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Effect of 2,4-D and BAP on the *Saurauia bracteosa* In Vitro Culture

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Abstract **Original Research Article**

Saurauia bracteosa DC. (Pirdot) is one of the endemic woody plant species that has medicinal properties. The declining population in natural habitats caused this plant as a vulnerable species that need in vitro culture to prevent further extinction. The combination of 2.4-D and BAP as an exogenous PGR influenced the success of woody plant in vitro culture. Therefore, this research aimed to determine the effect of 2.4-D and BAP concentrations on the in vitro response of the *S. bracteosa* leaf explant. This research used a complete randomized design (CRD) with two factors: (1) the concentration of 2.4-D (0.50; 0.75; 1 mg/L), and (2) the concentration of BAP (B) (1; 2; 3 mg/L). This research consisted of 16 treatments (including a control); each treatment was repeated three times. All data were analyzed statistically using ANOVA, and the significant results will be followed by Duncan's Multiple Range Test (DMRT). All PGR treatments could not produce a callus growth response, yet all explants showed various responses to browning intensity (0.08–1) and 100% browning. Further research still needs to be done to find the best type of explant and concentrations of PGR that can overcome the recalcitrant explant of Sauraia bracteosa. These results can be valuable information to promote the successful in vitro conservation of *Sauraia bracteosa*.

Keywords: Browning, in vitro culture, PGR, recalcitrant, *Saurauia bracteosa*.

INTRODUCTION

Saurauia bracteosa DC. or locally known as Pirdot is an endemic plant belongs to the Actiniadiacae family which is naturally distributed in several mountain areas in Central Java, West Java, Bali, North Sumatra, and North Sulawesi. This plant is categorized as a medium-sized woody plant species with a 3-15 meter height (Helmanto *et al*., 2020; Situmorang & Sunandar, 2019). The leaf parts of *S. bracteosa* are traditionally used by people in North Sumatra as herbal tea with medicinal properties to lower blood sugar, reduce blood cholesterol levels, prevent high blood pressure, as an anti-cancer and anti-inflammatory (Lubis *et al*., 2022; Situmorang & Sunandar, 2019; Situmorang *et al*., 2015). The pharmacological effects are attributed to phytochemical compounds contained in each part of this plant, such as flavonoids, phenolics and tannins and have very strong antioxidant activity ranging from 28.18 - 38.01 ppm (Lubis *et al*., 2022; Murtihapsari dkk., 2022). Based on IUCN Red List since 2019, the population of *S. bracteosa* in its natural habitat has been decreasing, thus it is considered as a vulnerable species (Helmanto *et al*., 2020). One of the attempts to overcome this problem is through ex situ conservation. In vitro culture has been widely applied for ex situ conservation as it can produce true to type plants in a large number of plants with a relatively short time and only requires a small part of the plant size to be used as explants (Radomir *et al.*, 2023; Dhiman *et al*., 2020). It is an effective tool for in vitro conservation of *S. bracteosa* plants in order to prevent its further extinction. Previous studies have reported ex situ conservation of an endangered species *Saurauia punduana* Wallich form India through callus propagation using nodal explants on MS media supplemented with BA and Kinetin (0-15 μM)(Deb & Gangmei, 2016) and shoot propagation using nodal explants on MS media supplemented with BA (9 μ M) and IBA (10 μ M) resulted in 43% of micro shoots (Deb & Gangmei, 2017). However, to the best of our knowledge, there are still limited studies regarding the in vitro conservation of *Saurauia bractoesa*. Therefore, this research is important to provide the in vitro propagation protocol of *S.bracteosa.*

One of the important factors that support the success of in vitro culture, particularly for woody plant

