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Induction of Different Morphological Types of calli from Leaf and Nodal Explants from *Pueraria tuberosa* (Roxb)

V. Sadguna, Md. Mustafa*

[1]Plant Tissue Culture and Molecular Taxonomy Laboratory, Deportment of Botany, Kakatiya University, Telangana State, India, Warangal – 506009, India

[2]Plant Tissue Culture and Molecular Taxonomy Laboratory, Deportment of Botany, Kakatiya University, Telangana State, India, Warangal – 506009, India

Abstract: ***For the development of an effective tissue culture system in Pueraria tuberosa leaf and node explants were used for the induction of callus. The explants were cultured on MS medium fortified with BAP, 2,4-D,Kn, IAA and NAA in different combinations. Interestingly different types of calli were formed. Bright green and compact green callus was formed from leaf explants on MS medium fortified with BAP + Kn combination and the growth of the callus was also recorded high. Green friable Embryogenic callus was observed from node explants on MS medium with BAP +IAA. Leaching of phenolic compounds was inhibited by increasing the concentration of sucrose from 25% - 35%. Both growth and nature of the callus was good from leaf explant, which is so useful for the regeneration studies and secondary metabolites production in further studies.***

Key words: MS medium, Callus, Nodular callus, friable callus, Phenolic compounds, Secondary metabolites.

1. Introduction

Pueraria tuberosa (Roxb. ex. wild.) DC belongs to Papilionaceae family, is the important Indian medicinal plant. The tuber of *Pueraria* is sweet in taste and used in indigenous system of Indian medicine as tonic, aphrodisiac, anti-rheumatic, diuretic and galactogogue and also used in Chywanprash, a popular tonic.

Pueraria tuberosa tuber was reported to contain Puerarin, daidzein, puerarone, coumestan, tuberosin, pterocarpintuberosin, puetuberosanol and hydroxytuberosone [1]. The isoflavones, Puerarin, are the most active constituent of *pueraria* tubers revealed wide range of pharmacological activities including hypoglycemic [2], anti-cancer [3], cardioprotective [4] neuroprotective [5], anti-allergic [6] and anti-arrhythmic [7] activity. Reproduction is primarily vegetative, threatened species by these grate efficacies urge. Present study describes the procedures for the establishment of different types of callus from leaf and nodal explants and callus growth in *Pueraria tuberosa*.

2. Material and Methods

Leaf and node explants of *Pueraria tuberosa* were collected from intact plant from our research field for tissue culture studies. These explants were thoroughly washed under running tap water for 10 minutes and surface sterilized with 0.1% $Hgcl_2$ for 7-8 minutes rinsed 4 times with sterilized distilled water. The sterilized nodes were cut into small pieces and inoculated on MS medium supplemented with BAP + 2,4-D, BAP +Kn and BAP individually in combination with 3.0% sucrose and 0.9% agar- agar, pH was adjusted to 5.8 and the medium was autoclaved at 121°c for 20 minutes. After inoculation these cultures were incubated at 25 ± 2°c under 16 hours day photoperiod for the induction of callus. These cultures were responded after one week of culture and results were recorded with different intervals of time after first subculture.

3. Results

The leaf and node explants of *Pueraria tuberosa* showed callus initiation after 7 days of inoculation and well developed callus was obtained after 20 days. Black and Brownish callus was obtained on MS medium supplemented with 2, 4-D and BAP from leaf explant and MS medium fortified with (1.0mg/l) 2,4-D + (1.0mg/l) BAP favored for the induction light green compact callus (Table-1,Fig a & b). However the callus become green by increasing the concentration of BAP from 1.0 mg/l to 2.0 mg/l, but callus turned to brown soon. Leaf explants positively responded on MS with BAP +Kn (Table-1). Callus was brown and white, but not black. Bright green friable callus was obtained on MS medium fortified with 1.0mg/l BAP +2.0 mg/l Kn. Green compact white callus was obtained on MS with 2.0mg/l BAP +2.0mg/l Kn (Table-1, Fig-c). MS medium supplemented with BAP alone did not show positive results. Whitish brown compact callus was obtained on MS supplemented with 2.0mg/l BAP (Table-1, Fig-d). Above all the three combinations (2.0mg/l) BAP + (2.0mg/l) Kn, is the best for the induction of regeneration callus from leaf explants and the calli obtained another combinations may be useful for the extraction of isoflavonoids.

