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# CLONAL MICROPROPAGATION OF *PAEDERIA FOETIDA* L. - A POTENT HERBAL MEDICINAL PLANT

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A protocol has been developed for complete plantlet regeneration from nodal explants by one step treatment of MS basal medium supplemented with a combination of three hormones viz. kinetin, BAP, and IBA (1.5+1.0+1.5 $mgL^{-1}$). Treatment with NAA and IBA individually in the range of 0.5 to 3.0 $mgL^{-1}$ also induced complete plantlet regeneration but the combination of three hormones was superior. NAA and IBA also induced callusing after 30 days of culture. BAP in the range of 0.5 to 5.0 $mgL^{-1}$ induced multiple shooting and promoted shoot growth while kinetin was found to be inefficient. 2,4-D induced only callusing.

**Keywords :** Hormone; Medicinal plants; *Paederia foetida*, Plantlet regeneration.

## Introduction

North East India is known to be an abode of large number of medicinal plant amidst its famed biodiversity of plants. While many plants are used as raw materials of pharmaceuticals industries, many more are used traditionally in ethnic medicine system since time immemorial. *Paederia foetida*, L. (Family : *Rubiaceae*) locally known as "Bhedia lata" in Assam is one such medicinal plant that grows in the wild and is part of ethnic tradition of Assam since time immemorial. The leaft extract is taken to overcome physical weakness and almost every type of stomach ailment. The juice of the plant is frequently used to remove pain after childbirth, particularly in rural areas. The leaf extract is also used as tonic, sperm stimulator and is known to be useful in joining broken bones. The extract is claimed to be useful in curing allergy and jaundice[1]. Apart from widespread use in ethnic medicine system, the leaf and tender shoots are used as leafy vegetables and as additive to food in preparing certain delicacies. Hence the leafy shoots are often sold in rural as well as urban markets of Assam. The plant is stem climber, which emits a characteristic pungent smell when the leaves are smeared. This pungent smell is due to the presence of volatile principle methyl mercaptan which is removed to a great extent due to cooking[2]. But it is not known whether any of the medicinal property is due to this compound. The palnt exhibit profuse growth during summer and also flowers profusely with the onset of winter. The plant normally grows in forests, forest margin and marginal land in countryside on medicum size trees as well as bushy shrubs. A forest survey of India report showed that between 1991 and 1993, North East India recorded a loss of 783 $km^2$ forest cover; Assam topping the list with 447 $km^2$ . Since the plant need mechanical support from trees and bushes, gradual loss of forest cover is gradually endangering the plants. The plant can be propagated vegetatively as well as by seed but the natural process of regeneration is slow and inefficient. No information is available about micropropagation of this plant and hence the present investigation was undertaken to develop a protocol for *in-vitro* regeneration.

## Material and Methods

MS medium was used as basal medium and it was supplemented with kinetin and BAP separately - the concentration being 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 $mgL^{-1}$. Likewise for NAA and IBA five concentrations were used to supplement the basal medium, viz 0.5, 1.0, 1.5, 2.0 ad 3.0 $mgL^{-1}$. For 2,4-D five concentrations were used viz 0.5, 1.0, 1.5, 2.0 and 3.0 $mgL^{-1}$. Apart from these hormones used individually, few combined treatments were also tried. These are BAP + IBA with BAP concentration fixed at 1.5 $mgL^{-1}$ and six IBA concentration from 0.5

**Table 1-a.** Effect of kinetin on organogenesis from nodal explants of *P. foetida* L.

| Treatment MS+ kin. ($mgL^{-1}$) | Shoot induction | | | | | | Root Induction | Callusing |
|---|---|---|---|---|---|---|---|---|
| | Shoot Number | | Shoot Length | | Leaf Number | | | |
| | After days | | After days | | After days | | | |
| | 30 | 45 | 30 | 45 | 30 | 45 | | |
| 0.0 | 1.50 | 2.00 | 1.25 | 1.75 | 2.50 | 3.00 | No rooting in any treatment | No callus in any treatment |
| 0.5 | 1.75 | 2.00 | 1.00 | 1.40 | 1.50 | 2.00 | | |
| 1.0 | 1.50 | 1.75 | 1.00 | 1.35 | 2.00 | 2.00 | | |
| 1.5 | 2.00 | 2.00 | 1.25 | 1.50 | 2.50 | 2.50 | | |
| 2.0 | 2.00 | 2.00 | 1.15 | 1.35 | 2.00 | 2.50 | | |
| 3.0 | 1.50 | 1.75 | 0.75 | 0.90 | 1.70 | 2.00 | | |
| 5.0 | 1.50 | 1.50 | 0.68 | 0.77 | 1.50 | 1.80 | | |
| C.D. at 5% | 0.296 | | 0.193 | | 0.433 | | | |
| C.D. at 1% | 0.418 | | 0.273 | | 0.612 | | | |

