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Review

# Polyamines and plant disease

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Dedicated to the memory of Professor Jeffrey B. Harborne

**Abstract**

The diamine putrescine and the polyamines spermidine and spermine are found in a wide range of organisms from bacteria to plants and animals. They are basic, small molecules implicated in the promotion of plant growth and development by activating the synthesis of nucleic acids. Polyamine metabolism has long been known to be altered in plants responding to abiotic environmental stress and to undergo profound changes in plants interacting with fungal and viral pathogens. Polyamines conjugated to phenolic compounds, hydroxycinnamic acid amides (HCAAs), have been shown to accumulate in incompatible interactions between plants and a variety of pathogens, while changes in the diamine catabolic enzyme diamine oxidase suggest a role for this enzyme in the production of hydrogen peroxide during plant defence responses. More recent work has suggested a role for the free polyamine spermine in the hypersensitive response of barley to powdery mildew and particularly in tobacco to TMV. The prospects for the genetic manipulation of HCAA levels in plants as a means of both defining their role in plant defence and in the generation of disease resistant plants is discussed briefly.


*Keywords:* Polyamines; Hydroxycinnamic acid amides; Plant disease; Resistance

**Contents**



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## 1. Introduction

The polyamines spermidine (**1**) and spermine (**2**) occur ubiquitously in the plant kingdom, together with their diamine precursor putrescine (**3**). These low molecular weight compounds are positively charged at physiological pH and because of this are known to bind to negatively charged molecules, e.g. nucleic acids, acidic phospholipids and various types of proteins (Cohen, 1998). In higher plants, polyamines occur in free form, bound electrostatically to negatively charged molecules, and conjugated to small molecules and proteins (Martin-Tanguy, 1997).

Polyamines are required for normal development of prokaryotes and eukaryotes (Tabor and Tabor, 1984). Work on higher plants suggests that polyamines play a critical role in a range of developmental processes e.g. root growth, somatic embryogenesis, floral initiation, and the development of flowers and fruits (Evans and Malmberg, 1989; Galston and Kaur-Sawhney, 1990; Slocum and Flores, 1991). This body of work has been supported by more recent studies showing that when polyamine levels are altered by genetic manipulation, plant growth and development can be profoundly affected (e.g. Kumar et al., 1996; Watson and Malmberg, 1998). Polyamines have also been implicated in plant responses to abiotic stress, with polyamine levels increasing several fold in plants responding to, for example, potassium deficiency, osmotic shock, drought and salt stress (Watson and Malmberg, 1996; Evans and Malmberg, 1989). Despite the correlation between abiotic stress and polyamine levels in higher plants, the physiological rationale for such alteration in polyamine levels is not known. However, work by Liu et al. (2000) showed that spermidine (**1**), spermine (**2**), cadaverine and putrescine (**3**) strongly inhibited opening and closing of stomata in *Vicia faba*. Their work suggests that polyamines target inward potassium channels in guard cells and modulate stomatal movements, so providing a link between abiotic stress, polyamine levels, and stomatal regulation.

Polyamine levels are also known to change in plants responding to biotic stress, e.g. pathogen infection (Walters, 2000). The purpose of this article is to review the alterations in polyamine levels and metabolism in plants interacting with pathogens, particularly incompatible interactions. However, before that is done, it is necessary to review briefly the biosynthesis and catabolism of polyamines, and formation of polyamine conjugates.

## 2. Biosynthesis and catabolism of polyamines

The first step in polyamine biosynthesis in higher plants is the formation of putrescine (**3**). Putrescine (**3**) is synthesized directly from ornithine (**4**) by ornithine decarboxylase (ODC; EC 4.1.1.17) and indirectly from arginine (**5**) by arginine decarboxylase (ADC; EC 4.1.1.19) and two aminopropyltransferases (Fig. 1). Spermidine (**1**) and spermine (**2**) are formed by the subsequent addition of an aminopropyl moiety to putrescine (**3**) and spermidine (**1**), respectively. These reactions are catalysed by the aminopropyltransferase enzymes spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22). The aminopropyl group (decarboxylated AdoMet) (**6**) is formed by the decarboxylation of *S*-adenosylmethionine (AdoMet) (**7**) in a reaction catalyzed by the enzyme AdoMet decarboxylase (AdoMetDC; EC 4.1.1.50). Evidence suggests that the activities of ODC and ADC are regulated in a developmental and tissue-specific manner (Minocha et al., 1995; Walden et al., 1997). ODC, ADC and AdoMetDC have short half lives, indicating that they are important metabolic control points in cells (Cohen, 1998). In recent years, manipulation of polyamine metabolism using transgenic approaches has been a useful tool in examining their physiological roles in higher plants, and polyamine levels in cells have been modulated by altering the expression of the genes coding for ODC (*odc*), ADC (*adc*) and AdoMetDC (*samdc*). This work has shown that overexpression of heterologous *odc* or *adc* cDNAs leads to elevated putrescine (**3**) levels (e.g. Capell et al., 1998; Kumar and Minocha, 1998; Bhatnagar et al., 2001), although in most cases this is accompanied by only small increases in levels of spermidine (**1**) and spermine (**2**). This suggests that spermidine (**1**) and spermine (**2**) levels in plants are under tight homeostatic regulation. Nevertheless, in some interesting recent work, Thu-Hang et al. (2002) found that overexpression of *samdc* in transgenic rice was translated into a direct increase in levels of spermidine (**1**) in leaves and in levels of spermidine (**1**) and spermine (**2**) in seeds.

