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Enhanced accumulation of phytosterols and phenolic compounds in cyclodextrin-elicited cell suspension culture of *Daucus carota*

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ABSTRACT

In this work, suspension-cultured cells of *Daucus carota* were used to evaluate the effect of β-cyclodextrins on the production of isoprenoid and phenolic compounds. The results showed that the phytosterols and phenolic compounds were accumulated in the extracellular medium (15100 $\mu g\,L^{-1}$ and 477.46 $\mu g\,L^{-1}$, respectively) in the presence of cyclodextrins. Unlike the phytosterol and phenolic compound content, β-carotene (1138.03 $\mu g\,L^{-1}$), lutein (25949.54 $\mu g\,L^{-1}$) and α-tocopherol (8063.82 $\mu g\,L^{-1}$) chlorophyll *a* (1625.13 $\mu g\,L^{-1}$) and *b* (9.958 (9958.33 $\mu g\,L^{-1}$) were mainly accumulated inside the cells. Therefore, cyclodextrins were able to induce the cytosolic mevalonate pathway, increasing the biosynthesis of phytosterols and phenolic compounds, and accumulate them outside the cells. However, in the absence of these cyclic oligosaccharidic elicitors, carrot cells mainly accumulated carotenoids through the methylerythritol 4-phosphate pathway.

Therefore, the use of cyclodextrins would allow the extracellular accumulation of both phytosterols and phenolic compounds by diverting the carbon flux towards the cytosolic mevalonate/phenylpropanoid pathway.

1. Introduction

Daucus carota L. (Umbelliferae), commonly known as carrot, is an important plant cultivated worldwide for its nutritive roots. Carrot has been used in traditional medicine due to a wide range of reported pharmacological effects, including antibacterial [1], antifungal [2], antihelminthic, and hepatoprotective [3] effects. Carrot roots contain a wide variety of high-value compounds including phenolic compounds such as coumarins [4] and *p*-hydroxybenzoic acid [5], volatile terpenoids [6] and several isoprenoid compounds like carotenoids [7], phytosterols [8] chlorophylls [9] and tocopherols [10]. The production of these high-value compounds increases under stress conditions. In fact, carrots respond to wounding stress by producing phenolic compounds such as caffeoylquinic acids [11], and elicited carrot cell cultures are able to produce *p*-hydroxybenzoic acid [12]. In addition, Sircar and Mitra [13] have shown that the presence of chitosan enhances the production of *p*-hydroxybenzoic acid after 36 h of treatment in hairy root cultures of *D. carota*. Light also provoke changes in the metabolic profile of green hairy root cultures of *D. carota*, increasing the production of volatile terpenoids, while the biosynthesis of *p*-hydroxybenzoic acid decreased [6].

As regards the biosynthesis of isoprenoid compounds, isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) constitute the two initial precursors of the isoprenoid biosynthetic pathway. These precursors can be obtained by two different pathways (Fig. 1): (i) the cytosolic mevalonic acid pathway (MVA), which is largely responsible for the generation of farnesyl diphosphate (FDP, C15), which, in turn, is converted in squalene (C30), the central precursor of all phytosterols, and (ii) the methylerythritol 4-phosphate (MEP) pathway localized in the plastids involved in the biosynthesis of geranylgeranyl diphosphate (GGDP, C20), which is used not only for the biosynthesis of tocopherols and phytol (C20), the hydrophobic tail of chlorophylls, but also for the biosynthesis of carotenoids (β-carotene and lutein, C40) [14] (Fig. 1). Moreover, tocopherol biosynthesis occurs as a result of a combination of the shikimate and isoprenoid pathways, which lead to the tocopherol precursors, homogentisic acid and phytyl diphosphate, respectively [15]. While the isoprenoid biosynthetic pathway has been well-characterized at molecular level, its regulation in plants

Abbreviations: DMADP, dimethylallyl diphosphate; DW, dry weight; FDP, farnesyl diphosphate; FW, fresh weight; GGDP, geranylgeranyl diphosphate; IDP, isopentenyl diphosphate; MEP, methylerythritol 4-phosphate; MVA, mevalonic acid pathway; PCV, packed cell volume; SCC, suspension cultured cells; CDs, β-cyclodextrins.