**Table 2-b.** Effect of BAP on organogenesis from nodal explants of *P. foetida* L.

| Treatment MS+ BAP ($mgL^{-1}$) | Shoot induction | | | | | | Root Induction | Callusing |
|---|---|---|---|---|---|---|---|---|
| | Shoot Number | | Shoot Length | | Leaf Number | | | |
| | After days | | After days | | After days | | | |
| | 30 | 45 | 30 | 45 | 30 | 45 | | |
| 0.0 | 1.5 | 2.0 | 1.25 | 1.75 | 2.5 | 3.0 | No rooting in any treatment | No callus in any treatment |
| 0.5 | 3.0 | 4.5 | 2.40 | 3.00 | 4.5 | 9.0 | | |
| 1.0 | 3.5 | 6.0 | 2.60 | 3.50 | 6.0 | 11.0 | | |
| 1.5 | 4.0 | 6.0 | 3.00 | 4.00 | 6.0 | 12.0 | | |
| 2.0 | 4.0 | 6.5 | 3.50 | 4.25 | 7.5 | 12.5 | | |
| 3.0 | 4.5 | 7.0 | 3.30 | 4.75 | 8.0 | 14.0 | | |
| 5.0 | 3.0 | 5.0 | 2.00 | 2.80 | 5.0 | 8.5 | | |
| C.D. at 5% | 0.471 | | 0.218 | | 1.930 | | | |
| C.D. at 1% | 0.665 | | 0.308 | | 2.730 | | | |

to 5.0 $mgL^{-1}$. Another combination of kinetin, BAP and IBA was tried with the concentration of kinetin fixed at 1.5 $mgL^{-1}$ and that of BAP fixed at 1.0 $mgL^{-1}$ while IBA concentrations were in the range of 0.5 and 3.0 $mgL^{-1}$ with five IBA concentrations. The media were fortified with 3% sugar (w/v) and pH was adjusted to 5.8 ± 0.2 using 1 N HCl/ 1 N NaOH before adding 0.8% agar (w/v), following which they were dispensed in test tubes and conical flasks. The media were autoclaved under 15 psi at a temperature of 121° C for 15 minutes. Nodal segments were used as explants that were collected from healthy plants maintained in the experimental garden of the Department of Biotechnology, Gauhati University. The explants (about 1.5 cm of nodal region) were first washed with running tap water followed by another washing with Tween 20 (5% v/v) for 5 minutes. Subsequently they were washed again with running tap water. Sterilization of explants were done with 2.0 (w/v) $HgCl_2$ solution for 3 to 4 minutes followed by thorough washing with sterile distilled water. Approximately 0.8 cm long explants were cut and inoculated. The cultures were maintained under 16 hour light and 8 hour dark cycle with light intensity of 2000 lux. Temperature was maintained at 24 ± 2° C. Observations were recorded at 15, 30 and 45 days interval.

## Results and Discussion

Shoot development was found to occur in MS medium without any hormone supplement 15 days after inoculation. Following kinetin treatment frequency of shoot development either remained same or marginally enhanced which was however,

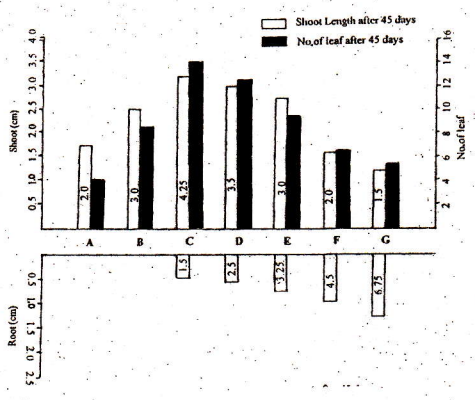
**Table 2-a.** Effect of NAA on organogenesis from nodal explants of *P. foetida* L.

