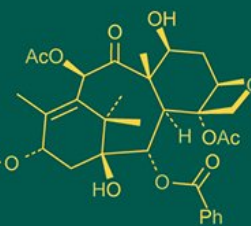
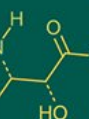
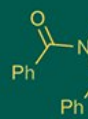


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Combined effect of antioxidants and adsorbents in controlling phenolic browning of *in-vitro* *Phalaenopsis* culture

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Abstract

Phenolic browning during tissue culture limits efficient micropropagation of *Phalaenopsis* orchids causing media discoloration, cytotoxic quinone formation ensuing explant mortality. To address this, effect of antioxidants viz. ascorbic acid (AA), citric acid (CA) and adsorbent polyvinylpyrrolidone (PVP) was evaluated either as culture media additive or as pre-soaking treatment of explants in separate experiments. In the first experiment *in-vitro* plantlets were cultured in MS medium supplemented with treatments: AA 0.1%, CA 0.1%, PVP 1%, AA 0.1% + CA 0.1%, PVP 0.5% + AA 0.05% + CA 0.05%, PVP 0.5% + AA 0.1% + CA 0.1%, PVP 1% + AA 0.1% + CA 0.1%. In experiment-2, flower-stalk nodal explants were soaked in 0.1% CA + 0.1% AA + 1% PVP for 0.5–3.0 hours prior to culture. Results showed that combined antioxidant–adsorbent treatments markedly reduced media browning and improved explant survival compared with single treatments or untreated controls. The best media additive (T7: 1% PVP + 0.1% AA + 0.1% CA) gave 82.50% survival and 15% browning at 30 DOI. Pre-soaking for 3 hours improved survival to 88.75% and reduced browning to 15%. Lower PVP or antioxidant doses and short pre-soakings were less effective. The findings indicate that managing phenolic exudation, with an optimal PVP–ascorbic–citric acids formulation, especially as a 3-hour pre-soak or as a media supplement significantly mitigates enzymatic browning and enhances establishment of *Phalaenopsis* explants *in-vitro*. Further optimization of PVP concentration and alternative antioxidant systems could refine protocols for large-scale micropropagation.

Keywords: *Phalaenopsis* orchid, enzymatic browning, phenols, anti-oxidants, adsorbents, tissue-culture

Introduction

Phalaenopsis, often known as moth orchid, are among the most recognized orchid species due to its beautiful and long-lasting flowers of extraordinary color and shape. *Phalaenopsis* orchids occupy the top most position in the international market as potted flowering plant (Kaveriamma *et al.*, 2019) [17] and have immense economic value as cut flower (Khatun *et al.*, 2020). Taiwan alone had exported *Phalaenopsis* to the tune of 130 million USD (Council of Agriculture, Taiwan, 2015). Since, *Phalaenopsis* is a monopodial plant, it is traditionally propagated by keikis and dividing off-shoots. However, these methods of multiplication are very slow, making them ineffective for large-scale production. On the other hand, seed propagation *in-vivo* is not possible due to lack of endosperm in the seeds. Moreover, heterozygous nature of parent plants results in high variability among seedlings. As orchids are declared as endangered by CITES, only tissue cultured plants are tradable. Hence, *Phalaenopsis* is commercially multiplied through tissue culture. Leaf and flower stalk are common explants used for *in-vitro* multiplication of *Phalaenopsis*. At present, plant tissue culture is mainly used in rapid propagation and mass multiplication of *Phalaenopsis*.

One of the major problems faced during the development of *Phalaenopsis* plantlets through tissue culture is the secretion of phenols from the explants which turn the media and the explant brown. Browning of explants hinders the growth of tissue and plantlets, and Millard reaction is the non-enzymatic cause of media browning. However, this is not detrimental to growth of plantlets and can be overcome through frequent subculturing. Enzymatic reaction of phenols, on the other eventually affect the success of rapid propagation (Ru Z2013; Xu

