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# Effect of Ascorbic Acid, Activated Charcoal and Dark Incubation on Browning Intensity of *Saurauia bracteosa* In Vitro Culture

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**Abstract.** *Saurauia bracteosa*, also known as Pirdot, is an endemic woody plant of the Actinidiaceae family with medicinal properties. Its population decline in nature has led to a vulnerable status, prompting conservation efforts, such as in vitro culture. Browning poses a significant challenge in the woody plant in vitro culture. Browning inhibitors such as ascorbic acid, activated charcoal and dark incubation can be used to address this issue. This study aims to determine the effect of ascorbic acid, activated charcoal and incubation conditions on browning inhibition of *S.bracteosa*. The study employed a Completely Randomized Design with 12 treatments on leaf explants, repeated five times. Observations, including the time of browning formation, percentage of browning, intensity of browning and percentage of live explant were carried out for 30 days and analyzed using ANOVA followed by DMRT. The results showed that the addition of activated charcoal in MS media with dark incubation delayed the browning formation time (12.40 DAI) while the adding ascorbic acid in MS media and dark incubation resulted in the lowest browning intensity (0.22). These research findings can serve as a foundational protocol for browning prevention, supporting the successful in vitro conservation of *S. bracteosa*.

**Keywords:** Ascorbic acid; active charcoal; browning; in vitro culture; *Saurauia bracteosa*

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## INTRODUCTION

Pirdot (*Saurauia bracteosa* DC.) is an endemic woody plant belonging to the Actinidiaceae family that is naturally distributed in several highlands in Indonesia, such as North Sumatera, Java, Bali, and North Sulawesi (Situmorang & Sunandar, 2019; Helmanto et al., 2020). Pirdot leaves contains various bioactive compounds such as polyphenols, flavonoids, tannins, triterpenoids, saponins and glycosides (Helmanto et al., 2020; Pasaribu et al., 2020). People in North Sumatra use Pirdot leaves to treat diabetes and prevent cancer by consuming boiling-extracted Pirdot herbal tea (Situmorang & Sunandar, 2019). Moreover, pirdot plant extract is known for several pharmacological effects such as antidiabetic (Helmanto et al., 2020), anti-inflammatory (Pasaribu et al., 2020), anti-cholesterol, antidiarrhea (Situmorang & Sunandar, 2019), antioxidant (Helmanto et al., 2020), and antibacterial (Pasaribu et al., 2020); therefore, this plant is considered as an important medicinal plant that can be used in herbal medicinal treatments. However, the use of pirdot leaves for herbal treatment is not followed by sustainable propagation, thus leading to population decline in nature and a vulnerable status for this plant (IUCN, 1998; Situmorang et al., 2015; Helmanto et al., 2020). Conventional propagation of woody plants has several constraints, such as stem cuttings which cannot produce large numbers of plants, long period of flowering and low regeneration of rooting (Deb & Gangmei, 2017; Helmanto et al., 2020). Hence, an alternative and effective propagation method is needed to produce a large number of plants in a short period of time by only using a small part of the plant (Radomir et

al., 2023).

The in vitro culture method is carried out in an artificial growth medium enriched with growth regulators (PGR) thus explant could grow and regenerate optimally into whole plants under sterile conditions (Habibah et al., 2023). Previous studies have reported the successful ex-situ conservation of endangered woody plants using in vitro culture such as *Stelechocarpus burahol* (Habibah et al., 2016), *Saurauia punduana* (Deb & Gangmei, 2016), *Justicia gendarussa* (Wahyuni et al., 2017), *Commiphora wightii* (Arnott) (Jakhar et al., 2019), woody plants of Georgia (Gaidamashvili & Benelli, 2021), and *Salvia dominica* (Al-Qatrani et al., 2021). However, there is unavailable information regarding in vitro culture of *Sauraia bracteosa*. Therefore, an establishment of *Saurauia bracteosa* in vitro culture protocol is important to support ex situ conservation attempt.

