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Polyamine biosynthesis inhibitors: New protectants against fungal plant diseases

M. V. Rajam

Department of Genetics, University of Delhi, South Campus, Benito Juarez Road, New Delhi 110 021, India

Polyamines (PAs) have been shown to be involved in a variety of growth and developmental processes in a wide range of organisms, including fungal systems. Because of their vital role in cell proliferation and differentiation, the specific inhibition of PA biosynthesis has provided a novel approach for new therapies. The PA biosynthesis inhibitors like α-difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase have been shown to be very effective protectants against various kinds of fungal plant diseases, especially rust infections and powdery mildews. DFMO is highly potent, persistent, fast acting and translocatable. Interestingly, DFMO is non-phytotoxic and has no effect on the growth and endogenous PA pools of the host plants. It thus affords a possible new means of controlling plant diseases of fungal origin in the future, and may generate a new class of target-specific pesticides for use in plant disease control. The current status of this newly emerging field is reviewed here.

POLYAMINES (PAs) are one of the most important and interesting groups of naturally occurring polycationic low molecular aliphatic nitrogenous compounds that are present in all cells[1]. The most common PAs are the diamines putrescine (PUT) and cadaverine (CAD), triamine spermidine (SPD) and tetraamine spermine (SPM) (Figure 1). In general, prokaryotic cells contain fairly large amounts of PUT, small quantities of SPD and no SPM, while eukaryotes have little PUT, more SPD and considerable SPM[2]. In addition to the above

a $H_2N-(CH_2)_4-NH_2$

b $H_2N-(CH_2)_5-NH_2$

c $H_2N-(CH_2)_4-NH-(CH_2)_3-NH_2$

d $H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$

Figure 1. Structure of common di-and polyamines· putrescine (*a*), cadaverine (*b*), spermidine (*c*) and spermine (*d*).

usual PAs, some novel and unusual PAs such as caldine, thermine, and caldopentamine have been described in a variety of organisms, especially thermophiles (Table 1)[3,4]. In higher plants, PAs are present in both free and bound forms (Table 1). The biochemistry of PA-conjugates synthesis and their degradation has not been studied in detail, although they play a potential role in plant developmental events[5].

The past two decades have witnessed a growing awareness of the importance of PAs for the normal functioning of the cells. Several investigations have emphasized the many and varied roles of PAs and associated enzymes in molecular and cellular functions such as regulation of cell division, growth and differentiation, nucleic acid synthesis and function, protein synthesis, embryo development, physical and chemical properties of membranes, hormones, senescence, several forms of stresses, modulation of enzyme activities and so on. A number of excellent reviews on function and biochemistry of PAs are available[6-12]. Many of the biological functions of PAs appear to be attributable to the cationic nature of these molecules, which are highly protonated at physiological pH and their electrostatic interaction with polyanionic nucleic acids and negatively charged functional groups of membranes and enzymatic or structural proteins in the cell[6]. These compounds have been described as endogenous growth regulatory compounds or intracellular second messengers mediating the effects of plant hormones[1,7,12,13]. Besides the usual PAs, some unusual PAs (Table 1) have been postulated to serve specific protective roles in different organisms, for example, protection against osmotic lysis of membranes and *in vitro* protein synthesis at elevated temperatures in bacteria, particularly thermophiles and the adaptation of higher plants to drought and high temperature stress[3,4,8]. Similarly, the bound PAs which are conjugated to various phenolic secondary metabolites like hydroxycinnamic acids (Table 1) also have significant roles in plant developmental processes, especially flower development and plant defense[5].

Although most of the work in the PA field has centered on the physiology and biochemistry in different organisms[6,7,14], in recent years increasing attention has been focused on the chemotherapeutic implications of inhibition of PA biosynthesis. Specific inhibitors of PA biosynthesis like α-difluoromethylornithine (DFMO) have been used in the chemotherapy of cancer[15-18] and protozoan infections[19-21]. DFMO and other inhibitors of PA biosynthesis have also been shown to be very effective protectants against various kinds of fungal plant diseases[22-25]. The present article attempts to summarize the recent developments on the potential uses of PA biosynthesis inhibitors in the control of fungal plant diseases and future prospects of this new and novel approach. The influence of PAs in plant stress reactions has not been covered in this review because of a lot of work on this topic[6,8,10], and it falls outside the scope of this article.