is the interaction of exogenous plant growth regulators (PGRs) added to the culture medium and endogenous hormones contained in the explants (Jayusman *et al*., 2022; Kumar & Jakhar, 2018). Among several types of PGRs, auxin and cytokinin are two important hormones for in vitro culture. 2,4-D (2,4-Dichlorophenoxyacetic Acid) and BAP (6-Benzylaminopurine) are types of PGRs from the auxin and cytokinin groups that are widely used for in vitro propagation of woody plants (Jayusman *et al.*, 2022; Sharma, 2017). The combination of auxin and cytokinin plays an important role in supporting cell division, cell elongation and morphogenesis. (Habibah *et al.*, 2023; Restiani *et al*., 2022). Previous studies have reported the success of endangered plants in vitro conservation using BAP and 2.4-D. Al-Qudah *et al*.(2023) reported that BAP 1.5 mg/L produce the optimum callus weight (5.81 g). Shirsat *et al*. (2021) also reported the combination of 2.5 mg/L 2.4-D and 2 mg/L BAP resulted in the highest fresh weight friable embriogenic callus (2.35 g) of threatened species *Caesalpinia bonducella*. According to previous studies, the optimal concentration of 2.4-D and BAP added to the culture media resulted in different responses for each type of explant although within the same species. Therefore, this research aimed to determine the effect of 2.4-D (0.50; 0.75; 1 mg/L) and BAP (1; 2; 3 mg/L) concentration on in vitro response of *S.bracteosa* leaf explant.

MATERIALS AND METHODS

Materials

The newly sprouting young leaves from old and mature *Saurauia bracteosa* tree branches obtained from "Eka Karya" Bali Botanical Garden collection with plant code XIX.D.7-7a, b, c, d were used as an explant. Pra-sterilization and sterilization of leaf explant was carried out prior to sterilization in Laminar Air Flow Liquid detergent, tween 80, sterile distilled water, bactericide, fungicide were used in explant pra-sterilization outside the Laminar Air Flow. 70% alcohol, 1,5% belaching solution (Clorox) and ascorbic acid were used in explant sterilization inside the Laminar Air Flow. Murashige and Skoog media, 30% (w/v) sucrose, agar, 2,4-D (0.50; 0.75; 1 mg/L) and BAP (1; 2; 3 mg/L) were used in media preparation.

Methods

Media Preparation

The Murashige and Skoog (MS) media used in this study were solidified using 8 g/L bacto agar, which was prepared according to Murashige and Skoog's (1962) formulation. MS media was supplemented with various concentrations of 2,4-D (0.50; 0.75; 1 mg/L) and BAP (1; 2; 3 mg/L). Subsequently, the pH of the media was adjusted to 5.7-5.8 using drops of either 1N NaOH or 1N HCl before adding bacto agar as a gelling agent. The media was autoclaved at 121℃ for 15 minutes. This research used a Complete Randomized Design (CRD) with two factors. The first factor was the concentration of 2.4-D (0.50; 0.75; 1 mg/L), and the second factor was the concentration of BAP (B) (1; 2; 3 mg/L). This research consisted of 16 treatments (including a control), each treatment was repeated three times.

Explant Preparation

Newly sprouting young leaves of *Sauraia bracteosa* were obtained from 2nd to 3rd leaves from the shoot apical. The leaves were kept in a coolbox to maintain humidity and temperature, then subsequently transferred to the biotechnology laboratory of Duta Wacana Christian University. Pre-sterilization was carried out by washing *S. bracteosa* leaf explants under running water for 60 minutes, immersing them in a solution containing liquid detergent and tween 80 for 60 minutes, and then rinsing them using distilled water. Subsequently, the leaf explants were immersed in a solution containing bactericide and fungicide for 15 minutes. The sterilization of leaf explants was carried out inside the LAF by immersing *S. bracteosa* leaf explants in 70% alcohol for 3 minutes, then washing by sterilized aquadest three times, and then immersing the explants in a 1.5% clorox solution for 15 minutes. Subsequently, the explants were washed by sterilized aquadest three times before being immersed in 200 ppm of ascorbic acid solution for 30 minutes. The sterilized explants were excised to a size of approximately 1 x 1 cm using a scalpel and then inoculated into MS media according to PGR treatments. The cultures were incubated for a 24-hour light period using a 20-watt TL lamp with a light intensity of 671 lux at a temperature of 22-23°C.

Observation and Data Analysis

The observation of culture was carried out for 60 days. Parameters were observed, including time of browning emergence, percentage of browning (%), browning intensity, percentage of contamination, and percentage of viable explants. The intensity of browning was determined by scoring of browning (Table 1) that refers to Admojo & Indrianto (2016).

