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REVIEW

POLYAMINES IN PLANTS

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Summary. The available information on plant polyamines is reviewed. The polycationic character of polyamines is suggested as a rationale of their properties and functions. Different aspects of polyamines are examined: chemistry, occurrence, localization, uptake, transport, interactions with nucleic acids, proteins, membranes and cell walls, and role in maintaining of pH, ion and osmotic cell homeostasis. The biosynthetic and catabolic pathways of polyamines and their relation to the overall metabolism, especially to the alcaloid formation, are outlined. The physiological functions of polyamines are discussed, namely their involvement in cell cycle, cell division, growth, development, senescence, as well as in stress phenomena. Alternative views on polyamines as a new class of plant growth regulators or second messengers are presented. An emphasis is laid on the phenylamides (conjugates of polyamines and hydroxy cinnamic acids) as specific plant molecules with regulatory and radical scavenging properties. The possibilities for practical application of polyamines are concerned.

Key words: polyamine chemistry, occurrence, localization, transport, interactions, biosynthesis, catabolism, physiological functions, growth regulation, stress

***Abbreviations*:** ACC – 1-aminocyclopropane-1-carboxylic acid; ADC – arginine decarboxylase; CAM – Crassulacean acid metabolism; DAO – diamine oxidase; DFMA – DL-α-difluoromethyl arginine; DFMO – DL-α-difluoromethyl ornithine; dSAM – decarboxylated S-adenosylmethionine; IAA – indolylacetic acid; LDC – lysine decarboxylase; ODC – ornithine decarboxylase; PA – polyamines; PAO – polyamine oxidase; PCA – perchloric acid; PhA – phenylamides; PO – peroxidase; PCT – putrescine N-caffeoyl transferase; SAM – S-adenosylmethionine; SAMDC – S-

adenosylmethionine decarboxylase; SHT – spermidine hydroxy cinnamoyl transferase; TCA – trichloroacetic acid

Introduction

Di- and polyamines* (PA) are organic molecules discovered in human semen by Antoni van Leewenhoek in 1678. They were considered as decomposition products of nitrogen containing organic compounds in animal organisms, but later proved to be important constituents ubiquitous to all pro- and eucaryotes, taking part in many biological processes (Smith, 1991). An impressive body of data is accumulated, reporting their occurrence, localization, chemical properties, metabolism and physiological functions, as well as their involvement in stress phenomena in plants.

In PA research four approaches are mainly used: *in vitro* experiments with model systems; study of plant responses to exogenous PA supply; following the variations of PA content and related enzyme activities; PA depletion. Depletion can be achieved by blocking PA biosynthesis with specific inhibitors; by using mutants, lacking loci important for PA biosynthetic pathways; by using organs temporarily deficient in PA. In these cases, the reversal of depletion effects by addition of PA is very indicative. Whereas most of the above approaches yield correlative evidence, the depletion approach may directly contribute to understanding of the metabolic role of PA. The exogenous supply of PA is thought to be less informative in view of the high internal concentration of PA; however, this approach is reasonably applied in systems expressing PA deficiency, such as senescence, and in the study of PA uptake, translocation and localization, using labelled products.

Definition. Occurrence. Localisation.

PA in plants are straight-chained C_3–C_{15} aliphatic hydrocarbons substituted with two primary (terminal) amino groups and in most cases – with one or more imino groups. They are widely distributed in both higher and lower plants. PA common to all plant species are putrescine (diamine), spermidine (triamine), and spermine (tetraamine) (Smith, 1970, 1991; Flores, 1990). Other PA occur only in some plant families: the diamine cadaverine and the triamine homospermidine are characteristic for Leguminosae (Flores, 1990), the diamine 1,3-diaminopropane is the predominant PA of Gramineae, the rare tetraamine canavalmine and two canavalmine – related pentaamines are found in seeds of *Canavalia gladiata*, sword bean (Matsuzaki et al., 1990). The

* For the sake of brevity only the term polyamines involving diamines and polyamines proper will be used

uncommon PA norspermidine (caldine) and norspermine (thermine) are identified in drought tolerant alfalfa genotypes in water deficit stress conditions, and in pollen and cell cultures of heat tolerant cotton after high temperature stress, where another unusual PA, caldopentamine, is also synthesized (Kuehn et al., 1990). Norspermidine, norspermine and caldopentamine are referred to as thermopolyamines and are suggested as being involved in thermotolerance (Kuehn et al., 1990). Closely related to them is the thermospermine, a tetraamine $NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$ which is reported in an extremely thermophilic microorganism, *Thermus thermophilus* (Takeda et al., 1982).

The formulae of the above mentioned PA are presented in Table 1.

PA are distributed in all vegetative and reproductive plant organs: roots, stems, leaves, flowers (pollen, stamens, pistils); they are found in seeds (embryo and endosperm), seedlings, tubers; in meristem, xylem, phloem, and parenchyma tissues (Vallee et al., 1983; Felix and Harr, 1987; Evans and Malmberg, 1989). In cells PA are localized mainly in vacuoles; they are also bound to nuclei, mitochondria, chloroplasts, ribosomes, cell walls, membranes, and are present in the apoplastic fluid (Goldberg and Perdrizet, 1984; Bagni, 1989; Evans and Malmberg, 1989; Bors et al., 1989; Slocum, 1991b; Bagni and Pistocchi, 1991).

Chemistry

PA are strongly basic in character; they are aliphatic polycations having two or more positively charged loci. The primary (terminal) amino groups are highly protonated at neutral (physiological) pH 7.0, their pK values being about 10–11. Imino groups are also protonated but to a lesser degree (pK 8–9) except those located in the vicinity of CH_2 or $(CH_2)_2$ (i.e. where CH_2 groups are less than three); these imino groups are not protonated. Protonation sites in PA are determined by reliable methods, such as nuclear magnetic resonance spectroscopy. Positive charges are unevenly distributed along the chain, depending on the chain length, the number and position of imino groups between methylene residues, the number of methylenes, and pH and ionic strength of the environment.Thus, pK values of terminal amino groups in —$(CH_2)_4$— chains are higher than those in —$(CH_2)_3$— chains, and pK in —$(CH_2)_3NH(CH_2)_4$— is higher than in —$(CH_2)_3NH(CH_2)_3$— chains (Takeda et al., 1983).

The specific structural and electrochemical characters of PA (as polycations, whose positive charges are distributed terminally and between small methylene residues in a short to medium chained aliphatic molecule) determine their interactions with anions and negatively charged loci in cell components, as well as their ability to form bridge structures between proximately disposed negative sites. These features are not shared by metal cations.

