

Plantlet regeneration from Callus culture of various explants of Winged bean *Psophocarpus tetragonolobus* variety NS 122

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ABSTRACT

Protein content in Psophocarpus tetragonolobus is comparable to soya bean, higher than that of other pulses, it is a high yielding legume crop in tropical countries like India. An in vitro plantlet regeneration protocol was developed from callus cultures of leaf, petiole and internode in P. tetragonolobus with combination of 2,4-D & Thidiazuron. Leaf callus show maximum percentage of plantlet regeneration in combination with 2,4-D + TDZ (1.5+0.5) petiole callus show moderate percentage of plantlet regeneration in combination with 2, 4-D+ TDZ, (1.5+0.75), internode callus show minimum percentage plantlet regeneration in combination with 2, 4-D+TDZ, (1.5+1.2). Leaf explant show maximum plantlet regenerated percentage (70-80) in combination with 2,4-D & Thidiazuron when compared to other explants such as petiole and internode.

Keywords: Callus, Plantlet regeneration, *Psophocarpus tetragonolobus*, explants

INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus*) commonly known as Goa bean, Four angled bean & Four corned bean, is present exploited legume plant¹. Winged beans can be grown as a green vegetable, tuber crops or forage cover crops², field crop in Myanmar and tuber production has become an important objective³. The winged bean contains significant amount of protein in all parts of the plant i.e. seeds, leaves, pods and roots all these are edible (Fig-1)^{4,5}. Protein content (29.65) in winged bean is comparable to soya bean (36.05) and higher than that of other pulses⁶. The young leaves can be picked and prepared as leaf vegetable curries & eaten raw. Tender leaves have higher protein content than mature leaves. Winged bean pods are most popular for protein⁷. As legume winged bean fix nitrogen, characterized by the ability to live in symbiotic relationship with a wide range of tropical *Rhizobium*⁸, it grows well in the hot humid equatorial countries where southeast Asia and the west pacific. In tissue culture successful application of *in vitro* methods is largely dependent on reliable organogenesis and regeneration system. In winged bean requires improvement in various characters such as plant architecture and reduction of anti nutritional factors in the seeds. Efficient *in vitro* regeneration systems are a free requisite for the application of genetic engineering and similar techniques. The present paper reports on plantlet regeneration via induced organogenesis from various explants (Leaf, Petiole & internode) of winged bean.

Abbreviation: MS Murashige and Skoog medium, 2, 4-D-dichlorophenoxyacetic acid, TDZ Thidiazuron, IAA indole-3-acetic acid & IBA, abscisic acid.

MATERIAL AND METHODS

Seeds of *P. tetragonolobus* variety NS 122 were collected from Nature Seeds Store, Malaysia, were surface sterilized with 90% ethanol for 1 minute, followed by treatment with 0.1% mercuric chloride for 10 minute. They were then rinsed thoroughly in sterile distilled water and germinated on MS basal media⁹ containing 0.8% agar under a 16h photoperiod at 25±1° C. Media were adjusted to p^H 5.6 - 5.8 before autoclaving at 121°C for 15 minute. Seed germinated within one week and after 10 days the explants were excised from the seedlings, cut transversely into 1-2 cm long sections and used as explants.

RESULT AND DISCUSSION

The present paper report on *in vitro* plantlet regeneration via indirect organogenesis pathway in leaf, petiole and internode explants cultured on MS+2,4-D, Kn, BAP, TDZ & IAA in their combinations of *P. tetragonolobus* variety NS 122, after six weeks of culture.

Callus was induced in leaf, petiole and internode explants on MS medium supplemented with different concentration of 2, 4-D (0.5-3 mg/L, IAA (0.5-3 mg/L) and IBA (0.5-3 mg/L) alone or in combinations with 2,4-D + Kn, 2,4-D + BAP and 2,4-D + TDZ in Winged Bean, after two weeks of culture. (Fig-2: a,d,g).

Plantlets regeneration from callus cultures:

Plantlet regeneration from callus culture of various explants such as leaf, petiole and internode respectively were observed in combination with 2,4-D & TDZ in winged bean.

