Journal of Cell and Molecular Biology **2**: 13-18, 2003.
Haliç University, Printed in Turkey.

Phenolic cycle in plants and environment

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Received 30 October 2002; Accepted 15 November 2002

Abstract

Phenolic substances are synthesized in plants and in the soil. They exist in the form of polymers and monomers. The latter group of phenolics is assembled within the chloroplasts of plant cells, whereas soil phenolics are associated with the process of humus formation on the alumino-silicate matrix of the soil micelle. As plants grow, phenolics accumulate in cell vacuoles, or polymerize into lignin, which strengthens the secondary cell walls. In addition to this, phenolics possess also some physiological functions as they regulate cell elongation. When they are excreted from plant root systems they exert inhibitory growth function within adjacent rhizospheres. This work presents the latest experimental evidence of phenolic synthesis and transformation in the environment, while providing an understanding of their effect in plant-soil relations.

Key words: Allelopathy, chloroplasts, humus, phenolics, soil micelle

Bitkilerde fenolik döngü ve çevre

Özet

Fenolik maddeler bitkilerde ve toprakta sentezlenir. Bunlar polimerler ve monomerler şeklinde bulunurlar. Fenoliklerin monomer grubu bitki hücresinin kloroplastlarında biraraya gelirken, toprak fenolikleri toprak misellerinin alumino-silikat matriksi üzerinde humus oluşum olayı ile uyumluluk gösterir. Bitki büyürken hücre vakuollerinde fenolikler birikir veya sekonder hücre çeperlerine sağlamlık kazandıran ligninlere polimerize olurlar. Bunlara ilave olarak fenolikler hücre uzamasını düzenleyerek bazı fizyolojik işlevlere de sahiptirler. Bitki kök sistemlerinden salındıkları zaman hemen yakınındaki rizosferlerde büyümeyi inhibe edici etki meydana getirirler. Bu çalışma fenolik sentezlerinin en son deneysel verilerini ve çevredeki dönüşümlerini sunarken, bitki-toprak ilişkilerindeki etkilerini anlamamıza yardım etmektedir.

Anahtar sözcükler: Allelopati, kloroplastlar, humus, fenolikler, toprak miseli

Introduction

Phenolics are very stable products in plant organisms. Generally, they are characterized by a benzene ring and one hydroxyl group (-OH). They can be converted into lignin which is the main phenolic polymer in plants. Microorganisms break down these molecules and their fragments contribute to the mineralization of soil nitrogen and humus formation. Thus, humus participates actively in fulfilling plants nutritional needs and growth. Light enhances the biosynthesis of phenolic substances in plant chloroplasts and these constitute in addition to soil micelles (humus) a second formation site for this diverse group of organic

molecules. It should be mentioned however, that phenolics tend to accumulate in plant vacuoles in relatively high amounts, or they deposit in the secondary cell wall as lignin.

Chloroplasts as centers of phenolics biosynthesis

Experiments with chloroplasts of willow (*Salix spp.*) leaves showed that the synthesis of phenol-carboxylic acids and flavonoids is strongly stimulated by light exposure. Metabolic inhibitors that depress photosynthetic activity (simazine, diurone, chloramphenicol), affect negatively the biosynthesis of flavonoids. Leaves chloroplasts have the capability to localize phenol compounds, some of which are specific to these organelles only. The chloroplasts of spring willow leaves contain more phenols than the chloroplasts of the same leaves in the autumn. Light is a mandatory condition to initiate phenolics synthesis and this is indicated also by the lack of such molecules in the protoplastids of etiolated willow shoots (Kefeli and Kalevitch, 2002). Light appears also to induce flavonols synthesis in the chloroplasts and cytoplasm. Chalcone and phenolcarbonic acid present in etiolated willow shoots can be considered metabolic precursors of light-synthesized flavonols. In certain cell compartments (vacuoles and cell wall) phenols are contained in significant amounts (Lewis and Yamamoto, 1990). However, it is not clear yet how phenols are translocated within plant cells and how they affect the function of cell organelles such as ribosomes and mitochondria. Phenolic substances that inhibit plant growth (hydroxy derivatives of cinnamic acid, coumarin and naringenin) are synthesized similarly to other phenolics. The synthesis of growth inhibitor derivatives of hydroxycinnamic acids follows the pathway: shikimic acid-chorismic acid-prephenic acid-cinnamic acid and p-coumaric acid. A theory of metabolism bifurcation among phenolic substances, some of which can inhibit growth and synthesis of indolic compounds has been proposed. According to this new approach, indolil-3-acetic acid (IAA) becomes the main natural auxin (Kefeli, 1978; Kefeli and Dashek, 1994; Kefeli and Kalevitch, 2002). Therefore, indole auxins (IAA, indoleacetonitrile) as well as phenolic inhibitors (p-coumaric acid, coumarin, naringenin and others) are derived from the common precursors, shikimic and chorismic acids (Figure 1).

