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Phenolic Exudation Control and Establishment of *In vitro* Strawberry (*Fragaria × Ananassa*) cv. Chandler

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Authors' contributions

This work was carried out in collaboration between all authors. Author HM designed the study, carry out the experiments, developed the efficient protocol and wrote the first draft of the manuscript. Authors RR and FA performed the statistical analysis and provided the guidance during the course of study. Authors AKS and SP managed the literature searches. Author VK supports in data recording. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

The rate of strawberry propagation through conventional technique is quite low and it is difficult to maintain planting material during the summer months under Bihar condition. Further, importing mother plants adds to the production cost. *In vitro* micro propagation has emerged as a potential alternative for supplying planting material for strawberry. Two type of explants viz., runner tip and nodal segment were used for the study. Phenol exudation was the major problem during establishment which caused death of majority explants. In our experiment, almost no phenolic exudation (+) and maximum percent regeneration for runner tip (55.2 ± 0.52%) and nodal segment (58.1 ± 0.54%) was observed when MS medium was supplemented with ascorbic acid 200 mg per liter. Phenolic exudation was recorded highest (++++) under control when no antioxidants were supplemented. Minimum number of days for runner tips (8.4 ± 0.23) and nodal segments (10.3 ± 0.33) taken for shoot proliferation was observed when MS medium was supplemented with

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activated charcoal 300 mg and 200 mg per liter, respectively**.** Though all other antioxidants used in our study including citric acid, PVP and activated charcoal significantly reduced oxidative browning, ascorbic acid was found to be most effective antioxidant in controlling lethal browning during *in vitro* establishment of strawberry. This protocol has a potential for allowing a large scale multiplication of this important crop.

Keywords: In vitro; strawberry; micropropagation; phenol; browning; antioxidants.

1. INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) belonging to rosaceae family is a popular fruit growing successfully in the Northern hemisphere in temperate and sub temperate environment [1]. Strawberry fruits are rich source of vitamin C, B1, B2, protein, calcium, potassium, copper and iron [2] and being rich in bioactive photochemicals, especially phenolic compounds with high antioxidant capacity are considered to be highly nutritious[3] .

Strawberry clones are conventionally propagated by runners. This method is relatively slow as it is limited to a finite number of plants produced on runners and depended on season and does not guarantees virus elimination and mass production. Commercial production of Strawberry relies heavily on a continuous production of disease free clones throughout the year. Tissue culture has been effectively used in this regard as it ensures availability of virus free clones as and when required [4].

Though it is a major fruit of temperate region, with advent of day neutral cultivars, it growswell in tropical and subtropical regions as well. In India it grows well in tropical and subtropical regions including Maharashtra, West Bengal, Punjab, Rajasthan, Haryana and Delhi. Recently, strawberry cultivation in northern India especially in Haryana, Punjab, Himachal Pradesh and parts of Uttar Pradesh is getting popular due to market demands. Keeping in mind its potentiality strawberry is now also being tried as an important cash crop in climatic conditions of Bihar. But rate of strawberry propagation through conventional technique is quite low and it is difficult to maintain planting material during summer months under Bihar condition. Further, importing mother plants is very expensive for the farmers. *In vitro* micro propagation has recently emerged as a potential alternative for supplying of planting material for strawberry.

A major problem during micropropagation of strawberry is lethal browning caused by the exudation of phenolic compounds (secondary metabolites) from excised portion of explants. Phenolic compounds or secondary metabolites in plants, being involved in defence mechanism of plants, are produced in response of biotic and abiotic stress. During explant preparation when explants are excised phenolic exudation is stimulated. High phenolic exudation causes activation of oxidative enzyme such as polyphenol oxidase (PPO) phenylalanine ammonia lyase (PAL) and peroxidase (POD) when explants are cut, causing lethal browning and death of explants (reduced explant initiation, growth, and development). Therefore, preconditioning of explants with media supplements such as ascorbic acid, citric acid, PVP and activated charcoal is necessary to limit production of these substances [5]. Keeping this in view an experiment was designed to study role of these antioxidants in controlling *in vitro* phenolic exudation and establishment of strawberry cv Chandler.

2. MATERIALS AND METHODS

Runner tips and nodal segment explants of variety Chandler were used for present study. The explants were collected from runner producing strawberry plants growing at Horticulture garden, BAU Sabour. The explants were first washed thoroughly with tap water 4-5 times followed by washing in solution containing 2- 3 drops detergent (tween -20) and 1-2 ml Dettol for about 10 minutes and subsequent washingwith sterilized water for 2-3 times. Furthermore, washed nodal segment and leaf explant were dipped in 0.2% Bavistin solution for 30 minutes to control the fungal contamination.