The diamine cadaverine can be formed from lysine either via the action of ODC or as a result of lysine decarboxylase activity (LDC; EC 4.1.1.18). In higher plants, most evidence suggests that cadaverine formation occurs via the action of LDC (Slocum, 1991).

The diamines cadaverine and putrescine (**3**) are oxidatively deaminated by the action of the copper-containing diamine oxidases (DAO; EC 1.4.3.6), while the polyamines spermidine (**1**) and spermine (**2**) are oxidized by the flavoprotein-containing polyamine oxidases (PAO; EC 1.5.3.3) (Smith, 1985; Cohen, 1998; Bagni and Tassoni, 2001). DAO converts putrescine (**3**) into $\Delta^1$-pyrroline (**8**), with the release of ammonia and hydrogen peroxide (Fig. 2). Pyrroline dehydrogenase then converts $\Delta^1$-pyrroline (**8**) to $\gamma$-aminobutyric acid (GABA) (**9**), which can be transaminated and oxidized to yield succinic acid (**10**), and can then enter the Krebs cycle (Flores and Filner, 1985). This ensures that carbon and nitrogen resulting from putrescine (**3**) breakdown is

recycled. Degradation of spermidine (**1**) by PAO yields $\Delta^1$-pyrroline (**8**) and 1,3-diaminopropane (**11**), while spermine (**2**) oxidation yields 1,3-aminopropylpyrroline (**12**), along with diaminopropane (**11**) and hydrogen peroxide (Smith, 1985; Bagni and Tassoni, 2001). Interestingly, the generation of transgenic rice cell lines exhibiting reduced expression of the genes coding for DAO led to increases in levels of putrescine (**3**) and spermidine (**1**) (Bassie et al., 2000). Less predictable perhaps was the finding of Bhatnagar et al. (2002) that in transgenic poplar cell lines overexpressing *odc* and thus containing 3- to 4-fold increases in levels of putrescine (**3**), increased putrescine (**3**) degradation occurred without significant alteration in DAO activity. The authors suggest that in this particular system, DAO is either functioning at a non-saturating level (i.e. high $K_m$ of the enzyme but low amount of available substrate) or the enzyme is present in non-saturating amounts (i.e. excess amount of the enzyme). In this way, any excess putrescine (**3**) in cells would be catabolised at a rate proportionate to its availability up to the level of saturation. Such studies using transgenic plants indicate that genetic manipulation of one step in the polyamine biosynthetic pathway has pleiotropic effects on both the downstream and upstream reactions in which the substrate of the transgene is used.

