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ORIGINAL ARTICLE

Optimization of Rosmarinic Acid Production in Basil (*Ocimum basilicum*) via Salicylic Acid and BAP Elicitation In vitro

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KEYWORDS

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ABSTRACT: Basil (*Ocimum basilicum*), a member of the mint family (Lamiaceae), is a significant medicinal plant with potential applications in treating various diseases. This plant contains diverse phenolic compounds, with rosmarinic acid being the most abundant. One method to enhance the production of secondary metabolites such as rosmarinic acid in tissue culture involves the use of biological and non-biological stimuli. In this study, the effects of different concentrations of salicylic acid (0, 100, 200, 300, 400, and 500 μM) and BAP (0, 1, 2, 3, 4, and 5 mg L^{-1}) on rosmarinic acid production were evaluated *in vitro* using a factorial design in a completely randomized format with three replications. The results revealed that all treatments significantly increased rosmarinic acid production compared to the control. The highest rosmarinic acid production (25.14 mg g^{-1} dry weight) was observed at 200 μM salicylic acid, while the lowest production occurred at 1, 2, and 3 mg L^{-1} BAP hormone levels. Overall, salicylic acid had a greater impact on rosmarinic acid production than BAP. It appears that hormonal treatments enhance this compound in basil by influencing growth processes, metabolism, and the activity of enzymes involved in rosmarinic acid biosynthesis.

INTRODUCTION

Despite the advancements in synthetic and chemical drugs, medicinal plants and their derived formulations continue to be extensively used and are considered an integral part of the medicinal and therapeutic systems in many countries [1]. Secondary metabolites are complex organic chemical substances produced by plants during their lifecycle. While these compounds do not play a direct role in growth or vital processes, they serve critical functions, such as repelling pests, attracting pollinators, and combating microbial diseases. Examples of secondary metabolites include aromatic substances, medicinally active compounds, seasonings, natural sweeteners, antimicrobial agents, pheromones, insecticides, herbicides, fungicides, plant hormones, and allelopathic compounds [2].

Plant tissue culture has emerged as a reliable alternative for producing biologically active compounds, offering a biotechnological approach to enhancing valuable plant characteristics. This method facilitates the rapid production of secondary metabolites, such as phenolic acids and flavonoids, within a short time frame.

Basil (*Ocimum basilicum* L.), a member of the mint family (Lamiaceae), is an annual aromatic herb renowned for its medicinal properties due to the high concentration of essential oils in its vegetative organs. Widely used in traditional medicine [3], basil contains

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diverse phenolic compounds, with rosmarinic acid being the most abundant [4]. Rosmarinic acid possesses numerous biological properties, including antioxidant, antiviral, antibacterial, anti-allergy, antipyretic, anti-inflammatory, and anticancer activities. Its antioxidant capacity surpasses that of vitamin E, offering protection against damage caused by free radicals and preventing lipoprotein oxidation. Additionally, reports highlight its anti-AIDS potential. Rosmarinic acid is recommended for treating arthritis, cataracts, cancer, rheumatic inflammation, and respiratory conditions [5].

The composition of basil essential oil varies among cultivars, with key components such as linalool, methyl chavicol, citral, eugenol, cineole, camphor, and methyl cinnamate. These components hold significant value in the global markets for essential oils, perfumes, medicines, and the food industry. Usable parts of the basil plant include the leaves, flowering branches, and seeds. In traditional medicine, basil seeds are regarded for their calming, tonic, aphrodisiac, and diuretic properties [6]. Research has shown that purple basil varieties are rich in anthocyanins, making them a potent source of antioxidants [7].

Tissue culture techniques enable the production of secondary metabolites by optimizing plant selection, nutrient composition, plant hormones, vitamins, and environmental factors such as temperature. These techniques, including the use of elicitors, are particularly valuable for producing economically significant anticancer compounds on a small scale and at reduced costs [8]. With numerous species and cultivars in the *Ocimum* genus, understanding genotype-specific responses to culture conditions and treatments can inform the development of effective protocols to enhance secondary metabolite production [9].

Various studies have investigated in vitro basil culture, including cell culture strategies to improve secondary metabolite production [10], cultivation of hairy roots [11], micropropagation [12–14], and the use of elicitors to increase rosmarinic acid content [15- 25].