In vitro propagation of woody plants is still challenging due to long regeneration, originating from an uncontrolled environment, have a hard tissue, and the presence of phenolic compounds, which can cause browning reactions (Liang et al., 2019). In the initiation stage of in vitro culture, wounds will be carried out by cutting parts of plant organs (explant) and sterilization. This stage can lead to a browning condition, a typical problem found in in vitro plant propagation due to the increased of the accumulation phenolic compounds in injured tissue and damage to cell walls. According to Moon et al. (2020), when explants are cut, the cells will be rupture, causing stress on the tissue characterized by accumulating phenolic compounds. Phenolic compounds that accumulate in the vacuole will interact with the polyphenol oxidase (PPO) enzyme in the chloroplast to produce o-quinone, creating a brown color in the explant. If it is not treated well, the growth process will be hampered, thus the browning reaction becomes an essential factor that needs to be considered in in vitro culture (Pushpraj & Patel, 2016). Therefore, it is important to use browning inhibitor to overcome browning problems in in vitro culture of woody plant species including Pirdot plant.

The alternatives that can be used to reduce browning formation in in vitro culture are using anti-browning compounds such as ascorbic acid and activated charcoal which can be added to the culture medium, immersing explant in ascorbic acid solution before inoculation, and incubation in dark conditions. Browning prevention methods could affect different responses in in vitro culture. Admojo & Indrianto (2016) reported that immersing *Hevea brasiliensis* midrib leaf explants in 100 mg/L ascorbic acid resulted in 30% browning percentage. In addition, Patel et al. (2018) also reported that immersing *Punica granatum* L. explants in 200 mg/L ascorbic acid reduced browning up to 75%. However, the immersion method was less effective when applied to *Castanopsis argantea* explants; conversely, adding ascorbic acid into the media could reduce higher browning level (Chika et al., 2022). Light conditions also affect in vitro culture. Light can increase enzyme activity, biosynthesis, and phenol oxidation, leading to browning reactions. In contrast, under dark conditions, the rate of PPO enzyme is reduced (Pushpraj & Patel, 2016). According to Tarampak et al. (2019), light conditions will affect ascorbic acid becoming unstable which is showed by Ironwood seed cultures incubated in dark conditions resulted in higher regeneration rate (56.25%) compared to incubation in light conditions (31.25%). Similar results were obtained from research by Admojo & Indrianto (2016), which reduced browning by up to 70 % by immersing explants in 100 mg/L ascorbic acid and incubated in dark conditions. Research conducted by Warakagoda & Subasinghe (2013) showed that adding 0.1% activated charcoal in the media for *Pterocarpus santalinus* L. plant cultures gave the best results by reducing the browning reaction by 100%. In the other hand, Tao et al.(2007) reported that immersing explants in activated charcoal was less effective in reducing browning.

Despite several previous studies reported on the bioactive components and bioactivity of Pirdot plant, research on the establishment on in vitro culture and browning prevention of *S.bracteosa* in vitro culture is still unavailable. Therefore, this research aims to determine the effect of ascorbic acid, activated charcoal and incubation conditions on browning inhibition of *S.bracteosa*. This research is needed to establish a browning prevention protocol in the initiation stage of *S. bracteosa* in vitro culture. In vitro culture of *S. bracteosa* could support the ex-situ conservation attempt to prevent the extinction of this plant.

## METHODS

The research was carried out in March – July 2023 at the Biotechnology Laboratory, Faculty of Biotechnology, Duta Wacana Christian University, Yogyakarta (UKDW). This research was carried out as a collaboration between Faculty

of Biotechnology Universitas Kristen Duta Wacana and Eka Karya Bali Botanical Gardens under the National Research and Innovation Agency (BRIN).

**Plant Material**

Pirdot (*S.bracteosa*) leaf explants were obtained from Eka Karya Bali Botanical Garden, which originates from its natural environment. Explants were excised at the stalk (petiole), with young leaves on the top shoot or the second leaf from the shoot. Young leaves were characterized by a bright color and trichome-covered leaf surfaces. The explants were kept in a plastic bag with a ziplock, placed in an ice box with controlled temperature and humidity, and then transported to Yogyakarta on the same day. Explant were directly prepared for inoculation soon after they arrived at the Laboratory of Biotechnology UKDW.