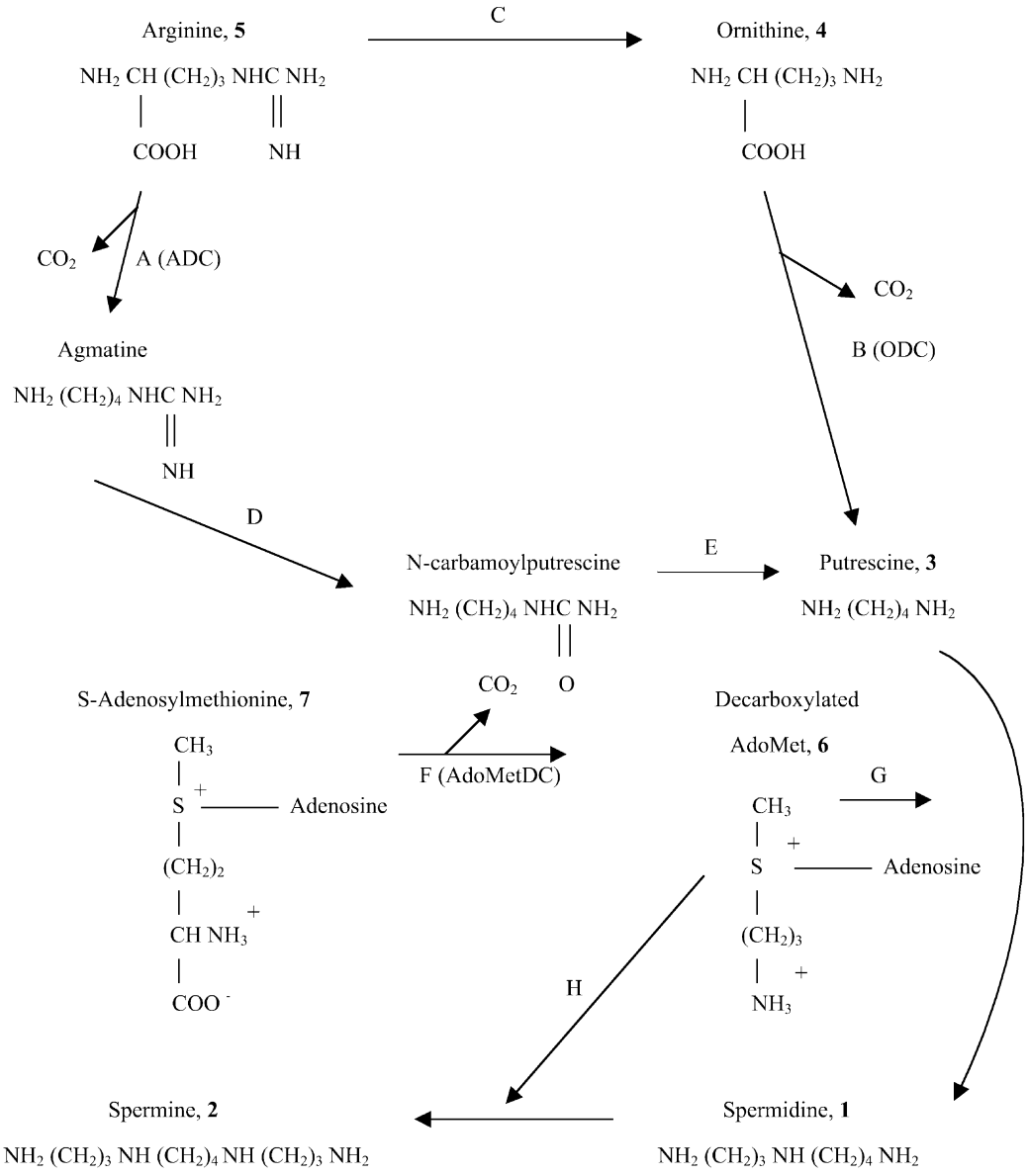



Fig. 1. Pathways of biosynthesis of the major plant polyamines (putrescine (**3**), spermidine (**1**) and spermine (**2**)). A, arginine decarboxylase; B, ornithine decarboxylase; C, arginase; D, agmatine iminohydrolase; E, *N*-carbamoylputrescine amidohydrolase; F, AdoMet decarboxylase; G, spermidine synthase; H, spermine synthase.

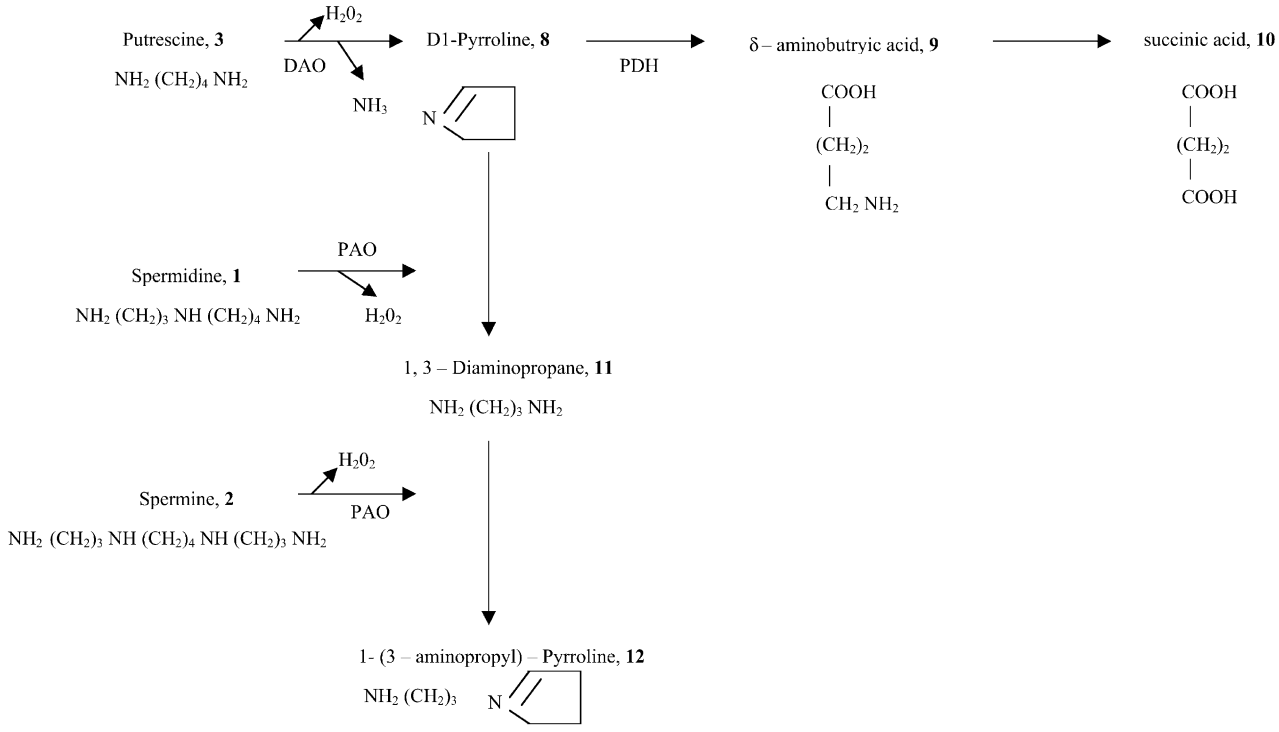



Fig. 2. Polyamine catabolism by amine oxidases; DAO, diamine oxidase; GABA, γ-aminobutryic acid; PAO, polyamine oxidase; PDH, pyrroline dehydrogenase.