Salicylic acid (SA), a plant signaling molecule, has been extensively studied for its ability to enhance the production of secondary metabolites in various plants. As an abiotic elicitor, SA plays a significant role in plant defense mechanisms by inducing systemic acquired resistance (SAR) and activating several biosynthetic pathways, including those responsible for the synthesis of phenolic compounds. Several studies have demonstrated the positive effects of SA on the production of secondary metabolites in basil. In particular, SA at a concentration of 200 μM has been shown to significantly enhance the production of rosmarinic acid in basil under in vitro conditions [26]. This effect has been attributed to SA's ability to activate the phenylpropanoid pathway, which is responsible for the biosynthesis of phenolic compounds like rosmarinic acid. These findings align with earlier studies where SA was found to increase the production of antioxidant compounds in various plants [27]. Salicylic acid has also been reported to stimulate the production of flavonoids and alkaloids in other medicinal plants, highlighting its role as an important elicitor in secondary metabolite biosynthesis [28]. Furthermore, SA's ability to act as a stress signal that promotes the synthesis of defensive metabolites makes it particularly useful in plant tissue culture, where controlled stress conditions can lead to higher yields of bioactive compounds.

Benzylaminopurine (BAP), a synthetic cytokinin, is commonly used in plant tissue culture to promote cell division, shoot proliferation, and overall growth. While BAP is primarily used to enhance vegetative growth, its effect on secondary metabolite production can vary depending on the concentration, species, and experimental conditions. In basil, BAP has been studied for its impact on both growth and metabolite production, but its effects on rosmarinic acid accumulation appear to be less pronounced compared to other growth regulators and elicitors like salicylic acid. In a study by Saha et al. [29], the application of BAP at concentrations of 1, 2, and 3 mg L^{-1} did not significantly increase rosmarinic acid production in basil, suggesting that BAP's impact on metabolite accumulation is secondary to other treatments like salicylic acid. These findings were echoed in studies on other medicinal plants, where BAP was found to promote overall growth but did not significantly enhance secondary metabolite production compared to treatments with other elicitors or hormones [30]. In contrast, BAP's role in promoting cell proliferation and shoot formation remains essential for generating large quantities of plant material for further metabolite analysis and extraction.

Elicitation is a well-established strategy used to enhance the production of secondary metabolites in plant tissue culture. Both biotic and abiotic elicitors, such as salicylic acid, jasmonic acid, and methyl jasmonate, have been shown to effectively stimulate the accumulation of bioactive compounds. Elicitors induce the plant's stress response pathways, which in turn activate the biosynthetic pathways responsible for secondary metabolite production. In a study by Liu et al. [31], the combination of SA with other growth regulators was found to enhance the production of secondary metabolites in medicinal plants. However, the results of the current study align with those of earlier studies that suggest salicylic acid has a more significant role in promoting rosmarinic acid production than BAP. According to Sharma et al. [28], the use of SA as an elicitor in basil not only enhances rosmarinic acid production but also improves the antioxidant activity of the plant tissue, making it a highly effective strategy for obtaining plants with higher therapeutic potential.

Biotechnological methods provide a means to produce significant quantities of plant material, often rare in the natural environment, while enhancing the concentration of biochemical compounds through optimized cultivation conditions [32]. Given the medicinal importance of basil and the high value of its secondary metabolites, particularly rosmarinic acid, identifying effective methods to stimulate their production is crucial. This study aims to evaluate the use of abiotic stimuli to enhance rosmarinic acid concentration in basil under tissue culture conditions.

MATERIALS AND METHODS

Plant materials and culture medium preparation

In this experiment, seeds of basil (*Ocimum basilicum*), Genovese variety—a modified cultivar imported from Italy—were used. The culture was established on Murashige and Skoog (MS) basal medium, supplemented with 3% sucrose and 0.7% agar. The pH of the medium was adjusted to 5.7, and the medium was autoclaved at 121°C under a pressure of 1.2 kg cm^{2-1} for 20 minutes.

The seeds were disinfected using 3% sodium hypochlorite and subsequently treated with 70% ethanol. To ensure better contact between the disinfectant and the seed coat, 1–2 drops of Tween 20 were added during the process. Ten seeds were planted in each jar, and the cultures were incubated in a controlled growth chamber at 25 ± 1°C with a 16-hour photoperiod under fluorescent light at an intensity of 2200 lux. After four weeks, terminal buds with four leaves were used as isolated explants. To ensure a sufficient number of contamination-free samples, subcultures were prepared every two weeks [33].

Plant regulator treatments

Two types of plant growth regulators were applied in this study. BAP (6-Benzylaminopurine) was used at concentrations of 0, 1, 2, 3, 4, and 5 mg L^{-1}, while salicylic acid was applied at concentrations of 0, 100, 200, 300, 400, and 500 µM. These treatments were incorporated into the MS basal culture medium. After one month, the samples were subcultured and grown for an additional month.

Preparation of methanol extracts of basil

Fresh samples (0.5 g) from aerial plant parts were ground with 3 mL of 96% ethanol. The resulting extract was transferred to a test tube, sealed with cotton, and wrapped in aluminum foil. The tubes were placed on a shaker for 24 hours to facilitate extraction, and the extracts were then purified.