Table 1: Browning Intensity Scoring

Scoring	Information
0 – 0.24	0 - < 1/4 of the explants experienced browning
0.25 – 0.49	1/4 - < 1/2 of the explants experienced browning
0.50 – 0.74	1/2 - < 3/4 of the explants experienced browning
0.75 – 0.99	3/4 - < 1 of the explants experienced browning
1.00	All parts of the explants experienced browning

All data were performed as mean ± standard deviation (SD) and analyzed statistically using the IBM SPSS Statistics V21 x 86 programs with an ANOVA test at a significant level of 5%. Significant results are obtained if a value of less than 5% is obtained. The significant ANOVA test results will be followed by a post hoc test using the Duncan's Multiplate Range Test (DMRT).

RESULTS

Effect of 2.4-D and BAP concentrations on browning response of *S.bracteosa* leaf explants

Based on data from Table 2, all treatments, including the control, resulted in a browning response of *S.bracteosa* leaf explants at 60 DAI. Despite various PGR treatments including control resulted in 100% browning of leaf explants, PGR-free media (control) resulted in the lowest browning intensity (0,08 ± 0,06a), while increasing concentrations of 2.4-D (1 mg/L) and BAP (3 mg/L) resulted in the highest browning intensity (1 ± 0^{fg}) (Table 2 and Figure 1). Furthermore, the increasing concentration of 2.4-D (1 mg/L) and BAP (3 mg/L) resulted in the fastest browning emergence (2 ± 0^{a} DAI), while the concentration of 2.4-D (1 mg/L) and BAP (2 mg/L) showed the slowest browning emergence (8 ± 0^{d}). However, based on the DMRT significance test, the effect of increasing level of BAP (1–3 mg/L) on MS media without 2.4-D showed no significant effect on browning intensity (0.08, 0.10, and 0.22). These treatments showed similar results. In addition, the increasing level of BAP (1–3 mg/L) on MS media containing the highest level of 2.4-D (1 mg/L) also showed no significant effect on browning intensity (0.70, 0.71, and 1) based on the DMRT significance test. These results indicated that the browning response of *S.bracteosa* leaf explants was influenced not only by the exogenous PGR added to culture media but also by the oxidation of phenolic compounds of the explants during excision at the isolation stage, as *S.bracteosa* is one of the woody plant species that contains a high level of phenolic compounds and strong antioxidant activity (Lubis *et al*., 2022; Pasaribu *et al*., 2020), which makes it prone to enzymatic browning (Chai *et al*., 2018). This is supported by the similar browning percentage and intensity of browning that resulted from the control (PGR-free media) and PGR treatments (Figure 1).

Table 2: Browning percentage, time of browning emergence and browning intensity of *Saurauia bracteosa* Leaf Explants at 60 DAI (Days after Inoculation)

Plant Growth Regulators		Treatment Code	Browning Percentage	Time of Browning Emergence (DAI)	Browning Intensity
2.4-D (mg/L)	BAP (mg/L)				
0	0	D0B0	100%	4 ± 0^{ab}	0,08 ± 0,06^{a}
0	1	D0B1	100%	5,33 ± 2,31^{bc}	0,10 ± 1,71^{ab}
0	2	D0B2	100%	5,33 ± 1,15^{bc}	0,22 ± 0,06^{abc}
0	3	D0B3	100%	4,67 ± 1.15^{bc}	0,92 ± 0,08^{fg}
0.5	0	D1B0	100%	6 ± 2^{bc}	0,33 ± 0,20^{abcd}
0.5	1	D1B1	100%	4,67 ± 1,15^{bc}	0,40 ± 0,15^{bcde}
0.5	2	D1B2	100%	4 ± 0^{ab}	0,50 ± 0,25^{cde}
0.5	3	D1B3	100%	4 ± 0^{ab}	0,65 ± 0,05^{def}
0.75	0	D2B0	100%	5,33 ± 1,15^{bc}	0,48 ± 0,25^{cde}
0.75	1	D2B1	100%	5,33 ± 1,15^{bc}	0,58 ± 0,10^{de}
0.75	2	D2B2	100%	4,67 ± 1,15^{bc}	0,45 ± 0,22^{cde}
0.75	3	D2B3	100%	4,67 ± 1,15^{bc}	0,43 ± 0,11^{cde}
1	0	D3B0	100%	4 ± 0^{ab}	0,53 ± 0,42^{cde}
1	1	D3B1	100%	6,67 ± 1,15^{cd}	0,70 ± 0,05^{efg}
1	2	D3B2	100%	8 ± 0^{d}	0,71 ± 0,10^{efg}
1	3	D3B3	100%	2 ± 0^{a}	1 ± 0^{fg}