Table 1. Polyamines occurring in plants

Polyamine	Formula	Number of amino+imino groups	Number of methylene groups	Occurrence
Putrescine	$NH_2(CH_2)_4NH_2$	2	4	common
Spermidine	$NH_2(CH_2)_3NH(CH_2)_4NH_2$	3	7	common
Spermine	$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	4	10	common
Cadaverine	$NH_2(CH_2)_5NH_2$	2	5	uncommon
Homospermidine	$NH_2(CH_2)_4NH(CH_2)_4NH_2$	3	8	uncommon
1,3-diaminopropane	$NH_2(CH_2)_3NH_2$	2	3	uncommon
Norspermidine (caldine)	$NH_2(CH_2)_3NH(CH_2)_3NH_2$	3	6	uncommon
Norspermine (thermine)	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$	4	9	uncommon
Caldopentamine	$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$	5	12	uncommon
Canavalmine	$NH_2(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$	4	11	uncommon
Aminopropyl canavalmine	$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$	5	14	uncommon
Aminobutyl canavalmine	$NH_2(CH_2)_4NH(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$	5	15	uncommon

Interactions of polyamines with macromolecules and subcellular structures. Involvement in the regulation of pH, ion and osmotic cell homeostasis

Interactions with nucleic acids

Data from crystallography X-ray diffraction experiments prove PA binding to DNA. Different types of interactions are established: electrostatic interactions of amino and imino groups of PA with negatively charged phosphate groups of DNA, and hydrogen and covalent bonding of PA with nucleotide bases. The interactions of PA are effectuated both intra- and inter-DNA chains, and obey stringent structural requirements where the size of CH_2 residues appears of crucial importance. Thus, the trimethylene portion of spermidine links phosphate groups in adjacent nucleotides on one chain of DNA, whereas the tetramethylene portion stretches across the minor groove of the double helix forming a bridge between phosphate groups on opposite DNA strands. Structural and functional consequences of these interactions are expressed at submolecular, molecular and higher levels of organization, namely: repulsive electrostatic forces between phosphate groups are neutralized, and attractive van der Waals forces are increased; bridge structures are built up, leading to important conformational changes, complexation and stabilization of DNA structure, and chromosome condensation. This results in a reduced thermic and X-ray denaturation of DNA, increased resistance to enzymatic breakdown, and facilitated B $\rightarrow$ Z conformational transition. *In vivo* experiments with PA-deficient mutants and inhibitors of PA biosynthesis, depleting PA, point to their involvement in ensuring a higher rate and reliability of transcription. PA modulate also mRNA, tRNA and rRNA conformation, and thus are involved in optimisation of post-transcriptional cell events (Tofilon et al., 1982; Slocum et al., 1984; Chroboczek, 1985; Yarigin, 1987; Mehta et al., 1991).

Interference in protein biosynthesis and enzyme activity

Modulation of protein biosynthesis is effectuated primarily via PA–nucleic acid interactions. Moreover, PA specifically modulate protein synthesis *in vivo* by controlling the formation of polysomes and the binding of aminoacyl tRNA to ribosomes; negatively charged loci in ribosomes are targets for binding of PA which act synergistically with Mg^{2+} to form and maintain functionally active polysome assembly. As a next step, PA enhance the rates of both peptide chain initiation and elongation, resulting in increased yield of full-length translational products, as shown in wheat germ cell–free translation system.

PA regulate enzyme activity in several ways: through modulation of phosphorylation–dephosphorylation pattern, involving size – charge interactions with protein

kinases catalyzing phosphorylation; through various types of ionic interactions, and through covalent binding. As protein phosphorylation–dephosphorylation is a universal signal transducing mechanism in living systems, the involvement of PA in this process points to their regulatory role in plants (Slocum et al., 1984; Veluthambi and Poovaiah, 1984; Data et al., 1987; Ye et al., 1994).

Interactions with membranes

Numerous data point that PA influence membrane structure and function. This is accounted for by interactions of PA with negatively charged sites located on phospholipid heads of membrane bilayer and on membrane bound proteins; this results in specific charge–density distribution and specific configurational states.

PA can lay parallel to membrane surface interacting with more than one negative charge. Thus PA could bridge membrane components through lipid–lipid and/or protein–lipid interactions forming "packed" surface structures (inner organization of membranes seems to be not perturbed by PA). As a consequence, complexation, rigidifying and stabilization of membranes occur, this leading to alterations of membrane permeability and active transport properties. Antiperoxidative action of PA (based on formation of ternary complex between PA, phospholipid heads and Fe^{3+}) also contributes to this end. By exogenous application of natural PA their stabilizing effect on membrane structure and function is well documented (Schuber, 1980; Srivastava and Smith, 1982; Tadolini, 1988; Flores, 1990; Tiburcio, 1994). Using a synthetic compound, diethylene triamine, Boyanov and Tenchov (personal communication) demonstrated that this PA analogue stabilizes the lamellar membrane phase and prevents the formation of crystal phase, i.e. interferes with dehydration provoked by extreme temperatures.

Besides, it is established that PA with longer chain, like spermidine, can bridge acidic phospholipid domains of closely apposed membranes. As a consequence, reduction of mutual electrostatic repulsive forces and increase of attractive forces between membrane vesicles occur, which results in membrane aggregation and fusion; this is an essential event taking part in many cellular processes: membrane flow during cell growth, secretion, endocytosis, cell division. In the above phenomena bivalent cations (Ca^{2+}, Mg^{2+}) are involved but PA do not mimic their action (Schuber, 1989).

Modulation of membrane-bound enzymes by PA can be a result of direct PA–protein interactions, as well as of overall PA-induced changes in membrane surface charges. In plants, a membrane-bound enzyme, β-1,3-glucan synthase, is strongly activated by PA, acting synergistically with Ca^{2+}; it contributes to the formation of β-1,3-glucan containing callose barrier structures deposited in cell walls as a response to stress (Kauss and Jeblick, 1986).

The function and structure of plant photosynthetic membranes also undergo electrostatic regulation by PA. Changes in the surface charge density are shown to in-

duce considerable conformational shifts and reorganization of pigment–protein complex (Barber, 1982). Exogenously applied, PA prevent chlorophyll loss and preserve the integrity of chloroplast membranes (Cohen et al., 1979; Tiburcio et al., 1994). They stabilize the lipid bilayer and maintain the primary chloroplast function – generation of proton gradient. PA–membrane interactions result in membrane stacking, separation of both photosystems and association of the light-harvesting complex II with the photosystem II core complex (Yordanov et al., 1989, 1990; Yordanov, 1995).