**Explant Preparation**

The leaves were processed for pre-sterilization and sterilization processes prior to inoculation. Pre-sterilization was carried out by gently brushing them with a toothbrush under running water to remove the trichomes and dirt from the leaves. Then, the leaves are soaked under running water for approximately 60 minutes to maximize the cleaning of sap and dirt from the leaves, which can initiate severe browning if not treated well before further sterilization. Subsequently, the leaf explants were immersed in a mixture of 45 mL of distilled water with 5 mL of liquid detergent (Sunlight) and three drops of Tween 80 for 30 minutes. The leaf explants were rinsed using distilled water three times, and the final rinse used sterile distilled water. Then, the leaves were immersed in a mixture of 3 g/L of bactericide containing the active ingredient *streptomycin sulfate* and 3 g/L of fungicide containing the active ingredient *carbendazim* with three drops of Tween 80 for 30 minutes, then rinsed using distilled water three times. After that, the leaves were placed in a sterile petri dish and transferred to the Laminar Air Flow for further sterilization. The explants were immersed in 70% alcohol for 3 minutes and then rinsed using sterile distilled water three times. Afterwards, the leaf explants were immersed in 1.5% NaOCl for 15 minutes (Ndakidemi et al., 2013) and then rinsed using sterile distilled water three times. The sterilized explants were dried in sterilized filter paper on a petridish before further treatments.

**Browning Prevention Treatment**

The browning inhibitor compounds used in this research were ascorbic acid and activated charcoal, with different treatments as presented in Table 1. All treatments were incubated in light and dark conditions. All explants were excised into 1 x 1 cm. Subsequently, all excised explants from each treatment were inoculated on MS media supplemented with 0.75 ppm 6-Benzylaminopurine (BAP) and 2 ppm 2.4-Dicholorphenoxyacetic acid (2.4-D). The culture will be incubated at a temperature of 22-23°C, in dark conditions covered with a black cloth and in bright conditions using 20 watt TL lamp with light intensity of 671 lux.

**Table 1.** Various Treatments for Browning Prevention of *S. bracteosa* In Vitro Culture

| Treatment | Treatment Code | Incubation | |
|---|---|---|---|
| Control | AA0 | Light | Dark |
| Immersion of the explant in ascorbic acid 200 ppm | AA1 | Light | Dark |
| Addition of ascorbic acid 150 ppm in MS media | AA2 | Light | Dark |
| Addition of ascorbic acid 250 ppm in MS media | AA3 | Light | Dark |
| Addition of activated charcoal 2000 ppm in MS media | AC1 | Light | Dark |
| Addition of activated charcoal 5000 ppm in MS media | AC2 | Light | Dark |

**Observation**

Observations were carried out at 30 days after inoculation (DAI) with parameters such as time of browning formation, browning percentage, browning intensity and percentage of contamination, and percentage of live explant.

**Table 2.** Browning Scoring Intensity (Admojo & Indrianto, 2016)

| Scoring | Information |
|---|---|
| 0 – 0.24 | 0<0.25 parts of the explant were browning |
| 0.25 – 0.49 | 0.25 <0.5 parts of the explant were browning |
| 0.50 – 0.74 | 0.5 <0.75 parts of the explant were browning |
| 0.75 – 0.99 | 0.75<1 parts of the explant were browning |
| 1.00 | All parts of the explant were browning |

### Data analysis

The data obtained were analyzed descriptively and statistically. The percentage of browning and live explant were analyzed descriptively. The time of browning formation and the intensity of browning were analyzed statistically using a randomized block design with five replications using the IBM SPSS Statistics 25 application using the Univariate Analysis of Variance test at a significance level of 5%. If the results are significant, the test will continue using Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

### Browning on Pirdot Leaf Explants (*Saurauia bracteosa* DC.)

Browning is a condition where the explant color changes to brown as a result of tissue wounding, which causes phenol accumulation in the tissue and further oxidation by the PPO enzyme to produce quinon, which results in a brown color (Araji et al., 2014). This condition is a common problem in the application of plant in vitro cultures. This research attempted to reduce browning formation using browning inhibitor compounds by immersing explants in browning inhibitor solutions and incorporating browning inhibitors into the media. Pirdot leaf explants were inoculated in MS media, which added anti-browning according to the treatment and plant growth regulators 2,4-D and BAP with concentrations of 0.75 ppm and 2 ppm, respectively, and then incubated under dark and light conditions.