et al., 2007; Zhao *et al.*, 2010) [15, 18]. The browning in tissue culture is reported to be due to enzymatic or non-enzymatic reactions. Pyrolysis of sugar or the hand, impede cell and tissue growth *in-vitro*. Phenolic compounds are present in healthy plant tissues and accumulate in the vesicles (Beckman, 2000) [12], they are produced in greater abundance and/or released as a defense response, especially following tissue wounding or stress (Dixon and Paiva., 1995; Lorenzo *et al.*, 2001) [3, 9]. Phenolics, secreted by the wounded tissue, get oxidized by enzymes like polyphenol oxidase (PPO) and peroxidase (POD) released from the cytoplasm and form quinones which, being electrophilic, are further polymerized and produce black brown substance that impart blackish-brown pigmentation in both culture media and tissue (Liu *et al.*, 2024 [8]; Holderbaum *et al.*, 2010 and Chen *et al.*, 2019) [19]. Ethylene accumulation in the culture medium resulting in low gas exchange, is also reported as a cause of browning of explants during *in-vitro* cultures (Gerszberg *et al.*, 2015). Overall, the tissue browning of *Phalaenopsis* was attributed to several factors like pH, temperature, phenol concentration of explant, enzyme activity of protease (polyphenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia lyase (PAL)), genotype, and so on (Xu and Li, 2006; Zhao *et al.*, 2006; 2009 and 2010) [15].

During the tissue culture process of *Phalaenopsis*, when explants such as inflorescence nodes, flower stalk meristems, and shoot tips are cut pre-existing polyphenol oxidase (PPO) is released. Shortly after culture initiation, cutting stress increases the synthesis of phenolic compounds and PPO which react and form quinones, which polymerize and turn brown; quinones are cytotoxic and damage explant tissue. (Mayer, 2006; Pandey & Ranade, 2005) [10, 14]. Certain varieties of *Phalaenopsis* are more likely to darken than others again mature or stressed explants typically acquire more phenolics and darken more (Leng *et al.*, 2009) [7].

To scavenge phenolics and prevent oxidation, addition of antioxidants such as - citric acid, ascorbic acid or comparable substances is recommended in *Phalaenopsis* (Kang *et al.*, 2026). For binding phenolics and lessen their toxicity, adsorbents like activated charcoal or polyvinylpyrrolidone (PVP) are recommended in *Phalaenopsis* (Tao *et al.*, 2007) [4]. Using younger and healthier explants and pre-culture treatments such as a quick soak in antioxidant solutions can lower the phenolic burden prior to plating (Murthy *et al.*, 2006) [13]. Oxidation is reported to be reduced by frequent subculturing, limiting extended exposure to light or heat, and adequate gas exchange (Liu *et al.*, 2024) [8]. A hybridization programme of *phalaenopsis* was taken up and since, tissue culture is the only avenue of multiplication, similar constraints of phenolic browning were experienced. To overcome the problem the present research explores the combined effect of antioxidants and adsorbent both as media additive and pre culture treatment to reduce browning in *in-vitro* culture of *Phalaenopsis* hybrids and expediting its growth and propagation rate.

Materials and methods

The present experiment was conducted during year 2022 to 2025 at Uttar Banga Krishi Viswavidyalaya, Micro-propagation laboratory under Rashtriya Krishi Vikash Yojana, Pundibari, Coochbehar, West Bengal. Two experiments were conducted to assess the efficiency of

antioxidant treatments in controlling browning. For the first experiment *in-vitro* plantlets were cultured in MS media with different combinations and concentrations of antioxidants such as Citric acid and Ascorbic acid and the adsorbent Polyvinylpyrrolidone while the second experiment encompassed pre-soaking of flower stalk explants in antioxidant solution of 0.1% Citric acid (CA) + 0.1% Ascorbic acid (AA) + 1% Polyvinylpyrrolidone (PVP) for different time durations.

Treatment details

In the first experiment *in-vitro* plantlets were cultured in MS media containing different combinations and concentrations of antioxidants. The treatments were, AA 0.1% as (T₁), CA 0.1% as (T₂), PVP 1% (T₃), 0.1% AA + 0.1% CA as (T₄), 0.5% PVP + 0.05% AA + 0.05% CA as (T₅), 0.5% PVP + 0.1% AA + 0.1% CA as (T₆), 1% PVP + 0.1% AA + 0.1% CA as (T₇) and Control without antioxidants (T₀).

In the second experiment single node segment of flower stalk were used as explants which were pre-soaked in aqueous solution of 0.1% Citric acid + 0.1% Ascorbic acid + 1% Polyvinylpyrrolidone for different time durations viz. 30 minutes (T₁), 1 hour (T₂), 2 hours (T₃), 2.5 hours (T₄) and 3 hours (T₅) which were considered as treatments and were tested against untreated control (T₀).

