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PLANT REGENERATION FROM *IN VITRO* CULTURED LEAF IN MOTHBEAN

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Plants were regenerated from the *in vitro* cultured leaf explants of Mothbean (*V. aconitifolia* Jacq.) cv. BMB-43. Leaf explants were excised from seven-day-old seedlings and cultured on Murashige and Skoog (MS) basal medium supplemented with 5.0µM Indole-3Acetic Acid (IAA) and 9.3 µM Kinetin (KN). Callus formed at the petiole ends. Shoot regeneration observed when this callus was transferred to MS Medium containing 9.3 µM Kinetin and 100mg l^{-1} Adenine sulfate (AS). Regenerated shoots were rooted on MS basal medium without growth regulators and were established in soil where they showed normal morphological characters.

Keywords : Callus; Growth regulator; Shoot regeneration; *Vigna aconitifolia.*

Introduction

The grain legumes are next to cereals as a source of human food and are known to contain rich protein than any other vegetable product and are also rich in minerals and B-vitamins. As all the plant parts of the legumes are rich in proteins, they are valuable too as field and forage crops. In addition legumes are crucial to the balance of nature, as many species are able to convert gaseous nitrogen from the air into ammonia, a soluble form of nitrogen which is readily utilized by plants. Since grain legumes are notoriously recalcitrant species so they are difficult to regenerate from tissue culture. Successful regeneration of legume species has been greatly aided by species-specific determination of critical regeneration parameters such as explant source, genotype and media constituents.

However, the successful regeneration of grain legumes from leaf explants has been reported in only a few species such as *Glycine max* (L)[1], *Arachis hypogea* (L)[2,3], *Vigna radiata* (L)[4], and *Vigna unguiculata* (L.)[5]. Regeneration of shoots from leaf explants is also reported in *Vigna aconitifolia* via somatic embryogenesis[6,7]. In the present paper we describe a method for successful regeneration of shoots from leaf explants via organogenesis may be ideal for *Agrobacterium* - mediated - genetic transformation.

Materials and Methods

Vigna aconitifolia (Jacq.) cv. BMB-43 seeds obtained from the University of Agricultural Sciences, Dharwad, Karnataka state, India were surface sterilized in 70% ethanol for 3 minutes followed by immersion in 0.1% mercuric chloride for 5 minutes. Seeds were rinsed three times with sterile double distilled water and were germinated aseptically at 26 ± 2^0 C on a 16 hour photoperiod with 50µ mol m^{-2} sec^{-1} under cool white fluorescent light with a relative humidity of 55-60% on Murashige and Skoog (MS) basal medium[8] containing 2.0% sucrose (Analar grade) and 0.7% agar (Difco-bacto) in 145 x 25mm glass culture tubes containing approximately 15 ml of the medium. One to two cm long leaves excised from seven-day-old seedlings were split longitudinally through the mid-vein and were cultured in 145x25 mm glass culture tubes containing approximately 15ml of MS basal medium containing 5.0µ M IAA + 9.3µ M KN. The explants were incubated in the light for the induction of callus. Total 25 leaf explants were cultured for the initiation of callus. For each set 3 replicates were maintained and mean was considered.

To test the effect of various concentrations of auxins, 9.3µ M KN was substituted with IBA, NAA and 2,4-D. The callus thus obtained were transferred to 145 mm x 25 mm culture tubes containing MS basal medium[8] supplemented with 9.3 µM

KN and 100 mgl^{-1} Adenine sulfate at 26 ± 2^0 C under 18 hour photoperiod (100-120 $\mu M\ m^{-2}\ sec^{-1}$) for the shoot induction. Light was provided by cool white fluorescent tubes. Shoots with fully expanded leaves were transferred to 145 mm x 25 mm glass culture tubes containing 15 ml of growth regulator free MS basal medium for rooting. Rooted plants were transferred to 9 cm pots filled with vermiculite for gradual hardening and finally transferred to soil. All the experiments were repeated for three times.

Results and Discussion

In the present study, the leaf explants with the intact petiole end expanded and produced profuse mass of callus with few roots from the petiole end of the leaves within fifteen days when cultured on MS basal medium supplemented with 5.0 µM IAA and 9.3µM KN (Callus Induction Medium) (Fig. 1 - A). The resulting callus was again subcultured for one week on the same medium for further growth. Regeneration of shoots was observed when this callus was transferred to shoot induction medium i.e. MS basal medium containing 9. 3 µM KN with $100mg^{-1}$ adenine sulfate (Fig 1-B). The calli obtained from other combinations of IAA and KN when transferred to shoot induction medium turned green but shoot organogenesis was not observed. Other auxins such as IBA, NAA and 2, 4-D when substituted with KN, gave rise to callus that turned brown and did not regenerate shoots when transferred to shoot induction medium. In another set of the experiment the callus was also subcultured on the shoot induction medium supplemented with KN and different concentration of adenine sulfate to promote shoot differentiation. Shoot regeneration occurred from the calli on all tested adenine sulfate concentrations with the highest percent (15-18%) produced on 100 mgl^{-1} adenine sulfate (Table 1). Two to four shoots per responding explant were induced within 25 days of transfer. The developed shoots formed roots within 20 days when transferred to growth regulator-free-MS basal medium. The rooted plants were subsequently transferred to pots and to the soil. The survival rate was 50%. All the regenerated plants were fertile and exhibited normal pod development.