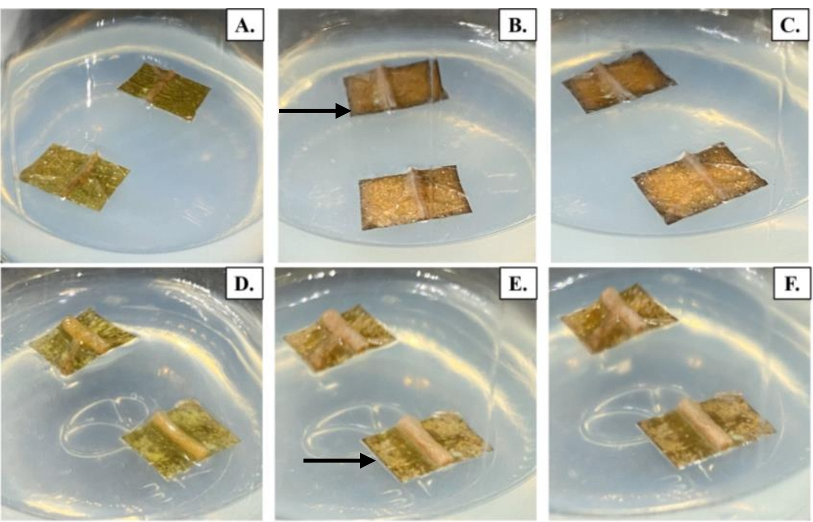


**Figure 1.** Browning formation process in Pirdot (*S. bracteosa*) leaf explants. Note: Control treatment in light incubation: A) 0 DAI; B) 15 DAI C) 30 DAI. Control treatment in dark incubation D) 0 DAI, B) 15 DAI C) 30 DAI

Figure 1 shows the process of browning formation in pirdot leaf explants in the control treatment (without the addition of browning inhibitors). An initial sign of browning formation is a contrasting color change at the edge of the cut explant (Figures 1B and 1E). Cutting the explant will trigger stress in the tissue, which activates the PPO enzyme as a defense reaction when cells are damaged. When the explant is cut, the cells will rupture (decompartmentation), increasing the respiration rate on the surface of the injured explant and causing interactions between enzymes and phenolic compounds, which have accumulated to form a brown pigment (Guan et al., 2023). Browning at the edge of the cut can interfere with nutrient absorption and slow down the growth of the explant due to blockage of vessels in the tissue (Chuanjun et al., 2015).

Light conditions also influence increased browning in pirdot leaf explants. The results obtained showed that there were differences in color in explants incubated in light and dark conditions. Explants incubated in light conditions observed for 30 DAI had a browner color and black at the edges compared to explants incubated in dark conditions, which had a brownish-green

color. This happens because the presence of light can increase the activity of the PPO enzyme, whereas in dark conditions, it will be inhibited (Ahmad et al., 2013). The result was similar to Baharan et al.(2015) who reported that the culture of date palm leaves (*Phoenix dactylifera* L.), which were incubated in dark conditions, produced lower browning intensity compared to light conditions.

### Effect of Browning Prevention Treatment on the Percentage of Browning and Live Explant of Pirdot (*Saurauia bracteosa* DC.) in Vitro Culture

All browning prevention treatments, including control, showed 100% browning on all live explants (Table 3). However, all live explants (no necrotic in the tissue) did not show any growth of callus after 30 days of observation. This result might be because the high content of phenolic compound in pirdot leaves explant which caused a down regulation of genes encoding hormones that involved in metabolism and nutrient transportation. This condition affected the inhibition of explant growth (Wang et al., 2016; Pasaribu et al., 2020). This condition indicated that high phenolic content in pirdot leaves allows an increase in the browning percentage and lower growth rate.

**Table 3.** Effect of browning prevention treatment on percentage of browning and live explant of *S.bracteosa* in vitro culture

| Treatment Code | Percentage of Browning (%) | Percentage of Live Explant (%) |
|---|---|---|
| (Control) AA0 Dark | 100 | 100 |
| (Control) AA0 Light | 100 | 100 |
| AA1 Dark | 100 | 100 |
| AA1 Light | 100 | 100 |
| AA2 Dark | 100 | 100 |
| AA2 Light | 100 | 100 |
| AA3 Dark | 100 | 100 |
| AA3 Light | 100 | 100 |
| AC1 Dark | 100 | 100 |
| AC1 Light | 100 | 100 |
| AC2 Dark | 100 | 100 |
| AC2 Light | 100 | 100 |

