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Research Article

An efficient protocol devised for rapid callus induction from leaf explants of *Biophytum sensitivum* (Linn)DC.

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Abstract

The Cell cultures are used extensively for *in vitro* secondary metabolite productions were obtained from callus tissue through cell suspension culture. The establishment of callus cultures has considerable potential for the production of known and novel secondary metabolites. The objective of the study was to scientifically assess callus culture of *Biophytum sensitivum* (L) DC. This was established from leaf explants with different growth regulators which greatly influenced the growth of callus cultures. The callus from leaf explants is induced by inoculating the young leaf bits on MS medium supplemented with various auxins (2, 4-Dichlorophenoxyacetic acid (2, 4-D), α-Naphthalene Acetic Acid (NAA) and Indole Buteric Acid (IBA), cytokinins (6-Benzyladenine (BA) Kinetin (KN) and cytokinin-auxin combination (BA+NAA) in different concentrations (0.5 to 5.0 mg/l) were used. BA 1mg/l, in combination with NAA (1.0 mg/l) also produced maximum amount of callus. So, this research concluded that the plant leaf explants cultured on MS medium with 1 mg/ l BA with 1 mg/l NAA was found most efficient for callus induction.
Keywords: *Biophytum sensitivum* callus, leaf explants, callus induction and growth regulators

1. Introduction

The Plant secondary metabolites have enormous potential for research and new drug development. The medicinal plants are the most important source of life saving drugs for the majority of the world population. A large number of medicinal plants are explored from the natural flora for the production of commercial drugs. It is a qualitative chemical evaluation, which indicates spectrum of chemical constituents present in a plant drug. Thus the phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc.[1] The qualitative analysis of the phytochemicals in the fresh plant tissue showed that phytochemical constituents such as alkaloids, anthroquinones, flavonoids, phenols, reducing sugars, saponins, phytosteroids and tannins are present in it. All the phyto components detected were known to support bioactive activities in medicinal plants.

Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests.[2] The accumulation of phytochemicals in the plant cell cultures has been studied from the past thirty years, and the generated knowledge helped in realizing the usage of cell cultures for production of desired phytochemicals.[3] Plants extracts of secondary metabolites have served as antioxidant in phytotherapeutic medicines to protect against various diseases for centuries.[4] So, cell suspension culturing is considered as one of the best approaches for studying the biosynthesis of natural products [5] and calli are the richest sources of cell mass when establishing such cultures.[1,6]

Biophytum sensitivum common name is life plant, little tree plant and sensitive plant belongs to Oxalidaceae. Attapatti, Chumi, Jala pushpa in Telugu, Lakshmana, Lajalu in Hindi. It possesses a wide spectrum of medicinal properties including positive effects in inflammatory diseases.[7,8] It possesses a wide spectrum of medicinal properties namely antiseptic properties, asthma, and anti-inflammatory activity.[9] The biological activity of the plant shows hypoglycemic,[10] immunomodulatory[11] effects. We previously reported that the *in vitro* pharmacological analysis using callus extracts of *B.sensitivum.*[12] Cell cultures which have been used extensively for *in vitro* secondary metabolite production were obtained from callus tissue through cell suspension culture. So, further going to production of secondary metabolites using with different cytokinin and auxin combinations of using leaf explants.

2. Materials and Methods

2.1 Plant material: The fresh matured 100 plants of *B.sensitivum* were collected from Acharya Nagarjuna University Campus, Guntur District, Andhra Pradesh, India during 2009-2010 and used as a source of explants. The leaf explants were excised into 1 cm long segments and were washed with liquid detergent (Teepol, Qualigens, India), Bavistin (1% w/v) for 3 min and mercuric chloride (0.1% w/v) for 1 min, followed by 70% ethanol. These are inoculated on Murashige and Skoog medium (1962)[13] supplemented with various concentrations and combinations of phyto hormones for induction of callus. The combination of NAA+BA (BA 1 mg/l, NAA 1 mg/l) produced callus.

2.2 Callus culture: The leaf explants were cultured on MS basal medium supplemented with various concentrations of BA+NAA for callus induction. BA 1.0 mg/l + NAA 1.0 mg/l is the best concentration for callus induction. After 30-60 days, old callus was collected and sub cultured

on fresh medium with same growth regulator combinations twice in four week time interval. All the cultures were incubated at 24±2º C under 16h photoperiod provided by cool white florescent lights.

2.3 Data analysis: All the experiments were repeated thrice with 15 replicates. The effect of different treatments was analyzed using one way analysis of variance (ANOVA), and means were compared using the Tukey test at the 0.05 level of significance.