Preparation of standard solution

A standard solution of rosmarinic acid at a concentration of 1×10^{-3} µM was prepared by dissolving 1 mg of rosmarinic acid in ethanol. Zirconium (IV) oxide chloride solution (1 µM) was prepared by dissolving 0.61 g of zirconium oxide chloride octahydrate ($ZrCl_2O \cdot 8H_2O$, sourced from Merck, Germany) in 10 mL of water [33].

Spectrophotometric test for rosmarinic acid

Portions of 50, 75, 100, 125, 150, 175, and 200 µL of the standard rosmarinic acid solution were separately transferred into 5 mL flasks. To each, 4.6 mL of ethanol, x µL of the rosmarinic acid solution, and [(200 − x) µL] of ethanol were added. Subsequently, 200 µL of zirconium (IV) oxide chloride solution was added to the mixture to make a final volume of 5 mL. After 5 minutes, the absorbance at 362 nm was recorded against a reagent blank using a UV–vis spectrophotometer equipped with quartz cuvettes.

A calibration curve of rosmarinic acid was generated by plotting absorbance against concentration, and the molar absorptivity was determined from the slope of the calibration line [33].

Determination of rosmarinic acid level

To determine the rosmarinic acid concentration in basil extracts, 200 µL of the extract was mixed with 4.6 mL of ethanol and 200 µL of zirconium (IV) oxide chloride solution in a test tube. The absorbance was measured at 362 nm at room temperature after 5 minutes. The concentration of rosmarinic acid in the extract was calculated using the following equation derived from the standard calibration curve:

$$\text{(Absorbance) } Y = 0.1003X - 0.1283$$
$$(R^2 = 0.9869)$$

Statistical analysis

The experiment was conducted using a completely randomized design, with three replications and five explants per treatment. Data were analyzed using SAS software, and mean comparisons were performed using Duncan's multiple range test at a 5% significance level. Correlations between traits were also evaluated.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) revealed that salicylic acid had a significant effect on the concentration of rosmarinic acid at the 1% significance level. Furthermore, the results of ANOVA indicated a significant difference among the various concentrations of BAP.

A comparison of the average salicylic acid levels in this study showed that the highest concentration of rosmarinic acid was observed at 200 µM. The differences between the various concentrations were highly significant in terms of effectiveness. While increasing the concentration to 500 µM resulted in a reduction in rosmarinic acid content, all tested concentrations produced higher levels of rosmarinic acid compared to the control.

According to Figure 1, the results showed that increasing the concentration of salicylic acid from 0 to 200 µM significantly increased rosmarinic acid production, rising from 2.31 to 25.14 mg g^{-1} dry weight. However, further increasing the salicylic acid concentration from 200 to 500 µM led to a decrease in rosmarinic acid production, dropping from 25.14 to 9.92 mg g^{-1} dry weight.

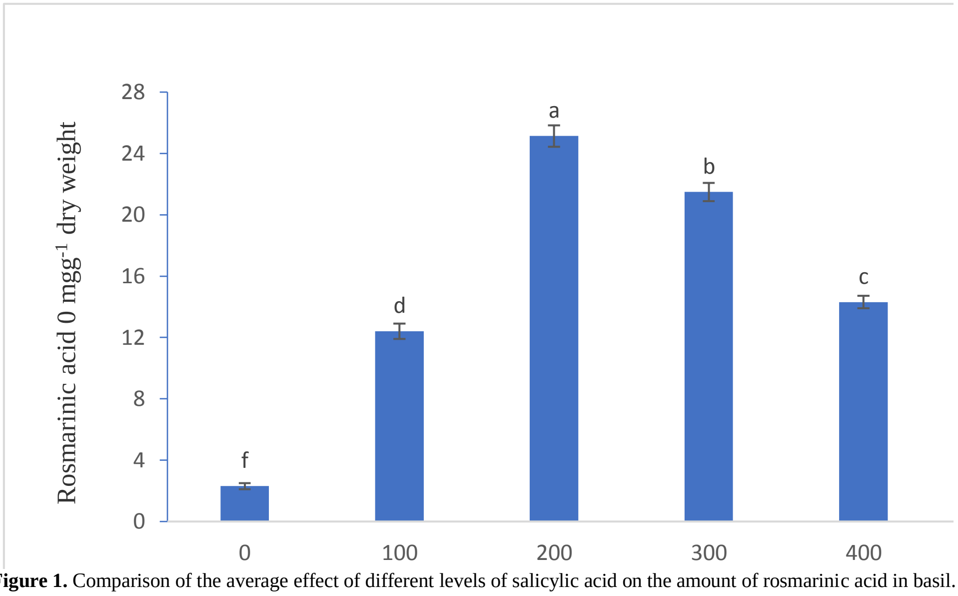

Figure 1. Comparison of the average effect of different levels of salicylic acid on the amount of rosmarinic acid in basil.

