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An efficient *in vitro* callogenesis from various explants of *Passiflora vitifolia* kunth: An important medicinal and ornamental plant

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Abstract

An efficient *In vitro* protocol was established for callogenesis from various plant parts (Leaf, stem, flower bud and tendril) of *Passiflora vitifolia* Kunth. On Murashige and Skoog (MS) basal medium supplemented with various concentration of auxins (NAA, IAA, 2,4-D) and cytokinins (BAP and Kn). Different concentrations of auxins (0.5 to 4 mg/L), IAA (0.5 to 2mg/L), NAA (0.5 to 2 mg/l) and cytokinin BAP (0.5 to 2 mg/L), Kn (0.5 to 2 mg/L) were assessed for callus induction. Growth regulators when supplemented individually on MS medium produced hard white callus in stem and tendril explant, soft friable callus in leaf and flower bud explants. The callogenic response was more in leaf, stem and flower explant on MS media supplemented with NAA (1.5mg/L) and the callogenic response was more in tendril explants on MS media supplemented with 2,4-D (1mg/L).

Keywords: Callus; *Passiflora*; Flower bud; Auxins

1. Introduction

Plants have been associated with the health of mankind from time immemorial. In the past, sickness was viewed as a punishment from the gods and hence was treated with prayers and rituals that included what may have been considered 'magic potion' prepared from local herbs. Medicinal plants have been playing a vital role on the health and healing of man since dawn of human civilization. In spite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine (Farnsworth and Soejarto 1991). India is one of the mega diversity country in the world. It has 166 species of agro – horticultural crop plant and about 324 species of wild relatives of crop plants. The world health organization (WHO) has compiled a list of 20,000 medicinal plants used in different parts of the globe (Gupta and Chadha, 1995).

Passiflora comes from Latin word "Passio" that was first time discovered by Spanish discoverers in 1529 and was described as a symbol for "Passion of Christ" (Kinghorn,2001; Dhawan etal.,2004). The genus Passiflora, comprising about 400 species, is the largest in the family Passifloraceae (Montanher et al., 2007;Be-ninca etal.,2007). A large genus of herbaceous or woody tendril climber, mostly distributed in the warm temperate and tropical regions of the world (Beninca etal.,2007). Many of the species are of ornamental value and a few are cultivated for their edible fruits. *Passiflora vitifolia*, the perfumed passion flower, is a species of *Passiflora*, native to southern Central America (Costa Rica, Nicaragua, Panama) and northwestern South America (Venezuela, Colombia, Ecuador, Peru). It is a vine with cylindrical stems covered in red-brown hairs when young. The leaves are serrate, three-lobed, up to 15 cm long and 18

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cm broad. The lobed leaves' resemblance to grape leaves gives this passion flower its specific epithet, "*vitifolia*" meaning "grape leaves" after the Latin for grape "*vitis*." The flowers are bright red, up to 9 cm diameter. The fruit is a berry 5 cm long and 3 cm broad, with green flesh speckled with white, slight downy hairs, containing numerous seeds. The fruit is quite sour still when it falls off the plant and can take a month to ripen to its full flavor of sour strawberries. Due to the fragrant fruit, it is in small-scale cultivation in the Caribbean for edible fruits.

2. Material and methods

2.1. Collection of plant materials

Passiflora vitifolia plant materials were collected from the Botanical Garden of Mysore university campus.

2.2. Preparation of media for callus induction

Stock solutions were prepared for MS media, by weighing the respective chemicals and dissolved in a known volume of distilled water and the solutions were stored in a refrigerator. For media preparation required amount of sucrose and growth regulators were added to the stock solution and allowed to dissolve. pH of the media adjusted to 5.6-5.8 using 0.1 N NaOH or 0.1 N HCL. Agar-agar was dispersed into sterile culture tubes, flasks or bottles after thorough mixing (Murashige and Skoog, 1962). All the media were sterilized in a pressure cooker for about 20 min at 15 PSI. The basal MS media supplemented with different concentrations of auxin (NAA, IAA, 2,4-D) Cytokinin (BAP and Kn) at 0.5 to 2 mg/l were used for callus initiation.

