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A COMPLETE PROTOCOL TO REDUCE BROWNING DURING COCONUT (*COCOS NUCIFERA* L.) TISSUE CULTURE THROUGH SHOOT TIPS AND INFLORESCENCE EXPLANTS

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Abstract

Browning of explants cultured *in vitro* is a severe problem that impairs successful micropropagation of coconut (*Cocos nucifera* L.). Seedlings and spadix were gathered from Coconut Research Institute of Chinese Academy of tropical agricultural sciences germplasm, Wenchang, Hainan, China to examine the diverse variables which influencing in browning, i.e., 1) type of antioxidants, 2) concentrations of antioxidants, 3) combinations of antioxidants, 4) time of treatment, 5) dark and light conditions during all the process (preparation, dipping, and incubation), 6) status of solution (fresh or kept), 7) media type, 8) concentration of 2,4-D and 9) adding some antioxidants to culture medium. Anyhow, results indicate that treating inflorescence and meristem explants in the antioxidant solution containing 1.5 g L^{-1} citric acid and 1 g L^{-1} ascorbic acid, for 90 and 120 minutes respectively, has reduced the browning significantly. Also, incorporation of these antioxidants in the culture medium along with 2,4-D (less than 25 mg L^{-1}) significantly reduced the browning.

***Keywords*:** *Cocos nucifera*, *In vitro*, Browning, Shoot tips, Inflorescences

Introduction

The commercial importance of Coconut (*Cocos nucifera* L.) has been increasing in all respects, with high-value, cultivated in several countries, contributing extensively to the improvement of nutrition, food security, employment, and income generation (Sáenz-Carbonell *et al*., 2016). The commercial importance of coconut has been becoming in all respects quickly in the course of the most recent fifteen years, with high-value products such as packed coconut water, virgin oil, coconut milk products, coco-biodiesel, fiber derivatives for the automobile industry and horticulture (Roolant 2014; Lao 2009). In the interim, the increase in production capabilities is difficult to achieve while depending only on propagation through seeds. Consequently, alternative means of propagation need to be considered, for example, *in vitro* propagation or micropropagation by somatic embryogenesis (Solís-Ramos *et al*., 2012). Also, in coconut amid tissue culture, the majority of the explants unable to grow and gradually die because of browning incidence. A membrane structure in tissue separates Polyphenol Oxidase (PPO) and phenolic compounds, and when something causes damage to this membrane, browning occurs. Vacuoles of healthy cells contain phenolic compounds, but Polyphenol Oxidase located in the cytoplasm. Therefore, until the tissue is still intact, no browning happens (Mayer, 1979).

A noteworthy limitation in tree tissue culture is browning of the explants and the medium that achieves the demise of the tissues. The cut surfaces start to discolor soon after excision and continue exuding phenolics even after culturing. Browning is due to a mixture of complex phenolic exudates, which are found liberally in woody plants. These phenols become lethal by being reversibly appended to proteins by their hydrogen holding and by their oxidation to form highly active Quinones, which then become cyclic or polymerized and oxidize proteins to form increasingly melanic compounds called polyphenols. Oxidative browning is a common problem in plant tissue culture; resulting in reduced growth (Krishna *et al*., 2008 and Uchendu *et al*., 2011), lower rates of regeneration or recalcitrance (Laukkanen *et al*., 2000, Aliyu 2005 and Parthasarathy *et al*., 2007), and can ultimately lead the cells, tissues or even the plant to death (Toth *et al*., 2007; Panaia *et al*., 2000 and Tabiyeh *et al*., 2006).

Therefore, many other anti-browning agents with various mechanisms have been presented. Ascorbic acid is a good antioxidant (Amorati *et al*. 2011; Ali *et al*. 2013) and the most anti-browning agent widely used today (Golan-Goldhirsh *et al*. 1992; Whitaker 1994; Rupasinghe *et al*. 2005). Some reports attributed its action to the reduction of the formed quinones to the original colorless diphenols (Nicolas *et al*. 1994; Son *et al*. 2001; Limbo and Piergiovanni 2006); others considered ascorbic acid as a PPO inhibitor (Altunkaya and Gökmen 2008); in any case, the condition required to possess each mechanism is not characterized.

Herein, browning of explants cultured in vitro is a severe problem that impairs successful micropropagation of woody plants (Thakur and Kanwar, 2008). For this the advancement of micropropagation protocols for coconut have advanced gradually as a monocot species has been challenging to regenerate especially through *in vitro* propagation by utilizing seedling as a source for shoot tip meristem, inflorescences, and this due the browning problem

that faces the scopes in coconut tissue culture during use any explant source with the exception of the embryo.

In light of browning, the shoot tips meristem and inflorescences turned necrotic and died within a few days after inoculation. It was consequently essential to devise a technique to decrease browning in coconut tissue culture. For this, the target of the present investigation was finding a complete protocol to reduce browning and avoid oxidation of polyphenolic compounds as an initial step to begin a coconut tissue culture by utilizing shoot tips and inflorescences.

Materials and Methods

Plant Material

Seedlings as a source for shoot tips and spadix as a source for inflorescences were gathered from the Coconut Research Institute of Chinese Academy of tropical agricultural sciences germplasm, Wenchang, Hainan, China. Thus, young seedlings and diverse stages from inflorescences (25- 50 cm) of coconut were selected to be healthy and in functional status.

