In Vitro Cellular & Developmental Biology - Plant (2023) 59:365–377
https://doi.org/10.1007/s11627-023-10341-9

PLANT TISSUE CULTURE

Optimization of sucrose concentration to promote root proliferation and secondary metabolite accumulation in adventitious root cultures of *Ocimum basilicum*

İlhami Karataş[1]

Received: 18 November 2022 / Accepted: 23 February 2023 / Published online: 12 April 2023 / Editor: Marco Buenrostro-Nava

Abstract

Ocimum basilicum L. (sweet basil) is a medicinal herb that contains valuable secondary metabolites belonging to different groups. Therefore, the plant displays a wide range of pharmacological activities. *In vitro* cultures of sweet basil are a very promising approach to produce bioactive compounds, but studies on optimization of essential medium components are very limited. To the current author's knowledge, there is no previous report on the determination of the optimal sucrose concentration for rosmarinic acid production in adventitious roots of sweet basil in airlift bioreactors. This study aimed to determine the effects of 2.0, 3.0, 4.0, and 5.0% sucrose on rosmarinic acid content, biomass accumulation, and antioxidant activities (DPPH, ABTS, and FRAP) in adventitious root culture of sweet basil under balloon-type bubble bioreactors. Also, phenylalanine ammonia lyase activities (PAL) and accumulation of total phenolic and flavonoid were measured. Additionally, the amounts of some stress parameters (proline, malondialdehyde, and hydrogen peroxide) and the activities of antioxidant enzymes (CAT, POD, and SOD) were measured to assess the levels of sucrose-induced stress and antioxidant defense system. UHPLC-HESI-MS/MS analysis showed that the maximum rosmarinic acid content was determined as 28.11 ± 0.61 mg g^{-1} DW at 2.0% sucrose, which was 1.28, 1.34, and 1.78 times higher than 3.0, 4.0, and 5.0% sucrose, respectively. Similarly, the highest accumulations of fresh weight, total phenolic, and flavonoid were obtained at 2.0% sucrose as 91.46 ± 1.91 g L^{-1}, 28.83 ± 0.70 mg g^{-1} DW, and 3.26 ± 0.07 mg g^{-1} DW, respectively. In addition, the highest levels of PAL activity and antioxidant activity (DPPH, ABTS, and FRAP) were achieved at 2.0% sucrose. On the other hand, stress parameters and antioxidant enzyme activities of adventitious roots were the lowest at 2.0% sucrose. These results indicated that determination of optimum sucrose concentration is an indispensable parameter for further enhanced production of rosmarinic acid and biomass in adventitious root culture of sweet basil through bioreactors.

Keywords Adventitious root · Antioxidant activity · *Ocimum basilicum* · Rosmarinic acid · Sucrose

Introduction

Ocimum basilicum L. (sweet basil) is an annual medicinal and aromatic herb that is used in many industries, such as food, medicine, cosmetics, and pharmaceuticals (Copolovici *et al.* 2021; Bajomo *et al.* 2022). The plant especially is used in traditional medicine due to its many biological activities, including headaches, influenza, diarrhea, colds, coughs, kidney malfunction, fevers, and flu. Some of the most important pharmacological activities of basil cultivars are antioxidant, antidiabetic, anticancer, antimicrobial, antistress, anti-inflammatory, and antiarthritic. These properties of the plant are related to the biologically active metabolites with different chemical structures found in its various parts. The plant contains a wide variety of valuable compounds belonging to different secondary metabolite groups, such as phenolic acid, flavonoid, anthocyanin, terpene, and essential oil (Skrypnik *et al.* 2019; Shahrajabian *et al.* 2020; Copolovici *et al.* 2021). With regard to phenolics, rosmarinic acid is the main compound of *Ocimum basilicum* (Prinsi *et al.* 2020; Zeljković *et al.* 2020). Also, studies conducted in the basil plant in recent years have demonstrated that the main phenolic acids are chicoric acid, ferulic acid, caftaric acid, and caffeic acid in addition to

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

[1] Department of Forestry, Almus Vocational School, Tokat Gaziosmanpaşa University, Almus, 60900 Tokat, Turkey

rosmarinic acid, and the amounts of these compounds vary according to the varieties (Skrypnik *et al.* 2019; Bajomo *et al.* 2022; Romano *et al.* 2022).

Rosmarinic acid, which is a potential candidate drug, shows many biological and pharmacological activities, such as antitumor, antidiabetic, antioxidant, antimicrobial, hepatoprotective, antiangiogenic, antiviral, antiallergic, anti-inflammatory, and neuroprotective (Kim *et al.* 2015; Guan *et al.* 2022). However, due to these activities, the high demand for rosmarinic acid cannot be met from plants alone due to its low level in plant organs. Also, many plants that produce the compound are under the threat of extinction due to various reasons, such as environmental changes, overharvesting, and unscientific harvesting. Furthermore, its chemical synthesis is complex and expensive. These difficulties can be eliminated with the contribution of biotechnological techniques (Swamy *et al.* 2018). Particularly, adventitious root cultures offer an alternative to plant harvesting and propagation to obtain secondary metabolites from plants. The cultures of adventitious roots (ARs), which are known as roots that emerge from any organ of the plants except roots, provide ecological and economic advantages in secondary metabolite production (Khanam *et al.* 2022).

The optimal level of culture nutrient medium in adventitious root culture is a critically important factor for the production of valuable compounds (Cui *et al.* 2013). In this context, there are many essential medium components, including types and concentration of carbohydrates, appropriate salt strength, and growth regulator levels, which could influence secondary metabolite production in cell and organ cultures of plants (Murthy *et al.* 2014). Carbohydrates need to be added to *in vitro* cultures of plants as energy and carbon sources for growth stages, such as organogenesis, shoot proliferation, root induction, and embryogenesis, as well as maintaining their osmotic potential. Various carbon sources are added to culture media according to genotypes and growth stage (Yaseen *et al.* 2013). Especially, sucrose is an important source of carbon and energy for adventitious root cultures, and its concentration can influence secondary metabolite yield and growth (Cui *et al.* 2010). Furthermore, the influences of sucrose concentration on growth and metabolite content vary according to many factors, such as compound types, plant species, culture method, and cell lines (Li *et al.* 2016). Also, the addition of sucrose above a certain amount to *in vitro* cultures may induce osmotic stress during which ROSs are produced (Baque *et al.* 2012).

The basil tissue cultures are useful procedures for improved production of biologically active compounds, but protocols need to be developed for the proliferation of basil cells and organs in large quantities (Jakovljević *et al.* 2022). Accordingly, for the evaluation of rosmarinic acid production, there are many studies on callus cultures, cell suspension cultures, *in vitro* propagated plants, and hairy root culture of *Ocimum basilicum.* Most of the studies conducted in recent years are related to the determination of the effects of various elicitor applications on rosmarinic acid accumulation in callus and suspension cultures of basil (Karataş 2022a). For this purpose, the effects of various elicitors, such as melatonin, cadmium chloride, copper oxide nanoparticles, manganese oxide nanoparticles, different spectral lights, yeast extract, methyl jasmonate, and silver nitrate, on the production of rosmarinic acid in basil callus and suspension cultures were investigated in previous studies (Duran *et al.* 2019; Pandey *et al.* 2019; Açıkgöz 2020; Nazir *et al.* 2020, 2021). The elicitor applications to *in vitro* cultures of basil are a suitable approach to increase rosmarinic acid production, and the efficiency of the applications may increase when performed under optimum medium conditions. However, the influences of essential medium ingredients on the production of valuable compounds have been very insufficiently investigated in *in vitro* cultures of *Ocimum basilicum* (Karataş 2022a). Also, there are only few publications on the cultivation of basil under bioreactor conditions (Jakovljević *et al.* 2022). Moreover, to the current author's knowledge, there is no available publication on the determination of the optimum sucrose concentration for rosmarinic acid production in ARs of this plant through bioreactors. In this study, it was aimed to evaluate the effects of different sucrose concentrations on biomass and rosmarinic acid accumulation in adventitious root culture of sweet basil under airlift bioreactors. In addition, total phenolic content and flavonoid quantity, PAL enzyme activity, and antioxidant capacity (DPPH, ABTS, and FRAP) of ARs grown under different sucrose concentrations were analyzed. Also, the amounts of some stress parameters (proline, malondialdehyde, and hydrogen peroxide) and the activities of antioxidant enzymes (CAT, POD, and SOD) were measured in ARs to evaluate the levels of sucrose-induced stress and antioxidant defense system.

