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IN VITRO CALLUS INDUCTION IN HORSE GRAM (*DOLICHOS BIFLORUS* LINN.) GROWING ON HEAVY METAL POLLUTED SOIL

K. JAGPAL AND S. VASANTHA PILLAY

Department of Botany University College of Science
Saifabad Hyderabad- 500063. (T.S) India
Email: kjagpalbot@gmail.com
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The present study was carried out to analyze the effects of different growth regulators on formation of callus from leaf explants of Horse gram. Leaf explants of non-polluted area plant and industrially contaminated (Polluted) area plants were cultured on M.S. medium supplemented with various concentrations of 2,4-D/NAA/IAA. Culture was incubated at 25 ± 2°C temperature and photoperiod of 16 hours. The results indicated that Heavy Metals affects growth, texture and color of callus. The overall morphological response of the leaf explants indicated that the interaction of these factors leads to callus formation.

Key Words: *D.biflorus,* Auxins, Cytokinins, Callus induction, Growth hormones.

The Leguminosae is one of the most cosmopolitan natural order, the second largest of flowering plants containing between 6000 and 7000 known species. It comprises a vast number of genera which are sub-grouped into three different subfamilies, viz., Mimosoideae, Caesalpinoideae, Papilionoideae.The Papilionoideae with about three-hundred and seventy five genera is the largest subfamily and to it belong the majority of legumes of temperate regions of both the northern and southern hemispheres. This subfamily is composed of ten tribes and is characterised by the usually gamosepalous calyx and the papilionaceous corolla.

Horse gram (*Dolichos biflorus*) is a minor, under- exploited legume of tropics and subtropics grown mostly under dry-land agriculture. It is an important source of protein, iron and molybdenum. It has been identified as one of the potential food sources for the future by the US National Academy of Sciences (1979). It is extensively grown in India, mainly for animal feed. The use of dry seeds of horse gram as human food is limited due to its poor cooking quality, presence of high levels of enzyme inhibitors and heamagglutinin activities (Ray 1969). The seed is reported to be high in tannins and polyphenols compared to other legumes (Kadam and Salunkhe 1985). Fujie *et al.* (2007) studied the kinetic properties of dissolving phosphorus by the phosphobacteria.

The bio fertilizers are live microorganisms that are able to colonize the rhizosphere or internal tissues of the plants. They promote the growth of host plant through increase supply or availability of nutrients, such as nitrogen and phosphorus (Richardson *et al.* 2009). Bacteria with capacity to release phosphate from insoluble phosphorus forms by organic acid production and enzymatic activity (i.e. phytase), also known as phosphobacteria, are currently studied and commercialized to improve the growth, yield and quality of crops (Jorquera *et al.* 2008).

Heavy metals have played a great role in the genesis of present-day civilization. Heavy metals are deposited in soils. Toxic heavy metals are mostly absorbed and get accumulated in various plant parts and adversely affect plant growth and metabolism. Human beings are affected when these metals are incorporated into the food chain. Though many of these are essential plant nutrients, but they become phytotoxic at higher concentrations.

Present paper is focused on the callus culture of *Dolichos biflorus*. There are very few references about the micro-propagation of

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Dolichos biflorus. Thus, this protocol provides a method for callus induction of *Dolichos biflorus*.

MATERIAL AND METHODS

Dolichos biflorus leaf explants were collected from two different areas-grown plants (Control) of Non-polluted area and grown plants (Polluted) of the industrially contaminated area by four heavy metals-Manganese, Chromium, Nickel and Magnesium in Hyderabad city. These two leaf explants were used for induction of callus cultures separately. For this MS media with various concentrations of PGRs (Plant Growths Regulators) were prepared. The explants from control showed better response than polluted plant on MS medium (Murashige and Skoog 1962).

The callus induction medium consisted of M.S basal medium supplemented with 2,4-D/NAA/IAA (0.5-5.0mg/L), and 30 g/L sucrose, medium was solidified with 0.8% (w/v) Agar –Agar. For sterilization, medium was autoclaved for 20 min. All cultures were performed in culture test tubes. All chemicals used were provided by Hi-media. Explants were inoculated with their axes in contact with the callus induction medium positioned up right on 25-30 ml of solid agar M.S. Medium.