Interaction with cell walls

Multiple negatively charged sites are available in cell wall components – uronic acid residues in pectins and phenolic residues in lignins (Varner and Lin, 1989). Electrostatic interactions of PA with these components underly their binding to cell walls (Goldberg and Perdrizet, 1984; Bors et al., 1989; Bagni, 1989; Slocum, 1991b). More detailed information is lacking, but regulation of cell wall rheology by PA is strongly possible.

Involvement of PA in maintaining of pH, ion and osmotic cell homeostasis

Being polyvalent cations, PA are implicated in metabolic buffering of cell pH and maintaining ion and osmotic homeostasis. This statement is exemplified by numerous data pointing to dramatic increase in PA (mainly putrescine) in situations where the pH, ion and osmotic balance of cell is disturbed, i.e. in mineral deficiencies and acidification of cell due to stress factors (Galston, 1989; Flores, 1990, 1991). PA are more specifically involved in maintaining of Ca^{2+} homeostasis – an important function, having in view the universal role of Ca^{2+} as second messenger. PA influence Ca^{2+} transmembrane transport and mobilization (Schuber, 1989). Competition between PA and Ca^{2+} for common entry site in membranes is admitted (Galston and Kaur-Sawhney, 1990).

Biosynthesis of polyamines

Putrescine and cadaverine

Putrescine can be synthesized by three main pathways: ornithine decarboxylase-, arginine decarboxylase-, and putrescine synthase-pathways (Slocum, 1991a; Palavan-Ünsal, 1995). The first pathway is an one-step process; the decarboxylation of L-ornithine, catalyzed by ornithine decarboxylase (ODC; E.C.4.1.1.17) results in putrescine formation. The ODC activity is the unique route to putrescine biosynthesis in animals and most fungi (Tabor and Tabor, 1985).

The second pathway is a three-step process: decarboxylation of L-arginine to agmatine catalyzed by arginine decarboxylase (ADC; E.C.4.1.1.19); hydrolysis and deamination of agmatine, resulting in N-carbamoylputrescine, catalyzed by agmatine iminohydrolase; and hydrolysis, deamination and decarboxylation of N-carbamoylputrescine to putrescine, catalyzed by N-carbamoylputrescine amidohydrolase. This pathway is predominant in plants. Specific suicide inhibitors of ODC and ADC are synthesized, namely DL-α-difluoromethylornitine and DL-α-difluoromethylarginine.

The putrescine synthase pathway is catalyzed by a multifunctional enzyme, putrescine synthase, discovered by Srivenugopal and Agida in 1981. Putrescine may also be synthesized through decarboxylation of citrulline catalyzed by citrulline decarboxylase but this pathway in plants is questioned (Slocum, 1991a).

Lysine decarboxylase (LDC; E.C.4.1.1.18) catalyzing the decarboxylation of lysine to cadaverine is reported in numerous plants. In most plant tissues LDC has a low activity, except for some alcaloid synthesizing plants, in which cadaverine is a metabolic precursor of alcaloids (Schoofs et al., 1983).

Spermidine and spermine

Spermidine is synthesized by addition of one aminopropyl residue to the amino group of putrescine catalyzed by the spermidine synthase (E.C.2.5.1.16). Spermine synthesis proceeds through the addition of one aminopropyl group to spermidine and is catalyzed by spermine synthase (E.C.2.5.1.22). A central metabolite supplying the aminopropyl groups is the decarboxylated S-adenosylmethionine (dSAM). The donor of aminopropyl groups, dSAM, is synthesized from L-methionine through the following pathway: L-methionine → S-adenosylmethionine (SAM) → decarboxylated S-adenosylmethionine (dSAM), catalyzed by S-adenosylmethionine synthase and S-adenosylmethionine decarboxylase, respectively (Slocum, 1991a). The key enzyme of this pathway is S-adenosylmethionine decarboxylase (SAMDC; E.C.4.1.1.50).

It is noteworthy that ethylene, a plant senescence hormone, is also derived from SAM; thus, PA and ethylene compete for common precursors which could explain their antagonistic relationships as inhibitors (PA) and promoters (ethylene) of senescence (Kushad and Dumbroff, 1991).

Catabolism of polyamines

Polyamine catabolism is paid relatively less attention as compared to other aspects of PA biochemistry. The main reaction of PA cleavage is the oxidative deamination giving rise to metabolically active products, subsequently involved in different biochemical interactions. Two enzymes catalyze the initial steps of PA catabolism: diamine oxidase and polyamine oxidase.

Diamine oxidase

The diamine oxidase (DAO; E.C.1.4.3.6.) acts on the primary amino groups of putrescine, cadaverine, spermidine and spermine releasing NH_3 and H_2O_2 as well as aminoaldehydes with the same number of carbon atoms as the corresponding PA. Further, the aminoaldehydes cyclize spontaneously into Δ^1-pyrroline, Δ^1-piperideine and 1,5-diazabicyclononane, products of putrescine, cadaverine and spermidine deamination, respectively (Fig.1) (Federico and Angelini, 1991). DAO has a broad amine substate specificity acting on aliphatic di- and polyamines, as well as on aromatic and aliphatic monoamines – agmatine, tryptamine, histamine, lysine, ornithine (Smith, 1985). It is loosely bound to cell walls and can be easily released in the apoplastic fluid.

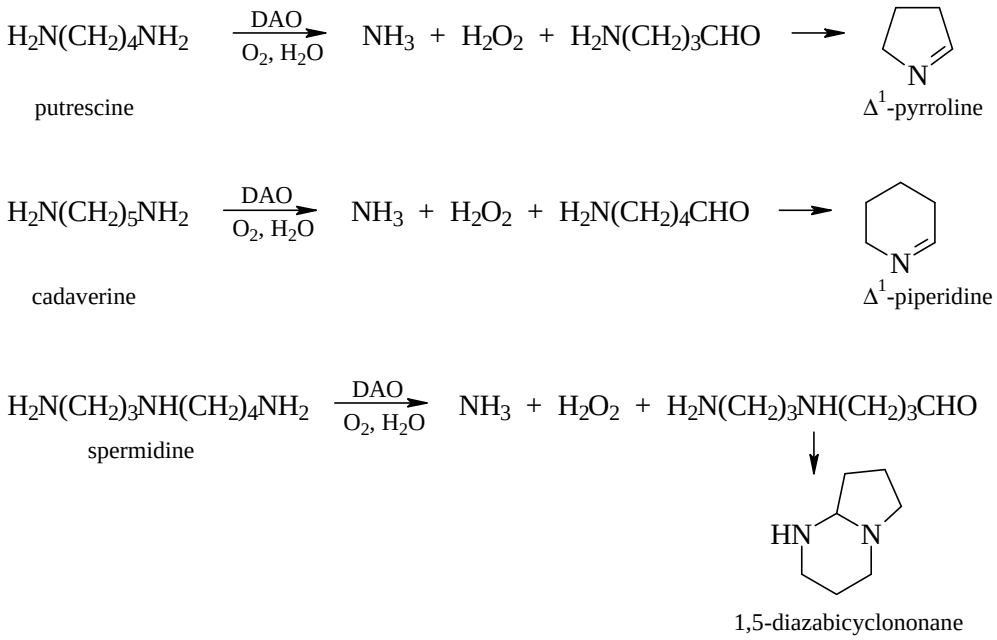

Fig. 1. Oxidative deamination of di- and polyamines by diamine oxidase (DAO) (Federico and Angelini, 1991)