Explant preparation:

In this concern, two explants sources were prepared as the next steps at the Coconut Research Institute (CRI) of the Chinese Academy of tropical agricultural sciences tissue culture lab Wenchang, Hainan, China. The experiment was carried out from October 2018 to February 2019 as pursuing:

A. Coconut Seedlings (as a source of shoot tip) from field to culture: -

1. After bringing, the seedlings from the field put it under running tap water for 1.5 – 2 hours.

2. Cut the seedlings to get the inside part, which has the shoot tip and the meristem through taking inside part of 10cm length and 2 cm width.

3. Rinse this inside part, which we get it under running tap water for 1 hour without Clorox (2.5% sodium hypochlorite) and 30 minutes with Clorox after making dilatation for it.

4. Rinsed the inside part in distilled water for two times.

5. Soaking the inside part in anti-oxidants solution.

6. Rinse the inside part once with sterile distilled water and transfer to 50% Clorox (2.5% sodium hypochlorite) for 20-25 minutes.

7. The inside part will surface sterilized by using ethyl alcohol for 1 min. to remove all traces of the used of disinfectant.

8. Soaking and rinse three times using autoclaved distilled water.

9. The isolation of the explants (meristem shoot tips with leaf primordia) will be under complete aseptic conditions in culture cabinet (laminar airflow hood).

10. Shoot tip meristem will prepare again by removing the outer leaves from the inside parts and excising of shoot tip containing the apical meristem with 6-8 leaf primordia (2-3 cm length and 1.5 cm width)

11. Soaking shoot tip meristem and rinse it three times using autoclaved distilled water.

12. Cutting the meristem with shoot tips and some leaf primordia to 20 – 30 parts and put (3-5 slices) in one jar.

B. Coconut spadix (as a source of inflorescences and flowers) from field to culture: -

1. After bringing the spadix (25-50cm) from the field in safety way sterilized.

2. The isolation of inflorescences after opening the spadix will be under complete aseptic conditions in the culture cabinet (laminar airflow hood).

3. Cut the inflorescences to little section 3-5 cm and soaking these parts in anti-oxidants solution.

4. Cutting the inflorescences to parts 0.5-1 cm and put (3-5 slices) in one jar (container).

The treatments based on the nine over-mentioned factors as follow: -

1- Three type of antioxidants (ascorbic acid, citric acid and polyvinylpyrrolidone).

2- Common concentration of the three types (0.1%, 0.15% and 0.5% of ascorbic acid, citric acid and polyvinylpyrrolidone , respectively.).

3- Eight possible combinations as follows: 1) Control: sterilized distilled water, 2) 0.1% Ascorbic acid. (100 mg/L), 3) 0.15% citric acid. (150 mg/L), 4) 0.5% P.V.P. (500mg/L polyvinylpyrrolidone), 5) 0.1% ascorbic acid + 0.15% citric acid, 6) 0.1% ascorbic acid + 0.5% P.V.P, 7) 0.15% citric acid +0.5% P.V.P and 8) 0.1% ascorbic acid + 0.15% citric acid + 0.5% P.V.P.

4- Four periods for dipping 30, 60, 90 and 120 minutes.

5- Effect of light or completely dark conditions during all process (preparation, dipping and incubation).

6- Two status of solutions, which used fresh solution or kept solution under light or completely dark conditions.

7- Three media types (Y3 Eeuwens 1976), (72 Karunaratne and Periyapperuma 1989) and (MS Murashige and Skoog 1962).

8- Five Concentrations of 2, 4-D in the culture media (15, 25, 50, 75 and 100 mg/L.).

9- Adding antioxidants to the culture medium (Y3+ 25 mg/L 2, 4-D) as shown in Table (1).

Table 1 : Effect of adding citric acid and ascorbic acid to the culture medium.

Medium type Y3	Citric acid and ascorbic acid concentrations
Y3 1	0.1g citric + 0.1 g ascorbic /L.
Y3 2	0.15g citric + 0.1 g ascorbic /L.
Y3 3	0.2g citric + 0.1 g ascorbic /L.
Y3 4	0.15g citric + 0.15 g ascorbic /L.

All previously mentioned treatments and factors were used with tow explants sources (shoot tip meristem and inflorescences).

Data and Calculation

Scores of browning were calculated as the degree of browning according to the status of explants after incubation this degree (start from 0 to 5) put by the authors, as shown in Table (2).

Table 2 : Degree of the intensity of browning

Degree of browning	Status of explants after incubation
0	Explant without browning
1	Explant has so light browning (light yellowish)
2	Less than half of the explant turned to brown
3	More than half of the explant turned to brown
4	Explant turned nearly fully brown
5	Explant turned fully brown

Experiment layout and statistical analysis

The complete randomized design with five replications was employed in arranging the investigated treatments. Whereas, each replicate was represented by two jars. According to Snedecor and Cochron (1980). However. The obtained data were statistically analyzed, and the means were differentiated according to Duncan's multiple range test at 1% level according to (Duncan, 1955).

Results

Seedlings and spadix of coconut were gathered from the field and arranged as the already previously mentioned techniques after getting the shoot tip meristem from the seedlings and the inflorescences from the spadix promptly soaked in the antioxidant solutions according to every factor. The explants after antioxidant treatment and the surface-sterilized as explained in materials and methods cultured on Y3 medium with 40g/L sucrose, gelled with 7.5 g/L agar as well as, active charcoal at 2.5 g/L. Besides, the Intensity of browning was noted at the end of 5, 10, and 15 days of incubation.