Materials and methods

Cultivation of plant material In the study, *Ocimum basilicum* (sweet basil, green colored) plant grown under *in vitro* culture conditions was used as plant material. The seeds of the plant were provided from Zengarden firm (İzmir, Turkey). For the sterilization of seeds, they were first soaked in ethanol (70.0%) for 30 s, and then agitated in NaClO solution (4.0%) for 30 min. Subsequently, the sweet basil seeds were washed with sterile distilled water to remove sterilizing agents. Following this, the seeds were sown into jars including 50 mL of Murashige and Skoog (MS; Murashige and Skoog 1962) medium supplemented with 3.0% sucrose (Duchefa Biochemie BV, Haarlem, The Netherlands) and 2.0 g L^{-1} phytagel. After the seeds germinated under dark

conditions for 8 d, the sprouting plants were grown in a 16-h light/8-h dark photoperiod for 25 d. Also, in the preparation stage of the culture medium, the pH was adjusted to pH 5.8 using sodium hydroxide or hydrochloric acid, and then, the medium was autoclaved at 121 °C for 20 min. Finally, the plants were cut to be used as explant source for the establishment of adventitious root cultures.

Induction and multiplication of adventitious roots Adventitious root induction was achieved from hypocotyl explants of sweet basil in 0.75 × MS (Duchefa Biochemie BV, Haarlem, The Netherlands) medium supplemented with 2.0 mg L^{-1} IBA (indole-3-butyric acid, Sigma-Aldrich Co, St. Louis, MO), 30.0 g L^{-1} sucrose, and 2.0 g L^{-1} phytagel under the dark conditions. The sterilization process and pH adjustment of the culture media were performed as indicated in the cultivation of plant material. Adventitious roots (ARs) induced in petri dishes were separated from the explants at 25 d and used to form suspension cultures. To propagate ARs, the suspension cultures were performed on a shaker (120 rpm) under dark conditions for 2 wk. In this context, the ARs (2.0 g) were cultured in 250-mL Erlenmeyer flasks containing 100 mL of nutrient medium with the same content as in Petri dishes, except for phytagel.

Establishment of bioreactor cultures To determine the optimum sucrose concentration, ARs propagated in the suspension culture were inoculated into an airlift balloon-type bubble bioreactor (BTBB; 1 L). For this purpose, 3.5 g of ARs was cultured at 0.75 × MS liquid medium (500 mL) containing different concentrations of sucrose (2.0, 3.0, 4.0, and 5.0%) and 2.0 mg L^{-1} IBA for 30 d (Karataş 2022a). The aeration volume of bioreactor culture was set to 0.1 vvm (air volume/culture volume per min). The stages of the study are summarized in Fig. 1 as a pictorial presentation. Finally, ARs were harvested and used in the expressed analyses (Fig. 2).

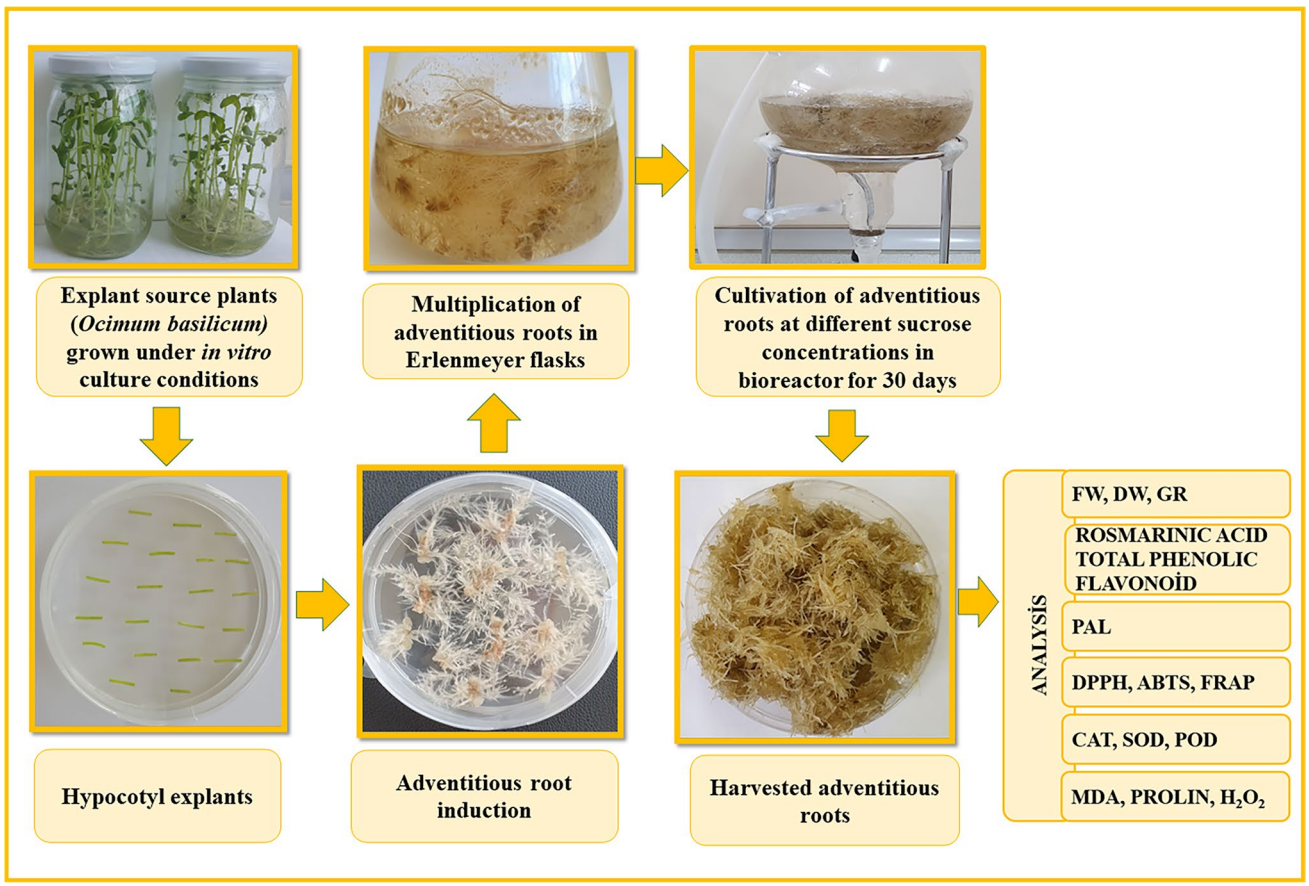

Figure 1. A pictorial presentation of the study of optimization of sucrose concentration in *Ocimum basilicum* L. (sweet basil) adventitious root culture under bioreactor conditions. FW, fresh weight; DW, dry weight; GR, growth rate; PAL, phenylalanine ammonia lyase activity; DPPH, DPPH· free radical scavenging activity; ABTS, $ABTS^{\cdot+}$ cation radical scavenging activity; FRAP, ferric reducing antioxidant power; CAT, catalase activity; SOD, superoxide dismutase activity; POD, peroxidase activity; MDA, malondialdehyde; H_2O_2, hydrogen peroxide.