Polyamine oxidase

Polyamine oxidase (PAO; E.C. 1.4.3.4.) acts on imino groups of spermidine and spermine producing 1,3-diaminopropane, H_2O_2, and aminoaldehydes: $H_2N(CH_2)_3CHO$ from spermidine and $H_2N(CH_2)_3NH(CH_2)_3CHO$ from spermine. The latter products cyclize spontaneously to form Δ^1-pyrroline and 1,5-diazabicyclononane, respectively.

PAO appears to be distributed only in monocots. Cell wall and apoplastic localization of PAO is established. Narrow substrate specificity, limited to spermidine and spermine, is demonstrated (Federico and Angelini, 1991).

Functions of polyamine catabolism

From the previous text it may be inferred that PA catabolism is not simply a degradative process, but a link in plant metabolism. Thus, DAO, via the cyclic products of its action, Δ^1-pyrroline or Δ^1-piperideine, is involved in the biosynthesis of alcaloids, containing a pyrrolidine or a piperidine ring (Smith and Wilshire, 1975). The oxidative deamination of tryptamine by DAO suggests the involvement of the enzyme in IAA biosynthesis. By the combined action of DAO and other enzymes, putrescine can be converted (via γ-aminobutyric acid, GABA) to succinic acid – a way to link PA catabolism with Krebs cycle (Flores and Filner, 1985). An important function could be accomplished by a coordinated operation of DAO/PAO and peroxidase (PO) localized in cell walls. The H_2O_2, generated by the amine oxidases in cell walls can be used *in situ* by the PO for processes regulating cell wall rigidity: oxidative polymerisation of phenol monomers to lignin and aromatic moiety of suberin, and of polysaccharide-bound phenols in cell wall matrix, as well as of tyrosine residues in the cell wall proteins. This results in cell wall cross-linking and rigidification, i.e. in modulation of cell wall properties (Fry, 1986; Iiyama et al., 1994). Finally, it is essential to emphasize that both biosynthesis and catabolic conversions of PA are closely related to nitrogen metabolism: amino acids are substrates in PA biosynthetic pathways, and numerous nitrogen containing compounds are formed in the different biosynthetic and catabolic steps, giving rise to series of nitrogen derivatives including alcaloids. The importance of these relationships must not be overlooked when interpreting experimental data.

Alcaloids derived from polyamines (pyrrolidine, tropane, pyrrolizidine, and quinolizidine alcaloids)

Several hundred alcaloids derived from PA and occurring in about 56 plant genera are reported. The putrescine-derived alcaloids include pyrrolidine alcaloids of *Nicotiana* (nicotine, nornicotine), tropane alcaloids of *Datura* and *Hyoscyamus* (scopolamine, hyoscyamine), and pyrrolizidine alcaloids of *Nicotiana* (anabasine) as well as quinolizidine alcaloids of *Lupinus*. Some plants synthesize alcaloids derived from putrescine, spermidine, and spermine. The specific pathways of alcaloid biosynthesis are studied using root cultures; recently, a molecular approach was successfully applied, namely the utilization of plant cells genetically transformed with *Agrobacterium rhizogenes*. The hairy roots obtained in this system grow at a much faster rate but

express the same metabolic pathways as normal roots. ADC and/or ODC pathways, as well as LDC are shown to be involved in the initial stages of alcaloid biosyntheses (Flores and Martin-Tanguy, 1991). The participation of ODC in nicotine biosynthesis is evidenced by using roots of transgenic *Nicotiana rustica* plants overexpressing the ODC gene (Malmberg and Bell, 1991). As alcaloids are of important pharmacological interest, researchers aim at selecting root and cell cultures overproducing alcaloid substances (Medina-Bolivar and Flores, 1995).

Conjugated polyamines

Definition

The term means PA, covalently bound to low molecular compounds. It should not be confounded with the so-called "bound PA". The classification "free" and "bound" corresponds to the differential solubility of PA in trichloracetic acid (TCA) or perchloric acid (PCA), namely free PA (TCA- or PCA-soluble) and bound PA (TCA- or PCA-insoluble). The first fraction covers free PA bases and their derivatives not precipitated in TCA or PCA; the second involves PA bound to high molecular compounds or subcellular structures and hence precipitated in the above media. Thus, according to their solubility, conjugated PA may fall in both fractions.

Chemistry

Three types of conjugated PA are reported: conjugates of PA to alcaloids, to fatty acids, and to hydroxy cinnamic acids (Smith, 1975, 1991).

A complex structure is ascertained for the alcaloid palustrine, a conjugate of spermidine (Fig. 2). This type of alcaloids where a spermidine-derived loop occurs are classified as macrocyclic. Fatty acids are conjugated to PA by an amidic bond. The same type of bonding is formed between PA and hydroxy cinnamic acids giving rise to conjugates named phenylamides (PhA) (Smith, 1975, 1991). Two kinds of PA are mainly observed: basic, where one primary amino group of PA is bound to one molecule hydroxy cinnamic acid, the other amino group being protonated; neutral, where both primary amino groups are bound to two hydroxy cinnamic acid molecules. Imino groups can also be involved in such interactions; recently, trisubstituted hydroxy cinnamic acid derivatives of spermidine are reported. Aromatic mono-

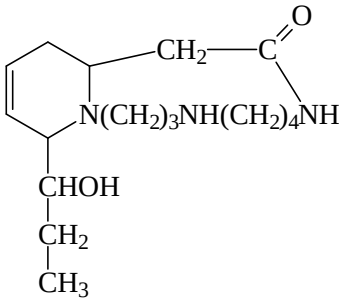

Fig. 2. Palustrine – a macrocyclic alcaloid, conjugate of spermidine (Smith, 1991)

amines (tryptamine, tyramine) also conjugate with hydroxy cinnamic acids but these amines and their derivatives are out of the scope of this paper. The hydroxy cinnamic acids involved in conjugation with PA to form PhA are mainly caffeic, ferulic and *p*-coumaric (Martin-Tanguy, 1985; Bokern et al., 1995). An example of basic and neutral conjugates of ferulic acid with putrescine is given in Fig. 3.