| Treatment MS+ NAA ($mgL^{-1}$) | Shoot induction | | | | | | Root Induction | | | | Callusing |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot Number | | Shoot Length | | Leaf Number | | Root Number | | Root Length | | |
| | After days | | After days | | After days | | After days | | After days | | |
| | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | |
| 0.0 | 1.25 | 1.5 | 1.00 | 1.25 | 2.0 | 2.5 | - | - | - | - | No callusing |
| 0.5 | 1.0 | 1.0 | 0.75 | 0.93 | 2.0 | 2.5 | 3.5 | 6.0 | 1.0 | 1.55 | Callus |
| 1.0 | 1.0 | 1.0 | 0.70 | 0.80 | 2.0 | 2.0 | 5.0 | 8.5 | 1.0 | 1.70 | growth |
| 1.5 | - | - | - | - | - | - | 12.5 | 21.0 | 1.3 | 2.0 | in all NAA |
| 2.0 | - | - | - | - | - | - | 22.0 | 29.5 | 1.36 | 2.25 | treatments |
| 3.0 | - | - | - | - | - | - | 28.0 | 34.75 | 1.45 | 2.56 | after 30 days. |
| C.D. at 5% | | | | | | | 3.114 | | 0.414 | | |
| C.D. at 1 % | | | | | | | 4.718 | | 0.585 | | |

**Table 2-b.** Effect of IBA on organogenesis from nodal explants of *P. foetida* L.

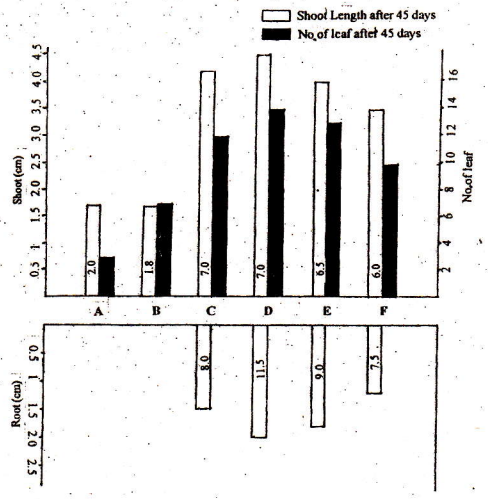
| Treatment MS+ IBA ($mgL^{-1}$) | Shoot induction | | | | | | Root Induction | | | | Callusing |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot Number | | Shoot Length | | Leaf Number | | Root Number | | Root Length | | |
| | After days | | After days | | After days | | After days | | After days | | |
| | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | 15 | 30 | |
| 0.0 | 1.25 | 1.5 | 1.0 | 1.25 | 2.0 | 2.5 | - | - | - | - | No callusing |
| 0.5 | - | - | - | - | - | - | 5.0 | 5.70 | 0.85 | 1.0 | Callus |
| 1.0 | 1.0 | 1.25 | 1.0 | 1.52 | 2.8 | 4.0 | 5.0 | 6.50 | 0.85 | 1.25 | growth |
| 1.5 | 1.25 | 1.25 | 1.16 | 1.87 | 3.5 | 5.25 | 6.0 | 8.25 | 1.05 | 1.56 | in all IBA |
| 2.0 | 1.25 | 1.25 | 1.00 | 1.45 | 3.0 | 5.0 | 6.25 | 8.50 | 0.90 | 1.32 | treatments |
| 3.0 | 1.00 | 1.00 | 0.85 | 1.25 | 2.5 | 3.5 | 4.75 | 5.25 | 0.80 | 1.15 | after 30 days. |
| C.D. at 5% | 0.321 | | 0.230 | | 0.971 | | 1.823 | | 0.194 | | |
| C.D. at 1 % | 0.454 | | 0.325 | | 1.373 | | 2.978 | | 0.275 | | |

**Fig. 1.** BAP+IBA induced shoot and root induction and growth in *P. foetida*. L.




Figures within parenthesis indicate number of shoot and root after 45 days BAP+IBA (mg/l) A-0.0+0.0 B-1.5+0.5 C-1.5+1.0 D-1.5+1.5 E-1.5+2.0 F-1.5+3.0 G-1.5+5.0

