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REVIEW

Basil (*Ocimum* L.) cell and organ culture for the secondary metabolites production: a review

Dragana Jakovljević[1] · Milan Stanković[1] · Marzena Warchoł[2] · Edyta Skrzypek[2]

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Abstract

Species from the genus *Ocimum* L. (basil) are among the most cultivated plants due to their large content of secondary metabolites, in particular essential oils and phenolic compounds. Still, different conditions of basil cultivation cause significant variations of quantitative and qualitative characteristics of material originating from basil plants. The application of plant tissue culture to produce biologically active compounds is a well-established alternative to the cultivation of whole plants. It provides the opportunity to obtain biotechnologically valuable plant characteristics, including a high content of secondary metabolites such as phenolic acids and flavonoids for the shortest period. The present paper summarizes the most cultivated basil genotypes worldwide and data about the mass propagation, somatic embryogenesis, basil cell and organ cultures, together with the main properties regarding the enhanced synthesis of secondary metabolites under tissue culture conditions. For most of the studies, increased synthesis of biologically active compounds (in particular phenolic acids, isoprenoids, and flavonoids) can be seen. However, for many of them, information regarding the used genotype or origin of plant material is missing. Considering the large number of species and cultivars belonging to the genus *Ocimum* L., appropriate utilization can provide maximal exploitation. Understanding how particular genotypes respond to specific conditions, treatments, and types of culture for enhancing the production of secondary metabolites could be the basis in designing protocols and further progress.

Key message

In this review, we described recent progress in secondary metabolites production of *Ocimum* L. species. The content of secondary metabolites, mainly phenolics and flavonoids, can be enhanced by various types of in vitro cultures.

Keywords *Ocimum basilicum* L. · Secondary metabolites · Callus · Cell suspension · Phenolic compounds

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	6-Benzylaminopurine
CMCs	Cambial meristematic cells
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
KIN	Kinetin
MeJ	Methyl jasmonate
MS	Murashige and Skoog medium
n.s.	Non-specified
NAA	1-Naphthylacetic acid
PAL	Phenylalanine ammonium lyase
PGRs	Plant growth regulators
SE	Somatic embryogenesis
SMs	Secondary metabolites
TDZ	Thidiazuron (1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea

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✉ Dragana Jakovljević
dragana.jakovljevic@pmf.kg.ac.rs

[1] Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia

[2] The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Kraków, Poland

Introduction

Basil (genus *Ocimum* L., family Lamiaceae) is a popular herb commonly used in many kinds of food and is one of the major essential oils producing species (Grayer et al. 2002). Basil extracts show antioxidant and antimicrobial activities due to their phenolic acids and aromatic compounds suggesting that they might have potential human health benefits (Gutierrez et al. 2008; Hussain et al. 2008). As basil plants are cultivated worldwide, the differences in the conditions of cultivation and commercial exploitation followed by the significant variations of quantitative and qualitative characteristics of material originating from basil plants can be seen. As a contemporary method for overcoming cultivation problems plant tissue culture provides the opportunity to obtain valuable plant characteristics such as high content of biologically active secondary metabolites. Furthermore, the application of new approaches to well-known tissue culture methods follows the increased demand for products with biological activities originating from natural sources.

The cultivation of basil plants is intensified globally. For example, in the last decades, estimated volume of basil essential oil produced globally was 50–100 tones, while indicative price of essential oils available on the market from basil originated from India was 40–45 € (Lubbe and Verpoorte 2011). As an aromatic plant grown for fresh herbs, basil is mainly cultivated in the system of conventional production in the open field, however, under these conditions it is impossible to control environmental factors such as light or temperature (Bączek et al. 2019). Furthermore, it is shown that basil plants can respond with better yield under soilless systems than conventional systems (Saha et al. 2016) with two times lower production costs (Khater et al. 2021).

Many decades of cultivation and traditional use of basil have contributed to variability among species and cultivars of the genus *Ocimum* L. Nowadays the number of basil cultivars is variable mostly because of a man interference with the hybridization, cultivation, and selection. According to www.theplantlist.org over 70 taxa are with accepted scientific names, whereas according to Labra et al. (2004) the *Ocimum* L. genus includes more than 150 species. Taking into account the number of species and cultivars, it is clear that the adequate utilization of the rich basil gene pool together with the application of modern in vitro methods can provide successful cultivation and maximal exploitation, and both are of multiple importance—scientific, as well as practical. However, in many cases, published papers do not provide information regarding used genotype, seed distributors or voucher specimen for particular cultivar, which is accompanied by interchangeable citations through the literature—for example, lemon basil cultivar is frequently cited as *O.* × *citriodrum*, *O. americanum*, or *O. basilicum* var. *citriodorum* (Pyne et al. 2018). It is clear that one who is dealing with basil tissue culture, particularly with the enhanced production of secondary metabolites, faces difficulties to compare obtained results or searching published data. Therefore, there is a need to summarize literature data about various cultures of basil, but with a special emphasis on the presence or the absence of sample origin.

The presented review will attempt to summarize basil plants available worldwide and cultivated in vitro (with a background of their in vitro multiplication and fast propagation) and to evaluate the advantages/disadvantages of developed in vitro systems. Although a large number of protocols is available, it is necessary to distinguish the protocols that can be regarded as successful, i.e., provides mass propagation and cultivation with an adequate number of shoots per initial explant, and relate them with a particular genotype. The various basil tissue cultures in terms of secondary metabolites production were also evaluated and discussed. The establishment of in vitro tissue culture of basil plants with the aim of production of compounds of interest in recent years has been intensified, primarily due to the efficient production of particular metabolites at significantly higher concentrations and in a shorter time (Jakovljević et al. 2017a; Jakovljević et al. 2019; Açıkgöz, 2020, 2021; Jakovljević et al. 2021). The difficulties that may arise during these processes are mainly related to the plant regulators and elicitor effects in the media and genotype used. Since in recent years great theoretical and practical importance has been related to plant secondary metabolites and their biological activity, both regarding the clarification of the plant response to stress factors and the synthesis of metabolites of interest, there is a need to have a unique presentation of methods used (callus, cell suspension, bioreactors, hairy roots and somatic embryogenesis) and to emphasis main biologically active components obtained via these methods in largest quantities, but with the critical point of view in terms of genotype-based selections, type of tissue culture applied, and key factors mediating success of the used method.

Basil—king of the herbs

Plants from the genus *Ocimum* L. (known as basil and „king of the herbs") are annual and perennial herbs and shrubs native to Asia, Africa, Central and South America (Labra et al. 2004). Basil is used in many countries around the world. The leaves and tops of shoots are used in the food and beverage industry and as aromatic spices, but also in traditional medicine due to the antiseptic, analgesic, antiulcer, antioxidant, antibacterial and antifungal, as well as anti-inflammatory and antituberculosis activities (Jakovljević 2018). The most valued components are essential oils, namely methyl-chavicol and linalool (Labra et al. 2004; Piras et al. 2018), and phenolic

components—rosmarinic and caffeic acid, rutin and isoquercetin (Jakovljević et al. 2019; Açıkgöz 2020). Previous research has shown that the content of important components of basil varies, and that the quantitative and qualitative composition of the main bioactive compounds is determined by genetic characteristics (Kwee and Niemeyer 2011) and various environmental factors including climate, season, and sampling period (Hussain et al. 2008; Baldim et al. 2018). Also, the time of sampling, plant parts from which the material originates, as well as processing and extraction of materials further affect the quantitative and qualitative composition of basil (Hussain et al. 2008; Jakovljević et al. 2019). Due to all these factors, the production of biologically active components of basil is either limited or requires significant investments. For detailed studies of basil, or quantitative–qualitative screening of different varieties that exist within the genus *Ocimum* L., the use of conventional plantations is a technique that is extensive and financially demanding. Therefore, it is necessary to establish a system that can be managed in smaller areas (Srivastava et al. 2014).

Taxonomically, according to Carović-Stanko et al. (2011), there are five main varieties of *O. basilicum*: var. *basilicum* L. (most popular cultivars are 'Genovese' and 'Sweet Basil'), var. *difforme* Benth., var. *purpurascens* Benth. (most popular cultivar is 'Dark Opal'), var. *thyrsiflorum* /L./Benth., and var. *minimum*. Among other species, *O. africanum* Lour. (syn. *O.* × *citriodorum* Vis.), *O. americanum* L. (syn. *O. canum* Sims.), *O. gratissimum* L., and *O. tenuiflorum* L. (syn. *O. sanctum* L.) are also commonly grown because of their economical and medicinal importance (Carović-Stanko et al. 2010). There are also several types of basil—citriodorum types with lemon flavour, purple types with sweet flavour, as well as large-leafed or small leafed types, and compact types (such as var. *thyrsiflora* commonly called 'Thai' basil). For detailed description of basil taxa classification see the study of Carović-Stanko et al. (2010), and Carović-Stanko et al. (2011). Regardless of the taxonomy, commercially grown basil varieties are most often classified based on the size and color of leaves (small or large, green to purple) and flowers (white, pink, red, purple). However, these characteristics due to intense cultivation and hybridization show a high degree of variability (Patel et al. 2016). Differences may be further determined by the method of cultivation and geographical origin of the sample (Jakovljević et al. 2017a). Controlled conditions of growth and development, with the absence of fluctuations of abiotic and biotic environmental factors, make tissue culture a suitable system for investigations of different basil varieties. Furthermore, the culture of plant cells, tissues, and organs includes methods that are widely used in the production of secondary metabolites, primarily because the production of bioactive substances in vitro is reliable and predictable, independent of geographical location, seasonal and external factors, allows content modification and obtaining products of appropriate qualitative and quantitative composition (Karuppusamy 2009; El-Salam et al. 2015; Açıkgöz 2020). Therefore, it should be obligatory to provide basic backup information's regarding seeds distributors, genotype used, geographic origin, or voucher specimen for the tested cultivar.