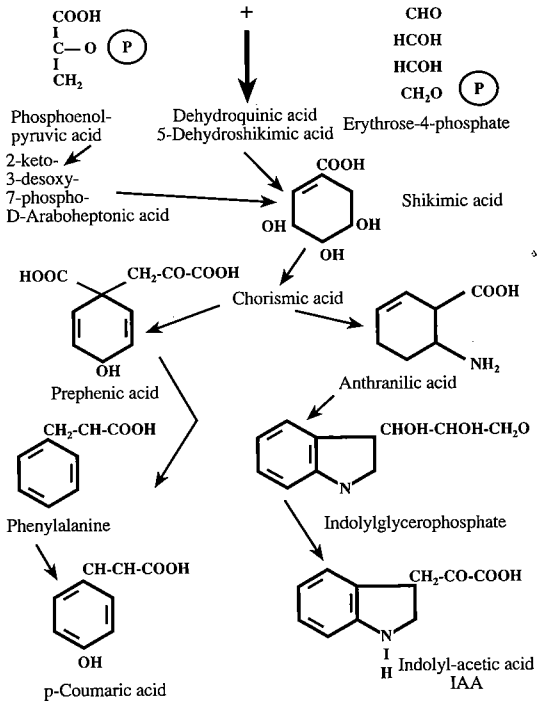

Figure 1: Phenol-propanoids in metabolic bifurcation.

Muzafarov and collaborators (1992) investigated the functions of some phenolics in chloroplasts. They assumed that the essence of the relationship between photosynthesis and phenolics biosynthesis is that phenolics exert a direct and an indirect effect on the process of solar accumulation itself. From our point of view, flavonoids as polyfunctional compounds in green plastids fulfill three major functions as:

- substrates (use polyphenols and their catabolic products for other kinds of biosynthesis);
- energy sources (electron and proton transport, ion exchange and membrane potential, radicals formation);
- regulators (involvement in enzyme reactions as inhibitors or activators).

During photosynthesis under light, flavonoids change the rate of electron transport and photophosphorylation, bringing about the change of ATP/NADPH ratio. In the reactions of carbon metabolism they can shift the dynamic equilibrium of pentosephosphate reduction cycle to enhance the synthesis of certain metabolites both due to the change in energy substrate intake and to the interaction with enzymes of the cycle. Additionally, flavonoids

exercise a feedback control over their own biosynthesis, although this phenomenon is not clearly understood. This questionable situation remains as the biosynthesis of the entire flavonoid structure within plastids has not been explained, nor the complete enzymatic package of their biosynthesis has been discovered yet. Lack of direct eveidence of flavonoids transport within the cell and through the whole plant constitues another challenge to a more accurate description of their functions. Noneless, a variety of phenolic compounds, present simultaneously within cells appear to be capable of influencing the rate and direction of plants metabolic activities. Thus, any change in the flavonoid structure, or qualitative composition of the phenol complex result in a change of the mechanism of its effect upon the processes of cell energy exchange.