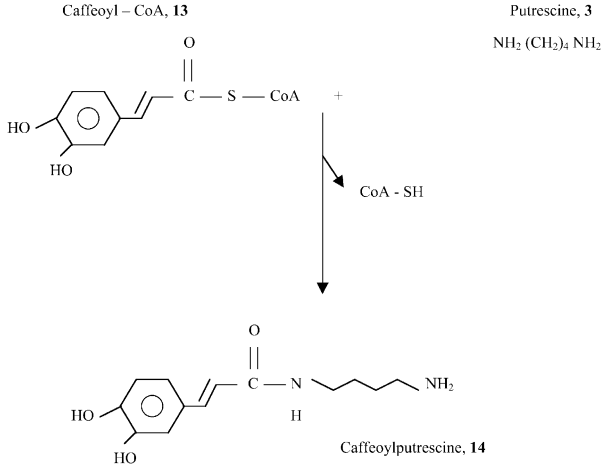


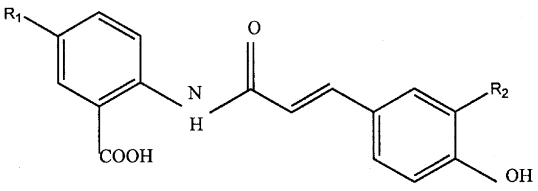

Fig. 3. Enzymatic reaction catalysed by PHT. Caffeoyl-CoA (R1 = OH, R2 = OH) (**13**) is the best phenolic substrate. The diamine in the figure is putrescine (**3**).

## 3. Polyamine conjugates

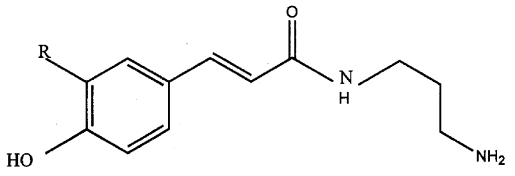
Polyamines occur in plant cells as free molecular bases, but they also occur in conjugated form, associated with small molecules like phenolic acids or with larger molecules like proteins. Polyamines are most commonly conjugated to cinnamic acids, e.g. *p*-coumaric, ferulic and caffeic acids and the resulting conjugates are known as hydroxycinnamic acid amides (HCAAs). This diverse group of low molecular weight compounds occurs in a wide range of plant families, and are the main phenolic constituents of reproductive organs and seeds of some 20 species of plant, representing 13 different families (Martin-Tanguy et al., 1978). Basic HCAAs, which typically contain the aliphatic di- and polyamines like putrescine (**3**), spermidine (**1**) and spermine (**2**), are water soluble, while neutral HCAAs, which contain aromatic amines like tyramine, octopamine and tryptamine, are not soluble in water (Facchini et al., 2002).

Polyamines are conjugated by the formation of an amide linkage, using esters of CoA for provision of the activated carboxyl groups (Negrel et al., 1992). Putrescine (**3**) and spermidine (**1**) are conjugated by distinct transferases that differ in their specificity towards hydroxycinnamoyl CoA derivatives. So, the enzyme putrescine hydroxycinnamoyl transferase (PHT; EC 2.3.1.-) catalyses the transfer of hydroxycinnamic acids between CoA and putrescine (**3**) (Fig. 3; Negrel, 1989). Thus, the reaction between caffeoyl-CoA (**13**) and putrescine (**3**) produces the HCAA caffeoylputrescine (**14**) (Fig. 3). Spermidine and spermine hydroxycinnamoyltransferases have also been isolated (e.g. Hedberg et al., 1996), while a similar enzyme, hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-(hydroxycinnamoyl) transferase (HHT), was isolated from oat leaves and is involved in the biosynthesis of avenanthamides (**15**–**17**; Fig. 4) (e.g. Ishihara et al., 1998).

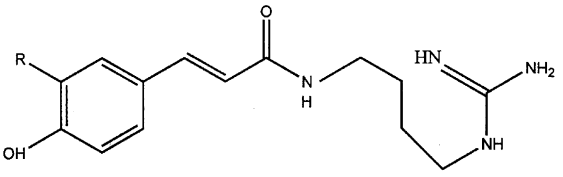
Although controversy continues surrounding the physiological relevance of HCAAs, they have been widely implicated in a variety of plant growth and



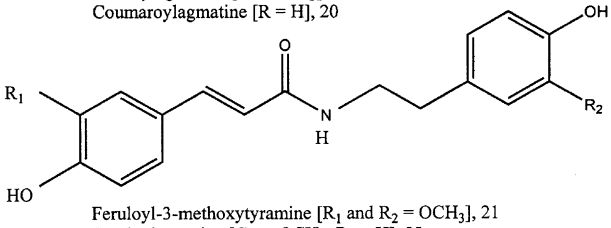

Avenanthramide A [R1 = OH; R2 = H], 15
Avenanthramide B [R1 = OH; R2 = $OCH_3$], 16
Avenanthramide C [R1 = H; R2 = H], 17




Feruloylputrescine [R = OCH3], 18
Caffeoylputrescine [R = OH], 14




Feruloylagmatine [R = $OCH_3$], 19
Coumaroylagmatine [R = H], 20




Feruloyl-3-methoxytyramine [$R_1$ and $R_2$ = $OCH_3$], 21
Feruloyltyramine [ $R_1$ = $OCH_3$; $R_2$ = H], 22

Fig. 4. Chemical structures of the HCAAs : avenanthramides A (**15**), B (**16**), C (**17**); feruloylputrescine (**18**), caffeoylputrescine (**14**); feruloylagmatine (**19**), coumaroylagmatine (**20**); feruloyl-3-methoxytyramine (**21**), feruloyltyramine (**22**).

developmental processes (Martin-Tanguy, 1985). More recently, HCAAs have began to attract renewed attention with several studies suggesting an important role for these compounds in plant defence responses to pathogens. This aspect will be discussed later in this review.

