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IN VITRO REGENERATION OF *ANNONA SQUAMOSA* LINN.

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ABSTRACT

In the present investigation various phytohormones and their combinations were used for the regeneration and callus from the explants of *Annona squamosa*. Readymade MS media was used for media preparation, and different types of hormones were used such as 2, 4-D, IBA, IAA, BAP, Kinetin and NAA. 0.2 ppm BAP and 0.2 ppm NAA is best concentration for shooting in *Annona squamosa* and 0.6 ppm BAP and 0.6 ppm Kinetin is best concentration for callus induction in *Annona squamosa*. The best result obtained in nodal explant and followed by leaf explant on the same hormonal concentration**.** The establishment of plant tissue culture can open the door for the analysis of the bioactive compounds and enhancement of bioactive compound production in the plant.

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INTRODUCTION

Advances in plant biotechnology research in last few decades have opened new areas in the propagation of plants with superior resistance to diseases, pests, herbicides and stress etc. Micro-propagation of fruit trees is an priceless abet in the production of best, disease free, breeding true breed of fruits in large numbers in short duration.

The clonal propagation in *Annona* species is carried out by grafting and budding which are time consuming, while seedlings rootstocks are highly variable resulting in the decreased productivity (George and Nissen, 1987). The seed propagation results immense variability affecting yield, size and quality of fruits. Due to the lack of germplasm of improved varieties its cultivation is limited. Micro-propagation has been used in other species of *Annona viz*. *Cherimoya A. cheimola* (Tizzari *et al*., 1990; Rasai *et al*., 1995) and *A. muricata* (Lemos and Blake, 1996), but the reports on *Annona squamosa* are scarce. Present study deals with the establishment of standard protocols for the micro-propagation of the large-scale rapid multiplication of improved genotypes, free of seed borne bacterial and viral diseases.

Annona squamosa Linn. is an evergreen tree is found throughout India for its fruits, different parts of *Annona squamosa* Linn. are used in folkloric medicine for the treatment of various disease (Suresh *et al*., 2006). This plant is commonly called *Custard apple* in English and *Sharifa* in Hindi and *Sitaphalam* in Telugu in India (Raj *et al*., 2009).

The said plant is very difficult to regenerate in lab conditions. The explants of the plant gone blackish during the course of time in the MS medium and dying gradually. So, an establish protocol is needed for the regeneration and callusing of *Annona squamosa*. The proposed investigation is done to establish regeneration and callusing protocol for the said plant.

MATERIALS AND METHODS

Readymade MS media (Murashige and Skoog, 1962) was used for media preparation, and different types of hormones were used such as 2, 4-D, IBA, IAA, BAP, Kinetin and NAA.

The plants were collected from places in and around Ranchi. The plant was identified and authenticated.

With several modifications the protocol of George *et al*. (2008) was followed for the plant tissue culture work.

The plant samples were washed in running water to remove all traces of dust and soil. Explants were cut from appropriate regions like nodes, internodes and leaf. Explants were washed in distilled water. The explants were sterilized with 70% ethanol for 1 minute. The explants were washed with distilled water again. Explants were treated with 0.1% $HgCl_2$ solution for 1 to 3 minutes. Explants were washed with distilled water 3 to 5 times before transfer.

After sterilizing the explants, whole surface of the laminar air flow was wiped

thoroughly with 70% ethyl alcohol, and then hands were washed with alcohol. Now the test tubes were wiped with alcohol and kept in laminar air flow. Now the explants were placed on the Whatmann filter paper, so that the filter paper absorbed the water and make the explants completely dried. Now the basal and the proximal ends of the explants where cut again and placed on nutrient medium in the test tubes having different rooting media with control. Cotton plugs were used to make the test tubes air tight. These tubes were stored in proper condition i.e., 25 ºC ± 3 ºC with appropriate light of 16 hrs light and 8 hrs dark with light intensity of 1000 – 3000 lux and 60% relative humidity.