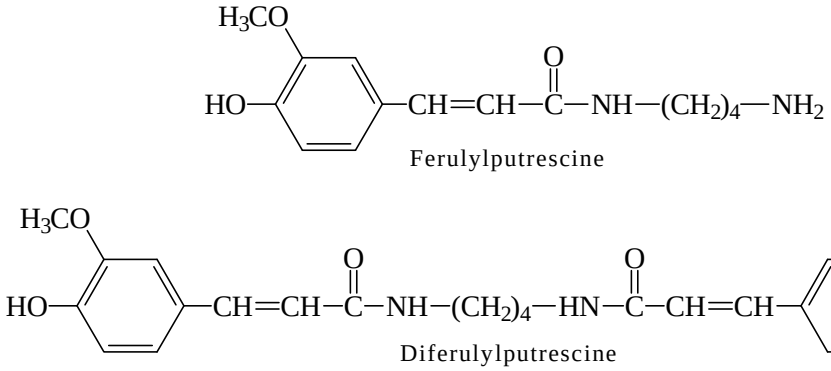

Fig. 3. An example of phenylamide compounds: ferulylputrescine, a basic conjugate of one molecule ferulic acid with one amino group of putrescine; diferulylputrescine, a neutral conjugate of two ferulic acid molecules with both amino groups of putrescine

Occurrence and localization

PhA are plant specific constituents, ubiquitous to plants. They are identified in roots, stems, leaves, tubers, seeds, flowers, as well as in tissue and cell cultures; reproductive organs are characterized by important amounts and various patterns of PhA, involving basic and high levels of neutral PhA derivatives (Flores and Martin-Tanguy, 1991). In cells PhA are localized in vacuoles, cell walls, membranes, and apoplastic fluid (Clarke, 1982; Vallee et al., 1983; Bors et al., 1989; Langebartles et al., 1991).

Interactions and functions

PhA are typical bifunctional compounds carrying properties of both amines and hydroxy cinnamic acids. Possible interactions of basic PhA with negatively charged sites in macromolecules and subcellular structures can be due to the protonated primary amino group. In these cases as well as by interfering in pH and ion balance, PhA can act as amine cations. The hydroxy cinnamic acid moiety of PA convey other important properties: an ability to form dimers catalyzed by the PO (Smith, 1985) and free radical scavenging activity (Bors et al., 1989). Thus, when bound to cell walls, PhA could bridge and cross-link polysaccharide chains, contributing to cell wall consolidation; as free radical scavengers, PhA interfere with the accumulation of toxic spe-

cies and maintain cell homeostasis. It is noteworthy that Bors et al. (1989) question the data of Drolet et al. (1986) that PA are free radical scavengers, proving that this is a property of hydroxy cinnamic acids, whereas PA are only antioxidants. Possibly, through conjugation of PA with hydroxy cinnamic acids, the characters of both types are coupled, and optimal defense functions are achieved. Moreover, in this way the cytotoxicity of free hydroxy cinnamic acids is regulated (Bors et al., 1989). These data were recently confirmed (Ohnishi et al., 1994; Volpert et al., 1995). It is possible that conjugation may also regulate PA level; hence, PhA may serve as a storage reserve from which PA could be released at the appropriate conditions. There are also data that the conjugation of putrescine with hydroxy cinnamic acids is a prerequisite for the putrescine oxidation to GABA, a key metabolite relating PA metabolism to Krebs cycle (Flores and Filner, 1985).

Biosynthesis

In the biosynthesis of PhA, enzymes of phenol biosynthetic pathways (phenylalanine ammonia lyase, *trans*-cinnamate-4-hydroxylase, *p*-coumarate lyase), as well as enzymes of PA biosynthesis are involved. Moreover, enzymes catalyzing the conjugation are described. They utilize hydroxy cinnamic acid – CoA thioesters as acyl donors. From tobacco callus Negrel (1989, 1991) isolated a caffeoyl-CoA putrescine N-caffeoyl hydroxy cinnamoyl transferase (PCT; E.C.2.3.1.-) and spermidine hydroxy cinnamoyl transferase (SHT) catalyzing the formation of putrescine- and spermidine-hydroxy cinnamic acid derivatives. Catabolic conversions of PhA catalyzed by DAO is suggested.

The above commented specific biochemical features of PhA suggest their regulatory and defense functions.

Uptake and transport of polyamines

The fact that PA bind to negatively charged cell substructures (cell walls, membranes) interfered with research on PA uptake and transport. Nevertheless, the occurrence of these phenomena was reliably demonstrated. By tracer experiments it was shown that PA are uptaken into the cells; PA efflux is also established. Hence, cellular transport is bidirectional and, at least in the inward direction, is energy- and Ca^{2+}-dependent; pH and hormone (IAA, cytokinin) dependence of PA uptake is shown as well (Bagni and Pistocchi, 1991). The long distance transport is a matter of discussion. Some authors question its existence (Young and Galston, 1983) but Bagni and co-workers (data summarized by Bagni and Pistocchi, 1991) present supportive evidence, based on several systems: in apple, labelled putrescine was translocated from leaves to fruitlets and vice versa; the translocation occurs via the peduncle and doesn't appear to be

polar; in maize and tomato seedlings, labelled putrescine fed to roots was translocated upwards, to the coleoptiles and cotyledons, respectively. A basipetal transport from primary leaf or cotyledons of tomato was also shown, although it was less intensive than the acropetal transport. Presence of PA in xylem and phloem exudates was demonstrated by Friedman et al. (1986). Hence, PA translocation proceeds mainly via the xylem, being transpiration dependent; phloem transport has a less important role as evidenced by the lower basipetel transport versus the acropetal one. In other words, in plants intercellular and interorgan translocation of PA takes place; the localization of PA in the apoplastic fluid (Langebartels et al., 1991) lends support to this assertion. The rate of process however is not high as well as the amount of the translocated PA (Bagni and Pistocchi, 1991).