**Fig. 2.** Kinetin+BAP+IBA induced shoot and root induction and growth in *P. foetida*. L.




Figures within parenthesis indicate number of shoot and root development after 45 days Kn+BAP+IBA (mg/l) A-0+0+0; B-1.5+1.0+0.5; C-1.5+1.0+1.0; D-1.5+1.0+1.5; E-1.5+1.0+2.0; F-1.5+1.0+3.0

insignificant. Moreover, kinetin did not enhance growth of shoot, rather inhibited the same significantly at higher concentration (Table 1-a). On the other hand BAP significantly enhanced multiple shoot formation. Number of shoots formed increased with increase in duration of culture. Highest number of shoots was observed after 45 days of culture. MS supplemented with 3 $mgL^{-1}$ BAP was found to be best with 7 shoots after 45 days of culture against 2 in MS basal medium. BAP also significantly enhanced shoot growth in all the concentrations tried; however the concentrations 2 $mgL^{-1}$ and 3 $mgL^{-1}$ BAP were the best. Highest shoot growth of 4.75 cm was observed in 3$mgL^{-1}$ BAP treatment after 45 days of culture whereas the corresponding value of MS basal medium was 1.75 cm. In all BAP treatments the number of leaves per shoot were much higher than those of MS basal medium (Table 1-b).

The effect of NAA was found to be different from both kinetin and BAP because it induced root growth in all the concentrations tried apart from induction of both root and shoot at low concentrations. For 0.5 and 1.0 $mgL^{-1}$ NAA treatment, single shoot was found to develop after 15 days of culture which remained same after 30 days. But shoot growth was retarded and lesser than those of MS basal medium. However, leaf sizes were bigger. On the other hand root induction was impressive. At 0.5 $mgL^{-1}$ treatment, number of root induced after 30 days of culture was 6 and there was a concomitant rise in the number of root induced (Fig. 3a) and the highest number of root 34 was observed in 3 $mgL^{-1}$ treatment, the highest dose of NAA in the present study. Root growth was also very good; however, there was not much difference among the treatments (Table 2-a). After 30 days of culture there was callus development and growth in all the treatments which arrested further induction and growth of root and shoot. The effect of IBA was to some extent similar to NAA in the sense that it could induce both root and shoot development at certain concentrations. Low frequency shoot induction occurred in

all the concentrations of IBA except in the lowest dose of 0.5 $mgL^{-1}$ after 15 days of culture. However, even after 30 days of culture the number of shoots remained the same. Shoot induction was little lesser than that of MS basal medium and there was no difference in shoot induction due to difference in concentration of IBA with the exception of the highest dose (3.0 $mgL^{-1}$) where shoot induction dropped marginally. However leaf development was impressive in all the treatments. As a whole IBA did not appreciably induced shoot development while growth was enhanced to certain extend in moderate concentrations. The effect of IBA on root induction and growth was very impressive for all the concentrations tried. The optimum concentrations were 1.5 (Fig. 3C) and 2.0 $mgL^{-1}$ where highest number of roots (8.25 and 8.50 respectively) developed after 30 days of culture. Root growth was found to be good in all the concentrations (Table 2b). It is noteworthy that like NAA, in case of IBA also, there was callus development and growth in all the treatments, after 30 days which arrested further induction and growth of root and shoot. Unlike other hormones 2-4D induced callusing after 15 days of culture in all the treatments. After 30 days of culture there were significant increase in callus growth in the treatments. Optimum concentrations were 1.0 and 1.5 $mgL^{-1}$ (Fig. 3b) with 0.600 and 0.750 gm fresh weight calli respectively after 30 days of culture. Even after prolonged period of culture there were no sign of organogensis in any of the treatments.

