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AGRONOMIC ASPECTS OF THE SUNFLOWER 7-HYDROXYLATED SIMPLE COUMARINS

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SUMMARY

Sunflower and other *Helianthus* ssp. produced, among other secondary metabolites, the coumarins scopoletin, scopolin and ayapin. In the most general sense they can be defined as stress metabolites, their synthesis being induced in response to adverse environmental condition, both biotic and abiotic. The pattern of coumarin synthesis and accumulation depends on plant variety, it is tissue dependent and developmentally regulated. Coumarin synthesis in sunflower seems to be part of the defence strategy against microorganisms, insect and parasitic plants. From an agricultural point of view the defensive potential of these compounds can be exploited in order to develop resistant varieties (either by classical plant breeding or by biotechnology) or crop protection strategies involving the use of chemicals which induced coumarin synthesis.

Key words: ***Helianthus* ssp., sunflower, coumarins, stresses, plant defence response, crop protection**

INTRODUCTION

Coumarins are a group of plant phenolic compounds bearing a benzopirone ring in their structure, and are defined as a lactones of 2-hydroxycinnamic acids (Figure 1). The group is diverse and individual components are classified in subfamilies according to their substitution pattern in both ring A and B. Our interest is focused on coumarins with a substitution pattern similar to those of cinnamic acids (6,7,8 hydroxylated and/or methoxylated coumarins; Figure 1). They are amply distributed in the plant kingdom, being the coumarin produced, characteristic of a species or botanical family. Thus, sunflower produces scopoletin, scopolin (the scopoletin glucosil-derivative) and ayapin (Figure 1).

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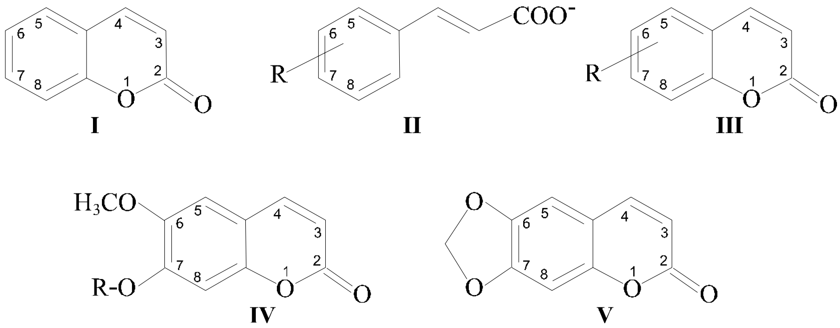

Figure 1: Chemicals structrures named in the text
(I) Coumarin (benzopirone ring)
(II) Cinnamic acids (R: -H, -OH, -OCH_3)
(III) 7-hydroxylated simple coumarins (R: -H, -OH, -OCH_3)
(IV) Scopoletin (R: -OH); Scopolin (R: -Glucose)
(V) Ayapin

Coumarins are a group of interesting natural products not only because of their importance for the producer plant itself (mainly as defence compounds) but also due to their practical uses either by themselves or as components of foodstuffs (*i.e.*, they possess interesting pharmacological or toxicological properties, have high anti-oxidant activity, protect against UV light, are components of colorants, flavors, aromas and can be used as analytical reagents). Extensive review on these compounds have appeared in the past (Matern, 1991; Murray *et al.*, 1982; Murray, 1989; 1995).

The coumarins can be synthesized de *novo*, from phenylalanine, or from pre-formed phenolics (storing forms) such as, in the case of sunflower, the chlorogenic acid (Figure 2). The exact coumarinization pathway from specific cinnamic acids (coumarate, caffeate or ferulate) is almost unknown, although a cinnamate-2-hydroxylase has been proposed as a key enzymatic step of the route. Different enzymes of the pathway (phenylalanine ammonia-lyase, cinnamate hydroxylase, methyl transferases, glycosyl transferases and peroxidases) are being studied in our laboratory.

Coumarins are stress inducible metabolites

In sunflower, the synthesis of coumarins is induced in response to an ample variety of stresses both biotic and abiotics, as well as in response to nutritional deficiencies, xenobiotics, agrochemicals, and plant metabolites (sucrose), as summarized in Table 1. The coumarin content in sunflower plants grown under optimal non-limiting conditions tends to be very low. For that reason their presence can be considered as a very good marker of a stress situation and these compounds can be defined, in the most general sense, as stress metabolites.

