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Somatic embryogenesis in *Vigna radiata* (L.) Wilczek

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Somatic embryogenesis was achieved from immature cotyledon derived callus of mungbean, *V.radiata* (L.) Wilczek in MS liquid medium. Embryogenic callus was induced on MS medium with NAA (5mg/L). Differentiation of somatic embryos was observed when embryogenic callus was transferred to MS liquid medium containing 2,4-D (1.5 mg/L) and L-proline (50 mg/L). The torpedo shaped embryos were transferred to MS liquid medium with BAP and ABA (1 mg/L each) for maturation and germination. Fifty per cent of torpedo shaped embryos were converted into tiny plants (8-9 plants out of 17) after one week of culture. The germinated embryos were isolated and transferred to MS half strength basal (solid) medium for further development.

Vigna radiata (L.) Wilczek is considered as an important pulse crop. Attempts made for improvement of this crop by conventional breeding methods met with limited success[1]. *In vitro* plant regeneration from cell suspension culture offers a suitable system for mass propagation of economically important plants. It could become a pivotal tool for the large-scale production of somatic embryos that would greatly aid the genetic manipulation[2] and production of transgenic legumes[3]. Somatic embryogenesis has been achieved in many legume species[4]. However, conversion of somatic embryos into plants has remained inefficient and has limited the application of somatic embryogenesis as a regeneration system in legumes. A variety of methods have been used to increase the frequency of somatic embryo conversion, including desiccation and plant growth regulator treatment[3]. Although Eapen and George[5] and Patel *et al.*[6] have been also able to induce somatic embryogenesis in *Vigna radiata* they could not achieve maturation and germination. Chen *et al.*[7] have an opinion that the ability of explants to differentiate into embryos is apparently associated with interspecific hybridity. The present study describes a repeatable protocol for plant recovery *via* somatic embryogenesis through suspension culture that is characterized by stages of embryo development, maturation and germination.

Seeds of *Vigna radiata* (cv. V1) were procured from Regional Pulses Research Centre, Tamil Nadu Agricultural University, Vamban, Pudukkottai, Tamil Nadu, India. Plants were grown in the field and immature pods were collected after 14 days of anthesis. They were surface sterilized by soaking in $HgCl_2$ (0.1%) for 10 min and then rinsed three times with sterile distilled water. Immature pods were aseptically dissected to remove immature cotyledons. The embryonic axis and adjoining cotyledonary tissues were removed. The remaining tissue was cultured on 20 mL of solid MS (Murashige and Skoog[8] basal medium with 3% of sucrose and 0.7% of agar (Hi-media, Mumbai, India) in test tubes (25×150 mm). The *p*H of the medium was adjusted to 5.6 to 5.8 before autoclaving at 121° C for 15 min. For callus induction, the explants (immature cotyledon) were cultured on MS medium supplemented with various concentrations of napthalene acetic acid (1-5 mg/L; NAA and 2,4- dichlorophenoxy acetic acid (2,4-D; 0.5-2.5 mg/L) individually to check their embryogenic callus induction efficiency. Two week old embryogenic calli were transferred to liquid medium for development of somatic embryos. The suspension cultures were initiated by transferring two week old embryogenic callus (2 g of fresh wt) to MS liquid medium (50 mL) containing various concentrations (0.5-2.5 mg/L) of 2,4-D in combination with different levels (25-100 mg/L) of L-proline in a Erlenmeyar flask (250 mL) and kept in gyratory shaker at 100 rpm. Suspension cultures were subcultured at weekly interval in the medium having same composition. After 15 days, somatic embryos of torpedo stage were transferred to MS liquid medium (50 mL) containing BAP (1 mg/L) and ABA at different concentrations (0.5 to 2.5 mg/L) and placed on a gyratory shaker at 80 rpm for maturation and germination. The tiny plants containing shoot and

root primordia were isolated individually and kept on sterile filter paper for 30 sec and transferred to MS half strength basal (solid) medium for further development. The cultures were incubated at 25° ± 2°C with a 16 hr photoperiod (cool white fluorescent lamp). Samples of suspension cultures were taken at random at the end of each subculture and number of somatic embryos were counted under a microscope. Counts were made from 20 different independent samples and the percentage of somatic embryos calculated was based on the total number of cells present in the field. For callus induction, 25 explants were used for each treatment and experiment was repeated thrice. Data were analysed using Duncan's multiple range test to separate the means.