Leaf callus show maximum percentage (70-80) of plantlet regeneration in combination with 2,4-D + TDZ (1.5+0.5). Petiole callus show Moderate percentage (40-55) of plantlet regeneration in combination with 2, 4-D+ TDZ, (1.5+0.75) Internode callus show minimum percentage (10-20) plantlet regeneration in combination with 2, 4-D+TDZ, (1.5+1.2) (Fig-2: c,f & i and Graph-1). Similar results were reported by Venketeswaran & Huhtinen,¹⁰ Venketeswaran *et.al.*,¹¹ Tran Thanh Van *et al.*,¹² Venketeswaran *et.al.*,¹³ using cotyledon explants for shoot organogenesis with different plant hormones in *P. tetragonolobus*. Bottino *et al.*, Venkateswaran *et al.*, reported multiple shoots regeneration from epicotyls explants.^{14, 13} Gregory *et al.*, reported multiple shoots regeneration from leaf explants,¹⁵ Mehta and Mohan Ram, reported multiple shoots regeneration from epicotyl explants¹⁶. Indirect somatic embryogenesis by Ahmed *et.al.*,¹⁷ Dutta,*et.al.*,¹⁸ Dutta,¹⁹. *In vitro* multiple shoot regeneration from nodal explants Naik and Naik.²⁰ Callus induction were reported by Chetna *et.al.*, in *P. tetragonolobus*²¹.

Graph-1: Graph represents maximum plantlet regeneration were observed in leaf callus in *Psophocarpus tetragonolobus* 2,4-D + TDZ (1.5+0.5)

