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Regeneration of *Solanum nigrum* L. from organogenic callus obtained from young leaves

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Abstract : An efficient protocol was devised for rapid callus induction and plantlet regeneration from young leaves of *Solanum nigrum. For in vitro* callus induction auxins such as 2, 4-D, in combination with cytokinin BAP were used. High frequency of green compact callus was obtained in leaf explants cultured on MS medium supplemented with 0.25 mg/l 2, 4-D +2.0 mg/l BAP. The present study also describes successful plant regeneration from in vitro derived callus of young leaves. BAP alone or in combination with 2,4-D was used for regeneration of plantlets from callus culture. High frequency and maximum number of multiple shoots were induced on MS medium supplemented with 2.0 mg/l BAP + 0.25 mg/l 2,4-D.

Keywords : Callus induction, Plant regeneration, Leaf, Inter node, 2, 4-D, BAP, Solanum nigrum (L.)

Abbreviations: 2, 4-D- 2, 4-Dichloro phenoxy acetic acid; BAP- 6-benzylaminopurine .

INTRODUCTION

Plants produce various secondary metabollites of great therapeutic values. Dependence of human being of plant products is increasing continuously. There are many problems associated with extraction of phytochemicals from yield grown plants that include changes in environmental condition natural hazards, extinction of endangered species etc. Thus to overcome these factors, it become imperative to go for tissue culture of the plant to obtain round the year uniform production of secondary metabolite. Moreover, some novel compounds are produced in the cell cultures which are not produces in plants growing in natural condition.

Solanum nigrum L. (Black night shade) a member of Solanaceae is a common herbaceous plant distributed everywhere. It is cultivated as a food crop, both for its leaves fruits. *Solanum* genus comprises about 1400 species. All parts of the plant contain steroidal alkaloid of which solanine and solasodine glycosides are major component. Tartaric acid, citric acid and malic acid are identified as the major organic acids in *S. nigrum*. High concentration of Solanine, a glycoalkaloid is found in most plant parts, however its concentration is highest in unripe berries, (4). Other components include steroidal saponins called *solanigroside* (11), spirostanol glycoside, quercitin (3) etc. The plant extract exhibit anti viral, anti-cancer (2), antioxidant, hepatoprotective, anti-tumour properties (7), anti-convulsant, anti-ulcerogenic, anti-inflammatory and anti-hyperglycaemic potentiaI (4). *Solanum nigrum* is used by the tribes of Jharkhand for curing ulcers, stomach ache, gastritis etc. Considering the high economic and medicinal importance of secondary metabolites plant tissue culture techniques can be used first its large scale production. Thus, the aim of present work was to study callus induction and multiple regeneration of shoots from leaf explants.

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METHODOLOGY

The healthy disease free plants of *Solanum nigrum* were selected from botanical garden of Ranchi college, Ranchi. Young leaves from these plants were used for inoculation. Young leaf were surface sterilized with 0.1 % HgCl2 for 5 min and repeatedly washed with sterile water for 3-4 times before inoculation on MS medium supplemented with various concentration of cytokinins (BAP) and auxin (2,4-D). Inoculated culture tubes were then transferred to Culture Room under the standard conditions of temperature (25 ± 2ºC) for 16 hours of daybreak and 8 hours night break under the cool white fluorescent light. Observations were recorded after every 10-15 days. Shoot regeneration from buds was observed after 3weeks. Well developed elongated shoots were excised and cultured on root induction medium (0.2 mg/l of 2,4-D+ 0.5-1 mg/l of BAP).

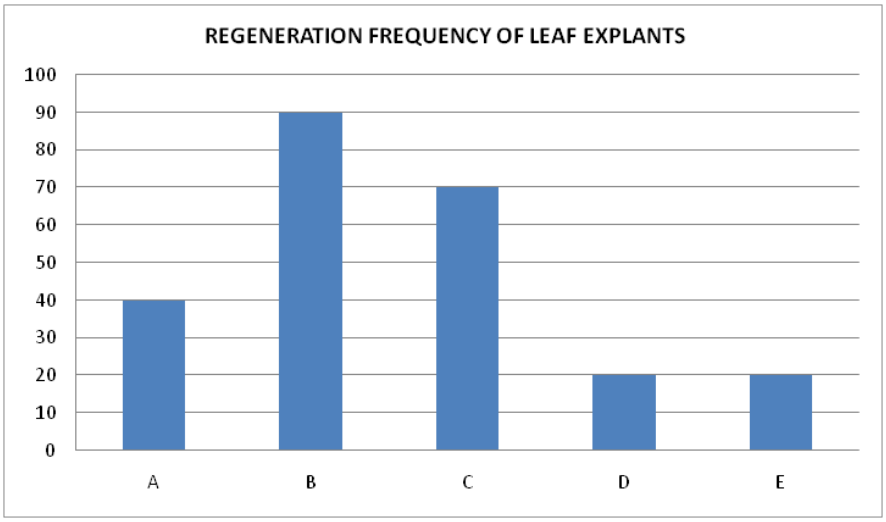
Plant growth hormone		Intensity of callus form	Nature of callus
2,4-D (mg/L)	BAP (mg/L)		
0	-	-	No callus formed
0.25	-	++	Brown callus
0.5	-	++	Dark brown callus
1.0	-	++	Dark brown callus
0.1	0.5	++	Compact yellowish green callus
0.2	1.0	+++	Compact green nodule callus
0.25	2.0	+++	Compact green organogenic callus.
0.5	1.0	++	Brown callus
1.0	1.0	+	Brown callus