Media preparation

Media with antioxidant and adsorbent

Plantlets were cultured in MS media supplemented with Kinetin @ 1.00 ppm, NAA @ 1.00 ppm was added with different antioxidants and adsorbent combinations as per treatments. Sucrose was added to the media at a concentration of 3.0% as source of carbon. Other additives like vitamin B₁ @ 5 ml/L, antibiotic like azithromycin @ 10 ml/L, myo-inositol @ 100mg/L and coconut water @ 10 ml/L were added to the media which was solidified using Agar @ 6 gm/L.

The pH of the media was adjusted to 5.6 with the help of KOH (Potassium hydroxide) and HCL (Hydrochloric acid) solutions. Into each jam bottle 100 ml of the medium was dispensed and sterilized in an autoclave at 121° C for 15 minutes. The sterilized bottles were stored in room temperature for one week before inoculation.

Media for pre-treated explants

The flower stalks were presoaked in solution for stipulated time period and cultured in MS media supplemented with Kinetin @ 1.00 ppm, NAA @ 1.00 ppm and sucrose @ 3% (w/v), vitamin B₁ @ 5 ml/L, azithromycin as antibiotic @ 10ml/L, Myo-inositol @ 100mg/L and Coconut water @ 10 ml/L. For solidifying, Agar was added at the rate of 6 gm/L and finally the pH of the media was adjusted to 5.6. Hundred milliliters of the media was dispensed into each jam bottle and was sterilized in autoclave at 121° C for 15 minutes.

Explants

In-vitro plantlets

Plantlets with minimum two or three leaves which were formed through *in-vitro* germination of seeds of *Phalaenopsis* orchid were used as explant in the experiment.

Flower stalk

Flower stalks were collected from mature flowering *Phalaenopsis* hybrid plants of cultivar Prima-Piano

maintained under a shade net house in the Instructional Farm of the Department of Floriculture, Medicinal and Aromatics Plants. When the flowers have withered, flower stalks with vegetative buds were cut from mother plants and brought to the laboratory.

Surface sterilization of explants

Plantlets

The explants were directly transferred from *in-vitro* seed culture bottles to different antioxidant treated media under the lamina air flow hood. As they were sourced from sterile cultures, sterilization of explants was not required.

Flower stalks

The flower stalks were cut in segments having a swelled vegetative nodal bud and were soaked in distilled water and the bracts on the nodes of the flower stalks were removed with the help surgical knife and then the nodal segments were kept in Tween-80 for 15 minutes and then thoroughly cleaned in running tap water till it was fully cleaned. Clean flower stalks were treated for 50 minutes with 0.2% (w/v) Bavistin solution and then were washed three times with distilled water.

Pre-soaking treatment in antioxidants

The sterilized flower stalks were treated with aqueous solution of 1% Citric acid + 0.1% Ascorbic acid + 0.1% Polyvinylpyrrolidone for different time durations such as 30 minutes (T₁), 1 hour (T₂), 2 hours (T₃), 2.5 hours (T₄) and 3 hours (T₅) which were considered as treatments and were tested against untreated control (T₀). Then the explants were surface sterilized with 0.1% HgCl₂ (mercury chloride) for 3 minutes in lamina air flow hood. Finally, the explants were rinsed with sterile distilled water for 2-3 times to eliminate residues of mercuric chloride. The operation was done inside lamina hood air flow.

Inoculation of flower stalk

Under the lamina air flow, the segments of flower stalk were again sterilized for the final time before inoculation with the help of HCL, which were diluted with 100 mg/100 ml of sterilized distilled water and was treated for 3 minutes time duration, after sterilization stalks were again properly rinsed 3 times with sterilized distilled water inside the lamina air hood flow before inoculation. The cultures were incubated under controlled conditions with fluorescent light of 3000 lux intensity with culture room temperature of 25±C and a relative humidity of 70%. The photoperiod in the culture room was 12-12 (day-night) hours.

Observations

Browning intensity

The browning intensity in media was assessed visually on the 7th day, 15th day, 30th day and 60th day of inoculation and was marked with following notations : Very low browning = *, Moderate Browning = **, High Browning = ***, Intense Browning = ****.

Appearance of explants

Appearance of explant was also observed at seven days interval till 60 days after inoculation and the visual observations on their colour were noted as Green = G, Slightly green = SG, Yellow = Y, Brown = B. The effects of different treatments were quantified as the mean percentage of blackening, percentage of survival and percentage of dead cultures. Data obtained in percentage were subjected to

angular transformation before analysis. The data were analyzed following Completely Randomized Design using OPSTAT statistical Software to find out the significance of variance.