Nodal explants responded differently on MS medium supplemented with the different combinations for the induction callus from node explants. Brown green compact callus was obtained on MS medium supplemented with NAA (1.0mg/l) + BAP (2.0mg/l), from the nodal explants (Table-1, Fig-e). Brown green compact callus was induced on MS medium with 1.0 mg/l IAA+ 1.0 mg/l BAP (Table-1, Fig-f). When the BAP concentration was increased from1.0 mg/l to 2.0mg/l blackish green callus was induced on MS medium with 1.0 mg/l IAA + 2.0 mg/l BAP (Table-1, Fig-f).

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However IAA (1.0mg/l) + BAP (2.0mg/l) favoured the formation of black green compact callus with higher percentage of response. Leaching of Phenolic compounds were more on this combination. Green nodular friable callus was obtained from the nodal explant on MS medium supplemented with 2.0mg/l BAP + 2.0mg/l Kn (Table-1, Fig-h). As the callus growth was significant obtained from leaf explant, the growth was measured after 28 days of subculture on the medium.

Table-1: Induction of different types of callus from Node of Pueraria tuberosa on MS medium fortified with different hormonal combinations.

2,4-D+BA P	% of response	Leaf explant Nature of callus
0.5 + 0.5	20	Black green compact callus
0.5 + 1.0	30	Light brown friable callus
0.5 + 2.0	50	Whitish, green compact callus
1.0 + 0.5	40	Blackish brown friable callus
1.0 + 1.0	**50**	**Light green compact callus**
1.0 + 2.0	70	Greenish brown compact callus
BAP +Kn		
0.5 + 0.5	20	Brown friable callus
1.0 + 0.5	30	Brown, white friable callus
2.0 + 0.5	50	White friable callus
0.5 + 1.0	30	Brown friable callus
1.0 + 1.0	40	Brown, green friable callus
2.0 + 1.0	52	Black, green compact callus
0.5 + 2.0	60	Whitish green friable callus
1.0 + 2.0	60	Light green compact callus
2.0 + 2.0	**80**	**Green compact callus**
BAP		
0.5	20	Blackish green friable callus
1.0	40	Brown friable callus
2.0	**60**	**White, brown compact callus**
Node explant		
NAA+BAP	**% of response**	**Nature of callus**
0.5 + 0.5	20	Brown friable callus
0.5 + 1.0	30	Blackish friable compact callus
0.5 + 2.0	42	Light green compact callus
1.0 + 0.5	54	Brown friable callus
1.0 + 1.0	60	White green compact callus
1.0 + 2.0	**74**	**Brown green compact callus**
IAA + BAP		
0.5 + 0.5	30	Black friable callus
0.5 + 1.0	40	Brown friable callus
0.5 + 2.0	40	Brown, green compact callus
1.0 + 0.5	30	Brown friable callus
1.0 + 1.0	50	Brown green compact callus
1.0 + 2.0	80	Blackish green compact callus
BAP + Kn		
0.5 + 0.5	20	Brown compact callus
1.0 + 1.0	30	Black and brown friable callus
1.0 + 2.0	52	Brown nodular callus
2.0 + 2.0	**60**	**Nodular green friable callus**

*Data was collected after four weeks of first subculture.

Callus growth:

Callus growth was recorded by measuring fresh and dry weight of callus on MS medium with BAP + 2, 4-D, BAP + Kn and BAP. Leaf explant derived callus was measured for fresh and dry weight as per procedure to measure the growth of the callus. The data was collected after the completion of four weeks. The high growth of callus was reported on MS medium fortified with 2.0mg/l BAP + 2.0mg/l 2, 4-D (Table – 2). Callus growth was higher on MS medium fortified with 2.0mg/l BAP + 2.0mg/l Kn. MS medium with BAP (2.0 mg/l) alone was also good. Among all the tested combinations MS medium with BAP + Kn was proved as the best for callus growth as per the fresh and dry weights.

Table - 2: Measurement of callus growth of leaf explant on MS medium fortified with by fresh and dry weight different hormonal concentrations in *Pueraria tuberosa* (Roxb).