All the aseptic manipulations such as surface sterilization of explants, preparation and inoculation of explants and subsequent sub culturing were carried out under aseptic conditions in the hood of clean laminar airflow chamber. For surface sterilization a separate experiment conducted earlier in which Ethanol (70%) for 30 sec + $HgCl_2$ (0.1%) for 2 min resulted less contamination of cultures, was

given to both the explants. The main study was carried out to see the effect of anti-oxidants such as ascorbic acid, citric acid, PVP and activated charcoal in the media at different concentrations on phenol exudation and *in vitro* establishment. Exudation of phenolics is not only a natural mechanism in plants but also produced in many plants after wounding during explant incision and can result in death of cells. Phenolic exudation inactivates growth of tissues in culture (Ahmed *et al.* 2013), thus reducing survival percentage.

Murashige and Skoog's [6] media consisting of 0.8% Agar, 2.5% sucrose and having pH 5.8 supplemented with 3.0 mg/l BAP+ 0.5 mg/l IAA was used as basal medium. The inoculated cultures were incubated at 25±2°C in an air-conditioned culture room with a light intensity of 2000-3000 lux from cool white fluorescent tubes. The light/dark cycles of photoperiod were maintained at 16/8 hours daily.

The observation recorded includes degree of phenolic exudation, percent regeneration of explants and number of days taken for shoot proliferation. Scoring was given as (++++) high browning, (+++) medium browning, (++) low browning, (+) almost no browning.for degree of phenolic exudation as per visual observation on colour intensity (browning) of media.

3. RESULTS AND DISCUSSION

The exudation of phenolic compounds from cut ends of plant tissues results in browning of explants and has proved lethal to establishment of cultures. Phenolic compounds are secondary metabolites released from plants, which are present in high amounts and being involved in defence mechanism of plants, are produced in response of biotic and abiotic stress. Browning in plants occurs mainly due to the oxidation of phenolic compounds by phenol oxidase. This phenomenon occurs when the compartmentalized phenolic compounds are released during explant incision and henceforth react with phenolic oxidases and release quinone. Quinone has negative effect on cell growth and can result in death/necrosis of cells. The results of present study demonstrated that phenolic exudation can be controlled by supplementing media with various antioxidants. In our experiment, almost no phenolic exudation (+) and maximum percent regeneration for runner tip (55.2 ± 0.52%) and nodal segment (58.1 ± 0.54%) was found when MS medium was supplemented with ascorbic acid 200 mg per liter. However, minimum number of days for runner tips (8.4 ± 0.23 days) and nodal segments (10.3 ± 0.33 days) taken for shoot proliferation was observed when MS medium was supplemented with activated charcoal 300 mg and 200 mg per liter, respectively. Phenolic exudation was recorded highest (++++) under control when no antioxidants were supplemented into the media.

Citric acid (300mg/l) also reduced browning to nil but it decreased survival percentage of explants and delayed shoot proliferation in both cases. Similar case was observed in case of ascorbic acid+ citric acid 150 mg/l + 150 mg/l where although no browning was observed but survival percentage reduced to 48.6 ± 1.06 in runner tip and 51.2 ±1.16 in nodal segment and number of days required for shoot proliferation increased to 13.9 ± 0.38 days. In this experiment PVP at all the tested concentrations for runner tip and nodal segments was found to be least effective antioxidant in controlling browning, reduced survival percentage and also delayed shoot proliferation. Prajapati et al. [7] also found PVP ineffective in controlling browning during *in vitro* propagation of *Curculigo orchioides.*

Though all the other antioxidants including citric acid, PVP and activated charcoal significantly reduced per cent oxidative browning, ascorbic acid (200 mg/l) was found to be most effective antioxidant followed by ascorbic acid (300 mg/l) in the media for both the explants.

Ascorbic acid has been used successfully in the past to inhibit the exudation of phenols and reduced oxidative browning in various fruit crops [8,9]. Ascorbic acid is able to scavenge oxygen radicals produced when the plant tissue is wounded, therefore protecting the cells from oxidative injury. The oxidative browning of explant tissue is reduced by ascorbic acid detoxifying these free radicals. Moreover, ascorbic acid is an antioxidant that is able to prevent or inhibit oxidation process. Besides its role as an antioxidant, ascorbic acid is involved in cell division and elongation. A similar result was reported by Poudyal et al. [10] who found that use of ascorbic acid in the growing medium was very effective to control browning and only 8% explants of Yali variety of pear were infected by browning and 92% of explants survived by using 100 mg/l ascorbic acid in the MS growing medium. Ahmad et al. [11] also reported that samples treated with ascorbic acid 250 mg/l for 5 hours prior to culturing showed least browning in micropropagation of guava.