Basil micropropagation

Different basil cultivars which are grown from seeds show significant variations in both quantitative and qualitative characteristics depending on cultivation conditions. Therefore, to achieve the desired characteristics it is often necessary to select and propagate selected plants vegetatively. Also, conventional propagation techniques for certain plant species may require a longer period and large financial investments, and for these plants propagation through in vitro tissue culture is of great practical importance. In addition to practical and economic applications, the mass propagation of plant material is important in the case of plants that can produce a significant amount of biologically active compounds since the method of in vitro propagation on a medium of appropriate composition enables the fast cloning and preservation of the desired genotypes in a short time, and multiplication of plants released from pathogens (Máthé et al. 2015; da Silva et al. 2017). Furthermore, the system of organized and controlled growth enables the reduction of various effects on natural populations of plant species that have different practical applications. When it comes to the in vitro propagation and regeneration of basil, an insufficient number of protocols are available, considering that the protocols in which the formation of a low number of shoots per initial explant has been observed cannot be applied for mass propagation and cultivation. Therefore, for a high regeneration rate, it is necessary to first develop a successful protocol with a high multiplication rate (Siddique and Anis 2008). This has practical importance when it comes to secondary metabolites (SMs) production having in mind the quantity of bioactive compounds produced by the basil plants.

Basil micropropagation can be established using cotyledons derived from in vitro germinated plants of *O. basilicum* L. in which lower levels of auxin in combination with different concentrations of cytokinin favored the development of calli (Dode et al. 2003). Briefly, the explants were placed on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 0.2 mg L^{-1} naphthylacetic acid (NAA) and 0.0–5.0 mg L^{-1} 6-benzylaminopurine (BAP). The highest efficiency of shoots formation (66.7%), as well as the most shoots per explant (3.46), was observed on medium with the addition of 5 mg L^{-1} BAP. The hormone-free MS medium promotes root development,

so more than 90% of the shoots form the root system after transfer to the hormone-free medium. Ekmekci and Aasim (2014) established a protocol for in vitro regeneration and successful acclimatization of sweet basil originated from Turkey. A maximum number of shoots from epicotyl (3.22) and shoot tip explants (3.58) were obtained on MS medium with 2.4 mg L^{-1} thidiazuron (TDZ) and 0.1 mg L^{-1} indole-3-butyric acid (IBA), while hypocotyl explant induced maximum number of shoots per explant (5.17) on MS medium with 2.0 mg L^{-1} TDZ. The authors developed an efficient method for micropropagation of basil and the established protocol can be used for isolation of secondary metabolites or genetic transformation studies of basil.

An efficient regeneration system for three *O. basilicum* L. varieties (named GPR—all green selection of Purple Ruffles; Sweet Dani lemon basil and PU SW69-3—methylcinnamate basil) was reported by Phippen and Simon (2000). By using the young leaf explant tissue, authors established shoot regeneration for these basil cultivars through indirect organogenesis. For callus induction MS medium with 16.8 µM TDZ was used, and the next obtained shoots were rooted on MS medium without plant growth regulators. It is shown that the individual shoots developed roots after 7 to 10 days and had sufficient root system for transplantation to ex vitro conditions after about 30 days. Basil plants obtained in this way were successfully acclimated with a survival rate of 98%. Still, it should be mentioned that the shoot regeneration efficiency varied significantly—from 28% for methylcinnamate basil to 85% for Sweet Dani. Moreover, all regenerated plants expressed anthocyanin pigments which were short-living in Sweet Dani and methylcinnamate basil, but in GPR basil variety resulted in stable purple basil plants.

Nodal explants as a starting material were used for successful in vitro micropropagation of *O. basilicum* L. by Shahzad et al. (2012). The best results for shoot growth were obtained by applying MS medium with 10.0 µM BAP and 30 mg L^{-1} L-glutamine where after 8 weeks of in vitro culture average of 13.4 shoots per initial explant were formed. Half strength of MS medium with 5.0 µM IBA was the most adequate for shoots rooting and plants developed in this way were successfully planted into the soil. According to Kiferle et al. (2014) the nodal segments obtained during the flowering period from basil grown under controlled conditions can also be used for propagation. The authors used 1 cm long nodal segments and MS medium with 0.25 mg L^{-1} BAP to initiate shoots and MS medium (50%) without growth regulators for rooting. This method of propagation proved to be successful for basil with green leaves ('Genovese'), as well as for basil with purple leaves ('Dark Opal'). On the other hand, Siddique and Anis (2008) established rapid, efficient and large-scale propagation of *O. basilicum* L. through the in vitro culture of nodal segments with axillary buds from mature plants. The highest multiplication rate was achieved on MS medium (50%) with 2.5 µM BAP and 0.5 µM indole-3-acetic acid (IAA). MS medium with 1.0 µM IBA was the most adequate for rooting compared to the medium where the IBA and NAA were used together. In this study, the survival rate of produced plants was very high (90%).

Bhuvaneshwari et al. (2016) propagated in vitro two basil species *O. basilicum* L. and *O. tenuiflorum* L. The nodal explants were cultured on MS medium with different concentrations of BAP alone or in combination with kinetin (KIN) (0.25–2.0 mg L^{-1}) and IAA (0.25–2.0 mg L^{-1}). Results showed that for both species 1 mg mL^{-1} BAP in combination with 0.5 mg mL^{-1} KIN resulted in the highest shoot induction (99 and 98%, respectively). Lately, Shukla et al. (2021) were micropropagated of *O. sanctum* L. ('Tulsi basil', line named Vrinda) on semisolid MS supplemented with 1.1 µM BAP, 0.3 µM gibberellic acid and 0.6% activated charcoal. The authors pointed out that the developed method have significant potential for the commercial-scale production of plant material free of biological contaminants and chemicals.

Basil in vitro cultures as a source of secondary metabolites

Various substances produced by plants can be classified according to their origin into primary and secondary metabolites. While primary metabolites are molecules essential for plant growth and development, secondary metabolites (SMs) are produced by a sophisticated mechanism, so plants can develop in response to adverse environmental conditions (Caretto et al. 2015; Yoshikawa et al. 2018). SMs are important non-enzymatic components of the plant defense system since they play a crucial role in plant adaptations to various factors. The components of plant secondary metabolism are derivatives of isoprenoid, phenylpropanoid, alkaloid, or fatty acid pathways. Plant phenolic compounds are products of shikimic or mevalonic acid pathways. In higher plants, the shikimic acid pathway (phenylpropanoid metabolism) is dominant compared to the mevalonic acid pathway, which is primarily important for the synthesis of bacterial and fungal metabolites (Manach et al. 2004; Mandal et al. 2010). Callus culture, cell suspension, and hairy root cultures are in recent years extensively used to evaluate the possibilities of plant-derived SMs production. By reviewing the literature regarding the basil SMs production under tissue culture conditions it can be seen that most of the investigations include phenolic acids (rosmarinic acid, caffeic acid, chicoric acid), isoprenoids (betulinic acid, ursolic acid, oleanolic acid), and flavonoids (rutin, isoquercetin, cyanidin and peonidin). Chemical structure of main phenolic compounds which are investigated in basil in vitro studies is presented in Figs. 1 and 2. Among essential oils most investigated are methyl

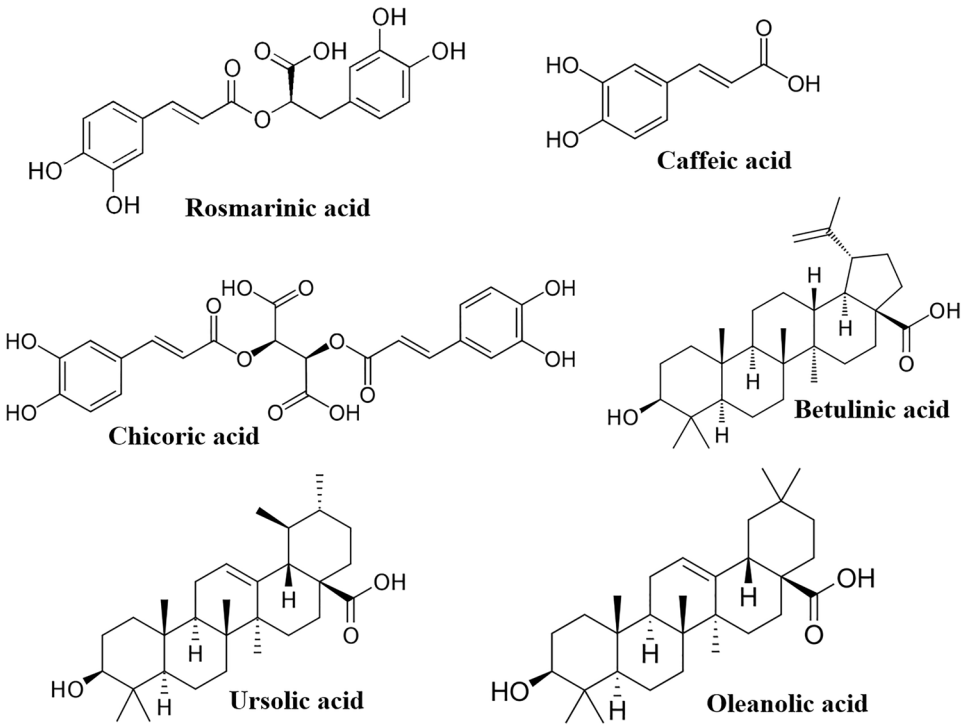


Fig. 1 Chemical structure of main basil phenolic acids (rosmarinic acid, caffeic acid and chicoric acid) and isoprenoids (betulinic acid, ursolic acid and oleanolic acid) induced under tissue culture conditions