Thus, the highest production of rosmarinic acid was observed at 200 µM, while the lowest was recorded at 500 µM. Based on these results, 200 µM salicylic acid is recommended for maximizing rosmarinic acid production in basil.

Statistical analysis of the data also revealed significant differences in rosmarinic acid production across different levels of BAP. Increasing BAP from 0 to 1 mg L^{-1} raised the average production of rosmarinic acid from 2.31 to 6.26 mg g^{-1} dry weight. Between 1 and 3 mg L^{-1}, rosmarinic acid production remained stable, as indicated by similar statistical groupings. However, increasing BAP from 3 to 5 mg L^{-1} significantly boosted rosmarinic acid production, rising from 7.49 to 15.4 mg g^{-1} dry weight. The highest production was achieved at 5 mg L^{-1} of BAP, while the lowest was observed at 1 mg L^{-1}, which was statistically equivalent to the levels at 2 and 3 mg L^{-1} (Figure 2).

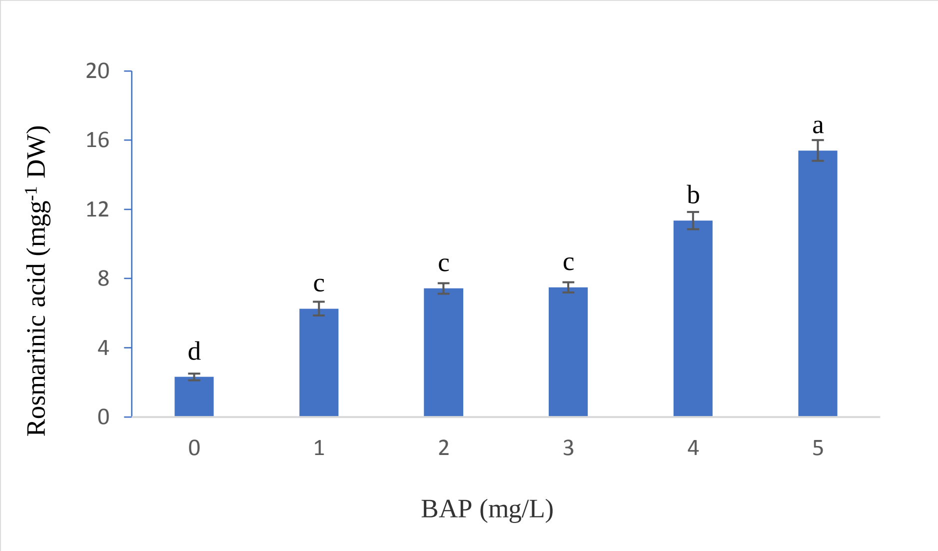

Figure 2. Comparison of the average effect of different levels of BAP on the amount of rosmarinic acid in basil.

DISCUSSION

The present study evaluated the impact of two plant regulators (salicylic acid (SA) and 6-Benzylaminopurine (BAP)) on the production of rosmarinic acid, a prominent secondary metabolite, in *Ocimum basilicum* (basil) grown in vitro. Secondary metabolites like rosmarinic acid are important due to their antioxidant, anti-inflammatory, antimicrobial, and anti-cancer properties. Enhancing the production of such metabolites is a major goal in medicinal plant biotechnology, as these compounds hold significant therapeutic value. In this study, the effects of various concentrations of salicylic acid and BAP on rosmarinic acid production were systematically investigated plant tissue culture; elicitors are external agents (either biological or abiotic) that stimulate the production of secondary metabolites. Both salicylic acid and BAP are known to affect plant growth and metabolic pathways. The results of this study confirm that both salicylic acid and BAP have significant effects on the accumulation of rosmarinic acid in basil, though the response varied between the two regulators.