Somatic embryos were induced from immature cotyledon derived callus of *Vigna radiata* (cv.V1). Immature cotyledons showed the high degree of embryogenic potential in several plant species[9] in general and pulses like soybean[10], Chick pea[11], Pigeon pea[12] in particular. In mungbean, plant development from somatic embryos has not yet been achieved[1].When immature cotyledons were cultured on MS medium fortified with NAA (5 mg/L) or 2,4-D (2 mg/L), four different types of calli were observed *viz.* white watery, green-compact, yellowish-nodular friable and brown friable calli. Factors affecting the induction of embryogenic callus are explant type, growth regulators and their concentrations. The promotive effects of NAA on induction of somatic embryos in cultures of legumes has been demonstrated earlier[13]. In our study, embryogenic competence was observed in immature cotyledon derived callus cultured on medium supplemented with NAA or 2,4-D, but the percentage of embryogenic callus was high (100%) in NAA (5 mg/L) containing medium in comparison with that of 2,4-D (85%; 2 mg/L). Similar result has been reported earlier in mungbean[5].

Among the four different types of calli produced, the yellowish nodular friable callus was found to possess the embryogenic potential (Table 1). Two weeks old callus (2 g of fresh wt) was found suitable to initiate the suspension culture. Auxin is known to be essential for induction of somatic embryogenesis and

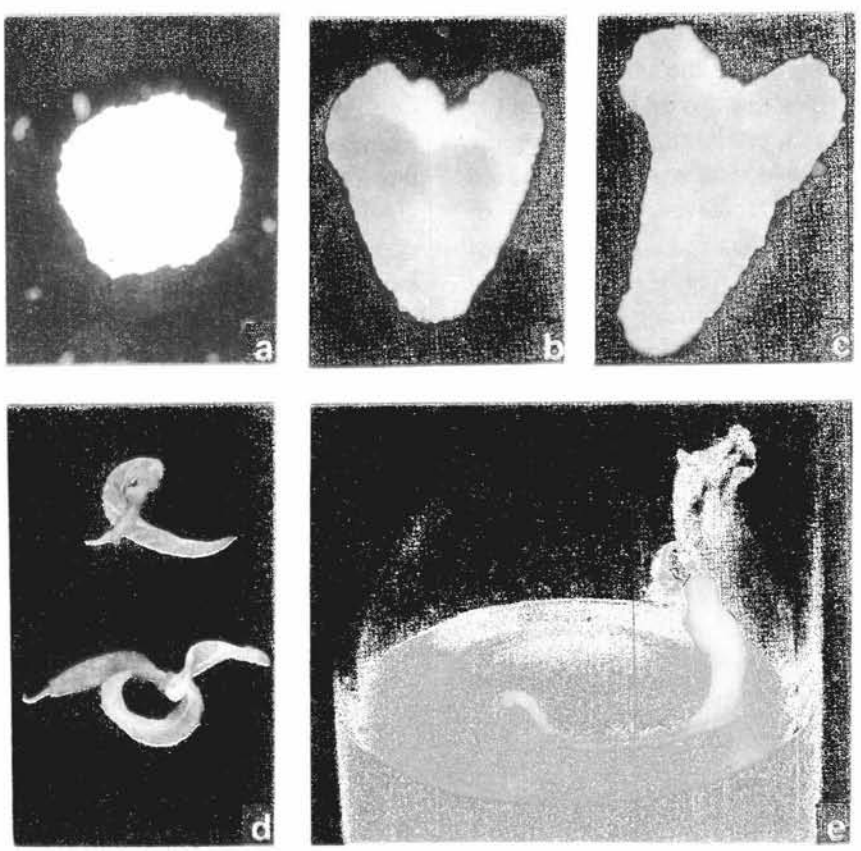

Fig. 1—Somatic embryogenesis in mungbean (a) Globular shaped embryo (bar=×15); (b) heart shaped embryo (bar=×25); (c) torpedo shaped embryo (bar=×25); (d) Isolated tiny plants from MS liquid medium; and (e) regenerated plant from somatic embryo on MS basal (Half strength) solid medium (sh—shoot, r—root).