Table 1. Effect of various treatments on *in vitro* phenolic exudation from runner tip of strawberry cv. Chandler

Treatment code	Treatments		Degree of phenolic exudation	No. of days taken for shoot proliferation	Percent regeneration of explants
T0	Control		+ + + +	10.4±0.75	5.6 (13.7 ± 0.43)
T1	Ascrobic acid	100 mg/l	+ +	10.7±0.29	27.4 (31.5 ± 0.76)
T2		200 mg/l	+	12.2±0.35	67.5 (55.2 ± 0.52)
T3		300 mg/l	+	13.2±0.38	60.4 (51.0 ± 0.42)
T4	Citric acid	100 mg/l	+ + +	11.2±0.32	28.7 (32.4 ± 0.29)
T5		200 mg/l	+ +	12.1±0.35	61.1 (51.4 ± 0.54)
T6		300 mg/l	+	13.4±0.38	56.4 (48.6 ± 0.49)
T7	PVP	100 mg/l	+ + + +	10.2±0.29	10.1 (18.5 ± 0.78)
T8		200 mg/l	+ + +	10.8±0.29	19.6 (26.2 ± 1.14)
T9		300 mg/l	+ +	12.2±0.35	24.3 (29.5 ± 1.30)
T10	Activated charcoal	100 mg/l	+ + +	9.0±0.26	16.2 (23.8 ± 0.41)
T11		200 mg/l	+ +	8.6±0.23	27.6 (31.7 ± 0.57)
T12		300 mg/l	+ +	8.4±0.23	31.7 (34.3 ± 0.63)
T13	Ascrobic acid+ citric acid	150 mg/l + 150 mg/l	+	13.9±0.38	56.3 (48.6 ± 1.06)
C.D.				1.066	2.121
SE(m)				0.366	0.728

Table 2. Effect of various treatments on *in vitro* phenolic exudation from nodal segment of strawberry cv. Chandler

Treatment code	Treatment		Degree of phenolic exudation	No. of days taken for shoot proliferation	Percent regeneration of explants
T0	Control		++++	11.4±0.64	4.9 (12.8 ± 0.38)
T1	Ascrobic acid	100 mg/l	++	11.9±0.53	37.4 (37.7 ± 0.94)
T2		200 mg/l	+	12.6±0.18	72.2 (58.1 ± 0.54)
T3		300 mg/l	+	13.6±0.18	65.4 (53.9 ± 0.47)
T4	Citric acid	100 mg/l	+++	11.9±0.18	31.1 (33.9 ± 0.28)
T5		200 mg/l	++	12.2±0.18	63.0 (52.5 ± 0.56)
T6		300 mg/l	+	13.2±0.22	56.3 (48.9 ± 0.49)
T7	PVP	100 mg/l	++++	11.1±0.92	14.1 (22.0 ± 0.92)
T8		200 mg/l	+++	11.0±0.90	19.5 (26.2 ± 1.13)
T9		300 mg/l	+++	11.2±0.92	23.6 (29.0 ± 1.27)
T10	Activated charcoal	100 mg/l	++++	11.2±0.37	23.7 (29.1 ± 0.51)
T11		200 mg/l	+++	10.3±0.33	29.6 (32.9 ± 0.60)
T12		300 mg/l	++	10.7±0.33	29.8 (33.1 ± 0.60)
T13	Ascrobic acid+ citric acid	150 mg/l + 150 mg/l	+	13.6±0.43	60.8 (51.2 ±1.16)
C.D.				1.5	2.2
SE(m)				0.53	0.77

++++: High browning; +++: Moderate browning; ++: Low browning; +: No browning

4. CONCLUSION

The results of the present study showed that almost all explants on the medium without antioxidants or with its lower concentration browned extremely. Almost no phenol exudation and maximum percent regeneration for runner and nodal segment was observed when MS medium was supplemented with ascorbic acid 200 mg per liter. The study revealed that ascorbic acid plays a significant role in reducing phenolic exudation and thus increase percent

regeneration by controlling oxidative lethal browning. At the same time it also increases growth rate thus requiring shorter time for shoot proliferation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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