2.3. Surface sterilization of explants

The leaves, stem, tendril and flower buds were used as explants. The explants collected were thoroughly washed in running tap water for ten minutes in order to remove the surface debris. Then explants were soaked in liquid detergent (Labolene) for five minutes and rinsed twice with sterile distilled water, again the explants were immersed in 1% of bavistin (Antifungal agent) solution. Further surface sterilized with 0.1 % mercuric chloride solution for about 2-3 min and finally rinsed with sterile distilled water for three to five times.

2.4. Inoculation and incubation

The surface sterilized explants were brought into the incubation chamber and washed with sterile distilled water 3-4 times to remove the traces of surface sterilent. Inoculation instruments were transferred to the alcohol swabbed inoculation chamber and were irradiated with UV-light for about 20 min. before inoculation. The explants were inoculated to the culture media near the flame. After transferring the explant, the inoculated tubes were sealed with non absorbent cotton and labeled appropriately before incubation. All the inoculated tubes were incubated in inoculation chamber temperature 25±2 ℃, relative humidity 60 to 70%, under the photoperiod with the illumination of 3,000 lux cool white fluorescent light maintained for the callus induction.

3. Results

3.1. Response of explant on basal medium

Explants remained dull on B5 basal medium. A response viz. leaf curling was noticed on MS medium was selected to carry out further work. Different growth regulators were supplemented to the medium.

3.2. Callogenesis

For the induction of callus from the selected plant species, different sterilized explants were inoculated on MS media, supplemented with auxins like 2,4-D, IAA, NAA and cytokinins like BAP and Kn in different concentrations andcombinations. The experiment was carried out in different modes of hormone supplementation and the explants responded better on MS medium compared to B5 medium.The frequency of the response of leafandstem explants were considered to be the best for callogenesis compared to response of tendril and flower bud. The MS media fortified with 2,4-D (1-1.5 mg/L), IAA(1mg/L), NAA(1.5mg/L), BAP (1.5mg/L) andKn (1mg/L) were found to the more suitable for the callus induction.

There was no callus proliferation evident during first few days (fig. 1A). After 9 days of incubation, callus initiation was observed from both the surfaces of the leaves and stem, (fig. 1,B C and D) profused callus was observed after 25 days of inoculation from both the explants. The maximum callogenesis response was observed in leaf and stem explants at a

concentration of 1-1.5mg/L (2, 4-D) and1.5mg/L (NAA)respectively. Initiated calli were observed for its colour and nature under a stereo binocular microscope. Observations were recorded and tabulated. Those which showed callusing was observed for the amount of callus proliferated. This was done by a scoring method where the fresh weight of the callus is used as the criterion.' - ' represents no callusing, '+' represents Low callusing,++ = Good, +++ = Very good, ++++ = excellent. The percent response of explants to callusing was calculated using the relation.

$$\text{Percent Response} = \frac{\text{No. of callus initiating explants}}{\text{No. of explants inoculated}} \times 100$$

3.3. Response on MS medium supplemented with growth regulators

3.3.1. Response on medium supplemented with 2, 4-D

MS medium was fortified with 2, 4-D at a concentration ranging from 0.5 to 4 mg/L. Leaf explants showed curling after one week of incubation. Subsequently, callogenesis was observed from the vein and cut ends of the leaf lamina. Brown translucent callus of non-friable nature was noticed .Callus induction was observed from internodal explant after three weeks of incubation (Fig. E, F, G and H). 2, 4-D at 1mg/L resulted in low callusing whereas 1.5mg/ml of 2, 4-D resulted in good callusing from stem explants. Callus from stem explant was brown and non-friable. Callus induction was observed from flower bud and tendril after three weeks of incubation supplemented with 1.5mg/l of 2.4-D (Fig. G - L).

Table 1 Response on medium supplemented with 2,4-D

Growth regulator	Concentration (mg/l)	Leaves		Stem		Flower bud		Tendril	
		Callus formation (%)	Response	Callus Formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response
2,4-D	0.5	50.0	++	45.0	++	65.0	+++	65.0	+++
	1.0	66.66	+++	66.0	+++	100.0	++++	100.0	++++
	1.5	87.0	++++	100.0	++++	85.0	++++	85.0	++++
	2.0	70.0	+++	55.0	++	55.0	++	55.0	++
	3.0	55.0	++	33.0	+	-	-	-	-
	4.0	33.33	+	-	-	-	-	-	-

Note: '-' no callusing, '+' represents Low callusing, ++ = Good, +++ = Very good, ++++ = excellent.