Table 1. Some common and uncommon polyamines and polyamine conjugates

Common polyamines
Putrescine
Cadaverine
Spermidine
Spermine
Uncommon polyamines
Norspermidine (Caldine)
Homospermidine
Norspermine (Thermine)
Thermospermine
Caldopentamine
Caldohexamine
Polyamine conjugates (bound polyamines conjugated to various phenolic secondary metabolites)
Hydroxycinnamoylputrescine
Alkylcinnamoylputrescine
p-Coumaroylputrescine
Feruloylputrescine
Caffeoylputrescine
Caffeoylspermidine
Coumarylagmatine

Polyamine biosynthesis

The essential diamine PUT is synthesized directly by the decarboxylation of ornithine via the rate-limiting enzyme ornithine decarboxylase (ODC) or indirectly by decarboxylation of arginine via arginine decarboxylase (ADC; Figure 2). Both pathways (ODC & ADC) operate in bacteria and plant cells[2,6,8]. By contrast, only the ODC pathway is involved in the synthesis of PUT in most fungal[2,26,28], mammalian[17,29,30] and most probably protozoan cells[17,20,21]. The higher PAs, SPD and SPM are synthesized from PUT by subsequent additions of aminopropyl groups donated by decarboxylated *S*-adenosylmethionine (SAM) (Figure 2). The aminopropyl group additions are catalysed by specific aminopropyltransferases commonly known as SPD and SPM synthases. The formation of the diamine cadaverine as a result of lysine decarboxylation is also found in certain organisms including higher plants[6,11]. In plants, arginine can be converted into ornithine by

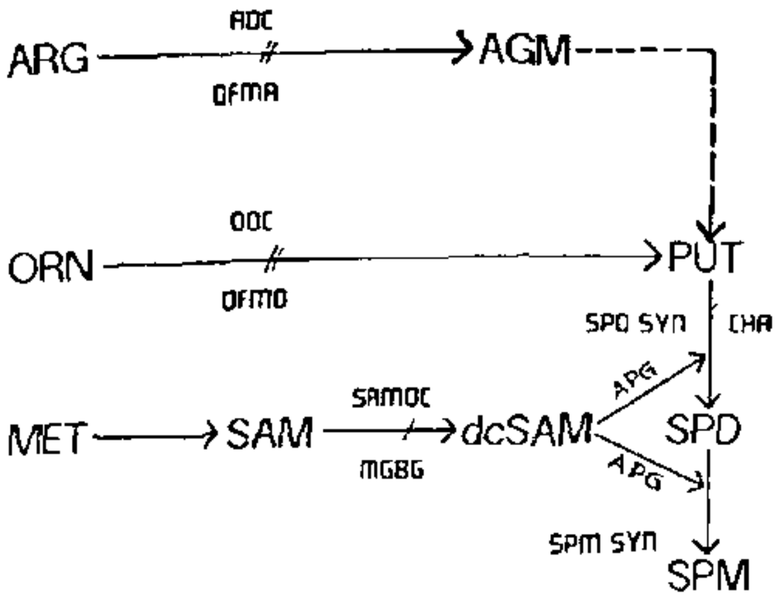

Figure 2. Polyamine biosynthesis in plants, ARG, arginine; AGM, agmatine, ORN, ornithine, MET, methionine; SAM, *S*-adenosylmethionine; PUT, putrescine; SPD, spermidine, SPM, spermine, ADC, arginine decarboxylase; ODC, ornithine decarboxylase, SAMDC, SAM decarboxylase; SPD SYN, SPD synthase, SPM SYN, SPM synthase, dcSAM, decarboxylated SAM, APG, aminopropyl group, DFMA, difluoromethylarginine; DFMO, difluoromethylornithine, MGBG, methylglyoxyl *bis*guanylhydrazone); CHA, cyclohexylamine

