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IN-VITRO PROPAGATION OF *ALPINIA GALANGA* L. VIA SOMTIC EMBRYOGENESIS – A MEDICINAL PLANT

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ABSTRACT

Unscientific collection of medicinal plants from the wild nature became many medicinal plants in endangered and threatened condition. Many members of the Zingiberaceae have became Vulnerable and threatened. Vegetative propagation of *Alpinia galanga* Linn (Zingiberaceae) cannot fulfill the current demand for planting material and so tissue culture offers an alternative means for mass propagation. Propagation of *A. galanga* via colonal propagation and organogenesis were successfully standardized and reported earlier by the same author. Propagation by somatic embryogenesis is not reported yet. This paper deals with propagation of *A. galanga* via somatic embryogenesis. Rhizome discs were inoculated in M.S. medium supplemented with various concentrations of BAP, KIN and 2, 4-D individually or in various combinations. Rhizome discs were produced embryogenic callus when it cultured in M.S. medium supplemented with 2Mg/L. BAP or KIN and 0.25 Mg/L.2,4-D. This embrogenic callus was transferred into a medium having higher concentrations of BAP or KIN + 2, 4-D (3Mg/L + 0. 25Mg/L), produced an average of 4.0 well developed globular embryos. On latter cultures, the auxin were completely removed, well rooted plantlets were emerged from the cultures. Fully developed plantlets were acclimatized successfully in a mixture of soil, sand and compost (1: 1: 1). Survival of the established plantlets was 40%. This method can be used for the large scale production planting material, and germplasm conservation of this threatened medicinal plant. Moreover, the callus can be further utilized for suspension cultures for secondary metabolite production.

KEYWORDS: *Alpinia galanga*, Embryogenic callus, Somatic embryo genesis, 6-Benzylaminopurine (BAP), Kinetin (KIN), 2, 4- Dichlorophenoxyacetic acid.

INTRODUCTION

Alpinia galanga Linn. (*Vern*: Mal. Aratha, Eng. Greater galanga) is an important endangered medicinal plant belongs to the family Zingiberaceae. It is used in traditional Ayurvedic and Unani systems of medicine for curing various ailments of human. Clinically, the dried and sliced rhizome of *A.galanga* is used in more than fifty ayurvedic preparations like *Aswagandharishta*, *Chavikaasava*, *Balathaila*, *Ashtavargham kashaya* etc (Sabu, 1991). Because of its wide range of medicinal usage, this plant collected from the nature by raw drug collectors and tribes in an unscientific manner. Moreover, the rhizome is the major useful part of the plant and during collection the collectors take complete rhizome from the plant without thinking the future need and automatically it gets completely root out from the nature. The conventional propagation is season depended and some soil pathogens affect the quality also. So plant has become very rare in natural population. This makes the alternative methods of generating sufficient planting material production, its germplasm protection and continuous supply of this drug in the market. Here the *in-vitro* propagation method plays a vital role.

There are many reports available for clonal propagation, callus induction and organogenesis of different genera of the same family. Some of them are in *Alpinia*, (Agretious *et al*., 1996, Anand & Hariharan, 1997), *Boesenbergia* (Yusef *et al*.,2011), *Elatteria* (Nadgauda *et al*.,1983), *Costus* (Roy and Pal,1991), *Kaempferia* (Vincent *et al*.,1991, Parida *et al*.,2010, Kochuthressia *et a., 2012)*, *Curcuma* (Srirat *et al*., 2008, Ferdous *et al*., 2012). The present study reveals the development of a protocol for the successful *in-vitro* propagation of *Alpinia galanga* Linn by somatic embryogenesis. No other reports are available yet on the somatic embryogenesis from rhizome derived callus of *Alpinia galanga* Linn.