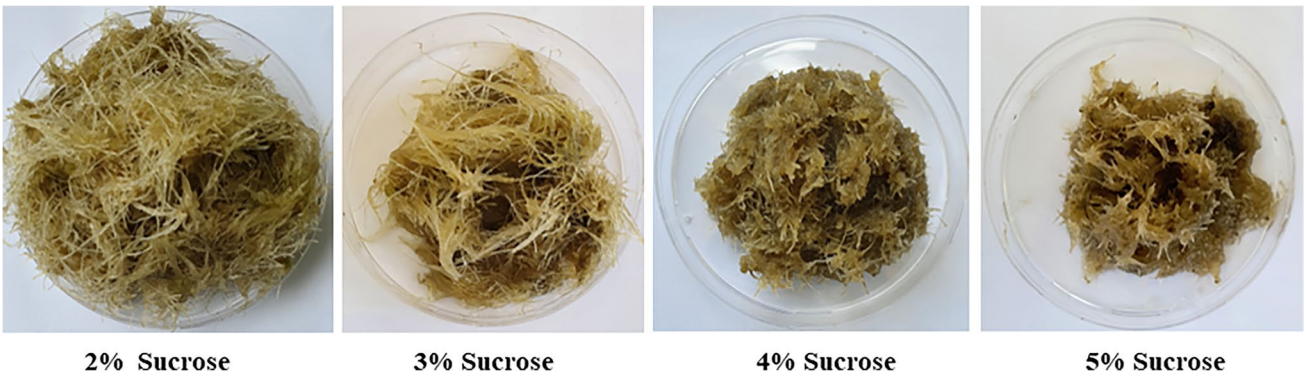

Figure 2. Post-harvest appearance of *Ocimum basilicum* L. (sweet basil) adventitious roots cultured at different sucrose concentrations for 30 d in bioreactor.

Analysis of biomass parameters ARs were harvested on the 30th day of bioreactor culture and filtered through a sieve to remove nutrient medium. Afterwards, the ARs were rinsed with distilled water, and excess water on the root was removed with blotting paper. ARs were weighed to assess the fresh weight (FW), and then the roots dried to a constant dry weight in an incubator at 50 °C for 2 d to determine dry weight (DW). Afterwards, the dry weight percentages (% of DW) of ARs were calculated. The growth rates (GR) of ARs were determined using the equation below.

$$\text{Growth rates} = \left[FW_{harvested} - FW_{inoculated}\right]/FW_{inoculated}$$

Extraction of adventitious roots The ARs (0.2 g DW) were extracted using methanol-dichloromethane solution (10.0 mL, 4:1) to analyze bioactive compounds and non-enzymatic antioxidant activities. The extracts were shaken vigorously with a vortex and then passed through a syringe filter (0.22 µm). Afterwards, the samples were stored at 4 °C until analysis was complete (Karataş 2022a, b).

UHPLC-HESI-MS/MS analysis of rosmarinic acid The rosmarinic acid amounts of the ARs were analyzed by using Dionex UltiMate 3000 UHPLC (ultra-high performance liquid chromatography; Thermo Fisher Scientific, Bremen, Germany) combined with a triple-quadrupole mass spectrometer (Thermo Fisher Scientific, TSQ Quantum Access Max) with a heated electrospray ionization (HESI) source according to the method described by Karataş (2022a). The assay was carried out at the HÜBTUAM Center of Hitit University (Çorum, Turkey). The identification of rosmarinic acid in ARs was accomplished by direct comparison with the fragmentation pattern and retention time of the standard compound. The rosmarinic acid contents of ARs were determined by the calibration curve of standard compound.

Determination of total phenolic content The total phenolic content of ARs was analyzed by the method of Slinkard and Singleton (1977) with a slight modification. First, the adventitious root extracts (200.0 µL), distilled water (4.4 mL), and Folin-Ciocalteu reagent (100.0 µL, 2N; Merck KGaA Darmstadt, Germany) were pipetted into the reaction test tube, respectively, and incubated for 5 min. Then, Na_2CO_3 (300.0 µL, 2.0% *w/v*) was pipetted to the test tubes and incubated for 2 h at room temperature. After that, the absorbance of the test tubes was measured with a spectrophotometer (Varian Cary 50 Bio UV–VIS, Victoria, Australia) at 760 nm. Total phenolic contents of the ARs were calculated by the equation of the calibration curve of gallic acid (Merck KGaA Darmstadt, Germany) used as a standard.

Assessment of flavonoid content The flavonoid amounts of ARs were detected according to the procedure (with some modifications) specified by Pekal and Pyrzynska (2014). First, 100.0 µL of adventitious root extract was poured into the test tubes. Then, $AlCl_3$ (100 µL, 10.0% *w/v*), distilled water (4.7 mL), and CH_3COONa (100.0 µL, 1 M) were added to these tubes, respectively. Afterwards, the tubes were vortexed vigorously and incubated for 30 min. The absorbance of the test tubes was read with a spectrophotometer (Varian Cary 50 Bio UV–VIS) at 425 nm. Flavonoid contents of the ARs were calculated by the equation of the calibration curve of quercetin (Sigma-Aldrich Co, St. Louis, MO) used as a standard.

DPPH radical scavenging activity DPPH· (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich Co, St. Louis, MO) free radical scavenging activities of ARs were analyzed according to the method expressed by Blois (1958). Firstly, 2960.0 µL of ethanol (100% *v/v*) was added to test tubes containing 40.0 µL (20.0 mg DW mL^{-1}) of adventitious extract. Then, 1.0 mL of the DPPH· solution (in ethanol, 0.26 mM) was pipetted into the tubes. After vigorous vortexing, the tubes

were incubated for 30 min in the dark, and their absorbances were read with a spectrophotometer at 517 nm. The activities of the ARs were detected using the following equation.

$$\text{Activity (\%)} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}}] \times 100$$

ABTS cation radical scavenging activity $ABTS^{\cdot+}$ (2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) cation radical scavenging activities of ARs were measured spectrophotometrically using the procedure expressed by Re *et al.* (1999). Firstly, 2980.0 µL of phosphate buffer (0.1 M, pH 7.4) was added to test tubes containing 20.0 µL (20.0 mg DW mL^{-1}) of adventitious extract. Afterwards, 1.0 mL of the ABTS-$K_2S_2O_8$ solution was pipetted into the tubes. After vigorous vortexing, the tubes were incubated for 30 min in the dark, and their absorbances were read with a spectrophotometer at 734 nm. For the preparation of the ABTS-$K_2S_2O_8$ solution, ABTS solution (2.0 mM, Sigma-Aldrich Co, St. Louis, MO) and $K_2S_2O_8$ solution (2.45 mM) were mixed in a 1:2 ratio and kept in the dark for 6 h. The activity results of the ARs were detected using the following equation.

$$\text{Activity (\%)} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}})/\text{Absorbance}_{\text{Control}}] \times 100$$

Ferric reducing antioxidant power (FRAP) The FRAP activities of samples were analyzed according to the method specified by Oyaizu (1986). Firstly, 1200.0 µL of phosphate buffer (0.2 M, pH 6.6) was added to test tubes containing 50.0 µL (20.0 mg DW mL^{-1}) of adventitious extract. Later, 1.25 mL of $K_3Fe(CN)_6$ (1.0%) solution was pipetted into the tubes and incubated at 50 °C for 20 min. After this step, 1.25 mL of trichloroacetic acid (TCA, 10.0%) and 0.25 mL of $FeCl_3$ (0.1%) were pipetted into the test tubes and vortexed vigorously. The absorbances of the test tubes were read with a spectrophotometer at 700 nm. The reducing power of the ARs was calculated by the equation of the calibration curve of Trolox used as a standard.

Assay of PAL activity To measure PAL (phenylalanine ammonia lyase) activity, 0.5 g of fresh adventitious root was homogenized in 5.0 mL of Tris–HCl buffer (50.0 mM, pH 8.5) including EDTA (0.2 mM), polyvinyl pyrrolidone (2.0% *w/v*), and 2-mercaptoethanol (15.0 mM). Then, the samples were centrifuged at 12,000 g at 4 °C for 15 min to obtain the supernatant (enzyme source). Subsequently, L-phenylalanine (0.5 mL, 10.0 mM), Tris–HCl buffer (1.0 mL), distilled water (0.4 mL), and supernatant (100.0 µL) were pipetted into test tubes, respectively. The tubes were incubated at 37 °C for 1 h, and then, 50.0 µL of HCl (5.0 N) was added to the tubes to terminate the reaction. The absorbances of the tubes were read with a spectrophotometer at 290 nm to determine the trans-cinnamic quantity. The PAL activity of the ARs was calculated as nmol of trans-cinnamic acid (nmol h^{-1} mg^{-1} protein) (Beaudoin-Eagan and Thorpe 1985). The protein amounts of ARs were analyzed according to the method expressed by Bradford (1976).

