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Direct organogenesis from cotyledon explants of *Psophocarpus tetragonolobus* variety NS 122 (Winged bean)

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Abstract:
The tuber and seed contain a high rich in protein. Direct multiple shoot bud induction was developed in *Psophocarpus tetragonolobus* used as cotyledon explants. MS media supplemented with various concentration of TDZ alone or along with IAA. MS media +TDZ (2.0 mg/L) minimum number of shoot buds were induced. MS media supplemented with TDZ (2.0 mg/L) in combination with IAA (0.4 mg/L) induced dark green compact callus with maximum number of shoot buds were obtained.

Keywords: *Psophocapus tetragonolobus*, Cotyledon explants, Direct organogenesis, MS Medium, TDZ and IAA

1. Introduction:
Psophocapus tetragonolobus belongs to Papilionaceae family, Commonly Known as Winged bean, is a one of the under exploited legumes, it has been traditionally grown as back yard crops with most potential for easing the problem of protein malnutrition throughout the hot humid countries (Amoo *et al.*, 2006, NAS 1979) [1, 8]. All part of the plant i.e. Seeds, immature pods, leaves and tuberous roots are edible (NAS 1975) [7]. It is a commercially important cultivated crop recently has been distributed one tropical area to another (Maxted 1990) [4]. Tuber production is important objective in Myanmar (Eagletone, 1999) [3]. Food production is essential for increasing global population which can be augmented by new technique and developing genetically improved tissue cultures of a wide range of legume crop (Roychowdhury *et al*., 2012a) [11]. In tissue culture successful application for *in vitro* methods is largely dependent on reliable organogenesis and regeneration. The present study report on direct organogenesis from cotyledon explants of winged bean.

Abbreviation:
Psophocapus tetragonolobus, Murashige and Skoog (1962) [6] MS medium, Cotyledon explants, Thidiazuron (TDZ), Indole- 3 acetic acid (IAA).

2. Material and Methods:
Dry Seeds of *Psophocapus tetragonolobus* variety NS 122 were obtain from Nature Seeds Store, Malaysia, which are surface sterilized for 15 min in 20% Blue Ram Bleach with two drop of Tween 20 for 3-5 minutes, then rinsed thoroughly three times with sterile distilled water. The seeds were then soaked in sterile distilled water for 24 hour. Then the seed coat was removed. Cotyledon explants were cut into small pieces (1.0 cm) and cultured on MS media (Murashige and Skoog 1962) [6] supplemented with various concentrations of TDZ alone or in combination with IAA containing 0.8% agar (High Media) and 25 g/L sucrose under a 16h photoperiod at 25±1 $^{\circ}$C. All media were adjusted to p^H 5.8 before autoclaving at 121°C for 15 minute and incubate under aseptic condition.

3. Results:
The objective of present study was to develop a protocol for *in vitro* direct organogenesis with cotyledon explants of *P. tetragonolobus* variety NS 122 cultured on MS+ TDZ alone or

in combinations of IAA.

Shoot bud induction: Multiple shoot buds were induced after three weeks of culture from cotyledon explants of *P. tetragonolobus* variety NS 122. The culture medium is MS+TDZ (0.5 mg/L) alone, single shoot were induced. Maximum number of shoot buds (5.22±0.43), were observed form cotyledon explants on MS+TDZ (2.0 mg/L). The number of shoot buds initiated, depending on concentration of TDZ (Table-1). MS medium supplemented with TDZ (2.0 mg/L) in combination with IAA (0.4 mg/L), induced maximum number of shoot buds (11.20±0.32) with dark green compact callus were observed in cotyledon explants (Plate-1 & Table-2).

4. Discussion

In the present study, multiple shoot buds were induced directly from cotyledon explants, cultured on MS+TDZ (2.0 mg/L). This is the first report related to *in vitro* directed organogenesis from cotyledon explants of *P. tetragonolobus* variety NS 122. Shoot buds developed in cotyledon explants were associated with dark green compact callus obtained from MS medium with TDZ (2.0 mg/L) in combination with IAA (0.4 mg/L).

