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BIOTECHNOLOGY

Effect of Silver Nitrate on Leaf Abscission in Culture during Establishment of Explants of Custard Apple (*Annona squamosa* l.) cv. Balanagar

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ABSTRACT

The present investigation was carried out on "Micropropagation in custard apple (*Annona squamosa* L.)" with objective to develop commercially feasible and economically viable protocol for micropropagation technique of custard apple. While standardization of micropropagation in custard apple, Effect of silver nitrate on leaf abscission in culture during establishment of explants of custard apple cv. Balanagar were examined. In order to control leaf abscission silver nitrate at concentrations of 0, 2.5, 5 and 10 mg/l was tested to medium MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 100mg/l CH. The results revealed that Silver nitrate significantly reduced leaf abscission in both shoot tip and axillary bud explants. The least leaf abscission was reported in treatment N_3 (5 mg/l) followed by treatment N_4 (10 mg/l) and N_1 (2.5mg/l) in both shoot tip and axillary bud explants. Whereas, in treatment N_1 (without silver nitrate) more than 80% leaf abscission was observed. The number of leaves produced by the explants was maximum in treatment N_3 (5 mg/l) in both shoot tip (7.5) and axillary bud (8.5) explants. Whereas, when silver nitrate was applied at 10 mg/l (Treatment N_4) this was phytotoxic and inhibited leaf production.

Highlight

- Silver nitrate can be used as effective inhibitors of leaf abscission in the establishment of *Annona squamosa* in culture. Silver nitrate is effective for reducing leaf abscission when used at 5 mg/l.

Keywords : Shoot tips, axillary buds, silver nitrate and leaf abscission

Annona squamosa L. (Custard apple) is a favourite table fruit which is widely grown in arid and semi arid region of the India. It is native to tropical America (George and Nissen 1987). It is widely distributed throughout the tropical regions of Central America and West Indies (Popenoe 1974). It is a drought tolerant, hardy plant and grows well even on shallow soil without much care. It is a deciduous in nature which sheds leaves during winter. The pulp surrounding the seeds of ripe fruit is very delicious and nutrition. The immature fruit, seeds, leaves and roots are of considerable medicinal value both in Ayurvedic and Yunani systems of medicine (Kirtikar and Basu 1933). Its fruits are good tonic, enrich the blood, increase the muscular strength, lessen the burning sensation and relieve vomiting (Ayurveda). It is commonly propagated by either seeds or vegetative methods, Propagation through seed method is easy and cheaper, but seeding plants are not true type, hence, this method is not desirable for commercial cultivation of this crop. Seed germination of *A. squamosa* in nature is only about 30-40%. The seed propagation results immense variability affecting yield, size and quality of fruits (George *et al.*, 1987). Alternatively, clonal propagation in custard apple

is carried out by grafting and budding (Rasai *et al.*, 1994). Although, grafted plants are true to type, this method is rather slow to meet the demand desirable genotype in short duration. Moreover, for almost all these species clonal propagation by cutting or air layering has not been very successful because of their low rooting potential (Rasai *et al.*, 1995).

Recently, micropropagation is the advanced technique for rapid multiplication on large scale in short period. While standardization of micropropagation in custard apple, Effect of silver nitrate on leaf abscission in culture during establishment of explants of custard apple cv. Balanagar were examined. *Annona squamosa* L. is a drought resistant tropical tree which has a physiological mechanism for leaf-shedding during dry seasons. Attempts to micropropagate custard apple from shoot tip and axillary bud explants have been hindered by of complete leaf abscission during establishment in culture. As consequence of leaf abscission, the growth was reduced and the explants became weak, until it was impossible to continue the micropropagation process. Hence this experiment was practiced to overcome this serious problem.

MATERIALS AND METHODS

Source of explant and types of explant

The present investigation was carried out at the Department of Biotechnology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India. Shoot tip and axillary bud from newly emerged shoots were collected from 6-8 month old grafted plants of custard apple cv. Balanagar.

Explant preparation

Leaves were removed leaving the petiole. They were washed thoroughly in running tap water for 15 minutes to remove traces of dirt. Shoot tip and axillary bud were kept in a solution of 0.2 per cent BAVISTIN (carbendazim 50 per cent WP) and 0.05 per cent streptomycin for approximately one hour. The solution was removed and explants were treated with 10 per cent solution of teepol for 10 minutes. All traces of teepol were removed by repeated washing in double glass distilled water. Explants were then dipped in antioxidant *viz.* ascorbic acid (150 mg/l) for 1 hour. The traces of antioxidants were washed out with sterilized double distilled water.

Media preparation and sterilization

MS (Murashige and Skoog 1962) medium supplemented with 0.5 mg/l BAP + 0.5 mg/l KIN + 100 mg/l CH, added 2% Sucrose and 0.8 % agar. Medium pH was adjusted to 5.8. Media was sterilized in autoclave at 121ºC and 15 psi for 20 min.

