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EFFICIENT PLANT REGENERATION FROM COTYLEDONARY NODE OF BLACKGRAM (*Vigna mungo (L.) HEPPER*)

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ABSTRACT

A simple and rapid regeneration protocol was developed from cotyledonary node of blackgram (*Vigna mungo* (L.) Hepper). Murashige and Skoog's (MS) medium supplemented with BAP at 4mg l^{-1} was more effective in producing shoots. The culture expressed maximum plant regeneration potential with four shoots per cotyledon on regeneration. Green shoots thus developed were successfully rooted within 20 days on MS media containing IBA 1mg l^{-1}. Over 86 % of rooted plants grew well and produced seeds normally when transferred to green house.

Keywords: Black gram, *in vitro* culture, plant growth regulator

INTRODUCTION

Legumes are mostly grown in nutrient poor soils, under rain fed conditions and suffer from a host of biotic constraints. This lead to the low productivity of legumes and improving the varietal performance against these constraints is need of the day. Black gram (*Vigna mungo* (L.) Hepper) is a widely cultivated pulse crop mainly for its protein rich content. Although India is the main producer of black gram, but production is limited due to various biotic and abiotic stresses. The high susceptibility of the crop to yellow mosaic virus (YMV), fungal pathogens, insects and drought result in significant yield losses [1,2]. Attempts to enhance genetic tolerance to these constraints through traditional breeding revealed limited success, due to availability of low genetic tolerance. The potentials of biotechnological tools can be explored by supplementing the breeding programes through insertion of genes of interest into elite genotypes of blackgram. For genetic transformation, however, a robust and high frequency regeneration system is needed. Legumes in general are recalcitrant to tissue culture and are highly genotype specific [3]. In this report, we present a simple and efficient protocol for rapid *in vitro* plant regeneration from cotyledons of black gram.

MATERIALS AND METHODS

2.1. Seed Disinfection

Surface sterilization of the seeds was done by rinsing them in 70% ethanol for 1 min, followed by 0.1% Mercuric chloride for 5 min. The seeds were then rinsed in sterile distilled water 3-4 times and soaked in sterile water for overnight. The imbibed seeds were decoated and two cotyledons were carefully separated [4].

2.2. Shoot Induction

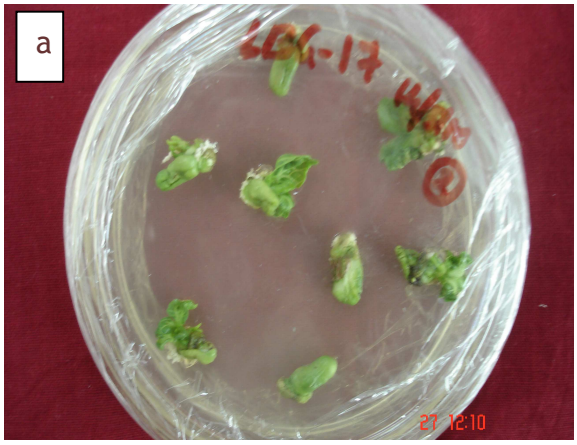
The embryo was excised. The cotyledons were placed in contact with the shoot induction medium. The medium used was MS containing, 3%sucrose, 0.8% agar and combination of different plant growth regulators i.e. Benzyl amino purine (BAP), NAA and IAA. The plant growth regulator combinations tested were BAP (1, 2, 3, 4 mg/l) alone and in combination with 0.5 mg/l IAA or with 1.0 mg/l NAA. Observations on regeneration frequencies, number of shoots per explant were recorded. Elongated shoots were rooted on MS+IBA(Indole-3-butyric acid) 1mg/l +3%Sucrose+0.8%Agar with ten different concentration of IBA (0.1 mg/l to 1.0 mg/l). Plantlets transferred to plastic buckets containing farm soil were irrigated with water and/or half strength Hoagland solution alternatively [5].