Chalcone and phenolcarbonic acids present in etiolated willow shoots can be viewed as the potential precursors of light synthesized flavonoids. However, the use of paper chromatography to investigate isosalidpurposide transformation products did not reveal the presence of any flavonols sensitive to conventional reagents. Therefore, the transformation of chalcone (isosalpurposide) in lightless vitro appears to terminate at a second stage. The synthesis of eriodyctiol and luteolin that occurred in willow leaves evidently took place *in vivo* and under light exposure. It should be pointed out however, that phloridzin and isosalipurposide were decomposed from aglycone and that phloridzin and phloretin produced yellow stains on the chromatogram as well as flavonoids. It is known that flavonoid glycosides are revealed as dark spots on chromatograms exposed to UV light. Therefore, our yellow stains were classified as flavanones, since they did not react with $AlCl_3$, nor Na_2CO_3 like flavonols, that also form yellow spots. At the same time, similar to chalcones and aurones, these floridzin transformation products are yellow colored and they turn into orange-pink when exposed to Na_2CO_3 or NH_4OH. Relatively easy transformations of isosalipurposide and phloridzin into compounds of other classes (flavanones, chalcones, or aurones) evidenced the role of these products in the general metabolism of flavonoids (Figure 2).

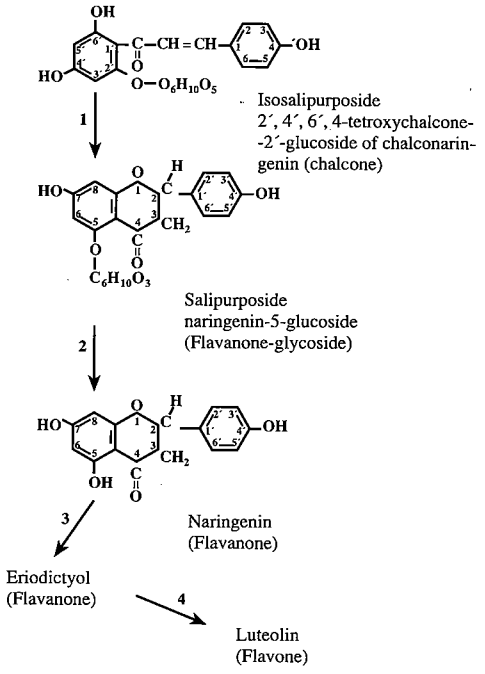

Figure 2: Flavonoid biosynthesis.

Phenolic substances secreted by roots and leached from leaves

Plants contain and secrete a diverse group of growth inhibiting substances that may affect other plants development, if grown in their vicinity (allelopathy). Leaf exudates of willow species such as *Salix rubra* or *Salix viminalis*, contain phenolic inhibitors like naringenin derivative isosalipurposide. Other species instead like apple trees (*Malus spp.*) contain phloridzin, which is a strong respiratory inhibitor. Roots and leaves of the wild plant *Nanaphyton* native to semi-desert regions of Mongolia contain also strong phenolic inhibitors. Seed as well may secrete allelochemicals. Tobacco seed (*Nicotiana tabacum*) for example suppress germination of its own seed when leachates come in contact with the seeds (Kefeli and Kalevitch, 2002). Although the inhibition of germination was observed at various levels of intensity, this phenomenon demonstrates the selectivity of these natural excreta, similar to the effect of synthetic herbicides. Therefore, increasing evidence indicates that phenolics and alkaloids play the role of selective agents. Secondary compounds can be modified in transgenic plants and genetic mutants.

Hence, molecular genetics becomes a tool, which may help to regulate the level of secondary metabolites in plants. Therefore, there is a need continue the search for botanical herbicides as a rise of ecological concerns has clearly identified the environmental impact of herbicides of synthesis.

