OPEN ACCESS at journalijcir.com

Research Article

ISSN: 2395 -5775

EFFICIENT PROTOCOL FOR IN VITRO PLANTLET REGENERATION FROM LEAF EXPLANTS OF GREEN GRAM (VIGNA RADIATA) L. WILCZEK

Viji Maluventhen* and Maruthupandian Arumugam

Abstract

Efficient protocols have been developed to induce highly proliferative callus subsequent to plantlet regeneration from green gram explants. The age of the seedlings and type of explants were found to influence the callusing response. 11 day old leaf explants were cultured on MS medium containing 2,4-D and Kn induced highly proliferative callus on 28th day. Subsequently plantlet regenerated was achieved on MS basal medium without plant growth hormones. Regenerated shoots showed 65 % rooting on full strength MS basal medium without any plant growth regulators.

Key Words: Green gram, Leaf explants, Callus, MS medium

INTRODUCTION

Generally legumes comprise a wide range of crop species including herbaceous annuals and perennial trees which are used as food, feed, forage, fiber, industrial and medicinal compounds (Somers *et al.*, 2003). Legumes are excellent sources of protein, low- glycemic index carbohydrates, essential micronutrients and fiber. Green gram is one of the most important pulse crops in India and cultivated in different parts of the world. Green gram is protein rich legume and its sprouts are rich in vitamins and amino acids and also it is widely used as forage.Despite large area of cultivation of this crop, total productivity is found to be low because of several biotic and abiotic stress factors. Consequently, there is a dire need to improve the crop productivity; nutritive value and stress tolerance of this species which have better protein quality and quantity are needed. Micropropagation is an alternative to the conventional method of vegetative propagation. Most of the green gram *in vitro* micropropagation focuses on different explants including

Department of Botany, Thiagarajar College, Madurai -09, Tamil Nadu, India
Correpondence and Reprint Requests: Viji Maluventhen
Received: November 20 , 2015 | **Accepted:**December 12, 2015 | **Published Online:** December 28 , 2015

Conflict of interest: None declared | **Source of funding:** Nil

cotyledonary node multiplication, which is ideal for high clonal fidelity and efficiency (Khatun *et al.,* 2008). We are interested to develop efficient protocol for high frequency plantlet regeneration in green gram from leaf explants.

MATERIALS AND METHODS

Collection and germination of seeds

Seeds were obtained from Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu. India. Seeds were washed with distilled water 3-4 times, followed by treatment with 70 % ethanol for 30 sec and 0.1% (w/v) $HgCl_2$ for 4 min and rinsed with sterile distilled water for 5 times to remove the fine particles. Seeds were germinated on semi-solid 0.7% agar water substratum. Seedlings and cultures were maintained at 25 ± 1 °C under 16h L/8hD.

Preparation of explants

Leaf explants were placed on Petridishes lined with two layers of moist sterile Whatmann No.1 paper to collect explants. Explants were cultured in the medium supplemented with specified concentrations of plant growth hormones. Culture responses were routinely determined in 28th d followed by inoculation.

Effect of media, carbohydrates and additives on SE induction

The MS medium supplemented with vitamins, 30 g/l sucrose, 0.7% agar and different growth regulators, different concentrations of 2,4-D (1-10 µM) and kinetin (5-15 µM) were used for callus induction. The pH of the media was adjusted between 5.6-5.8. Cultures initiated from explants were maintained at 25±2°C under 16hL/8hD photoperiod.

Data presentation

At least 50 explants were employed in each of the experiments and each experiment was repeated thrice. Values of experimental determinations for the different culture response parameters indicate mean ± SD.

RESULT AND DISCUSSION

Effect of seedling age and explant types on callus induction

In order to optimize callus production as a function of seedling age and explant types, a set of experiments was undertaken. Primary explants were prepared from seedlings of different age and optimized for callus formation and also seedlings at the age of 3, 8, 9, 10 and 11 days were employed. Primary explants such as embryonal axis, cotyledonary node, shoot tip, epicotyl and leaf were used. The aim was to develop highly proliferative callus which could subsequently be induced to develop plantlet regeneration. Results showed that callusing response differed with the explants used in relation to the age of source seedlings. In contrast to explants prepared from the seedlings in the age group of 8 to 10 days, leaf explants

prepared from 11 d old was found to produce highly proliferative callus at a higher level. Explants prepared from seedlings beyond 11-d were found to accumulate phenolics in culture which inhibited callus production besides browning of the callus. Hence explants prepared from 11-d old were included to be the best for callus proliferation and the explant was used for further experiments. In general, legume plant tissue culture protocols involve a variety of explant types for inducing plantlet regeneration (Manoharan *et al*., 2005). Several workers reported the *in vitro* response of seedling derived explants from different legume species such as cowpea, black gram and pigeonpea (Aasim *et al*., 2008; Muruganantham *et al*., 2010; Geetha *et al*., 1998).