Polyamines in plant growth and development

The structural–functional interactions of PA with nucleic acids, proteins and cell substructures can be a rationale for their implication in plant growth and development. Numerous data unequivocally recognize that PA are required for these important physiological functions. However, no agreement is available about the mechanisms of PA involvement in these phenomena, and conflicting results as well as diverse hypotheses are evolved (Smith, 1985; Evans and Malmberg, 1989; Bagni, 1989; Galston and Kaur-Sawhney, 1990). This may be due to the large variations in the experimental design employed – from the choice of model to the concentration range. In plants, another cause may be the lack of synchronous cell material, as well as the diversity of biosynthetic routes to PA biosynthesis.

Bagni (1966) and his colleagues at Bologna University were the first to report growth promoting properties of PA. Since then, a cascade of papers on PO implication in growth and development phenomena are published.

Cell cycle

The model used in this research is an organ naturally deficient in PA, namely dormant tubers of *Helianthus tuberosum*. Dormancy is characterized by very low levels of PA, hormones, DNA, RNA and protein metabolism, and high levels of ABA. Release from dormancy giving rise to sprouting is related to dramatic perturbation in cell morphology and metabolism. The entering the early G_1 phase of cell cycle is accompanied by a sharp and rapid increase of PA biosynthesis, preceding the degradation and the new synthesis of protein, RNA and non-chromosomal DNA. In the late G_1 phase PA and protein syntheses continue. Further on, two maxima of PA are observed: upon entering the S phase (preceding chromosomal DNA synthesis), and in the middle of D (cell division) phase. Inhibitors of PA biosynthesis affect both DNA

replication and cell cycling. The importance of PA for these events is also substantiated by the fact that tuber dormancy is broken up by PA application; hormones exert the same effect (Serafini-Fracassini, 1991). Similar results are obtained in *N. tabacum* cell suspension cultures (Pfosser et al., 1990).

Cell division

In plants, cell division occurs in developing embryos and fruits, in meristematic tissues, and in cell and tissue cultures. Cell division is correlated with an increased PA level observed in some systems (apple and tomato fruits) premitotically. Depletion of PA by biosynthetic inhibitors interfere with cell division, and exogenous PA supply promote this process (Biasi et al., 1988; Egea-Cortines and Mizrahi, 1991).

Growth

Plant growth, as a resultant of cell division, is also PA-dependent, as reported in numerous papers employing various approaches (Smith, 1985; Evans and Malmberg, 1989; Galston and Kaur-Sawhney, 1990). Abnormally growing tissues such as tumours are used as convenient models for research on growth–PA relationships. A correlation between growth rate and PA level is demonstrated, and the predominant participation of ODC-pathway is ascertained (Bagni, 1989).

Cell differentiation

Cell differentiation, underlying developmental and morphogenetic phenomena, is strongly suggested as being PA related. In this aspect many developmental processes are reported, such as embryogenesis, root initiation, flower induction, tuberisation, sprouting etc. A large body of data on this subject is available, reviewed by the authors cited above (“Growth”); some spectacular systems can be examined with more details. Montague et al. (1978) first demonstrated a rise in ADC activity and putrescine level when carrot cultures are shifted from callus medium to embryogenesis medium. Inhibiting of PA biosynthesis blocked this transition, but the addition of putrescine restored the embryogenic potential. In a non-embryogenic line of carrot a rise in ADC and PA does not occur (Evans and Malmberg, 1989). Kaur-Sawhney et al. (1988) used tobacco PA-mutants, expressing flowering abnormalities, to show that cultures producing flowers were converted to the vegetative state by applying cyclohexylamine, a spermidine synthase inhibitor, which depletes spermidine titer; contrariwise, cultures producing only vegetative buds were induced to flower by addition of spermidine. Recent *in vitro* experiments on inductive rooting phase of poplar shoots (Hausman et al., 1994), tuberisation of *Solanum tuberosum* (Mader, 1995), and flower induction in soybean (Caffaro and Vicente, 1995) lend support to the above data.

Conjugated polyamines in growth and development

Like PA, their conjugates with hydroxy cinnamic acids are involved in cell multiplication and organogenesis. They induce cell multiplication of an *in vitro* cultivated tobacco mutant, and promote callus formation (Martin et al., 1985). Distinct levels and patterns of PhA are observed in vegetative and reproductive organs, and dramatic shifts are observed upon transition from vegetative to reproductive state. Additional experiments lead to the idea that PhA are "a driving force from vegetative to reproductive meristem" (Flores and Martin-Tanguy, 1991). PhA are closely related to flower induction, to fertility expression in male and female reproductive organs (pollen, ovules), and to tuberisation. They are proposed as biochemical markers of pollen fertility in maize and of tuberisation in *Solanum* species (Martin-Tanguy, 1991). Possibly, mechanisms similar to those in PA underly the above phenomena; moreover, the specific characters of PhA may also be of importance.

Antisenescence effect of polyamines

It is recognized (De Vecchi, 1971; Kaur-Sawhney and Galston, 1979; Cohen et al., 1979; Dhindsa et al., 1981) that plant senescence is accompanied by a cascade of degradative events: increase of RNA-ase and protease activity, nucleic acid and protein breakdown, peroxidation of membrane lipids, disintegration of thylakoid membranes and chlorophyll loss. A decline of PA level and enzymes of their biosynthesis was also observed during ageing and at the onset of senescence which suggest the involvement of PA in the above phenomena (Galston and Kaur-Sawhney, 1987).

The antisenescence properties of PA were first observed in oat protoplast cultures (Brenneman and Galston, 1975) and later demonstrated in leaves and other plant tissues and organs, where induced or natural senescence occur; PA, exogenously supplied, retard senescence and the associated metabolic events. The mechanisms underlying the antisenescence effect of PA are proposed to be mainly related to their polycationic nature, namely stabilization of membranes (and modulation of membrane-bound enzymes), and interactions with nucleic acids and proteins, respectively involvement in the regulation of macromolecular biosynthesis. Inhibition of membrane lipid peroxidation by PA can also be of importance. Interfering with the biosynthesis of ethylene, a senescence hormone, is another possible PA antisenescence mechanism. It may be explained in terms of competition of PA and ethylene for common precursor, ACC (1-aminocyclopropane-1-carboxylic acid). Non-specific effect of PA on ethylene biosynthesis is also admitted; it may flow from PA-membrane interactions leading to inhibition of ethylene-synthesizing enzymes (Kaur-Sawhney and Galston, 1991).

Responsiveness of polyamines to light and hormones. Polyamines: hormone-like substances, second messengers, or factors, correlated to growth and development?