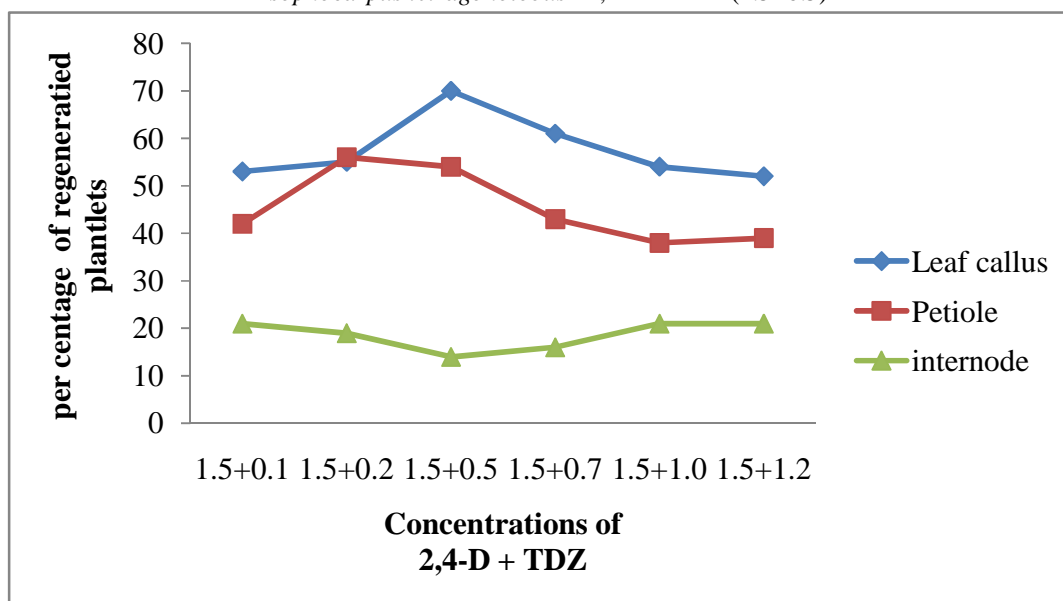
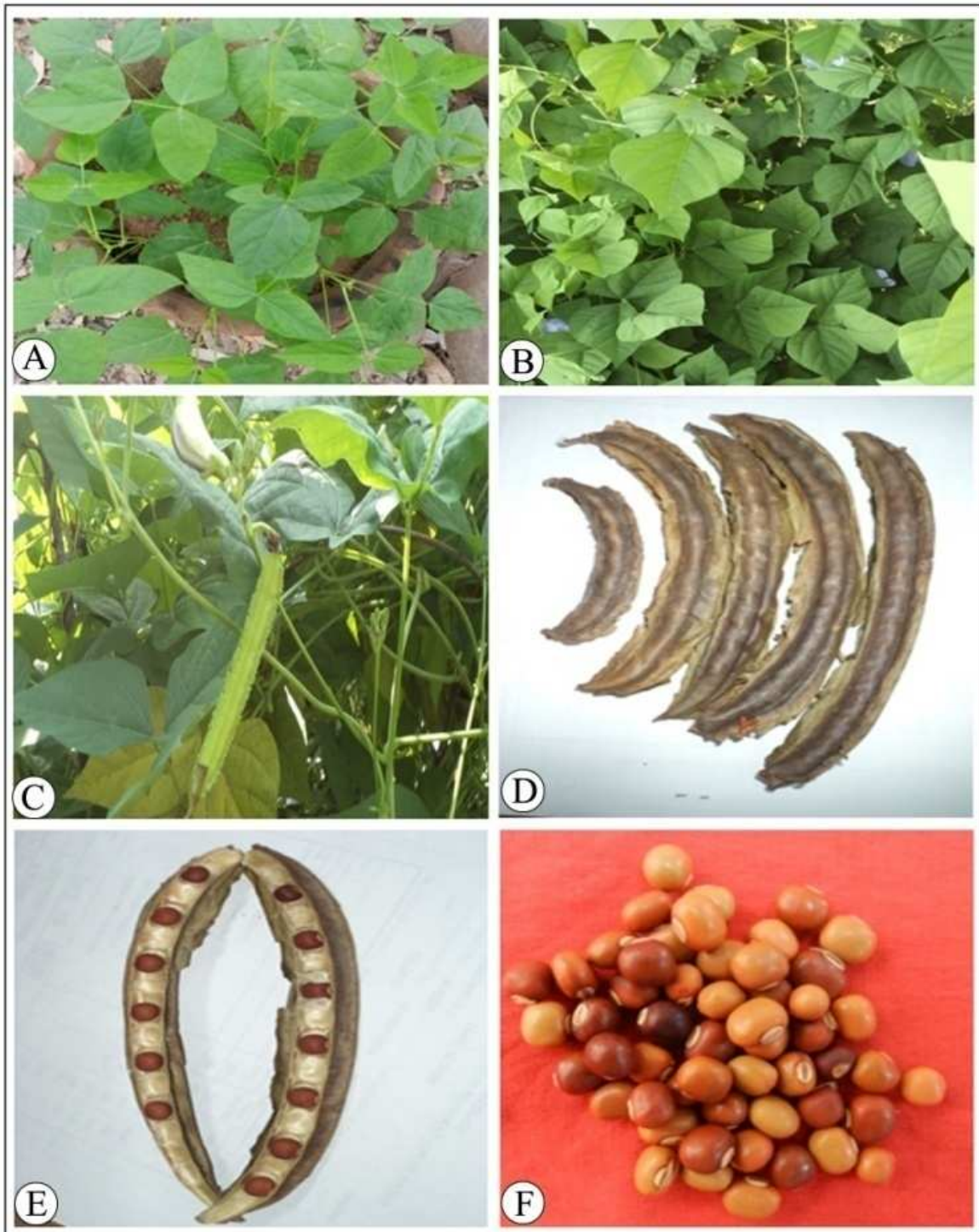


Fig.1:**Plant morphology**

- Fig. A.** One month old plant
- Fig. B.** Flowering plant
- Fig. C.** Pod with plant
- Fig. D.** Dry pods
- Fig. E.** Ripened dry fruit with seeds
- Fig. F.** Seeds