These studies focused on direct plantlet regeneration, organogenesis *via* callus and somatic embryo formation (Ramakrishnan *et al*., 2005; Shiva Prakash *et al*., 1994; Viji *et al*., 2012). In general, the source and choice of explants is known to be a critical factor that determines the success of most tissue culture experiments.

The young tissues and organs such as embryonal axis from young seedlings, hypocotyl, epicotyl, cotyledonary node and immature and mature cotyledons are found to be more responsive explants. There is an overwhelming evidence that organogenesis is high in the cotyledonary node explants (Gulati and Jaiwal 1994; Shiva Prakash *et al*., 1994; Thibaud-Nissen *et al*., 2003).

Table 1 Effect of concentration of phytohormones on the callusing response and nature of callus raised from 11-d old leaf explants of green gram

S.No	Phytohormone/growth factor concentration	Callus produced per culture tube (mg dry wt)*	Nature of callus
1	1 µM 2,4-D	12 ± 0.5	Compact, brown callus
2	2 µM 2,4-D	28 ±1.06	Compact callus
3	3 µM 2,4-D	43 ±1.72	Creamy callus
4	4 µM 2,4-D	66 ±2.2	Creamy callus
5	5 µM 2,4-D	81 ±2.7	Friable, creamy callus
6	6 µM 2,4-D	74± 2.8	Creamy callus
7	7 µM 2,4-D	43 ± 1.35	Compact callus
8	8 µM 2,4-D	54±2.0	Compact, green callus
9	9 µM 2,4-D	22±0.9	Compact, green callus
10	10 µM 2,4-D	104±3.9	Friable, creamy callus
11	2,4-D 10 µM + Kn 1µM	34±1.4	Friable, yellowish green callus
12	10 µM 2,4-D +2 µM Kn	38±1.7	Friable, green callus
13	2,4-D 10 µM + 3µM Kn	43±1.6	Compact callus
14	2,4-D 10 µM +4 µM Kn	52±1.9	Compact callus
15	2,4-D 10 µM +5 µM Kn	60±2.0	Friable, brown callus
16	2,4-D 10 µM + 6 µM Kn	65±2.2	Friable, brown callus
17	2,4-D 10 µM +7 µM Kn	70±2.4	Friable brown callus
18	2,4-D 10 µM +8 µM Kn	78±2.8	Compact callus
19	2,4-D 10 µM +9 µM Kn	83±3.4	Friable brown callus
20	2,4-D 10 µM +10 µM Kn	188±4.7	Friable, greenish callus
21	2,4-D 10 µM + 5 µM Kn	122±3.9	Friable, green callus

In the present study, it has been found that 11-d old leaf explants showed better callusing response as compared to cotyledonary node explants.

Effect of phytohormones and growth factors on callusing response of explants

Subsequent to the production of highly proliferative callus, a set of experiments was carried out to produce high frequent plantlet regeneration. Phytohormones such as 2,4-D (5 to 15 µM) and kinetin (kn) (5 to 15 µM) were used in different combinations for callus induction. There was a significant difference observed in callus induction and the friability of the callus also varied. In the present study, supplementation of 2,4-D along with kn showed better callusing response at high frequency. Observations showed that the production of high amount of proliferative callus was achieved in MS + 2,4-D (10 µM) + kn (10 µM; Tables 1). Kn supplemented (10 µM) cultures supported organogenesis. When the concentration of kn was increased along with the presence of 2,4-D at 10 µM there was a linear decrease in the amount of callus produced. Different concentrations and combinations of plant hormones are involved in the induction of callus which leads to organogenesis. Ganasen *et al.*, 2006 reported shoot organogenesis in cotton by the supplementation of polyamines.

Previous reports showed that supplementation of BA induced multiple shoots has been reported in mung bean (Gulati *et al.,* 1994; Vats *et al.,* 2014). Supplementation of 2-iP also proved best in multiple shoot induction in green gram (Gulati *et al.,* 1994).

In this set of experiments, nutrient media formulation of MS (Murashige and Skoog, 1962) medium was employed and the callusing response was determined. It was observed that MS medium was found to be optimal for callus induction in leaf explants. It was also observed that 16hL/8hD incubation showed high frequency callusing response and yielded high amount of callus when compared to continuous dark. However the callus cultures incubated in 16hL/8hD cycle conditions were more friable on subculturing which is the desirable callus morphology. In the present study 3% of sucrose showed best response for high frequency of shoot multiplication.

