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RESEARCH ARTICLE

Efficient plant regeneration of Green gram (*Vigna radiata* (L.) Wilczek) via cotyledonary explant

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Abstract

The seeds of mung bean variety Vamban$_2$ were treated with different dose/conc. of physical (Gamma rays) and chemical mutagens (Ethyl methane sulphonate) to induce mutagenesis. The phenotypic responses were studied in M_1 and M_2 generations and spectrum of chlorophyll mutation were worked out. There were four types of chlorophyll mutation was observed, i.e. albina, xantha, chlorina and viridis. While analyzing the result, it was observed that the mutation frequency increased with increase in the dose/concentration of mutagen. In general, the chlorophyll mutant was higher in EMS than the gamma rays treated plants. Among the various dose/conc. of mutagens, EMS showed more frequency was observed in 50mM and gamma rays at 60kR had higher mutation frequency.

INTRODUCTION

Grain legumes are major source of protein and green gram (*Vigna radiata* (L.) Wilczek) is third mostly grown grain legumes contributes nearly 15% of total pulse production. Though the crop is most preferred for their earliness and demand 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 (Sahoo *et al*., 2002).

Attempts to enhance genetic tolerance through traditional breeding revealed limited success due to availability of low genetic tolerance (Jaiwal and Gulati, 1995). Transgenic development through the introduction of alien genes of recognized relevance into elite germplasm of greengram in order to enhance the tolerance of these stresses is need of the day. Legumes in general are recalcitrant to tissue culture and are highly genotype specific (Somers *et al*., 2003) The direct *in vitro* organogenesis from explants had been reported to be a rapid multiplication method for true to elite strain and is preferred for developing transgenic plants to avoid somaclonal variations. In this report, we present a simple and efficient protocol for rapid *in vitro* plant regeneration from cotyledon of green gram.

MATERIALS AND METHODS

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. 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 regulator i.e BAP and IAA. Besides BAP, other growth hormones (Murashige, T. and Skoog, F., 1962) also evaluated including Kn, NAA. The plant growth regulator combinations tested were BAP (0.5, 1.0, 2.0, 3.0, 4.0 mg/l) alone and in combination with 0.5 mg/L IAA .Observations on regeneration frequencies, number of shoots per explants (Chandra, M. and Pal, A., 1995) were recorded. Elongated shoots were rooted on MS+IBA 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.

RESULTS AND DISCUSSION

In response to different combinations of growth hormones cotyledonary node explant responded for shoot initiation (Khera, G. S. and Mathias, R. J., 1992) was ranging from 40-90%.(Table.1).The frequency of shoot initiation seemed to depend more on concentration of BAP. The shooting frequency (Brill, L. M. and Hirsch, A. M., 1999) was ranged from 23.4% to 83.3%, when BAP was used alone or in combination with IAA.Other growth hormones (D. Aurovinda and P. Rajendra, 2004) Kn, NAA did not show any shoot intiaion response and hence did not present in the table. Besides Maximum shoot regeneration frequency (83.3%) (R.K.Singh and S.S.Raghuvanishi, 1988) was observed on 4.0 mg/l BAP medium with 4.0mg/l shoots/explant without any exogenous addition (Sharma, K. K. and Thorpe, T. A., 1990) of other growth regulators. Benzyl adenine is the most widely used and effective cytokinin for various legumes including *Vigna species* (Gulati and Yadav, 2010).However by increasing the concentration of BAP with other harmones (M.C.Polanco and M.L Ruiz, 2001) did not show additional benefit.

Green healthy shoots regenerated (Patel, M. B. and Joshi, A., 1991) within 24 days were transferred on to rooting media of MS medium with ten concentrations of IBA (0.1mg/l – 1.0mg/l).High concentration IBA (1.0mg/l) observed (M.V. Polanco and M. L. Ruiz, 1997) highest rooting frequency of 95% in 25 days compared to all other concentrations (Table.2; Fig.1).Similar results have been described for *Vigna mungo*, where elongated shoots (S. Amutha and A.Ganapathi *et al*., 2006) obtained from callus were rooted on B5 medium with 14.7µm IBA. The IBA was an efficient auxin to produce the roots (Patel *et al* ., 2006).

After 25 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 (M. C. Polanco and M.L Ruiz, 2001) shoot and root initiation from shoottip of *Vigna radoata* (R. K. Singh and S.S.Raghuvanishi, 1988) has been standardized.