## 4. Polyamine metabolism in plant pathogen interactions

### *4.1. Polyamines in compatible interactions between plants and pathogens*

Despite the continued interest in polyamine metabolism in plants exposed to abiotic stress (Bouchereau et al., 1999), little information exists on polyamine metabolism in the compatible interactions between plants and pathogens. Much of the early work in this area focussed on changes in polyamines in green islands that form on cereal leaves infected with biotrophic fungal pathogens like rust and powdery mildew (Walters, 2000). Green islands surround the infection sites of powdery mildew and rust fungi and are thought to represent regions in which a juvenile condition is maintained (Walters, 1989). The presence of green islands can thus ensure that the fungal pathogen is surrounded by metabolically active cells from which it can absorb nutrients. Greenland and Lewis (1984) showed that polyamine levels and particularly the level of spermidine (**1**), increased greatly in leaves of barley infected with the brown rust fungus *Puccinia hordei*. In view of the association of polyamines with the regulation of plant senescence and the maintenance of juvenility in plant tissue (Kaur-Sawhney et al., 1982), these workers suggested that the increased polyamine concentrations in rust-infected barley might be related to green island formation. Later work showed that infection of barley with the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* led to increased concentrations of putrescine (**3**), spermidine (**1**) and spermine (**2**) in infected leaves and that these changes were accompanied by increased activities of the biosynthetic enzymes ODC, ADC and AdoMetDC (Walters et al., 1985; Walters and Wylie, 1986). Further work using a detached leaf system for the generation of green islands on powdery mildew infected barley leaves showed that several-fold increases in polyamine concentrations occurred in green islands (Coghlan and Walters, 1990).

However, although increased polyamine concentrations were found in compatible interactions between cereals and biotrophic fungal pathogens, this was not the case for other compatible interactions between plants and fungal pathogens. Thus, infection of tobacco leaves with *Peronospora tabacina*, *Erysiphe cichoracearum* and *Alternaria tenuis* resulted in decreased concentrations of free putrescine (**3**) and spermidine (**1**) (Edreva, 1997). Since the greatest reduction in polyamine concentrations was found in leaves infected with pathogens causing the most severe damage to tissue, the author suggested that reduction in polyamine levels in these interactions was a non-specific response to tissue damage (Edreva, 1997). This is compatible with the view that high polyamine levels are characteristic of young, metabolically active tissue, while low polyamine levels are more typical of plant tissue when metabolism has slowed down, e.g. senescing leaves (Galston and Kaur-Sawhney, 1990).

In some interesting work on sugarcane infected with the smut fungus *Ustilago scitaminea*, concentrations of free and conjugated forms of putrescine (**3**) and spermidine (**1**) were found to decrease, while an increase in free and conjugated forms of spermine (**2**) was detected (Legaz et al., 1998). Since a linear correlation exists

between the concentrations of phenolic compounds produced in sugarcane tissue and infection (Lloyd and Naidoo, 1983), it was suggested that susceptibility of sugarcane to smut is the result of the conjugation of the phenolics to polyamines, thus reducing the fungitoxic properties of the phenols (Legaz et al., 1998).

It is well known that virus infection of plant tissue can lead to a large accumulation of polyamines e.g. infection of turnip with the turnip yellow mosaic virus (TYMV; Torget et al., 1979). A regulatory role for polyamines in virus replication has been suggested (Morch and Benicourt, 1980) and indeed, it was shown that in turnip protoplasts infected with TYMV, the newly formed virus particles contained predominantly newly synthesized spermidine (**1**) and spermine (**2**) (Balint and Cohen, 1985a). These authors later suggested that spermidine (**1**) and spermine (**2**), synthesized from methionine in the cytosol of turnip cells, are initially present in a transient pool, which is distinguishable from a more stable pool of spermidine (**1**) and spermine (**2**) which is associated with cellular structures and polymers (Balint and Cohen, 1985b).

Infection of tomato and *Gynura aurantiaca* with the citrus exocortis viroid (CEVd) led to stunting and leaf epinasty, increased production of ethylene and of pathogenesis-related (PR) proteins (Belles and Conejero, 1989; Belles et al., 1989; Belles et al., 1991). Viroid infected plants also exhibited a marked reduction in putrescine (**3**) concentration and application of putrescine (**3**) suppressed the development of symptoms and the production of PR proteins (Belles et al., 1991). Evidence was provided to suggest that the increased production of ethylene in CEVd infected plants was responsible for the reduced putrescine (**3**) concentration, and later, that the reduction in putrescine (**3**) was brought about by reduced ODC activity (Belles et al., 1993).