Data Analysis: At least 12 replicates were maintained for each treatment and data were recorded after 4 weeks of cultures. Each experiment was repeated at least twice with similar results and data presented are of one representative experiment. All the data were statistically analyzed.

OBSERVATION AND RESULTS

Callus induction ability of different explants such as healthy and polluted leaves were investigated by using varying concentrations of different auxins individually. Callus proliferation was initiated at the cut surfaces of the explants studied and later it covered the entire surface. Both color and texture of the callus also varied with growth regulators. The results are presented in Table-1 and shown in Plate 1. The leaf explants cultured on MS medium supplemented with different concentrations (0.5-5.0mg/L) of auxin such as 2,4-D/NAA and IAA, individually exhibited ignition of callus after 15 days of incubation while it took 12-15 days in leaf explants. Callus

Table 1: Morphogenetic response of Leaf explants (Control & Polluted) of Horse gram *Dolichos biflorus* L on MS medium with different concentrations of 2, 4-D/NAA and IAA

Hormone cone (mg/L)	% of cultures responding		Morphology		Callusing response	
2.4 – D	Control	Polluted	Control	Polluted	Control	Polluted
0.5	75	60	W. F	W. F	++	++
1.0	66	54	W. F	W. F	++	+
2.0	85	50	W. F	W.C	+++	+
3.0	90	45	W.F	W.C	+++	+
4.0	70	40	W.F	W.C	++	+
5.0	50	35	W. F	W.C	+	+
NAA						
0.5	73	58	W. F	W. F	++	+
1.0	80	50	W. F	W. F	++	+
2.0	82	48	W.F	W.C	++	+
3.0	86	43	W.F	W.C	+++	+
4.0	78	40	W. F	W.C	+++	+
5.0	70	38	W. F	W.C	++	+
IAA						
0.5	73	58	B.F	B.C	++	+
1.0	80	50	B.F	B.C	++	+
2.0	82	48	B.F	B.C	++	+
3.0	86	43	B.F	B.C	+++	+
4.0	78	40	B.F	B.C	+++	+
5.0	70	38	B.F	B.C	++	+

W. F= White Friable, W.C= White Compact, B. F= Brown friable, B.C= Brown Compact: - = No, + = low, ++ = moderate, +++ = high

