



REGENERATION OF MULTIPLE SHOOTS FROM COTYLEDONS OF *VIGNA RADIATA* (L.) WILCZEK.

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Vigna radiata (L.) Wilczek is economically important as a dietary constituent of people in subtropics. This leguminous plant has been reported to be recalcitrant to tissue culture. In the present investigation, protocols for induction of callus and multiple shoots were standardised. These can subsequently be used for improvement of this crop.

Key words: Callus, in vitro, Multiple shoot regeneration, *Vigna radiata*, Green gram.

Vigna radiata (L.) Wilczek., commonly known as "Green gram", is one of the most important pulse crops. This leguminous crop is a source of dietary protein, especially for the vegetarian population of subtropics. According to earlier reports organogenesis and regeneration of the whole plantlets was found to be difficult in *Vigna* species as well as in other legumes. However, limited success has been achieved on morphogenetic response in different legume species (Gill et al. 1986 & Davey et al. 1994). Keeping in view the importance of the plant, the present investigations were taken up to standardize protocols for induction of callus and multiple shoots which could be used for the improvement of this crop through tissue culture techniques.

MATERIAL AND METHODS

MS (Murashige & Skoogs, 1962) medium was prepared as per standard methodology suggested by Dodds & Roberts (1985). An authenticated seeds of *Vigna radiata* (L.) Wilczek. Cultivar Ps-16 were obtained from Agricultural Research Station, Gulbarga (Karnataka). Observations were recorded daily and continued up to 45 days. Data obtained

were statistically analyzed (Nageswara Rao, 1983).

RESULTS AND DISCUSSION

The seeds showed signs of germination, in vitro, within 3 days of incubation and 90% germination rate was recorded. The effects of 2,4-D, NAA and Kn on callus induction of *Vigna radiata* cotyledons were observed. The results indicate that 2,4-D and NAA in the concentration of 0.5 mg / l and 1 mg / l respectively, showed little response towards callusing. However, at 2 mg / l concentration 2,4-D and NAA alone, the growth of the callus was maximum. Supplementing Kn to 2, 4-D and NAA at 2 mg / l, further enhanced the growth of callus. Increasing the concentration of Kn from 0.5 to 1.0 mg / l in combination with 2,4-D and NAA separately, caused increase in the callus production. However, increase in the concentration of Kn to 2 mg / l slightly inhibited the growth of callus.

Perusal of Table-1 clearly reveals the effect of different plant growth regulators on cotyledon culture of *Vigna radiata* (L.) Wilczek for multiple shoot formation. The direct multiple shoot formation was recorded on MS medium fortified with BAP at 2.25 mg / l and 5.0 mg / l in combination with IAA and Kn (0.5 mg / l each) as shown in Table-1. When BAP was used alone at 2.25 mg / l concentration, it resulted into an average 2.0 number of shoot buds, as the BAP concentration was increased to 5.0 mg / l, it enhanced formation of number of shoot buds, up to an average of 5.6 in number. Addition of IAA and Kn (0.5 mg / l each) to BAP, further enhanced the number of shoot buds. As shown in Table 1, when IAA (0.5

Table No. 1 Response of cotyledon explants to various combinations of BAP, Kn and IAA in MS medium

MS Medium + concentration of phytohormones (mg /l)	Percentage of Explant response	Mean number of shoot buds / explant	Mean number of shoot / explant	Mean length of shoot/ explant (cm)	Mean length of internode / explant (cm)	Mean number of leaf / explant	Days required
BAP 0.5	60	-	-	-	-	-	30-35
BAP 1.0	65	-	-	-	-	-	30-35
BAP 2.25	70	2.0±0.8	1.5±0.4	1.5±0.3	0.75±0.07	3.0±1.2	40-42
BAP 5.0	71	5.6±1.8	3.2±1.2	2.5±0.9	1.3±0.2	3.5±1.3	40-45
BAP 2.25 + IAA 0.5	72	3.8±1.4	3.0±1.0	3.4±1.2	1.3±0.2	3.2±1.1	40-46
BAP 2.25 +Kn 0.5	76	4.2±1.5	3.4±1.3	3.3±1.2	1.4±0.3	3.4±1.2	40-45
BAP 5.0 + IAA 0.5	80	8.6±2.1	6.1±1.8	3.8±1.4	1.4±0.2	3.5±1.3	40-45
BAP 5.0 + Kn 0.5	85	29.1±2.2	6.4±1.9	3.6±1.4	1.5±0.2	3.6±1.1	40-45

± = Standard error

mg/l) added to the medium containing BAP (2.25 mg / l), the number of shoot buds increased up to 3.8. where as addition of Kn (0.5 mg / l), proved slightly better than IAA in multiple shoot formation i.e. an average number of 4.2 shoot buds were noticed on the medium containing BAP (2.25 mg / l) + Kn (0.5 mg / l). Similarly, BAP (5.0 mg / l) in combination with IAA (0.5 mg / l) induced 8.6 number of shoot buds.

Protocols for plant regeneration from in vitro cultures using various explants have been developed for all Indian grain legume species. However, less success has been achieved towards establishment of totipotent cultures using cotyledon as an explant due to its low regeneration efficiency and reproducibility. In the present studies, it has been observed that 2,4-D followed by NAA are most suitable phytohormones for callus induction in *Vigna radiata*. The optimum concentration of phytohormone being 2 mg /l and below this, the explant did not produce much callus, whereas concentration more than this concentration, inhibited growth of the callus. In most of the legumes, the required auxins and cytokinin ratio is high for callus initiation than do other plants [Flick et al.1983] but in the present investigations, it was noticed

that Kn in the range of 0.5 to 1 mg /l, when added to 2,4-D or NAA enhanced callus formation and BAP enhanced shoot bud formation. Similarly, on other legumes also viz, *Glycine max* (Cheng et al.,1980) and in *Vigna mungo*, (Gill et al.,1986) BAP enhanced shoot bud formation. Experiments aimed at *Agrobacterium* transformation of *Vigna radiata* was possible through shoot regeneration from cotyledonary nodes, where a total of ten shoots were recovered. Similarly, multiple shoot formation from cotyledonary node of *Vigna mungo* was achieved on B5 (MS +B5 vit.) + BAP (13.3 µm) (Ignacimuthu et al.,1997).

In the present investigations, it was found that, addition of IAA (1mg /l) in MS medium supplemented with BAP (5 mg / l) was essential for the foermentation of multiple shoots. Similarly, it has demonstrated the totipotency and efficiency of callus induction and multiple shoot formation from cotyledon explant. The best medium for callus formation was found to be MS + 2,4-D (2mg /l) + Kn (1mg /l). The maximum, 9.1 shoot buds, were induced from cotyledons, when cultured on MS + BAP (5 mg / l) + Kn (0.5). Thus, above techniques and protocol of multiple shoot formation could be applied for the development of an efficient

transformation method for the improvement cum breeding of this crop.

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