RESULTS

The type and concentration of plant growth regulators strongly influenced the organogenic potential of the cotyledonary node explant of black gram. In response to different combinations of growth hormones cotyledonary node explant responded for shoot initiation was ranging from 70-90 %.(Table.1a).The frequency of shoot initiation seemed to depend more on concentration of BAP [6]. The shooting frequency was ranged from 2.34% to 87.5%, when BAP was used alone or in combination with NAA or IAA. Maximum shoot regeneration frequency (87.5%) was observed on 3.0 mg/l BAP medium with 4.2 shoots/explant without any exogenous addition of other growth regulators (Fig-1) Though BAP at 4.0 mg/l with 0.5 mg IAA/L responded similar results, but regeneration frequencies were low. Inclusion of NAA and IAA in shoot induction medium found not useful in improving either shooting frequency or (Table.1b) number of shoots/explants [3]. Benzyl adenine is the most widely used and effective cytokinin for various legumes including Vigna species [7].

Green healthy shoots regenerated within 24 days were transferred on to rooting media of MS medium with ten concentrations of IBA (0.1 mg/l – 1.0 mg/l).High concentration IBA (1.0mg/l) observed highest rooting frequency of 95% in 20 days compared to all other concentrations (Table.2; Fig.1). Similar results have been described for vigna mungo, [8] where elongated shoots obtained from callus were rooted on B5 medium with 14.7 mM IBA. The IBA was an efficient auxin to produce the shoots [9].

After 20 days, healthy plant lets with good root systems, were transferred to sterile soil and maintained in controlled conditions in the growth room itself. After 10 days the plants were transferred to green house. In conclusion, using plant growth regulators ,the efficient shoot and root initiation from cotyledonary node of *vigna mungo* [10] has been standardized. The cotyledon could serve as an ideal explant material for developing an efficient blackgram transformation system.