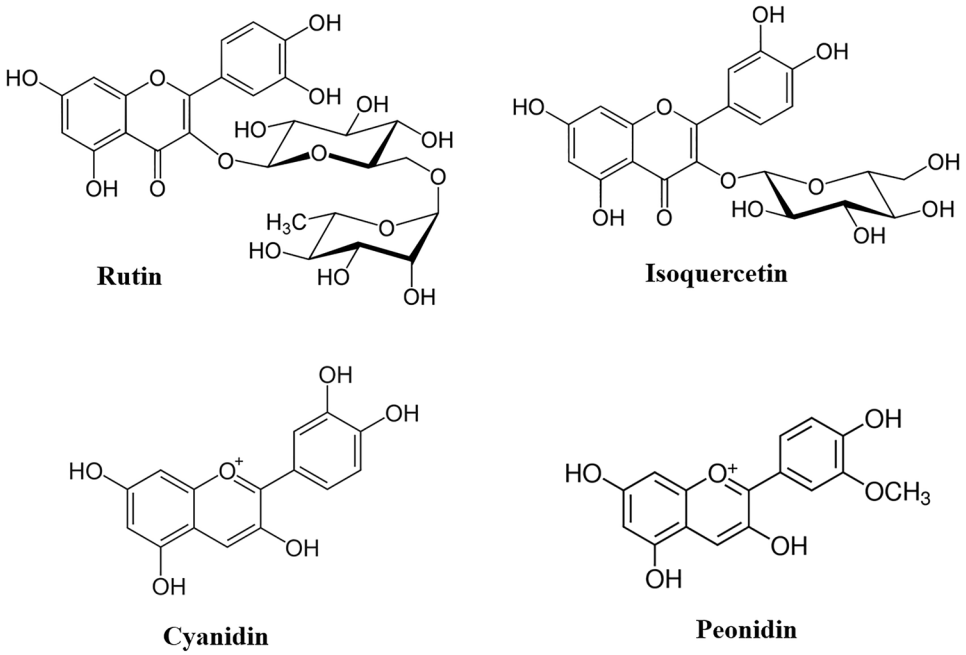
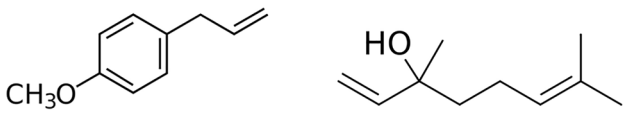


Fig. 2 Chemical structure of main basil flavonoids induced under tissue culture conditions

chavicol and linalool (Fig. 3) with only a few records of these SMs. Besides the fact that basil plants are rich in essential oils, these bioactive compounds are least investigated in vitro possibly due to the low yield of essential oils under tissue culture conditions. Consequently, extensive research regarding the essential oil production of basil cells cultured in vitro should be emphasized in the future.

When it comes to the basil phenolic compounds synthesized through the phenylpropanoid metabolism, it is known that the first step in this phenylpropanoid pathway is catalysed by an enzyme phenylalanine ammonium lyase (PAL). The reaction catalysed by the PAL enzyme is based on the deamination of phenylalanine to cinnamic acid and ammonium (Fraser and Chapple 2011). In this way, the synthesis



Methyl chavicol **Linalool**

Fig. 3 Chemical structure of main essential oil components of basil induced under tissue culture conditions

of different aromatic metabolites (phenolic compounds) can begin, which can be further classified into classes (or subclasses) including lignins, coumarins, flavonoids, anthocyanins, stilbene, phenolic acids, etc. Plant polyphenols (phenolic compounds) are one of three main groups of secondary metabolites, primarily classified based on their chemical structure (Larbat et al. 2014; Yoshikawa et al. 2018) which includes an aromatic ring with several hydroxyl groups. Phenolic compounds are used in the pharmaceutical and food industry primarily due to antioxidant, anticancer, antimicrobial, and various therapeutic activities (Tapas et al. 2008; Jakovljević et al. 2013). Flavonoids are phenolic compounds located in the vacuoles of plant cells. The synthetic pathway of flavonoids is part of phenylpropanoid metabolism where different groups of flavonoids are formed, including isoflavones, flavones, flavonols, anthocyanins, and others (Mierziak et al. 2014). Flavonoids formed by phenylpropanoid metabolism undergo further modifications that lead to various changes in solubility, stability, and reactivity. The antioxidant capacity of flavonoids is based on the ability to scavenge free radicals and is correlated with the number and position of hydroxyl groups attached to the phenolic ring. Due to the diverse chemical structure resulting from the heterogeneity of substituents, flavonoids show a large number of biological activities (Mierziak et al. 2014; Stanković et al. 2019). Phenolic acids are synthesized by phenylpropanoid metabolism by esterification of cinnamic and benzoic acid derivatives and their incorporation into the cell wall. Cinnamic acid derivatives are ubiquitous phenolic acids and studies of these secondary metabolites have been intensified, primarily due to the significant biological activities they show. The most important hydroxycinnamic acids in basil are presented in many food sources, drinks and attracted interests due to antioxidant, antitumor, anti-inflammatory, and antimicrobial functions (Jakovljević et al. 2019).

The antioxidant activity of basil extracts is the consequence of the defensive function of phenolic compounds, i.e., their high reactivity as a hydrogen or electron donor, the ability of an aromatic phenolic radical to localize an unpaired electron, as well as the ability to remove superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals. Additionally, phenolic compounds can also react with metal ions (Rohma et al. 2010; Falleh et al. 2011; Jdey et al. 2017). The antioxidant capacity of SMs (the ability of SMs to neutralize free radicals) and overall biological activity are related to their chemical structure. It has been confirmed that simple, monomeric polyphenols are weaker antioxidants compared to more complex polyphenols, as well as that a higher degree of polymerization inhibits lipid peroxidation, allowing better metal reduction and better ability to neutralize free radicals (Moure et al. 2001). In recent years the use of plant antioxidants in the form of additives, functional foods, and various forms of medical and pharmaceutical material increasing (Povichit et al. 2010) since it is believed that plant polyphenolic components have biologically active properties that may be important in various treatments (Sousa et al. 2015; Zengin et al. 2015). Antioxidant activity of basil extracts obtained through the tissue culture conditions is confirmed in the case of *O. basilicum* L. (var. *basilicum* cv. 'Genovese', var. *minimum,* var. *purpurascens* cv. 'Dark Opal'*)* and *O.* × *citriodorum* L. seedling cultures (Jakovljević et al. 2019), callus culture from *O. sanctum* L. (Hakkim et al. 2007), *O. basilicum* L. var. *purpurascens* (Nazir et al. 2019, 2020a, 2020b) and *O. basilicum* L. cv.'Thai basil' (Nazir et al. 2020c, 2021), as well as in hairy roots from three *O. basilicum* L. cultivars (B3 Subja, B12 Holy Green, B13 Red Rubin), and in cell suspensions from *O. basilicum* L. (Açıkgöz, 2020, 2021).

Seedling culture

Seedling culture (culture of whole, intact young plants derived from seeds) can be established through the germination of surface-sterilized seeds which are put on appropriate media. This method finds significant application in seeds from plant species characterized by a low percentage of seed germination, or in seeds for which the additional environmental requirements exist regarding the germination process. Additionally, this type of tissue culture could be a source of aseptic explants for both solid and liquid in vitro cultures with minimal loss of funds and materials. Still, seeds of different *O. basilicum* L. cultivars (var. *basilicum* L. cv. 'Genovese', var. *minimum* L., var. *purpurascens* Benth. cv. 'Dark Opal') and *O.* × *citriodorum* Vis. can demonstrate significant differences in germination characteristics (speed, uniformity, rate of germination, and germination percentage) depending on the availability of nutrients (quarter, half or full-strength), and nitrogen form used (Jakovljević et al. 2017a; Jakovljević et al. 2020). Therefore, to obtain a high level of germinated seeds with significant rate and uniformity of germination under in vitro conditions, culture media composition must be adjusted according to the investigated genotype. Da Silva et al. (2017) showed that seedling of *O. basilicum* L. cv. 'Red Rubin' differed due to alterations of culture medium (concentration of MS salts, charcoal, sucrose, and potassium

iodine). Furthermore, the inappropriate composition of the medium can inhibit the induction of calli.