The responsiveness of PA to light and hormones, indicated previously in this paper, is a well documented phenomenon. White and red-far red light, as well as auxins, cytokinins, gibberellins, abscisic acid and ethylene induce modulations in the level of PA and the enzymes of their metabolism (Smith et al., 1985; Evans and Malmberg, 1989; Rastogi and Davies, 1991; Sergiev et al., 1995; Mader, 1995; Caffaro and Vicenle, 1995). On the other hand, as well known, hormones and light are regulators of growth and development in plants; PA also provoke growth and/or developmental effects. Taken together, all these findings give rise to different hypotheses concerning PA regulatory functions.

PA – second messengers? The fact that PA respond to light and hormones, and, moreover, that this may precede the growth and/or development responses to these factors, substantiate the idea that PA are mediators of hormone action in cells, i.e. PA function as second messengers. The implication of PA in protein phosphorylation (Ye et al., 1994) is in accordance with this view.

PA – factors correlated to growth and development? In other systems, PA response may merely accompany growth and/or development responses; fluctuation in PA level may also accompany important key events in growth and development; hence, in these cases the evidence for PA involvement in these phenomena is of a correlative type.

PA – hormone-like factors? Like hormones, PA induce growth and provoke developmental changes; are light-dependent; interfere with senescence; are translocated in plants. However, PA do not meet three essential criteria for hormones, namely the low cellular concentration and the high rate of long-distance transport, as well as the low effect-producing dose. The topic is a matter of permanent discussions by outstanding authors in the last decade, but no decisive evidence of either views is presented (Smith, 1985; Evans and Malmberg, 1989; Bagni, 1989; Galston and Kaur-Sawhney, 1990). Nevertheless, PA may be considered as a new class endogenous growth regulators.

Involvement of polyamines and phenylamides in stress phenomena

In the last decade, the involvement of PA in stress phenomena is comprehensively reviewed by Shevyakova (1981), Flores et al. (1985), Galston (1989), Flores (1990, 1991), and recently by Palavan-Ünsal (1995). Summarizing data of previous researchers and her own results, the latter author states that putrescine accumulation may be taken as an indicator of stress metabolism in plants, for it is a response to a wide ar-

ray of abiotic stress factors: low pH, nutrient deficiency, especially K^+ deficit, high osmolarity, water deficit, low temperature, high NH_4^+ concentration, metal toxicity, high salinity, ozone and SO_2 pollutants. Additional information points that other abiotic stresses produce the same effect, namely flooding of tobacco plants (Hurng and Kao, 1993), anaerobiosis of rice (Reggiani et al., 1989), high temperature stress in bean (Edreva and Yordanov, unpublished), and treatment of pea plants with the herbicide atrazine (Zheleva et al., 1993a). The few data concerning pathogenic stress factors show putrescine accumulation in tissues infected by biotrophic fungi: wheat leaves inoculated with *Puccinia graminis* f. sp. *tritici* (Machatschke et al., 1990), and barley leaves infected with *Puccinia hordei* and *Erysiphe graminis*, respectively (Greenland and Lewis, 1984; Walters and Wylie, 1986). In these systems putrescine accumulation is related to the formation of "green islands" – metabolically active zones around the infection sites. Contrarily, pathogenic stress factors causing tissue damage provoke decline of PA titer in tobacco leaves (Edreva and Hadjiiska, 1985).

In most of the stress situations the putrescine rise is due to an enhanced ADC activity; thus, ADC is referred to as "stress enzyme". In some cases, ODC pathway is involved in putrescine response. The regulation of these enzymes in stress situations is not fully understood. Synthesis *de novo* of ADC is strongly suggested; an "antizyme" (Koromilas and Kyriakidis, 1988) post-translational mechanism for ODC is admitted. The possibility is also discussed that the putrescine increase may be due to blocking its conversion to spermidine, spermine and other products, i.e. to inhibition of spermidine and spermine synthases (Negrel, 1984; Flores et al., 1985; Galston, 1989; Galston and Kaur-Sawhney, 1990; Kramer and Wang, 1990; Flores, 1990, 1991; Zheleva et al., 1993b). The problem needs further experiments using molecular approaches.

In this connection it must be emphasized that in many stress situations spermidine and spermine behave differently from the putrescine; this suggests not identical mechanisms of their action depending on the type of stress.

Thus, according to Flores (1991) developing Smith's (1985) pH-stat hypothesis, putrescine is predominantly involved in the so-called ion stresses, related to cation–anion imbalance and lowering cell pH: high salt, ammonia and H^+ concentrations, high osmolarity, cation deficit. In these situations the massive, rapid increase of putrescine (which is a short-chained dication) can prevent cell acidification and contribute to the maintaining of pH and ion homeostasis. Similarly, Slocum (1984) claims that stresses, generating excessive H^+ provoke putrescine accumulation. The synchrony between the fluctuations of putrescine and malic acid in plants expressing Crassulacean acid metabolism (CAM) (Morel et al., 1980) is in accordance with the above ideas. Another hypothesis (Lovatt, 1990) relates the putrescine response to nitrogen metabolism, postulating that the primary stress event is the production of ammonia excess; a way to detoxify ammonia is its conversion to arginine and via ADC – to putrescine. These considerations find support in Galston's finding (1989) that

the light-dependent arginine availability is a prerequisite for the expression of putrescine response. In line with these views, Smith (1985) examines the overproduction of putrescine in stress as a way to sequester nitrogen in an innocious storage product from which it may be reversibly released. Polyamines proper, spermidine and spermine, as mentioned above, are less responsive to ion stresses. This could be accounted for by their specific characters, distinguishing them from diamines: the presence of more than two positively charged loci, distributed on a longer carbon chain. This determines their ability for structural and conformational interactions with complex cell components carrying multiple negative sites (macromolecules, membranes, cell walls). Flores (1991) evolves the view that such interactions can take place in another group of stress phenomena known to produce membrane damage, namely chilling, drought, heat, ozone stresses. The rise of spermidine and spermine reported in these situations could contribute to stabilization of the membranes and the macromolecular constituents. Several findings underly this view: unusual long-chained tri-, tetra- and pentaamines are synthesized following drought and high temperature stress in drought and heat tolerant alfalfa and cotton, respectively (Kuehn et al., 1990); chilling tolerance in *Cucurbita pepo*, and ozone tolerance in tobacco and barley are associated with an increase of spermidine and spermine (Kramer and Wang, 1989; Rowland-Bamford et al., 1989; Langebartels et al., 1991). Recent data are in accordance with the above results: atrazine and high temperature stress damaging chloroplast membranes induce higher spermidine levels in pea and bean plants, respectively (Zheleva et al., 1993a; Edreva and Yordanov, unpublished). In some of the above stress situations a rise of putrescine is also observed indicating that ion imbalance may also occur; however, the response of the higher PA is generally more expressive.

