



Production of rosmarinic acid and biomass from adventitious root cultures of *Ocimum basilicum* by optimization of medium components in airlift bioreactors

İlhami Karataş¹

Received: 5 April 2022 / Accepted: 24 June 2022 / Published online: 25 July 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract

Optimization of medium components for each species is critically important to produce valuable bioactive compounds at high yields in plant tissue and organ culture methods using bioreactor systems. Rosmarinic acid production in different in vitro culture methods of *Ocimum basilicum* L. (sweet basil) was evaluated in previous studies, however, to our knowledge, there was no available literature on adventitious root culture under bioreactor culture conditions. The aim of this study was to evaluate the effects of indole-3-butyric acid (IBA) concentrations (0.50, 1.0, 2.0 and 4.0 mg L⁻¹) and Murashige and Skoog (MS) medium salt strengths (0.50, 0.75, 1.0 and 1.5) on biomass, accumulation of bioactive compounds (rosmarinic acid, total phenolic and flavonoid) in adventitious root cultures of sweet basil using a balloon-type bubble bioreactor. In addition to, antioxidant capacities (DPPH, ABTS and FRAP) and phenylalanine ammonia lyase activities (PAL) of adventitious roots were assessed. Also, the changes caused by these medium components in antioxidant enzymes activities (catalase, superoxide dismutase, peroxidase) and some stress parameters (malondialdehyde, hydrogen peroxide and proline) were investigated. The rosmarinic acid content of the adventitious roots was analyzed using UHPLC-HESI-MS/MS. Among the used medium components, 0.75 MS and 2 mg L⁻¹ IBA were found to be the most appropriate quantities for biomass, PAL activities, accumulation of rosmarinic acid, phenolics and flavonoids, and activities of DPPH, ABTS and FRAP. The maximum accumulation of rosmarinic acid was determined as 20.98 ± 1.38 mg g⁻¹ DW at 2 mg L⁻¹ IBA which was 1.45, 1.19 and 4.02 times higher than 0.5, 1 and 4 mg L⁻¹ IBA, respectively. Moreover, the content of rosmarinic acid at 0.75 MS was 1.94, 2.66 and 5.99-fold greater than 0.5, 1 and 1.5 MS, respectively. At these optimum conditions, the activities of antioxidant enzymes and the levels of stress parameters were generally determined to be lower. Overall, the results of our study make an important contribution to the mass production of rosmarinic acid in adventitious root cultures of sweet basil.

Key message

Optimum IBA concentration and MS medium salt strength are critically important determinants for production of rosmarinic acid and biomass in sweet basil adventitious root cultures through a balloon-type bubble bioreactor.

Keywords Adventitious roots · Antioxidant activities · Bioreactor culture · Medium salt strength *Ocimum basilicum* · Rosmarinic acid

Abbreviations

ABTS	2, 2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)
AR	Adventitious root
BTBB	Balloon-type bubble bioreactor
CAT	Catalase
DPPH	2,2-Diphenyl-1-picrylhydrazyl
IBA	Indole-3-butyric acid
MS	Murashige and Skoog
PAL	Phenylalanine ammonia lyase

Communicated by Christophe Hano.

✉ İlhami Karataş
ilhami.karatas@gop.edu.tr

¹ Department of Forestry, Almus Vocational School, Tokat Gaziosmanpaşa University, 60250 Tokat, Turkey

POD	Peroxidase
RA	Rosmarinic acid
SOD	Superoxide dismutase

Introduction

Ocimum basilicum L. (basil) is a medicinal and aromatic plant used in traditional medicine for the treatment of kidney problems, coughs, colds, malaria, asthma, fevers, bronchitis, flu, influenza, diarrhea, and stomach aches. This plant is also an important essential oil source that is used in food, health, and fragrance industries (Shahrajabian et al. 2020). In addition, thanks to the various compounds found in the basil, it can show variety of activities such as antioxidant, anti-inflammatory, antimutagenic, antibacterial, antiviral, antifungal, antitoxic, antihyperglycemic, hypolipidemic, immunomodulatory, hepatoprotective, insect repellency and depigmenting (Khair-ul-Bariyah et al. 2012). The chemical constituents of *O. basilicum* include various pharmaceutically active substances belonging to the class of flavonoids, alkaloids, phenolics and terpenoids (Açıkgöz 2020). In terms of phenolics, basil contains different types of phenolic compounds, and its main phenolic compound is rosmarinic acid (Zeljkočić et al. 2020; Teofilović et al. 2021). In addition to the rosmarinic acid, the most recent studies suggest that main phenolic compounds are also chicoric acid, caftaric acids (Prinsi et al. 2020; Bajomo et al. 2022) and caffeic acid (Bajomo et al. 2022), depending on the cultivar.

Rosmarinic acid is a phenolic compound that is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid, and it is synthesized from L-tyrosine and L-phenylalanine amino acids by the catalysis of eight enzymes including phenylalanine ammonia lyase (PAL). This molecule exhibits a multitude of biological and pharmacological activities including antioxidant, antibacterial, antiviral, anti-inflammatory, antiallergic, antitumor, neuroprotective and antiangiogenic effects (Petersen 2013; Kim et al. 2015). However, many plants producing this compound are threatened with biodiversity loss and extinction due to environmental changes, over-collecting and unscientific harvesting (Swamy et al. 2018). On the other hand, the chemical synthesis of rosmarinic acid is complex, and natural resources of compound are limited (Jiang et al. 2016). These problems can be overcome with alternative approaches using biotechnological methods (Swamy et al. 2018). The biotechnological approaches play a critical role in the production of high value pharmaceuticals to contribute to industrial production and to reduce the overuse of natural resources (Isah et al. 2018). In line with this purpose, the culturing of plant cells, tissues and organs is an alternative biotechnological method to plant cultivation to produce valuable bioactive metabolites with high yield

and consistent quality (Li et al. 2016; Jakovljević et al. 2022). In this context, to meet the growing demand for rosmarinic acid, various biotechnological methods have been developed using plant cell and hairy root cultures (Khojasteh et al. 2014). For this purpose, several studies were carried out using different cell, tissue and organ cultures in many plant species such as callus culture of *Satureja hortensis* (Tepe and Sokmen 2007a), cell suspension culture of *Satureja khuzistanica* (Sahraroo et al. 2016) and *Mentha × piperita* (Krzyzanowska et al. 2012), in vitro shoot cultures of *Salvia officinalis* (Kračun-Kolarević et al. 2015) and hairy root culture of *Coleus blumei* (Bauer et al. 2009) and *Dracocephalum moldavica* (Weremczuk-Jeżyna et al. 2013). In case of *O. basilicum*, there are many publications evaluating rosmarinic acid production in callus cultures (Nazir et al. 2019; Duran et al. 2019), cell suspension cultures (Açıkgöz 2020), hairy root culture (Srivastava et al. 2016), in vitro root cultures (Biswas 2020) and in vitro propagated plants (Verma et al. 2016), however, no reports are available on adventitious root culture under bioreactor culture conditions. Therefore, optimization of culture conditions is needed for rosmarinic acid production in adventitious root of *O. basilicum* using bioreactors.

Adventitious roots are defined as roots that develop from non-root tissues of plants such as leaves and stems (Steffens and Rasmussen 2016). Adventitious root culture is an effective and attractive method to produce valuable plant secondary metabolites and biomass. Compared to other plant cell, tissue and organ culture methods, adventitious root cultures have several advantages including stable metabolite productivity, fast growth rates, high root proliferation, high biomass accumulation and high genetic stability (Baque et al. 2013; Lee et al. 2015; Murthy et al. 2016). In addition, these cultures enable the production of plant bioactive compounds for commercial applications through bioreactors (Rahmat and Kang 2019). Successful production of the valuable metabolites under in vitro culture conditions requires consideration of culture conditions and individual plant species together with different genotypes (Ho et al. 2019). Moreover, optimization of the culture medium is a critical determinant for stable commercial production of plant secondary metabolite in adventitious root cultures (Cui et al. 2013). In this context, some basic components of culture medium which could influence accumulation of biomass and compounds consist of suitable medium, amount and types of carbohydrates, growth regulator concentration and levels of nitrate and phosphate (Murthy et al. 2014). Among these ingredients, medium salt strength is one of the most important factors in regulating secondary metabolite accumulation and root growth (Yin et al. 2013). Similarly, plant growth regulators in the culture medium play a critical role in metabolite production, growth and proliferation of adventitious roots (Murthy et al. 2014).

We summarized above some of the previous studies on rosmarinic acid production in callus, suspension, in vitro shoot, in vitro root, and hair root cultures of basil plants. However, to our knowledge, there was no available literature regarding the optimization of medium components in adventitious root culture of *O. basilicum* for rosmarinic acid production under bioreactor culture conditions. Therefore, the aim of this study was to optimize MS salt strength and IBA concentration for biomass and rosmarinic acid production in adventitious root culture of *O. basilicum* using balloon-type bubble bioreactors (BTBBs). In addition, some basic biochemical parameters were analyzed to understand the underlying reasons of differences in compound and biomass levels caused by the medium components. Also, antioxidant capacities of adventitious roots were evaluated according to three different methods. In this context, total phenolic and flavonoid contents, enzymatic (catalase, superoxide dismutase, peroxidase) and non-enzymatic antioxidant activities (DPPH, ABTS and FRAP), PAL activities and some stress parameters (malondialdehyde, hydrogen peroxide and proline) of adventitious roots were assessed.

Materials and methods

Plant materials

Sweet basil (*Ocimum basilicum*, green colored) used as plant material was grown from mature seeds under in vitro culture conditions. The seeds of sweet basil were obtained from Zengarden traditional seed company (Izmir, Turkey). The surface sterilization of the seeds was achieved by soaking in a 70% ethanol (v/v) for 30 s and then gently mixing in a 4% sodium hypochlorite solution (v/v) for 30 min. Afterwards, the seeds were rinsed four times using sterile distilled water. Upon completion of the sterilization process, the seeds were planted into glass jars containing 50 mL of nutrient medium consisting of 4.4 g L⁻¹ MS (Murashige and Skoog 1962), 30 g L⁻¹ sucrose and 2 g L⁻¹ phytagel. In preparation of the medium, the pH was adjusted to 5.8 using 1 M NaOH or 1 M HCl before autoclaving at 121 °C for 20 min. After the 8-day germination period of the seeds in the dark, the sprouting plants were transferred to a photoperiod of 16 h light/8 h dark for 22 days and then cut for using adventitious root culture studies.

Induction and proliferation of adventitious roots

Adventitious roots were induced from the stem segment explants (the stem region between the cotyledon leaves and root) of sweet basil plants grown in vitro culture conditions. The explants were cultured in nutrient medium containing 4.4 g L⁻¹ MS (Murashige and Skoog 1962; Duchefa

Biochemie BV, Haarlem, The Netherlands), 30 g L⁻¹ sucrose and 2 g L⁻¹ phytagel, and 2 mg L⁻¹ IBA at 25 ± 2 °C under the dark conditions. In preparation of the culture medium, the pH of nutrient solutions was adjusted to 5.8 using 1 M NaOH or 1 M HCl and then autoclaved at 121 °C with 1.2 atmospheres pressure for 20 min. The adventitious roots grown in semi-solid culturing conditions were excised from the explant sources after 25 days of culture and used to initiate suspension culture. The suspension cultures were established for two weeks to propagate adventitious roots obtained from semi-solid nutrient medium. Adventitious roots (2 g) were inoculated into Erlenmeyer flasks containing 100 mL of nutrient medium consisting of 4.4 g L⁻¹ MS, 30 g L⁻¹ sucrose and 2 mg L⁻¹ IBA and incubated on a shaker operating at 120 rpm under dark conditions (Fig. 1).