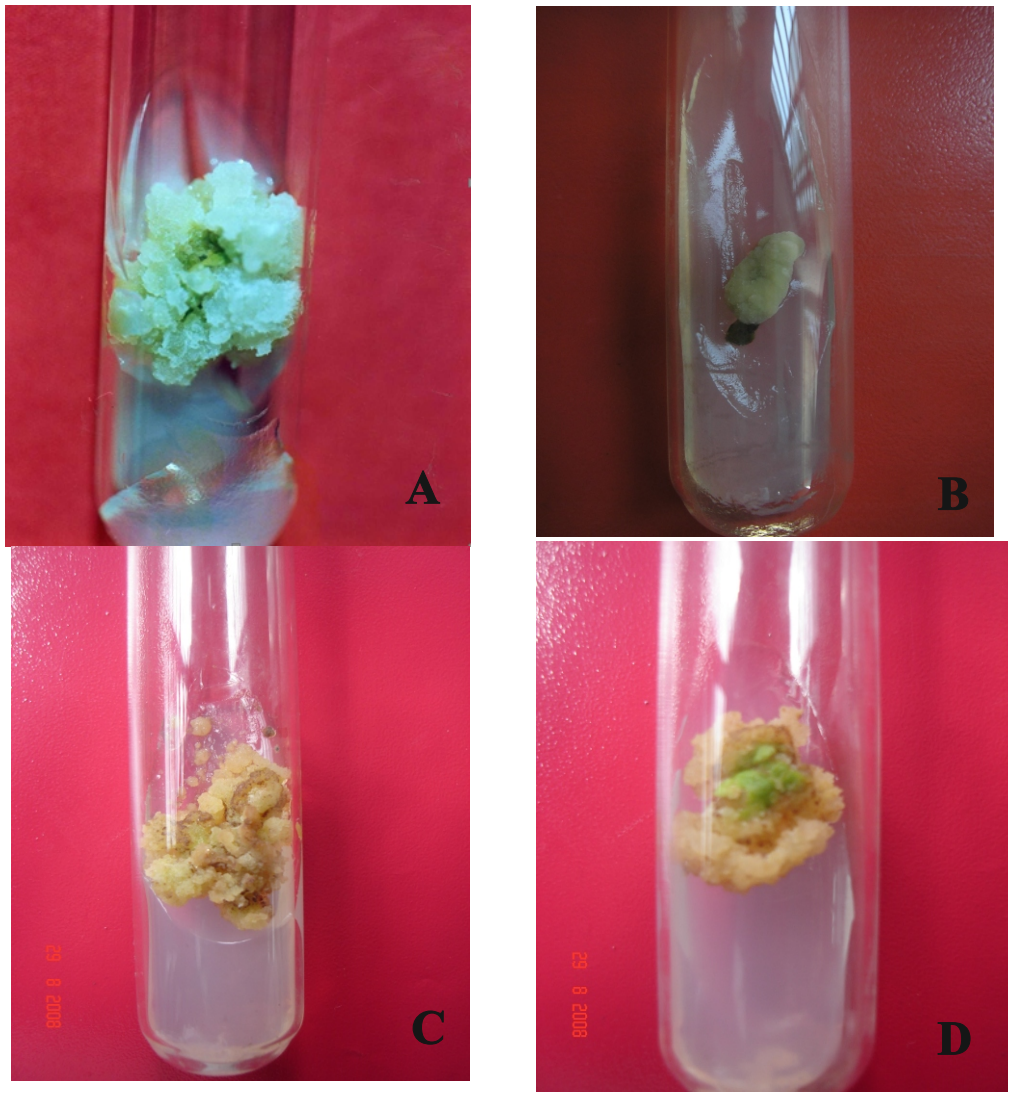

Plate 1 (A-D): Effect of heavy metals on *in vitro* callus induction in Horse gram Callus ability of *Dolichos biflorus* after 6 weeks of culture **A .** White friable callus on MS +1.0 mg/L 2,4-D from control leaf explants. **B.** White compact callus on MS +3.0 mg/L 2,4-D from polluted leaf explants **C.** Brown friable callus on MS + 3.0mg/L IAA from control leaf explants. **D.** Brown compact callus on MS+1.0mg/L IAA from polluted leaf explants.

proliferation was initiated at the cut surfaces of the explants studied and later it covered the entire surface.

Effect of 2,4-D: On 2,4-D supplemented medium early induction of callus was observed in all the concentrations of 2,4-D. 90% callusing response was recorded in control leaf explants at (3.0 mg/L) 2,4-D. Polluted explants callusing response decreases from 1.0 mg/L to 5.0mg/L. Morphology of callus was not similar at different levels of 2,4-D. White compact callus was induced at 2.0 to 5.0mg/L from polluted explants while 1.0 and 2.0mg/L, 2,4-D. induced White friable callus. In Control leaf explants, white friable callus was obtained in 2.0 and 3.0 mg/L, 2,4-D.

Effect of NAA: Effect of NAA on callusing ability leaf explants of both control and polluted is shown in Table-1. Highest percentage (86%) of response was observed at 3.0 mg/L NAA in control leaf explant culture. 50% callus percentage was observed at 1.0mg/L in polluted leaf explants. White compact callus was induced in 2.0-5.0mg/L

NAA in polluted leaf explants. The callus induction was moderate to high in all concentrations of NAA in Control leaf explants. White friable callus was observed at all concentrations of NAA in Control leaf explants. White compact callus was obtained in 3.0-5.0mg/L NAA in polluted leaf explants. (Plate-1).

