A REVIEW ON *Ocimum basilicum* L. VARIETIES CULTIVATED *IN VITRO* FOR ENHANCING ACTIVE CONSTITUENTS CONTENT USING ELICITORS

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Abstract. The **a**ntioxidant and biotechnological potential of basil related to different cultivars is reviewed in this paper. Particularities of elicitors added in different proportions to the *in vitro* culture medium in order to increase the content in essential oils are listed.

Keywords: basilcultivars, elicitors, antioxidant activity.

Rezumat. Un review al soiurilor de *Ocimum basilicum* L. cultivați *in vitro* pentru stimularea conținutului de compuși activi folosind elicitori. Potențialul antioxidant și biotehnologic al busuiocului legat de diferite soiuri este revizuit în această lucrare. Sunt enumerate particularitățile elicitorilor adăugați în diferite proporții la mediul de cultură *in vitro* pentru a crește conținutul în uleiuri esențiale.

Cuvinte cheie: soiuri de busuioc, elicitori, activitate antioxidantă.

INTRODUCTION

Plants with medicinal properties are of special interest for *in vitro* culture methodology. *In vitro* culture of basil offer rapid multiplication (KASEM, 2017) and avoid environmental stress that affect the basil in field (JAKOVLJEVIC et al., 2021).

Antioxidant potential of basil. The *Ocimum* genus includes about 160 species and varieties (ONOFREI et al., 2015). *Ocimum basilicum* L. (sweet basil) covers a broad field of applicability in the area of medicine, aromatherapy, cosmetics, culinary art, according to biological properties. Among *Ocimum* species, Tulsi (holy basil) stands out in Ayurvedic medicine for more than 3000 years (UPADHYAY, 2017). The phytoconstituents and pharmacological activities of basil are well documented by a number of reviews and studies (CASTAÑO et al., 2016; JAMSHIDI & COHEN, 2017; KUMAR, 2020; SHAHRAJABIAN et al., 2020; KULDEEP et al., 2021). Phytochemical, ethnobotanical and nutritional analyses of basil varieties confirm their importance as remedy for a series of maladies, as alimentary supplement (KPÈTÈHOTO et al., 2017). The anthocyanin concentration determines the classification of different basil varieties into different groups (FLANIGAN & NIEMEYER, 2014). The yield and the quality of the secondary metabolite profiles of numerous *Ocimum* species beside metabolic engineering (GURAV et al., 2021) are improved by tissue cultures. Antioxidant activity of basil is measured by different chemometric techniques, and different extraction solvents (EL-SOUD et al., 2015; TEOFILOVIĆ et al., 2017; ALHASNAWI, 2019).

Different locations of the world correlated with climatic conditions and seasonal variation are variables that influence *Ocimum* species composition in essential oils (YAMANI et al., 2016). Experimental studies revealed that developmental stage and cultivar type determine also the antimicrobial and biotechnological potential of basil (VOICU et al., 2020). Basil herb content is rich in polyphenols such as flavonoids and anthocyanins (PISAREV et al., 2015; DEINEKA et al., 2019). A wide variability of compounds as phenylpropanoids and terpenoids (MAURYA & SANGWAN, 2019) are encountered in the inflorescences, leaves and stems of basil plant as linalool, methyl cinnamate and methyl chavicol (WESOLOWSKA & JADCZAK, 2016).

Also, basil presents a wide variety of chemotypes with rich content in essential oils as *O. basilicum* var. *purpureum*, *O. basilicum* var. *thyrsiflora*, and *O. citriodorum* vis. (AVETISYAN et al., 2017). Basil cultivar has a significant effect on the total anthocyanin level and antioxidant properties (FLANIGAN & NIEMEYER, 2014). Also, the chemotype, environmental conditions, and origin of the plant influence the chemical composition of basil cultivar (ILIĆ et al., 2019). Assessing the antioxidant capacity of basil cultivars 'italiano classico', purple 'red rubin' and 'dark opal' basil varieties reveals components yet unknown and scarcely described, beside rosmarinic acid, like members of the salvianolic acid family.