Bioreactor cultures

To optimize IBA concentration and strength of MS medium, adventitious roots of sweet basil were cultured in 1 L balloon-type bubble bioreactor (BTBB) containing 0.5 L of culture medium for 30 days. Bioreactor cultures were initiated by inoculating 3.5 g of adventitious root that were obtained from suspension cultures, and airflow rate of cultures were adjusted to 0.1 vvm (air volume/culture volume per min). The adventitious roots were applied with different strengths of MS medium (0.5 MS, 0.75 MS, 1 MS and 1.5 MS) supplemented with 2 mg L⁻¹ IBA and 30 g L⁻¹ sucrose. After determining the suitable strength of MS medium, adventitious roots were cultured in medium augmented with different concentrations of IBA (0.5, 1, 2 and 4 mg L⁻¹), 0.75 MS and 30 g L⁻¹ sucrose (Fig. 1).

Determination of adventitious root biomass

Adventitious roots of *Ocimum basilicum* were harvested after 30 days of the culture and separated from the medium by filtration through a sieve. Subsequently, these roots were rinsed three times with double distilled water. After removing unwanted surface water on the roots with blotting paper, fresh weights (FW) were determined by weighing. To determine dry weights (DW), the fresh adventitious roots were dried in an incubator at 50 °C for 48 h to a constant dry weight. The growth ratio (GR) was calculated using the following equation:

$$GR = [\text{harvested FW(g)} - \text{inoculated FW(g)}] / \text{inoculated FW(g)}$$

Preparation of adventitious root extracts

For the determination of secondary metabolite contents (rosmarinic acid, total phenolic and flavonoid,) and antioxidant

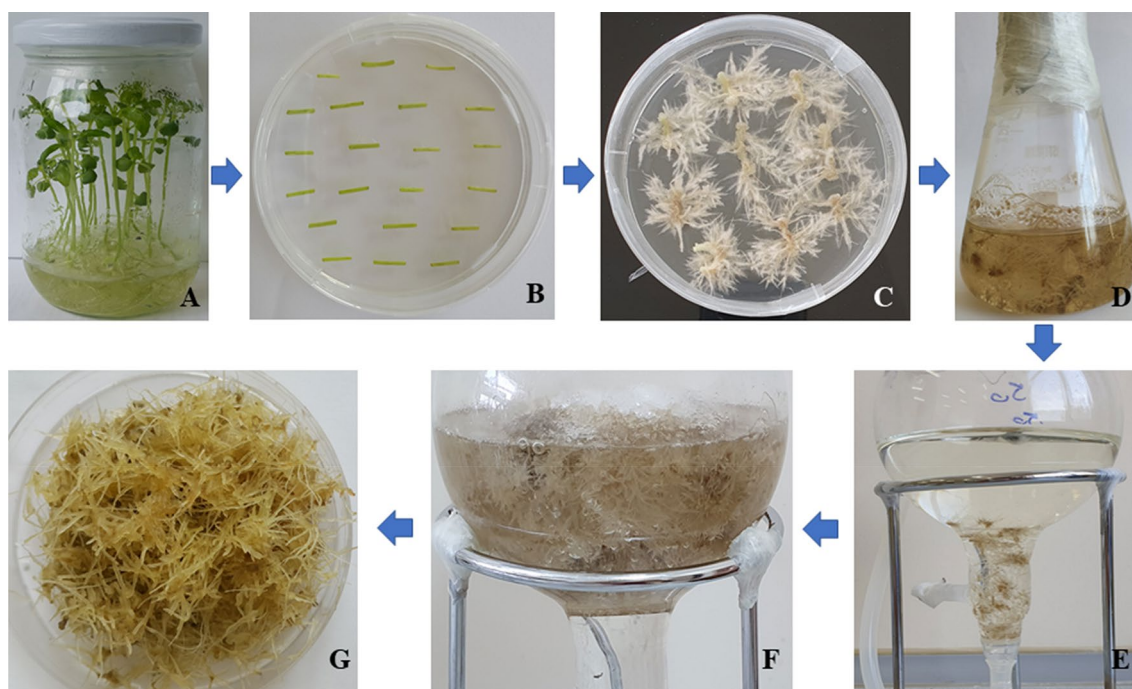


Fig. 1 Schematic representation of adventitious root culture stages of *Ocimum basilicum* in balloon-type bubble bioreactors. In vitro cultured plants for explant sources (A). Hypocotyl explants (B). Adventitious root induction from hypocotyl explants in petri-dish culture (C). Proliferation of adventitious roots in Erlenmeyer flasks (D). Cultiva-

tion of adventitious roots in bioreactors containing different concentrations of IBA and MS medium (at the beginning of culture) (E). Proliferation of adventitious roots in bioreactor for 30 days (F). Harvested adventitious roots (G)

activities (DPPH, ABTS and FRAP), 0.20 g powder of dried adventitious root was extracted in 10 mL of a methanol/dichloromethane solution (4:1). The mixture was vortexed and then filtered using a syringe filter (0.22 μm pore size). The extracts were kept in the dark at 4 $^{\circ}\text{C}$ until further analyses.

Rosmarinic acid analysis by UHPLC-HESI-MS/MS

Rosmarinic acid contents of adventitious roots were determined according to the method described by previous studies with some modifications (Kračun-Kolarević et al. 2015; Katanić Stanković et al. 2020). Quantification and identification of rosmarinic acid in adventitious root extracts were performed by using Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Germany) coupled with TSQ Quantum Access Max (triple-quadrupole mass spectrometer, Thermo Fisher Scientific). Elution was executed at 30 $^{\circ}\text{C}$ on Hypersil ODS column (250 \times 4.6 mm) with 5 μm particle size (Thermo Fisher Scientific, USA). The mobile phase consisted of 0.1% formic acid in water (A) and methanol (B), which were applied in the following gradient program: 0–1.00 min 100% A, 1.01–25.00 min 5% A and 95% B and 25.01–34.00 min 100% B. The injection volume and flow rate were set as 20 μL and 0.7 mL min^{-1} , respectively. The

mass spectrometry data was acquired in negative ionization mode on a TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with heated electrospray ionization (HESI) source. The operating conditions of the mass spectrometer were set as follows: capillary temperature of 300 $^{\circ}\text{C}$, vaporizer temperature of 350 $^{\circ}\text{C}$, sheet gas pressure of 30 Arb, aux gas pressure of 13 Arb, discharge current of 4.0 μA and spray voltage of 2500 V (negative polarity). The collision energy was set to 44 eV and 20 eV, and the collision gas was used argon. Rosmarinic acid was identified by direct comparison with collision-induced fragmentation patterns of standard compound and retention time. The total amount of rosmarinic acid was evaluated using the calibration graph of standard compound.

Content of total phenolic compound

The content of total phenolic compound of adventitious roots was determined spectrophotometrically using Folin–Ciocalteus reagent. Briefly, 4.5 mL of the distilled water and 100 μL of Folin–Ciocalteus reagent (2 N) were added to 100 μL of the adventitious root extracts, respectively. After 5 min, 300 μL of Na_2CO_3 (2% w/v) was added to reaction mixture and incubated under room conditions for 2 h. The absorbance of the reaction mixture was recorded at 760 nm using

a UV–Vis spectrophotometer. The content of compounds of the roots were determined using the calibration graph prepared from gallic acid (Slinkard and Singleton 1977).

Flavonoid content

The flavonoid content of adventitious roots was analyzed by the method of Pekal ve Pyrzyńska (2014) with a slight modification. Briefly, 1.5 mL of ethanol, 100 μL of AlCl_3 (10% w/v), 100 μL of NaCH_3COO (1 M) and 3.1 mL of distilled water were pipetted onto 200 μL of the adventitious root extracts, respectively. Then, the reaction mixtures were strongly shaken and incubated at room conditions for 30 min, their absorbance was measured with a spectrophotometer at 427 nm. The flavonoid contents of the adventitious roots were determined using the calibration graph prepared from quercetin.

DPPH radical scavenging activity

The determination of free radical scavenging activity (DPPH \bullet) of adventitious roots was carried out according to the procedure described by Blois (1958). Briefly, the final volumes of the tubes containing different concentrations of extract (50–400 $\mu\text{g mL}^{-1}$) were filled to 3 mL with ethanol. Afterwards, 1 mL of DPPH \bullet solutions (0.26 mM) prepared in ethanol were added to the tubes and were vortexed vigorously. After 30 min incubation in the dark, the absorbance of the reaction mixture was measured in a spectrophotometer at 517 nm. The activity results were expressed as the IC_{50} value, which was concentration of the adventitious roots that scavenged 50% of the DPPH \bullet radical. IC_{50} values were calculated using the graph equations of sample concentration versus activity (%). The activity of adventitious roots was determined using the equation given below.

$$\text{Activity \%} = [(\text{control absorbance} - \text{extract absorbance}) / \text{control absorbance}] \times 100.$$

ABTS cation radical scavenging activity

The determination of ABTS $^+$ (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) cation radical scavenging capacity of adventitious roots was carried out according to the procedure described by Re et al. (1999). Briefly, the final volumes of the tubes containing different concentrations of extracts (50–200 $\mu\text{g mL}^{-1}$) were completed to 3 mL with 0.1 M phosphate buffer (pH 7.4). Then, 1 mL of ABTS- $\text{K}_2\text{S}_2\text{O}_8$ solution, which was prepared by mixing 2 mM ABTS solution and 2.45 mM $\text{K}_2\text{S}_2\text{O}_8$ solution at a ratio of 1:2 and incubating for six hours in dark, was added to these tubes. After 30 min incubation in the dark, the absorbance of the reaction mixture was measured in a spectrophotometer at 734 nm. The activity results were expressed as the IC_{50}

value, which was concentration of the adventitious roots that scavenged 50% of the ABTS cation. IC_{50} values were calculated using the graph equations of sample concentration versus activity (%). The activity of adventitious roots was determined using the equation given below.

$$\text{Activity \%} = [(\text{control absorbance} - \text{extract absorbance}) / \text{control absorbance}] \times 100.$$

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP)

The reducing power activities of the adventitious roots were determined spectrophotometrically according to the procedure described by Oyaizu (1986). Briefly, different amounts of extracts (100–400 $\mu\text{g mL}^{-1}$) were taken, and their final volumes was completed to 1.25 mL with 0.2 M phosphate buffer (pH 6.6). Afterwards, 1.25 mL of $\text{K}_3\text{Fe}(\text{CN})_6$ (1%) solutions were added to this mixture and incubated for 20 min at 50 $^\circ\text{C}$. At the end of the incubation period, 1.25 mL of TCA (10%) and 0.25 mL of FeCl_3 (0.1%) were added to the mixture, and their absorbance at 700 nm was measured by a spectrophotometer. The results of the reducing power were determined using the equation of the standard graph prepared from trolox standard antioxidant compound ($\mu\text{mol TE g}^{-1}$).

Determination of antioxidant enzyme activities

For the determination of antioxidant enzyme activities (CAT, POD and SOD), 0.25 g of adventitious roots was homogenized in 2 mL of 50 mM KH_2PO_4 (pH 7.0) buffer containing 1% polyvinyl pyrrolidone (w/v) and 0.1 mM EDTA. The supernatant used as the enzyme source was obtained by centrifuging the homogenate at 12,000 g at 4 $^\circ\text{C}$ for 15 min.

For the measurement of CAT activity, 3 mL of reaction mixture was prepared consisting of 50 mM KH_2PO_4 (pH=7) buffer, 15 mM H_2O_2 and 3 μL enzyme extract. The decrease in absorbance of H_2O_2 at 240 nm was measured in the spectrophotometer for two min. One unit of CAT activity (EU) was expressed as the amount of enzyme that provides the decomposition of 1 $\mu\text{mol H}_2\text{O}_2$ per minute and was calculated using the extinction coefficient (0.036 $\text{cm}^2 \mu\text{mol}^{-1}$) of H_2O_2 (Havir and Mchale 1987).

To determine the activity of POD, 3 ml of reaction mixture was prepared consisting of 50 mM KH_2PO_4 (pH=6.5) buffer, 15 mM H_2O_2 , 10 mM guaiacol and 5 μL enzyme extract. Then, the increase in the absorbance of tetra guaiacol was measured in a spectrophotometer at 470 nm for two min. One unit of enzyme (EU) was expressed as the amount of enzyme catalyzing the oxidation of 1 μmol guaiacol per minute and was calculated using the extinction coefficient (26.6 $\text{mM}^{-1} \text{cm}^{-1}$) of tetra guaiacol (Angelini et al. 1990).