MS medium with Hormones	I Week Mean + S.E		IV Week Mean ± S.E	
BAP + 2, 4-D	**Fresh weight (gm)**	**Dry Weight (gm)**	**Fresh weight (mg)**	**Dry weight (mg)**
0.5+0.5	0.14±0.05	0.013±0.04	0.19±0.09	0.013±0.07
1.0+1.0	0.15±0.05	0.014±0.06	0.21±0.11	0.012±0.05
2.0+1.0	0.18±0.08	0.017±0.05	0.26±0.18	0.014±0.10
2.0+2.0	0.20±0.10	0.020±0.08	0.29±0.21	0.015±0.12
BAP + Kn				
0.5+0.5	0.17+0.03	0.017±0.05	0.20±0.08	0.011±0.06
1.0+0.5	0.21±0.11	0.020±0.09	0.23±0.15	0.013±0.09
1.0+1.0	0.25±0.13	0.022±0.11	0.27±0.19	0.016±0.12
2.0+2.0	0.31±0.17	0.025±0.16	0.31±0.24	0.019±0.11
BAP				
0.5	0.16±0.09	0.016±0.09	0.19±0.08	0.013±0.01
1.0	0.17±0.11	0.015±0.11	0.20±0.13	0.015±0.14
1.5	0.18±0.13	0.017±0.14	0.21±0.16	0.017±0.17
2.0	0.21±0.14	0.020±0.17	0.24±0.21	0.018±0.15

*Data was collected after four weeks.

4. Discussion

The main effect of explant and plant growth regulators and their interactions were highly significant for the induction of callus. Greenish brown compact callus was achieved on MS medium fortified with 1.0 mg/l 2, 4-D + 2.0 mg/l BAP, when BAP concentration was reduced, light green compact callus was induced. The combination of BAP + NAA was better for callus induction from leaf explant. Thus 2, 4-D plays prominent role in the induction of green compact callus. Similarly MS medium supplemented with2.0 mg/l BAP + 2.0 mg/l Kn favoured the induction of green compact callus, which is coinciding the results of [8] , where hypocotyl explants of Cowpea exhibited the highest mean callus was induced on MS medium with Kn. However BAP 2.0 mg/l alone induced white compact callus soon turned to black. Green nodular compact callus was achieved from nodal explants of *Pueraria tuberosa* on MS medium with 1.0 mg/l NAA + 2.0 mg/l BAP. The embryogenic callus cultures were compact or friable, nodular and similar in appearance to those obtained for gramineous species [9]. Nodular green compact callus was obtained from nodal explants cultured on MS medium supplemented with 2.0 mg/l BAP + 2.0 mg/l Kn. Similar results have been obtained in soybean (Glycine

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max. L.) [10]. Similar to these finding callus was reportedly induced from cotyledon explants on MS basal medium containing 2, 4-D in combination with Kn + BAP [11]. Thus whatever type of callus is induced from any explant is not useful for regeneration and somatic embryogenesis but also useful in the extractions of medicinally useful secondary metabolites.MS medium supplemented with 2.5 mg/l BAP and 0.5 mg/l NAA was chosen as the best medium for leaf explants due to high fresh weight, callus percentage and its effective formation of embryogenic stages in combination of BAP and NAA [12], in *Onobrychis sativa*. The concentrations of 2, 4-D (18µM) + Kinetin (2.0µM) was sufficient to induce callus in 95% of cultures from leaf segments [13]. In our finding whatever different types of calli are formed may be useful for both further tissue culture studies and extraction of useful secondary metabolites in this plant.



Figure 1: Induction of different types of calli from Leaf and Node Nodal explants from *Pueraria tuberosa* (Roxb).

a & b Light green compact callus from leaf explants on MS medium with 1.0 mg/l 2, 4-D +1.0BAP
c. Green compact callus from leaf explants on MS medium with 2.0 mg/l BAP + 2.0 mg/l Kn
d. Whitish brown compact callus from leaf explant on MS medium with 2.0 mg/l BAP
e. Light green compact callus from node explant on MS medium with 1.0 mg/l NAA+ 2.0 mg/l BAP
f. Brown green compact callus from node explant on MS medium with 1.0 mg/l IAA + 1.0 mg/l BAP
h. Nodular green friable callus from node explant on MS medium with 2.0 mg/l BAP+ 2.0 mg/l Kn

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