The evaluation of the antioxidant activity and phytochemical screening of basil revealed high content of polyphenols (1.9630 ± 0.1340 g% tannic acid) and rosmarinic acid (620.952 mg%) of *O. basilicum* var. *purpurascens* Benth (GIRD et al., 2015). A wide range of studies have characterized the basil cultivars essential oils content. Between basil cultivars, qualitative and quantitative differences are obvious regarding chemical compounds (KUMAR et al., 2020). Also, *Ocimum* species can be identified using the chemotype criterium and analytical techniques easy to use for the qualitative control of *Ocimum* species. Different genotypes differ in terms of polyphenolic content (CIRIELLO et al., 2020).

Comparative studies performed regarding different parts of the plant and different basil cultivar types revealed that leaves have the higher rosmarinic acid content for *Ocimum basilicum* cultivars - green (cinnamon) and purple (red ruby) basil, by cloning genes related to rosmarinic acid biosynthesis (KWON et al., 2019); also, green basil accumulates more rosmarinic acid compared to purple basil.

Therefore, *O. basilicum* var. *thyrsiflora* has a pronounced antioxidant activity with 68.0% linalool content, beside *O. basilicum* var. *purpureum* with 57.3% methyl chavicol. The nerol (23.0%) and citral (20.7%) content of *O. citriodorum* cultivar triggers a considerable antimicrobial activity. The implication of secondary plant products in the defence system of the plant is well known (KASEM, 2017).

The suspension of beneficial bacteria isolates *Bacillus licheniformis* B40 and *Bacillus amyloliquefaciens* increases the antioxidant activity of the plant and activates mechanisms of defence as lignin deposition on epidermal and cortical cells (HELEPCIUC et al., 2014). *In vitro* cultures of *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. are of special importance for eugenol extraction.

Essential oils extracted from *O.basilicum* var. *genovese, O. gratissimum* and *O. tenuiflorum* have antibacterial activity (ŞTEFAN et al., 2013). Also, related to its antioxidant capacity, basil shows antimicrobial activity, being more active against gram-positive bacteria as culinary basils - 'genovese' or 'italian large leaf' (TRETTEL et al., 2018; VOICU et al., 2020).

The aqueous extracts of *Ocimum* prevented the proliferation of pathogenic bacteria (DAS, 2020). An active constituent of *Ocimum basilicum* is rosmarinic acid. Rosmarinic acid absorbs UV-B light, and this process has a favourable effect on its accumulation in basil leaves (MOSADEGH et al., 2021).

The accumulation process of rosmarinic acid is more pronounced in green basil cinnamon in the hairy root *in vitro* system than in purple basil (var. *purpurascens*) (KWON et al., 2019; 2021).

MATERIAL AND METHOS

The biotechnological potential of basil. Advances in genetic engineering as chloroplast genome engineering to *Ocimum* provide a series of benefits, namely enzyme cocktails derived from chloroplast, increasing biomass (RASTOGI & SHASANY, 2018). A high nutritional quality of the protein, sources of fibres, the content in carbohydrates with decreasing lipogenesis activity and the elevation of the level of satiety hormones are functions that underline the nutritional value of basil seeds for human health (BRAVO et al., 2021). The quantities of the metabolites of the plant can be amplified by applying biotechnological approaches for enhancing accumulation of these compounds (SHARAN et al., 2019). *In vitro* basil cultures are initiated and maintained by phytohormones that act as signalling molecules and elicitors. Optimal concentrations of phytohormones supplementing micropropagation culture media are different for different basil cultivars.