SOD activity was determined using the procedure described by Beyer and Fridovich (1987). The assay medium comprised of 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 60 mM nitroblue tetrazolium (NBT) and 100 μ L enzyme extract in a final volume of 3 mL. Then, 60 mM riboflavin was added to these mediums and the reaction mixtures were illuminated under two cool white fluorescent lamps (60 W) for 30 min. The reaction mixture without enzyme was used as the control. The reaction was stopped by turning off the light and its absorbance was measured in a spectrophotometer at 560 nm. One unit of SOD (EU) activity was expressed as the amount of enzyme that inhibited 50% of NBT photoreduction.

Proline content

The proline content of adventitious roots was measured spectrophotometrically using the method of Bates et al. (1973). Adventitious roots (0.2 g) were extracted in 4 mL of 3% sulfosalicylic acid and then centrifuged at 10,000 g for 10 min. One milliliter of supernatant was mixed with 1 mL of ninhydrin reagent and 1 mL of glacial acetic acid (96%), and reaction mixtures were incubated at 100 °C for one hour. Then, the reaction was stopped by placing the test tubes in an ice bath and 2 mL of toluene was added into mixture. The absorbance of toluene phase was recorded at 520 nm and the content of proline was determined with a standard graph.

Malondialdehyde (MDA) content

To determine the MDA content, adventitious roots (0.4 g) were extracted in 4 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution and then centrifuged at 10,000 g for 20 min. The reaction mixture was prepared from 0.5 mL of the supernatant and 1 mL of TBA (0.5%) in 20% TCA. The sample tubes were placed in boiling water for 30 min and then cooled in an ice bath. After this stage, the samples were rapidly cooled in an ice bath and centrifuged at 10,000 g for 10 min. The absorbance of the mixtures was recorded at 532 nm, and then the absorbance measured at 600 nm was subtracted from this absorbance value to correct for nonspecific turbidity. The amount of MDA was determined utilizing the extinction coefficient of 155 $\text{mM}^{-1} \text{cm}^{-1}$ (Velikova et al. 2000).

H₂O₂ content

To evaluate the H₂O₂ content, adventitious roots (0.25 g) were extracted in 2.5 mL of 0.1% (w/v) TCA solution in ice bath and then centrifuged at 12,000 g for 20 min. Briefly, 0.5 mL of 10 mM KH₂PO₄ buffer (pH 7.0) and 1 mL of 1 M KI were pipetted onto 0.5 mL of the supernatant, respectively. The absorbances of the mixtures at 390 nm were

read and the amount of H₂O₂ was calculated using standard graphics (Velikova et al. 2000).

Determination of phenylalanine ammonia lyase (PAL) activity and protein content

The PAL activities of adventitious root were determined by the method of Beaudoin–Eagan and Thorpe (1985) with some modification. Adventitious roots (0.5 g) were homogenized in a mortar at 4 °C with 5 mL of 50 mM Tris–HCl buffer (pH 8.5) containing 2% polyvinyl pyrrolidone(w/v), 15 mM 2-mercaptoethanol and 0.2 mM EDTA. The supernatant used as the enzyme source was obtained by centrifuging the homogenate at 12,000 g for 15 min at 4 °C. The reaction mixture was prepared from 0.5 mL of 10 mM L-phenylalanine, 1 mL of 50 mM Tris- HCl buffer (pH 8.5), 0.4 mL of distilled water and 0.1 mL of enzyme extract and incubated at 37 °C for 60 min. The reaction was terminated by the addition of 50 μ L of 5 N HCl, and then the absorbance of the samples at 290 nm was measured to determine the content of trans-cinnamic. The activities were expressed as nmoles of trans cinnamic acid formed in $\text{mg}^{-1} \text{protein h}^{-1}$. The protein content in the adventitious roots was determined as described by Bradford (1976) method utilizing bovine serum albumin as the standard.

Statistical analysis

All experiments in this study were performed in triplicates, and statistical analyzes of the data were performed using the SPSS 20 (Armonk, NY: IBM Corp.) program. The differences between the values for experimental groups were determined by one-way ANOVA according to Duncan's multiple range test (Duncan, 1955). The results were expressed as the mean \pm standard error (SE). The correlation analysis was conducted by bivariate (Pearson's) correlation test at $p < 0.05$ significance level.

Results and discussion

The effects of MS medium salt strength on biomass, secondary metabolite accumulation and PAL activity in *O. basilicum* adventitious roots

The optimum concentration of medium salt strength has a critical impact on the growth of the roots and the production of bioactive compounds in plant cell and organ cultures. To determine the optimum MS medium salt strength for production of rosmarinic acid and biomass, adventitious roots of sweet basil were cultured for 30 days in a 1 L bioreactor at different salt strengths of MS medium (0.50 MS, 0.75 MS, 1 MS and 1.5 MS). The fresh weight (FW), dry weight (DW)

and growth ratio (GR) in adventitious roots of *O. basilicum* were significantly affected by MS medium salt strength. Among the applied MS medium salt strengths, 0.75 MS was found to be appropriate medium for biomass accumulation (Table 1). Notably, the highest FW ($87.0 \pm 5.56 \text{ g L}^{-1}$), DW ($4.93 \pm 0.32 \text{ g L}^{-1}$), and GR (11.42 ± 0.79) of *O. basilicum* adventitious root cultures were obtained at 0.75 MS medium. FW, DW and GR of adventitious roots increased up to 0.75 MS salt strength, then decreased with increasing salt strength. The effect of MS salt strength on adventitious root growth may differ according to the plant species. The results obtained from present study are consistent with those of Wu et al. (2018) in which 0.75 MS medium provided the maximum FW, DW and GR in bioreactor cultures of *Echinacea pallida* and *E. purpurea* adventitious roots. In addition, in previous studies, it was determined that different MS strengths such as 0.25 MS (Cui et al. 2013), 0.5 MS (Baque et al. 2013), and 1 MS (Yin et al. 2013) were the most suitable concentrations for the growth of adventitious roots. On the other hand, the root growth was inhibited in medium stronger than 1 MS and, this may be due to high osmotic stress (Cui et al. 2013; Yin et al. 2013). Ho et al. (2021) stated that concentrations exceeding 1 MS strengths may reduce the water potential, which inhibited the intake of water and mineral substances from medium, thereby inhibiting root growth. In our study, root growth was significantly reduced at 1.5 MS strength, in accordance with previous studies.

The rosmarinic acid content of the adventitious roots was analyzed using UHPLC-HESI-MS/MS (liquid-chromatography tandem mass spectrometry). The identification and quantification of this compound was evaluated by comparing the retention time and MS spectra with commercial standard compound. Rosmarinic acid produced a $[M-H]^-$ ion at m/z 359.18 as a major parent ion, and its fragment ions were at m/z 134.30 and 162.20. In addition, the retention time for this compound was determined as 17.82 min. The values obtained from present study are consistent with those of Kračun–Kolarević et al. (2015) in which parent ion was found as a $[M-H]^-$ ion at m/z 359.061.

MS medium salt strength significantly affected the accumulation of rosmarinic acid, phenolics, and flavonoids, and the results were similar to adventitious root biomass findings. The contents of rosmarinic acid and phenolics at low MS salt strength (0.5 and 0.75 MS) was higher than at high MS salt strength (1 and 1.5 MS). The maximum accumulation of rosmarinic acid, phenolics and flavonoids were achieved at 0.75 MS salt strength as $18.51 \pm 1.43 \text{ mg g}^{-1}$ DW, $19.47 \pm 1.18 \text{ mg g}^{-1}$ DW and $2.41 \pm 0.06 \text{ mg g}^{-1}$ DW respectively. The content of rosmarinic acid in the adventitious roots at the 0.75 MS medium was 1.94, 2.66 and 5.99 times higher than 0.5, 1 and 1.5 MS medium, respectively. As shown in Table 1, 0.75 MS salt strength was determined to be the most suitable medium not only for root growth but also for rosmarinic acid production. Similar results were also observed by Wu et al. (2018) who stated that the maximum phenolic and flavonoid content was obtained at 0.75 MS salt strength. However, some previous studies reported that 0.5 MS medium was appropriate concentration for phenolic and flavonoid accumulation in adventitious root cultures of *Echinacea angustifolia* (Wu et al. 2006), *Hypericum perforatum* L. (Cui et al. 2010), and *Morinda citrifolia* (Baque et al. 2013). In hairy root cultures of *Dracocephalum moldavica*, the rosmarinic acid content at 0.5 MS salt strength was found by Weremczuk–Jeżyna et al. (2013) to be higher than 1 MS. Considering all these studies, the optimum MS salt strength for secondary metabolite production varies depending on the target compound, culture type and plant species.

PAL catalyzes the first step of the phenylpropanoid pathway, one of the most important secondary metabolic pathways of plants, in which a wide variety of phenolic compounds with different structural and defense-related functions are produced (Ejtahed et al. 2015). The phenylpropanoid pathway is triggered in various stress situations resulting in productions of different phenolic compounds which support the plant to overcome environmental constraints. Phenolic compounds also play a critical role in the developmental stages of plants such as nutrient mineralization, photosynthetic activity, cell division,

Table 1 Effects of MS medium salt strength on biomass, secondary metabolite accumulation and PAL activity in bioreactor cultures of sweet basil adventitious roots

MS salt strength	Fresh weight (g L ⁻¹)	Dry weight (g L ⁻¹)	Growth ratio	Rosmarinic acid (mg g ⁻¹ DW)	Total phenolics (mg g ⁻¹ DW)	Flavonoids (mg g ⁻¹ DW)	PAL activity nmol h ⁻¹ mg ⁻¹ protein
0.50	69.2 ± 6.04 b*	3.73 ± 0.24 b	8.88 ± 0.86 b	9.5 ± 0.40 b	12.45 ± 0.29 b	1.69 ± 0.11 b	11.80 ± 1.22 b
0.75	87.0 ± 5.56 a	4.93 ± 0.32 a	11.42 ± 0.79 a	18.51 ± 1.43 a	19.47 ± 1.18 a	2.41 ± 0.06 a	18.92 ± 1.32 a
1.0	53.1 ± 3.51 b	3.19 ± 0.23 b	6.57 ± 0.50 b	6.94 ± 1.19 b	10.06 ± 0.65 bc	1.75 ± 0.04 b	6.67 ± 0.83 c
1.5	31.0 ± 2.45 c	1.86 ± 0.16 c	3.48 ± 0.35 c	3.09 ± 0.61 c	7.63 ± 1.62 c	1.56 ± 0.03 b	4.17 ± 0.65 c

*The different letters within the same column show significant differences according to Duncan's multiple range test ($p < 0.05$). The data are expressed as the mean ± standard error (n = 3)

hormonal regulation, and reproduction (Sharma et al. 2019). As shown in Table 1, the PAL enzyme activity of sweet basil adventitious roots was significantly affected by MS salt strength. The activity increased with the elevation at MS salt strength up to 0.75 MS salt strength, decreased thereafter. The activity of adventitious roots at 0.75 MS medium, where the highest PAL enzyme activity was observed, was 1.60, 2.83 and 4.53 times higher than 0.5 MS, 1 MS and 1.5 MS, respectively. In addition, the stimulation of PAL enzyme activity by MS salt strength showed strong positive linear relationship with accumulation of rosmarinic acid ($R^2=0.972$), phenolics ($R^2=0.979$) and flavonoids ($R^2=0.820$) (Fig. S2). Similarly, Baque et al. (2010a) stated that there was a strong and positive correlation between PAL activity and phenolic compound accumulation in *Morinda citrifolia* adventitious root culture applied at different MS salt strengths.