Surface-sterilization of the explant and Inoculation

Further, sterilization procedures were carried out under aseptic conditions in laminar air flow cabinet. Surface sterilization is done using 0.1% $HgCl_2$ solution for 5 minutes for shoot tips and for 8 minutes for axillary bud explants under aseptic conditions. Shoot tips and axillary buds were then thoroughly rinsed at least three times with autoclaved de-ionized double distilled water. The sterilized explants were then, cut and trimmed into small explants of 1.5 cm length. Immediately, the explants were inoculated individually on MS medium containing 0.5 mg/l BAP, 0.5 mg/l KIN and 100mg/l CH. Silver nitrate at concentrations of 0, 2.5, 5 and 10 mg/l in medium were tested to control the leaves abscission. The observations regarding percent leaf abscission and no. of leaves produced were recorded 4 weeks after inoculation. The method was followed as per cited by Singh and Patel (2014).

Culture conditions

All the cultures were incubated in a culture room at a temperature of 26 $\pm$ 2ºC with relative humidity at 55 $\pm$ 5 per cent. Cultures were provided with light using fluorescent tubes with 16:8 hours light/dark cycle kept 50 cm above bench surface (3000 lux).

Statistical analysis

All the experiments were setup in the completely randomized design and repeated four times, each treatment consisted of 50 explants and the means separation were done according to Least Significant Differences (LSD) at 5% level.

RESULTS AND DISCUSSION

Effect of silver nitrate on leaf abscission of culture of custard apple cv. Balanagar

Annona squamosa L. is a drought resistant tropical tree which has a physiological mechanism for leaf-shedding during dry seasons. As consequence of leaf abscission, the growth was reduced and the explants became weak, until it was impossible to continue the micropropagation process (Lemos and Blake 1994).

After 4 weeks of culture, silver nitrate significantly reduced leaf abscission in both shoot tip and axillary bud explants (Table 1). The least leaf abscission was reported in treatment N_3 (5 mg/l) followed by treatment N_4 (10 mg/l) and N_1 (2.5mg/l) in both shoot tip and axillary bud explants. Whereas, in treatment N_1 (without silver nitrate) more than 80% leaf abscission was observed. Similar results were also reported by Lemos and Blake (1996) in *Annona squamosa* L.

Table 1: Effect of silver nitrate levels on leaf abscission in explants of custard apple cv. Balanagar

Treatment No. Silver Nitrate (mg/l)	Leaf abscission (%)		No. of leaves Produced	
	Shoot Tip	Axillary bud	Shoot Tip	Axillary bud
N_1: 0.0	86.00 (68.03)	84.00 (66.43)	1.50	2.00
N_2: 2.5	38.00 (38.06)	35.00 (36.27)	5.50	7.00
N_3: 5.0	5.75 (13.84)	5.00 (12.89)	7.50	8.50
N_4: 10.0	11.55 (19.87)	10.75 (19.13)	3.00	3.75
S.Em. ±	0.37	0.40	0.32	0.35
C.D. at 5%	1.13	1.23	0.99	1.07
C.V. %	2.11	2.39	14.75	13.03

** Figures in parentheses are arc sine transformed values*

Medium: *MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 100mg/l CH*

Incubation: *4 weeks*

Explants: *Shoot tips and Axillary buds*

Growth regulators are presumed to be involved in regulating abscission in the leaves of many plants (Addicott and Wiatr 1976). Ethylene is particularly important in the sequence of abscission phenomena (Abeles *et al.* 1992). Low levels of ethylene can promote abscission of fruits, petals, leaves and buds in many plants (Sisler and Yang 1984). Burg (1968) has reported that ethylene is a hormonal regulator at 0.1-10µl/l. In some tissues, however, concentrations as low as 0.01µl/l are effective in inducing hormonal responses (Salveit and Yang 1987). In micropropagation conditions, because of the nature of the closed environment to avoid infections and reduce evapotranspiration, the ethylene produced by the tissues may accumulate to physiologically active levels.

Leaf Abscission (Control)

MS + 0.5 mg/l BAP + 0.5 mg/l KIN + 100 mg/l CH + 5 mg/l Silver nitrate

Fig. 1: Effect of silver nitrate on leaf abscission of culture of custard apple cv. Balanagar

The number of leaves produced by the explants was maximum in treatment N_3 (5 mg/l) in both shoot tip (7.5) and axillary bud (8.5) explants. Whereas, when

silver nitrate was applied at 10 mg/l (Treatment N_4) this was phytotoxic and inhibited leaf production.

Species of *Annona* are known to be ethylene sensitive (Lemos and Blake 1994). Microcuttings of *Annona squamosa* in culture have expressed leaf abscission which may be related to the ability of the tissues to produce ethylene under stress (Abeles *et al.*, 1992). The process of harvesting twigs, sterilization, cutting and culture in a restricted environment are stressful for the tissues and ethylene is likely to be produced as a response. Low level of ethylene produced by *Annona squamosa* explants in culture (0.1µl/l/day) was enough to produce physiological effect like leaf abscission and under such conditions; it was very difficult for plantlets to become establishment (Lemos and Blake 1994).

CONCLUSION

The study for controlling leaf abscission has demonstrated that silver nitrate can be used as effective inhibitors of leaf abscission in the establishment of custard apple (*annona squamosa* l.) cv. Balanagar in culture. Silver nitrate is effective for reducing leaf abscission when used at 5 mg/l.

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