3. Results

The callus from leaf explants is induced by inoculating the young leaf bits on MS medium supplemented with various auxins (2, 4-D, NAA and IBA), cytokinins (BA and KN) and cytokinin-auxin combinations (BA+NAA) in different concentrations (0.5 to 5.0 mg/l) (Table-1) were used. Results indicated that all the growth regulators alone not able to induce a callus from *Biophytum sensitivum* leaf explants. While the combinations of growth regulators show maximum callus production. Callus from leaf segments showed initiation of vigorous, proliferating, soft and green colored tissue.[14]

The results indicated that 1.0 mg/l BA and 1.0 mg/l NAA (Fig 1A) on MS medium induced high amounts of callus with high frequency of regeneration interms of their fresh weight and dry weight (Table 1), (Fig 7). 2, 4-D (0.5 mg/l) with 3.0 mg/l BA (Table 1), (Fig 1 B, Fig 8), 2.0 mg/l IBA and 2.0 mg/l NAA (Table 1), alone BA 3mg/l (Fig 2), alone KN 0.5 mg/l (Fig 3), 2 mg/l of NAA and 0.5 mg/l 2,4-D was suitable for callus induction. The leaf explants when planted on the MS medium containing the combination of BA and NAA the young moderate sized leaf explants were well responded for rapid callogenesis after incubation period of about 3- 4 weeks. Green and healthy compact callus observed after 3-4 weeks of inoculation with 1 mg/l BA and 1 mg/l NAA combinations.

Table 1: Effect of different concentrations of auxin and cytokinins on leaf callus induction

Plant Growth Regulators (mgL $^{-1}$)	Different concentr-ations (mgL $^{-1}$)		Nature of callus	Intensity of callus formation	Results	% of response	Fresh weight (mg)(SD)	Dry weight (mg) (SD)
Control	0	-	0	0	0	0	0	0
BA	0.5	-	Green callus, compact	+	Callus	23	230 ± 1(21)	22 ± 3(53)
	1.0	-	Green callus, compact	+	Callus	35	280 ± 1(32)	36 ± 2(10)
	2.0	-	Green callus, compact	++	Callus	42	320 ± 2(14)	40 ± 2(41)
	3.0	-	Green callus, compact	+++	Callus	58	440 ± 1(83)	42 ± 2(12)
	4.0	-	-	-	No Callus	-	-	-
	5.0	-	-	-	No Callus	-	-	-
KN	0.5	-	Soft green	++	Callus	55	560 ± 1(41)	62 ± 3(53)
	1.0	-	Soft green	+	Callus	44	480 ± 1(32)	56 ± 2(10)
	2.0	-	Soft green	+	Callus	41	360 ± 2(12)	50 ± 2(41)
	3.0	-	Soft green	+	Callus	32	230 ± 1(82)	42 ± 2(12)
	4.0	-	Soft green	+	Callus	27	200 ± 1(21)	24 ± 2(14)
	5.0	-	Soft green	+	Callus	32	280 ± 1(32)	26 ± 1(11)
IBA	0.5	-	Soft, green	+	Callus	32	220 ±1(51)	22 ± 3(53)
	1.0	-	Soft, green	++	Callus	45	340 ± 1(42)	36 ± 2(10)
	2.0	-	Soft, green	+++	Callus	58	520 ± 2(28)	40 ± 2(41)
	3.0	-	-	-	No Callus	-	-	-
	4.0	-	-	-	No Callus	-	-	-
	5.0	-	-	-	No Callus	-	-	-
NAA	0.5	-	Green compact	++	Callus	43	420 ± 1(31)	22 ± 3(53)
	1.0	-	Green compact	+++	Callus	57	580 ± 1(22)	36 ± 2(10)
	2.0	-	Green compact	+++	Callus	58	660 ± 2(24)	40 ± 2(41)
	3.0	-	Green compact	++	Callus	42	230± 2(12)	24± 2(16)
	4.0	-	-	-	No Callus	-	-	-
	5.0	-	-	-	No Callus	-	-	-
2.4-D	0.5	-	Light green, friable	+++	Callus	70	440 ± 1(0)	58 ± 2(10)
	1.0	-	Light green, friable	+++	Callus	65	360 ± 2(30)	50 ± 2(41)
	2.0	-	Light green, friable	++	Callus	52	240 ± 1(41)	32 ± 2(12)
	3.0	-	Light green, friable	+	Callus	47	200 ± 1(21)	24 ± 2(14)
	4.0	-	-	-	No Callus	-	-	-
	5.0	-	-	-	No Callus	-	-	-
BA+ NAA	0.5	1.0	Green, compact	++	Callus	52	320 ± 1(11)	28 ± 3(23)
	1.0	1.0	Green, compact	+++	Callus	82	1290 ± 11(26)	86 ± 2(25)
	2.0	1.0	Green, compact	++	Callus	42	860 ± 2(46)	20 ± 2(21)
	3.0	1.0	Green, compact	+	Callus	40	560 ± 1(12)	38 ± 2(10)
	4.0	1.0	-	-	No Callus	-	-	-
	5.0	1.0	-	-	No Callus	-	-	-
BA+2,4-D	0.5	0.5	Green, friable	+	Callus	43	330 ±1(41)	12 ± 3(23)
	1.0	0.5	Green, friable	++	Callus	55	450 ± 1(31)	26 ± 2(10)
	2.0	0.5	Green, friable	++	Callus	57	470 ± 2(24)	30 ± 2(21)
	3.0	0.5	Green, friable	+++	Callus	68	670 ± 1(14)	48 ± 3(43)
	4.0	0.5	Green, friable	+++	Callus	65	530 ± 1(15)	52 ± 2(35)
	5.0	0.5	-	-	No Callus	-	-	-