The tissue culture of *Ocimum basilicum* is a very promising approach to produce valuable metabolites. However, for mass propagation of basil cell and organ cultures, various factors must be considered such as genotype, cost, and protocol development (Jakovljević et al. 2022). Moreover, the optimization of the culture medium is a critical determinant for stable commercial production of plant secondary metabolite in adventitious root cultures (Cui et al. 2013). In accordance with this, in our study, the optimum MS medium salt strength (0.75 MS provided an increase in rosmarinic acid content up to 5.99-fold compared to other applied MS medium salt strengths. Nevertheless, the effects of essential medium components on metabolite production have not been extensively studied in in vitro cultures of basil. On the other hand, in recent years, studies on in vitro cultures of basil cultivars have focused on the evaluation of the effects of various elicitors on the biosynthesis of valuable compounds, including rosmarinic acid. Most of these studies were performed in the callus and suspension culture. In this context, rosmarinic acid contents of callus cultures obtained from different cultivars of *Ocimum basilicum* increased with the application of suitable levels (or forms) of melatonin (Duran et al. 2019), copper oxide nanoparticles (Nazir et al. 2021a), different spectral lights (Nazir et al. 2020a), yeast extract (Zaman et al. 2022), different LEDs (light emitting diodes) (Nadeem et al. 2019), light regime (with salicylic acid) (Nazir et al. 2021b), and UV-C radiation (Nazir et al. 2020b). As for the cell suspension cultures of basil cultivars, previous studies have demonstrated that rosmarinic acid content increased with the appropriate levels of elicitors such as methyl jasmonate (Pandey et al. 2019), sorbitol (Açıkğöz 2021), yeast extract, cadmium chloride and silver nitrate (Açıkğöz 2020). Overall, elicitor application is an effective strategy to obtain high amounts of rosmarinic acid in in vitro cultures of *Ocimum basilicum*, and the effectiveness of these treatments may be greater when the studies are combined

with optimum medium conditions, including MS medium salt strength.

The effects of MS medium salt strength on the activities of CAT, POD and SOD enzyme, and contents of MDA, H₂O₂ and proline in *O. basilicum* adventitious roots

Medium salt strength remarkably influenced the activities of SOD, CAT and POD antioxidant enzymes (Fig. 2). In general, a gradual increase in the activities of these enzymes was detected as the salt strength increased from 0.5 to 1.5 MS. Only, the POD activity of adventitious roots at 0.5 MS medium was higher than at 0.75 MS, but the difference between them was not statistically significant. The activities of enzymes at high MS strengths (1 and 1.5 MS) were significantly higher than those at low MS strengths (0.5 and 0.75 MS). The highest activities for three enzymes were determined in adventitious roots cultured at 1.5 MS.

Reactive oxygen species (ROS) production increases as a result of exposure of plants to a wide variety of stresses such as mineral deficiency, drought, salinity, temperature extremes. In case of overproduction of ROS, the balance between the antioxidant defense system and ROS levels is disrupted and oxidative damage occurs (Kusvuran et al. 2016). ROS derivatives such as free radicals (superoxide anion, hydroperoxyl radical, alkoxy radical and hydroxyl radical) and nonradical molecules (hydrogen peroxide and singlet oxygen) can interact with cellular macromolecules including DNA, proteins, lipids, and pigments. The scavenging and detoxification of overproduced ROS is provided by an effective antioxidant defense system consisting of enzymatic (ascorbate peroxidase, catalase, peroxidase, superoxide dismutase, glutathione reductase) and non-enzymatic (ascorbic acid, phenolics, flavonoids, α -tocopherol, carotenoids, glutathione and proline) antioxidants (Ozturk et al. 2012; Sen and Alikamanoglu 2013; Hasanuzzaman et al. 2020). Especially, antioxidant enzymes such as POD, SOD and CAT are effective protection mechanism against oxidative stress, and the activities of these enzymes markedly increase under stress conditions (Karataş et al. 2014). In our study, it was determined that the activities of antioxidant enzymes enhanced in parallel with the increase in the amount of H₂O₂ and MDA, particularly at high MS strengths (1 and 1.5 MS). These increases are thought to be due to osmotic stress that may occur in adventitious root culture at high MS salt strength (≥ 1 MS). Similarly, Cui et al. (2013) stated that osmotic stress was provoked in adventitious root culture at high MS salt strength (≥ 1 MS). Furthermore, Lee et al. (2014) reported that the water potential reduced essentially with increasing MS medium strength and this potential of the medium decreased sharply at higher than 1 MS. Similar results were also observed by Baque et al.

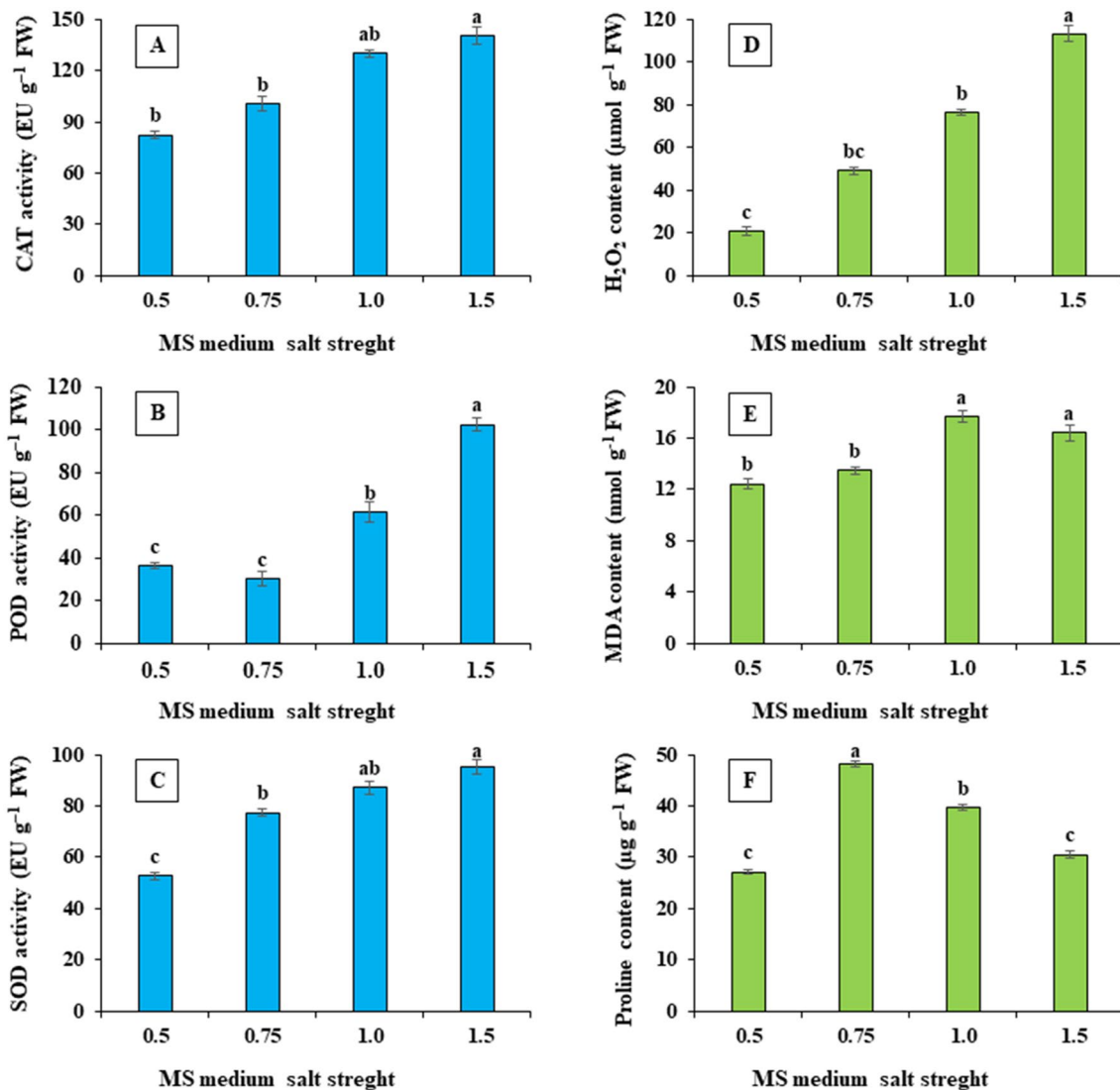


Fig. 2 The effects of MS medium salt strength on the activities of CAT (A), POD (B) and SOD (C) enzymes and contents of H₂O₂ (D), MDA (E) and proline (F) in bioreactors cultures of sweet basil aden-

titious roots. The different letters in each graph show significant differences according to Duncan's multiple range test ($p < 0.05$). Bars represent means \pm standard error ($n = 3$)

(2010a) who determined that osmotic stress was triggered at higher salt strength (1.5 and 2 MS), and CAT, G-POD and SOD activities of adventitious roots elevated especially at 2 MS medium.

The level of MDA, the main final product of membrane lipid peroxidation, is frequently used as an indicator of oxidative stress. One of the other important parameters used to evaluate the stress levels of plants is the accumulation of H₂O₂, a type of reactive oxygen species (ROS) (Ozturk et al. 2012). In sweet basil adventitious roots, the content of MDA and H₂O₂ were significantly affected by MS salt strength (Fig. 2). The quantities of MDA and H₂O₂ gradually increased with increasing MS salt strength. Thus, the highest amounts of H₂O₂ and MDA were observed in

adventitious roots cultured at high MS concentrations (1 and 1.5 MS), while the lowest amounts were detected at lower MS salt strengths (0.5 and 0.75 MS). Consistent with these results, Baque et al. (2010a) observed that the content of H₂O₂ and MDA in the adventitious roots increased progressively with elevating MS medium strength. Likewise, in another study, a sharp increase in MDA and H₂O₂ levels was also reported for the roots of chickpea under osmotic stress, heat stress, and their combination. However, a pre-treatment (coronatine, a jasmonate analog) that increased the activities of H₂O₂-scavenging enzymes such as APX, CAT, POX protected the roots against lipid peroxidation (Ceylan et al. 2013). Moreover, it has been reported that there was a negative correlation between SOD, POX and CAT activities

and MDA content in in vitro propagated beet shoots treated with PEG 6000 (polyethylene glycol) but increase in antioxidant enzymes were insufficient to prevent lipid peroxidation (Sen and Alikamanoglu 2013). Similarly, in our study, the increase in amount of MDA and H_2O_2 at a high MS salt strength (1 and 1.5 MS) indicated that the antioxidant enzymes were not sufficient to alleviate the deleterious effects of oxidative stress in adventitious roots.

Proline, a useful parameter to evaluate the stress tolerance capacity, accumulates in many plants in response to the imposition of various abiotic and biotic stresses. Against the adverse effects of stress, the main functions of proline include the protection of proteins and membranes, osmotic adjustment, stabilize subcellular structures and scavenge free radicals (Ozturk et al. 2012; Karataş et al. 2014). However, proline cannot be considered from some publications as a marker for salt tolerance due to the decrease in its level in many salt-stressed plants unlike the increase in many types of stress such as heat, drought, and starvation (Sairam et al. 2002). As shown in Fig. 2, the accumulation of proline increased up to 0.75 MS salt strength and thereafter decreased with increasing MS salt strength. Contrary to the general approach concerning the increase in proline content under stress conditions, in this study, the proline content was significantly decreased at 1 and 1.5 MS strengths, where oxidative stress parameters were significantly increased. Also, the highest proline content was determined at 0.75 MS medium where the levels of these stress parameters were significantly lower compared to those with high MS strengths (1 and 1.5 MS). Findings from the present study are consistent with those published in a previous study in which the accumulation of proline at low MS strengths such as 0.25 and 0.5 MS was significantly higher than at 1 MS medium in callus culture (Cingöz and Karakaş 2016). On the other hand, another study reported that the amount of proline noticeably increased with elevating MS salt strength in adventitious root culture (Baque et al. 2010a). Considering the findings obtained from this study and previous studies, proline accumulation varies considerably according to the type and severity of stress in addition to plant species.