### 4.2. Polyamines in incompatible interactions between plants and pathogens

#### 4.2.1. Incompatible interactions between plants and fungal pathogens

In recent work on barley reacting hypersensitively to the powdery mildew fungus *B. graminis* f. sp. *hordei*, Cowley and Walters (2002a) found that levels of free putrescine (**3**) and spermine (**2**), and conjugated forms of putrescine (**3**), spermidine (**1**) and spermine (**2**) were greatly increased 1–4 days following inoculation. These changes in free and conjugated polyamines were accompanied by increased activity of the biosynthetic enzymes ODC, ADC and AdoMetDC, and of the catabolic enzymes DAO and PAO. Interestingly, in this hypersensitive reaction of barley to powdery mildew, activities of two enzymes involved in conjugating polyamines to hydroxycinnamic acids, putrescine hydroxycinnamoyltransferase (PHT) and tyramine feruloyl-CoA transferase (TFT) were also observed to increase 1–4 days following inoculation (Cowley and Walters, 2002a). Although the work did not specifically determine HCAAs in the hypersensitive response (HR), data suggest that HCAAs may accumulate in this interaction. This would be worthy of further investigation since HCAAs are known to accumulate in a number of incompatible plant–pathogen interactions (e.g. von Ropenack et al., 1998; McClusky et al., 1999) and they have also been shown to exhibit direct antifungal properties (Walters et al., 2001).

In an incompatible interaction between barley and powdery mildew, where the resistance was penetration based (*mlo* allele), levels of free spermidine (**1**) and of conjugated forms of putrescine (**3**) and spermidine (**1**) were found to increase 1-3 d after inoculation (Cowley and Walters, 2002b). Here, as in the HR of barley to powdery mildew (Cowley and Walters 2002a), activities of the catabolic enzymes DAO and PAO were increased. The activities of DAO and PAO produce hydrogen peroxide ($H_2O_2$) which is required for polysaccharide–protein cross linkings and lignification (Pellegrini et al., 1994). Indeed, DAO activity increases in plant cell walls in response to wounding (Scalet et al., 1991) and enhanced DAO activity has been linked to resistance in chickpea to the fungal pathogen *Ascochyta rabiei* (Angelini et al., 1993). Certainly in barley, one of the mechanisms involved in penetration based resistance to powdery mildew infection is the oxidative burst which occurs directly beneath the region of attempted penetration (e.g. Vanacker et al., 2000). This leads to the rapid accumulation of $H_2O_2$ at the site of eventual papilla formation and may well be involved in oxidative cross-linking of components such as phenolics and protein into the papilla and associated cell wall region. It is possible that the enhanced activities of DAO and PAO observed in the penetration based resistance of barley to mildew (Cowley and Walters, 2002b) could be involved in production of $H_2O_2$ destined for defence as described above. However, it is yet to be determined whether DAO and PAO activities are enhanced in the period 12–24 h after inoculation, which covers the critical phase when mature powdery mildew appressoria initiate their attack on leaf epidermal cells and when plant responses determine the outcome of attempted infection.

But DAO and PAO activity can also lead to the formation of reactive oxygen species (ROS; Allan and Fluhr, 1997). Both $H_2O_2$ and ROS are involved in signalling in programmed cell death (PCD; Mittler et al., 1997). They also play a key role in the cross linking of polysaccharides and proteins (Yang et al., 1997) and have a direct antimicrobial effect (Peng and Kuc, 1992). Moreover, work on *Arabidopsis* has shown that DAO activity and reduced polyamine levels initiate PCD-driven changes in development (Moller and McPherson,

1998). It seems possible therefore that formation of $H_2O_2$ and ROS, resulting from enhanced activities of DAO and PAO, could be a cause of the HR observed in barley to powdery mildew infection (Cowley and Walters, 2002a). Another possibility is also worthy of further examination as a cause of the HR is this barley/mildew interaction. Cowley and Walters (2002a) showed that levels of free spermine (**2**) increased substantially in barley reacting hypersensitively to mildew infection. Studies of apoptosis, the most well-characterised form of animal PCD, have led to the identification of a central tripartite death switch, the enzymatic component of which is a family of cysteine proteases known as caspases (Lam and Pozo, 2000). Interestingly, it has been shown that spermine (**2**) accumulation triggers caspase activation in leukaemia cells (Stefanelli et al., 1998). Walters and Cowley (2002a) suggest that, in view of the recent evidence for the existence of caspases in higher plants, it would be valuable to examine the possibility that spermine (**2**) accumulation is associated with caspase activation in barley reacting hypersensitively to powdery mildew infection.

Alterations in polyamine metabolism have also been detected in plants expressing systemically induced resistance. Thus, treatment of the first leaves of barley seedlings with methyl jasmonate (MJ) led to significant reductions in powdery mildew infection in second leaves (Walters et al., 2002). This systemic protection against mildew was accompanied by increased activation of the defence-related enzymes phenylalanine ammonia lyase (PAL) and peroxidase, but also by significant increases in levels of soluble conjugates of putrescine (**3**) and spermidine (**1**) and increased activity the biosynthetic enzymes and of DAO. Increased polyamine levels and activities of biosynthetic and catabolic enzymes were also found in MJ-treated first leaves. Jasmonates are known to increase the formation of phenolic compounds by stimulating the phenylpropanoid pathway. Thus, exposure of various plant cell cultures to MJ increased PAL activity (Gundlach et al., 1992), while jasmonic acid-induced accumulation of acid soluble polyamine conjugates could be reduced by prior treatment with a PAL inhibitor (Mader, 1999). Interestingly, MJ has recently been shown to increase the expression of the ADC2 gene in *Arabidopsis* and ADC gene expression was also induced by wounding (Perez-Amador et al., 2002). Wounding was also shown to increase DAO activity both locally and systemically in chickpea (Rea et al., 2002). When these workers inhibited DAO activity in chickpea resistant to the fungal pathogen *A. rabiei* using 2-bromoethylamine, resistance to the pathogen was greatly reduced. This work demonstrates the importance of DAO in defence against *A. rabiei* in chickpea, and highlights the need for a more wide ranging examination of DAO and resistance to pathogens in other systems.