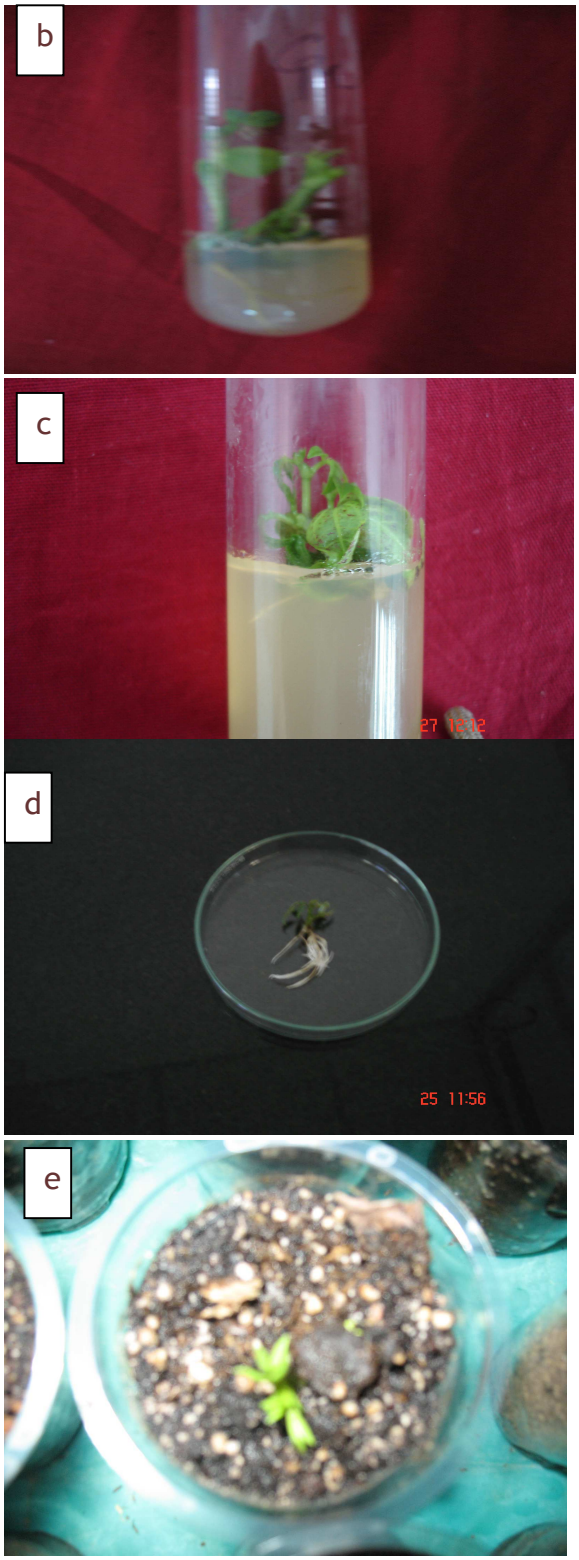


Fig1. Regeneration of black gram
a) Cotyledonary response b) Shooting c) Rooting d) rooted plant e) hardening

[Table-1a].

Growth regulators (mg/l)	Responding Explant
1.0 BAP	70±5.77
2.0 BAP	70±5.77
3.0 BAP	90±6.77
4.0 BAP	80±6.77
1.0 BAP+0.5 IAA	60±8.82
2.0 BAP+0.5 IAA	70±3.33
3.0 BAP+0.5 IAA	90±3.33
4.0 BAP+0.5 IAA	70±3.33
1.0 BAP+1.0 NAA	70±11.55
2.0 BAP+1.0 NAA	70±8.82
3.0 BAP+1.0 NAA	80±6.67
4.0 BAP+1.0 NAA	80±5.77

[Table - 1a] : Cotyledonary node explant response to growth hormones for shoot initiation

[Table-1b].

Growth regulators (mg/l)	Regeneration frequency	Number of shoots per explant
1.0 BAP	23.44±5.77	1.3±0.67
2.0 BAP	53.3±3.33	1.3±0.33
3.0 BAP	87.5±3.33	4.2±0.33
4.0 BAP	76.7±3.33	2.7±0.33
1.0 BAP+0.5 IAA	23.44±5.77	NS
2.0 BAP+0.5 IAA	33.44±5.77	1.3±0.67
3.0 BAP+0.5 IAA	66.3± 5.77	1.3±0.67
4.0 BAP+0.5 IAA	33.44±5.77	2.3±0.67
1.0BAP+1.0 NAA	NR	NR
2.0BAP+1.0 NAA	NR	NR
3.0BAP+1.0 NAA	NR	NR
4.0BAP+1.0 NAA	NR	NR

[Table - 1b] : Cotyledonary node explant shooting frequency or number of shoots/explants

[Table-2].

Growth regulator IBA mg/l	Rooting frequency	Days of root
0.1	-	-
0.2	-	-
0.3	-	-
0.4	-	-
0.5	40	17
0.6	50	17
0.7	70	19
0.8	90	21
0.9	NR	-
1.0	95	20

[Table - 2] : Root formation after 20 days in an medium with varying concentration IBA.

DISCUSSION

From this we can come across rapid regeneration from cotyledonary node other then any explants [11]. Cotyledonary node has highly meristematic tissue [12] and has responded short time than other explants. Among various concentrations, benzyl amino acid with 3mg/l has show best response. Within 25 days then plants are transported to rooting which contain 1mg/l Indole Butyric Acid. Then greeny healthy plant were kept under the plastic cups containing garden soil and soilrite (3:1) mixture of about 15 days so as to maintain 100% relative humidity.

In recent years, nodal explants have been preferred to produce large number of genetically identical clones [13, 14]. Then these plants showed 92 % survival rate, equal growth and good yield. These were disease resistant, stress tolerant and grown in drought conditions.

CONCLUSION

In this present report the efficient and rapid regeneration protocol has best regeneration with explant cotyledonary node of blackgram (*vigna mungo*) by the growth hormone benzyl amino purine .This can be helpful with agrobacterium tumeficienes for development of transgenics.

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