The effects of IBA concentration on biomass, secondary metabolite accumulation and PAL activity in *O. basilicum* adventitious roots

To determine the optimal IBA concentration for efficient production of rosmarinic acid and biomass, adventitious roots were cultured in media containing different concentrations of IBA (0.5, 1, 2 and 4 mg L⁻¹) for 30 days in a 1 L bioreactor. As shown in Table 2, the concentration of IBA significantly influenced the fresh weight (FW), dry weight (DW) and growth ratio (GR) in adventitious root cultures of *O. basilicum*. FW, DW and GR of adventitious roots increased up to 2 mg L⁻¹ IBA, then decreased with increasing hormone concentrations. The maximum FW (96.50 ± 8.43 g L⁻¹), DW (5.26 ± 0.46 g L⁻¹) and GR (12.78 ± 1.20) were achieved at 2 mg L⁻¹ IBA. On the other hand, considering the decrease in FW, DW and GR at 4 mg L⁻¹ IBA, it was clearly seen that high IBA concentration strongly inhibited the growth of sweet basil adventitious roots. Earlier studies showed that the effect of auxin group hormones on adventitious root growth varies according to the concentration and type of auxin in addition to the plant species. In this context, the results obtained from present study are consistent with those of Jiang et al. (2015) and in which FW and DW of adventitious elevated increasing the IBA concentrations from 2 to 3 mg L⁻¹ but IBA concentrations higher than 3 mg L⁻¹ (4 and 5 mg L⁻¹) showed an inhibitory effect on biomass parameters. In another previous study examining the effects of IBA concentrations on adventitious root growth, the highest FW, DW and growth index were obtained from the culture medium containing 2 mg L⁻¹, consistent with our study (Ho et al. 2021). Similarly, Wu et al. (2006) reported that among the studied IBA concentrations, the highest FW, DW and GR in adventitious roots were obtained from the medium containing 2 mg L⁻¹ IBA. However, in some previous studies, it was reported that high IBA concentrations such as 5 mg L⁻¹ (Baque et al. 2010b) and 7 mg L⁻¹ (Lulu et al. 2015) were appropriate concentrations for the growth of adventitious roots.

Table 2 Effects of IBA concentration on biomass, secondary metabolite accumulation and PAL activity in bioreactor cultures of sweet basil adventitious roots

IBA (mg L ⁻¹)	Fresh weight (g L ⁻¹)	Dry weight (g L ⁻¹)	Growth ratio	Rosmarinic acid (mg g ⁻¹ DW)	Total phenolics (mg g ⁻¹ DW)	Flavonoids (mg g ⁻¹ DW)	PAL activity nmol h ⁻¹ mg ⁻¹ protein
0.5	65.00 ± 5.85 b*	3.86 ± 0.26 b	8.28 ± 0.83 b	14.37 ± 0.72 c	15.31 ± 0.79 b	1.81 ± 0.09 c	9.04 ± 0.57 c
1	74.50 ± 7.28 b	4.48 ± 0.37 ab	9.64 ± 1.04 b	17.62 ± 0.63 b	19.49 ± 0.84 a	2.29 ± 0.10 b	11.44 ± 0.72 b
2	96.50 ± 8.43 a	5.26 ± 0.46 a	12.78 ± 1.20 a	20.98 ± 1.38 a	21.55 ± 1.77 a	2.70 ± 0.06 a	14.57 ± 1.29 a
4	25.08 ± 2.11 c	1.50 ± 0.11 c	2.57 ± 0.30 c	5.21 ± 0.55 d	7.31 ± 0.13 c	2.21 ± 0.11 b	4.34 ± 0.26 d

*The different letters within the same column show significant differences according to Duncan's multiple range test ($p < 0.05$). The data are expressed as the mean ± standard error (n = 3)

IBA concentration significantly affected the production of rosmarinic acid, phenolics and flavonoids in adventitious root cultures of sweet basil, and results for the accumulation of these metabolites showed a similar trend to the results obtained from the biomass parameters. A gradual increase in the accumulation of these metabolites was detected as the IBA concentration increased from 0.5 to 2 mg L⁻¹, and then a sharp decrease in metabolite content was determined. As it can be seen from the Table 2, the maximum accumulation of rosmarinic acid, phenolics and flavonoids were determined in medium containing 2 mg L⁻¹ IBA as 20.98 ± 1.38 mg g⁻¹ DW, 21.55 ± 1.77 mg g⁻¹ DW and 2.70 ± 0.06 mg g⁻¹ DW respectively. As for the PAL enzyme activities of sweet basil adventitious roots, the activity varied significantly with the studied IBA concentration. The activity increased with increasing IBA concentration up to 2 mg L⁻¹ IBA, then decreased. The PAL enzyme activity of adventitious roots at 2 mg L⁻¹ IBA, where the highest activity was obtained, was 1.61, 1.27 and 3.35 times higher than 0.5, 1 and 4 mg L⁻¹, respectively. Also, the induction of PAL enzyme activity by IBA concentration showed strong positive linear relationship with accumulation of rosmarinic acid (R²=0.978) and phenolics (R²=0.968) but weakly correlated with the flavonoid content (R²=0.342) (Fig. S2). Findings from current study agree with those declared in previous research in which the highest phenolic and flavonoid content was determined at 2 mg L⁻¹ IBA in adventitious roots of *Echinacea angustifolia*. Also, IBA concentrations higher than 2 mg L⁻¹ IBA markedly suppressed metabolite accumulation (Wu et al. 2006). Similarly, Ho et al. (2021) demonstrated that the highest contents of phenolics and flavonoids in adventitious root culture were obtained in medium supplemented with 2 mg L⁻¹ IBA. However, in previous publications, it was reported that the highest accumulation of phenolic and flavonoid compound in adventitious root cultures occurred at very different IBA concentrations such as 1 mg L⁻¹ (Baque et al. 2010b), 3 mg L⁻¹ (Jiang et al. 2015) and 9 mg L⁻¹ (Lulu et al. 2015).

When it comes to adventitious root culture and in vitro root culture (formed from root explants) of *Ocimum basilicum*, the effects of auxin hormones on production of rosmarinic acid, phenolic and flavonoid have not been comprehensively investigated in previous studies. However, the effects of hormone types and concentrations on these metabolites were evaluated in callus cultures and in vitro propagated plants of basil. In this context, Nazir et al. (2019) reported the effects of some plant growth regulators, including NAA (α -naphthalene acetic acid), on production of phenylpropanoid metabolites in callus culture of purple basil. Among NAA concentrations applied alone (0.1, 1.0, 2.5, 5, 10 and 20 mg L⁻¹), it was observed that the maximum accumulation of rosmarinic acid, total phenolic and flavonoid was achieved at 2.5 mg L⁻¹ NAA. In addition, rosmarinic

acid content of purple basil callus cultures increased up to 2.5 mg L⁻¹ NAA, then decreased with increasing hormone concentrations, except for 20 mg L⁻¹ NAA. Likewise, in the current study, the rosmarinic acid content of sweet basil adventitious roots increased with increasing IBA concentration up to 2 mg L⁻¹ IBA, then decreased. In a previous study on callus culture of Thai basil, the effects of different concentrations of 6-benzylaminopurine (BAP), gibberellic acid (GA₃) alone or along with 1 mg L⁻¹ NAA on phenolic compounds were studied. As a result of this study, it was found that the highest accumulation of rosmarinic acid and phenolics was obtained under optimal callus culture conditions consisting of a combination of BAP: NAA (5 mg L⁻¹:1 mg L⁻¹) (Nazir et al. 2020c). Similarly, in current study, the optimum IBA concentration (2 mg L⁻¹ IBA) increased rosmarinic acid content up to 4.02-fold compared to other applied concentrations. Also, the effects of 6-benzyladenine (BA) on the rosmarinic acid content of three different cultivars of *O. basilicum* grown in vitro conditions were evaluated in another study. It was reported that BA application decreased the content of rosmarinic acid in purple-leaf cultivar (Dark Opal) but increased in the green-leaf cultivars (Genovese and Superbo) (Kiferle et al. 2011). Moreover, in an earlier study on *Ocimum basilicum*, the root parts of axenic plants grown in in vitro were cultured in MS liquid medium including 1 mg L⁻¹ NAA. As a result of this study, Tada et al. (1996) stated that the main phenolic compound of these root cultures was rosmarinic acid, and its amount increased continuously during the culture period. Overall, production of rosmarinic acid in basil in vitro cultures is affected by the type, concentration and combination of the hormone used. Another important point in terms of rosmarinic acid production is that different cultivars of the basil are affected differently by hormones applied. Consequently, the optimum IBA concentration is a critical determinant to produce rosmarinic acid in the adventitious root culture of sweet basil under bioreactor conditions.

The effects of IBA concentration on the activities of CAT, POD and SOD enzyme and contents of MDA and proline in *O. basilicum* adventitious roots

The activities of SOD, CAT and POD enzyme were significantly affected by the concentration of IBA in adventitious root culture of sweet basil. As the IBA concentration increased from 0.5 to 2 mg L⁻¹ IBA, a gradual decrease in the activities of these enzymes was determined (Fig. 3). The lowest activities for the SOD, CAT and POD enzymes were detected in adventitious roots cultures supplemented with 2 mg L⁻¹ of IBA. On the other hand, the H₂O₂ content limitedly increased with the increase of IBA concentration. This increase in H₂O₂ content is probably due to a decrease in antioxidant enzyme activities. Also, there was no

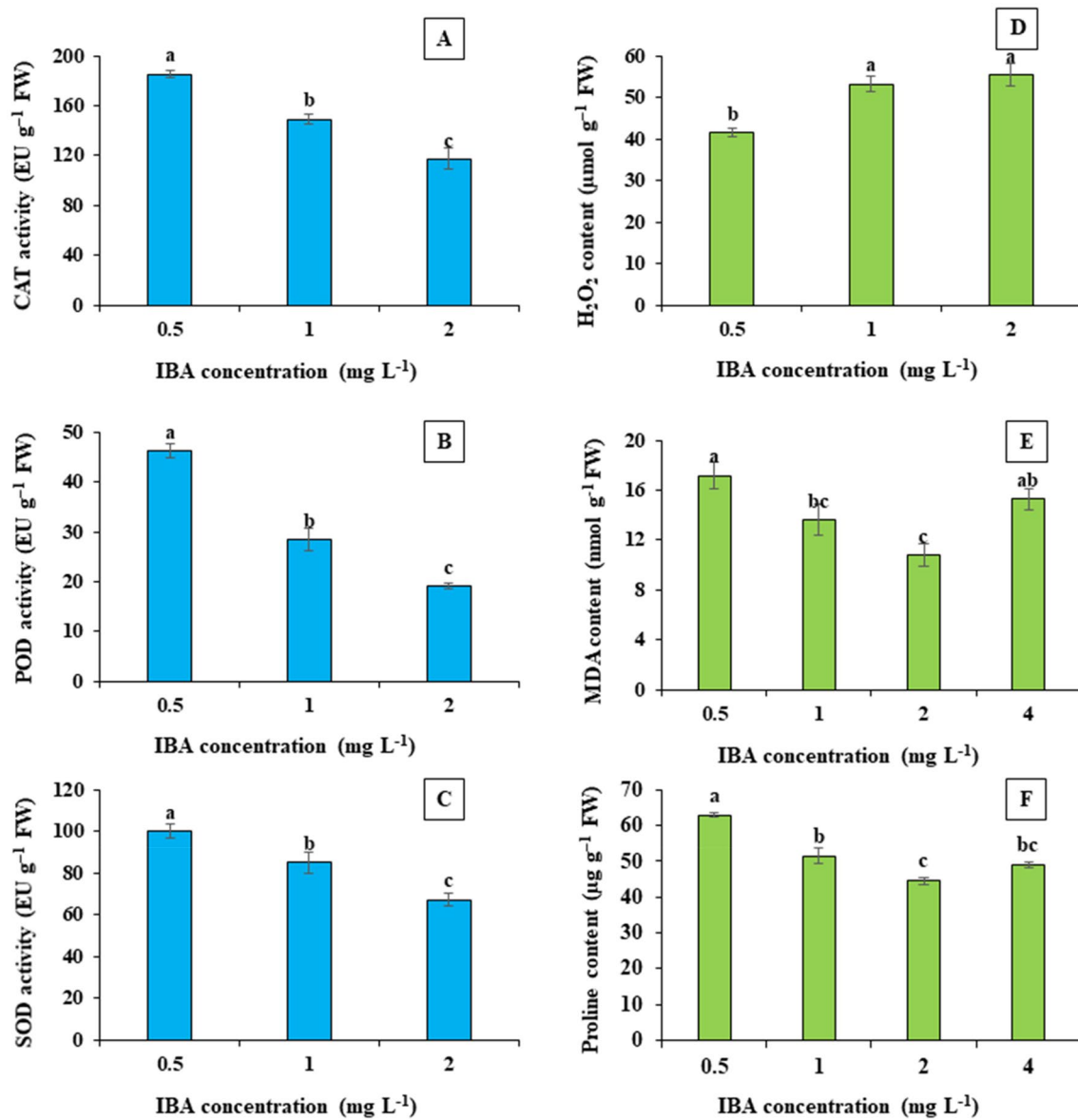


Fig. 3 The effects of IBA concentration on the activities of CAT (A), POD (B) and SOD (C) enzymes and contents of H₂O₂ (D), MDA (E) and proline (F) in bioreactors cultures of sweet basil adventitious