As an example, for *O. americanum*, optimal concentrations are 0.25 mg/L of BA and 0.5 mg/L of GA3. For *O. basilicum* and *O. gratissimum*, the optimal level of BA and GA in the culture medium recorded is 0.5 mg/L and 0.3 mg/L, respectively. In *O. sanctum*, high frequency sprouting of the axillary buds and development of multiple shoots is best elicited on MS with 1.0 mg/L BA and 0.5 mg /L GA3. Callus cultures initiated from *O. basilicum* L. (var. *purpurascens*) have antioxidant potential (LONDHE et al., 2015; LUNA et al., 2015; KAROMATOV & PULATOV, 2016; PEDRO et al., 2016; MAJDI et al., 2020; NAZIR et al., 2020). It is known that the variety and season of cultivation have a significant effect on the content (extraction yield) and the qualitative as well as quantitative profile of basil essential oils.

In vitro culture is meant to overcome the challenge of climate changes that influence the crop of the plant in the natural environments for *Ocimum basilicum* species. Climatic changes condition the plants from the natural environment or cultivated in field, as water stress (KALAMARTZIS et al., 2020); cinnamon has physiological properties to counter drought stress. Different cultivars can face with drought in different manner and thus we can use the appropriate variety in such a climate.

The quantities of the secondary metabolites of the basil plant can be amplified by applying biotechnological approaches by plant tissue culture *in vitro* using elicitors (SHARAN et al., 2019; HUANG et al., 2021), divided into biotic elicitors - bacterial extracts, compounds acquired from microbial cell wall and fungal extracts, (HELEPCIUC et al., 2014; 2019) and abiotic substances such as heavy metals (TRETTEL et al., 2018) and UV radiations.

RESULTS AND DISCUSSIONS

Elicitors of basil active constituents. Elicitors exert favourable effects not only on chemical composition in volatile compounds but also on *in vitro* developmental processes. Plant interactions with pathogenic or non-pathogenic bacteria *in vitro* are stimulatory by increasing antioxidant activity and activation of mechanisms of defence like lignin deposition on epidermal and cortical cells (HELEPCIUC et al., 2014).

Substances as methyl jasmonate and melatonin can be intercalated on the biosynthetic pathway of *in vitro* plants. It seems that biotic (fungal extracts, bacterial extracts) and abiotic stress (including exposure of the plant tissue cultures to extreme temperatures, ultraviolet light radiations, antibiotics, heavy metals salts, fungicides) enhances their synthesis. Regarding cell elicitors administration, ithe term of vaccination of plant cell cultures is used. Secondary metabolite production is different at elicited and non-elicited cell cultures, callus cultures and field grown herbs. The growth-stimulatory effect of melatonin and its protective effect against diverse abiotic (drought, salinity, waterlogging, cold, heat, metal toxics, herbicides, UV radiation) and biotic (bacteria, fungi, virus) stressors has been widely studied (BACK et al., 2016; ARNAO & HERNÁNDEZ-RUIZ, 2017; 2020).

Melatonin also functions as a signalling molecule beside ethylene in abiotic stress as salt tolerance (XU et al., 2019). Exogenous melatonin increases peroxydase activity and protects against pathogens in the rice plant (CHEN et al., 2020). Hairy root cultures are preferred for eliciting the production of secondary metabolites because of their stability between the types of tissue culture (HALDER et al., 2019).

Signalling molecule abscisic acid (ABA), called stress hormone, in plants synthesized in plastids has a distinct role in plant abiotic stress tolerance (DAR et al., 2017), activating the leaf plants' anti-perspiring activity, in order to avoid water loss in conditions of dehydration environment (WILKINSON et al., 2012). Methyl ijsmonate (MeJA) treatment produces a higher fresh weight of the cultures. The addition of methyl jasmonate elicitor in shake flasks with the suspension of cambial meristematic cells is an efficient alternative to biomass accumulation and increasing triterpenoid productivity (MEHRING et al., 2020). An active constituent of *Ocimum basilicum* is rosmarinic acid. The level of this compound was compared in a series of studies, as these of KWON et al. (2019, 2021) considering the green basil (cinnamon) and purple basil (purpurascens).