2,4-D is the most commonly used auxin[14]. Embryogenic callus when transferred to MS liquid medium containing 2,4-D (0.5-2.5 mg/L) along with L-proline (50 mg/L) produced globular, heart and torpedo (Fig 1a, b, c) shaped embryos after 15 days. Liquid cultures were subcultured in the same medium at weekly intervals for the development of somatic embryos. The initial dependence of callus for 2,4-D to initiate embryogenic culture has been reported in a number of species[15]. Incorporation of L-proline (50 mg/L) along with 2,4-D (1.5 mg/L) in liquid medium was essential for the induction of somatic embryos and yielded large number of somatic embryos. This result is in agreement with that of Stuart and Strickland[16] who have tested the effects of nine amino acids in alfalfa somatic embryogenesis and found that L-proline is the most effective one. Maximum numbers of somatic embryos were observed at 1.5mg/L of 2,4-D in combination with L-proline (50mg/L; Table 2). No further development of embryos was achieved when the cultures were maintained in the medium containing same concentration of 2,4-D. Although, somatic embryogenesis has been obtained in a number of species, low efficiency of embryo germination and their conversion to plantlets remains a major hurdle[17]. This may be due to presence of exogenous auxins. Sankara Rao has reported that presence of auxin is required for determination of somatic embryos in sandalwood[18], but once determination occurs, auxin is inhibitory for further development of embryos. It is necessary to remove or reduce the 2,4-D from the embryo induction medium for further development of somatic embryos. Different treatments have been used to improve the maturation of embryos and subsequent development and germination. As ABA affects many developmental stages in zygotic embryos and maturation of somatic embryos[19], combination of benzylaminopurine (1.0 mg/L) with different concentrations (0.5-2.0 mg/L) of abscisic acid has been tested. Similar combination has also been attempted in sunflower[20]. In the present study, BAP + ABA combination (1 mg/L of each) favoured the maturation and conversion of torpedo shaped embryos into plants with distinct shoot and root primordia (Fig. 1 d, e) after one week of subculture.

An average of 8-9 embryos converted into plants from 17 torpedo shaped embryos. The germinated embryos showed further development only upon transfer to half strength MS basal (solid) medium (Fig. 1e), thereby indicating the inhibitory effect of high concentration of inorganic elements on plant development. The present protocol may be helpful in further improvement of the crop.

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Table 1—Effect of NAA and 2,4-D on callus induction from immature cotyledons of *Vigna radiata*
[Values are mean of 25 replicates of 3 experimen ts]

Hormone Conc (mg/L)	Callus Induction (%)	Nature of calli
2,4-D		
0	—	—
0.5	45e	White watery
1.0	66d	White watery
1.5	78b	Brown friable
2.0	85a	Yellowish nodular friable*
2.5	76bc	Brown friable
NAA		
0	—	—
1.0	69d	White watery
2.0	80c	Brown yellowish friable
3.0	94b	Brown friable
4.0	100a	Green compact
5.0	100a	Yellowish nodular friable*

*Indicates embryogenic calli.
Mean value having the same letter are not significant at 5% level according to Duncan's multiple range test.

Table 2—Effect of 2,4-D on percentage of somatic embryo development in liquid media after 15 days

2,4-D conc. (mg/L) L-proline (50 mg/L)	Percentage of somatic embryos		
	Globular	Heart	Torpedo
2,4-D			
0.5	13d	8d	5d
1.0	16c	11c	9c
1.5	27a	21a	17a
2.0	20b	17b	13b
2.5	15c	10c	7c

Each observation was calculated as a percentage of somatic embryos to total cells present per microscope field. Values are average of 20 random samples.
Mean value having the same letter are not significant at 5% level according to Duncan's multiple range test.

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