Among the individual hormone treatment, BAP remarkably induced shoot development and growth while IBA was the best in inducing high frequency rooting as well as low frequency shoot development. Hence a combination of both was attempted for complete planlet regeneration in one step. BAP concentration was kept consant at 1.5 $mgL^{-1}$ in all the combinations, while IBA concentrations varied from 0.5 to 5.0 $mgL^{-1}$ with a total of 6 combinations. Complete plantlet regeneration was found in all the treatments except MS basal and combinations with 0.5 to 1.0 $mgL^{-1}$ IBA. Shoot induction and growth was very good in the combinations involving medium concentrations of IBA. Optimum shoot induction, shoot growth and leaf development were found in BAP 1.5 + IBA 1.5 $mgL^{-1}$ combination (Fig. 3d). However, in this combination root did not develop. Combination involving high concentrations of IBA (3.0 and 5.0 $mgL^{-1}$) were not good for shoot development and growth but found to be best for root induction and growth. The combination BAP 1.5 + IBA 2.0 $mgL^{-1}$ resulted in sub-optimum root and root induction and growth and hence best for complete plantlet regeneration.

The combination involving kinetin and IBA were found to be the best of all treatments for regeneration of complete plantlet. In this combined treatment kinetin concentration was fixed at 1.5 $mgL^{-1}$, BAP fixed at 1.0 $mgL^{-1}$ while IBA concentration varied from 0.5 to 3.0 $mgL^{-1}$ with a total of 5 combinations. The triple combination induced highest multiple shooting among all the treatments in the present study. Particularly the combination involving medium concentration of IBA (1.0 and 1.5 $mgL^{-1}$) induced 7 shoots which is the highest of all against 2 in MS basal after 45 days in culture. However, the combination involving lowest concentration of IBA (0.5 $mgL^{-1}$) slightly retarded shoot development and shoot growth compared to basal medium. Except this, shoot growth for other combinations were very good and at par with BAP treatment alone. Leaf number and leaf sizes were very impressive and comparable to that of BAP. The combination involving 0.5 $mgL^{-1}$ IBA failed to induce root but in all other combinations there were reasonably good induction and growth of root. In combinations involving medium concentrations of IBA, root induction and growth were highest . As a whole, in the triple combination root induction and root growth were better than