The absence of explant growth in Pirdot in vitro culture indicates that Pirdot is considered as a recalcitrant species unable to respond to the in vitro culture condition, preventing the explant form regeneration and growth in the in vitro culture. According to Abdalla et al. (2022), recalcitrant explants are one of the major problems in implementing in vitro culture for micropropagation, including woody plant species. In addition, the combination and concentration of PGR can be the reason for the regeneration capacity of explants. The choice of PGR is one of the important factors that stimulates the regeneration rate of explants. Jayusman et al. (2022) reported that BAP and NAA are better for woody plant in vitro culture than BAP and 2.4-D. This is because using BAP and 2.4-D together often leads to the necrotic growth of woody explants. The results of this study were similar to those of Sitinjak et al. (2015), who found that adding 2.4-D and kinetin to MS media in *Typhonium* sp. in vitro culture did not help the explants grow, but they did turn brown and were still alive. Plants exposed to stress conditions have different adaptations to reduce the damage caused by the stress, one of which is browning, making it possible that browning does not necessarily indicate death; it just slows down explant growth. This is in line with research by Wang et al. (2016), who reported that the expression of enzymatic browning genes significantly down-regulated the expression of genes associated with carbohydrate metabolism and nutrient transport that inhibited the growth of *M. formosana* after cutting the explant.

### Effect of Browning Prevention Treatment on The Time of Browning Formation in Pirdot (*Saurauia bracteosa* DC.) In Vitro Culture

The browning appearance time parameter is used to see the effect of anti-browning effectiveness on the speed of browning appearance (Handayani et al., 2022). Figure 2 shows the average time for browning in all treatments on Pirdot leaf explants. Based on the results, the slowest average time for browning to formed was in the dark AC1 (12.40 DAI). This result indicated that the time of browning

formation is slower when compared to the light control and dark control. On the other hand, the fastest average time for browning to appear was in the ascorbic acid (AA1) pre-treatment (2.80 DAI), which was incubated in light conditions. These results show the fastest time of browning formation compared to the dark and light control. This is indicated that the immersion of explant in certain time not efficiently inhibit the phenolic oxidation due to short time of antioxidant contact with the explant (Das & Srivastav, 2015).

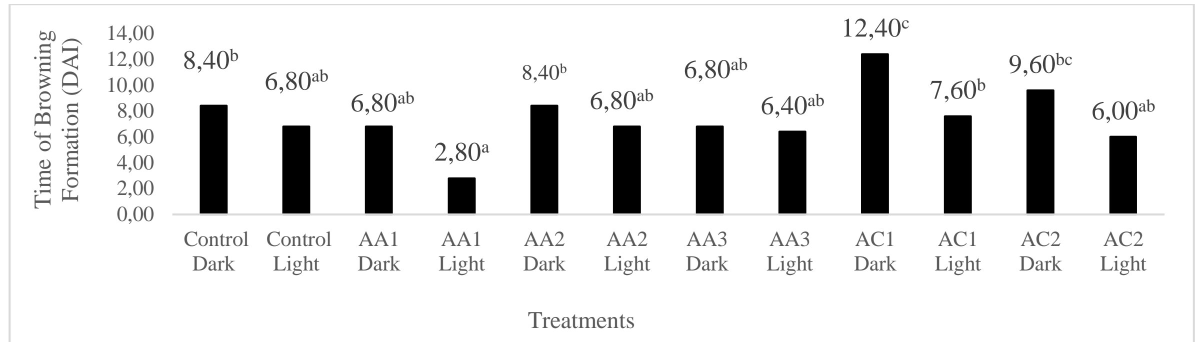


**Figure 2.** Average time of browning formation of *S.bracteosa* in vitro culture at 30 DAI. Note: AA1 (Immersion of explant in 200 ppm ascorbic acid); AA2 (Addition of 150 ppm ascorbic acid in MS media); AA3 (Addition of 250 ppm ascorbic acid in MS media); AC1 (Addition of 2000 ppm activated charcoal in MS media); AC2 (Addition of 5000 ppm activated charcoal in MS media). Means with the same letter are not significantly different at p=0.05.

Ascorbic acid acts as an antioxidant, reducing the o-quinone into a colorless phenolic compound (o-diphenol) (Permadi et al., 2023). In addition, ascorbic acid provides acidic conditions with a pH ranging from 2.5-3.0 so that PPO enzyme activity can be inhibited. According to Zhou et al. (2019), the optimal activity of the PPO enzyme ranges from pH 6.0-7.0, and its activity is significantly inhibited at pH < 4 due to structural changes in the enzyme. According to Ndakidemi et al. (2014), the optimal concentration of ascorbic acid is around 100-250 mg/L. However, in this study, immersion of pirdot leaf explants in 200 ppm ascorbic acid was ineffective and resulted in a faster browning appearance. The minimal oxidation resistance is might be due to the concentration of ascorbic acid is still less than optimal for Pirdot leaves. This study was not in line with Desai et al. (2018) that reported the immersion of *Punica granatum* L. explants in 200 mg/L of ascorbic acid resulted in low browning percentage. In addition, Tarampak et al. (2019) also reported that immersion of *Eusideroxylon zwageri* explant with a low pH setting, were able to overcome browning up to 100%. This difference in results may be due to the higher phenolic content of pirdot compared to *Punica granatum* L., which was soaked with the same concentration and duration in this study. Hence, immersing of explant is a less optimal method for controlling browning in in vitro pirdot culture.