Determination of some stress parameters The proline amounts of ARs were detected according to the method expressed by Bates *et al.* (1973). The proline content was quantified by the equation of the calibration curve of proline used as a standard.

The malondialdehyde (MDA) amounts of ARs were analyzed according to the procedure expressed by Velikova *et al.* (2000). The MDA contents were calculated by means of the extinction coefficient (155.0 mM^{-1} cm^{-1}).

The hydrogen peroxide (H_2O_2) content of ARs was determined according to the method stated by Velikova *et al.* (2000). The H_2O_2 levels were detected by the equation of the calibration curve of H_2O_2 used as a standard.

Assay of antioxidant enzyme activities To measure the activities of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), the homogenization of ARs (0.25 g) was performed in 2.0 mL of phosphate buffer (50.0 mM, pH 7.0) including EDTA (0.1 mM) and polyvinyl pyrrolidone (1.0% *w/v*). Then, the samples were centrifuged at 12,000 g at 4 °C for 15 min to obtain the supernatant (enzyme source).

The CAT activities of ARs were measured according to the procedure expressed by Havir and Mchale (1987). The enzyme unit (EU, 1 unit) was described as the enzyme quantity that achieves the decomposition of 1.0 µmol H_2O_2 per min (EU mg^{-1} protein).

The POD activities of ARs were assayed using the procedure stated by Angelini *et al.* (1990). The enzyme unit (EU, 1 unit) was described as the enzyme quantity that catalyzes the oxidation of 1.0 µmol guaiacol per min (EU mg^{-1} protein).

The SOD activities of ARs were analyzed according to the method stated by Beyer and Fridovich (1987). One unit of SOD enzyme (EU) was described as the enzyme amount that inhibits 50% photoreduction of nitroblue tetrazolium (EU mg^{-1} protein).

Statistical analysis The study was performed in a completely randomized design with three replicates. All data obtained in this study were statistically analyzed with the SPSS 20 computer software (SPSS 20, IBM Crop, Armonk, NY). The differences between applications were detected by one-way analysis of variance (ANOVA) using Duncan's multiple range test ($p < 0.05$). Data are shown as the mean ± standard error (Duncan 1955). In addition, correlation analyses were performed using Pearson's correlation test.

Results and discussion

The influences of sucrose concentration on biomass parameters, secondary metabolite contents, and PAL enzyme activities To determine the optimum sucrose concentration, ARs of *Ocimum basilicum* were cultured at different sucrose concentrations (2.0, 3.0, 4.0, and 5.0%) for 30 d. The post-harvest appearance of ARs is presented in Fig. 2. As listed in Table 1, the sucrose concentration significantly influenced the biomass parameters of ARs. The FW and GR of ARs declined significantly with increasing sucrose concentration from 2.0 to 5.0%. Among the applied sucrose concentrations, 2.0% sucrose (20.0 g L^{-1}) was found to be the appropriate concentration for FW and GR. In this context, FW and GR of ARs at 2.0% sucrose were determined to be the highest as 91.46 ± 1.91 g L^{-1} and 12.06 ± 0.27, respectively. However, unlike FW and GR, the dry weight percentage (% of DW) of ARs increased significantly with elevating sucrose concentration from 2.0 to 5.0%. As it can be seen from Table 1, the highest dry weight percentage was determined as 8.29 ± 0.20 at 5.0% sucrose. Also, the highest accumulation of DW was observed as 4.77 ± 0.14 g L^{-1} at 3.0% sucrose-treated culture. In the literature, 3.0% (30.0 g L^{-1}) sucrose concentration is commonly used in *in vitro* plant cultures, but the optimum sucrose concentration for biomass parameters varies according to the culture method and the plant species. A similar outcome was determined by Rajesh *et al.* (2014) who expressed that 2% sucrose supplementation provided the maximum biomass production in adventitious root cultures of *Podophyllum hexandrum*. Likewise, in a different study conducted on hair root culture of *Withania somnifera*, the appropriate sucrose concentration for biomass production was found to be 2% (Sivanandhan *et al.* 2012). Also, in early research, various sucrose concentrations, such as 3% (Cui *et al.* 2010), 4% (Yin *et al.* 2013), and 5% (Baque *et al.* 2012), were reported to be the proper concentration for biomass accumulation in adventitious root cultures. Moreover, the optimum sucrose concentration for biomass varies according to the *in vitro* culture method used. For instance, in *in vitro* cultures of *Withania somnifera*, it was reported that the optimum sucrose concentration was 2% for adventitious root culture, 3% for hairy root culture, and 4% for cell suspension culture (Nagella and Murthy 2014).

In the current study, considering the decline in FW and GR results at sucrose concentrations higher than 3.0%, it was clearly understood that high sucrose levels greatly hampered the development of ARs. Similarly, Cui *et al.* (2010) demonstrated that sucrose concentrations higher than 3% inhibited the biomass accumulation of ARs due to the higher osmotic pressure. Furthermore, according to the findings in Fig. 3, oxidative stress parameters increased significantly in ARs treated with sucrose concentrations above 3.0%. These findings showed that determination of optimum sucrose concentration in adventitious root culture is a critical factor in terms of biomass parameters.

The rosmarinic acid amounts of the ARs were determined using UHPLC-HESI-MS/MS. The identification of rosmarinic acid in ARs was accomplished by direct comparison with the fragmentation pattern and retention time of the standard metabolite. The retention time for rosmarinic acid was detected as 17.73 min. As for the mass spectrum, the parent ion of rosmarinic acid was found at m/z 359, and its product ions were determined at m/z 162 and 134. These values are consistent with the findings reported by Karataş (2022a). In a previous study, it was stated that the characteristic ion fragments (m/z) for rosmarinic acid were 359, 161, and 133 (Ruan *et al.* 2012).

The sucrose concentration considerably influenced the production of rosmarinic acid, total phenolics, and flavonoids, and the findings of secondary metabolite accumulation showed a similar phenomenon with the biomass results. The accumulation of the compounds in ARs decreased gradually as the sucrose ratio elevated from 2.0 to 5.0%. As shown in Table 2, the highest rosmarinic acid, total phenolic, and flavonoid contents were obtained from the MS medium supplemented with 2.0% sucrose as 28.11 ± 0.61 mg g^{-1} DW, 28.83 ± 0.70 mg g^{-1} DW, and 3.26 ± 0.07 mg g^{-1} DW, respectively. In addition, the highest yield of rosmarinic acid was detected as 116.65 ± 3.82 mg L^{-1} DW at 2.0% sucrose concentration. The results of the present study are consistent with a previous study in which the maximum rosmarinic acid content was observed at 2.0% sucrose concentration in cell suspension cultures of *Anthoceros agrestis* (Vogelsang *et al.* 2006). Also, in some early studies, the optimum sucrose concentration for rosmarinic acid production was determined as 3% in cell suspension culture of *Anchusa*

Table 1. The influences of sucrose concentration on biomass parameters in adventitious root cultures of *Ocimum basilicum* L. (sweet basil) after 30 d of bioreactor cultures

Sucrose (%)	Fresh weight (FW) (g L^{-1})	Dry weight (DW) (g L^{-1})	% DW	Growth ratio
2	91.46 ± 1.91 a	4.15 ± 0.13 b	4.54 ± 0.05 d	12.06 ± 0.27 a
3	86.83 ± 2.16 a	4.77 ± 0.14 a	5.49 ± 0.08 c	11.40 ± 0.30 a
4	62.60 ± 4.15 b	4.45 ± 0.35 ab	7.10 ± 0.11 b	7.94 ± 0.59 b
5	50.70 ± 3.43 c	4.19 ± 0.18 b	8.29 ± 0.20 a	6.24 ± 0.49 c

The different *letters* within the same *column* demonstrate significant differences according to Duncan's multiple range test ($p < 0.05$)