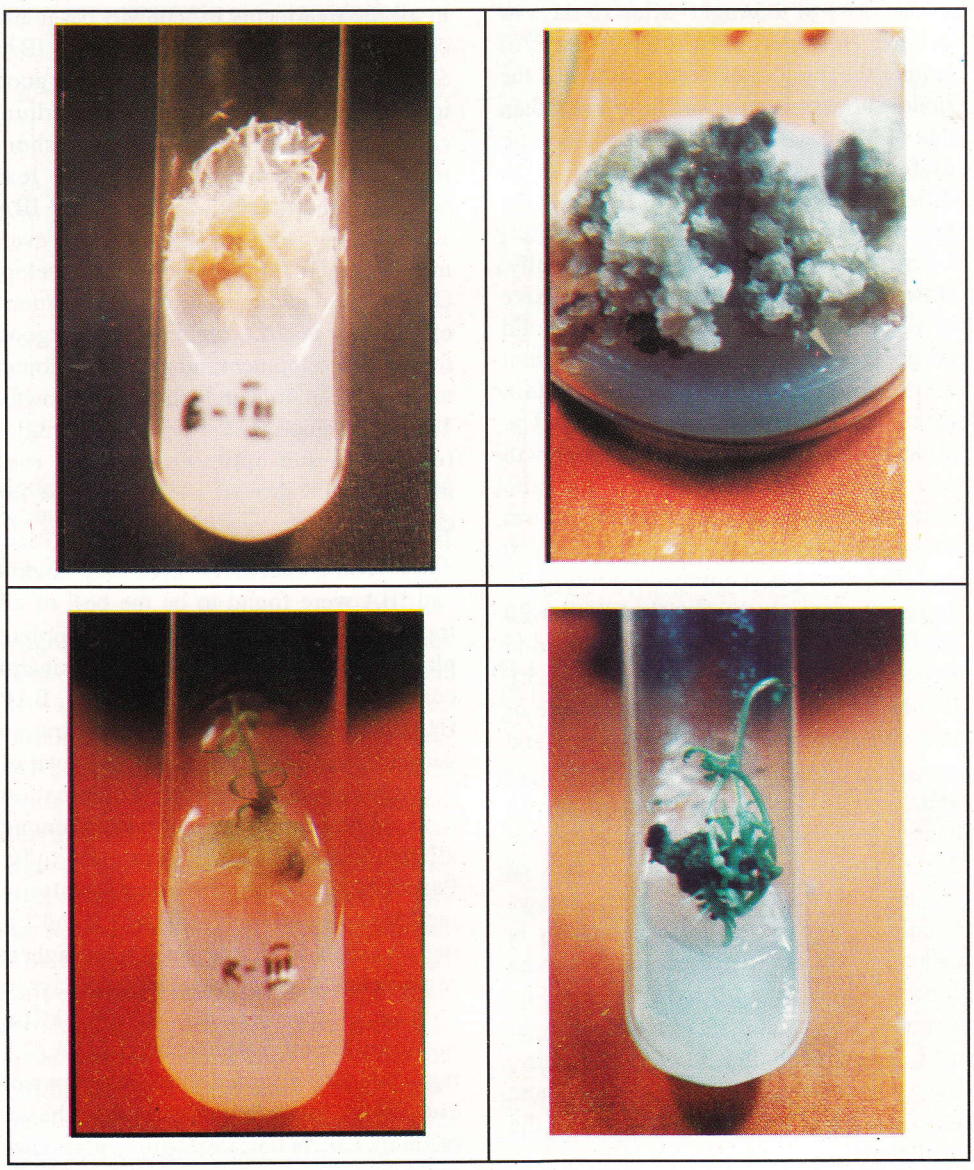


**Fig. 3.** a-d : 3a - NAA 2.5 $mgL^{-1}$; 3b - 2,4 - D 1.5 $mgL^{-1}$; 3c - IBA 1.5 $mgL^{-1}$; 3d - BAP 1.5 $mgL^{-1}$ + IBA 1.5 $mgL^{-1}$.

IBA alone but little lesser than NAA. The combinations involving Kin 1.5 + BAP 1.0 + IBA 1.0 + IBA 1.0 $mgL^{-1}$ and Kin 1.5 + BAP 1.0 + IBA 1.5 $mgL^{-1}$ have been found to best of all the treatments in the present study for complete plantlet regeneration in one step treatment.

The present study shows that 2,4-D is ineffective so far as organogenesis is concerned in *P.foetida* L. Even prolong culture there were callusing and no sign of

organogenesis which is in agreement with the often reported fact that 2,4-D is a callus inducing growth regulator[4]. But response to a hormone varies from species to species and there are instances of callus induction by hormones other than 2,4-D like NAA, IBA, kinetin etc[5], while in *Bixa orellana* 2,4-D fails to induce callusing[6]. Kinetin and BAP are generally regarded as shoot inducing hormones, which is confirmed for many species[7]. But in the present study only BAP was found to be very effective while kinetin was not. Sharon and D'Souza[6] also reported that kinetin fails to induce shoot formation in *Bixa orellana* but induces callusing at high concentration. However, the observations with BAP are in conformity with many other workers who reported multiple shoot formation in glory lily[7], African marigold[8] etc. Neither kinetin nor BAP induced root or callus which is in conformity with the findings of Ahuja *et al.*[9] that BAP and kinetin suppress both root and callus initiation. The observations with NAA and IBA are quite interesting in the sense that both could induce root, shoot as well as callus depending upon concentration and duration of culture, although in general they are root inducing hormones [7,10]. Sharon and D'Souza[6] reported that in *Bixa orellana* NAA could induce and promote growth of both root and shoot depending upon concentration and basal medium. The present study also showed that concentration is a critical factor for organogenesis.

Although NAA and IBA could generate complete plantlet yet it can not be considered efficient because shoot initiation and growth was poor; however, root induction and growth was good. On the other hand the combined treatments were superior and very effective with multiple shooting and root development. In the combined treatment involving low concentration of IBA, rooting did not take place, which may be due to the suppression of root initiation by shoot inducing hormones BAP and kinetin that were in higher concentration. Likewise, in the treatment involving IBA alone, low concentration of IBA suppressed shoot initiation. In the combined treatment involving higher concentration of all the three hormones, there were no such interference and hormones exerted their respective effects resulting in healthy, vigorously growing complete plantlet. Philip *et al.*[11] showed that in *Piper longum* BAP alone had no effect, IAA alone induced callus, while a triple combination involving BAP+IAA+kinetin induced shoot and root growth.

The present study has established in *P.foetida*, a triple combination involving kinetin, BAP and IBA can efficiently regenerate complete plantlet in one step treatment instead of sequentially culturing in shoot inducing medium and rooting medium.

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