MATERIALS AND METHODS

Since the explants taken from underground rhizome, establishment of contamination free culture was a major task. The surfaces of the explants usually carry a wide range of microbial contaminants. Thus, the explants must be thoroughly surface sterilized before inoculating on culture medium to avoid infection. The usage of commercial bleach (1-10% sodium hypochlorite), ethanol and mercuric chloride solution (0.5-1%) have

been used as effective sterilizing agent in establishing aseptic culture. This method was agreed with the opinion of earlier researchers like Sundram *et al*. (2012), Chan and Tong (2004), Yusef *et al*. (2007). The healthy rhizomes collected of *in-vitro* studies were washed thoroughly in running tap water followed by 3% solution of sodium hypochlorite then rinsed in double distilled water and were surface sterilized with 0.5% of mercuric chloride. Again the explants were washed with DD water. These explants were taken into Laminar Air Flow chamber. Explants were inoculated M.S Medium (Murashige and Skoog 1962) supplemented with different concentration of Kinetin, BAP or 2, 4-D singly or in various combinations (Table 1). Cultures were incubated with 25±2ºC and 16 – 8 Photoperiod (1000 – 2000 Flux). Observations were taken at regular intervals of two weeks.

Enbryogenic callus induction was noticed in MS medium with high auxin – low cytokinin ratio and embryogenesis and plantlets development were occurred in medium with less auxin – high cytokines ratio. Fully developed plantlets were transferred to pots containing 1:1:1 sand, soil and coir pith.

RESULT AND DISCUSSION

Somatic embryogenesis has been reported earlier in certain members of the family *Zingibraceae*. The resent reports include *Kaempferia galanga* (Vincent *et al.* 1992), Lakshmi and Mythili (2003), *Hedychium* (Verma and Bansal 2012) and in *Zingiber* (Babu *et al.*, 1992&1996) In *Kaempferia rotunda in-vitro* somatic embryogenesis has been reported by the same author in (2014).

Embryogenic callus were induced from the rhizome discs of explants *Alpinia galanga* Linn. After two weeks of incubation on MS medium supplemented with 2.0 Mg/L BAP + 2, 4-D 0.25 Mg/L. 30% of the culture responded positively. Up on slight increasing the quantity of BAP or KIN (3.0Mg/L), in the medium, produced well developed globular embryos. The percentage of response was 40 % (Table 1 & Plate 1). This is in corroboration with the findings of Babu *et al.* (1992 and 1996). They found that the combination of 2, 4-D along with BAP very suitable for embryogenic callus induction in *Zingiber*. Same was opined by Vincent *et al.* (1992) while working with *Kaempfria galanga*. Agretius *et al*. (1996) also reported the role of 2, 4-D along with BAP for callus induction in *Alpinia calcarata.* Srirat (2008) also explained the role of BA along with auxin, while working with *Curcuma longa* L.

The fully developed embryos were transferred to MS medium supplies with only 4.0Mg/L or 5Mg/L BAP or KIN, 30% and 40% of the cultures produced well developed plantlets with an average of 3.9 and 3.7 respectively (Table.1 & Fig. E&G). This observation was in corroboration with observation made by the same author while working with *Kaempferia rotunda* (Anand, 2014). Again, Samsudeen (1996) opined that the transfer embryogenic callus from a medium having auxin and cytokinin into a medium containing high concentration BAP only resulted well developed plantlets from ginger callus. This study also proved that the complete removal auxin from the medium and the presence of increased quantity of BAP (4.0 Mg/L) resulted fast growth of plantlets from the somatic embryos.

In this study, the results showed that combined effect of 2, 4-D and KIN produced embryonic callus. Same was explained by Rahman *et al.* (2004). He got the highest percentage of embryogenic callus in *Kaempferia galanga*, when medium supplied with 2, 4-D and BA. Again, Saensouk (2011) explained that, the synergetic action of auxin and cytokinin for the production of embrogenic callus in *Cornukaempferia aurantiflora* .The present study also once again proved embryogenetic callus induction is easy in medium having combination of auxin and cytokinin.

The plantlets produced through somatic embryogenesis were transferred after two months of total growth into the field after proper hardening (Plate 1.H). During hardening the pots were kept under room temperature .For maintaining the humidity, pot was covered with polythene bag for one week then made holes on cover to easily accustom with natural condition. The plantlets were established in the soil with 40% survival.