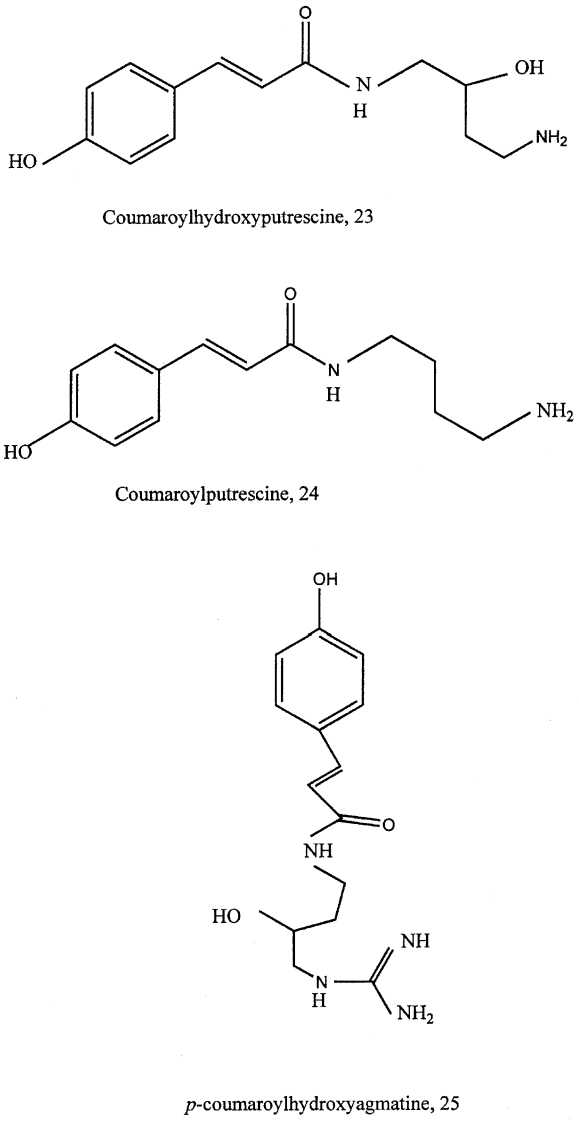
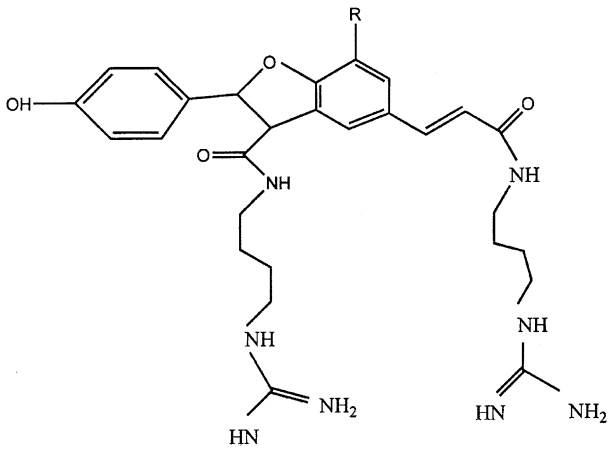



Fig. 5. Chemical structures of the HCAAs : coumaroylhydroxyputrescine (**23**); coumaroylputrescine (**24**) and *p*-coumaroylhydroxyagmatine (**25**).

As indicated above, relatively few studies have focussed on changes in free polyamines in plant–pathogen interactions. However a number of studies have examined changes in HCAAs in plants responding to fungal infection. Thus, feruloylputrescine (**18**; Fig. 4) was shown to accumulate in potato tubers infected with *Phoma exigua* (Malmberg, 1984), while HCAAs of tyramine and octopamine were synthesized and integrated into cell walls of potato tubers as a very early response to attempted fungal infection (Clark, 1982). In the latter work, the HCAAs were thought to contribute to the formation of a phenolic barrier, making the cell walls more resistant to enzymic hydrolysis. In cereals, Samborski and Rohringer (1970) found that coumaroylhydroxyputrescine (**23**; Fig. 5) accumulated in wheat challenged with rust, while in barley, coumaroylagmatine (**20**; Fig. 4) was present in considerable




Hordatine A [R = H], 26
Hordatine B [R = $OCH_3$], 27

Fig. 6. Chemical structures of Hordatines A (**26**) and B (**27**).

quantities in seedlings, together with the antifungal compounds hordatine A (**26**) and B (**27**) (Fig. 6; Smith and Best, 1978). Hordatine A (**26**) is a dimer of *p*-coumaroylagmatine (**20**), while hordatine B (**27**) is an analogous conjugate of *p*-coumaroyl and feruloylagmatines (**20, 19**; Fig. 4). A novel HCAA, *p*-coumaroyl-hydroxyagmatine (**25**; *p*-CHA; Fig. 5) was identified in barley and shown to accumulate to significant levels in a resistant variety challenged with powdery mildew (von Ropenack et al., 1998). *p*-CHA (**25**) started to accumulate in the resistant barley variety as early as 14 h after inoculation and was shown to exhibit significant activity against the powdery mildew fungus both in vitro and in vivo (von Ropenack et al., 1998). In addition to the above, avenanthramides (**15–17**), a series of HCAAs with hydroxyanthranilates, have been well characterized as phytoalexins in oats (Mayama et al., 1981, 1982). Avenanthramides (**15–17**) accumulate in oat leaves expressing resistance to crown rust (*Puccinia coronata* f. sp. *avenae*) infection and in oat leaves treated with various elicitors, e.g. *N*-acetylchitooligosaccharides (Bordin et al., 1991). More recently, activity of anthranilate synthase (EC 4.1.3.27), an enzyme thought to play a rate-limiting role in the biosynthesis of avenanthramides (**15–17**), was shown to increase greatly in oats in response to treatment with oligo-*N*-acetyl-chitooligosaccharides (Matsukawa et al., 2002).