The methyl jasmonate (MeJA) elicitor increased the rosmarinic acid content of *in vitro* treated cultures of basil developed on one fourth strength of Murashige and Skoog (MS) liquid medium supplemented with 1.0 mg/L α-naphthaleneacetic acid (NAA) (BISWAS, 2020). The accumulation process of rosmarinic acid is more pronounced in green basil, in hairy root *in vitro* system.The eElicitor treatment of *O. sanctum* cultures with MeJa of 50 µM concentration for 8 h produces a higher fresh weight. The amount of rosmarinic acid and total phenolic compounds is significantly increased by the 100 µM melatonin concentration (DURAN et al., 2019).

Rosmarinic acid was identified and quantified as the major compound of the callus samples followed by caffeic acid, cinnamic acid, and p-coumaric acid as minor phenolic acids. A total of 200 (46.83%) calluses were induced from 427 explants within 4 to 5 wk. An evaluation of the melatonin effects on the callus induction and phenolic compound production of *Ocimum basilicum L.* sustains that melatonin decreased the callus induction in the concentrations tested but melatonin in concentration of 100 or 200 µM supplementation stimulated the highest total phenolic acid content. Sucrose concentration up to 5 % improves the accumulation of rosmarinic acid compared with 3 % sucrose administration. Beside elicitor type, culture type and incubation time have an significant effect on rosmarinic acid accumulation (ABDELRAHMAN et al., 2019).

The different time exposure of purple basil callus cultures to UV - C (10 min) increases rosmarinic acid dry weight (134.5 mg/g DW) and (1 mg/L) melatonin determines a 79.4 mg/g DW maximum accumulation of rosmarinic acid. The combined action of melatonin (4 mg/L) + UV - C irradiation (for 20 min) is effective for caffeic acid elicitation (NAZIR et al., 2020). Another enhancer is phenylalanine (0.25 g/l) triggering high rosmarinic acid accumulation. Yeast extract increased rosmarinic acid content in concentration of 0.5 g/L. Concentrations of compounds like rosmarinic acid were stimulated regarding their content with substances like chitosan, sucrose (5 %), phenylalanine (0.2 g/l), yest extract (0.5 g/l), 100M methyl jasmonate, etc. It is known that sucrose concentration up to 5 % improves the accumulation of rosmarinic acid compared with 3 % sucrose administration (HAKKIM et al., 2011).

Another enhancer was found to be phenylalanine (0.25 g/l), triggering high rosmarinic acid accumulation. Yeast extract increased rosmarinic acid content in concentration of 0.5 g/L. The studies showed that NAA produced the highest botulinic acid yield in the callus cells. Elicitation with 200µM methyl jasmonate after 48h duplicated botulinic acid yield in the growth medium contained NAA of *O. basilicum* callus. 0.25 mg/L kin, 5 g/l yeast extract enhance the rosmarinic acid content (GIURGIS et al., 2007) in callus cultures.

There are optimal concentrations of elicitors for active ingredients increasing in basil cell cultures suspensions as 25 µM of silver nitrate, 50 - 200 mg/L of yeast extract (ACIKGÖZ, 2020). Basil callus cultures offer an alternative source to plant for the production of secondary metabolites. The rich content in polyphenols such as flavonoids and anthocyanins of basil herb is attested by a series of scientific papers. Cell suspension cultures can be initiated from callus cultures; in association with 3.0 mg/L 6-benzyladenine (BA) and 2 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) included in the basal culture medium of Murashige and Skoog, MeJa, eliciting the accumulation of total phenols, flavonoids, and acacetin, (MANIVANNAN et al., 2016; ACIKGÖZ, 2020).

Abiotic elicitors such as arachidonic acid, jasmonic acid and b-aminobutyric acid stimulate most of the anthocyanins compounds concentration in purple basil and enhance anti-inflammatory activity (SZYMANOWSKA et al., 2015). ALHASNAWI et al. (2019) included salinity between the abiotic elicitors (factors) which influence the plant metabolism; β-glucans expresses a protective role against stress saline conditions, and protect the plants against the salinity stress, without affecting the DNA-markers and genetic variations; B - glucans interactions with salt stress are favourable on seeds and seedlings. Artificial light has an stimulating effect on phenolic compounds level, determining the best content of these (DÖRR et al., 2020). It is well known the implication of secondary plant products in the defence system of the plant (KASEM, 2017). It seems that biotic (fungal extracts, bacterial extracts) and abiotic stress (including exposure of the plant tissue cultures to extreme temperatures, ultraviolet light radiations, antibiotics, heavy metals salts, fungicides) enhances their synthesis. Regarding cell elicitors administration, the term of vaccination of plant cell cultures is used.