RESULTS

In this investigation initially explant from leaf, node, stem and bud were cultured on MS medium with different concentration of hormones such as 1 ppm 2,4-D, 2 ppm 2,4-D, 3 ppm 2,4-D, 4 ppm 2,4-D, 5 ppm 2,4-D, 6 ppm 2,4-D, (3 ppm IAA + 0.5 ppm BA), (3 ppm NAA + 0.5 ppm BA), (0.5 ppm 2,4-D + 0.5 ppm BA + 0.5 ppm IBA), (0.6 ppm KIN + 0.6 ppm BA). After complete result analysis it has been cofirmed that 0.2 ppm BA and 0.2 ppm NAA is best concentration for shooting in *Annona squamosa* and 0.6 ppm BAP and 0.6 ppm Kinetin is best concentration for callus induction in *Annona squamosa*. The best result obtained in nodal explant and followed by leaf explant on the same hormonal concentration (Table 1 and Fig. 1 and 2).

DISCUSSION

In this present investigation different hormonal concentration were used to find out the best combination for callusing in this medicinal plant. The ultimate aim of this investigation is to find out those hormonal concentration which are best for callus induction.

The Annonaceae is an ancient family of plants. The most important species at commercial level (nourishment, pharmacy industry) belong to the genera *Annona*, being the most interesting and used species are *A. squamosa*, *A. muricata*, *A. cherimola* and the hybrid *A. squamosa x A. cherimola* (George *et al.*, 1993).

The vegetative propagation of these species present problems of different degree, being its sexual propagation of limited agronomic value due to the high degree of heterozygosis of these species and preventing their propagation by seed.

In order to fix the Annonaceae vegetative propagation problems, *in vitro* methods have been applied.

In this investigation initially explant from leaf, node, stem and bud were cultured on MS medium with different concentration of hormones such as (2,4-D), (IAA + BA), (NAA + BA), (2,4-D + BA + IBA) and (KIN + BA). After complete result analysis it has been cofirmed that 0.2 ppm BAP and 0.2 ppm NAA is best concentration for regeneration in *Annona squamosa*. This hormonal concentration is used with 100 ppm Riboflavin and 50 ppm Rifampicin. The best result obtained in nodal explant and followed by leaf explant on the same hormonal concentration. Callus obtain from these explant were dark brown and very soft in nature.

The stimulating effects of BAP on bud break and multiple shoot formation has been reported earlier for several medicinal and aromatic plant species including *Rauvolfia* (Banerjee and Modi, 2010), *Chlorophyllum borivilianum* (Purohit *et al.*, 1994). *Ocimum* (Patnaik and Chand, 1996) and *Withania somnifera* (Sen and Sharma, 1991). Composition of the culture medium is an important factor in the successful establishment of tissue culture.

Table 1: Regeneration and Callus induction from inter-nodal segment cultured in MS media supplemented with different concentrations of Phyto-hormones

Plant sample: Sl. No.	*Annona squamosa* Hormonal Concentration (mg/l)						Observation	Comments
	2,4-D	IAA	BAP	IBA	NAA	Kinetin		
1.	1.0	×	×	×	×	×	-	No response
2.	2.0	×	×	×	×	×	-	No response
3.	3.0	×	×	×	×	×	-	No response
4.	4.0	×	×	×	×	×	-	No response
5.	5.0	×	×	×	×	×	-	No response
6.	6.0	×	×	×	×	×	-	No response
7.	3.0	×	×	×	×	×	-	No response
8.	1.0	×	×	×	×	×	-	No response
9.	2.0	×	×	×	×	×	-	No response
10.	3.0	×	×	×	×	×	-	No response
11.	4.0	×	×	×	×	×	-	No response
12.	5.0	×	×	×	×	×	-	No response
13.	6.0	×	×	×	×	×	-	No response
14.	×	3.0	0.5	×	×	×	-	No response
15.	×	×	0.2	×	0.2	×	+ + +	Regeneration observed (shoot)
16.	0.5	×	0.5	0.5	×	×	+	Swelling observed
17.	×	×	5.0	0.5	×	×	+	Swelling observed
18.	2.0	×	0.5	×	×	×	+	Swelling observed
19.	×	0.5	×	×	×	5.0	+	Swelling observed
20.	×	×	0.6	×	×	0.6	+ + +	Callus formation

Figure 1: Regeneration of shoot in 29 days old explant of *Annona squamosa* in MS media supplemented with 0.2 ppm BAP + 0.2 ppm NAA

Figure 2: Regeneration of shoot in 19 days old explant of *Annona squamosa* in MS media supplemented with 0.2 ppm BAP + 0.2 ppm NAA

Concentrations of different growth regulators in the medium significantly influenced shoot regeneration and callus formation from various explants. By simply changing the type, concentration and combination of growth regulator in the medium explants responded in different way (Mehta and Mahato, 2012).

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