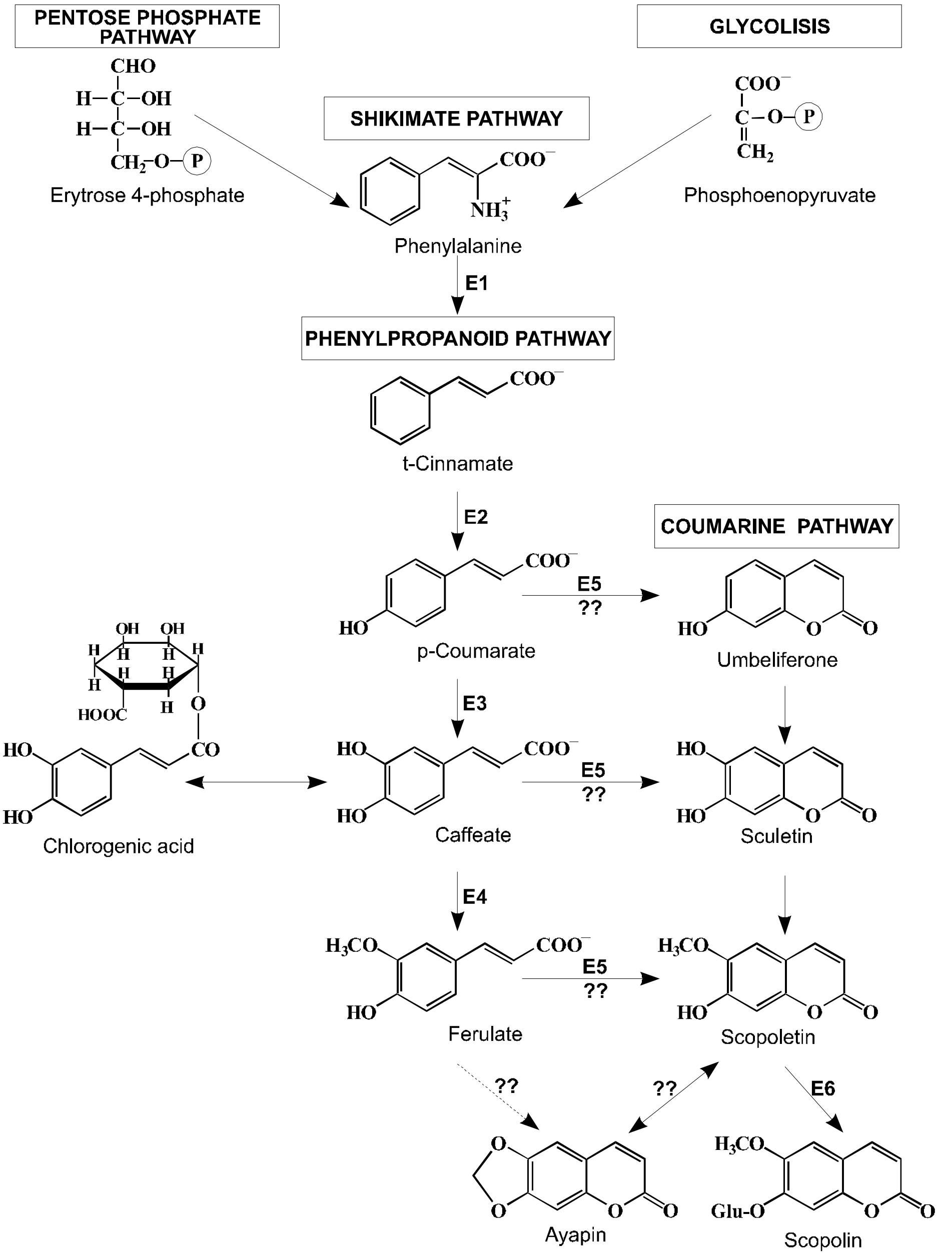

Figure 2: Proposed route of coumarin biosynthetic in plants

E1: Phenilalanine ammonia liase *E2: Cinnamate 4-hydroxylase*

E3: 4-Coumarate 3-hydroxylase *E4: O-methyl transferase*

E5: Cinnamate 2-hydroxylase *E6: Glycosyl transferase*

Table 1: Factors which have proven to induce coumarin biosynthesis in sunflower (data come from the authors´ laboratory)

Biotic	Fungi (*Plasmopara halstedii*; *Sclerotinia sclerotiorum*), parasitic plants (*Orobanche cernua*)
Abiotic	Drought, cold, salinity, UV light, mechanical impedance
Nutritional deficiencies	Potassium
Xenobiotics	Copper
Agrochemicals	Herbicides (paraquat), fungicides (metalaxyl), SAR inducers (BTH)
Plant metabolites	Sucrose

Coumarin synthesis depends on plant variety, it is tissue dependent and developmentally regulated

In sunflower, the pattern of coumarin synthesis and accumulation depends on the developmental stage of the plant, being higher in old mature plant than in young active growing ones. Coumarins are mainly synthesized in the aerial part (leaves) from where they can be translocated to the root. Coumarins can either be accumulated intracellularly (mainly as scopolin), incorporated to the cell wall (as is the case of scopolin, in a reaction catalyzed by a scopoletin peroxidase), or excreted (as is the case of ayapin). There are differences in the capacity of coumarin synthesis among plant varieties; these differences could explain in specific cases, as will be commented, differences in resistance to biotic stresses. Such differences (either pre or post-infectionally) are being evaluated in our laboratory by using sunflower lines with different levels of resistance to *Orobanche cernua* (sunflower broomrape) and *Sclerotinia sclerotiorum* (sunflower head rot).