1. Effect of antioxidants type and concentrations.

Referring to the influence of differential investigated antioxidants compounds on browning degree, Fig. (1) Displays obviously that the response was pronounced, in this concern, three antioxidants compounds were tested, i.e., ascorbic acid, citric acid, and polyvinylpyrrolidone with three standard concentrations of the three types 0.1%, 0.15% and 0.5% for ascorbic acid, citric acid, and PVP, respectively. Herein, the superiority of citric acid and ascorbic acid more than PVP in reducing the browning with different explants.

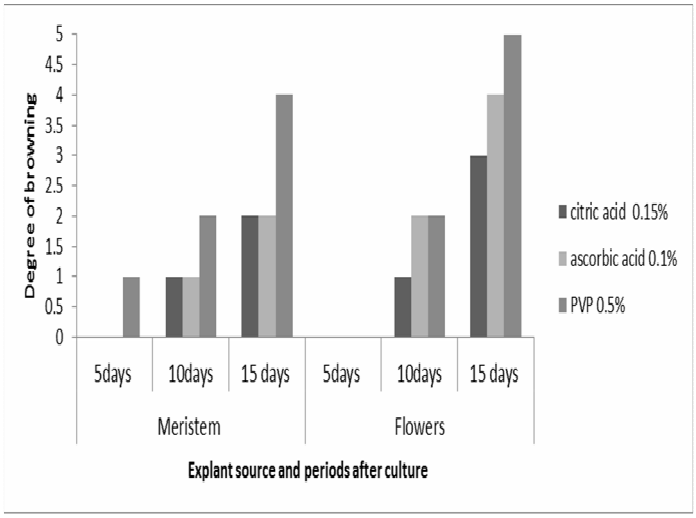

Fig. 1 : Degree of browning after culture 5, 10, and 15 days at different antioxidants type with two explant sources for 2 hours.

2. Effect of possible combinations.

Table (3) displays obviously that seven investigated antioxidants combinations decreased the degree of browning over control (sterilized distilled water) significantly. However, the fifty blends, i.e., 0.1% ascorbic acid + 0.15% citric acid, was statistically superior in this regard. However, eighth combinations (8. 0.1% ascorbic acid + 0.15% citric acid + 0.5% P.V.P.) ranked second in their effect on reducing the browning with different explants sources.

Table 3 : Degree of browning after 5, 10 and 15 days at different antioxidants combinations with two explants sources for 2 hours

Antioxidants combinations	Explant sources, Periods after culture and degree of browning					
	Meristem			Flowers		
	5days	10days	15 days	5days	10days	15 days
1- Control: sterilized distilled water.	1 a	3 a	5 a	1 a	4 a	5 a
2. 0.1% Ascorbic acid. (100 mg/L).	0 b	1 c	4 b	0 b	1 c	4 b
3. 0.15% citric acid. (150 mg/L).	0 b	1 c	3 c	0 b	2 b	4 b
4. 0.5% P.V.P. (500m g/L polyvinylpyrrolidonepolyvinylpyrrolidone).	1 a	2 b	4 b	0 b	2 b	5 a
5. 0.1% ascorbic acid + 0.15% citric acid.	0 b	0 d	1 e	0 b	1 c	2 d
6. 0.1% ascorbic acid + 0.5% P.V.P.	0 b	2 b	4 b	0 b	1 c	3 c
7. 0.15% citric acid +0.5% P.V.P.	0 b	2 b	4 b	0 b	2 b	3 c
8. 0.1% ascorbic acid + 0.15% citric acid + 0.5% P.V.P.	0 b	1 c	2 d	0 b	1 c	3 c

3. Effect of different periods dipping 30, 60, 90 and 120 minutes with the best combinations from the previous results in this concern two combinations gave the highest values in reducing browning, i.e., (0.1% ascorbic acid + 0.15% citric acid) and (0.1% ascorbic acid + 0.15% citric acid + 0.5% P.V.P.).

It is quite evident, as shown in Fig (2) that the combination (0.1% ascorbic acid + 0.15% citric acid) was superior in reducing browning with the different explant sources. Meanwhile, the dipping periods 120 minutes and 90 minutes were the best periods for dipping for shoot tip meristem and inflorescences, respectively.

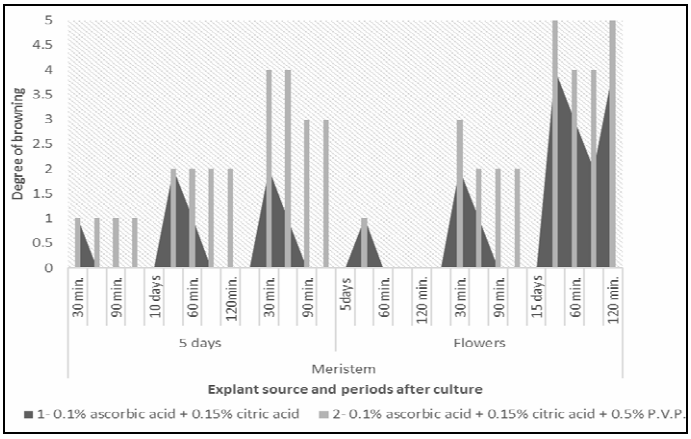

Fig 2. Degree of browning at the end of 5, 10 and 15 days from incubation after dipped at antioxidants combinations with two explants sources for 30, 60, 90 and 120 minutes.