Note: The numbers followed by the same letter show no significant difference between treatments based on DMRT test at α 5% level (DAI = Day After Inoculation)

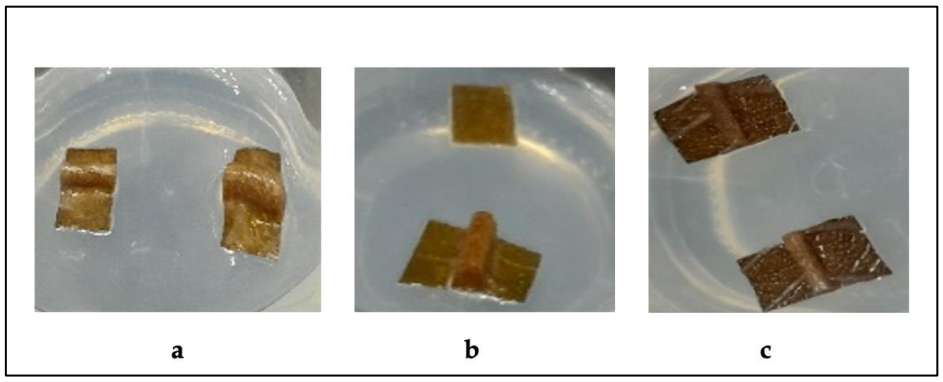

Figure 1: Browning intensity response of *Saurauia bracteosa* leaf explants at 60 DAI (a) MS media without PGR (control) (b) MS media + 0.75 mg/L 2.4-D + 2 mg/L BAP and (c) MS + 1 mg/L 2.4-D + 3 mg/L BAP

Effect of 2.4-D and BAP concentrations on growth response of *S.bracteosa* leaf explants

Based on the results in Table 3, all treatments, including the control, resulted in live explants (100%), as

they did not show any necrotic symptoms in the *S. bracteosa* leaf explant (Figure 1). However, all live explants did not result in callus growth at all concentrations of 2.4-D and BAP in MS media. Callus is one of the explant growth indicators in in vitro culture. Callus can be stimulated by the wound signal when cutting the explant, which subsequently modulates the expression of gene-regulating hormones such as auxin and cytokinin in explant cells (Habibah *et al*., 2023; Ikeuchi *et al*., 2013). The addition of exogenous hormones or PGR, such as 2.4-D (auxin) and BAP (cytokinin), in appropriate concentration could interact with the endogenous hormones already contained in the explant, which could enhance the capability of the explant growth response. However, in this research showed that all concentrations of PGR could not stimulate the callus growth of the *S.bracteosa* leaf explant. This might be correlated with the percentage and intensity of browning from all treatments, as previously explained. Browning is one of the major problems in woody plant in vitro culture due to the oxidation of high level of phenolic compounds in each part of plants, which causes an explant to become recalcitrant to in vitro culture. Explants from recalcitrant species are difficult to propagate or could not readily respond to nutrients and hormones in culture media, so the explants could not regenerate or resulting the death explants.

Table 3: Percentage of live explant and callus response of *Saurauia bracteosa* Leaf Explants at 60 DAI (Days After Inoculation)

Plant Growth Regulators		Treatment Code	Live Explant	Callus Growth
2.4-D (mg/L)	BAP (mg/L)			
0	0	D0B0	100%	0 %
0	1	D0B1	100%	0 %
0	2	D0B2	100%	0 %
0	3	D0B3	100%	0 %
0.5	0	D1B0	100%	0 %
0.5	1	D1B1	100%	0 %
0.5	2	D1B2	100%	0 %
0.5	3	D1B3	100%	0 %
0.75	0	D2B0	100%	0 %
0.75	1	D2B1	100%	0 %
0.75	2	D2B2	100%	0 %
0.75	3	D2B3	100%	0 %
1	0	D3B0	100%	0 %
1	1	D3B1	100%	0 %
1	2	D3B2	100%	0 %
1	3	D3B3	100%	0 %