Results and discussion

Experiment-1: Effect of antioxidants and adsorbent additives in culture media on browning of *in-vitro* plantlets of *Phalaenopsis*.

The media browning due to phenolic exudation was reduced with increase in the concentration of antioxidants such as citric acid, ascorbic acid, and polyvinylpyrrolidone (Table-1). A gradual browning was noted in all the treatments with advancement of time except for T₄ and T₇. The control media started to show moderate browning within 7 days of inoculation which turned intense brown within 60 days (Figure 1, 2, 3). Similar indications were noted in culture media added with ascorbic acid (T₁) or Citric acid (T₂) at 0.1% concentration or PVP alone at 1.0 % concentration. Combination treatments of the three chemicals at all the levels were effective in reducing the browning effect till 7days of inoculation. After 15 days of inoculation, control media and media with T₁, T₂ and T₃ showed high browning while the combination treatment of the antioxidants (T₄) was able to keep the browning level to very low and the effect was similar when PVP @ 1% was added to the antioxidant combination. However, combinations of the antioxidants along with the adsorbent at lower dose were not so effective. Intense browning of media was noted in culture media of control, T₁, T₂ and T₃ after 30 days of inoculation; in T₅ and T₆ the browning effect arrested to moderate level. Very low browning was noted in the culture media of the treatments T₄ and T₇ even after a month of inoculation (Figure 4, 5, 6). The colour of the explant was highly affected by the media treatment. The most of the explants in control media turned brown within 30 days of inoculation and died within 60 days. Explants in T₇ retained their green colour even after 30 days of inoculation and survived, while in the other treatments, the plantlets showed signs of yellowing and discolouration.

In comparison to the untreated control, antioxidant treatments dramatically decreased explant browning and increased survival, indicating their efficacy in reducing oxidative stress brought on by phenolic exudation in plant tissue culture (Table 2). With 82.50 % survival and barely 15% Browning, the treatment T₇ (1% PVP + 0.1% AA + 0.1% CA) produced the best outcomes. The synergistic action of PVP, which absorbs phenolic compounds, ascorbic acid (AA), which operates as a reducing agent, and citric acid (CA), which acts as a chelator, is probably responsible for this better performance (Kang, B.S *et al.*, 2026)

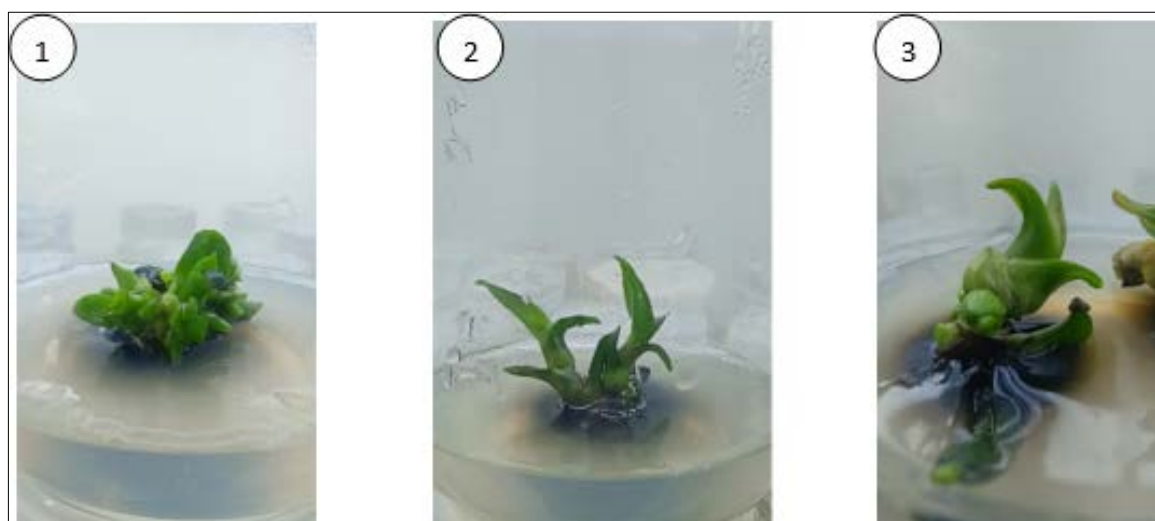
With survival rates ranging from 30% to 31.3% and browning ranging from 45% to 70%, single antioxidant treatments (T₁ AA 0.1 -T₃ PVP 1%) provided only modest benefits. Even though the dual combination treatment (T₄) outperformed singles, the triple combinations that contained PVP still performed better. As in T₅, lower antioxidant concentrations resulted in lower outcomes (32.5% survival, 71.3% browning), emphasizing the significance of ideal dose to prevent toxicity or insufficient protection.