4. Effect of dipping in 0.1% ascorbic acid + 0.15% citric acid under light (commonly conditions) or completely dark conditions for 120 minutes for the shoot tip and 90 minutes for the inflorescences during the preparation, dipping and incubation whereas the Intensity of browning was noted at the end of 5, 10, 15, 20, 25 and 30 days after incubation .

Fig 3 (A and B) shows obviously that the influence of dipping in antioxidants solution under light (commonly conditions) or completely dark conditions during all the process (preparation, dipping, and incubation). The most considerable reduce in browning coupled with entirely dark conditions, which showed the lowest degree of browning with the different explant sources. On the contrary, the highest degree of browning induced by dipping under light conditions or common conditions.

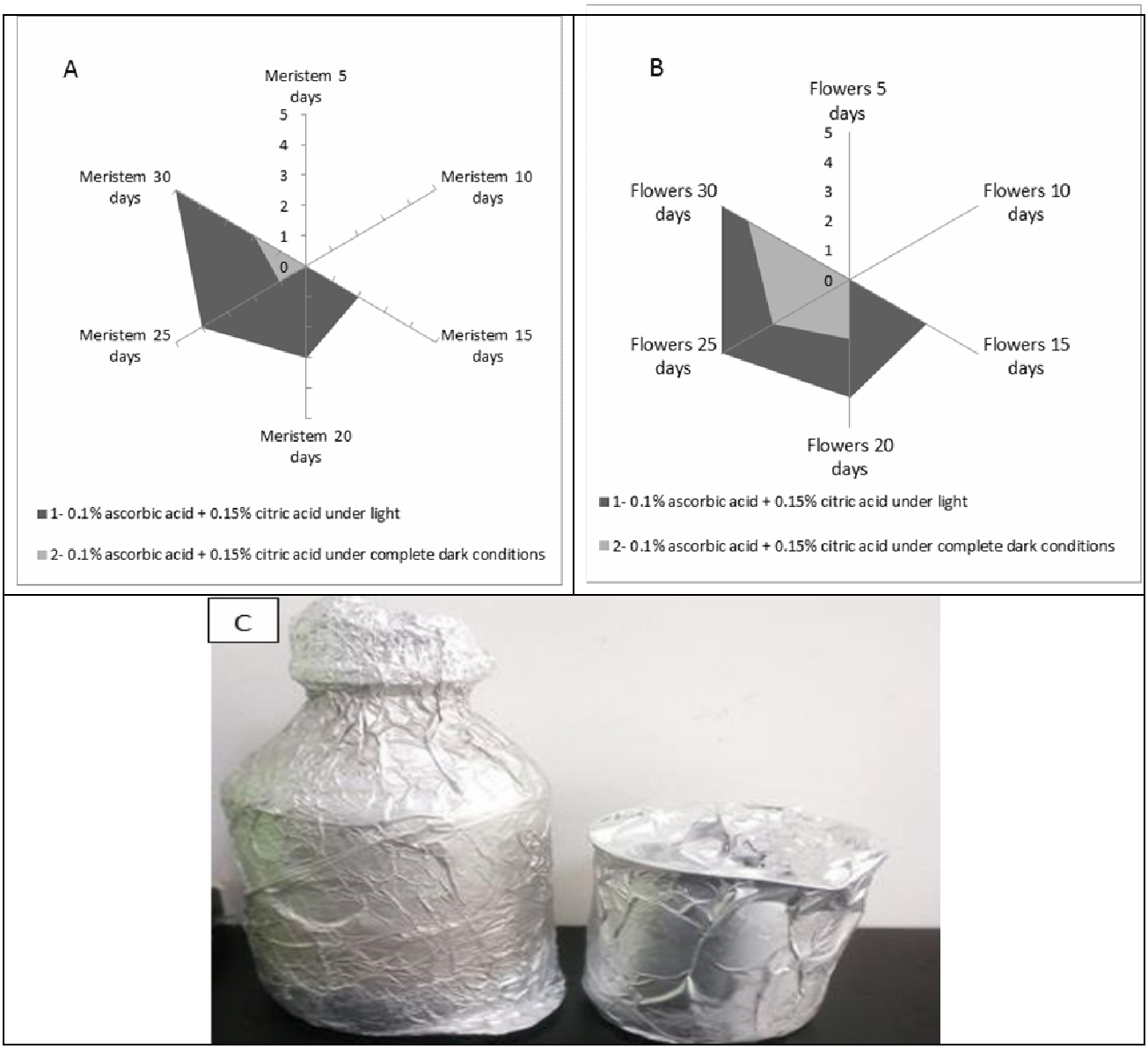

Fig 3. Degree of browning at the end of 5, 10, 15, 20, 25, and 30 days from incubation dipped in antioxidants combination (0.1% ascorbic acid + 0.15% citric acid). Where, A= shoot tip meristem dipped in antioxidants combination (0.1% ascorbic acid + 0.15% citric acid) for 120 minutes under light (commonly conditions) or completely dark conditions, B= inflorescences dipped in antioxidants combination (0.1% ascorbic acid + 0.15% citric acid) for 120 minutes under light (commonly conditions) or completely dark conditions and C= completely dark conditions during dipping and preparation of antioxidants.