Salicylic acid (SA) is a plant hormone that plays a key role in plant defense responses, particularly in inducing the biosynthesis of phenolic compounds. Previous research has established that SA acts as a signal molecule, activating stress-related pathways and triggering the production of secondary metabolites like flavonoids, alkaloids, and phenolic acids [34]. The significant increase in rosmarinic acid production at 200 µM SA observed in this study supports the notion that SA serves as an effective elicitor in basil tissue culture. At this concentration, the production of rosmarinic acid reached 25.14 mg g^{-1} dry weight, which represents a substantial increase compared to the control. The positive response of basil to SA treatment can be attributed to the activation of specific enzymes involved in the phenylpropanoid pathway, which is the biosynthetic route leading to the production of phenolic compounds such as rosmarinic acid. In particular, phenylalanine ammonia lyase (PAL), a key enzyme in this pathway, is known to be upregulated by SA [35, 36]. The activation of PAL by SA promotes the conversion of phenylalanine into trans-cinnamic acid, the precursor for rosmarinic acid and other phenolic compounds [37, 38]. This enzymatic activity results in enhanced production of secondary metabolites, as observed in this study. Furthermore, SA has been shown to enhance the plant's defense mechanisms by inducing the production of phytoalexins and antioxidants, which may also contribute to the increased production of rosmarinic acid [39, 40]. Similar findings were reported from the research of Tortosa et al. [41], who demonstrated that increasing the concentration of salicylic acid up to 10 µM significantly elevated the levels of rosmarinic acid and total phenolic compounds in thyme. This increase in phenolic compounds also enhanced the antioxidant capacity of the thyme plant. However, when the concentration of SA was raised to 100 µM, the levels of rosmarinic acid and phenolic compounds declined, suggesting a concentration-dependent effect.

Weitzel and Petersen [42] observed a direct relationship between increased activity of phenylalanine ammonialyase enzyme and rosmarinic acid production in lemongrass. Jiao et al. [43] reported that the application of salicylic acid at a concentration of 6.2 mg L^{-1} significantly increased rosmarinic acid content in sage plants. These findings align with the results of the current study, where optimal concentrations of SA elicited a marked increase in rosmarinic acid production.

However, it is crucial to note that the effectiveness of SA is highly concentration-dependent. While 200 µM SA induced the highest yield of rosmarinic acid, higher concentrations (400 µM and 500 µM) resulted in a decline in metabolite production. This decrease may be attributed to an overactivation of stress pathways, leading to metabolic disturbances. Excessive levels of SA can suppress metabolic processes, potentially through the inhibition of enzyme activity or gene expression [44]. This highlights the importance of optimizing elicitor concentrations in plant tissue culture to avoid detrimental effects on plant metabolism [45].

6-Benzylaminopurine (BAP), a synthetic cytokinin, is widely used in plant tissue culture for promoting cell division and shoot proliferation. Cytokinins like BAP can influence the accumulation of secondary metabolites by altering plant growth and modulating enzyme activities. The results of this study suggest that BAP at higher concentrations (3-5 mg L^{-1}) was effective in enhancing rosmarinic acid production, with the highest yield observed at 5 mg L^{-1} BAP (15.4 mg g^{-1} dry weight). This response could be attributed to the role of BAP in promoting cellular growth and differentiation, which may indirectly enhance the production of secondary metabolites. Cytokinins like BAP have been shown to increase the activity of PAL in various plant species, thereby facilitating the biosynthesis of phenolic compounds [46, 47]. The increase in rosmarinic acid at higher BAP concentrations observed in this study is consistent with previous reports that cytokinins can enhance the production of secondary metabolites by stimulating the phenylpropanoid biosynthetic pathway. Karalija and Paric [48] showed that the use of benzyl adenine at a concentration of 0.5 mg L^{-1} produced the highest amount of phenolic compounds in thyme plants. Kintzios et al. [49] in salvia plant concluded that increasing the concentration of Tofordi and Kintin at a concentration of 4.5 μM produced the highest amount of rosmarinic acid. The type of cytokinin, or the ratio of auxin to cytokinin, changes the formation and accumulation of secondary metabolites in cultured plant cells and has had a significant effect on the amount of compounds and secondary plant metabolites in *Aconitum violaceum*. Benzyladenine was more effective than thidiazrone, and the presence of auxin in the culture medium in combination with cytokinin increased the secondary metabolite of acetone [50]. However, the study also showed that lower concentrations of BAP (0-2 mg L^{-1}) did not significantly increase rosmarinic acid production compared to the control, indicating that there may be a threshold effect for the elicitor's efficacy. This suggests that the concentration of BAP needs to be optimized to balance cell growth and secondary metabolite production effectively. The study focused on two different classes of plant regulators, SA and BAP, which work through distinct signaling pathways. Salicylic acid is primarily involved in plant defense and stress response, while BAP acts as a growth regulator that promotes cell division and differentiation. The contrasting modes of action of these regulators suggest that they might act synergistically or antagonistically when combined in the same culture system. However, this study did not directly assess the combined effects of both elicitors, but based on the results, it is clear that both have distinct roles in influencing rosmarinic acid production.