Intensity of callus - + (low), ++ (moderated), +++(high)

Table 1 -Effect of different hormones on young leaf culture of Solanum nigrum.

Plant growth regulator		Regeneration frequency (%)	Mean no. of shoot per callus
2,4-D (mg/L)	BAP (mg/L)		
0.25	0.5	40	6
0.25	1.0	90	30
0.25	2.0	70	5
0.5	1.0	20	3
1.0	1.0	20	4

Table 2 -Effect of cytokinnin alone or along with 2,4-D on shoot regeneration .



Graph showing percentage regeneration of leaf explants cultured on the following media -

Where -

A = MS+2,4-D (0.25mg/l) +BAP (0.5mg/l)

B = MS+2,4-D (0.25mg/l) +BAP (1.0mg/l)

C = MS+2,4-D (0.25mg/l) +BAP (2.0mg/l)

D = MS+2,4-D (0.5mg/l) +BAP (1.0mg/l)

E = MS+2,4-D (1.0mg/l) +BAP (1.0mg/l)

RESULT AND DISCUSSION

Young leaf were cultured on MS media supplemented with various concentration of 2,4-D and BAP. Callusing was initiated two weeks after the inoculation.

Effect of auxin: cytokinin on callus induction

Callusing was observed on MS media supplemented with 2,4D alone or in combination with BAP (Table 1). Callus was formed along the cut portion of the leaves. Leaves showed infolding and callus were formed later. The calli obtained were of different kinds depending upon the combination of hormones. 2,4-D alone at lower concentration gave yellowish to white fragile callus(fig-a). At higher concentration of 2,4-D large brown calli (fig-b) were obtained. When in combination with BAP, the nature of callus changes from brown to light green (fig-c). Light green compact callus were obtained in explants cultured on MS supplemented with 2,4-D 0.25mg/ml + BAP 0.5 mg/ml and 2,4-D 0.25mg/ml + BAP 1 mg/ml). Interestingly media supplemented with 2,4-D 0.25mg/ml + BAP 1 mg/ml showed less callus formation as compared to media where BAP was 2 mg/ml.

Plant regeneration from callus

Well developed callus derived from leaves were sub cultured on fresh MS media supplemented with BA and 2,4-D (table 2). After two weeks of subculture, shoot emerged from the callus. Regeneration frequency was less (40%) at lower concentrations of BAP. Regeneration frequency enhanced to 90% at concentration of 1 mg/ml. High frequency of regeneration and maximum numbers of shoot (fig-e) were obtained at 1 mg/ml. Interestingly it was observed that the leaves kept on MS medium supplemented with 2,4-D 0.25mg/ml + BAP 1 mg/ml shows less callus formation and shows highest regeneration frequency in terms of number of shoots formed(fig-f). On the other hand leaves inoculated on MS media supplemented with 2,4-D 0.25mg/ml + BAP 2 mg/ml show more green and compact nodular organogenic callus. These nodular green calli when subcultured on 2,4-D 0.25mg/ml + BAP 1 mg/ml gave rise to many shoots. Cytokinin along with auxin plays very important role in callus formation and indirect regeneration in plants. Auxin alone results in callus formation. In 2,4-D alone medium prpfuse callusing has been seen. Callus is yellowish white at lower concentration of 2,4-D and turns brown at higher 2,4-D concentration. Brown colour is due to presence of phenolics produced by the cells. Explants inoculated on MS supplemented with 2,4-D and varying concentration of BAP showed light to dark green compact calli. Nodular organogenic calli were observed when BAP was 1mg/ml and 2 mg/ml. Presence of cytokinin alog with auxin is important for indirect organogenesis.