3.4. Response on medium supplemented with IAA

Table 2 Response on IAA supplemented medium

Growth regulator	Con. (mg/L)	Leaves		Stem		Flower bud		Tendril	
		Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response
IAA	0.5	-	-	-	-	30.0	+	-	-
	1.0	32.0	+	66.66	+++	-	-	-	-
	1.5	70.0	+++	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-

Note: '-' no callusing, '+' represents Low callusing,++ = Good, +++ = Very good, ++++ = excellent.

MS medium was supplemented with IAA at the concentrations ranging from 0.5 to 2 mg/L. leaf explants showed curling after ten days of incubation. Subsequently, callogenesis was observed from the cut ends of the leaf lamina at 1.5 mg/L. Brown translucent callus of non-friable nature was observed. Callus induction was observed from internodal explant

after two weeks of incubation. IAA at 1 mg/L resulted in meager callusing where as 1mg/L of IAA resulted in good callusing from stem explants. Callus from stem was brown and non-friable. Callus induction was not observed from flower bud and tendril.

3.5. Response on medium supplemented with NAA.

MS medium was supplemented with NAA at a concentrations ranging from 0.5 to 2.0 mg/L Leaf explants showed initial response after ten days of incubation. Subsequently, callus development was noticed from the margins of the leaf lamina at 1.5mg/L. Brown translucent non-friable nature was noticed in the callus. In internodal explants, callus induction was observed after two weeks of incubation. NAA at 1.5 mg/L resulted in promising callusing where as 1 mg/L of NAA resulted in moderate callusing from stem explants. The nature of callus from stem explant was brown and non-friable. There was no callus induction from flower bud and tendril in the medium supplemented with NAA.

Table 3 Response on supplemented medium NAA

Growth regulator	Concen-tration (mg/l)	Leaves		Stem		Flower bud		Tendril	
		Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response
NAA	0.5	30.0	+	70.0	+++	-	-	-	-
	0.1	57.14	++	80.0	+++	53.33	++	-	-
	1.5	100.0	++++	100.0	++++	100.0	++++	-	-
	2.0	55.0	++	45.0	++	76.0	+++	-	-

Note: '-' no callusing, '+' represents Low callusing,++ = Good, +++ = Very good, ++++ = excellent.

3.6. Response onsupplemented medium BAP

MS medium was supplemented with BAP at a concentrations ranging from 0.5 to 2.0 mg/L leaf explants showed early responsce after ten days ofincubation. Subsequently, callus development was observed from the vein and cut ends of the aboral surface of theleaflamina was noticed from 1,1.5 and 2.0 mg/L. Brown, translucent and non-friable type of callus was noticed . Callus induction was observed from internodal explants after two weeks of incubation. BAP at 0.5mg/Lresulted in excellent callusing where as 1mg/L of BAP resulted in good callusing from stem explants. Callus induction was observed from flower bud at 0.5, 1.0 and 1.5 concentrations, But high level of callus induction was seen in 1.5mg/L concentration. There was no callus induction fromtendril explants.

Table 4 Response on BAPsupplemented medium

Growth regulator	Concentration (mg/l)	Leaves		Stem		Flower bud		Tendril	
		Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response
BAP	0.5	28.57	+	75.0	+++	50.0	++	-	-
	1.0	42.00	++	57.14	++	62.0	+++	-	-
	1.5	60.0	++	-	-	75.0	+++	-	-
	2.0	57.14	++	-	-	-	-	-	-

Note: '-' no callusing, '+' represents Low callusing,++ = Good, +++ = Very good, ++++ = excellent.

3.7. Response on medium supplemented with Kinetin.

MS medium was supplemented with Kn at a concentrations ranging from 0.5 to 2.0 mg/L. leaf explants showed curling after a weekof incubation. Subsequently, callus growth was observed from the vein and cut ends of the leaf lamina at Kn concentration of1,1.5 and 2.0 mg/L. Callus induction was observed from internodal explants after two weeks of

incubation. Kn at 1mg/L resulted in very good callusing from stem and leaf explants. Callus from stem and leaf was brown and non-friable. There was no callus induction from flower bud and tendril.