5. Effect of dipping in 0.1% ascorbic acid + 0.15% citric acid under complete dark conditions for 90 minutes for the flowers and 120 minutes for the meristem with the periods after culture 5, 10, 15, 20, 25 and 30 days with two status of solution fresh or kept solution.

Table 4 : Shows clearly that the variances were relatively too few to be taking into consideration from the statistical point of view. Herein, it could be declared that explants of treated with fresh antioxidants solution relatively to be slightly in reducing the degree of browning as compared to the kept antioxidant solution. Generally, it could be safely said that using of fresh solution reduced the browning degree with different explant source to the end of the incubation period (30 days).

Table 4 : Degree of browning after 5, 10, 15, 20, 25, and 30 days of incubation with two Explant sources under complete dark conditions with two statuses of solution fresh or kept solution.

Antioxidants combinations status	Explant sources, Periods after culture (days), and degree of browning											
	Meristem dipping for 120 minutes						Flowers dipping for 90 minutes					
	5 days	10 days	15 days	20 days	25 days	30 days	5 days	10 days	15 days	20 days	25 days	30 days
1- 0.1% ascorbic acid + 0.15% citric acid fresh solution	0 a	0 a	0 b	0 b	1 a	2 a	0 a	0 a	0 b	2 b	2 b	4 b
2- 0.1% ascorbic acid + 0.15% citric acid kept solution	0 a	0 a	1 a	1 a	2 a	2 a	0 a	0 a	1 a	3 a	3 a	5 a

6. Effect media types on browning degree with different explant sources (shoot tip meristem and inflorescences).

In this concern, the Intensity of browning was noted at the end of 5, 10, 15, 20, 25 and 30 days after incubation with two explant sources under complete dark conditions with dipping as a pretreatment in 0.1% ascorbic acid + 0.15% citric acid for 120 minutes with meristem and 90 minutes with inflorescences in fresh solution with three types of media (Y3 (Eeuwens, 1976), 72 (Karunaratne and Periyapperuma, 1989) and MS (Murashige and Skoog, 1962) every media have 50 mg/L. 2, 4-D, with 40g/L sucrose, gelled with 7.5 g/L agar as well as active charcoal at 2.5 g/L. Anyhow, Referring to the influence of various media types on the degree of browning with different explant sources, it was quite evident, as shown from tabulated data in Table (5), that a positive response was detected with Y3 compared to 72 and MS medium. However, the Y3 medium was superior, reduced the browning during all the incubation periods, whereas the difference was so slight, and could be safely neglected between 72 and MS medium.

Table 5 : Degree of browning at the end of 5, 10, 15, 20, 25 and 30 days after incubation with two Explants sources under complete dark conditions with dipping as a pretreatment in 0.1% ascorbic acid + 0.15% citric acid for 120 minutes with meristem and 90 minutes with flowers in fresh solution with three types of media (Y3, 72 and MS) .

Medium type +50 mg/L. 2,4-D	Explant sources, Periods after culture (days), and degree of browning											
	Meristem dipping for 120 minutes						Flowers dipping for 90 minutes					
	5 days	10 days	15 days	20 days	25 days	30 days	5 days	10 days	15 days	20 days	25 days	30 days
Y3	0 b	0 c	1 b	2 b	4 b	5 a	0 a	1 b	2 c	3 c	4 b	5 a
72	1 a	2 b	4 a	5 a	5 a	5 a	0 a	1b	3 b	4 b	5 a	5 a
MS	1 a	3 a	4 a	5 a	5 a	5 a	0 a	2 a	4 a	5 a	5 a	5 a

7. Effect of 2, 4-D concentration in Y3 medium:

In this regard, the Intensity of browning was noted at the end of 5, 10, 15, 20, 25 and 30 days after incubation with two explant sources under complete dark conditions with dipping as a pretreatment in 0.1% ascorbic acid + 0.15% citric acid for 120 minutes with meristem and 90 minutes with inflorescences in fresh solution cultured inY3 medium with different 2, 4-D concentrations (15, 25, 50, 75 and 100 mg/L) with 40g/L sucrose, gelled with 7.5 g/L agar as well as, active charcoal at 2.5 g/L. Concerning the influence of 2, 4-D concentrations on browning degree at the end of 5, 10, 15, 20, 25, and 30 days after incubation, Table (6) displays a considerable higher browning degree with more top 2, 4-D concentrations. However, both 75 and 100 mg/L resulted in a relative higher browning degree. Also, using low 2, 4-D concentrations (15 and 25 mg/L) reduced browning degree with the different explant sources. It could be generally concluded that using of 2, 4-D concentrations start from 25 mg/L and lower than that decreased the degree of browning, and high 2, 4-D concentrations more than 25mg/L increased the degree of browning.

Table 6 : Degree of browning at the end of 5, 10, 15, 20, 25 and 30 days after incubation with two Explants sources under complete dark conditions with dipping as a pretreatment in 0.1% ascorbic acid + 0.15% citric acid for 120 minutes with meristem and 90 minutes with flowers in fresh solution with types Y3 medium with different 2, 4-D concentrations.