Effect of IAA: Early induction of callus was observed in all the concentrations of IAA. 86% callusing response was recorded in control. High content of callus was obtained at 3.0 and 4.0 mg/L IAA in Control leaf explants. All the concentrations of IAA induced very low amount of callus in polluted leaf explants. (Plate-1). Brown compact callus was induced at 0.5to 5.0mg/L IAA from polluted Explants. Brown friable callus was obtained in all concentrations from control leaf explants

DISCUSSION

Callus was directly initiated from control and polluted leaf explants of *Dolichos biflorus* cultured on MS media supplemented with plant growth regulators (2,4-D, NAA and IAA). At high concentration of 2,4-D and NAA, the morphogenetic response was high in control leaf explants and low in polluted leaf explants. This difference in callusing ability suggests the presence of different levels of endogenous hormones in the tissue.

However, in the present investigation calli were initiated from leaf explants of *Dolichos biflorus* after four weeks of culture on MS medium fortified with different auxins-2,4-D, NAA and IAA (0.5-5.0mg/L). About 90% callusing response with white friable callus was observed in control leaf explants cultured on MS medium supplemented with 2.4-D (3.0 mg/l). Variations in the callus forming ability of different explant types, has been reported in many plants (Ishii *et al.* 2004). Among the auxins tested, 2.4-D induced the high yield of callus followed by NAA and IAA. Similarly, Omar (1988) observed the same findings with NAA in *Rhazya stricta* a medicinal plant. Skoog and Miller (1957) recorded the higher callogenesis with auxins and cytokinins both together act synergistically to promote either cell division or expansion depending upon other factors within the cell which reacts with these hormones (Settler field 1963).

Presence of 2, 4-D has been shown to be essential for callus formation in *Capsicum annum* (Gupta *et al.* 1990). NAA played an important role in callus formation in *Withania somnifera* (Kannan *et al.* 2005). The auxin 2,4-D is a potent callus inducing phytohormone in studies with *Capsicum* (Gunay and Rao 1978). *Cucumis sativus* (Rajasekharan *et al.* 1983). In the present investigation 2,4-D induced less amount of callus proliferation compared to other auxins. Praveen *et al.*(2001) have studied the callusing ability of different explants in *Strychnos prolatorum* on various growth substances viz. IAA, NAA and 2,4-D. They observed that the maximum callus growth on MS medium containing 2,4-D in contrast to our present findings.

Tejavathi and Bhuvana (1998) have also observed the callusing ability of different explants of *Solanum viarum* using auxins NAA and 2,4-D. Among these auxins NAA (2.0 mg/L) was found to be the best to induce callus from hypocotyl while 2.4-D (3.0mg/L) elicited callus formation from root, stem and leaf explants. Kumari and Kumar (1995) have observed the friable callusing and Rhizogenesis in the explants cultured on a medium containing IAA, NAA and 2,4-D at 1.0-25.0 μM ranges of concentration in *Thevetia peruniana.* Shahzad *et al.* (1999) have observed the callus induction on MS medium supplemented NAA (2.0mg/L) and 2,4-D (2.0mg/L) leaf cultures of *Solanum nigrum.*

Callus produced from different explants showed variability in texture, form and coloration. This difference is dependent to various growth promoting substances. Thus, successful callus induction depends upon various factors such as composition of the nutrient medium hormonal balance besides the

type, age and genotype of the explant (Hung and Murashige 1976; Narayana Swamy, 1977).

CONCLUSION

Horse gram is a plant with phytoremedi and commercial importance. It is a hyper accumulating plant and can survive in higher concentrations of heavy metals. This work provides a useful protocol for callus culture of *Dolichos biflorus* and its micro propagation which may be used to generate heavy metal tolerating *Dolichos biflorus* plants in more numbers. It is an excellent example of removal of heavy metal pollution from soil through phytoremediation.

Phytoremediation exploits natural plant mechanisms against the industrial pollution. Tissue culture studies on *D. biflorus* provide a rapid culture protocol in the form of *in vitro* propagation by callus culture. These types of tissue cultured plants could be used for phytoremediation of industrially contaminated soil. Most experiments used to establish phytoremediation techniques were done with hydroponic culture or plants grown on normal soil. In today's scenario, future efforts must be directed toward research to improve the performance of plants in remediation technologies.

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