To further increase survival rates, future studies could concentrate on maximizing PVP concentrations or investigating other antioxidants.

Table1: Browning intensity and appearance of ex-plants as influenced by antioxidants and adsorbent in media

Treatment	Replication	At 7 DOI		At 15 DOI		at 30 DOI		At 60 DOI Survival
		Browning intensity	Appearance of ex-plants	Browning intensity	Appearance of ex-plants	Browning intensity	Appearance of ex-plants	
T ₀	1	*	Y	***	Y	****	B	D
	2	**	Y	***	Y	****	B	D
	3	*	G	**	Y	****	B	S
	4	**	SG	***	Y	****	Y	D
T ₁	1	*	G	**	SG	**	SG	D
	2	**	Y	***	Y	****	Y	D
	3	**	Y	***	Y	****	Y	D
	4	**	Y	***	Y	****	Y	D
T ₂	1	**	G	***	SG	****	Y	D
	2	*	G	**	G	****	SG	S
	3	*	G	**	G	****	SG	D
	4	**	G	***	SG	****	Y	D
T ₃	1	**	SG	***	Y	****	Y	S
	2	*	SG	**	Y	****	B	S
	3	**	G	**	SG	****	SG	S
	4	**	SG	***	Y	****	B	D
T ₄	1	*	G	*	G	*	G	S
	2	*	G	*	G	*	SG	S
	3	*	G	*	SG	**	SG	S
	4	*	G	*	SG	*	SG	S
T ₅	1	*	SG	***	Y	****	B	D
	2	*	G	**	SG	**	Y	S
	3	*	G	**	SG	**	Y	S
	4	*	G	**	G	**	SG	S
T ₆	1	*	SG	**	SG	**	Y	S
	2	*	SG	**	SG	**	Y	S
	3	*	G	**	SG	**	SG	S
	4	*	G	**	G	**	SG	S
T ₇	1	*	G	*	G	*	G	S
	2	*	G	*	G	*	SG	S
	3	*	G	*	SG	*	G	S
	4	*	G	*	G	*	G	S

Browning intensity - *Very low, ** Moderate Browning, *** High Browning, **** Intense Browning, S= survived, D= dead, G=Green, SG=Slightly Green, Y=Yellow, B=Brown DOI: Days of Inoculation, T₀= Control, T₁= AA 0.1%, T₂= CA 0.1%, T₃= PVP 1%, T₄= 0.1% AA + 0.1% CA, T₅= 0.5% PVP+ 0.05% AA+ 0.05% CA, T₆= 0.5% PVP+ 0.1% AA+ 0.1% CA, T₇= 1% PVP +0.1% AA+ 0.1% CA

**Fig 1, 2, 3:** *In-vitro* plantlets 60 days after inoculation in control media (T₀), evidence of browning

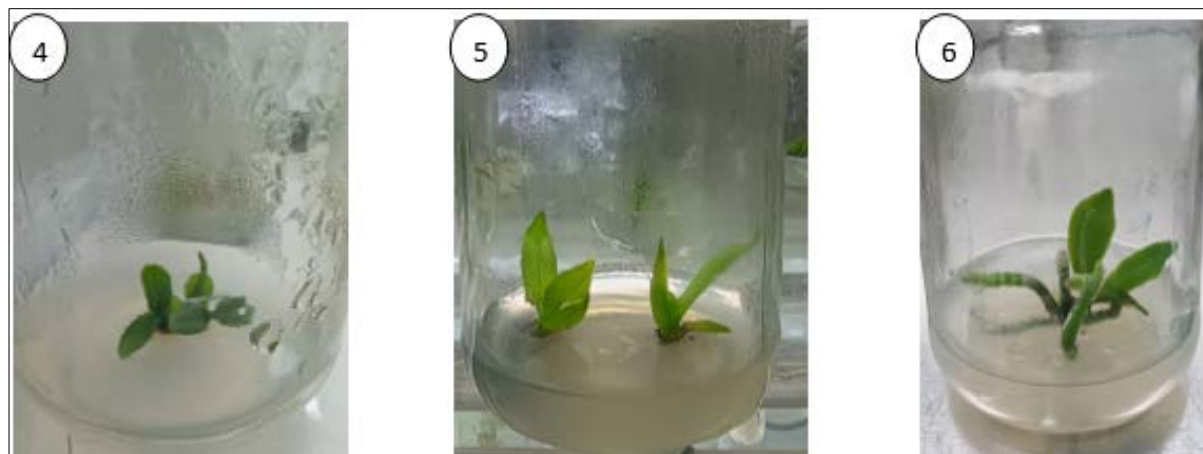


Fig 4, 5, 6: *In-vitro* plantlets 60 days after inoculation in media supplemented with 1% PVP +0.1% AA+ 0.1% CA (T₇) evidence of browning controlled