Values represent means ± standard error of 15 replicate per treatment in three repeated experiments. Mean followed by the same letter not significantly differently different by the Tukey Test at 0.05%. Intensity of callus: (+) low; (++) moderate; (+++) high.

Fig 1 (A) *In vitro* regenerated callus of *Biophytum sensitivum* after eight weeks of culture with BA 1.0 mg/l + NAA 1.0 mg/l. (B) Callus after eight weeks of culture with BA 3.0 mg/l + 2,4-D 0.5 mg/l

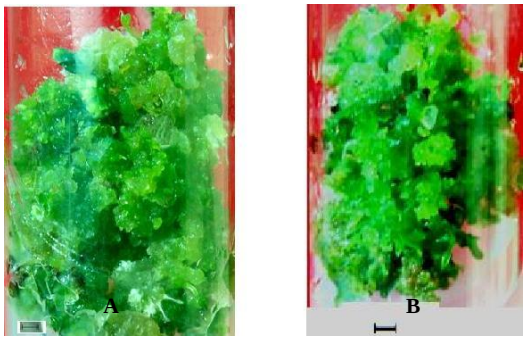


Fig 2: The callus growth variation with BA.

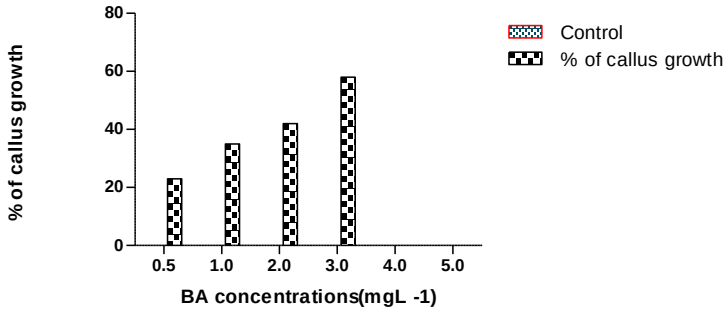


Fig 3: The callus growth variation with KN.

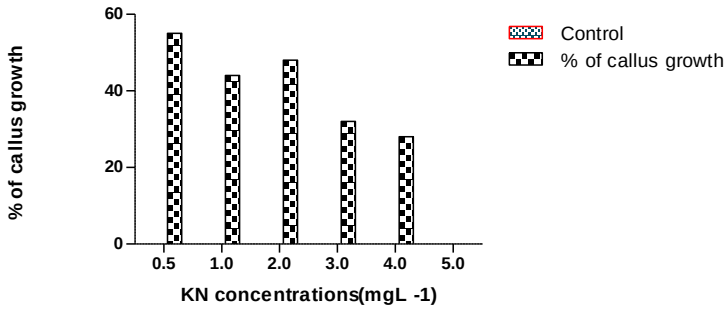


Fig 4: The callus growth variation with IBA.

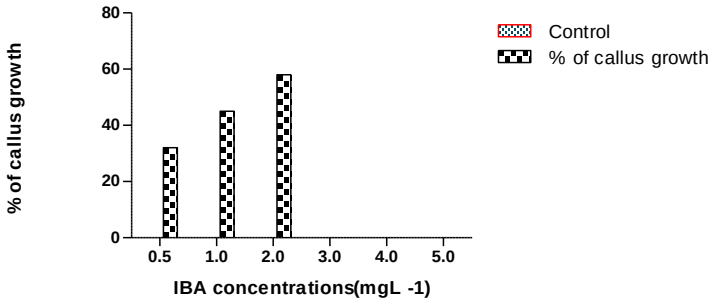


Fig 5: The callus growth variation with NAA.