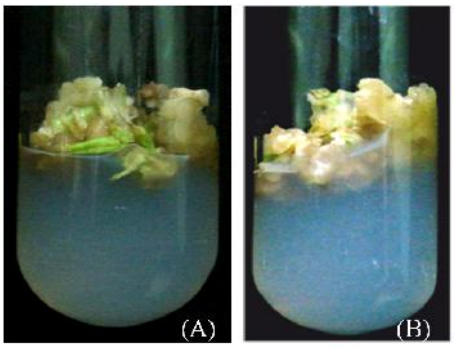

Figure 1 A & B – 28 d old callus raised from 11 d old leaf explants on MS medium supplemented with 2,4-D (10 µM) and Kn (10 µM)

Germination and transplantation

The highly proliferative callus was transferred to germination medium (MS basal medium) containing 3% sucrose and 0.7% agar. Where as, full strength MS medium consisted of 30 g/l sucrose, and 2,4-D led to the development of senesced

plants. Plantlet regeneration was achieved on MS basal medium. Sixty five percentage of rooting was achieved in MS basal medium without any plant growth regulators.

Figure 2 C – Plantlet regenerated from 28 d old callus on MS basal medium

Acknowledgement

The author is thankful to the Management and faculty members in the Department of Botany, Thiagarajar College, Madurai for their support and suggestions to complete this research work.

References

1. Somers DA, Samac DA & Olhoft PM (2003) Recent advances in legume transformation, Plant Physiol, 131: 892
2. Khatun MK, Haque MS, Islam S and Nasiruddin KM (2008). *In vitro* regeneration of mungbean (*Vigna radiata*L.) from different explants. Progressive Agriculture 19 (2) 13-19.
3. Aasim M, Khawar K, Ozcan S (2008) *In vitro* micropropagation from shoot meristems of turkish cowpea (*Vigna unguiculata* L.) cv. Akkiz. Bangladesh J Bot 37: 149-154
4. Muruganantham MS, Amutha S & Ganapathi A (2010) Somatic embryo productions by liquid shake culture of embryogenic calluses in *Vigna mungo* L, *In Vitro* Cell Dev Biol Plant, 46: 34-40
5. Manoharan K., Mishra G., Sathishkumar R., Pritam Bala Sinha, Karuppanapandian T., Agrawal S., Jagannathan V. (2005). Induction of embryogenic callus and direct plantlet regeneration in black gram (*Vigna mungo* (L.) Hepper). J Swamy Bot-Cl. 22: 39-46.
6. Shiva prakash N, Pental D, Bhalla-Sarin N (1994). Regeneration of pigeonpea (*Cajanus cajan*) from cotyledonary node *via* multiple shoot formation. Plant Cell Rep 13: 623-627
7. Thibaud-Nissen F, Shealy RT, Khanna A, Vodkin LO (2003) Clustering of microarray data reveals transcript patterns associated with somatic embryogenesis in soybean. Plant Physiol 131: 118-136
8. Geetha N, Venkatachalam P, Prakash V, Lakshmi Sita G (1998) High frequency induction of multiple shoots and plant regeneration from seedling explants of pigeonpea (*Cajanus cajan* L.). Curr Sci 75: 1036-1041
9. Ganesan M and Jeyabalan N (2005) *In vitro* plant regeneration from the callus callus of shot tip in cotton (Gossipian hirsutum L. cv. SVPR2). Iran J Botechnol 3: 144- 151
10. Ramakrishnan K, Gnanam R, Sivakumar P, Manickam A (2005) *In vitro* somatic embryogenesis from cell suspension cultures of cowpea (*Vigna*

unguiculata L.). Plant Cell Rep 24: 449–461

11. Gulati A and Jaiwal P (1994) Plant regeneration from cotyledonary node explants of mung bean [Vigna radiata (L.) Wilczek] .Plant Cell Reports 13 523 – 527.
12. Vats S, Solanki P and Alam A (2014) Efficient *in vitro* regeneration of *Vigna radiata* (L.) Wilczek. *Researcher* 6 12–15.
13. Murashige T & Skoog F (1962) Revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol Plant, 15: 473.
14. Viji M, Maheswari P, Manoharan K and Karuppanapandian T (2012) Induction of somatic embryogenesis by polyethylene glycol and mannitol in cultures of pigeonpea, *Cajanus cajan* (L.) Mpillsp. African Journal of Biotechnology: 10340–10349

****