While salicylic acid was more effective at inducing rosmarinic acid at lower concentrations (200 µM), BAP demonstrated its potential to increase metabolite production at higher concentrations (3-5 mg L^{-1}). The synergy between growth stimulation (via BAP) and stress-induced secondary metabolite biosynthesis (via SA) could be explored further to understand how these two regulators might work together to enhance both growth and metabolite accumulation in basil. Further research could focus on testing combined treatments to explore potential additive or synergistic effects, which could lead to more efficient production protocols for rosmarinic acid. The findings from this study underscore the importance of optimizing culture conditions to maximize secondary metabolite production. The concentration of elicitors, the type of plant growth regulator used, and the nutrient composition of the culture medium all play crucial roles in influencing the yield of secondary metabolites. In the case of basil, salicylic acid was identified as a particularly effective elicitor at a concentration of 200 µM, while higher concentrations of BAP (3-5 mg L^{-1}) also significantly increased rosmarinic acid production.

Further optimization could involve testing additional factors such as light intensity, temperature, and the composition of the culture medium to enhance metabolite yields. Additionally, exploring the interaction between various elicitors, including biotic elicitors (such as fungal or bacterial treatments) and abiotic ones like SA and BAP could provide new insights into enhancing the production of bioactive compounds in basil. The ability to increase the production of rosmarinic acid in basil using in vitro culture techniques has practical implications for medicinal plant biotechnology. Rosmarinic acid’s wide range of biological activities makes it a valuable compound in pharmaceutical, cosmetic, and nutraceutical applications. Enhancing its production through tissue culture and elicitation can help meet the growing demand for rosmarinic acid while reducing dependence on natural harvests, which are limited by environmental and seasonal factors. Furthermore, using elicitors like salicylic acid and BAP in controlled culture systems offers a sustainable and cost-effective method for producing high-quality basil-derived products. The results of this study can inform industrial-scale production of rosmarinic acid, contributing to the commercialization of medicinal plant-based products.

CONCLUSIONS

This study demonstrates the effectiveness of salicylic acid and BAP as elicitors of rosmarinic acid production in basil under in vitro conditions. Salicylic acid, in particular, was found to significantly increase the yield of rosmarinic acid at concentrations of 200 µM, while higher concentrations of BAP (3-5 mg L^{-1}) also enhanced production. The differential effects of these elicitors highlight the need for careful optimization of culture conditions, including elicitor concentration, to maximize metabolite yields. The findings have important implications for the production of rosmarinic acid in basil for medicinal and commercial use.

Future research should explore the combined effects of salicylic acid and BAP, as well as the impact of additional environmental factors, to further improve the production of secondary metabolites in basil tissue cultures. Moreover, studies on the mechanistic pathways involved in elicitor-induced metabolite biosynthesis could provide deeper insights into how these compounds enhance plant defense and secondary metabolite production.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Emad M., 2018. Identification of forest and pasture medicinal and industrial plants and their uses. Rural Development Institute: Iran.

2. Kumar J., Gupta P.K., 2008. Molecular approaches for improvement of medicinal and aromatic plants. Plant Biotech Rep. 2, 93-112.

3. Omid Beigi R., 2000. The production and processing of medicinal plants, Vol 3. Astan Quds Razavi: Iran.

4. Javanmardi J., Stushnoff C., Locke E., Vivanco J.M., 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum accessions*. Food Chem. 83, 547-550.

5. Park S.U., Uddin M.R., Xu H., Kim Y.K., Lee S.Y., 2008. Biotechnological applications for rosmarinic acid production in plant. Afr J Biotechnol. 7(25), 4959-4965.

6. Pushpagandan P., Bradu L., Basil. In: Chadha K.L., Gupta R., 1995. eds. Medicinal and aromatic plants, India: Malhotra publishing House. pp. 627-658.

7. Bicca Dode L., Bobrowski V.L., Braga E.J.B., Seixas F.K., Schuch M.W., 2003. In vitro propagation of *Ocimum basilicum* L. (Lamiaceae). Acta Sci. 2, 435-437.

8. Yavarian M., Behrouznam B., Zakerin A.R., 2012. The role of in vitro culture on secondary metabolites of medicinal plants. The third national conference of agricultural science and food industry. Fasa, Iran.

9. Jakovljević D., Stanković M., Warchoł M., Skrzypek E., 2022. Basil (Ocimum L.) cell and organ culture for the secondary metabolites production: a review. Plant Cell, Tissue Organ Cul. 149 (1-2), 61-79.

10. Mathew R., Sankar P.D., 2013. Plant cell culture technology and its entree into the world of *ocimum*. Int J Pharm Pharm Sci. 5(2), 975-1491.

11. Pistelli L., Giovannini A., Ruffoni B., Bertoli A., Pistelli L., 2010. Hairy root cultures for secondary metabolites production**.** Adv Exp Med Biol. 698, 167-184. https:// doi. org/10.1007/978-1-4419-7347-4_13.