Similar results were reported in winged bean, Mehta and Mohan Ram (1981) [5] direct shoot bud induction from cotyledon explants of *Psophocarpus tetragonolobus* cultured on B_5+BAP (5×10^{-6} mg/L). Mature cotyledon Dias *et al*., (1986) [2], epicotyl (Venketeswaran *et al*., 1992, Vinayak Singh *et al*., 2014) [12, 13], callus mediated regeneration from leaf explants (D.S.R Naik *et. al*., 2015) [10], protoplast (Wilson *et al.*, 1993) [14]. Some related work reported Rao *et al.,* (2005) [9] multiple shoot induction from hypocotyls explants of cultured on MS+BAP in *V. radiata*.

Table -1: Induction of multiple shoot buds in cotyledon explants cultured on MS medium supplemented with various concentrations of TDZ, in *P. tetragonolobus*

Explants	MS+TDZ mg/L	Shoot Phenotype (Single or Multiple)	No. of Shoot buds
Cotyledon	0.5	Single shoot bud	1.12±0.31
	1.0	Multiple shoot bud	2.11±0.26
	1.5	Multiple shoot bud	4.23±0.21
	2.0	Multiple shoot bud	5.22±0.43
	2.5	Multiple shoot buds	2.40±0.32
	3.0	Multiple shoot buds	3.35±0.29

Table -2: Induction of multiple shoot buds in cotyledon explants cultured on MS medium supplemented with combination of TDZ+IAA, in *P. tetragonolobus*

Explants	MS+TDZ+IAA mg/L	Shoot Phenotype (Single or Multiple)	No. of Shoot buds
Cotyledon	0.5+0.1	Multiple shoot bud	3.22±0.11
	1.0+0.2	Multiple shoot bud	5.11±0.23
	1.5+0.3	Multiple shoot bud	8.13±0.24
	2.0+0.4	Multiple shoot bud	11.20±0.32
	2.5+0.5	Multiple shoot buds	6.21±0.43
	3.0+0.6	Multiple shoot buds	4.32±0.24

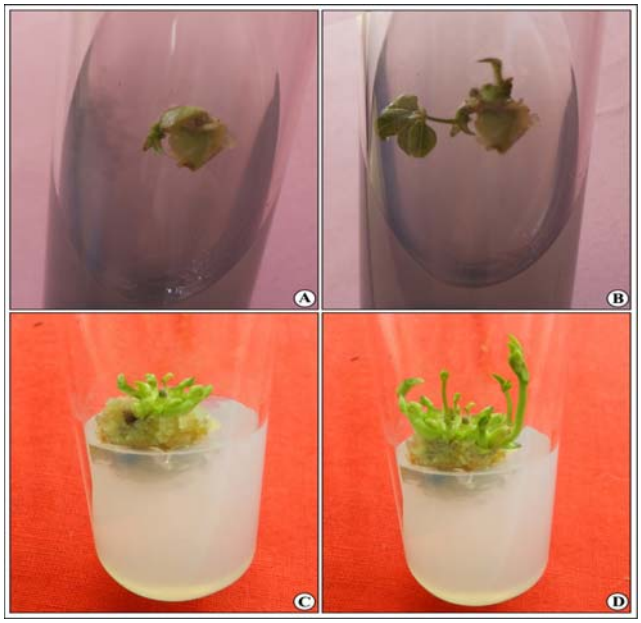

Plate -1: Multiple shoot buds induction from cotyledonary explants of *P. tetragonolobus*

A. Initiation of shoot but from cotyledonary explants
B. Formation of multiple shoot buds on MS+TDZ (2.0 mg/L)
C. Induction of multiple shoot buds on MS+ TDZ (2.0 mg/L) + IAA (0.4 mg/L)
D. Elongation of Shoot buds after 3 weeks of culture

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