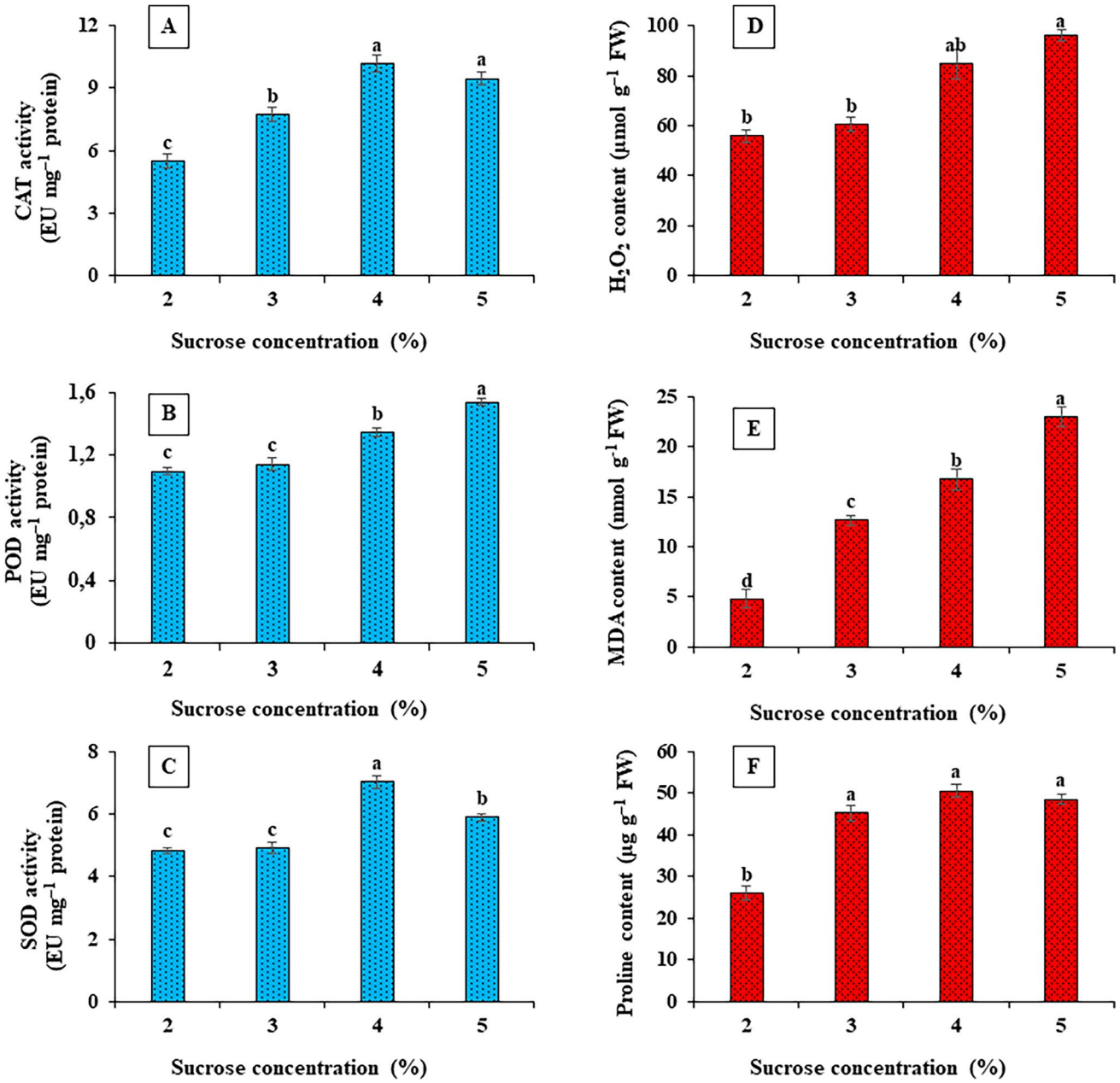

Figure 3. The influences of sucrose concentration on the activities of CAT (***A***), POD (***B***), and SOD (***C***) and quantities of H_2O_2 (***D***), MDA (***E***), and proline (***F***) in adventitious root cultures of *Ocimum basilicum* L. (sweet basil) after 30 d of bioreactor culture. The different *letters* in the figure indicate significant differences according to Duncan's multiple range test ($p < 0.05$). *Bars* express means ± standard error ($n = 3$). CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde.

officinalis (De-Eknamkul and Ellis 1985) and callus culture of *Satureja hortensis* L. (Tepe and Sökmen 2007). However, higher sucrose concentrations, such as 4% (Karam *et al.* 2003), 5% (Hakkim *et al.* 2011), and 7% (Ilieva and Pavlov 1997), were reported to be suitable for rosmarinic acid accumulation in callus and suspension cultures. On the other hand, the effects of sucrose concentration on rosmarinic acid quantity were reported mostly in callus and cell suspension culture in the literature, but they have not been adequately investigated in adventitious root culture. Therefore, the findings obtained from this study were not compared with adventitious root cultures. As for the effects of sucrose on total phenolics and flavonoids, a similar trend was detected as in the rosmarinic acid results. In this context, it was reported in previous publications that the highest quantities of phenolic and flavonoid in ARs were determined at different sucrose ratios, such as 1% (Baque *et al.* 2012) and 5% (Wu *et al.* 2018).

PAL has a crucial role in the phenylpropanoid pathway where various phenolic compounds are produced and catalyzed the first step of the pathway (Kim and Hwang 2014). PAL activities were significantly affected by sucrose concentrations in ARs of sweet basil. The activity gradually decreased as the sucrose concentration increased from 2.0 to 5.0% (Table 2). The maximum PAL activity was found as 12.65 ± 0.71 nmol h^{-1} mg^{-1} protein at 2.0% sucrose. In this context, the activity in the culture treated with 2.0% sucrose was 1.40, 1.50, and 2.29 times higher than the activity at the 3.0, 4.0, and 5.0% sucrose, respectively. Likewise, it was demonstrated that sucrose application to the embryo axes of *Lupinus luteus* stimulated PAL activity and phenylpropanoid metabolism (Morkunas *et al.* 2005). Furthermore, Morkunas *et al.* (2011) demonstrated that the application of sucrose alone highly induced the expression of flavonoid biosynthetic genes in the embryo axes of *L. luteus*, including PAL. Consistent with these studies, Tauzin and Giardina (2014) stated that regulation of PAL expression is associated with sugar. On the other hand, in this study, the PAL activity demonstrated a strong positive correlation with the accumulations of total phenolic ($r = 0.904$), rosmarinic acid ($r = 0.821$), and flavonoid ($r = 0.884$) (Table 3).

Sucrose, which is a source of carbon and energy for the plant cell, markedly affects the accumulation of compounds of the phenylpropanoid pathway (Kikowska *et al.* 2012). Also, sucrose concentration is a critically important regulatory chemical factor in metabolite production (Li *et al.* 2016). On the other hand, the addition of sucrose above a certain amount to *in vitro* cultures may induce osmotic stress (Baque *et al.* 2012). In this context, it was reported that sucrose concentrations higher than 3% (5, 7, and 9% *w/v*) caused osmotic stress in ARs of *Hypericum perforatum*, and the amounts of phenols, flavonoids, and some individual phenolic compounds increased at high sucrose concentrations (Cui *et al.* 2010). In a previous study, Ferri *et al.* (2011) stated that increasing sucrose concentrations in cell suspension culture of *Vitis vinifera* promoted cell growth and accumulation of phenylpropanoids. However, in this study, fresh weight, growth ratio, PAL activity, and accumulation of total phenolic, rosmarinic acid, and flavonoid decreased with increasing sucrose concentration from 2.0 to 5.0%. Also, in the current study, the levels of stress parameters at high sucrose concentrations (4.0% and 5.0%) were considerably higher than those of low sucrose concentrations (2.0% and 3.0%) (Fig. 3). Consistent with the results of this study, Karataş (2022a) expressed that the PAL activity, biomass, and rosmarinic acid amount decreased in sweet basil adventitious root cultures at high MS medium strengths (≥ 1 MS) where the oxidative stress parameter was higher. Likewise, Copolovici *et al.* (2021) demonstrated that total phenolic and flavonoid content decreased in the leaves of *Ocimum basilicum* subjected to flooding and drought stresses. Moreover, Shahivand *et al.* (2021) reported that phenylpropanoids biosynthetic genes showed different expression patterns in the Iranian red and green cultivars of sweet basil under different stresses, such as heat, cold, light, drought, and salt, and the expression dependent on the cultivar. In this context, Toscano *et al.* (2019) stated that each of the abiotic stresses triggers a specific biosynthetic pathway leading to the production of specific secondary metabolite.