DISCUSSION

This research showed that the supplementation of 2,4-D and BAP at various concentrations could not stimulate the growth of explants through callus initiation. However, all explants exhibited various responses to browning intensity and the time of browning emergence. This research used the leaf explant of *S. bracteosa*, which was isolated from young leaves. *S. bracteosa* is one of the woody plant species in the Actinidiaceae family, which contains a relatively high phenolic compound. The excision of the explant at the initiation stage could release the phenolic compound from the explant cells and be oxidized by the PAL or PPO enzyme to produce quinon, which causes brown color in explants and becomes toxic. This result was in accordance with research by Sitinjak *et al*., (2015) that reported the supplementation of 2.4-D (0.5–1.5 mg/L) and Kinetin (0.3–0.5 mg/L) in MS media could not produce callus growth in *Typhonium* sp. leaf explants, yet all explants were browning due to the cutting of the explant. Another study reported that the browning of stem explants of *Citrus reticulata* caused the deterrence of the explant and that the explant could not produce a callus (Prihastanti *et al*., 2020). Furthermore, Mayerni *et al*., (2020) also reported that the combination of relatively low and high concentrations of 2.4-D and BAP could not stimulate callus growth from *Pogostemon cablin* explants. These results indicated that the regeneration of explants could be inhibited by the browning problem, which reduced the regeneration ability of explants, and the inappropriate concentration of exogenous PGRs that interact with the endogenous hormones of explants.

In vitro regeneration of explants strongly depends on type of explants, genotype, developmental stage of explant, physiological states of explant including the status of endogenous hormone inside the explant, nutrient on media, exogenous hormones (plant growth regulators), light, and temperature (Ćosić *et al*., 2023; Guo & Jeong, 2021). However, most species of woody plants are recalcitrant to in vitro culture conditions, meaning that they are difficult to propagate or respond to the exogenous PGR on culture media (Abdalla *et al*., 2022; Ochatt *et al*., 2010). In addition, enzymatic browning caused by the oxidation of relatively high phenolic compounds in woody explants still becomes

one of the major problems, which mostly reduces or inhibits the regeneration ability of explants (Abdalla *et al*., 2022; Amente & Chimdessa, 2021). According to Gao *et al.*, (2020) and Wang *et al*., (2016), browning of explants induces down-regulation of genes involved in carbohydrate metabolism and nutrient transport, which could affect the growth stunting of explants. It is suggested that enzymatic browning of explants could directly inhibit the regeneration ability of explants, particularly for woody plants that are prone to enzymatic browning. This problem could be overcome by adding anti-browning to the in vitro culture, optimizing in vitro explants rather than grown field explants, and optimizing other types and concentrations of PGRs.

CONCLUSION

This research concluded that various concentrations of plant growth regulators (2,4-D and BAP) could not induce callus growth from explants; however, they did affect the browning intensity (0.08 – 1), browning percentage (100%), and time of browning emergence (2 – 8 DAI) in *Sauraia bracteosa* leaf explants. Further research is still needed to investigate and determine the best type of explant and concentrations of PGR that can overcome the recalcitrant condition of *Sauraia bracteosa* explants. These results provide valuable information to support the successful in vitro conservation of *Sauraia bracteosa.*