In an examination of the attempted penetration of onion epidermis by the fungal pathogen *Botrytis allii*, McClusky et al. (1999) observed the accumulation of feruloyl-3′-methoxytyramine (**21**; FMT; Fig. 4) and feruloyltyramine (**22**; FT; Fig. 4) in challenged tissues. FMT (**21**) and FT (**22**) were the major components of reaction material formed in the onion epidermal cells and were bound by ether linkage on to the cell wall as well as being present in methanol soluble granules. These workers could find no antifungal activity in FMT (**21**) or FT (**22**), but rather proposed that they have a key role in resistance by preventing fungal degradation of the cell wall (McClusky et al., 1999).

*4.2.2. Incompatible interaction between plants and viruses*

A sizeable body of information now exists on changes in polyamine metabolism during the HR of tobacco to infection by tobacco mosaic virus (TMV). Thus, a 20-fold increase in ODC activity was detected in tobacco leaves exhibiting a HR to TMV infection (Negrel et al., 1984), and in the tobacco cv Xanthi n.c., there was an accumulation of a number of HCAAs, including feruloylputrescine (**18**) and feruloyltyramine (**22**) during the HR to TMV infection (Martin-Tanguy et al., 1973, 1976). In the latter work, the HCAAs appeared at the time of lesion formation and greatest accumulation occurred in the living cells surrounding the necrotic lesion. Using the tobacco cv Samsun NN to study changes in polyamines during the development of the HR to TMV infection, Torrigiani et al. (1997) found that levels of free and conjugated putrescine (**3**) and spermidine (**1**) increased, with the greatest concentration occurring in the necrotic area. They suggested that high levels of polyamine conjugates might be required for the necrotic lesion to develop, so limiting virus movement and preventing systemic infection. In fact, Martin-Tanguy et al. (1976) have shown that treatment of tobacco leaf discs with coumaroyl- (**24**; Fig. 5) and caffeoylputrescine (**14**) reduced local lesion formation by 90% following TMV inoculation. These workers used this information, together with the inverse correlation between the rate of virus multiplication and HCAA formation, to suggest that HCAAs were involved in virus resistance.

Despite these correlations between levels of polyamines and HCAAs during the HR of tobacco to TMV infection, the mechanism (s) by which these compounds might exert their effects remained elusive. Hypotheses put forward to explain the role of polyamines and HCAAs in HR to TMV include (a) inhibition of viral replication by HCAAs (Martin-Tanguy et al., 1976) and (b) that polyamines might be involved in inducing apoptosis during HR, since apoptosis is rapidly induced in animal cells which have accumulated spermidine (**1**) due to overexpression of ODC (Poulin et al., 1995). In a very interesting study, Yamakawa et al. (1998) found that in the HR of tobacco to TMV infection, there was a 20-fold increase in spermine (**2**) levels in the intercellular spaces of the necrotic lesion-forming leaves. Significantly, they showed that spermine (**2**) was a salicylate-independent endogenous inducer of both acidic PR proteins and resistance to TMV. Further evidence for a role for spermine (**2**) in the HR of tobacco to TMV comes from a study by Hiraga et al. (2000), who found that a HR-induced peroxidase gene (*tpoxC*$_1$) is responsive to spermine (**2**), but not to salicylate or MJ.

## 5. Concluding remarks

From the above, it is clear that good correlations exist between the accumulation of HCAAs and pathogen resistance. One means of attempting to determine whether HCAAs have a role in disease resistance is to engineer HCAA metabolism in order to alter levels of HCAAs in plants. Should this approach work, it could be used to improve plant disease resistance. However, successful genetic manipulation of HCAA levels in plants requires a thorough understanding of the biochemical and molecular regulation of HCAA biosynthesis. Although our knowledge of this area is increasing (see Facchini et al., 2002), it is still far from complete. Nevertheless, some interesting work by Facchini et al. (1999) highlights the potential for the genetic engineering of HCAA levels in plants. In this work, they transformed *Brassica napus* with genes coding for tyrosine decarboxylase (TYDC; EC 4.1.1.25). TYDC catalyzes the decarboxylation of tyrosine to tyramine. The transgenic plants generated exhibited a 3- to 4-fold increase in TYDC activity and a 2-fold increase in cell wall-bound tyramine compared with wild type plants.

But we should not focus all of our attention on HCAAs! The recent work showing substantial increases in spermine (**2**) in the HR of barley to powdery mildew (Cowley and Walters, 2002a) and the work of Yamakawa et al. (1998) showing that spermine (**2**) induces PR proteins and resistance to TMV in tobacco, suggests that the role of spermine (**2**) in plant defence is an area ripe for investigation.

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