Table 5 Response on Kn supplemented medium

Growth regulator	Concentration (mg/l)	Leaves		Stem		Flower bud		Tendril	
		Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response	Callus formation (%)	Response
Kn	0.5	33.33	+	30.0	+	-	-	-	-
	1.0	66.0	+++	57.14	++	-	-	-	-
	1.5	56.66	++	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-

Note: '-' no callusing, '+' represents Low callusing,++ = Good, +++ = Very good, ++++ = excellent.

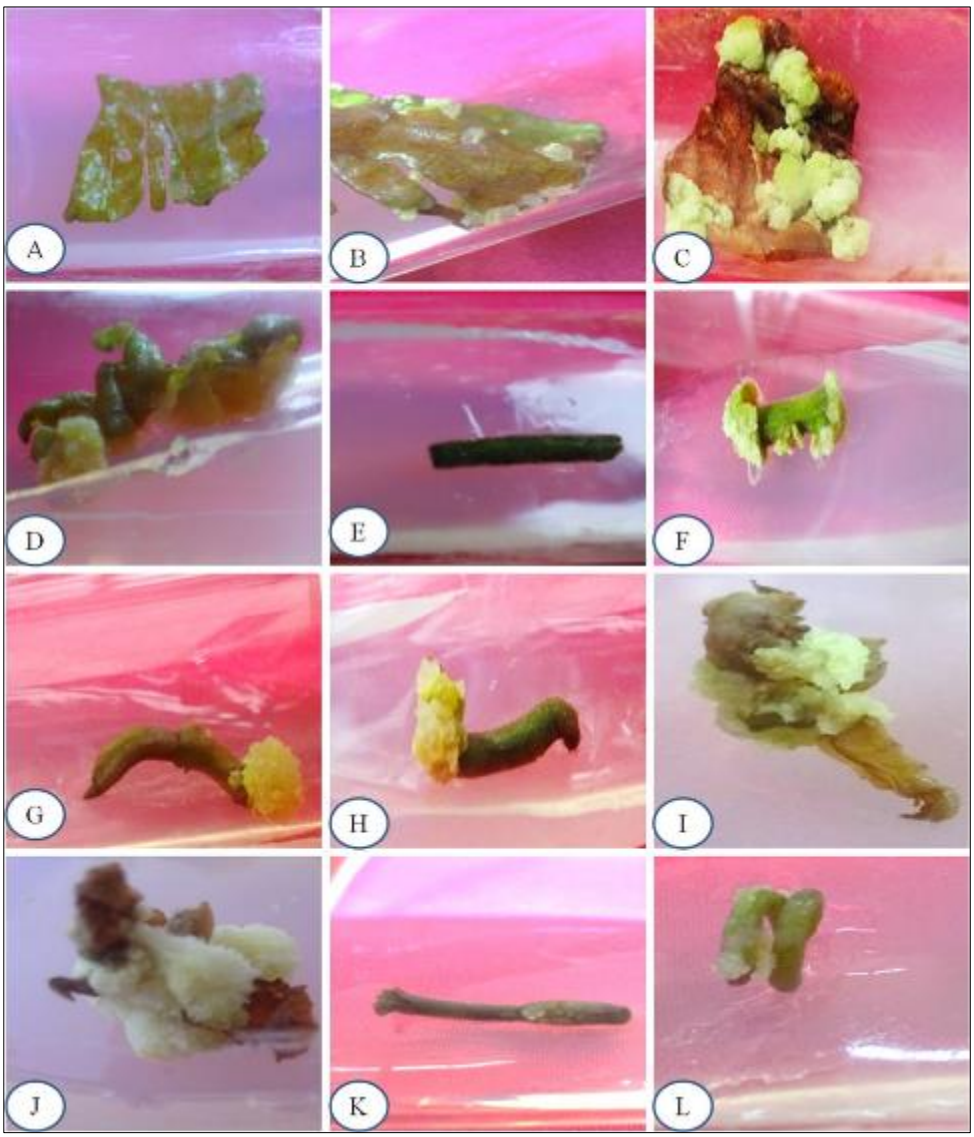

Figure 1A: Leaf inoculation on MS basal medium +24-D(1mg/L). 1B: Leaf curling on MS basal medium + NAA (1.5mg/L). 1C&1D: Brown translucent nature ofnon-friable callus from leaf on MS medium + NAA(1.5mg/L). 1D: Brown translucent nature of non-friable callus from leaf on MS medium + BAP(1.5mg/L).1E: Inoculation of internode