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REFERENCES

- Abdalla, N., El-Ramady, H., Seliem, M. K., El-Mahrouk, M. E., Taha, N., Bayoumi, Y., ... & Dobránszki, J. (2022). An academic and technical overview on plant micropropagation challenges. *Horticulturae*, *8*(8), 677. https://doi.org/10.3390/horticulturae8080677
- Admojo, L., & Indrianto, A. (2016). Pencegahan browning fase inisasi kalus pada kultur midrib daun klon karet (Hevea brasiliensis Muell Arg) Pb 330. *Indonesian Journal of Natural Rubber Research*, *34*(1), 25-34. https://doi.org/10.22302/ppk.jpk.v34i1.220
- Al-Qudah, T. S., Shibli, R. A., Zatimeh, A., Tahtamouni, R. W., & Al-Zyoud, F. (2023). A Sustainable Approach to In Vitro Propagation and Conservation of Salvia dominica L.: A Wild Medicinal Plant from Jordan. *Sustainability*, *15*(19), 14218. https://doi.org/10.3390/su151914218
- Ali, C., & Aminah, A. (2017). Perkembangan bunga dan buah pirdot (Saurauia bracteosa DC.) DI Arboretum aek nauli. *Jurnal Penelitian Hutan Tanaman*, *14*(2), 103-113.
- Amente, G., & Chimdessa, E. (2021). Control of browning in plant tissue culture: A review. *J. Sci. Agric*, *5*, 67-71. https://doi.org/10.25081/jsa.2021.v5.7266
- Chai, J., Gao, Y., Dong, Y., Kong, L., & Zhang, Y. (2018, December). Browning Treatment in Tissue Culture of 'Hongyang'Kiwifruit. In *IOP Conference Series: Materials Science and Engineering* (Vol. 452, p. 022075). IOP Publishing. https://doi.org/10.1088/1757-899X/452/2/022075
- Ćosić, T., Raspor, M., Motyka, V., Cingel, A., & Ninković, S. (2023). In Vitro Growth and Regeneration of Brassica oleracea var. gongylodes: A Decade of Research. *Horticulturae*, *9*(6), 674. https://doi.org/10.3390/horticulturae9060674
- Deb, C. R., & Gangmei, P. K. (2016). Ex situ conservation of endangered Saurauia punduana Wallich (Actinidiaceae) by in vitro propagation via callus generated from nodal segments and leaves. *Pleione*, *10*(1), 43-52.
- Deb, C. R., & Gangmei, P. K. (2017). Shoot regeneration from nodal segments of Saurauia punduana Wallich: A threatened therapeutically important plant. *Plant Cell Biotechnol. Mol. Biol*, *18*(3&4), 90-100.
- Dhiman, M., Sharma, L., Singh, A., & Sharma, M. M. (2020). Ex situ conservation using in vitro methods of an endangered plant Sterculia urens Roxb.: a high volume trade plant for Gum Karaya. *Industrial Crops and Products*, *158*, 113015. https://doi.org/10.1016/j.indcrop.2020.113015
- Gao, J., Xue, J., Xue, Y., Liu, R., Ren, X., Wang, S., & Zhang, X. (2020). Transcriptome sequencing and identification of key callus browning-related genes from petiole callus of tree peony (Paeonia suffruticosa cv. Kao) cultured on media with three browning inhibitors. *Plant Physiology and Biochemistry*, *149*, 36-49. https://doi.org/10.1016/j.plaphy.2020.01.029
- Guo, G., & Jeong, B. R. (2021). Explant, medium, and plant growth regulator (PGR) affect induction and proliferation of callus in Abies koreana. *Forests*, *12*(10), 1388. https://doi.org/10.3390/f12101388
- Habibah, N. A., Lutfiah, A., Liana, A., Tunjung, W. A. S., Indrowati, M., & Pa'ee, F. (2023). Callogenesis of Dayak Onion (Eleutherine palmifolia) Bulb in response of Picloram, 2, 4-D, and Kinetin. *Biosaintifika: Journal of Biology & Biology Education*, *15*(2), 270-280. https://doi.org/10.15294/biosaintifika.v15i2.46501
- Helmanto, H., Zulkarnaen, R. N., Fikriyya, N., & Robiansyah, I. (2020, July). Population status of Saurauia spp. in Slamet Mountain, Central Java. In *IOP Conference Series: Earth and*

Environmental Science (Vol. 528, No. 1, p. 012009). IOP Publishing. https://doi.org/10.1088/1755-1315/528/1/012009

- Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant callus: mechanisms of induction and repression. *The plant cell*, *25*(9), 3159-3173. https://doi.org/10.1105/tpc.113.116053
- Hakim, L., & Dalimunthe, A. (2022, December). Season, basal media and plant growth regulators effect in wood plant in vitro propagation: a comprehensive review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1115, No. 1, p. 012051). IOP Publishing. https://doi.org/10.1088/1755-1315/1115/1/012051
- Kumar, R., & Jakhar, M. L. (2018). Factor Affecting Tissue Culture Experiments of Woody Plant: A Review. *Journal of Cell & Tissue Research*, *18*(2), 6467–6471.
- Lubis, M. F., Hasibuan, P. A. Z., Syahputra, H., Surbakti, C., & Astyka, R. (2022). Saurauia vulcani (Korth.) as herbal medicine potential from North Sumatera, Indonesia: A literature review. *Heliyon*, *8*(4), 4–9. https://doi.org/10.1016/j.heliyon.2022.e09249
- Mayerni, R., Satria, B., Wardhani, D. K., & Chan, S. R. O. S. (2020, October). Effect of auxin (2, 4-D) and cytokinin (BAP) in callus induction of local patchouli plants (Pogostemon cablin Benth.). In *IOP Conference Series: Earth and Environmental Science* (Vol. 583, No. 1, p. 012003). IOP Publishing. https://doi.org/10.1088/1755-1315/583/1/012003
- Murtihapsari, B. S., Suparman, A. R., Parubak, A. S., Yogaswara, R., Susilawati, Y., & Herlina, T. (2022). Aktivitas Antidiabetes dari Fraksi Tumbuhan Pirdot Saurauia bracteosa pada Tikus dengan Metode Induksi Aloksan. *Jamb J Chem*, *4*(1), 1-9.
- Ochatt, S. J., Patat-Ochatt, E. M., Jacas, L., & Conreux, C. (2010). Competence versus recalcitrance for in vitro regeneration. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *38*(2), 102-108.
- Prihastanti, E., Hastuti, E. D., & Suedy, S. W. (2020). Comparing the growth of stem explants between Citrus reticulata var. Tawangmangu and C. reticulata var. Garut using in vitro culture methods. *Biodiversitas Journal of Biological Diversity*, *21*(12), 5845–5849. https://doi.org/10.13057/biodiv/d211248
- Radomir, A. M., Stan, R., Florea, A., Ciobotea, C. M., Bănuță, F. M., Negru, M., ... & Sumedrea, D. I. (2023). Overview of the Success of In Vitro Culture for Ex Situ Conservation and Sustainable Utilization of Endemic and Subendemic Native Plants of Romania. *Sustainability*, *15*(3), 2581. https://doi.org/10.3390/su15032581
- Restiani, R., Dolonseda, A. C., Kaban, S. M. P., Hutabarat, C. T., Sekar, A. A., Meliana, F. A., ... & KY, A. (2022). Efficient Callus and Shoot Induction Protocol from Leaf and Node Explants of Javanese Ginseng (Talinum paniculatum (Jacq.) Gaertn.). *Scholars Journal of Agriculture and Veterinary Sciences*, *9*(12), 223-231. https://doi.org/10.36347/sjavs.2022.v09i12.003
- Sharma, H. (2017). Role of Growth Regulators in Micropropagation of Woody Plants-a Review. *International Journal of Advanced Research*, *5*(2), 2378–2385. https://doi.org/10.21474/ijar01/3421
- Shirsat, R., Kengar, A., & Rai, A. (2021). In-vitro callogenesis and screening of antimicrobial activity of callus and seed of Caesalpinia bonducella F.: A threatened medicinal plant of Western Ghats. *Journal of Pharmaceutical Research International*, *33*(31A), 76-89. https://doi.org/10.9734/jpri/2021/v33i31a31666
- Sitinjak, M. A., Isda, M. N., & Fatonah, S. (2015). Induksi kalus dari eksplan daun in vitro keladi tikus (Typhonium sp.) dengan perlakuan 2, 4-D dan kinetin. *Al-Kauniyah: Jurnal Biologi*, *8*(1), 32-39.
- Situmorang, R. O., Harianja, A. H., & Silalahi, J. (2015). Karo's local wisdom: The use of woody plants for traditional diabetic medicines. *Indonesian Journal of Forestry Research*, *2*(2), 121-130. https://doi.org/10.20886/ijfr.2015.2.2.830.121-130
- Situmorang, R. O., & Sunandar, A. D. (2019, October). Pirdot (Saurauia bracteosa DC) Leaf Processing Technique for Making Herbal Tea. In *IOP Conference Series: Earth and Environmental Science* (Vol. 359, No. 1, p. 012004). IOP Publishing. https://doi.org/10.1088/1755-1315/359/1/012004
- Wang, Y., Wang, Y., Li, K., Song, X., & Chen, J. (2016). Characterization and comparative expression profiling of browning response in Medinilla formosana after cutting. *Frontiers in Plant Science*, *7*, 1897. https://doi.org/10.3389/fpls.2016.01897