It was emphasized in a previous study that the effects of sucrose concentration on growth and metabolite content vary according to compound type, plant species, culture method, and cell line (Li *et al.* 2016). On the other hand, under the same culture conditions, the accumulations of individual phenolic compounds are affected differently by sucrose concentrations. For instance, Modarres *et al.* (2018) showed that in cell suspension culture of *Salvia leriifolia*, the highest rosmarinic acid content was found in the medium

Table 3. Correlations between bioactive compound accumulations and activities of ABTS, FRAP, DPPH, and PAL

	DPPH	ABTS	FRAP	PAL
Rosmarinic acid	0.917**	0.889**	0.897**	0.821**
Total phenolic	0.878**	0.883**	0.871**	0.904**
Flavonoid	0.956**	0.964**	0.914**	0.884**

**Correlation is significant at the 0.01 level (2-tailed). *DPPH*, DPPH· free radical scavenging activity; *ABTS*, $ABTS^{\cdot+}$ cation radical scavenging activity; *FRAP*, ferric reducing antioxidant power; *PAL*, phenylalanine ammonia lyase activity

Table 2. The influences of sucrose concentration on secondary metabolite contents and PAL activities in adventitious root cultures of *Ocimum basilicum* L. (sweet basil) after 30 d of bioreactor cultures

Sucrose (%)	Rosmarinic acid (mg g^{-1} DW)	Yield of rosmarinic acid (mg L^{-1} DW)	Total phenolics (mg g^{-1} DW)	Flavonoids (mg g^{-1} DW)	PAL activity (nmol h^{-1} mg^{-1} protein)
2	28.11 ± 0.61 a	116.65 ± 3.82 a	28.83 ± 0.70 a	3.26 ± 0.07 a	12.65 ± 0.71 a
3	21.87 ± 0.31 b	104.31 ± 3.25 ab	22.32 ± 0.46 b	2.71 ± 0.06 b	8.99 ± 0.69 b
4	20.96 ± 0.45 b	93.27 ± 4.42 b	21.51 ± 0.51 bc	2.59 ± 0.02 b	8.38 ± 0.84 bc
5	15.78 ± 0.43 c	66.11 ± 2.86 c	16.93 ± 0.48 c	2.31 ± 0.02 c	5.51 ± 0.49 c

The different *letters* within the same *column* demonstrate significant differences according to Duncan's multiple range test ($p < 0.05$). Yield = dry weight (g L^{-1}) × rosmarinic acid content (mg g^{-1} DW). *PAL*, phenylalanine ammonia lyase activity

containing 50 g L^{-1} sucrose while the highest content of caffeic acid was determined at 40 g L^{-1} sucrose. Also, in *in vitro* cultures of *Withania somnifera*, the appropriate sucrose concentration was 2% for adventitious root culture, 3% for cell suspension culture, and 4% for hairy root culture to produce withanolide A (Nagella and Murthy 2014). Overall, the determination of the optimum sucrose concentration to be added to the culture medium is an indispensable parameter for further enhanced production of biologically active compounds and biomass in adventitious root cultures *via* bioreactors.

The influences of sucrose concentration on stress parameters and antioxidant enzyme activities To assess sucrose-induced stress and antioxidant defense system, the amounts of MDA, H_2O_2, and proline and the activities of CAT, POD, and SOD antioxidant enzymes were analyzed (Fig. 3). MDA is a major end product of lipid peroxidation, and its amount is commonly analyzed as an essential marker of oxidative damage. Another parameter analyzed to assess the stress level is H_2O_2, a compound belonging to reactive oxygen species (ROS). Additionally, proline amount is mostly measured to evaluate the stress tolerance capacity. On the other hand, antioxidant enzymes, such as SOD, POD, and CAT, are induced to protect the subcellular components of plants from ROS accumulation (Ozturk *et al.* 2012; Karataş *et al.* 2014).

MDA and H_2O_2 contents of ARs increased progressively with increasing sucrose concentration (Fig. 3). In accordance with this, the lowest amounts of H_2O_2 and MDA were determined in ARs obtained from the medium supplemented with 2.0% sucrose while the highest values were determined at 5.0% sucrose-treated culture. Similarly, the lowest proline content was determined in ARs grown at 2.0% sucrose. However, no statistically significant differences were found between the proline contents of ARs cultured at 3.0, 4.0, and 5.0% sucrose. As for antioxidant enzyme activities, the findings generally showed a similar pattern with the results of H_2O_2, MDA, and proline, except for minor individual differences. The SOD, CAT, and POD activities of ARs were markedly influenced by the sucrose ratios applied to the culture medium. In general, the activities of these antioxidant enzymes tended to increase with increasing sucrose concentration. However, a decrease in CAT and SOD activities was observed at a sucrose concentration higher than 4.0%. The lowest SOD, CAT, and POD activities were determined in ARs obtained from nutrient medium containing 2.0% sucrose.

The findings from this research are similar to those obtained from adventitious roots of *Hypericum perforatum* by Cui *et al.* (2010). In the mentioned study, the roots were greatly affected by water-deficit stress at sucrose concentrations above 3%, and the amounts of H_2O_2, MDA, and proline increased with increasing sucrose concentration. In another study, MDA and H_2O_2 levels were analyzed to evaluate the extent of oxidative damage in ARs of *Morinda citrifolia* grown at 1, 3, 5, 7, and 9% sucrose. It was reported that the H_2O_2 and MDA contents were significantly lower at lower sucrose concentrations (1 and 3%) but increased excessively at higher sucrose concentrations ($\geq 5\%$). In addition, they also stated that the high activity of CAT and G-POD was insufficient to alleviate the harmful effect of H_2O_2 (Baque *et al.* 2012). Accordingly, in the current study, antioxidant enzymes were insufficient to eliminate the excessive increase in H_2O_2 levels at high sucrose concentrations (5%). Likewise, Lee *et al.* (2014) demonstrated that water potential at high sucrose concentrations ($> 5\%$ sucrose) was significantly reduced in adventitious root culture of *Eleutherococcus koreanum*, and the various defense mechanisms against the sucrose-induced osmotic stress were stimulated. In the mentioned study, antioxidant system responses were induced against ROS, such as H_2O_2, which elevated with increasing sucrose concentrations. Also, the proline accumulation increased at high sucrose concentrations. To sum up, sucrose added to the nutrient medium of adventitious root cultures caused significant changes in oxidative stress parameters and antioxidant defense systems, and their levels varied according to the sucrose concentration.

The influences of sucrose concentration on DPPH, ABTS, and FRAP activities The antioxidant activities of ARs according to DPPH, ABTS, and FRAP methods were strongly influenced by the sucrose concentration applied to the culture medium. The activities of DPPH, ABTS, and FRAP gradually decreased as the sucrose concentration increased from 2.0 to 5.0% (Fig. 4). For the three analysis methods, the highest activities were observed in adventitious root cultures treated with 2.0% sucrose. On the other hand, the correlations between antioxidant activities and secondary metabolite accumulation were analyzed using Pearson's correlation test. As shown in Table 3, the antioxidant activities of ARs demonstrated a strong positive correlation with the accumulations of total phenolic, rosmarinic acid, and flavonoid. Likewise, in a previous study, the maximum DPPH activities in cell suspension cultures of *Prunella vulgaris* were reached at 20 to 25 g L^{-1} sucrose concentrations where the highest quantities of phenolic and flavonoid were obtained (Fazal *et al.* 2016). Similarly, in early research on hairy root culture of *Ocimum basilicum*, the antioxidant activity showed a high positive correlation with the amount of rosmarinic acid (Srivastava *et al.* 2016). Karataş (2022a) reported that the DPPH, ABTS, and FRAP activities demonstrated a very strong correlation with rosmarinic acid content in the ARs of sweet basil cultured at different MS salt strengths. Similarly, Baque *et al.* (2012) expressed that high DPHH activities