12. Dode L.C., Bobrowski V.L. Braga E.J.B., Seixas F.K., Schunch W., 2003. In vitro propagation of *Ocimum basilicum* L. Maringa. 25, 435-437.

13. Sadeghian S., Ranjbar G.A., Kazemitabar S.K., 2014. Consideration and Selection of Suitable Hormonal Composition for in vitro Shoot Regeneration and Propagation of *Ocimum basilicum* L. J Crop Breed. 6(13), 40-48.

14. Kumar A., Jhanwar B., 2013. In Vitro Micropropagation of *Ocimum Americanum* (L.): A Wild But Important Medicinal Plant. J Pharm Bioanal Sci. 2(3), 29-32.

15. Ghanati F., Rajabbeigi E., Sefidkon F. Abdolmalek P., 2006. Investigating the Changes of Essential Oil of *Ocimum basilicum* L. in Response to Electromagnetic Field. Iran J Med. Aroma Plants. 22(3), 341- 350.

16. Bais H.P., Walker T.S., Schweizer H.P., Vivanco J.M., 2002. Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum*. Plant Physiol Biochem. 40, 983–995.

17. Kintzios S., Makri O., Panagiotopoulos E., Scapeti M., 2003. *In vitro* rosmarinic acid accumulation in sweet basil (*Ocimum basilicum L.*). Biotech Lett. 25, 405–408.

18. Kim H., Chen F., Wang X., Rajapakse N.C., 2005. Effect of Chitosan on the Biological Properties of Sweet Basil (*Ocimum basilicum L.*). J Agri Food Chem. 53(9), 696-701.

19. Hakkima F.L., Kalyani S., Essa M., Girija S., Song H., 2011a. Production of rosmarinic acid in *Ocimum sanctum* (L.) cell suspension cultures by the influence of growth regulators. Int J Biol Medi Res. 2(4), 1158 -1161.

20. Hakkima F.L., Kalyani S., Essa M., Girija S., Song H., 2011b. Production of Rosmarinic in *Ocimum sanctum* cell cultures by the influence of sucrose, phenylalanine, yeast extract, and ethyl jasmonate. Int J Biol Med Res. 2(4), 1070 -1074.

21. Mathew R., Sankar P.D., 2012. Effect of methyl jasmonate and chitosan on growth characteristics of *Ocimum basilicum* L., *Ocimum sanctum* L. and *Ocimum gratissimum* L. cell suspension cultures. Afr J Biotech. 11(21), 4759-4766.

22. Mathew R., Sankar P.D., 2014. Comparison of major secondary metabolites quantified in elicited cell cultures, non- elicited cell cultures, callus cultures and field grown plants of ocimum. Int J Pharm Pharm Sci. 6(2), 975-1491.

23. Raoufa A. I., Abdel Rahman R.A.I., El-Wakil H., Abdelsalam N.R., Elsaadany R.M.A., 2015. In vitro production of rosmarinic acid from basil (*Ocimum basilicum* L.) and lemon balm (*Melissa officinalis* L.). Middle East J Appl Sci. 5(1), 47-51.

24. Acıkgoz M.A., 2020. Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. Ind Crop Prod. 148, 112278. ttps:// doi. org/ 10. 1016/j. indcr op. 2020. 112278.

25. Acıkgoz M.A., 2021. Effects of sorbitol on the production of phenolic compounds and terpenoids in the cell suspension cultures of *Ocimum basilicum* L. Biologia. 76(1), 395–409. https:// doi. org/ 10. 2478/

s11756- 020- 00581-0.

26. Yuan Y., Wang Z., Zhang, X., 2020. Enhancing rosmarinic acid production in basil (*Ocimum basilicum*) through salicylic acid treatment. Plant Cell, Tissue Organ Cul. 142(3), 507–516. https://doi.org/10.1007/s11240-020-01834-0.

27. Mishra A., Pandey P., Gupta, R., 2018. Salicylic acid as a potent elicitor for enhancing secondary metabolite production in medicinal plants. J Plant Growth Regul. 37(3), 888–900. https://doi.org/10.1007/s00344-018-9814-5.

28. Sharma S., Dubey S., Kumar, A., 2019. Elicitation strategies for enhanced production of bioactive compounds in medicinal plants. Biotechnol Adv. 37(1), 30–45. https://doi.org/10.1016/j.biotechadv.2018.11.001.

29. Saha P., Jha S., Ghosh S., 2020. The impact of salicylic acid on secondary metabolite production in basil (*Ocimum basilicum*). Phytochem Rev. 19(4), 717–730. https://doi.org/10.1007/s11101-019-09612-1.