Root exudates affect the germination of seeds of different crops: monocots and dicots (Table 1 and Table 2). However, it must be pointed out that only some phenolics were studied in the exudates of willow roots (1) which have no analogues in the roots (2) and leaves found among the common allelopathogens. Although some of these substances could be retained by willow roots, others where excreted into an external medium. Chromatography of these water exudates and a subsequent investigation of their chromatograms with UV-B light showed that most of these substances are polyphenols such as coumarin, or phenolic acids. The phenolic substances retained by cells had different chemical properties than those located in the root exudates. Thus, the data confirm the hypothesis that excreted substances had an allelopathic nature and were involved in developing ecological relationships with adjacent plants of different species.

During the composting process water extracts contain many inhibiting substances that might form toxic exudates (Kefeli et al., 2001). Paper chromatography reveals the presence of phenolic acids and coumarins in water extracts. The highest concentrations of these inhibitors was measured in abscised leaves of red maple (*Acer rubrum L.*). One gram of dry leaves was mixed in 29 ml of water to prepare the extracts. The pH of the solution was between 5.4 and 5.6 and the extracts were incubated for a week at room temperature while the pH raised to 7.2. Further observations revealed that during composting the amount of phenolics was drastically reduced. Seed germination tests were performed with these water extracts and pure water (control) on lettuce and wheat seeds. Germination rate and seedling lengths were measured to demonstrate that phenolics decreased inhibiting properties after dilution, or after

Table 1: Effect of root exudation on germination of crop seeds (Non-concentrated exudates).

Variant	% to tap water (control)			
	wheat	clover	lettuce	mustard
Tap water	100	100	100	100
Spider plants (Chlorophytum) exudates	54	93	75	100
Willow (*Salix vitaminalis*) exudates	58	79	74	138
	Stem length (5 tallest plants, mm)			
Tap water	29	23	18	25
Spider plants (Chlorophytum) exudates	15	21	14	2
Willow (*Salix vitaminalis*) exudates	7	18	13.5	3.5

Table 2: Biological activity of willow root exudates after paper chromatography (Biological activity in % to control (water)).

Rf	Colour in UV-B light	Clover		Lettuce	
		Germination	Stem length	Germination	Stem length
0	Blue	91	76	90	64
0.14	Blue	94	68	98	58
0.3	Violet	86	80	93	76
0.5	Blue	56	52	71	76
0.67	Yellow	87	68	89	88
0.88	Yellow	52	56	63	64

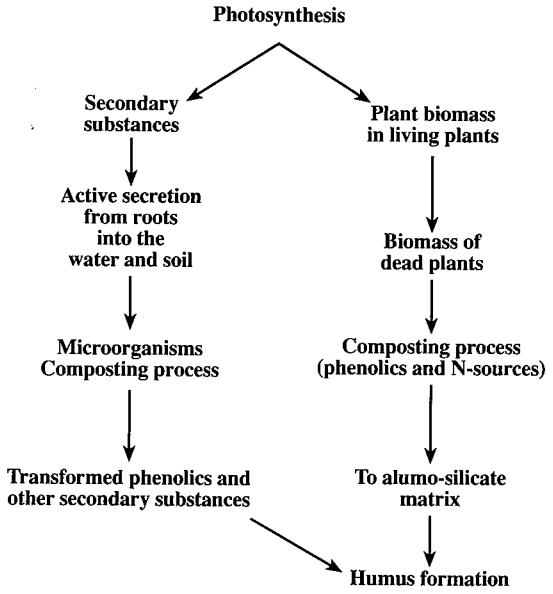

Figure 3: Secondary substances, plant biomass accumulation and humus formation during allelopathic effects.

contact with fungi. Therefore, the whole process of allelopathogens formation in the environment could be tightly connected with the formation of secondary substances and plant biomass accumulation (Figure 3).