Table 2: Survival and browning percentage of ex-plants as influenced by antioxidants and adsorbent in media

Soaking time	Survival at 30 DOI		Browning at 30 DOI	
	Percentage	SE	Percentage	SE
(T ₀)	22.50 (28.27)	0.99	87.5 (75.22)	8.96
(T ₁)	30.00 (33.01)	3.03	51.3 (45.67)	3.21
(T ₂)	31.25 (33.82)	2.86	45.0 (42.05)	3.34
(T ₃)	31.25 (33.52)	4.41	70.0 (57.51)	5.58
(T ₄)	67.50 (55.59)	3.92	25.0 (29.50)	3.69
(T ₅)	32.50 (34.70)	1.50	71.3 (59.69)	4.01
(T ₆)	52.50 (46.41)	0.82	45.0 (42.05)	3.34
(T ₇)	82.50 (65.99)	4.22	15.0 (22.12)	3.69
SE(m)	3.04**		4.84**	
C.D. at 1%	8.93		14.21	
C.V. %	14.69		20.72	

Note: Figures in parentheses are angular transformed data

Experiment 2: Effect of pre-soaking time of explants in antioxidant and adsorbent solution on browning of *in-vitro* flower stalk culture of *Phalaenopsis*.

The data regarding effect of pre-soaking of explants (Figure 7) in antioxidant + adsorbent solution (citric acid @ 0.1% ascorbic acid 0.1 % and polyvinylpyrrolidone @1.0%) for varying time period (0., 0.5, 1.0, 2.0, 2.5 and 3.0 hours) in controlling phenolic browning of culture media and survival of explants is represented in Table 3. The data indicates that the browning intensity was controlled effectively with increase in soaking time from 0 to 3 hours in the antioxidant + adsorbent solution. Moderate browning was noted in culture media of T₀, T₁, T₂, while low browning in T₄ and almost no browning was seen in T₅ after 7 days of inoculation.

After 15 days of inoculation untreated control (T₀) and pre-soaking for 0.5 hours (T₁) showed high browning but as the time duration was increased to 2.5 to 3 hours the intensity of browning was reduced drastically. Finally, after 30 days of inoculation T₀ (control) showed intense browning in all the replications and most of the explants were dead by 60 days of inoculation (Figure 8, 9, 10, 11). Pre-soaking for 0.5 to 2 hours (T₁, T₂, T₃) failed to exert any significant positive effect on phenolic browning over the untreated control. But, with increment of soaking period to 2.5 hours and 3 hours the antioxidant + adsorbent solution was visibly effective in controlling the enzymatic browning as the media showed no or very low sign of browning even after 30 to 60 days of inoculation (Figure 12 and 13).

The colour of the explants was also influenced by the pre-soaking duration in the antioxidant + adsorbent solution

(Table-3). When the explants were treated for 3 hours, they retained their green colour event after 30 days of inoculation. However, yellowing of explants was evident when the pre-soaking duration was less. The untreated control turned brown within 30 days of inoculation whereas, the explants lesser treatment durations (T₁, T₂, T₃) exhibited prominent signs of yellowing. Within 60 days of inoculation, most of the untreated control explants and those soaked for 0.5 hours died. The survival rate increased with increment in soaking duration.

The results showed that the time duration for which the explants were pre-soaked in antioxidant + adsorbent solution had a substantial impact on their survival and browning at 30 days after inoculation (Table 4). Explants pre-soaked for 3 hours (T₅) had the highest survival rate (88.75%) among the treatments, followed by 2.5 hours (T₄) with 67.5% survival and 2 hours (T₃) with 55% survival. Pre-soaking for 0.5 hour (T₁) failed to increase the survival rate (31.25 %) over control (15.0%), however, treatment for 1 hour (T₂) showed a moderate survival rate of 47.5%. On the other hand, media browning had the opposite pattern, with the control group exhibiting the greatest amount of browning (85.0 %). Pre-soaking for 0.5 hours failed to reduce the phenolic browning significantly, however, when the pre-soaking time was increased to 1 hour, the effect was statistically significant. The 3 hours treatment (T₅) was most effective with only 15.0 % browning.