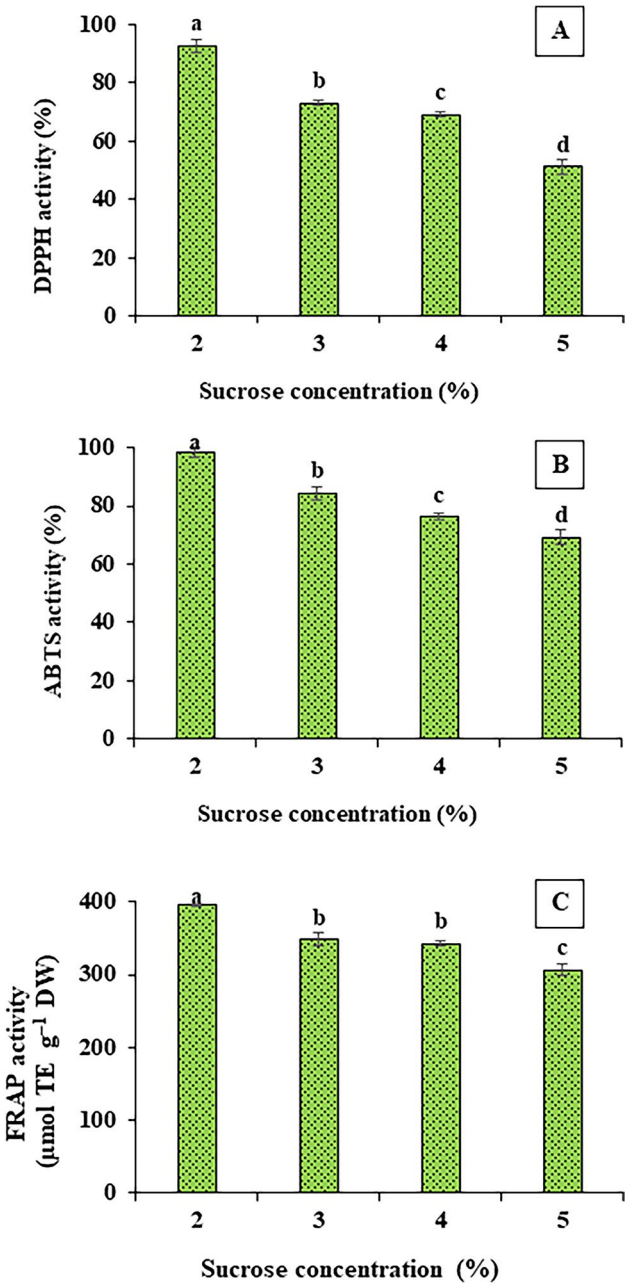

Figure 4. The influences of sucrose concentration on the activities of DPPH (***A***), ABTS (***B***), and FRAP (***C***) in adventitious root cultures of *Ocimum basilicum* L. (sweet basil) after 30 d of bioreactor culture. The different *letters* in the figure indicate significant differences according to Duncan's multiple range test ($p < 0.05$). *Bars* express means ± standard error ($n = 3$). DPPH, DPPH· free radical scavenging activity; ABTS, $ABTS^{\cdot+}$ cation radical scavenging activity; FRAP, ferric reducing antioxidant power.

were found at lower initial sucrose concentrations (1 to 3%) in ARs of *Morinda citrifolia* (up to 3 wk). On the other hand, it was reported that the maximum DPPH and reducing power activities were determined at 50 g L^{-1} sucrose in co-cultured adventitious roots of *Echinacea pallida* and *E. purpurea* (Wu *et al.* 2018). Consequently, the effect of sucrose on antioxidant activity varies according to the applied concentration, culture type, and plant species. The differences in antioxidant capacity are due to the variation of the amounts of antioxidant compounds according to the culture conditions. In this context, in the current study, it was found that ARs of sweet basil exhibited potent antioxidant activity, and the activity demonstrated a strong correlation with accumulation of the biologically active metabolites.

Conclusion

In this study, the effects of different sucrose concentrations applied to sweet basil adventitious root cultures on rosmarinic acid accumulation, biomass parameters, PAL activity, antioxidant capacity, stress levels, and antioxidant enzyme activities were investigated under balloon-type bubble bioreactors (BTBBs). The results obtained from this study showed that the optimum sucrose concentration was 2.0% (*w/v*) for biomass and secondary metabolite production in adventitious root culture of sweet basil. At optimum sucrose concentration, the highest values were reached for fresh weight, growth rate, rosmarinic acid accumulation, PAL activity, and non-enzymatic antioxidant capacities (ABTS, FRAP, and DPPH). The maximum amounts of rosmarinic acid and FW were obtained as 28.11 ± 0.61 mg g^{-1} DW and 91.46 ± 1.91 g L^{-1} at 2.0% sucrose, respectively. On the other hand, the levels of stress parameters and antioxidant enzyme activities were determined to be the lowest at 2.0% sucrose. Consequently, this study shows that determination of optimum sucrose concentration is of great importance to enhance rosmarinic acid accumulation and root proliferation in sweet basil adventitious roots cultured in bioreactors. In addition, the outcomes of the research will make a valuable contribution to the large-scale production of bioactive compounds.

Funding This work was funded by Tokat Gaziosmanpaşa University, Tokat, Turkey (Project Number: 2019/38).

Declarations

Conflict of interest The author declares no competing interests.

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

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 J 26:1–9. https://doi.org/10.1016/j.nfs.2022.01.001

Baque MA, Elgirban A, Lee EJ, Paek KY (2012) Sucrose regulated enhanced induction of anthraquinone, phenolics, flavonoids biosynthesis and activities of antioxidant enzymes in adventitious root suspension cultures of *Morinda citrifolia* (L.). Acta Physiol Plant 34:405–415. https://doi.org/10.1007/s11738-011-0837-2

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

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

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

Copolovici L, Lupitu A, Moisa C, Taschina M, Copolovici DM (2021) The effect of antagonist abiotic stress on bioactive compounds from basil (*Ocimum basilicum*). Appl Sci 11:9282. https://doi.org/10.3390/app11199282

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

Cui XH, Murthy HN, Wu CH, Paek KY (2010) Sucrose-induced osmotic stress affects biomass, metabolite, and antioxidant levels in root suspension cultures of *Hypericum perforatum* L. Plant Cell Tiss Org Cult 103:7–14. https://doi.org/10.1007/s11240-010-9747-z

De-Eknamkul W, Ellis BE (1985) Effects of macronutrients on growth and rosmarinic acid formation in cell suspension cultures of *Anchusa officinalis*. Plant Cell Rep 4:46–49. https://doi.org/10.1007/BF00269203

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

Fazal H, Abbasi BH, Ahmad N, Ali M, Ali S (2016) Sucrose induced osmotic stress and photoperiod regimes enhanced the biomass and production of antioxidant secondary metabolites in shake-flask suspension cultures of *Prunella vulgaris* L. Plant Cell Tiss Org Cult 124:573–581. https://doi.org/10.1007/s11240-015-0915-z

Ferri M, Righetti L, Tassoni A (2011) Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. J Plant Physiol 168:189–195. https://doi.org/10.1016/j.jplph.2010.06.027

Guan H, Luo W, Bao B, Cao Y, Cheng F, Yu S, Fan Q, Zhang L, Wu Q, Shan M (2022) A comprehensive review of rosmarinic acid: from phytochemistry to pharmacology and its new insight. Molecules 27:3292. https://doi.org/10.3390/molecules27103292

Hakkim FL, Kalyani S, Essa M, Girija S, Song H (2011) Production of rosmarinic in *Ocimum sanctum* cell cultures by the influence of sucrose, phenylalanine, yeast extract, and methyl jasmonate. Int J Biol Med Res 2:1070–1074

Havir EA, Mchale NA (1987) Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol 84:450–455

Ilieva M, Pavlov A (1997) Rosmarinic acid production by *Lavandula vera* MM cell-suspension culture. Appl Microbiol Biotechnol 47:683–688. https://doi.org/10.1007/s002530050995

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 Tiss Org Cult 149:61–79. https://doi.org/10.1007/s11240-022-02286-5