In recent years seedling culture was used for investigations of antioxidant responses of various basil cultivars under stressful conditions and it is shown that this tissue culture method could be successfully used for fast screening and evaluation of tolerance to abiotic stresses (temperature, salinity, nutrient deprivation) for various basil cultivars (Jakovljević et al. 2017a, 2017b, 2019, 2021). Furthermore, seedling culture are regarded as a promising method to obtain a high concentration of secondary metabolites with antioxidant activity (particular phenolic acids) in the shortest time. To investigate the possibilities of induced synthesis of secondary metabolites under conditions of nutrient limitation four different basil cultivars (*O. basilicum* L. var. *basilicum* L. cv. 'Genovese', var. *minimum* L., var. *purpurascens* Benth. cv. 'Dark Opal') and *O.* × *citriodorum* Vis.) were exposed to stress treatments with solid medium mineral deficiency applied with two forms of nitrogen—nitrate alone or in combination with ammonium (Jakovljević et al. 2019). Obtained results demonstrated major differences of tested cultivars grown under the conditions of nutrient-deprived seedling cultures, whereas the type of cultivar influenced the level of produced compounds. Briefly, after 1 month of in vitro germination and growth of seedlings, roots from tested basil cultivars were able to produce a high concentration of total phenolics, flavonoids, together with rosmarinic and caffeic acid with significant antioxidant activities. Additionally, the authors pointed out that the concentration of these metabolites in basil roots was several times higher under the described method of in vitro cultivation compared to the leaves from basil grown under standard open-field methods of cultivation. Different concentrations of obtained metabolites for particular genotype are presented in Table 1. Having in mind aseptic, strictly controlled cultivation and uniformity of plant material free of pathogens, which could be obtained through the seedling culture, this in vitro method of basil cultivation could be regarded as suitable to produce economically significant biologically active secondary metabolites in a short time. However, to test the limits of this approach more studies with various basil genotypes under different stress conditions are needed.

Callus culture

Callus culture allows the maintenance of undifferentiated plant cells on a solid medium for a long period of time. There are several phases in the establishment of callus culture which is accompanied by the changes in the size of cells and tissues, as well as the changes in structure and metabolic activity of cells. The success of callus culture establishment and maintenance mainly depends on the correct choice of initial explant together with the concentration of growth regulators and nutrients in the medium. Although callus may retain the characteristics of the plant or plant organ from which it was developed, callus cells may be characterized by a different metabolism. Therefore, placement of callus in conditions of intensive metabolic processes in cells and synthesis of bioactive components today finds great application in the synthesis of biologically active metabolites of interest. The biotechnological advantage of secondary metabolites production through the callus culture is mainly related to yield and biomass conservation (Nazir et al. 2019, 2020a). According to Efferth (2019), the major advantages of solid cell cultures are related to the generation of compounds of choice independently of environmental factors, cultivation of cells not attacked by microorganisms and insects, as well as reduction of costs and improvement of production through the regulation of SMs production. Still, commercial-scale production demands the successful establishment of callus culture which is related to the proper balance of plant growth regulators (PGRs) and controlled environmental conditions (Adil et al. 2019). Furthermore, SMs synthesis could be linked to the phytohormonal control regulation

Table 1 Production of phenolic compounds in shoots and roots of seedlings from different basil genotypes grown in vitro (according to Jakovljević et al. 2019)

	O. basilicum L. var. *minimum* L.		*O. basilicum* L. var. *basilicum* L. cv. 'Genovese'		*O. basilicum* L. var. *purpurascens* Benth. cv. 'Dark Opal'		*O.* × *citriodorum* Vis.	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Total phenolic content (mg GA g^{-1} DW)	47.41	184.76	92.82	152.16	107.05	152.59	108.86	110.40
Flavonoid concentration (mg RU g^{-1} DW)	23.88	6.91	19.01	7.11	11.14	11.10	19.13	7.89
Rosmarinic acid (mg g^{-1} DW)	21.00	38.80	44.20	61.20	44.30	42.40	32.50	39.20
Caffeic acid (mg g^{-1} DW)	1.10	1.45	0.573	0.759	0.316	0.351	0.301	0.289

GA gallic acid, *RU* rutin

under developmental as well as under stressful conditions (Hashim et al. 2021). Although a large number of protocols regarding the callus establishment in various basil genotypes are available online, they will not be discussed in this review, since we focused on secondary metabolites produced by callus cultures of different basil genotypes. The summary of genotype-specific secondary metabolites production in callus cultures of basil together with the amount of produced compounds is presented in Table 2.

According to Hakkim et al. (2007) callus cells from *O. sanctum* L. grown on media with 2,4-D (1 mg L^{-1}) and KIN (0.1 mg L^{-1}) can produce a high concentration of rosmarinic acid and this is in correlation with callus biomass production. Abdel Rahman et al. (2015) demonstrated that in the culture of *O. basilicum* L. cells on solid MS medium with 5 mg L^{-1} BA and 1 mg L^{-1} NAA rosmarinic acid can be produced in high concentrations. Rady and Nazif (2005) showed that the addition of 1 mg L^{-1} BA and 0.25 mg L^{-1} IAA to MS medium leads to the accumulation of rosmarinic acid in the higher levels (3.01 mg g^{-1} DW) in callus culture of *O. americanum* L. var. *pilosum* (Wills.) Benth. grown in MS-medium containing 1 mg L^{-1} BA and 0.25 mg L^{-1} IAA, while the lowest content (1.18 mg g^{-1} DW) was recorded in intact plant. Authors concluded that the addition of 1 mg L^{-1} BA to the culture medium caused a considerable increase in rosmarinic acid accumulation than that of intact plant.

Additionally, Wongsen et al. (2015) showed that callus obtained from leaves of *O. basilicum* L. could be a significant source of antioxidant compounds such as ß-carotene, ascorbic acid, phenolics and flavonoid. These bioactive compounds can be produced 7 days after callus induction. The effective protocol for callus culture of *O. basilicum* L. cv. 'Thai basil' with high production of biomass and antioxidants was developed by Nazir et al. (2020c). MS medium with 5 mg L^{-1} BAP and 1 mg L^{-1} NAA was most effective for the fresh and dry biomass accumulation, as well as for the enhanced production of phenolic compounds. According to the authors, under the optimized culture conditions 'Thai basil' callus culture can be used as a suitable, low-cost source for valuable phytochemicals (including rosmarinic and chicoric acid) with significant antioxidant activity. Moreover, the synthesis of main metabolites in the callus culture of 'Thai basil' could be several times higher compared to leaves obtained from commercial sources if the optimized protocol is used.

The antioxidant activity of basil calli can be increased by the application of gamma radiation. Guirgius et al. (2007) exposed seeds of *O. basilicum* L. to different doses of gamma radiation and further callus culture was established through the shoot tips of mutants grown on MS with 1 mg L^{-1} of 2,4-D, 0.25 mg L^{-1} KIN, and with yeast extracts. Results showed that some mutants have increased concentrations of rosmarinic acid. The addition of yeast extract (5 g L^{-1}) to the media increased content of rosmarinic acid 3.4 times.

The possibility of induced synthesis of betulinic acid was investigated in the callus cultures from *O. basilicum* L. (CIM-Soumya), *O. sanctum* L. (CIM-Ayu), *O. grattisimum* L., and *O. kilimandscharicum* Gürke from India (Pandey et al. 2015). Although 2,4-D led to the best callus growth, the synthesis of betulinic acid could be induced by NAA. The highest content of betulinic acid was recorded in callus from *O. basilicum* L. (2.59%) compared to *O. kilimandscharicum* Gürke (1.87%) and *O. sanctum* L. (0.39%), whereas in callus culture from *O. grattisimum* L. betulinic acid was not recorded. Moreover, due to the presence of methyl jasmonate (MeJ) at 200 µM, the concentration of betulinic acid in *O. basilicum* L. callus is doubled.

Recent studies on callus culture of purple basil (*O. basilicum* L. var. *purpurascens*) testify about the effects of plant growth hormones and abiotic elicitors on the biosynthesis of phenylpropanoid metabolites. Nazir et al. (2019) established purple basil callus culture by culturing the leaf explants on MS medium with different concentrations of PGRs, and the highest biomass accumulation together with the highest total phenolic and flavonoid production was obtained with 2.5 mg L^{-1} NAA. Additionally, different accumulations of caffeic acid, rosmarinic acid, and chicoric acid, together with anthocyanins cyanidin and peonidin was related to the various treatments of PGRs. Nazir et al. (2020b) further demonstrated that the light quality strongly influences phenylpropanoid metabolites biosynthesis along with the antioxidant potential of callus culture of purple basil (*O. basilicum* L. var. *purpurascens*). Callus treatments with blue light resulted in the highest total phenolic and flavonoid content, and antioxidant activity. Dark conditions resulted in higher content of rosmarinic acid and anthocyanins (cyanidin and peonidin), whereas red light caused maximum production of chicoric acid. The application of light-emitting diodes (LEDs) for enhanced production of biologically active metabolites was also a promising strategy in the case of callus cultures of *O. basilicum* L. from Pakistan (Nadeem et al. 2019), still, the results were different compared to those obtained for purple basil. The highest accumulation of total flavonoids, including anthocyanins peonidin and cyanidin, was recorded in callus culture grown under red light. The highest accumulation of rosmarinic acid and eugenol was recorded under blue light conditions while the synthesis of chicoric acid was induced under the white light.