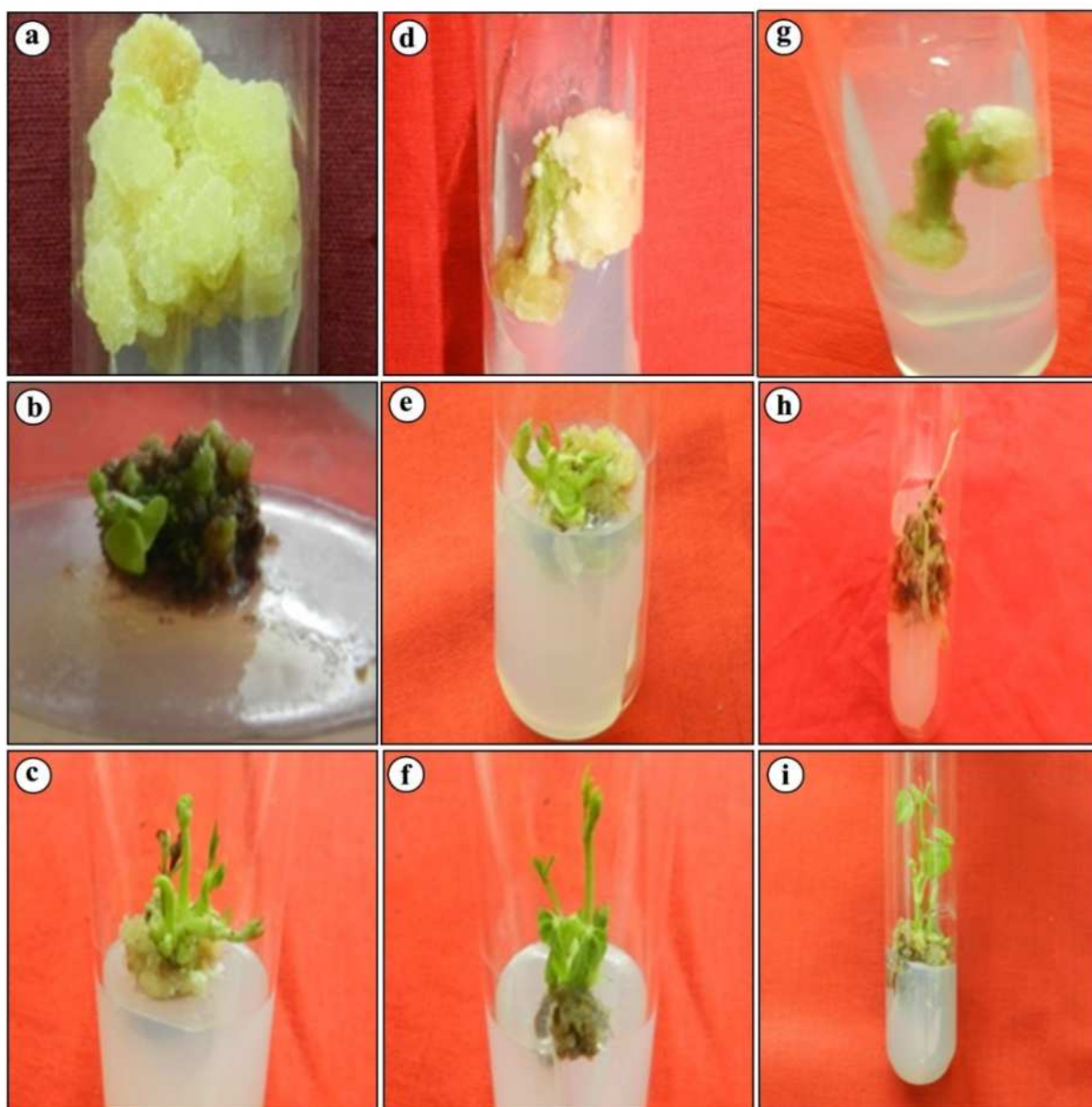
Fig.2: *In vitro* plant regeneration from Leaf, petiole & internode callus of *P. tetragonolobus*

Fig. a: leaf callus, Fig. b & c: regenerated plantlets from leaf (2,4-D + TDZ (1.5+0.5).), Fig. d: petiole callus, Fig. e & f: regenerated plantlets from petiole (2, 4-D+ TDZ, (1.5+0.25), Fig. g: internode callus, Fig. h & i: regenerated plantlets from internode (2, 4-D+TDZ, (1.5+1.2),

CONCLUSION

Leaf callus is the best material for plantlet regeneration in combination with 2, 4-D+TDZ, followed by petiole and internode explants in winged bean.

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