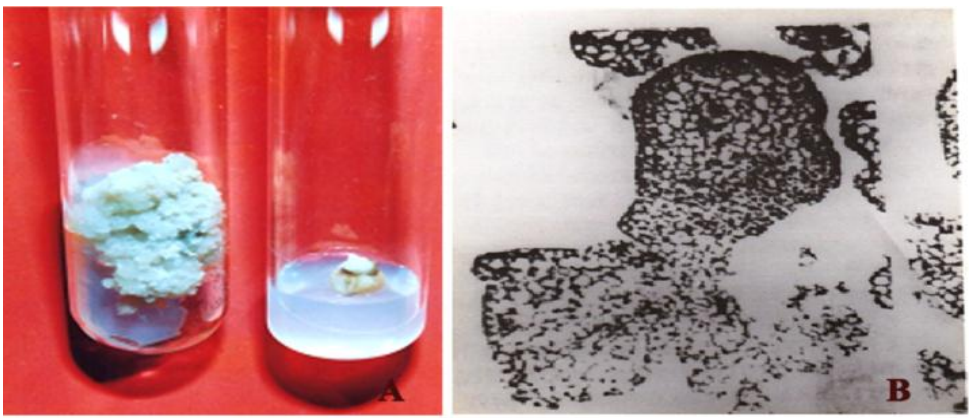

figure 1: A- Callus formation; B & C- Embryogenesis; D- Single units; E, F & G- Culture in clumps; H- Hardening in the nursery

Table 1: Effect of 2, 4-D, BAP and KIN on rhizome disc cultures of *A. galanga* after 60 days incubation.

Growth regulators	Concentration in mg./L.	Morphogenetic response	Percentage of response	Average Number of embryos (±)SD
M.S.+BAP	1.0	Slow callus growth	50	NIL
"	1.5	Slow callus growth	40	"
"	2.0	Slow callus growth	60	"
"	2.5	Slow Shoot buds induction	30	"
"	3.0	Only shoot buds developed well	30	"
M.S.+KIN	1.0	Disc turned to green	30	"
"	1.5	Slow callus growth	40	"
"	2.0	No callusing tendency	30	"
"	2.5	Shoot buds produced	60	"
"	3.0	Slow shoot buds induction	30	"
M.S. +2,4-D	0.5	Slow callus growth	30	"
"	1.0	Callus induced	40	"
"	1.5	White callus	30	"
"	2.0	Callus and roots	50	"
"	3.0	Callus and roots	40	"
M.S.+ BAP+ 2,4-D	0.5 + 0.25	Moderate callus growth	30	"
"	1.0 + 0.25	Moderate callus growth	50	"
"	1.5 + 0.25	creamy globular callus	20	2.6 ± 1.29
"	2.0 + 0.25	Callus compact and nodular	30	2.5 ± 1.6
"	3.0+0.25	Globular embryos produced	40	4.0 ± 1.7
M.S.+BAP ONLY	4.0	Fast plantlets growth	30	3.9 ± 1..8
"	5.0	Fast growth of plantlets	40	3.7 ± 1.0
M.S+KIN+2,4-D	1.0 + 0.25	callus developed	10	NIL
"	1.5 + 0.25	Moderate callus	10	NIL
"	2.0 + 0.25	Globular embryos	20	2.0 ± 0.16
"	3.0 + 0.25	More embryos developed	30	2.2 ± 0.18
M.S+KIN ONLY	4.0	fast plantlets growth	30	3.5 ± 0.29
"	5.0	fast plantlets growth	40	3.6 ± 0.18

MS +3% Glucose, + 0.8% Agar Growth rate were measured by visual observation.

CONCLUSION

The protocol developed here is a commercially feasible one and could easily be adopted for the large scale multiplication of this plant with view to sustainable preservation and eco-friendly exploitation for the wellbeing of the society. Moreover, such protocols are very simple to be instructed and practiced even for a layman.

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