The current study showed that pre-soaking explants in an antioxidant solution significantly reduced phenolic oxidation, increasing the *in vitro* survival rates. The progressive decrease in blackening and improvement in

explant survival from 30 minutes to 3 hours of therapy can be explained by extending the soaking time, which increased the antioxidant solution's ability to counteract oxidative processes. The 3-hour treatment produced the highest survival rate (88.75%) and the lowest blackening rate (15%), indicating that prolonged exposure to antioxidants and adsorbent offers better defense against oxidative stress and phenolic exudation. The lack of antioxidant therapy caused severe phenolic oxidation, which

is harmful to explant growth and establishment, as evidenced by the control treatment's poor survival and maximum browning. Higher degrees of tissue darkening and moderate survival were the results of shorter soaking times (30 minutes and 1 hour), which were insufficient to totally suppress oxidative browning. Thus, the best method for reducing browning and enhancing explant survival *in-vitro* was to pre-soak explants in an antioxidant solution for three hours.

Table 3: Visual scoring of browning intensity and appearance of ex-plants pre-soaked in antioxidants and adsorbent solution for variable duration

Treatment	Repli-cation	At 7 DOI		At 15 DOI		At 30 DOI		Condition at 60 DOI
		Browning intensity	Appearance of ex-plants	Browning intensity	Appearance of ex-plants	Browning intensity	Appearance of ex-plants	
T ₀ (Control)	1	*	SG	***	B	****	B	D
	2	**	B	***	B	****	B	D
	3	**	Y	***	Y	****	Y	S
	4	**	Y	***	B	****	B	D
T ₁ (30 mins)	1	*	SG	**	SG	***	SG	S
	2	**	Y	***	Y	****	Y	D
	3	**	Y	***	B	****	B	D
	4	**	SG	***	SG	***	SG	D
T ₂ (1 hours)	1	**	SG	**	Y	***	Y	S
	2	*	G	*	SG	**	SG	S
	3	**	SG	**	SG	****	Y	S
	4	**	SG	**	Y	***	Y	D
T ₃ (2 Hours)	1	*	G	**	G	**	SG	S
	2	**	G	**	Y	***	Y	S
	3	*	SG	*	Y	****	Y	D
	4	**	G	**	G	**	G	S
T ₄ (2.5 hours)	1	*	G	*	G	*	G	S
	2	*	G	*	SG	**	Y	S
	3	*	G	*	G	*	G	S
	4	**	G	***	SG	***	SG	D
T ₅ (3 hours)	1	*	G	*	G	**	G	S
	2	*	G	*	G	*	SG	S
	3	*	G	*	G	*	G	S
	4	*	G	*	G	*	G	S

Note: Browning intensity - Very low *, Moderate Browning **, High Browning ***, Intense Browning ****

G=Green, SG=Slightly green, Y=Yellow, B=Brown, T₀= Control, T₁= 30 mins, T₂= 1 hours, T₃= 2 hours, T₄= 2.5 hours, T₅= 3 hours

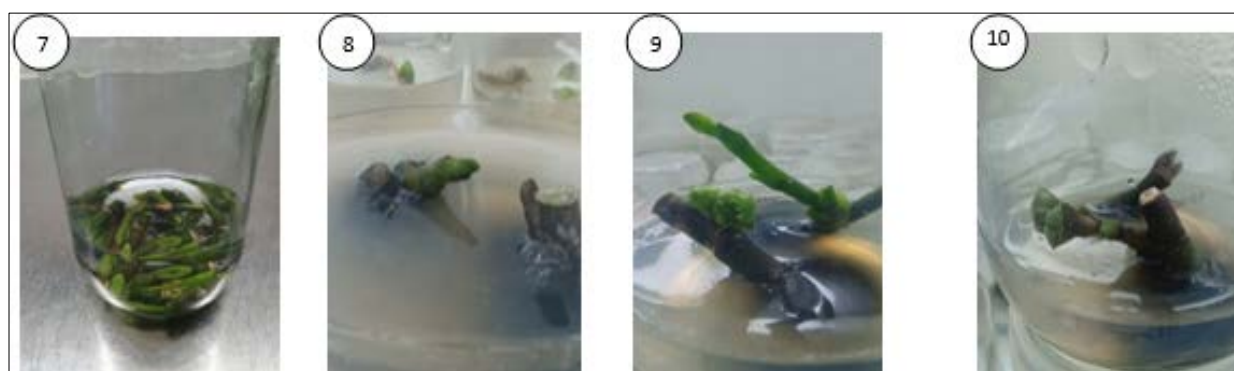


Fig 7: Soaking of flower stalks in 1% PVP +0.1% AA+ 0.1% CA solution. Figure 8, 9, 10: Untreated flower stalks affected with browning after 60 days of inoculation