The research of Nazir et al. (2021) showed that in callus of *O. basilicum* L. var. *thyrsiflorum* salicylic acid (10 µM) in combination with continuous light significantly increased antioxidant potential, total phenolic and flavonoid content (18.7 and 7.2 mgg^{-1} DW, respectively), eugenol (0.56 mg/g DW), rosmarinic and chicoric acid (54.35 and 64.46 mg/g DW, respectively), as well as cyanidin (0.42 mgg^{-1} DW) and

Table 2 In vitro studies on genotype-specific synthesis of secondary metabolites in callus cultures of basil

Species	Additional information	Medium and plant growth regulators (mg L^{-1})	Induced synthesis of secondary metabolites	Reference
O. basilicum L.	n.s.	MS with NAA 2.0, KIN 0.25, 100 mg L^{-1} ascorbic acid and 5 g L^{-1} yeast extract	23.02 mg g^{-1} rosmarinic acid	Guirgius et al. (2007)
O. sanctum L.	from India	MS with 2,4-D 1.0 and KIN (0.1–0.5)	4.0 mg mL^{-1} of extract total phenolics, 0.38 mg g^{-1} DW carnosic acid, 2.7 mg g^{-1} DW rosmarinic acid in leaf-derived callus; 3.1 mg mL^{-1} of extract total phenolics, 0.27 mg g^{-1} DW carnosic acid, 2.2 mg g^{-1} DW rosmarinic acid in stem-derived callus; 2.6 mg mL^{-1} of extract total phenolics, 0.22 mg g^{-1} DW carnosic acid, 1.4 mg g^{-1} DW rosmarinic acid in stem-derived callus	Hakkim et al. (2007)
O. sanctum L.	CIM- Ayu	MS with NAA (1.0–3.0) and 0.25 KIN	0.39% DW betulinic acid	Pandey et al. (2015)
O. kilimandsharicum Gürke	from India	MS with NAA (1.0–3.0) and 0.25 KIN	1.87% DW betulinic acid	Pandey et al. (2015)
O. basilicum L.	CIM-Soumya	MS with NAA (1.0–3.0) and 0.25 KIN	2.59% DW betulinic acid in callus grown on medium without and 5.10% DW betulinic acid in callus grown on medium with MeJ	Pandey et al. (2015)
O. basilicum L.	n.s.	MS with BA 5.0 and NAA 1.0	13.2 mg g^{-1} DW rosmarinic acid	Abdel Rahman et al. (2015)
O. basilicum L.	from Turkey	MS with NAA 2.0, BAP 1.0 and 100 µM melatonine	784.6 µg g^{-1} total phenolics, 754.2 µg g^{-1} rosmarinic acid	Duran et al. (2019)
O. basilicum L.	from Pakistan	MS with NAA 2.0	18.7 mg g^{-1} DW total phenolics, 96 mg g^{-1} DW rosmarinic acid and 0.273 mg g^{-1} DW eugenol in callus under blue light; 5.46 mg L^{-1} total flavonoids, 0.127 mg g^{-1} DW peonidin and 0.1216 mg g^{-1} DW cyanidin in callus under red light; 81.40 mg g^{-1} DW chicoric acid in callus under continuous white light	Nadeem et al. (2019)
O. basilicum L.	var. *purpurascens*	MS with NAA 2.5	210.7 mg L^{-1} total phenolics, 196.4 mg L^{-1} flavonoids	Nazir et al. (2019)
O. basilicum L.	var. *purpurascens*	MS with TDZ 5.0	44.67 mg g^{-1} DW caffeic acid, 43.89 mg g^{-1} DW chicoric acid, 52.22 mg g^{-1} DW rosmarinic acid, 16.39 mg g^{-1} DW cyanidin, 10.77 mg g^{-1} DW peonidin	Nazir et al. (2019)
O. basilicum L.	var. *purpurascens*	MS with NAA 2.5	20.1 mg g^{-1} DW total phenolics and 378.3 mg L^{-1} flavonoids in callus under blue light; 87.62 mg g^{-1} DW rosmarinic acid, 0.15 mg g^{-1} DW cyanidin and 0.13 mg g^{-1} DW peonidin in callus under dark; 14.65 mg g^{-1} DW chicoric acid in callus under red light	Nazir et al. (2020b)
O. basilicum L.	var. *purpurascens*	MS with NAA 2.5	134.5 mg g^{-1} DW rosmarinic acid, 51.52 mg g^{-1} DW chicoric acid, 0.50 mg g^{-1} DW cyanidin in callus exposed to UV-C; 79.4 mg g^{-1} DW rosmarinic acid, 39.99 mg g^{-1} DW chicoric acid, 0.45 mg g^{-1} DW cyanidin and 0.22 mg g^{-1} DW peonidin in callus grown on medium with melatonin	Nazir et al. (2020a)
O. basilicum L.	cv.'Thai basil'	MS with BAP 5.0 and NAA 1.0	132.6 mg g^{-1} DW total phenolics, 35.77 mg g^{-1} DW chicoric acid and 7.35 mg g^{-1} DW rosmarinic acid	Nazir et al. (2020c)

Table 2 (continued)

Species	Additional information	Medium and plant growth regulators (mg L^{-1})	Induced synthesis of secondary metabolites	Reference
O. basilicum L.	cv.'Thai basil'	MS with BAP 5.0 and NAA 1.0 and salicylic acid	18.7 mg g^{-1} DW total phenolic content, 7.2 mg g^{-1} DW flavonoid content, 0.56 mg g^{-1} DW eugenol, 54.35 mg g^{-1} DW rosmarinic acid, 64.46 mg g^{-1} DW chicoric acid, 0.42 mg g^{-1} DW cyanidin and 0.32 mg g^{-1} DW peonidin in callus grown on continuous light and with 10 µM salicylic acid; maximum caffeic acid production (0.54 mgg^{-1}DW) with salicylic acid treatment (25 µM) under photoperiod	Nazir et al. (2021)

n.s. non-specified; *DW* dry weight

peonidin (0.32 mgg^{-1} DW) production. Salicylic acid treatment (25 µM) under photoperiod showed maximum caffeic acid production (0.54 mgg^{-1} DW) in calli.

In addition to PGRs and light quality, the biosynthesis of phenolic compounds in the callus culture of purple basil can be affected by the application of melatonin or UV-C radiations (Nazir et al. 2020a). The 10 min treatment with UV-C increased the content of rosmarinic acid in purple basil callus culture, whereas, after 50 min of treatment, the content of chicoric acid, and anthocyanins cyanidin and peonidin were several times higher compared to the control. The presence of melatonin also affected the content of named metabolites, and the results were concentration-dependent. Positive effects of melatonin were also reported in the case of *O. basilicum* L. from Turkey (Duran et al. 2019). Different concentrations of melatonin were applied (0, 100 and 200 µM) on callus culture obtained from basil leaves. It was confirmed that the high content of total phenolics (particularly rosmarinic acid) in callus cells can be due to melatonin (100 µM) since the concentration of rosmarinic acid was five times higher compared to the control calli (melatonin-free MS medium). Besides the quantitative changes in secondary metabolism, the presence of melatonin as an elicitor could cause changes in the quality of secondary metabolites from sweet basil callus since Duran et al. (2019) demonstrated that the addition of melatonin to the MS medium leads to changes in essential oil content. The authors concluded that this elicitor could have several advantages regarding the isolation, quantity, and quality of basil secondary metabolites.

It can be concluded that basil plants are suitable for establishment of callus culture with the aim of SMs production. Since various plant parts can be successfully used as explant sources with high concentration of secondary metabolites produced, this could have significant economical and practical importance. Furthermore, stable concentration of metabolites of interest, in particular phenolic compounds including rosmarinic, chicoric and caffeic acid, can be enhansed (for example through the application of melatonin, salicylic acid or various physical agents) which makes basil callus culture a promising platform for sustainable and continuous production of high-valued phenolic compounds.

Cell suspension culture

The number of previous studies pointing on plant cell culture as a platform for SMs production is increasing mainly since this approach to produce valuable pharmaceuticals, which includes a unique combination of physical and chemical environments, provides a continuous, reliable, and large-scale production of natural products, particularly those with complex structures (Koul and Mallubhotla 2020). The culture of cells in a liquid medium can be obtained by

transferring friable calli to the appropriate medium with PGRs. High metabolic rate in cell suspension culture and rapid proliferation of cell mass are among the most important advantages of cell cultures when it comes to the production of secondary metabolites. Compared to callus culture, the synthesis of biologically active secondary metabolites in plant cell suspension culture can be more effective primarily due to the applicability, rapid response to various stimulants that induce increased synthesis of secondary metabolites, as well as rapid cell division (Kintzios et al. 2003; Açıkgöz, 2020). Multiple reports of different basil genotypes and concentration of SMs obtained through the basil in vitro cultures maintained in liquid medium are presented in Table 3.