roots. The different letters in each graph show significant differences according to Duncan's multiple range test ($p < 0.05$). Bars represent means \pm standard error ($n = 3$)

significant difference between 1 and 2 mg L⁻¹ IBA concentrations in terms of H₂O₂ accumulation. Similarly, Šípošová et al. (2021) expressed that exogenous application of IBA to maize plant affected antioxidant enzyme activities such as SOD, CAT and APX depending on concentration. Also, IBA at low concentration (10⁻¹¹ M) did not affect H₂O₂ contents or antioxidant enzymes activities, while the higher IBA concentration (10⁻⁷ M) only increased the H₂O₂ contents. In another study, it was reported by Li et al. (2009) that CAT, an H₂O₂-catalyzing enzyme, was inactivated by H₂O₂ during adventitious root development of mung bean seedlings. Also, H₂O₂ treatments of seedlings significantly reduced

POD activity. On the other hand, in the same study, POD activity in IBA-treated seedlings significantly decreased at the beginning of the incubation period (3 h and 12 h) compared to the control group but increased significantly at 36 h of incubation. In our study, due to insufficient biomass in adventitious root cultures at 4 mg L⁻¹ IBA, the activities of antioxidant enzymes and H₂O₂ content could not be determined. As for the MDA and proline content in adventitious root cultures of sweet basil, the results agree with those from the antioxidant enzyme activities. The amount of MDA and proline significantly decreased with increasing IBA concentration up to 2 mg L⁻¹ IBA, then increased with elevating

IBA concentration (Fig. 3). The results obtained from present study are consistent with those of Sarropoulou et al. (2013) in which proline content decreased with increasing IBA concentrations from 1 to 2 mg dm⁻³. As with antioxidant enzymes, the lowest amount of MDA and proline was determined in adventitious root culture including 2 mg L⁻¹ of IBA. Also, the findings obtained from the stress parameter are compatible with the findings obtained from biomass (Table 2). The highest biomass parameters were achieved at 2 mg L⁻¹ IBA concentration, where activities of CAT, POD, and SOD enzyme and contents of MDA and proline were observed as the lowest.

The effects of MS medium salt strength and IBA concentration on the activities of DPPH, ABTS and FRAP in *O. basilicum* adventitious roots

Antioxidant capacities of sweet basil adventitious roots were evaluated using DPPH, ABTS and FRAP methods. The activity results of DPPH and ABTS were expressed as the IC₅₀ value, while FRAP results were calculated as equivalent to trolox, a standard antioxidant compound. The activities of DPPH, ABTS and FRAP in adventitious roots of *O. basilicum* were significantly affected by IBA concentration and MS salt strength. The antioxidant activities for these three methods increased gradually as the IBA concentration enhanced from 0.5 to 2 mg L⁻¹, and then a sharp decrease in antioxidant activities was determined. As shown in Fig. 4, the highest DPPH, ABTS and FRAP activities in adventitious root cultures were determined at 2 mg L⁻¹ IBA. However, there was no statistically significant difference between 1 mg L⁻¹ IBA and 2 mg L⁻¹ IBA for three antioxidant methods. As for the effects of MS salt strength, activities of DPPH, ABTS and FRAP increased up to 0.75 MS salt strength, then decreased with increasing salt strength. The highest activities for the three methods were observed in adventitious roots grown in medium supplemented with 0.75 MS salt strength. Similarly, Wu et al. (2018) demonstrated that the highest activities of DPPH and reducing power in adventitious root were obtained in 0.75 MS medium. Contrary to the findings from our study, Baque et al. (2010a) stated that DPPH activity increased with increasing medium salt strength in adventitious root culture of *Morinda citrifolia*.

The correlation analysis between secondary metabolite content and antioxidant activity in adventitious roots obtained from different culture media was performed with bivariate (Pearson's) correlation test (Table 3). A very strong positive correlation between FRAP activity and content of rosmarinic acid and total phenolic was found in adventitious roots grown at different MS salt strengths and IBA concentrations. Also, the rosmarinic acid and total phenolic contents of adventitious roots treated with different MS salt

strengths and IBA concentrations were strongly negatively correlated with IC₅₀ values of ABTS and DPPH. The reason for the negative direction of correlation is due to the determination of DPPH and ABTS activity results as IC₅₀. The IC₅₀ values, which was amount of the adventitious roots that scavenged 50% of the initial radical concentration, are inversely related to the antioxidant activity. Thus, as the IC₅₀ values decrease, the antioxidant capacity of adventitious roots increases. In our study, the lowest IC₅₀ values for ABTS and DPPH, that is, the highest antioxidant activity, were determined in medium with high metabolite content. In addition, there was a very strong correlation between flavonoid content and antioxidant activity in adventitious roots obtained from different MS salt strengths, while a weak or moderate correlation was found in those obtained from different IBA concentrations. Findings from the present study are consistent with those published in a previous study in which high correlation was observed between rosmarinic acid and total antioxidant potential in hairy roots of *Ocimum basilicum* (Srivastava et al. (2016). Similarly, Tepe et al. (2007) stated that rosmarinic acid and its derivatives are more probably responsible for most of the antioxidant activity determined in *Salvia* species, and there was a powerful correlation between the content of rosmarinic acid and antioxidant capacities. In a different study, Weremczuk-Jezyna et al. (2013) demonstrated that the activity of DPPH and reducing power in hairy roots of *Dracocephalum moldavica* were well correlated with total phenolic compounds and less with rosmarinic acid. On the other hand, Gülçin et al. (2007) analyzed the antioxidant activity of basil herb with various methods including DPPH and reducing power and reported that it has strong antioxidant activity. Considering this and previous studies, the antioxidant capacity varies significantly according to the culture media, applications to the culture and the antioxidant test method. The reason for the differences in antioxidant activities is probably due to the change in the amount and composition of secondary metabolites depending on the growth conditions of plant cells. Our study showed that sweet basil adventitious roots have high antioxidant activity, and this activity is strongly correlated with accumulation of rosmarinic acid and total phenolic.

Conclusion

Adventitious root cultures under the bioreactor conditions provide an alternative and efficient production of medically important secondary metabolites. In this study, the optimization of MS salt strength and IBA concentration for biomass and bioactive compound accumulation in adventitious root of *Ocimum basilicum* was performed in bioreactor systems. Also, the effects of these media components on antioxidant enzyme activities, some stress parameters and PAL activity

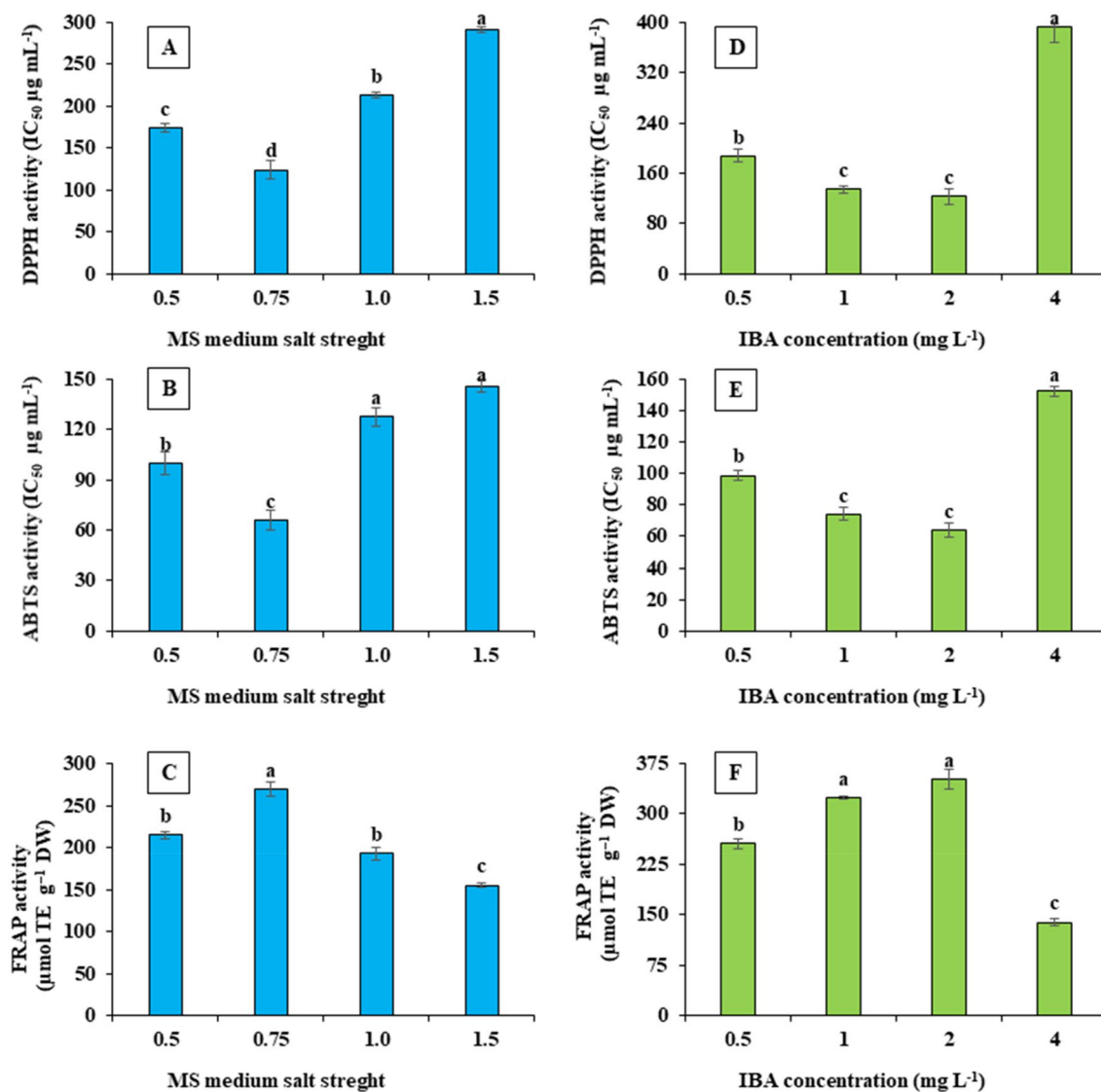


Fig. 4 The effects of MS salt strength and IBA concentration on the activities of DPPH (A and D), ABTS (B and E) and FRAP (C and F) in bioreactors cultures of sweet basil adventitious roots. The different

letters in each graph show significant differences according to Duncan's multiple range test ($p < 0.05$). Bars represent means \pm standard error ($n = 3$)