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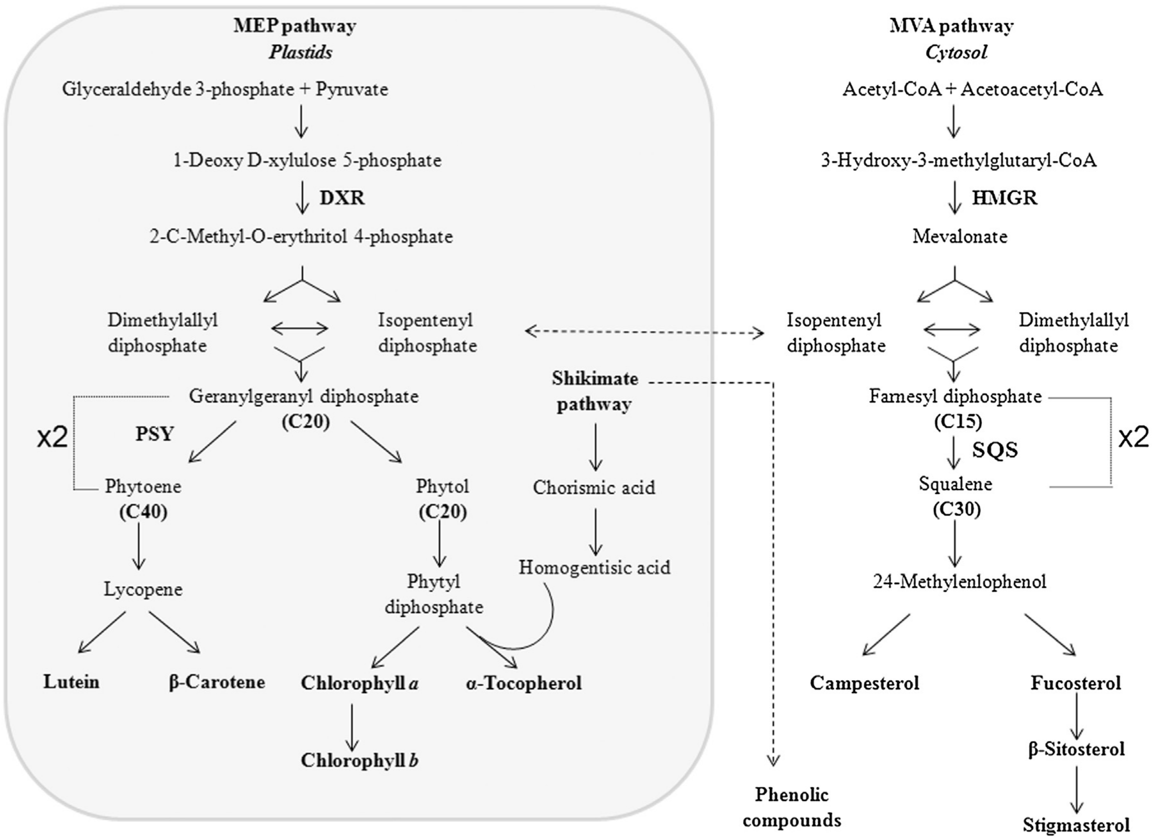

Fig. 1. Metabolic origins of the metabolites determined in this study. Isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) can be obtained by the cytosolic mevalonic acid pathway (MVA) involved in the biosynthesis of phytosterols, and the methylerythritol 4-phosphate (MEP) pathway localized in the plastids involved in the biosynthesis of tocopherols, phytol (C20), and carotenoids (β-carotene and lutein, C40). Likewise, phenolic compounds are formed from phenylalanine via the shikimate pathway. Phenylalanine biosynthesis takes place in plastids, but its conversion to phenolic compounds occurs outside this organelle.

during defence responses to elicitors is still poorly understood, and has mainly been studied in *Arabidopsis thaliana* [16].

All these metabolites have been described as bioactive compounds because of their human health benefits. For example, β-carotene and lutein prevent UV-induced erythema formation [17]. In addition, carotenoids are precursors of vitamin A and have a high antioxidant activity, so the intake of carotenoid-rich food can help prevent some kinds of cancer, cardiovascular diseases and macular degeneration [18].

For their part, phytosterols have received much attention in recent decades mainly for their ability to reduce cholesterol levels [19,20], and for their effects on several types of cancer and antioxidant activity [21,22]. α-Tocopherol is also an important bioactive compound been associated with the prevention of several human diseases, including coronary heart disease [23] atherosclerosis, diabetes, Parkinson's and Alzheimer's diseases, vision maladies, and impaired immune function [24]. Moreover, α-tocopherol is the most active form of vitamin E, which has strong antioxidant activity and therefore possesses a strong protective effect against lipid oxidation. Likewise, chlorophylls have therapeutic properties since they can form complex structures with certain chemicals that cause cancer, neutralizing them, while being a good source of antioxidant compounds [25].

As regard the biotechnological production of phenolic compounds, suspension cultured cells (SCC), immobilized cells and hairy root cultures have traditionally been used for the production of food additives like vanillin [26]. Vanillin, a mixture of flavor compounds obtained from the pods of *Vanilla planifolia*, is widely used for enhancing flavor in food and beverages, and as biopreservative because of its antimicrobial and antioxidant properties. Chemically isoeugenol is an isomer of eugenol, which is sometimes used as precursor in the production of vanillin [27]. Eugenol also has several pharmacological properties as an antioxidant, anti-inflammatory and anticarcinogenic agent, as well as other properties including antimicrobial and deterrent effect. This versatile compound is also a high value ingredient in perfumes and cosmetics.

As regards the biosynthesis of eugenol and isoeugenol, these compounds share the initial biosynthetic steps with lignins, until the formation of coniferyl alcohol. Then, coniferyl alcohol is transformed into coniferyl acetate by the action of coniferyl alcohol acetyltransferase before its reduction to eugenol and isoeugenol using eugenol synthase or isoeugenol synthase, respectively [28]. In a similar way, vanillin is synthesized from phenylpropanoid precursors, for which various biosynthetic pathways have been proposed. Anwar [29] indicated that vanillin could be synthesized from coniferin, a precursor of lignin by shortening of the C3 side chain and hydrolysis of the glucoside, while Zenk [30] suggested that vanillin was formed from ferulic acid through shortening of the side chain by β-oxidation. Yazaki et al. [31] reported the existence of a non-oxidative reaction by shortening of the phenylpropanoid side chain and the formation of 4-hydroxybenzaldehyde from 4-coumaric acid (C6-C3 compound) in cell-free extracts of *Lithospermum erythrorhizon* cell cultures. However, the complete biosynthetic pathway of vanillin from phenylpropanoids has not yet been demonstrated.

Due to the high value of carotenoids, tocopherols, phytosterols and phenolic compounds, great efforts have been made to improve the levels of these compounds plant *in vitro* cultures using biotechnological approaches.