The rosmarinic acid accumulation in cell suspension cultures of *O. sanctum* L. was investigated by Hakkim et al. (2011a). After stabilization of cell suspension with five subcultures, the highest rosmarinic acid content and biomass growth was observed in the cultures supplemented with 1 mg L^{-1} 2,4-D and 0.1 mg L^{-1} KIN. The concentration of rosmarinic acid was two-fold higher than that found in leaf callus induced on MS solid medium.

To stabilize the production of rosmarinic acid and anthocyanins, Strazzer et al. (2011) induced mechanical stress in cell suspension of *O. basilicum* L. cv. 'Dark Opal'. It was demonstrated that mechanical stress (applied through the increased agitation) can increase the production of secondary metabolites—rosmarinic acid, hydroxycinnamic acid, and flavonoids, but increased production of these phenolics was accompanied with the decrease in cell biomass production. Opposite, in the case of *O. basilicum* L., synthesis and accumulation of rosmarinic acid is in correlation with the growth of cell suspension (Kintzios et al. 2003). A high concentration of rosmarinic acid was obtained through the addition of PGRs (2 mg L^{-1} 2,4-D and 2 mg L^{-1} NAA) together with 5 g L^{-1} sucrose, 10 mg L^{-1} ascorbic acid, 0.1 g L^{-1} meso-inositol and 0.5 g L^{-1} L-phenylalanine to MS medium. It is proved that phenylalanine can be efficient elicitor in the case of *O. sanctum* L. from India (Hakkim et al. 2011b) but in the concentration of 0.25 g L^{-1}. Additionally, for this basil species, an increase in sucrose content in the MS medium (up to 5%) led to an increase in rosmarinic acid accumulation compared to the control medium containing 3% sucrose. The highest concentration of this phenolic acid was recorded when cells were cultivated with the addition of MeJ. According to Pandey et al. (2019), MeJ plays a major role in the accumulation of pharmacologically significant triterpenoids in cell suspension of *O. basilicum* L. (genotype CIM-Soumya), since the concentration of betulinic acid, ursolic acid, together with rosmarinic acid, was several times higher in cell suspension elicited with MeJ compared to the in vivo control leaves. The effect of various elicitors—biotic (yeast extract), and abiotic ($CdCl_2$ and $AgNO_3$) on growth and synthesis of secondary metabolites were tested in cell suspension in *O. basilicum* L. from Turkey (Açıkgöz 2020). This study confirmed the positive effects of yeast extracts on the content of phenolic compounds (total phenolics and total flavonoids, rutin and isoquercetin, rosmarinic acid and chicoric acid), however, the accumulation of these biologically active compounds varied depending on yeast extract concentration. The highest values of estragole and linalool were obtained with $AgNO_3$ treatment. Still, since the elicitor treatments decreased cell dry weight, a number of cells and cell viability, the author indicates that elicitor doses and application times should be carefully adjusted. In the most recent study on cell suspension of *O. basilicum* L. from Turkey (Açıkgöz 2021) osmotic pressure caused by sorbitol as elicitor was studied. Although the number of cells, cell viability, and dry weight decreased at the various concentration levels of sorbitol, this elicitor was effective in the accumulation of total phenolics and total flavonoids, rosmarinic and chicoric acid, as well as rutin, isoquercetin, linalool and methyl chavicol. In recent study Berim and Gang (2020) analysed neopetoidin A and B from calli and cell suspension of *O. basilicum* L. cultivars named 'SW' and 'Sweet Dani'. The authors pointed out that these antioxidant polyphenols accumulate at high levels under tissue culture conditions and suggested the model for biosynthesis studies which is from particular interest having in mind that neopetoidins often remain undetected because of their instability, poor extractability or coextraction with the rosmarinic acid.

Hairy root culture

Agrobacterium rhizogenes (soil-borne gram-negative bacterium) can introduce Ri plasmid into plant cells through chemotactic response from wounded plant cells. The integration of Ri T-DNA to the plant genome is followed by the formation of plagiotropic hairy roots caused by the root-inducing *rol* genes (Vyas and Mukhopadhyay 2014). This procedure can be carried under laboratory conditions and represents an effective strategy for induction and enhanced accumulation of SMs (Gangopadhyay et al. 2010; Wawrosch and Zotchev 2021). Hairy root culture is among the most promising approaches for in vitro SMs production because of the genetic stability and fast growth in media without hormones. More than 155 plant species have been transformed with different *A. rhizogenes* strains and many of the transgenic root cultures were scaled up for industrial production of diverse secondary metabolites (Tian 2015; Hanafy et al. 2016; Sharan et al. 2019).

To the best of our knowledge first report for hairy root culture of *O. basilicum* L. was recorded by Tada et al. (1996). By testing two *A. rhizogenes* strains (ATCC 15834 and MAFF 03-01724) and three hormone-free media (MS, Gamborg B_5 (Gamborg et al. 1968), and Woody Plant), authors showed that the maximum yield of rosmarinic acid

Table 3 In vitro studies on genotype-specific synthesis of secondary metabolites in different types of basil cultures grown in liquid medium

Species	Additional information	Induced synthesis of secondary metabolites	Type of culture	Reference
O. basilicum L.	n.s.	14.1% DW rosmarinic acid in hairy roots from MS medium and 14% DW rosmarinic acid in hairy roots from Gamborg B_5 medium	Hairy roots	Tada et al. (1996)
O. basilicum L.	n.s.	0.98% g FW in untransformed roots, three-fold increase in rosmarinic acid in hairy roots and 2.67-fold increase of rosmarinic acid in hairy roots upon elicitation with fungal cell walls elicitors	Hairy roots	Bais et al. (2002)
O. basilicum L.	n.s.	10 mg g^{-1} DW rosmarinic acid	Cell suspension	Kintzios et al. (2003)
O. basilicum L.	n.s.	4 µg g^{-1} DW rosmarinic acid in cell suspension in 250 ml flask; 29 µg g^{-1} DW rosmarinic acid in cell suspension in 2.5 L bioreactor; 178 µg g^{-1} DW rosmarinic acid in micropropagated nodal explants in 2.5 L bioreactor	Cell suspension	Kintzios et al. (2004)
O. basilicum L.	n.s.	betulinic acid, 3-epimaslinic acid, alphitolic acid and euscaphic acid	Hairy roots	Marzouk (2009)
O. basilicum L.	cv. Dark Opal	1944.63 µg g^{-1} rosmarinic acid and 1105 µg g^{-1} hydroxycinnamic acid under mechanical stress in the light	Cell suspension	Strazzer al. (2011)
O. sanctum L.	n.s.	3.01 mg g^{-1} DW rosmarinic acid in cell suspension supplemented with 1 mg L^{-1} 2,4-D and 0.1 mg L^{-1} KIN (two-fold higher compared to leaf callus)	Cell suspension	Hakkim et al. (2011a)
O. sanctum L.	from India	5.7 mg L^{-1} rosmarinic acid in non-supplemented cells; 4.1-fold higher accumulation of rosmarinic acid after supplementation with 0.25 g L^{-1} phenylalanine; up to 7-fold higher content of rosmarinic acid after supplementation with yeast extract; up to 8.6-fold higher content of rosmarinic acid after MeJ supplementation	Cell suspension	Hakkim et al. (2011b)
O. basilicum L.	cultivars B3 Subja; B12 Holy Green, and B13 Red Rubin	378.8 mg GAE g^{-1} DW total phenolics, 76.41 mg g^{-1} DW rosmarinic acid and 1.74 mg g^{-1} DW caffeic acid	Hairy roots	Srivastava et al. (2016)
O. basilicum L.	CIM-Soumya	15.73 mg g^{-1} DW rosmarinic acid, 14.63 mg g^{-1} DW betulinic acid, 4.71 mg g^{-1} DW ursolic acid and 0.91 mg g^{-1} DW oleanolic acid	Cell suspension	Pandey et al. (2019)
O. basilicum L.	CIM-Soumya	1.67-fold enhancement in rosmarinic acid, 1.41-fold enhancement in betulinic acid, 1.07-fold enhancement in ursolic acid and 2.74-fold enhancement in oleanolic acid production after MeJ addition	Cell suspension	Pandey et al. (2019)
O. basilicum L.	CIM-Soumya	1.66-fold enhancement in rosmarinic acid, 1.3-fold enhancement in betulinic acid and 1.8-fold enhancement in oleanolic acid production in bioreactors compared to cell suspension	Cell suspension	Pandey et al. (2019)
O. basilicum L.	n.s.	3.02 mg L^{-1} oleanolic acid and 4.79 mg L^{-1} ursolic acid from cambial meristematic cells	Cell suspension	Mehring et al. (2020)
O. basilicum L.	from Turkey	6.45 mg g^{-1} DW chicoric acid, 21.28 mg g^{-1} DW rosmarinic acid, 6.54 mg g^{-1} DW rutin and 3.72 mg g^{-1} DW isoquercetin after yeast extract treatment	Cell suspension	Açıkgöz (2020)
O. basilicum L.	from Turkey	4.37 µg g^{-1} DW linalool and 3.3 µg g^{-1} DW after $AgNO_3$ treatment	Cell suspension	Açıkgöz (2021)
O. basilicum L.	from Turkey	4.52 mg g^{-1} DW chicoric acid, 12.32 mg g^{-1} DW rosmarinic acid, 4.58 µg g^{-1} DW linalool and 4.4 µg g^{-1} DW methyl chavicol	Cell suspension	Açıkgöz (2021)