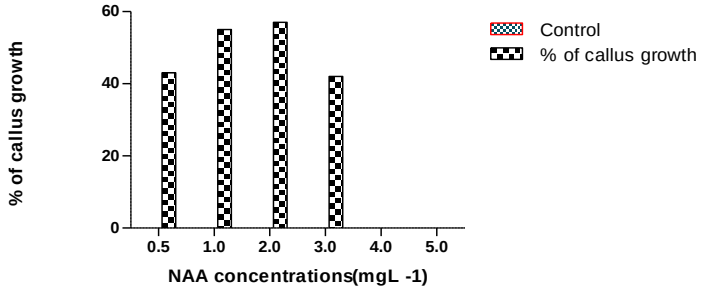


Fig 6: The callus growth variation with 2, 4-D.

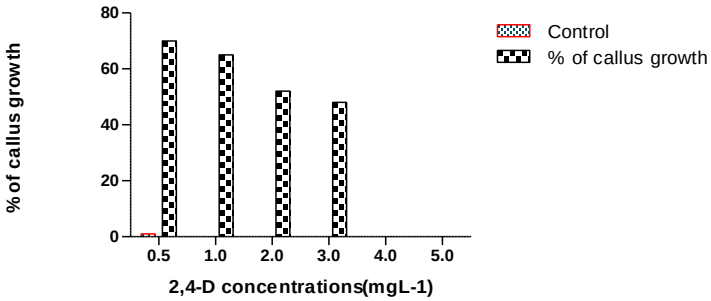


Fig 7: The callus growth variation with BA+NAA.

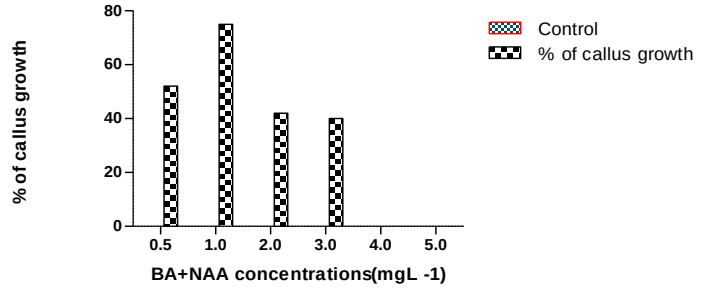
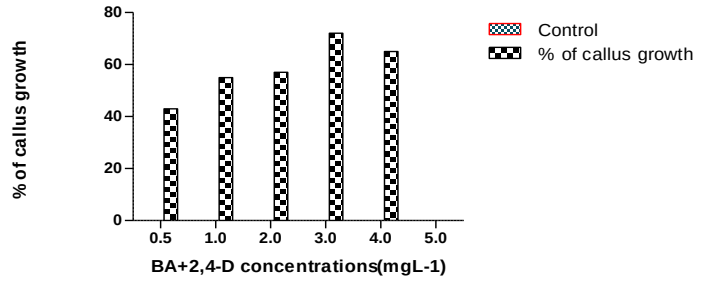


Fig 8: The callus growth variation with BA+2,4-D.



4. Discussion

Callus tissue is a good source of genetic variability and adventitious shoot formation. Regeneration of plants from callus tissue by organogenesis or somatic embryogenesis is an alternative method of shoot multiplication for cloning plant species.[15] *Biophytum sensitivum* is most important medicinal plant widely used in indigenous systems of medicine in India. Callus tissue was not uniform. Both friable and compact calli were obtained. Some were of fine texture and some were of nodular. These differences can be attributed to various factors like type of the explant, constituents of the medium and cultural environment. This is because of cells in callus cultures are undifferentiated and may not be under specific control.

The auxin and cytokinin ratio proved their importance for callusing in various explants. Further increase in the concentration of plant growth regulators did not show any significant improvement in callusing. These results agree with induced callus from the leaves of *Solanum tuberosum*.[16] Although cell cultures offer a suitable biological system in a controlled environment where in the morphogenic events can be maintained and regulated by growth regulators in the nutrient medium which shows a rapid production of plant metabolites of pharmaceutical value.[17]

5. Conclusion

In the present study, *in vitro* technique was chosen to develop and conserve this medicinally important plant for future. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents present in a drug or plant play a significant role in the identification of crude drug.

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