The essential oil of *Ocimum basilicum* L. has *in vitro* antifungal activity against *Aspergillus flavus* mycelial growth at 1000 ppm oil concentration and aflatoxin B1 production (El - Soud et al, 2015). The main components of essential oils, revealed by gas chromatography (GC) and GC coupled with mass spectrometry (GC/MS) are as follows: linalool (48.4%), 1,8-cineol (12.2%), eugenol (6.6%), methyl cinnamate (6.2%), α-cubebene (5.7%), caryophyllene (2.5%), β-ocimene (2.1%) and α-farnesene (2.0%).

Many studies reports that the compositions of EOs might be affected by the developmental stage of the plant (NEGAHBAN et al., 2015); regarding this aspect, essential oils of *Ocimum sanctum* L. (Tulsi) are obtained from aerial parts at different harvest stages (the vegetative, flower bud formation, and full flowering stages); eugenol reach a high content at flower bud formation stage and full flowering stage of the plant. The component β-bisabolen, the main component of the vegetative stage, declines at the flower bud formation stage. Also, the component 1, 8-cineole gradually decline along the different developmental stages. SAHARKHIZ et al. (2015) confirmed eugenol as the predominant compound of the EOS at all developmental stages, with a maximum level at second stage.

SHARMA et al. (2016) developed a new strategy to enhance the *Ocimum sanctum* eugenol content by adding to the tissue culture media a precursor (phenylalanine) in a concentration of 5mg/L. SHARAN et al. (2019) enhanced accumulation of ursolic acid and eugenol (phenolic compound) using three elicitors namely yeast extract, methyl jasmonate, and salicylic in hairy root cultures of *Ocimum tenuiflorum* L.

Most recent studies regarding *in vitro* methodologies to regenerate *Ocimum basilicum* of various cultivars using elicitors revealed that in the following are the most efficient in stimulating best response as multiple shoot regeneration and accumulation of secondary metabolites: benzylaminopurine alone or associated with thidiazuron or naphtylacetic acid or gibberellic acid and yeast extract, methyljasmonate, melatonin, salicylic acid, the predominant elicitors (Table 1).

Table 1. Comparative methodologies of *Ocimum* species and varieties in *in vitro* culture.

Reference	**Concentration and type of elicitor**	**Bioactive compounds accumulated in basil *in vitro* elicited cultures**
Biswas, 2020	methyl jasmonate (MeJA) in ¼ MS liquid medium supplemented with 1 mg/L NAA	rosmarinic acid
Nazir et al., 2020	2 mg/L melatonin (N-acetyl - 5-methoxytryptamine - an indole compound derived from serotonin) and UV	phenylpropanoid in purple basil calli
Mehring et al., 2020	methyl jasmonate (MeJA) added in shake flasks cultures suspension	biomass accumulation stimulated by increased triterpenoid activity
Duran et al., 2019	100 μM melatonin concentration	rosmarinic acid, caffeic acid, cinnamic acid, p-coumaric acid
Sharan et al., 2019	yeast extract, methyl jasmonate, salicylic acid in hairy root cultures of *Ocimum tenuiflorum* L.	ursolic acid and eugenol

CONCLUSIONS

The results highlighted that the age of the culture, the different exposure time, and concentration of elicitors are important parameters that determine the successful accumulation of the secondary metabolites. Variety and season of cultivation have a significant effect on the extraction yield as well as the qualitative and quantitative profile of basil essential oils. The highest amounts of active principles and antioxidant potential are correlated with elicitors and cultivar type. This review highlights the advantage of basil *in vitro* culture to modulate the content of active compounds by using different elicitor.

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