Fig1. Regeneration of greengram ,shoot and root from matured cotyledons

a) Cotyledon b) Shooting c) Rooting d) Harding

Table1.Effect of various concentrations of growth hormones shoots regeneration from cotyledonary explant in green gram (Vigna radoata)

Growth regulators (mg/l)	Responding explant	Regeneration frequency	Number of shoots per explant
0.5 BAP	NR	NR	NR
1.0 BAP	40±5.77	23.3±3.33	1.3±0.33
2.0 BAP	70±6.77	57.5±3.33	2.2±0.33
3.0 BAP	80±6.77	57.5±3.33	3.3±0.33
4.0 BAP	90±6.77	83.7±3.33	4.7±0.33
0.5 BAP+0.5 IAA	60±8.82	NR	NR
1.0 BAP+0.5 IAA	70±3.33	33.44±5.77	1.3±0.67
2.0 BAP+0.5 IAA	90±3.33	66.3± 5.77	1.3±0.67
3.0 BAP+0.5 IAA	70±3.33	33.44±5.77	2.3±0.67
4.0 BAP+1.0 NAA	70±11.55	NR	NR

Table 2.Effect of various concentrations of growth hormones root regeneration from cotyledonary explant in green gram (Vigna radoata)

Growth regulator IBA mg/l	Rooting frequency	Days taken for rooting
0.1	-	-
0.2	-	-
0.3	-	-
0.4	-	-
0.5	-	-
0.6	50	27
0.7	60	22
0.8	80	28
0.9	80	26
1.0	95	25

Reference

1. Amutha.S.,M.Muruganantham,A.Ganapathi.(2006).Thidiazuron-induced high-frequency axillary and adventitious shoot regeneration in Vigna Radiata (L.),In vitro cellular and Developmental Biology –Plant 42(1):26-30.
2. Aurovinda,D and P.Rajendra .(2004).Effect of plant growth regulators on greengram (phaseolusradiatus).IndianJ.Agric.sciences.74(5):271-272.
3. Brill L MandHirsch A M, (1999) Stable transformation of T1 and T2 Transgenic alfaifa with antisense-lectin constructs,Smbiosis,2717-32.
4. Chandra M andPal A ,(1995), Differential response of the two cotyledons of Vigna radiata in vitro,Plant cell R ep,15248-253.
5. Gulati AandJaiwal PK , (1983) Culture conditions affecting plant regeneration from cotledons of Vigna radiate(l.)Wilczek,plant cell Rep,247-50.
6. Jaiwal P KandGulati A, (1995)Current status and future strategies of in vitro culture techniques for genetic improvement of mungbean[vigna radiate(L.)Wilczek],Euphtica,86167-181.
7. Khera G S andMathias R J, (1992)The interaction of genotype ,explant and media on the regeneration of shoots from complex explants of brassica napus L.,J Exp Bot,431413-1418.
8. Murashige TandSkoog F, (1962)A revised medium for rapid growth and bioassays with tobacco tissue cultures,Phsiol plant,15473_497.

9. Patel M B,Bharadwaj R and Joshi A, (1991) Organogenesis in Vigna radiate (L.)Wilczek,Indian J Exp Biol ,29619-622.
10. Polanco. M.C., Ruiz. M.L (2001).Factors that affect plant regeneration from in vitro culture of immature seeds in four lentil cultivars,Plant cell,Tissue and Organ culture.66(2):133-139.
11. PolancoM.V..,M.L.Ruiz.(1997).Effect of BAP on In vitro and in vivo root development in Lens culinaris Medik,Plant cell Reports.17(1):22-26.
12. Sahoo L,Sugla T andJaiwal P K, (2002)In Vitro regeneration and genetic transformation of Vigna species,in biotechnology for improvement of legumes ,edited b P K Jaiwal andR P Singh (Kluwer Academic Publishers ,Dordrecht,The Netherlands).,1-48.
13. Sharma K K,Bhojwani S S andThorpe T A, (1990) Factors affecting high frequency differentiation of shoot and roots cotyledon from cotyledon explants of Brassica juncea(L.)Czern andcoss,plant sci,66247-253.
14. Singh. R.K., Raghuvanishi. S.S., (1988).Plantlet regeneration from nodal segment and shoottip derived explants of lentil(Lens culinaris).Lens Newsletter(ICARDA):Lentil Experimental news service. 16(1):33-35.
15. Singh.R.K.,S.S.Raghuvanishi.(1988).Plantlet regeneration from nodal segment and shoot tip derived explants of lentil(Lensculinaris).LensNewsletter(ICARDA):lentilexperimental news service.16(1):33-35.