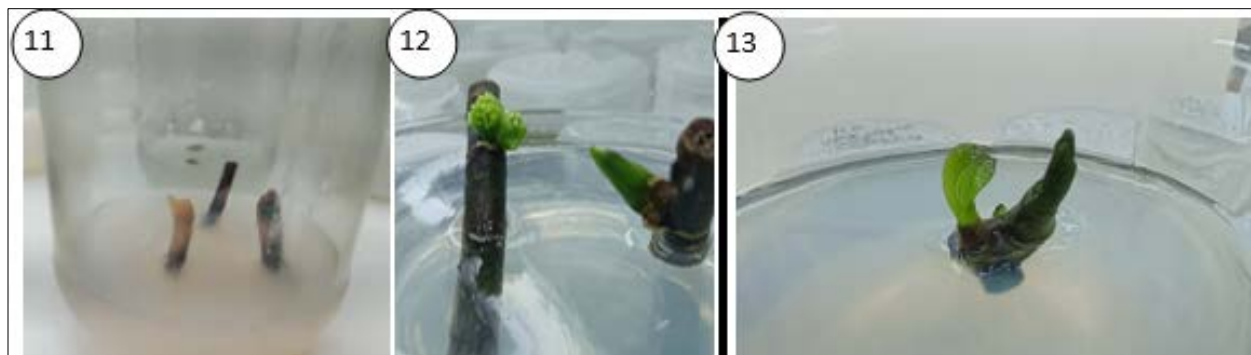


Fig 11: Dead flower stalks after 60 days of inoculation

Fig 12, 13: Browning controlled in flower stalks treated with antioxidant + absorbent solution for 3 hours (T₅), 60 days after inoculation

Table 4: Survival and browning percentage of ex-plants pre-soaked in antioxidants and adsorbent solution for varying duration

Pre-soaking Duration	Survival Percentage at 30 DOI		Browning at 30 DOI	
	Percentage	SE	Percentage	SE
(T ₀)	15.00 (22.12)	3.69	85.00 (68.74)	5.526
(T ₁)	31.25 (33.76)	3.19	65.00 (54.14)	4.560
(T ₂)	47.50 (43.47)	3.49	45.00 (42.08)	2.367
(T ₃)	55.00 (48.01)	4.21	42.50 (40.48)	4.440
(T ₄)	67.50 (55.74)	5.31	31.25 (33.16)	5.682
(T ₅)	88.75 (73.26)	6.20	15.00 (22.12)	3.693
SE(m)	4.48**		4.52**	
CD at 1%	13.42		13.53	
C.V. %	19.46		20.80	

Note: Figures in parentheses are angular transformed data

Conclusion

Together, experiment-1 and experiment-2 demonstrate that adequate pre-soak time and antioxidant therapies significantly increase explant life and decrease tissue browning after 30 DOI. Antioxidant formulations had notable effects in experiment-1. The triple combination T₇ (1% PVP + 0.1% AA + 0.1% CA) generated the highest results (82.5% survival, 15% browning). Suboptimal (lower) doses decreased efficacy, multiple mixtures were intermediate, and single antioxidants only slightly improved the effects. Longer pre-soak times of explants considerably improved survival and reduced browning in experiment-2, with 3 hours treatment yielding the maximum survival rate (88.75%) and lowest blackening (15%).

Overall, explant viability is significantly increased and browning is reduced in *in-vitro* when phenolic exudation and oxidative stress are managed with an ideal combination of PVP, ascorbic acid, and citric acid either as a media additive or as a pre-soaking for 3 hours. To increase longevity while preventing toxicity, future research could investigate other antioxidants or delivery systems and adjust PVP concentration and antioxidant dosages.

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Declarations

Conflict of interest: The authors declare no conflict of interest.

Consent to participate: Not applicable.

Ethical approval: Not applicable.

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