Karam NS, Jawad FM, Arikat NA, Shibli RA (2003) Growth and rosmarinic acid accumulation in callus, cell suspension, and root cultures of wild *Salvia fruticose*. Plant Cell Tiss Org Cult 73:117–121. https://doi.org/10.1023/A:1022806420209

Karataş İ (2022a) Production of rosmarinic acid and biomass from adventitious root cultures of *Ocimum basilicum* by optimization of medium components in airlift bioreactors. Plant Cell Tiss Org Cult 151:235–251. https://doi.org/10.1007/s11240-022-02347-9

Karataş İ (2022b) Reyhan bitkisinin (*Ocimum basilicum* L.) adventif kök kültürlerinde rosmarinik asit üretim olanaklarının ve antioksidan kapasitenin araştırılması. KSÜ Tarım ve Doğa Derg 25:459–466. https://doi.org/10.18016/ksutarimdoga.vi.947605

Karataş İ, Öztürk L, Demir Y, Ünlükara A, Kurunç A, Düzdemir O (2014) Alterations in antioxidant enzyme activities and proline content in pea leaves under long-term drought stress. Toxicol Ind Health 30:693–700. https://doi.org/10.1177/0748233712462471

Khanam MN, Anis M, Javed SB, Mottaghipisheh J, Csupor D (2022) Adventitious root culture-an alternative strategy for secondary metabolite production: a review. Agronomy 12:1178. https://doi.org/10.3390/agronomy12051178

Kikowska M, Budzianowski J, Krawczyk A, Thiem B (2012) Accumulation of rosmarinic, chlorogenic and caffeic acids in *in vitro* cultures of *Eryngium planum* L. Acta Physiol Plant 34:2425–2433. https://doi.org/10.1007/s11738-012-1011-1

Kim DS, Hwang BK (2014) An important role of the pepper phenylalanine ammonia-lyase gene (*PAL1*) in salicylic acid-dependent signalling of the defence response to microbial pathogens. J Exp Bot 65:2295–2306. https://doi.org/10.1093/jxb/eru109

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

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. https://doi.org/10.1007/978-94-017-9223-3_10

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

Modarres M, Bahabadi SE, Yazdi MET (2018) Enhanced production of phenolic acids in cell suspension culture of *Salvia leriifolia* Benth. using growth regulators and sucrose. Cytotechnology 70:741–750. https://doi.org/10.1007/s10616-017-0178-0

Morkunas I, Marczak Ł, Stachowiak J, Stobiecki M (2005) Sucrose-induced lupine defense against *Fusarium oxysporum*: sucrose-stimulated accumulation of isoflavonoids as a defense response of lupine to *Fusarium oxysporum*. Plant Physiol Biochem 43:363–373. https://doi.org/10.1016/j.plaphy.2005.02.011

Morkunas I, Narożna D, Nowak W, Samardakiewicz S, Remlein-Starosta D (2011) Cross-talk interactions of sucrose and *Fusarium*

oxysporum in the phenylpropanoid pathway and the accumulation and localization of flavonoids in embryo axes of yellow lupine. J Plant Physiol 168:424–433. https://doi.org/10.1016/j.jplph.2010.08.017

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 Tiss Org Cult 118:1–16. https://doi.org/10.1007/s11240-014-0467-7

Nagella P, Murthy HN (2014) Production of withanolides from cell and organ cultures of *Withania somnifera* (L.) Dunal. In: Paek KY, Murthy HN, Zhong JJ (eds) Production of biomass and bioactive compounds using bioreactor technology. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-9223-3_12

Nazir M, Ullah MA, Younas M, Siddiquah A, Shah M, Giglioli-Guivarc'h N, Hano C, Abbasi BH (2020) Light-mediated biosynthesis of phenylpropanoid metabolites and antioxidant potential in callus cultures of purple basil (*Ocimum basilicum* L. var *purpurascens*). Plant Cell Tiss Org Cult 142:107–120. https://doi.org/10.1007/s11240-020-01844-z

Nazir S, Jan H, Zaman G, Khan T, Ashraf H, Meer B, Zia M, Drouet S, Hano C, Abbasi BH (2021) 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 49:625–633. 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 17:7227–7236

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 Tiss Org Cult 137:65–75. https://doi.org/10.1007/s11240-018-01552-9

Pekal A, Pyrzynska K (2014) Evaluation of aluminium complexation reaction for flavonoid content assay. Food Anal Methods 7:1776–1782

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

Rajesh M, Sivanandhan G, Arun M, Vasudevan V, Theboral J, Girija S, Manickavasagam M, Selvaraj N, Ganapathi A (2014) Factors influencing podophyllotoxin production in adventitious root culture of *Podophyllum hexandrum* Royle. Acta Physiol Plant 36:1009–1021. https://doi.org/10.1007/s11738-013-1479-

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 26:1231–1237

Romano R, De Luca L, Aiello A, Pagano R, Di Pierro P, Pizzolongo F, Masi P (2022) Basil (*Ocimum basilicum* L.) leaves as a source of bioactive compounds. Foods 11:3212. https://doi.org/10.3390/foods11203212

Ruan M, Li Y, Li X, Luo J, Kong L (2012) Qualitative and quantitative analysis of the major constituents in Chinese medicinal preparation Guan-Xin-Ning injection by HPLC–DAD–ESI-MS^n. J Pharm Biomed Anal 59:184–189. https://doi.org/10.1016/j.jpba.2011.10.007

Shahivand M, Drikvand RM, Gomarian M, Samiei K (2021) Key genes in the phenylpropanoids biosynthesis pathway have different expression patterns under various abiotic stresses in the Iranian red and green cultivars of sweet basil (*Ocimum basilicum* L.). Plant Biotechnol Rep 15:585–594. https://doi.org/10.1007/s11816-021-00697-y

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

Sivanandhan G, Rajesh M, Arun M, Jeyaraj M, Dev GK, Manickavasagam M, Selvaraj N, Ganapathi A (2012) Optimization of carbon source for hairy root growth and withaferin A and withanone production in *Withania somnifera*. Nat Prod Commun 7:1271–1272

Skrypnik L, Novikova A, Tokupova E (2019) Improvement of phenolic compounds, essential oil content and antioxidant properties of sweet basil (*Ocimum basilicum* L.) depending on type and concentration of selenium application. Plants 8:458. https://doi.org/10.3390/plants8110458

Slinkard K, Singleton VL (1977) Total phenol analysis: automation and comparison with manual methods. Am J Enol Viticult 28:49–55

SPSS 20. IBM SPSS Statistics for Windows, IBM Corp., Version 20.0, Released 2011, Armonk, NY

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 Tiss Org Cult 126:19–32. https://doi.org/10.1007/s11240-016-0973-x

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

Tauzin AS, Giardina T (2014) Sucrose and invertases, a part of the plant defense response to the biotic stresses. Front Plant Sci 5:293. https://doi.org/10.3389/fpls.2014.00293

Tepe B, Sokmen A (2007) Production and optimisation of rosmarinic acid by *Satureja hortensis* L. callus cultures. Nat Prod Res 21:1133–1144

Toscano S, Trivellini A, Cocetta G, Bulgari R, Francini A, Romano D, Ferrante A (2019) Effect of preharvest abiotic stresses on the accumulation of bioactive compounds in horticultural produce. Front Plant Sci 10:1212. https://doi.org/10.3389/fpls.2019.01212

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

Vogelsang K, Schneider B, Petersen M (2006) Production of rosmarinic acid and a new rosmarinic acid 3′-O-β-D-glucoside in suspension cultures of the hornwort *Anthoceros agrestis* Paton. Planta 223:369–373. https://doi.org/10.1007/s00425-005-0089-8

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

Yaseen M, Ahmad T, Sablok G, Standardi A, Hafiz IA (2013) Review: role of carbon sources for *in vitro* plant growth and development. Mol Biol Rep 40:2837–2849. https://doi.org/10.1007/s11033-012-2299-z

Yin S, Gao W, Liang Y, Wang J, Liu H, Wei C, Zuo B (2013) Influence of sucrose concentration and phosphate source on biomass and

metabolite accumulation in adventitious roots of *Pseudostellaria heterophylla*. Acta Physiol Plant 35:1579–1585. https://doi.org/10.1007/s11738-012-1199-0

Zeljković SĆ, 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