Furthermore, adding ascorbic acid to the media generally has a slower average time for browning to appear compared to immersing an explant. This shows that using ascorbic acid in the media is sufficient to slow down browning. This result aligns with research by Jakhar et al. (2019) where adding 150 ppm ascorbic acid to the media reduced browning. The addition of ascorbic acid after sterilization of the media is thought to maintain its presence, thus providing a more stable effect and longer contact time for reducing phenolic compounds compared to the immersion of explants, which contact more quickly in acidic conditions. This research proves that the pre-treatment method shows a faster day of browning compared to the growth media method.

The treatment of adding 2000 ppm activated charcoal to the media resulted in the slowest browning appearance time with an average of 12.40 DAI. This is because activated charcoal provides dark conditions in the growing medium, which helps reduce light exposure to the explants so that phenol oxidation can be reduced (Tarampak et al., 2019). In general, the research results show that adding anti-browning compounds to the media tends to be more effective than only immersing an explant in antioxidant solution.

**Effect of Browning Prevention Treatment on Browning Intensity in Pirdot (*Saurauia bracteosa* DC.) In Vitro Culture**

Browning intensity parameters were observed for 30 days visually on the explants based on the browning intensity scoring table (Table 2). Despite the research results showed a browning percentage of 100%, each explant had a different browning intensity. After 30 days of observation, the *S. bracteosa* leaf explant images will be categorized based on the browning intensity scoring measurement in Table 4.

**Table 4.** *Browning* Intensity Scoring of *Saurauia bracteosa* DC. in vitro culture

| Score of Browning | Browning of Explant | Information |
|---|---|---|
| 0 – 0.24 | | The explant does not experience browning |
| 0.25-0.49 | | Browning occurs in half part of the explant |
| 0.50-0.74 | | Browning occurs more than half of the explants |
| 0.75-0.99 | | Browning occurs almost entirely in explant |
| 1.00 | | Browning occurs in all parts of the explant |

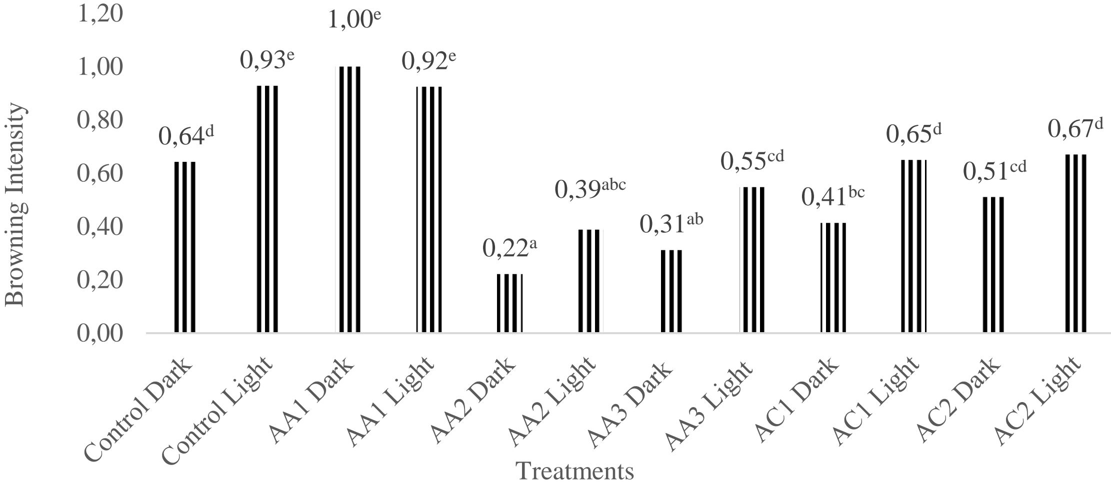


**Figure 3.** Average level of browning intensity of *S.bracteosa* in vitro culture at 30 DAI. Note: AA1 (Immersion of explant in 200 ppm ascorbic acid); AA2 (Addition of 150 ppm ascorbic acid in MS media); AA3 (Addition of 250 ppm ascorbic acid in MS media); AC1 (Addition of 2000 ppm activated charcoal in MS media); AC2 (Addition of 5000 ppm activated charcoal in MS media). Means with the same letter are not significantly different at p=0.05.