on MS medium. 1F: Light Brown and non-friable callus from internode on MS medium +2,4-D(1.5mg/L). 1G&1H : Brown and non friable Callus initiation on MS medium. 1I: Callus initiation from flower bud on MS medium+2,4-D (1.5mg/L). 1J: White and non-friable callus from flower bud on MS medium + 2,4-D(1.5mg/L). 1K: callus intiation from Tendril on MS medium + 2,4-D (1mg/L)& 1L: White and friable callus from Tendrilon MS medium +2,4-D (1mg/L).

4. Discussion

Tissue culture consists of growing plant cells as relatively on organized masses of cells on agar medium (callus culture) or as a suspension of free cells and small cell masses in a liquid medium (Archana *et al.,* 2015). Plant tissue culture is the technique of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. Plants tissue culture give various benefits to human being such as it can produce many copies of the true to true type plants, which may be used to produce plants with better flowers, odors, fruits or any other properties of the plants. Several endogenous and exogenous factors on the plant were effective in callus formation and growth. The chemical factors, mineral elements and growth regulators are the most important growth factors that influence plant growth initiation and differentiation. In cell culture, growth and morphogenesis are similarly controlled by the type and concentration of plant hormones and the mutual relationship between the hormones (Monemi et al., 2014).

The plantlets used in tissue culture are very small and their growth requires an exogenous sugar in the cultural medium. Although the plantlets may appear "fully functional'' physiologically, they are unlikely to be actively photosynthesizing-simply because it is unnecessary. Even if chlorophyll is present in the leaves, it is probable that the enzymes responsible for photosynthesis are inactive or absent. Younger tissue contains a higher proportion of actively dividing cells and is more responsive to a callus initiation programme (Molnar *et al.,* 2014).

The plant growth regulators are widely used for callus, shoot and root induction in tissue culture studies. During present investigation, the plant growth regulators are used for callus induction purpose. MS media is the best culture medium for the establishment of callus culture in *Passiflora vitifolia* compared to B5 Media. The plant tissue can be cultured by using different media; there are several reports to emphasize on varied responses obtained from different *Passiflora species* like *P. edulis* and *P. flavicarpa* (Otahola, 2000). Initially explants were cultured on MS and BS basal medium. The response on MS medium was achieved soon. Hence, MS medium was chosen as the nutrient medium.

Lower auxin, high cytokinin content, stimulates shooting but at higher auxin lower cytokinin content stimulates the rooting (Skoog and Miller, 1957). Callus induction was observed on the both leaf and nodal segments on MS medium augmented with NAA. The degree of callus production varied with reference to the supplementation of the plant growth regulators in the medium. The Highest degree of callus induction was observed on MS medium augmented with 2-4D (1-1.5mg/L) and NAA (1.5mg/L) respectively. The efficacy of exogenous 2, 4-D found in this experiment, was also been reported with other medicinal plants by various authors. Results described by Rani et al. (2003); Thomas and Maseena (2006); Hassan et al. (2009) and Davallo et al. (2014) were also in agreement with our result for using this synthetic plant growth regulator in the culture medium for callus induction of *Withania somnifera*, *Cardiospermum halicacabum*, *Abrus precatorious* and *Jasminum sambac* respectively.

5. Conclusion

This is the first report has an efficient protocol developed for callus induction for the *in vitro* propagation of *Passiflora vitifolia*. The solid MS medium containing 1.5mg/L NAA shows the high% of callus initiation form young leaf and internode, while flower bud and tendril shows the callogenesis on 2,4-D at 1.0 mg/L. The internodal explants showed the better response then the leaf explants. This technique could be used as a tool for the large scale multiplication program. The present work could be a base for further research on this plant.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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