n.s. non-specified; *DW* dry weight

was produced by *A. rhizogenes* ATCC 15834 in MS medium at week five. According to the authors, to produce SMs in *O. basilicum* L. hairy root cultures, it is very important to select appropriate strain and culture conditions. Bais et al. (2002) established transformed roots from *O. basilicum* L. with *A. rhizogenes* (ATCC 15834). The established roots showed stable and fast growth and produced rosmarinic acid constitutively. Furthermore, the concentration of rosmarinic acid was 3 times higher in hairy roots compared to untransformed roots. Besides the antimicrobial activity of hairy roots, elicitation with various fungal pathogens caused exudation of rosmarinic acid by root culture in droplet form. From transformed hairy roots from *O. basilicum* L. obtained by infecting with *A. rhizogenes* strain LBA 9420 six triterpene acids were identified—betulinic, oleanolic, ursolic, 3-epimaslinic, alphitolic and euscaphic acid (Marzouk 2009). These compounds demonstrated hepatoprotective activity in albino rats' livers. The *A. rhizogenes* strain ATCC 15834 was used for the transformation of red and green forma of *O. tenuiflorum* L. (Vyas and Mukhopadhyay 2014). Green forma showed high transformation frequency and leaves were the best explant choice (comparing to shoots and hypocotyls). Authors pointed out that green plants accumulated more signalling phenolics comparing to red forma (accumulated more anthocyanins), and that these compounds might influence hairy root formation. Srivastava et al. (2016) reported transformation of three *O. basilicum* L. cultivars (B3, Subja; B12, Holy Green and B13, Red Rubin) with four strains of *A. rhizogenes* (A4, ARqua1-pTSC5, 8196 and 11325). Hairy root development was explant-specific and virulence-dependent. Furthermore, the production of rosmarinic acid was age-dependent and cultivar-specific. Sharan et al. (2019) investigated the effects of different elicitors (yeast extract, MeJ, and salicylic acid) on hairy root culture of *O. tenuiflorum* L. induced by two *A. rhizogenes* strains (A4 and LBA 9402). While *A. rhizogenes* A4 was unable to induce hairy roots in *O. tenuiflorum* L., in hairy root culture obtained by *A. rhizogenes* strain LBA 9402 enhanced productions of ursolic acid and eugenol in the presence of yeast extract was obtained for 8 days. Still, elicitor-mediated accumulation of these compounds was age, dose, and duration dependent. In the most recent study of *O. basilicum* L. and hairy root production (Kwon et al. 2021) it has been shown that hairy root production improves when *O. basilicum* L., named as green basil—'Cinnamon' is cultured under light conditions, whereas for *O. basilicum* L. named as purple basil—'Purpurascens' greater hairy root production was under dark conditions. Furthermore, the accumulation of rosmarinic acid was higher in hairy roots obtained from green basil compared to purple basil under both dark and light conditions.

Despite the enhanced production of secondary metabolites through the hairy root culture, infection of basil plants with *A. rhizogenes* could result in the emergence of several different hairy root lines which may vary in SMs content and accumulation (Table 3). Additionally, a strong cultivar-dependent differential induction of hairy roots can be seen. Considering these issues, estimation and utilization of mass scale cultivation of basil hairy roots for the production of SMs must be carefully established so this type of basil culture may become cost-effective.

Somatic embryogenesis

Two different types of somatic embryogenic routes are generally engaged in plants: direct somatic embryogenesis (SE) and indirect SE (Williams and Maheswaran 1986). In direct SE, somatic embryos are induced from the surface of explants, without the callus stage, while indirect SE is a multistep regeneration process that includes the following steps: (i) initiation of callus, (ii) proliferation of embryogenic callus (production of proembryogenic mass—PEM), (iii) maturation of somatic embryos and (iv) regeneration of developed plants (von Arnold 2002). Although the SE pattern for many plants is similar, each species, and often even a variety, requires developing a detailed method of propagation protocol.

It seems to be difficult to establish an efficient protocol for somatic embryogenesis of basil plants compared to the other species. Hence, until now there are few reports published on this topic (Gopi and Ponmurugan 2006; Mathew and Sankar 2011; Ibrahim 2017; Ibrahim et al. 2019). Inductive conditions, such as source and physiological state of the explant, exogenous plant growth regulators (PGRs) added into medium and stress treatment, determine the competence to the dedifferentiation of plant cells and activation of the embryogenic pathway (Quiroz-Figueroa et al. 2006).

In basil, SE occurred indirectly, where callus tissue was formed and subsequently, competent cells were transitioned from somatic to embryogenic state. Different types and sizes of explants are used for generating somatic embryos. In the pioneering work, Gopi and Ponmurugan (2006) used leaf explants from the field-grown young plant of *O. basilicum* L. for indirect SE induction via callus. In addition to leaves as explants for in vitro study of *O. tenuiflorum* L., *O. basilicum* L., and *O. sanctum* L., stems and inflorescences were used (Hakkim et al. 2011c; Bhuvaneshwari et al. 2016). Next, leaves and cotyledonary leaves from plants germinated in vitro have been commonly used for SE induction (Table 4).

Already the first observations on reprogramming of callus cells seemed to be biochemical in nature, this was confirmed by the analysis of callus exudates in the medium. In it, sugars, growth regulators, amino acids, and vitamins were found. Therefore, it could be proven that the combined effect of growth regulators and other components of the medium

Table 4 In vitro studies on species, explant, media, and plant growth regulators in somatic embryogenesis of basil

Species	Explant	Medium and plant growth regulators (mg L^{-1})				Reference
		Callus induction	Callus proliferation	Embryo maturation	Conversion into plantlets	
Ocimum basilicum L.	Leaves	MS BAP 1.0 2,4-D 0.5	MS BAP 1.0 2,4-D 0.5	MS BAP 1.0 Kinetin 0.5 NAA 1.0	MS BAP 1.0 Kinetin 0.5 NAA 0.1	Ibrahim et al. (2019)
Ocimum basilicum L.	Leaves	MS BAP 1.0 2,4-D 0.5	MS BAP 1.0 2,4-D 0.5	MS BAP 1.0 Kinetin 0.5 NAA 1.0	MS BAP 1.0 Kinetin 0.5 NAA 0.1	Ibrahim (2017)
Ocimum basilicum L. *Ocimum sanctum* L. *Ocimum gratissimum* L.	Cotyledonary Leaves	MS BAP 0.5 2,4-D 0.5–2.0	–	MS BAP 1.0–5.0 Kinetin 2.5–5.0 IAA 1.25, 2.5	–	Mathew and Sankar (2011)
Ocimum basilicum L.	Leaves	MS 2,4-D 0.2–2.0 BAP 0.5–2.0	MS BAP 1.0 2,4-D 0.5	MS BAP 1.0–3.0 Kinetin 0.2–1.0 NAA 1.0–5.0	MS BAP 1.0–3.0 Kinetin 0.2–1.0 NAA 1.0–5.0	Gopi and Ponmurugan (2006)

influence both, callus production and establishment as well as cell polarity and the subsequent cellular processes leading to the formation and development of somatic embryos (Deo et al. 2010).

In many systems for indirect SE, primary explants are first cultured on medium at a high concentration of auxin to activate cell division and induce the generation of embryogenic cells. Then the proembryogenic cell mass (PEM) is transferred on medium without or with a reduced level of auxin, on which somatic embryos development occurs (Thorpe and Stasolla 2001). The embryogenic callus in the studies of Ibrahim (2017) and Ibrahim et al. (2019) was induced on the medium with a higher concentration of cytokinins compared to auxins (C>A). They used BAP and 2,4-D at a concentration of 1.0 mg L^{-1} and 0.5 mg L^{-1}, respectively. In contrast, Mathew and Sankar (2011) and Hakkim et al. (2011c) used A>C. Additionally, Bhuvaneshwari et al. (2016) only used 2,4-D from 0.25 to 2.0 mg L^{-1} concentration (Table 4). Gopi and Ponmurugan (2006) reported the highest frequency of callus induction (75%) and a number of somatic embryos (36.5 ± 2.17) on medium supplemented with BAP, NAA and KIN at 1.0 mg L^{-1}, 1.0 mg L^{-1} and 0.5 mg L^{-1} concentration, respectively. Moreover, Hakkim et al. (2011c) found that the same concentration and combination of NAA, BAP and KIN is essential for the greater embryogenesis potential in *O. sanctum* L. cultures, especially for globular embryos development.