Table 3 Correlation between secondary metabolite content and antioxidant activity in sweet basil adventitious roots obtained from different media conditions

	MS salt strength			IBA concentrations		
	FRAP	DPPH	ABTS	FRAP	DPPH	ABTS
Rosmarinic acid	0.916**	-0.922**	-0.934**	0.940**	-0.955**	-0.979**
Total phenolic	0.860**	-0.886**	-0.915**	0.986**	-0.965**	-0.975**
Flavonoid	0.852**	-0.796**	-0.818**	0.411	-0.28	-0.423

**Correlation is significant at the 0.01 level (2-tailed)

were evaluated. Overall, in our study, 0.75 MS medium salt strength and 2 mg L⁻¹ IBA concentration in culture medium were found to be optimal conditions for production of rosmarinic acid and biomass. In these optimum conditions, the levels of biomass, secondary metabolite, non-enzymatic

antioxidant activities (DPPH, ABTS and FRAP) and PAL activity were determined to be high, while the levels of antioxidant enzyme activities and stress parameters were found to be low. In this context, the highest accumulations of rosmarinic acid, flavonoid and fresh weigh were achieved

as $20.98 \pm 1.38 \text{ mg g}^{-1} \text{ DW}$, $2.70 \pm 0.06 \text{ mg g}^{-1} \text{ DW}$ and $96.50 \pm 8.43 \text{ g L}^{-1}$ at 2 mg L^{-1} IBA concentration, respectively. As for 0.75 MS medium salt strength, the amounts of rosmarinic acid, flavonoid and fresh weigh were determined as $18.51 \pm 1.43 \text{ mg g}^{-1} \text{ DW}$, $2.41 \pm 0.06 \text{ mg g}^{-1} \text{ DW}$ and $87.0 \pm 5.56 \text{ g L}^{-1}$, respectively. The results of this optimization study can be used for the large-scale production of rosmarinic acid in adventitious root cultures of sweet basil and may also be beneficial for research on the physiology and biochemistry of plants.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11240-022-02347-9>.

Acknowledgements The author would like to thank Hitit University Scientific Technical Application and Research Center (Çorum, Turkey) for the analysis of rosmarinic acid by UHPLC-HESI-MS/MS.

Funding This study was supported by Research Fund of the Tokat Gaziosmanpaşa University (Project Number: 2019/38).

Declarations

Conflict of interest The author declares that there is no conflict of interest.

Ethical approval None.

References

- Açıkgöz MA (2020) Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. *Ind Crops Prod* 148:112278. <https://doi.org/10.1016/j.indcrop.2020.112278>
- Açıkgöz MA (2021) Effects of sorbitol on the production of phenolic compounds and terpenoids in the cell suspension cultures of *Ocimum basilicum* L. *Biologia* 76:395–409. <https://doi.org/10.2478/s11756-020-00581-0>
- Angelini R, Manes F, Federico R (1990) Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. *Planta* 182:89–96
- Bajomo EM, Aing MS, Ford LS, Niemeyer ED (2022) Chemotyping of commercially available basil (*Ocimum basilicum* L.) varieties: cultivar and morphotype influence phenolic acid composition and antioxidant properties. *NFS Journal* 26:1–9. <https://doi.org/10.1016/j.nfs.2022.01.001>
- Baque MA, Hahn EJ, Paek KY (2010a) Growth, secondary metabolite production and antioxidant enzyme response of *Morinda citrifolia* adventitious root as affected by auxin and cytokinin. *Plant Biotechnol Rep* 4:109–116. <https://doi.org/10.1007/s11816-009-0121-8>
- Baque MA, Lee EJ, Paek KY (2010b) Medium salt strength induced changes in growth, physiology and secondary metabolite content in adventitious roots of *Morinda citrifolia*: the role of antioxidant enzymes and phenylalanine ammonia lyase. *Plant Cell Rep* 29:685–694. <https://doi.org/10.1007/s00299-010-0854-4>
- Baque MA, Shiragi MHK, Moh SH, Lee EJ, Paek KY (2013) Production of biomass and bioactive compounds by adventitious root suspension cultures of *Morinda citrifolia* (L.) in a liquid-phase airlift balloon-type bioreactor. *In Vitro Cell Dev Biol-Plant* 49:737–749. <https://doi.org/10.1007/s11627-013-9555-3>
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207
- Bauer N, Kiseljak D, Jelaska S (2009) The effect of yeast extract and methyl jasmonate on rosmarinic acid accumulation in *Coleus blumei* hairy roots. *Biol Plant* 53(4):650–656
- Beaudoin-Eagan LD, Thorpe TA (1985) Tyrosine and phenylalanine ammonia lyase activities during shoot initiation in tobacco callus cultures. *Plant Physiol* 78:438–441
- Beyer WF Jr, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal Biochem* 161:559–566
- Biswas T (2020) Elicitor induced increased rosmarinic acid content of in vitro root cultures of *Ocimum basilicum* L. (Sweet Basil). *Plant Sci Today*. 7(2):157–163
- Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 181:1199–1200
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Ceylan HA, Türkan I, Sekmen AH (2013) Effect of coronatine on antioxidant enzyme response of chickpea roots to combination of PEG-induced osmotic stress and heat stress. *J Plant Growth Regul* 32:72–82. <https://doi.org/10.1007/s00344-012-9277-5>
- Cingöz G, Karakaş FP (2016) The effects of nutrient and macronutrient stress on certain secondary metabolite accumulations and redox regulation in callus cultures of *Bellis perennis* L. *Turk J Biol* 40:1328–1335. <https://doi.org/10.3906/biy-1603-73>
- Cui XH, Chakrabarty D, Lee EJ, Paek KY (2010) Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L. in a bioreactor. *Bioresour Technol* 101:4708–4716
- Cui HY, Baque MA, Lee EJ, Paek KY (2013) Scale-up of adventitious root cultures of *Echinacea angustifolia* in a pilot-scale bioreactor for the production of biomass and caffeic acid derivatives. *Plant Biotechnol Rep* 7:297–308. <https://doi.org/10.1007/s11816-012-0263-y>
- Duncan DB (1955) Multiple range and multiple F-tests. *Biometrics* 11:1–42
- Duran RE, Kilic S, Coskun Y (2019) Melatonin influence on in vitro callus induction and phenolic compound production in sweet basil (*Ocimum basilicum* L.). *In Vitro Cell Dev Biol-Plant* 55:468–475. <https://doi.org/10.1007/s11627-019-10006-6>
- Ejtahed RS, Radjabian T, Tafreshi SAT (2015) Expression analysis of phenylalanine ammonia lyase gene and rosmarinic acid production in *Salvia officinalis* and *Salvia virgata* shoots under salicylic acid elicitation. *Appl Biochem Biotechnol* 176:1846–1858. <https://doi.org/10.1007/s12010-015-1682-3>
- Gülçin I, Elmastaş M, Aboul-Enein HY (2007) Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. *Phytother Res* 21:354–361. <https://doi.org/10.1002/ptr.2069>
- Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V (2020) Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* 9:681. <https://doi.org/10.3390/antiox9080681>
- Havir EA, Mchale NA (1987) Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol* 84:450–455
- Ho TT, Jeong CS, Lee H, Park SY (2019) Effect of explant type and genotype on the accumulation of bioactive compounds in adventitious root cultures of *Polygonum multiflorum*. *Plant Cell Tissue Organ Cult* 137:115–124. <https://doi.org/10.1007/s11240-018-01556-5>

- Ho TT, Le KC, Kim SW, Park SY (2021) Culture condition optimization and FT-IR analysis of *Polygonum multiflorum* Thunb. adventitious root cultures grown in an air-lift bioreactor system. *Plant Cell Tissue Organ Cult* 144:371–381. <https://doi.org/10.1007/s11240-020-01961-9>
- Isah T, Umar S, Mujib A, Sharma MP, Rajasekharan PE, Zafar N, Frukh A (2018) Secondary metabolism of pharmaceuticals in the plant in vitro cultures: strategies, approaches, and limitations to achieving higher yield. *Plant Cell Tissue Organ Cult* 132:239–265. <https://doi.org/10.1007/s11240-017-1332-2>
- Jakovljević D, Stanković M, Warchol M, Skrzypek E (2022) Basil (*Ocimum* L.) cell and organ culture for the secondary metabolites production: a review. *Plant Cell Tissue Organ Cult* 149:61–79. <https://doi.org/10.1007/s11240-022-02286-5>
- Jiang YJ, Piao XC, Liu JS, Jiang J, Lian ZX, Kim MJ, Lian ML (2015) Bioactive compound production by adventitious root culture of *Oplopanax elatus* in balloon-type airlift bioreactor systems and bioactivity property. *Plant Cell Tissue Organ Cult* 123:413–425. <https://doi.org/10.1007/s11240-015-0845-9>
- Jiang J, Bi H, Zhuang Y, Liu S, Liu T, Ma Y (2016) Engineered synthesis of rosmarinic acid in *Escherichia coli* resulting production of a new intermediate, caffeoylphenyllactate. *Biotechnol Lett* 38:81–88. <https://doi.org/10.1007/s10529-015-1945-7>
- Karataş İ, Öztürk L, Demir Y, Ünlükara A, Kuruç A, Düzdemir O (2014) Alterations in antioxidant enzyme activities and proline content in pea leaves under long-term drought stress. *Toxicol Ind Health* 30(8):693–700. <https://doi.org/10.1177/0748233712462471>
- Katanić Stanković JS, Srećković N, Mišić D, Gašić U, Imbimbo P, Monti DM, Mihailović V (2020) Bioactivity, biocompatibility and phytochemical assessment of lilac sage, *Salvia verticillata* L. (Lamiaceae) - A plant rich in rosmarinic acid. *Ind Crops Prod* 143:111932. <https://doi.org/10.1016/j.indcrop.2019.111932>
- Khair-ul-Bariyah S, Ahmed D, Ikram M (2012) *Ocimum basilicum*: a review on phytochemical and pharmacological studies. *Pak J Chem* 2(2):78–85
- Khojasteh A, Mirjalili MH, Hidalgo D, Corchete P, Palazon J (2014) New trends in biotechnological production of rosmarinic acid. *Biotechnol Lett* 36:2393–2406. <https://doi.org/10.1007/s10529-014-1640-0>
- Kiferle C, Lucchesini M, Mensuali-Sodi A, Maggini R, Raffaelli A, Pardossi A (2011) Rosmarinic acid content in basil plants grown in vitro and in hydroponics. *Cent Eur J Biol* 6(6):946–957. <https://doi.org/10.2478/s11535-011-0057-1>
- Kim GD, Park YS, Jin YH, Park CS (2015) Production and applications of rosmarinic acid and structurally related compounds. *Appl Microbiol Biotechnol* 99:2083–2092. <https://doi.org/10.1007/s00253-015-6395-6>
- Kračun-Kolarević M, Dmitrović S, Filipović B, Perić M, Mišić D, Simonović A, Todorović S (2015) Influence of sodium salicylate on rosmarinic acid, carnosol and carnosic acid accumulation by *Salvia officinalis* L. shoots grown in vitro. *Biotechnol Lett* 37:1693–1701. <https://doi.org/10.1007/s10529-015-1825-1>
- Krzyżanowska J, Czubačka A, Pecio L, Przybys M, Doroszevska T, Stochmal A, Oleszek W (2012) The effects of jasmonic acid and methyl jasmonate on rosmarinic acid production in *Mentha × piperita* cell suspension cultures. *Plant Cell Tissue Organ Cult* 108:73–81. <https://doi.org/10.1007/s11240-011-0014-8>
- Kusvuran S, Kiran S, Ellialtıoglu SS (2016) Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. In: Shanker AK, Shanker C (eds) *Abiotic and biotic stress in plants - recent advances and future perspectives*. Intech Open, London
- Lee EJ, Moh SH, Park SY (2014) Production of biomass and bioactive compounds in adventitious root cultures of *Eleutherococcus koreanum* Nakai. In: Paek KY, Murthy HN, Zhong JJ (eds) *Production of biomass and bioactive compounds using bioreactor technology*. Springer, Dordrecht
- Lee EJ, Park SY, Paek KY (2015) Enhancement strategies of bioactive compound production in adventitious root cultures of *Eleutherococcus koreanum* Nakai subjected to methyl jasmonate and salicylic acid elicitation through airlift bioreactors. *Plant Cell Tissue Organ Cult* 120:1–10. <https://doi.org/10.1007/s11240-014-0567-4>
- Li SW, Xue L, Xu S, Feng H, An L (2009) IBA-induced changes in antioxidant enzymes during adventitious rooting in mung bean seedlings: The role of H₂O₂. *Environ Exp Bot* 66:442–450. <https://doi.org/10.1016/j.envexpbot.2009.03.005>
- Li H, Piao XC, Gao R, Jin MY, Jiang J, Lian ML (2016) Effect of several physicochemical factors on callus biomass and bioactive compound accumulation of *R. sachalinensis* bioreactor culture. *In Vitro Cell Dev Biol-Plant* 52:241–250. <https://doi.org/10.1007/s11627-016-9758-5>
- Lulu T, Park SY, Ibrahim R, Paek KY (2015) Production of biomass and bioactive compounds from adventitious roots by optimization of culturing conditions of *Eurycoma longifolia* in balloon-type bubble bioreactor system. *J Biosci Bioeng* 119(6):712–717. <https://doi.org/10.1016/j.jbiosc.2014.11.010>
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
- Murthy HN, Lee EJ, Paek KY (2014) Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell Tissue Organ Cult* 118:1–16. <https://doi.org/10.1007/s11240-014-0467-7>
- Murthy HN, Dandin VS, Paek KY (2016) Tools for biotechnological production of useful phytochemicals from adventitious root cultures. *Phytochem Rev* 15:129–145. <https://doi.org/10.1007/s11101-014-9391-z>
- Nadeem M, Abbasi BH, Younas M, Ahmad W, Zahir A, Hano C (2019) LED-enhanced biosynthesis of biologically active ingredients in callus cultures of *Ocimum basilicum*. *J Photochem Photobiol B: Biol* 190:172–178. <https://doi.org/10.1016/j.jphotobiol.2018.09.011>
- Nazir M, Tungmunnithum D, Bose S, Drouet S, Garros L, Giglioli-Guivarc'h N, Abbasi BH, Hano C (2019) Differential production of phenylpropanoid metabolites in callus cultures of *Ocimum basilicum* L. with distinct in vitro antioxidant activities and in vivo protective effects against UV stress. *J Agric Food Chem* 67:1847–1859. <https://doi.org/10.1021/acs.jafc.8b05647>
- Nazir M, Ullah MA, Mumtaz S, Siddiquah A, Shah M, Drouet S, Hano C, Abbasi BH (2020b) Interactive effect of melatonin and UV-C on phenylpropanoid metabolite production and antioxidant potential in callus cultures of purple basil (*Ocimum basilicum* L. var *purpurascens*). *Molecules* 25(5):1072. <https://doi.org/10.3390/molecules25051072>
- Nazir M, Ullah MA, Younas M, Siddiquah A, Shah M, Giglioli-Guivarc'h N, Hano C, Abbasi BH (2020a) Light-mediated biosynthesis of phenylpropanoid metabolites and antioxidant potential in callus cultures of purple basil (*Ocimum basilicum* L. var *purpurascens*). *Plant Cell Tissue Organ Cult* 142:107–120. <https://doi.org/10.1007/s11240-020-01844-z>
- Nazir S, Jan H, Tungmunnithum D, Drouet S, Zia M, Hano C, Abbasi BH (2020c) Callus culture of Thai basil is an effective biological system for the production of antioxidants. *Molecules* 25:4859. <https://doi.org/10.3390/molecules25204859>
- Nazir S, Jan H, Zaman G, Ahmed N, Drouet S, Hano C, Abbasi BH (2021b) Synergistic effects of salicylic acid and light stress on bioactive metabolites in basil callus cultures. *Biocatal Agric Biotechnol* 37:102176. <https://doi.org/10.1016/j.bcab.2021.102176>
- Nazir S, Jan H, Zaman G, Khan T, Ashraf H, Meer B, Zia M, Drouet S, Hano C, Abbasi BH (2021a) Copper oxide (CuO) and manganese oxide (MnO) nanoparticles induced biomass accumulation,