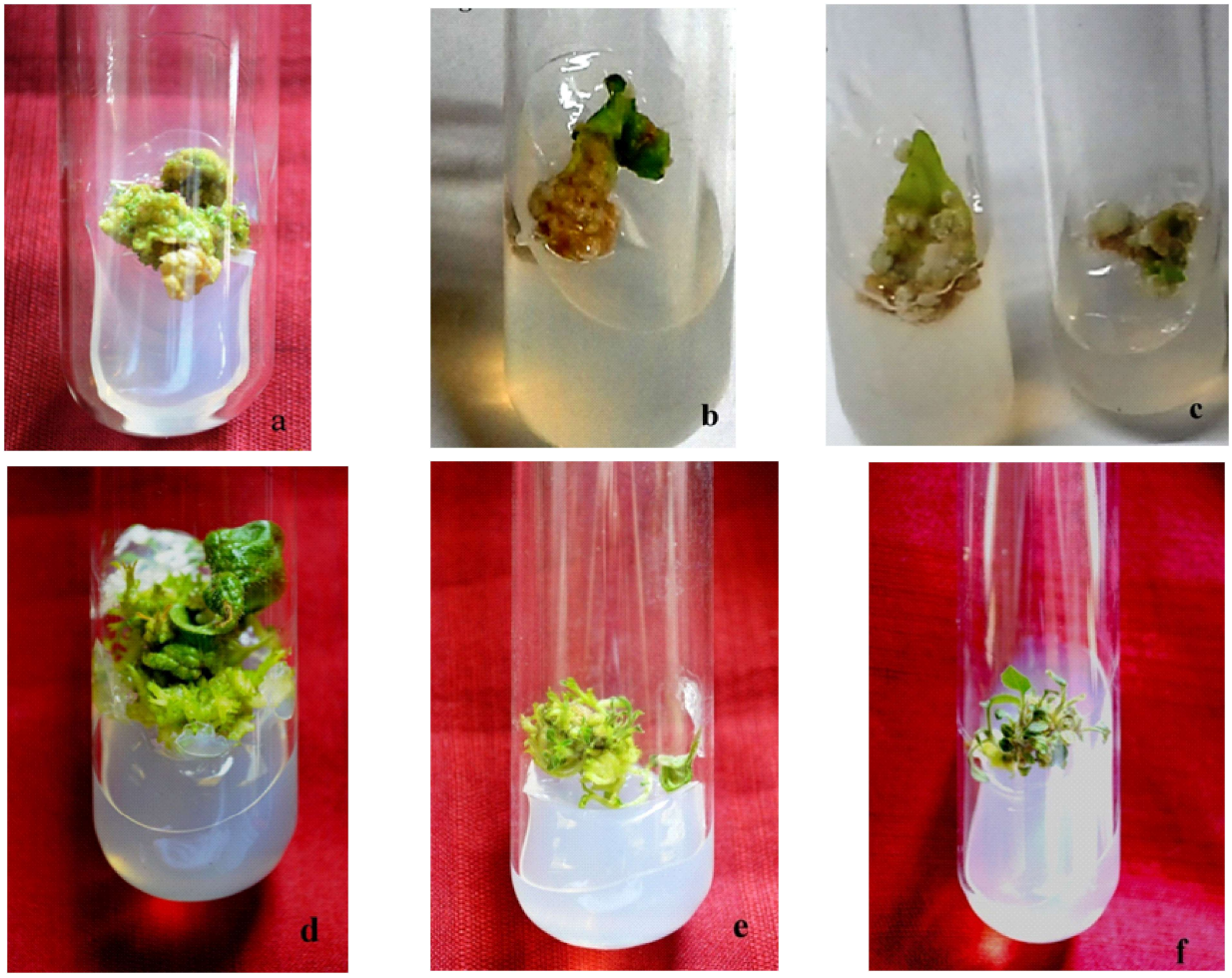


Fig a- yellowish green callus, Fig b- Brown callus, Fig c- brown and green callus
Fig d-compact green organogenic callus, Fig e and fig f -regenerating shoots

CONCLUSION

In the present investigation a protocol for rapid clonal propagation of Solanum spp. through mature leaves have been reported. As observed in the present study the young leaf may be used for the mass propagation of Solanum nigrum through micropropagation technique. The protocol promises a high regeneration and high survival rate for the large scale propagation of Solanum. These tissue culture raised plants may be utilized for production of secondary metabolites and it will be a more reliable, predictable and simpler process as compared to extraction from in vivo raised plants. Extraction from the in vitro tissues is much simpler than extraction from organized, complex tissues of a plant. Plant tissue culture techniques offer the rare opportunity to tailor the chemical profile of a phytochemical product, by manipulation of the chemical or physical microenvironment, to produce a compound of potentially more value for human.

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