In the last years the free radical scavenging properties of PhA are suggested as a rationale of defensive stress responses in plants (Bors et al., 1989). Thus, it was established that ozone fumigation of plants leads to the formation of cytotoxic free radicals (Alscher and Amthor, 1988). Ozone-tolerant tobacco genotypes respond to this stress situation by early and intensive accumulation of caffeoyl putrescine in the leaves parallel to the rise of free PA bases; contrarily, in the ozone-sensitive genotype this response is late and weak, and coincides with the symptom appearance. The assumption for defensive role of PhA in this system is substantiated by the fact that PhA are predominantly localized in the apoplastic fluid, the main site of generation of free radical species, having a short life-time (Langebartels et al., 1991). PhA may also be involved in structure consolidating events in which cell wall- and membrane-bound PhA, PO and H_2O_2 are implicated.

Possibly, all these properties of PhA account for their involvement in the resistance expression in several plant–pathogen systems where a hypersensitive mechanism occurs: tobacco–TMV (Martin-Tanguy, 1985); wheat – *Puccinia recondita* and *Puccinia graminis* (Sambroski and Rohringer, 1970); potato–*Phythophthora infestans* (Clarke, 1982). However, in susceptible plant–pathogen combinations, such as po-

tato–*Phoma exigua* (Malmberg, 1984) PhA are also synthesized as well as in abiotic stresses: O_3 fumigation, K^+, Mg^{2+}, Ca^{2+} and P deficiency, water excess and S-starvation in tobacco and tobacco cell cultures (Deletang, 1974; Klapheck, 1983; Langebartels et al., 1991; Edreva et al., 1995) and high temperature treatment of bean (Edreva et al., 1995). Hence, the formation of PhA is not associated uniquely with the expression of the hypersensitive resistance to pathogens, but may be part of a common defense mechanism in plants to a wider array of stress factors.

The protective effect of PA in stress is demonstrated by applying of exogenous PA. Experiments with atrazine-treated pea plants (Zheleva et al., 1994b) and ozone fumigated tomato and tobacco plants (Ormrod and Beckerson, 1986; Bors et al., 1989) show that exogenous PA reduce damage and increase endogenous PA levels, especially the content of conjugated PA in tobacco; in PA treated pea plants *de novo* protein synthesis is established (Zheleva and Karanov, 1994a), as well as stabilizing effect of PA on structural and functional state of chloroplast membranes (Zheleva et al., 1994).

Nevertheless, the interpretations of the role of PA in stress are not unequivocal. Along with the view of their protective function, some authors admit that PA accumulation following stress may be a cause of injury leading to symptom expression. Another possibility may be that it is an accompanying phenomenon, of no causal relation to stress (Galston, 1989; Evans and Malmberg, 1989; Flores, 1991).

Practical application of PA and inhibitors of their biosynthesis

The practical application of PA is still quite limited due to the insufficient understanding of fundamental problems related to the regulation of their biosynthesis and metabolism, uptake, translocation, as well as molecular bases of their regulatory functions, including signal transduction. Nevertheless, as being implicated in cell division and proliferation (Serafini-Fracassini, 1991), in growth and development phenomena, PA can be connected with the abnormal growth of tumour cells in both plant and animal organisms, where ODC-catalyzed pathway of PA biosynthesis is predominantly involved (Bagni, 1989). On this basis, the suicide ODC inhibitor (DFMO) is used in human cancer therapy; moreover, the ODC-dependent depletion of PA can modify the chromosome damaging effect of some anticancer drugs. DFMO is also used as an anti-protozoal product (Tofilon et al., 1982; West and Walters, 1988).

In plants, attempts are made to utilize PA as antisenescence agents (Srivastava et al., 1983; Kaur-Sawhney and Galston, 1991; Alexieva, 1994). Promising results are obtained in Karanov's laboratory by using natural and synthetic PA as antidotes of herbicide action. The results with the photosynthesis-inhibiting herbicide, atrazine, are of special interest (Alexieva, 1993; Zheleva et al., 1994; Zheleva and Karanov, 1994b).

The application of inhibitors of PA biosynthesis is a novel approach for control of fungal disease in plants. It is based on the finding that ODC is the only route to

PA biosynthesis in most fungi (Tabor and Tabor, 1985) whereas plants dispose of alternative PA biosynthetic pathways along with ODC (Slocum, 1991a). Thus, treatment of plants with ODC inhibitors will not interfere with plant metabolism but will provoke delay, arrest or abnormalities in growth and development of pathogenic fungi parasitizing on plants. The inhibitory effect of DFMO on several plant pathogenic fungi grown in culture was first demonstrated by Rajam and Galston (1985). Moreover, *in vitro* action of DFMO spray in a plant–obligatory fungus system (bean–*Uromyces phaseoli*) was shown (Rajam et al., 1985); prevention of infection with no negative effects for plants was established. These experiments were reviewed and successfully continued by Walters et al. (West and Walters, 1988; Foster and Walters, 1990); the novel, ecological approach in plant protection avoiding the use of chemicals, toxic to the environment deserves support and further efforts, including the search for new inhibitors.

Methods for determination of polyamines and related enzyme activities

The methods for identification and quantitation of PA are comprehensively reviewed by Mary Smith (1991). Thin layer chromatography, reversed phase high performance chromatography and ion exchange chromatography are examined, as well as the precedent steps of extraction, purification, dansylation and radiolabelling. Enzymatic methods, using maize PAO specifically oxidizing spermidine and spermine, and DAO from *Euphorbia characias* latex, reacting only with putrescine and cadaverine, are also recommended (Federico et al., 1991).

The assay methods for enzymes of polyamine metabolim are recently examined by Birecka (1991).

Conclusion

PA were a white spot in plant science, but in the last two decades they are paid an increasing attention. It was shown that PA metabolism and functions in plants share many elements in common with PA in animal and microbial systems, namely biosynthetic pathways and involvement in cell cycle, cell division, and morphogenesis. However, plant PA have some specific features, namely: the occurence of conjugates with alcaloids and hydroxy cinnamic acids, as well as of several hundred PA-derived alcaloids; the interference with senescence and the biosynthesis of the senescence hormone, ethylene; the responsiveness to light and hormones and to a wide array of abiotic and pathogenic stress stimuli. All these topics are far from being fully understood.

In 1987 Dale Walters stated that PA are “the Cinderellas of cell biology”. Future efforts are needed to disprove this metaphor.

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