- antioxidants biosynthesis and abiotic elicitation of bioactive compounds in callus cultures of *Ocimum basilicum* (Thai basil). *Artif Cells Nanomed Biotechnol* 49(1):625–633. <https://doi.org/10.1080/21691401.2021.1984935>
- Oyaizu M (1986) Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr* 44:307–315
- Ozturk L, Demir Y, Unlukara A, Karatas I, Kurunc A, Duzdemir O (2012) Effects of long-term salt stress on antioxidant system, chlorophyll and proline contents in pea leaves. *Rom Biotechnol Lett* 17(3):7227–7236
- Pandey P, Singh S, Banerjee S (2019) *Ocimum basilicum* suspension culture as resource for bioactive triterpenoids: yield enrichment by elicitation and bioreactor cultivation. *Plant Cell Tissue Organ Cult* 137:65–75. <https://doi.org/10.1007/s11240-018-01552-9>
- Pekal A, Pyszynska K (2014) Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal Methods* 7:1776–1782
- Petersen M (2013) Rosmarinic Acid: New Aspects. *Phytochem Rev* 12:207–227. <https://doi.org/10.1007/s1101-013-9282-8>
- Prinsi B, Morgutti S, Negrini N, Faoro F, Espen L (2020) Insight into composition of bioactive phenolic compounds in leaves and flowers of green and purple basil. *Plants* 9:22. <https://doi.org/10.3390/plants9010022>
- Rahmat E, Kang Y (2019) Adventitious root culture for secondary metabolite production in medicinal plants: a review. *J Plant Biotechnol* 46:143–157. <https://doi.org/10.5010/JPB.2019.46.3.143>
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26(9–10):1231–1237
- Sahraroo A, Mirjalili MH, Corchete P, Babalar M, Moghadam MRF (2016) Establishment and characterization of a *Satureja khuzistanica* Jamzad (Lamiaceae) cell suspension culture: a new in vitro source of rosmarinic acid. *Cytotechnology* 68:1415–1424. <https://doi.org/10.1007/s10616-015-9901-x>
- Sairam RK, Rao KV, Srivastava GC (2002) Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci* 163:1037–1046
- Sarropoulou V, Dimassi-Therios K, Therios I (2013) Indole-3-butyric acid and myo-inositol impacts on *in vitro* rooting of the cherry rootstocks CAB-6P and Gisela 6. *Biol Plant* 57(4):613–619. <https://doi.org/10.1007/s10535-013-0352-y>
- Sen A, Alikamanoglu S (2013) Antioxidant enzyme activities, malondialdehyde, and total phenolic content of PEG-induced hyperhydric leaves in sugar beet tissue culture. *In Vitro Cell Dev Biol-Plant* 49:396–404. <https://doi.org/10.1007/s11627-013-9511-2>
- Shahrajabian MH, Sun W, Cheng Q (2020) Chemical components and pharmacological benefits of Basil (*Ocimum basilicum*): a review. *Int J Food Prop* 23:1961–1970. <https://doi.org/10.1080/10942912.2020.1828456>
- Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M, Zheng B (2019) Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules* 24:2452. <https://doi.org/10.3390/molecules24132452>
- Šípošová K, Labančová E, Kučerová D, Kollárová K, Vívodová Z (2021) Effects of exogenous application of indole-3-butyric acid on maize plants cultivated in the presence or absence of cadmium. *Plants* 10:2503. <https://doi.org/10.3390/plants10112503>
- Slinkard K, Singleton VL (1977) Total phenol analysis: automation and comparison with manual methods. *Am J Enol Viticulture* 28:49–55
- SPSS 20. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. Released 2011
- Srivastava S, Conlan XA, Adholeya A, Cahill DM (2016) Elite hairy roots of *Ocimum basilicum* as a new source of rosmarinic acid and antioxidants. *Plant Cell Tissue Organ Cult* 126:19–32. <https://doi.org/10.1007/s11240-016-0973-x>
- Steffens B, Rasmussen A (2016) The Physiology of adventitious roots. *Plant Physiol* 170:603–617. <https://doi.org/10.1104/pp.15.01360>
- Swamy MK, Sinniah UR, Ghasemzadeh A (2018) Anticancer potential of rosmarinic acid and its improved production through biotechnological interventions and functional genomics. *Appl Microbiol Biotechnol* 102:7775–7793. <https://doi.org/10.1007/s00253-018-9223-y>
- Tada H, Ikeda Y, Omoto T, Shimomura K, Ishimaru K (1996) Rosmarinic acid and related phenolics in adventitious root cultures of *Ocimum basilicum* L. *Plant Tissue Cult Lett* 13(1):69–71
- Teofilović B, Grujić-Letić N, Karadžić M, Kovačević S, Podunavac-Kuzmanović S, Gligorića E, Gadžurić S (2021) Analysis of functional ingredients and composition of *Ocimum basilicum*. *S Afr J Bot* 141:227–234. <https://doi.org/10.1016/j.sajb.2021.04.035>
- Tepe B, Sokmen A (2007) Production and optimisation of rosmarinic acid by *Satureja hortensis* L. callus cultures. *Nat Prod Res* 21(13):1133–1144
- Tepe B, Eminagaoglu O, Akpulat HA, Aydin E (2007) Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and *S. verticillata* (L.) subsp. *amasiaca* (Frey & Bornm.) Bornm. *Food Chem* 100:985–989. <https://doi.org/10.1016/j.foodchem.2005.10.062>
- Velikova V, Yordanov I, Edrava A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Sci* 151:59–66
- Verma SK, Sahin G, Das AK, Gurel E (2016) In vitro plant regeneration of *Ocimum basilicum* L. is accelerated by zinc sulfate. *In Vitro Cell Dev Biol-Plant* 52:20–27. <https://doi.org/10.1007/s11627-015-9739-0>
- Weremczuk-Jeżyna I, Grzegorzczak-Karolak I, Frydrych B, Królicka A, Wysokińska H (2013) Hairy roots of *Dracocephalum moldavica*: rosmarinic acid content and antioxidant potential. *Acta Physiol Plant* 35:2095–2103. <https://doi.org/10.1007/s11738-013-1244-7>
- Wu CH, Dewir YH, Hahn EJ, Paek KY (2006) Optimization of culturing conditions for the production of biomass and phenolics from adventitious roots of *Echinacea angustifolia*. *J Plant Biol* 49(3):193–199
- Wu CH, Tang J, Jin ZX, Wang M, Liu ZQ, Huang T, Lian ML (2018) Optimizing co-culture conditions of adventitious roots of *Echinacea pallida* and *Echinacea purpurea* in air-lift bioreactor systems. *Biochem Eng J* 132:206–216. <https://doi.org/10.1016/j.bej.2018.01.024>
- Yin S, Liang Y, Gao W, Wang J, Jing S, Zhang Y, Liu H (2013) Influence of medium salt strength and nitrogen source on biomass and metabolite accumulation in adventitious root cultures of *Pseudostellaria heterophylla*. *Acta Physiol Plant* 35:2623–2628. <https://doi.org/10.1007/s11738-013-1270-5>
- Zaman G, Farooq U, Bajwa MN, Jan H, Shah M, Ahmad R, Andleeb A, Drouet S, Hano C, Abbasi BH (2022) Effects of yeast extract on the production of phenylpropanoid metabolites in callus culture of purple basil (*Ocimum basilicum* L var *purpurascens*) and their in vitro evaluation for antioxidant potential. *Plant Cell Tissue Organ Cult*. <https://doi.org/10.1007/s11240-022-02303-7>
- Zeljšković SČ, Komzáková K, Šišková J, Karalija E, Smékalová K, Tarkowski P (2020) Phytochemical variability of selected basil genotypes. *Ind Crops Prod* 157:112910. <https://doi.org/10.1016/j.indcrop.2020.112910>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.