Y3 medium+ different 2,4-D concentrations (mg/L.)	Explant sources, Periods after culture (days), and degree of browning											
	Meristem dipping for 120 minutes						Flowers dipping for 90 minutes					
	5 days	10 days	15 days	20 days	25 days	30 days	5 days	10 days	15 days	20 days	25 days	30 days
15 mg/L. 2,4-D	0 b	0 c	0 d	0 e	1 d	2 b	0 b	0 d	1 d	2 c	3 c	5 a
25 mg/L. 2,4-D	0 b	0 c	0 d	1 d	2 c	2 b	0 b	0 d	2 c	3 b	5 a	5 a
50 mg/L. 2,4-D	0 b	0 c	1 c	2 c	4 b	5 a	0 b	1 c	2 c	3 b	4 b	5 a
75 mg/L. 2,4-D	0 b	2 b	3 b	4 b	5 a	5 a	1 a	2 b	4 b	5 a	5 a	5 a
100 mg/L. 2,4-D	1 a	3 a	4 a	5 a	5 a	5 a	1 a	4 a	5 a	5 a	5 a	5 a

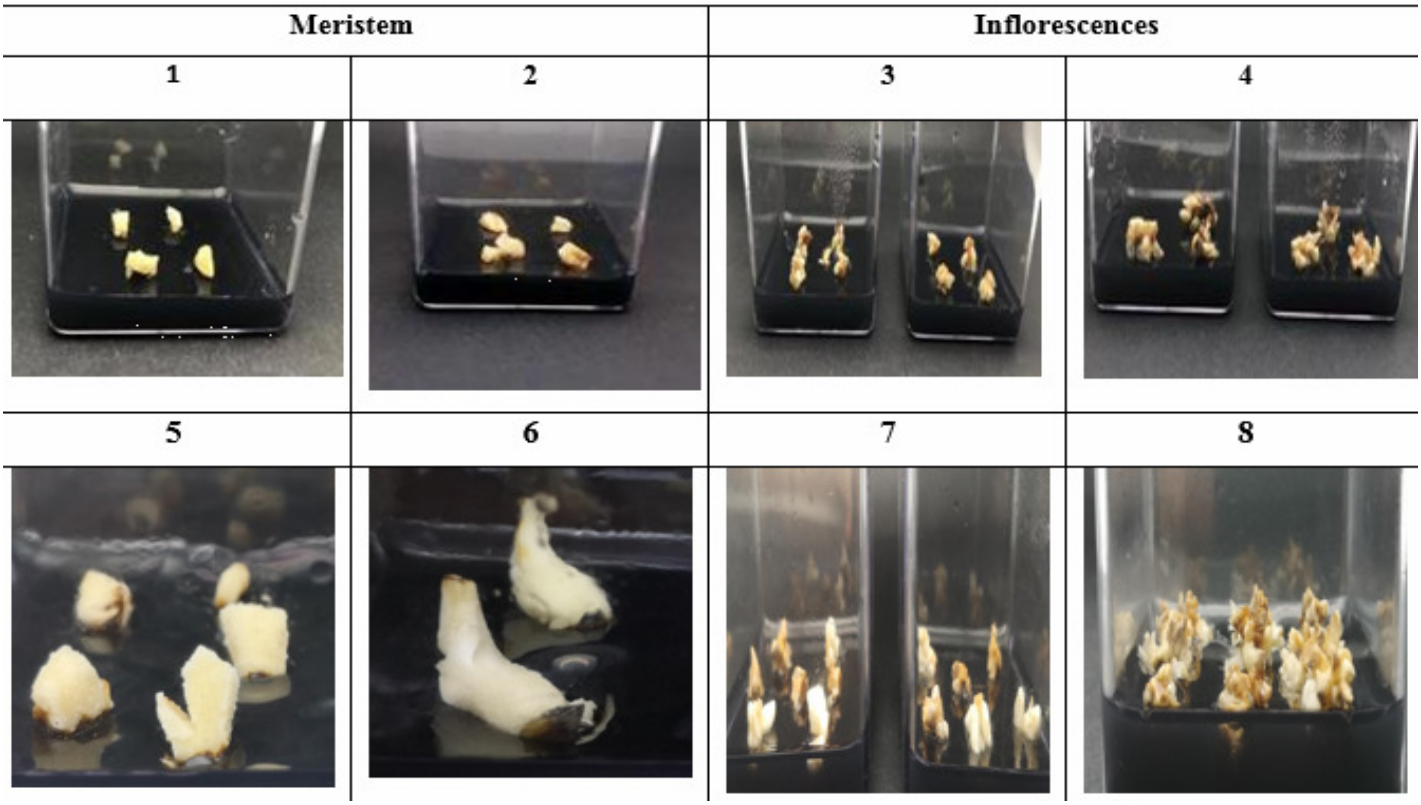

Photo 1. Degree of browning at the end of 15, 20, 25 and 30 days after incubation with two Explants sources under complete dark conditions with dipping as a pretreatment in 0.1% ascorbic acid + 0.15% citric acid for 120 minutes with meristem and 90 minutes with inflorescences in fresh solution culture at Y3 which have 25 mg/L 2,4-D. whereas, 1= meristem after 15 days, 2 = meristem after 20 days, 3 = inflorescences after 15 days, 4= inflorescences after 20 days, 5= meristem after 25 days, 6= meristem after 30 days, 7= inflorescences after 25 days and 8 = inflorescences after 30days