30. Chakraborty D., Ghosh S., Das P., 2017. Influence of plant growth regulators on the in vitro production of secondary metabolites. Plant Biotechnol Rep. 11(3), 273–281. https://doi.org/10.1007/s11816-017-0426-7.

31. Liu Y., Wang Y., Zhang X., 2019. Synergistic effects of plant growth regulators and elicitors on secondary metabolite production in medicinal plants. Ind Crops Prod. 141, 111804. https://doi.org/ 10.1016/j. indcrop.2019.111804.

32. Assaf M., Korkmaz A., Karaman Ș., Kulak M., 2022. Effect of plant growth regulators and salt stress on secondary metabolite composition in Lamiaceae species. S Afr J Bot. 144, 480–493. https://doi.org/10.1016/j.sajb.2021.10.030.

33. Öztürk M., E. Duru M., Ince B., Harmandar M., Topçu G., 2010. A new rapid spectrophotometric method to determine the rosmarinic acid level in plant extracts. Food Chem. 123, 1352–1356.

34. Vlot A. C., Klessig D. F., Park S. W., 2009. Salicylic acid and its function in plant defense. Plant Signal Behav. 4(6), 593-605.

35. Shah J., 2003. The salicylic acid loop in plant defense. Curr Opin Plant Biol. 6(4), 365-371.

36. Mueller M.J., Brodschelm W., Spannagl E., Zenk M.H., 1993. Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. Proceedings of the National Academy of Sciences of the United States of America. 90, 7490–7494.

37. Raman V., Ravi S., 2011. Effect of salicylic acid and methyl jasmonate on antioxidant systems of *Heamatococcus pluvialis*. Acta Physiol Plant. 33, 1043-1049.

38. Bagal U.R., Leebens mack J.H., Walter Lorenz W., Dean J.F.D., 2012. The phenylalanine ammonia lyase (PAL) gene family shows a gymnosperm specific line age. BMC Gen. 13(3), 1471- 2164.

39. Klessig D.F., Malamy J., 1994. The salicylic acid signal in plants. Plant Mol Biol. 26(3), 1439-1452.

40. Abdekhani S., Solouki M., 2016. Changes in Phenolic Compounds, Anthocyanin and Antioxidant Enzymes in Different Growth Stages Basil (*Ocimum basilicum* L), Using Growth Regulators. J Med Plants. 15(58), 164-175.

41. Tortosa V.P., Lopes-orenes A., Martinez_perez A., Ferre M.A. Calderon A.A., 2012. Antioxidant activity and rosmarinic acid changes in salicylic acid-treated *Thymus membranaceus* shoots. Food chem. 130(2), 362-369.

42. Weitzel C., Petersen M., 2010. Enzymes of phenylpropanoid metabolism in the important medicinal plant *Melissa officinalis* L. Planta. 232, 731–742.

43. Jiao M., Cao R., Chen H., Hao W., Dong J., 2012. Effects of salicylic acid on synthesis of rosmarinic acid and related enzymes in the suspension cultures of Salvia miltiorrhiza. Sheng Wu Gong Cheng Xue Bao. 28(3), 320-328.

44. Khan M.I.R., Fatma M., Per T.S., Anjum N.A., Khan N.A., 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci. 6, 462.

45. Zhang Y., Li, X., 2018. Impact of salicylic acid on secondary metabolite production in medicinal plants. Front Plant Sci. 9, 1971.

46. Nadeem M., Hameed M., 2019. Impact of cytokinin and other growth regulators on medicinal plant production. Plant Growth Regul. 88(3), 245–260. https://doi.org/10.1007/s10725-019-00505-6.

47. Aremu A.O., Bairu M.W., Szuˇcova L., Doleˇzal K., Finnie J.F., Van Staden J., 2012. Assessment of the role

of meta-topolins on in vitro produced phenolics and acclimatization competence of micropropagated 'Williams' banana. Acta Physiol Plant. 34, 2265–2273.

48. Karalija E., Paric A., 2011. The effect of BA and IBA on the secondary metabolite production by shoot culture of *Thymus vulgaris* L. Biol Nyssana. 2(1), 29-35.

49. Kintzios S., Nikolaou A., Skoula M., 1999. Somatic embryogenesis and in vitro rosmarinic acid accumulation in *Salvia officinalis* and *S. fruticosa* leaf callus cultures. Plant Cell Rep. 18(6), 462-466.

50. Rawat J.M., Rawat B., Chandra M., Nautyal S., 2013. Influence of plant growth regulators on indirect shoot organogenesis and secondary metabolite production in *Aconitum violaceum* Jacq. Afr J Biotechnol. 12 (44), 6287-6293.