants when the medium was added with 3 mg/l of BA (table 1; Plate1a,b,c & d) In recent years, shoot tip and nodal explants have been preferred to produce large number of genetically identical clones [2]. It was reported that BA was proved to be an ideal hormone for shoot multiplication of shoot tip culture in grain legumes [19]. BA found to enhance the regeneration frequency as reported by [7,4]. Nodal explants were also used to get higher rates of shoot multiplication of several plants [18]. Immature cotyledonary node explants produced a high frequency of plant regeneration in several species [22]. Multiple shoot formation from cotyledonary node was obtained on MS medium supplemented with 4.44μM BA, [21]. In the present investication 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 [13] in our study results were observed.

The *in vitro* developed from nodal cultures were harvested and transplanted in to half strength MS medium containing various concentration of IAA and IBA (Table 2). Among the growth regulators used, IBA at the concentrations of 3 mg/l showed maximum number of roots of about. 1 mg/l of IBA showed the effective results in *Cajanus cajan* L. [8]. Similar results were well documented in *Cicer arietinum* L. [17]. The slow movement and slow degradation of IBA facilitates its location near the site of application and, thus, it functions better in inducing roots. The root developed from the cut end of the shoot without callus proliferation after 7-8 weeks (Plate f and g). The rooted plantlets were subjected to hardening process. Hardening is a

crucial step prior to transplantation of plants to the soil. The *in vitro* plantlet need 100% relative humidity and they also depend on the medium for the supply of sugar and other nutrients. After rooting the *in vitro* regenerated plants were taken out carefully from the culture medium and the adhered agar was removed to avoid contamination. Plantlets were then washed in sterile distilled water and transferred to small plastic cups. For transferring plant from the *in vitro* to *ex vitro* environment during the weaning process. The plant were kept under the plastic cups containing garden soil and coco peat (3:1) mixture of about 15 days so as to maintain 100% relative humidity (Plate k). But after 2 weeks the humidity was lower down by making perforation in plastic cups. During this period, the plants developed an efficient root system, build up new leaves and photo synthetically active and enabling them to develop typical terrestrial water control. The regenerated plants were transferred to soil with 80 % survival. The data were statistically analyzed. The analysis of variance and mean separation were carried out using Duncan's Multiple Range Test (DMRT). And the significance was assessed at 5% level.

a

b

c

d

e

f

g

h

i

j

k

Fig 1 Micropropagation of *Vigna radiata*, (a-d) - growth of cotyledonary node in shooting medium, e – subculture, f & h – rooted plantlet, i – root generation, j – multiple shoot formation, k – hardening.

Table 1: Effects of NAAand BAP on the shoot formation from cotyledonary nodal explants of. *Vigna radiata* (L.) Wilczek on MS medium. Values are mean ± SE of 7 replicates in each in each treatment.

Growth regulators (mg/l) BA	Mean number of shoots/ explants	Morphogenetic response (Shoots)
0.5	4.142 ± 2.410	+
1.0	5.285 ± 1.496	+
2.0	6.428 ± 1.718	+
3.0	7.285 ± 1.496	+
4.0	5.571 ± 1.618	+
5.0	3.142 ±1.676	+
NAA+BA		
0.5+ 0.5	3.571 ± 0.975	+
0.5+ 1.0	3.857 ± 0.899	+
0.5+ 2.0	5.142 ± 1.345	+
0.5+ 3.0	3.142 ± 1.345	+
0.5+ 4.0	3.714 ± 1.112	+
0.5+ 5.0	4.571 ± 0.975	+
1.00+ 0.5	3.857 ± 0.899	+
1.00+ 1.0	4.428 ± 1.272	+
1,00+ 2.0	2.142 ± 1.069	+
1.00+ 3.0	4.285 ± 1.496	+
1.00+ 4.0	4.285 ± 1.799	+
1.00+ 5.0	3.285 ± 1.605	+

Table 2: Effects of growth regulators on root induction of *In vitro* developed shoots of *Vigna radiata* (L.) Wilczek On half strength MS medium. Observations were made after 7 weeks. Values are mean ± SE of 7 replicates in each in each treatment.

Growth regulators (mg/l)		Mean number of roots/ explants	Morphogenetic response (Roots)
IBA	0.5	2.142 ± 0.690	+
	1.0	3.142 ± 0.899	+
	2.0	3.714 ± 0.755	+
	3.0	4.714 ± 0.755	+
	4.0	3.428 ± 0.975	+
	5.0	2.142 ± 1.345	+
IAA	0.5	0.571 ± 0.786	+
	1.0	0.714 ± 0.487	+
	2.0	1.428 ± 1.133	+
	3.0	1.571 ± 0.975	+
	4.0	1.285 ± 0.755	+
	5.0	0.857 ± 0.690	+

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