8. Effect of adding antioxidants to the culture medium:

In this concern, Intensity of browning was noted at the end of 40 days after incubation with two explant sources under complete dark conditions with dipping as a pretreatment in 0.1% ascorbic acid + 0.15% citric acid for 120 minutes with meristem and 90 minutes with inflorescences in fresh solution cultured inY3 medium with one 2, 4-D concentration (25 mg/L) with 40g/L sucrose, gelled with 7.5 g/L agar as well as, active charcoal at 2.5 g/L. About the influence adding antioxidants (citric acid and ascorbic acid) to the culture media on browning degree at the end of 40 days after incubation. Anyhow, four combinations were used in this concern 0.1g citric + 0.1 g ascorbic /L, 0.15g citric + 0.1 g ascorbic /L, 0.2g citric + 0.1 g ascorbic /L and 0.15g citric + 0.15 g ascorbic /L. Fig (4) and photo (2) displays obviously that the response was pronounced, whereas all the antioxidants combinations resulted in decreasing the degree of browning compared to the previous results. However, combination number two (0.15g citric + 0.1 g ascorbic /L) was superior. The reverse was true with combination number four (0.15g citric + 0.15 g ascorbic /L).

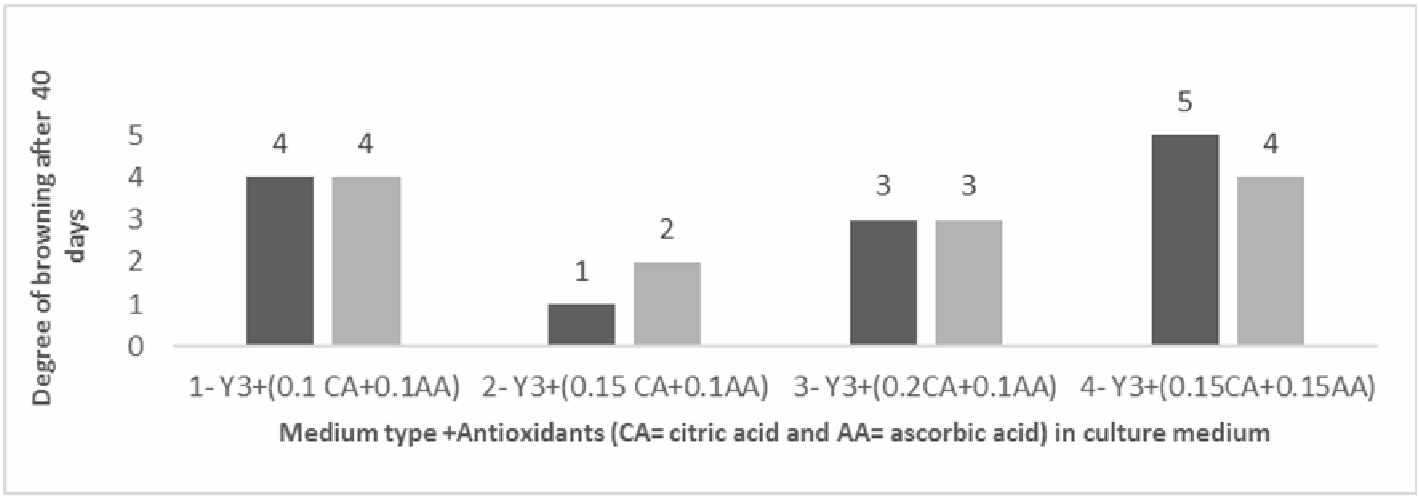

Fig. 4 : Effect of adding antioxidants to the culture medium, which have 25 mg/L 2, 4-D

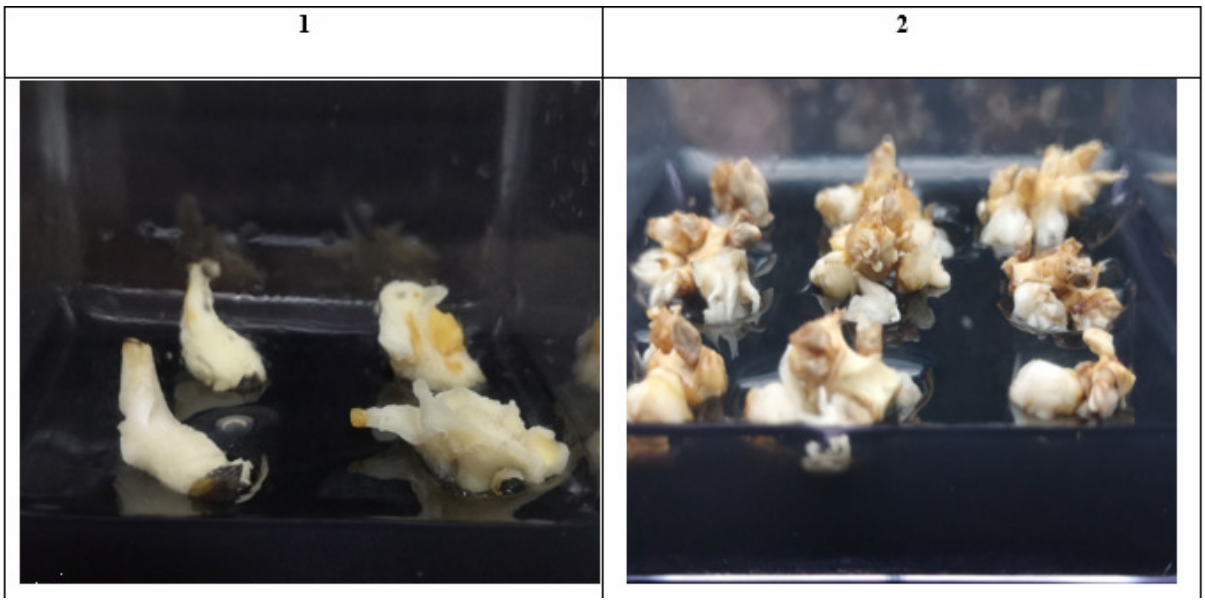

Photo 2. Effect of adding antioxidants to the culture medium, which have 25 mg/L 2, 4-D. Whereas, the 1= status of shoot tip meristem at the end of 40 days from incubation with second antioxidant combinations and 2= status of inflorescences at the end of 40 days from incubation with second antioxidant combinations.