The present study demonstrates the successful shoot regeneration from the *in vitro* cultured leaf explants of mothbean *Vigna aconitifolia* cv. BMB-43. Only the callus produced on MS basal medium supplemented with 5.0 µM. IAA + 9.3 µM KN were able to form shoots when subcultured to shoot induction medium. Hence the concentration of adenine sulfate (AS) in the shoot induction medium effects shoot regeneration. The highest percentage of shoot regeneration was observed from the callus when transferred to shoot induction medium containing 100 mgl^{-1} of adenine sulfate. Adenine in the form of adenine sulfate can stimulate cell growth and greatly enhance shoot formation[9]. It provides an available source of nitrogen to the cell and can generally be taken up more rapidly than inorganic nitrogen[10]. A similar or other concentration has been effectively used for regeneration of plants from primary leaves of Cow pea, *V. ungiuculata L.*[5] and a mature leguminuous liana (*Bauhinia vahlii* Wight and Arnott)[11]. Shoot regeneration observed from the calli produced at the petiolar end of the leaf explants of *Vigna aconitifolia*, which indicates that the callus produced from the petiolar end are having high shoot regeneration potential under the tested conditions. This was also reported in Soybean[1] and Cow pea[5].

In conclusion mothbean can be regenerated from the cultured leaf explants. Shoots were regenerated within 2-3 weeks of the transfer to shoot induction medium. This protocol is very simple and is an added advantage for propagating a large number of regenerated plants.

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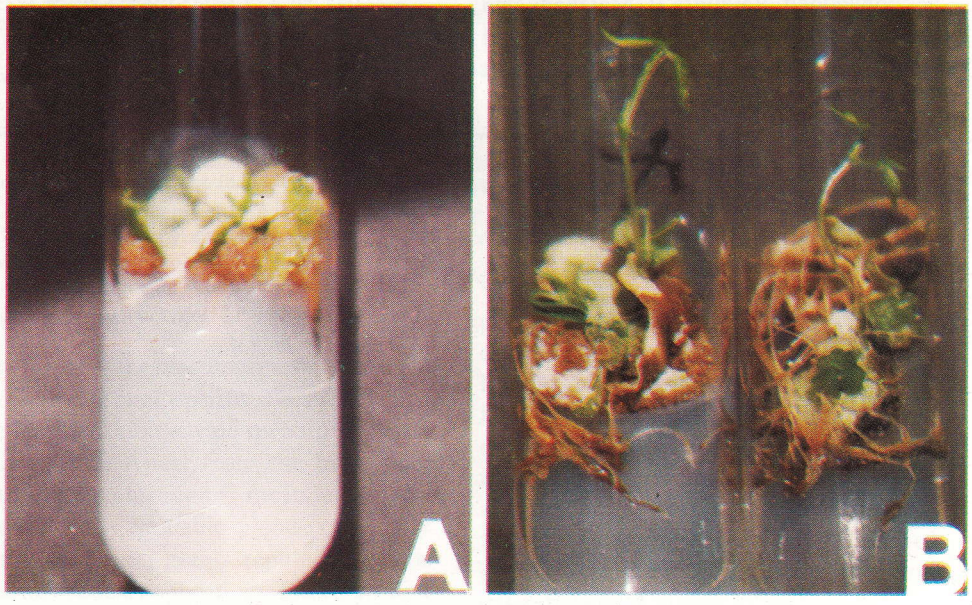

Fig. 1. A-Leaf explant cultured on MS + IAA (5.0 µM) + KN (9.3 µM) (Callus Induction Medium) showing proliferation of callus from petiole end.
B- Regeneration of shoots on MS + KN (9.3 µM) with adenine sulfate (100 mg^{-1}) (Shoot Induction Medium).

Table 1. Effect of various concentrations of Adenine sulfate in combination with KN (9.3 µM) on shoot regeneration of callus derived from leaf explants of *Vigna aconitifolia* (Jacq.) Cv. BMB-43 cultured on MS supplemented with IAA (5.0 µM) + KN (9.3 µM). Each value represents the mean ± SE of 3 replicates each with callus derived from 25 explants.

Nutrient Medium MS+KN (9.3 µM) with Adenine sulfate Concentration ($mg1^{-1}$)	% of Explants showing shoot regeneration	Mean No. of Shoots per explant
0	- Nil -	- Nil -
50	5 ± 0.1	2.8 ± 0.1
100	18 ± 0.4	4 ± 0.2
150	6 ± 0.2	2 ± 0.7
200	1.4 ± 0.4	1.2 ± 0.2

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References

1. Wright MS, Ward DV, Hinchee MA, Carnes MG and Kaufman RJ 1987, *Plant Cell Rep.* **6** 83
2. Mc Kently AH, Moore GA and Gardner FP 1991, *Crop Sd.* **31** 833
3. Eapen S and George L 1993, *Plant Cell Tissue Organ Cult* **35** 223.
4. Mendoza and Futsuhara 1990, *Jap. J. Breed* **40** 457
5. Muthukumar B, Mariamma M and Gnanam A 1995, *Plant Cell Tissue Organ Cult.* **42** 153.
6. Eapen S and George L, 1990, *Annals of Botany* **66** 210
7. Malabadi R B 1997, *Tissue Culture Studies of some commercially Important plants.* Ph.D. thesis submitted to the Karnatak University Dharwad, Karnataka - State, India.
8. Murashige T and Skoog F 1962, *Physiol. Plant.* **15** 473
9. Murashige T 1974 *Annu Rev Plant Physiol.* **25** 135
10. Thom MA, Maretzki Komer E and Sakai WS. 1981, *Plant Cell Tissue Organ Cult.* **1** 3
11. Dhar U and Upreti J 1999, *Plant Cell Rep.* **18** 664