The efficiency in regenerating plants via SE is strongly dependent on the plant species or even genotype, showing a different response during in vitro culture. Three different species of basil: *O. gratissimum* L., *O. basilicum* L. and *O. sanctum* L. were tested for SE efficiency in the Mathew and Sankar (2011) study. The maximum weight of *O. gratissimum* L. callus was received on medium with 0.5 mg L^{-1} 2,4-D, whereas the increasing concentration of 2,4-D to 1.0 mg L^{-1} was found suitable for *O. basilicum* L. and *O. sanctum* L. callus induction. The addition of KIN to the medium alone or in combination with IAA provided the highest percentage of embryogenic callus for all three species. Although *O. basilicum* L. produced less callus compared to the other two species. Somatic embryos regeneration efficiency of *O. basilicum* L. and *O. tenuiflorum* L. were studied by Bhuvaneshwari et al. (2016). Among all explants used, the leaves showed similarly high response (about 95%) of callus induction for both species and were found to be the most suitable for SE induction. However, the tested species showed significant differences in the effectiveness of somatic embryos development. Seventy per cent of developed green callus of *O. basilicum* L. cultured in liquid medium with NAA (1 mg L^{-1}) and BAP (1.5 mg L^{-1}) produced somatic embryos, while only 25% of *O. tenuiflorum* L. callus induced embryos.

To initiate embryos formation, treatment with various stresses, for example, heavy metal ions, NaCl, temperature, ultraviolet radiation, extreme concentrations of plant growth regulators, or mechanical treatments, are greatly effective (Fehér et al. 2015). Ibrahim et al. (2019) conducted a study showing the influence of copper nanoparticles (Cu-NPs) and copper sulphate $CuSO_4 \cdot 5H_2O$ on somatic embryos production. The results indicated that the addition of Cu-NPs (5 µM) significantly increased the percentage of explants producing somatic embryos (from

15 to 84%) and the average number of regenerated plantlets (from 4.3 to 18.7). Ibrahim (2017) examined the toxic effects of chloride on regeneration of somatic embryos. Obtained results confirmed that the addition of 5.99 mM $CaCl_2 \cdot 2H_2O$ into the medium significantly inhibited callus induction, the development of somatic embryos, and consequently the regeneration capacity of *O. basilicum* L. Whereas the enhancement of callus induction and regeneration was achieved using the medium containing the low level of chloride, 0.1 µM $CaCl_2 \cdot 2H_2O$ and 2.99 mM of $Ca(NO_3)_2$. It significantly led to an increase in the percentage of callus induction (from 77 to 99%), the percentage of explants with somatic embryos (from 6 to 15%), and the average number of plantlets of *O. basilicum* L. (from 2.10 to 4.32).

The overexploitation and reduction of natural resources to obtain substances used in medicine is one of the global problem today. The recent developments in the genetics and biotechnology of medicinal plants, lay stress on the use of in vitro cultures for secondary metabolites production (Nazir et al. 2020b). Rapid proliferation of cell mass via organogenesis or somatic embryogenesis leads usually to increase metabolic rates of developed cells and is one of the most important advantages of cell cultures for secondary metabolites production.

The recent progress in in vitro secondary metabolites production of *Ocimum* L. species was reviewed by Kasem (2017). Secondary metabolites such as rosmarinic and betulinic acid, β-carotene, ascorbic acid, phenolics, flavonoids and eugenol were enhanced and produced by the roots, callus and cell suspension culture (Strazzer et al. 2011; Wongsen et al. 2015). Although, *Ocimum* species has been in vitro propagated via SE and successfully regenerated, there is only a few reports concerning the use of somatic embryos for secondary metabolites isolation in this species (Hakkim et al. 2011c; Bhuvaneshwari et al. 2016).

The first time accumulation of rosmarinic acid in embryogenic callus and cell suspension culture of *O. sanctum* L. was investigated by Hakkim et al. (2011c). The analysis of the matured somatic embryos and callus revealed considerable rosmarinic acid accumulation on solid medium during the whole culture period. The highest dry weight (11.6 g L^{-1}) of cell suspension culture corresponds with the highest rosmarinic acid (82.1 mg L^{-1}) content. The authors emphasize that the rosmarinic acid content in cell suspension culture was 40-times higher compared to the callus obtained on the solid medium. In study of Bhuvaneshwari et al. (2016) extractions of eugenol were carried out from field and in vitro grown *O. basilicum* L. and *O. tenuiflorum* L. plants. Leaves, stems, inflorescences, roots, callus, and somatic embryos in globular and heart-shaped stage from both species were collected. In vitro grown leaves and somatic embryos of both species were found to contain similar concentration of eugenol (85 µg g^{-1} approximately), however higher than in field-grown leaves (30.2 µg g^{-1} and 25.1 µg g^{-1} respectively). This study demonstrated two important relationships: (i) the secondary metabolites detected in basil have different tissue-specific distribution patterns, (ii) the amount of eugenol in in vitro plants was higher than in the field grown plants. The knowledge about plant cultivation and specific localization in each individual organs of the secondary metabolites can be applied for large-scale commercial production of eugenol.

Research conducted so far on the basil in vitro cultures has shown great potential not only in micropropagation of this species, but also in the production of secondary metabolites. Bhuvaneshwari et al. (2016) showed that suspension cultures of *O. basilicum* L. and *O. tenuiflorum* L. could be a good source of eugenol. Application of basil SE and development of a protocol for its large scale propagation using bioreactors will be a key step towards commercial production of active compounds. In this context, the research conducted by Hakkim et al. (2011c) on accumulation of rosmarinic acid in somatic embryogenic callus and cell suspension culture of *O. sanctum* L. seems promising. It is worth noticing, that in vitro cultures overcome some limitations assigned to the field production of secondary metabolites for example, climatic condition, the soil problems, pests, diseases, and correlation with the planting season (Kasem 2017).

Production of basil secondary metabolites in bioreactors

Having previous investigations in mind, the opportunity for the large-scale production of SMs seems promising. Still, future work should focus on establishment and adjustment of appropriate protocols if the culture will be maintained in bioreactors, in order to avoid biomass decrease. On the other hand, the technology of plant cell culture nowadays can be regarded as the most convenient plant in vitro system for the large-scale biosynthesis of SMs in simplified bioreactor construction (Marchev et al. 2020). The goal of establishing a successful protocol for in vitro production of SMs of interest in bioreactors implies achieving ideal conditions for cellular metabolism and physiology through the management of various factors of the cellular environment (Kasem 2017). To the best of our knowledge there is only few reports regarding the basil cultivation in bioreactors. Kintzios et al. (2004) maintained a culture of nodal explants with lateral buds and leaf-derived suspension culture of *O. basilicum* L. in bioreactors (5 L disposable presterilized plastic airlift bioreactors) and demonstrated increased content of rosmarinic acid. Kiferle et al. (2014) indicated that the rosmarinic acid production in the in vitro plants cultured in bioreactor was up-regulated

and associated to a reduction of the biomass production. Authors confirmed the close relationship between the environment of the in vitro culture (culture medium, exchange of air, etc.) and the plants metabolism which can lead to an increased production of SMs when it is suitably modified. Pandey et al. (2019) found that the cultivation of *O. basilicum* L. (CIM-Soumya) cell suspension in a bioreactor with a volume of 10 L (working volume 7 L; stirred-tank), in the presence of MeJ as a stimulant, improves the cumulative productivity of metabolites of interest. The authors concluded that optimized bioreactor cultivation could be a more reliable and predictable mode of bioactive product cultivation. On the other hand, the cultivation of cambial meristematic cells (CMCs) could be one of the basil tissue culture alternatives. In the study of Mehring et al. (2020) CMCs were established for the first time on *O. basilicum* L. plants and cultivated in disposable bioreactors. According to the authors, the productivities of oleanolic acid and ursolic acid were consistently higher compared to dedifferentiated cells. These studies suggest that basil plants may be suitable for new approaches in tissue culture for both secondary metabolites production and extraction. As it is stated by Pandey et al. (2019) the practical functionality of this approach over the field grown basil plants can consequentially boost up its future utilization in realising the global industrial demand of the pharmacologically important secondary metabolites to ensure industrial sustainability.

Conclusion and future perspectives

There are numerous basil cultivars commercially available, and for in vitro studies, most of them belong to the species *O. basilicum* L. from which green or purple forms stand out by the use and amount of produced phenolic compound, in particular rosmarinic acid. Furthermore, *O. tenuiflorum* L.(syn. *O. sanctum* L.), *O. grattisimum* L., and *O. kilimandscharicum* Gürke can be regarded as good sources of valuable bioactive compounds and may therefore be desirable for various in vitro techniques in which it will be possible to optimize levels of produced secondary metabolites. Basil callus culture can be regarded as a promising platform for sustainable and continuous production of high-valued phenolic compounds in large quantities. In conclusion it can be said that the basil tissue culture is, without a doubt, a very promising approach for enhanced production of valuable bioactive compounds. However, several factors must be considered, such as genotype-dependent, cost-effective multiplication of plants in in vitro cultures, and development of protocols for large-scale propagation of cell and organ cultures of basil. Nevertheless, a clear evaluation of economical feasibility is still missing. With the progress in novel technologies such as elucidation of biosynthetic pathways and genome engineering, further progress in production of basil secondary metabolites through the in vitro culture can be expected.

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Data availability All data generated or analysed during this study are included in this article.

Declarations

Conflict of interest No conflict of interest is observed between any of the authors.

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