Discussion

Browning of cultured tissues, this phenomenon results from physiological changes inside the cultured tissues that lead to progressive browning and eventual death of tissues. The browning shows up because of the oxidation of phenols inside the tissues (Alkhateeb and Ali-Dinar, 2002). Tissue browning is a problem frequently observed during the in vitro establishment of explants from woody plants (Block and Lankes, 1996). The issue of phenolic browning was mainly limited by leaching of phenolic compounds due to agitation in antioxidants solution and by proper drying of explants before inoculation (Meghwal *et al*., 2001). Receiving specific measures, specifically: culturing of plant parts during winter and spring seasons, incubation of tissues in the dark especially in the first three months, and adding charcoal to the medium can reduce this phenomenon (Al Kaabi *et al*., 2005).

A few advances have been made towards decreasing oxidative browning by changing natural conditions utilized in tissue culture. For instance, tissues cultured in the dark often display lower levels of browning than those developed in the light (Lainé and David 1994; Ochoa-Alejo and Ramirez-Malagon, 2001). Changing the fundamental media structure and the type/concentration of plant growth regulators can likewise diminish the level of browning. A more focused on the methodology of pre-treating explants and/or additionally, culture media with compounds specially selected that decrease tissue browning is also often employed (Toth *et al*., 2007). Besides, utilizing antioxidants, i.e., ascorbic acid, melatonin, or citric acid, that decrease oxidative stress and prevent oxidation of phenolic compounds adsorbents that bind phenolic compounds rendering them less toxic such as activated charcoal or PVPP (Uchendu *et al*., 2011; Thomas 2008).

Numerous analysts have announced the connection between phenolic substances and antioxidant activity. In specific investigations, they found an association between's phenolic material and antioxidant activity (Velioglu *et al*., 1998). At any rate, The impact of antioxidants (ascorbic and citric acid) and phenol binding agents (polyvinyl pyrrolidone and activated charcoal) on explant browning amid various seasons demonstrated that in spring season most astounding explant survival percentage (81.91%) was observed with citric acid (1000 mg per liter), and it was on a par with ascorbic acid (1000 mg per liter), citric acid (500 mg per liter) and polyvinyl pyrrolidone (2500 and 5000 mg per liter). In the rainy season, the maximum explant survival (64.59%) was observed with ascorbic acid (1000 mg per liter) incorporation in the explant establishment medium (Thakur and Kanwar, 2008).

Plant regulators or PGR likewise influence the generation and action of the (Lebon *et al*., 2004) Plant growth regulators assume an essential job in the development and creation of optional metabolites in the plant tissue and cell culture. For instance, type and grouping of the auxin or cytokinin and auxin-cytokinin ratio have significant effects on both the growth and accumulation of secondary metabolites in plants (Ozyigit 2008). Besides, the sort of plant growth regulators influenced phenolic substances creation. The generation of phenolic substances diminished in a medium with the most concentration of growth regulators, four and 2.5µM concentration of benzylaminopurine, and 4.9 µM of auxin. The creation of phenolic contents expanded in the free-plant hormone medium (Azam Sedighi *et al*., 2014).

Negligible browning happens. Injuring an explant in the shoot-tip procedure causes the discharge of phenolic compounds into the culture medium, keeps the development of the initial explant, and eventually prompts demise. By the by, it very well may be constrained by incubation in complete darkness and activated charcoal in the media, especially during the initial stage. Nevertheless, the whole organ (spikelet) is regularly utilized in this technique (Abul-Soad and Mahdi, 2010).

Zaid *et al*., (2011) revealed that the frequency of tissue browning diminished when date palm axillary shoots were presoaked in an antioxidant solution (150 mg/l citric acid and 100 mg/l ascorbic acid). In addition, Meziani *et al*., (2015) noticed that a PGR-free medium accentuated tissue browning (95%) in “Mejhoul”, though the least tissue browning (20%)

was seen in a medium comprising of 0.2 mg/l NOAA, 0.2 mg/l IAA, 0.4 mg/l Kin and 0.4 mg/l 2iP, when the shoot tips were utilized as explants. In date palm, browning would thus be able to be constrained by varying the levels of PGRs in the medium.

Conclusion

The outcomes displayed here demonstrate that, it is currently possible to micro-propagate coconut with highly efficient through shoot tip meristem and inflorescences starting from this complete protocol as follows: anti-oxidants fresh solutions contents 1.5g citric acid + 1.0 g ascorbic acid per liter for 90 minutes and 120 minutes with inflorescences and meristem (shoot tip), respectively. Under complete dark conditions during preparation, dipping, and incubation with Y3 medium at 2, 4-D concentrations not more than 25mg/L. As well as, Efficiency increases with addition 0.15g citric acid + 0.1 g ascorbic acid per liter to culture media.

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