Coumarins and resistance to phytopathogenic microorganisms, insects, parasitic plants and weeds

According to data obtained in our laboratory and other published in the literature, we can establish that, at least in sunflower, although probably also in other coumarin-producing crops like tobacco and citrics, coumarin biosynthesis is part of the plant defence strategy against phytopathogenic microorganisms, insects, parasitic plants and weeds, being defined as phytoalexins, insect deterrents or allelochemicals. Their synthesis and cell accumulation or excretion to the surrounding media protect the plant by preventing fungal spore, weed or parasitic plant seed germination (first defense line), inhibiting the development of infective structures (fungi, parasitic weeds) or preventing host-tissue invasion (second defense line), and, hence, localizing and preventing parasite spread. In the case of insects, coumarins may act as antifeeding compounds. In Table 2 are listed a number of fungal and parasitic weed diseases as well as insect attack in which coumarin synthesis can be proposed as a resistance mechanism.

Table 2: Sunflower coumarins and resistance to fungi, insects and parasitic weeds

Fungi	*Alternaria helianthi*[1], *Phoma macdonaldii*[1], *Helminthosporium carbonum*[1], *Plasmopara halstedii*[2], *Botrytis cinerea*[2], *Puccinia helianthi*[2], *Sclerotinia sclerotiorum*[2]
Parasitic plants	*Orobanche cernua*[2]
Insects	*Zygogramma exclamationis*[3]

[1]Tal & Robeson (1986 a,b),
[2]Data from the authors´ group,
[3]Olson & Roseland (1991)

Most of the evidence in favor of the defensive role of coumarins comes from laboratory experiments in which these compounds have proven to act as inhibitors of fungal spore or parasitic plant or weed seed germination, and their differential accumulation in resistant and susceptible plant varieties in response to parasitism or insect attack has been observed. In general, and as a rule, coumarins are acccumulated earlier and in a greater amount in resistant than in susceptible varieties. In any case, coumarin accumulation does not preclude the existence of other resistance mechanisms and, what is most important, this point of view does not consider genetic aspects of the resistance, and whether resistance is a monogenic (qualitative) or multigenic (quantitative) character. Unfortunately we are not yet close to having isogenic plants, either mutants or transgenics, which could be used to prove the contribution of coumarins to resistance.

How can the coumarin potential be exploted in order to establish crop protection strategies?

Intensive research on secondary metabolism, as the case of the reported sunflower 7-hydroxylated simple coumarins, can help us to develop crop protection strategies. Firstly, coumarin production can be used as a molecular marker in plant breeding program directed towards obtaining resistant varieties. Molecular analysis, including metabolites, enzymes or corresponding genes, must be optimized. We are successfully using these assays in characterizing sunflower varieties. Secondly, coumarin production can be managed. There are a number of chemicals which have proven to induce their synthesis in sunflower, including xenobiotics (copper), environmental friendly compounds (sugars), and agrochemicals (fungicides, herbicides, SAR inducers). Finally, and with the advance of molecular genetic and plant transformation techniques, it is posible to obtain crop transgenic lines with a controlled overproduction of these defensive compounds. But before that, and in the case of coumarins, it is necessary to know in detail their metabolism and the genes involved.

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ASPECTOS AGRONÓMICOS DE LAS CUMARINAS SIMPLES 7- HIDROXILADAS EN GIRASOL

RESUMEN

El girasol y otras especies del género *Helianthus* produce entre otros metabolitos secundarios, las cumarinas escopoletina, escopolina y ayapina. De modo general pueden ser definidas como metabolitos de estrés, estando su síntesis inducida ante condiciones de estrés tanto biótico como abiótico. El patrón de síntesis y acumulación de cumarinas depende de la variedad, del tejido y del estado de desarrollo. La síntesis de cumarinas en girasol parece formar parte de una estrategia de defensa contra microorganismos, insectos y plantas parásitas. Desde el punto de vista agrícola el potencial de defensa de estos compuestos se puede explotar para desarrollar variedades resistentes (por mejora clásica o biotecnología) o estrategias de protección de cultivos que incluyan el uso de productos químicos que inducen su síntesis.

ASPECTS AGRONOMIQUES DE 7 COUMARINES HYDROXYLES SIMPLES CHEZ LE TOURNESOL

RÉSUMÉ

Le tournesol et d'autres espèces de la famille *Helianthus* ssp. produisent, entre autres produits du métabolisme secondaire, les coumarines scopoletine, scopoline et ayapine. Au sens le plus large, ces produits peuvent être définis comme des métabolites de stress car leur synthèse se fait en réaction à des conditions défavorables de l'environnement, autant biotiques qu'abiotiques. La dynamique de synthèse et d'accumulation de la coumarine dépend des sortes, c'est-à-dire du tissu et du degré de développement de la plante. Il semble que la synthèse de la coumarine chez le tournesol soit une partie de la stratégie de défense contre les micro-organismes, les insectes et les plantes parasites. Du point de vue agronomique, le potentiel de défense de ces composés pourrait être utilisé lors de la création de sortes résistantes (que ce soit par la sélection classique ou par les méthodes biotechnologiques) ou lors du développement des stratégies de protection des récoltes fondées sur l'utilisation de produits chimiques et qui provoquent la synthèse de ces composés.