Based on the results, the lowest average browning intensity (0.22) resulted by adding 150 ppm ascorbic acid to the media incubated in dark conditions (Figure 3). This shows that adding ascorbic acid to the media while incubating in dark conditions has more optimal results when compared to pre-treatment and control methods. The results of this research was align with research conducted by Ndakidemi et al. (2014) that reported the additon of 250 ppm ascorbic acid to the media reduced the formation of browning in *Brahylaena huillensis* cultures. Similar results were obtained by Jakhar et al. (2019), who used 150 mg/L ascorbic acid in the media can reduce the browning process in *Commiphora wightii* (Arnott) cultures. Ascorbic acid is an antioxidant compound that is susceptible to damage by light or heat. Damage to ascorbic acid will interfere with its effectiveness in carrying out its anti-browning action. Research by Patel et al. (2018), which added ascorbic acid to the media, could not reduce the browning reaction. It is suspected that the ascorbic acid in the media was damaged during the media sterilization process. In this study, ascorbic acid was added aseptically in the LAF after the media was autoclaved. Based on the results of this research, it has been proven that adding ascorbic acid after the media has been sterilized provides more optimal results in reducing browning.

Apart from ascorbic acid, using activated charcoal in the media is optimal in reducing browning because activated charcoal will have delicate and specific pores to absorb phenol exudation and inactivate the PPO enzyme ( Das & Srivastav, 2015; Amente & Chimdessa, 2021). In general, the results of this study show that the AC2 (5000 ppm) treatment has a higher average intensity when compared to the AC1 (2000 ppm) treatment both in dark and light conditions. These results are in line with research by Poniewozik et al. (2022) who reported that the addition of 2 g/L of activated charcoal to the media in *Paphiopedilum insgine* culture provided the most optimal results in reducing browning reactions, while a concentration of 4 g/L had no effect in reducing browning. Jakhar et al. (2019) stated that the higher the use of activated charcoal in the media produce the higher the browning level. However, other research shows that adding 10 g/L activated charcoal to the media in *Vacia faba* culture can reduce browning and help explant regeneration (Abdelwahd et al., 2008). There are differences in browning intensity in several studies due to variations in phenolic concentrations contain in explant tissues, thus the browning responses to each plant can be different.

The research results also showed that all additions of anti-browning compounds to media incubated in dark conditions had a lower average browning intensity when compared to light conditions. The increase in the average browning intensity value in bright conditions is caused by light being able to induce PPO enzyme activity

and increasing the synthesis of phenolic compounds, which will accumulate, whereas in dark conditions, the enzyme activity is inhibited (Ahmad et al., 2013; Desai et al., 2018). Activated charcoal provides dark conditions in the media to reduce the intensity of light exposure so phenol oxidation can be inhibited. Tarampak et al. (2019) reported that *Eusideroxylon zwageri* cultures incubated in light conditions could overcome browning with a success rate of 31.25%.

To the best of our knowledge, this research is the first report to investigate the effectiveness of browning prevention methods using ascorbic acid, activated charcoal, and dark incubation. There is no previous report about the protocol for *S. bracteosa* in vitro culture. This finding can significantly help establish a browning prevention protocol in the initiation stage of *S. bracteosa* in vitro culture, thus supporting the in vitro conservation attempt of this plant.

## CONCLUSION

Based on the results, it can be concluded that the addition of 150 ppm ascorbic acid to the media and incubation in dark conditions had a significant effect on the lowest browning intensity (0.22) of *S.bracteosa* leaf explant, meanwhile the addition of 2000 ppm activated charcoal to the media and incubation in dark conditions significantly slowed down the browning formation (12.40 DAI). Based on this research, it is suggested to conduct further research regarding the combination of immersion of the explant in an ascorbic acid solution and the best concentration of ascorbic acid and activated charcoal in the media, using young explants from in vitro seed germination that contain fewer phenolic compounds and increasing concentrations of 2.4-D and BAP in the media to induce explant regeneration. This research is expected to produce the lowest browning intensity and percentage of browning also the highest regeneration rate.

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