Soil-microbial complex for phenolic decomposition

Phenolic substances are the most resistant metabolites produced by plants. They undergo further transformation in the soil, forming humus molecules, strongly linked to the alumino-silicate matrix. Humus is more or less a stable fraction of soil organic matter; it adsorbs mineral elements that serve as important nutrients for plant growth and development (Kefeli, 2002). The alumino-silicate matrix and humus form primary soil units. Humus is formed by carbon-nitrogen interaction. Potential sources of carbon include cellulose and polyphenols from plant leaves, or transformed lignin polymers.

In order to verify the efficacy of microbial activity during the humification process, four different soil horizons in a Grashem soil at the Macoskey Center of Slippery Rock University of Pennsylvania, USA were investigated. The presence and number of colonies of heterotrophic soil microflora were determined in each horizon (TSA (triple-soya-agar, 48 hours, room temperature). The topsoil (horizon A, 0-28 cm) was dark gray in color, sandy, high organic matter content (5.6%), with slightly alkaline pH=7.5. This horizon was also high in potassium, low in available nitrogen, and medium in phosphates content, while very high was the microbial activity. Horizon E (28-52 cm) was ochric in color, it contained more loam, less organic matter, lower microbial activity and pH=7.7. Horizon B (52-62 cm) had no organic matter, microbial activity was the lowest and pH=7.8. Water permeability was also measured for each horizon to evaluate penetration times. The fastest penetration rate was measured in horizon A (11 minutes), whereas it took 47 minutes for horizon B and longer (more than 6 hours) below horizon B. Soil fertility conditions were also assessed with a wheat/clover germination test. A sand substrate was used as control, which yielded 30-50% germination. Horizon A had a germination of 80-82%, horizon B 40-60%, horizon E/B (with lowest microbial activity) yielded 30-70% germination rate (Kalevitch et al., 2002). The results of these experiments appear to indicate that topsoil (for its highest microbial activity)

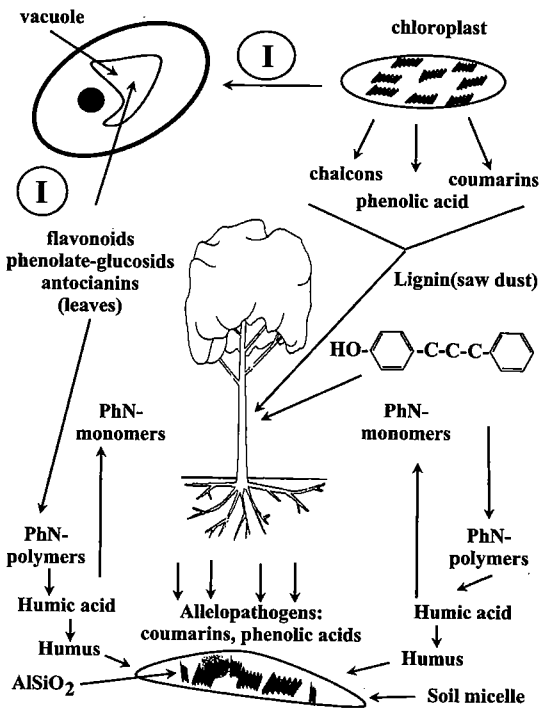

Figure 4: Phenolic cycle.

is an effective medium usable to facilitate composting of maple and sumac leaves, contaning nature phenolic compounds.

Conclusion

Microorganisms have the capability to decompose phenolic compounds to their monomers, being deglicosidation of phenolic molecules, followed by lignin decomposition the biochemical pathways of the process. Leaves become a primary substrate for soil microorganisms, while woody materials and sawdust serve as secondary type of biomass and these substrates play a major role in humus formation (Figure 4).

The biosynthesis of phenolic substances within chloroplasts and its further transformation on the alumino-silicate matrix of soil micelles led us to conclude about the existence of phenolics cycle in the plant-soil system. Although many aspects remain unknown, the ecological relevance of phenolic substances in the environment has been amply demonstrated as this cycle embrace lithosphere, microsphere and biosphere.

These emerging concepts facilitate the understanding of complexity within our living systems and their physical habitat while reinforcing the idea of interconnectedness among living species and ecosystems.

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