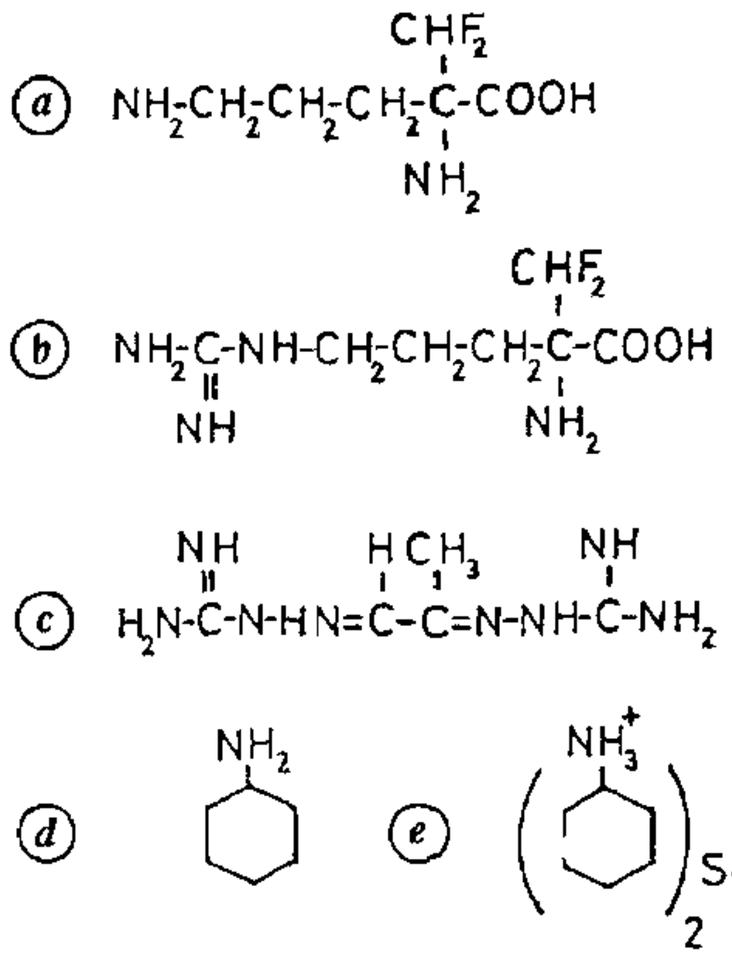

Figure 3. Structure of polyamine biosynthesis inhibitors commonly used for the control of plant pathogenic fungi. DFMO (an inhibitor of ODC) (*a*), DFMA (an inhibitor of ADC) (*b*), MGBG (an inhibitor of SAMDC) (*c*), CHA (an inhibitor of SPD synthase) (*d*), and BCHA (an inhibitor of SPD synthase) (*e*).

arginase and similarly ornithine to arginine through the ornithine cycle[6]. The degradation of PAs in plants is less well understood, although diamine and PA oxidases have been characterized in some plants like cereals and legumes[6,11].

Polyamine biosynthesis inhibitors

A major milestone in the development of PA field occurred in 1978, when scientists from Merrell Dow Research Institute, Cincinnati, USA synthesized specific, irreversible and enzyme activated inhibitor (substrate–based), DFMO for a PA biosynthetic enzyme ODC[31]. Subsequently, a number of competitive inhibitors (both substrate and product-analogues) for major enzymes of PA biosynthesis like DL-α-difluoromethylarginine (DFMA, an inhibitor of ADC) have been prepared[32–34].

Since the synthesis of specific and potent inhibitors of PA metabolism (Figure 3), rapid strides have been made to understand the close relationships between PAs and the physiological processes as well as of biochemical mechanisms at the molecular level in many systems[7,35]. In addition, chemotherapeutic implications of PA biosynthesis inhibition were also realized[13,15,25].

Inhibition of fungal polyamine biosynthesis and disease prevention

Since PAs play a potential role in cell proliferation and differentiation, the selective inhibition of PA biosynthesis has provided a novel and promising approach for new therapies. In other words, all the enzymes for PA biosynthesis have been targets for disease prevention[36–38]. In fact, the past few years have been an exciting time for scientists involved in PA research. Several breakthroughs in this field have been achieved only recently. Perhaps the most exciting and far reaching development in this area is the recent possibility of control of some dreaded diseases in human beings like cancer[15–19] and protozoan infections[17–20], and the control of fungal plant diseases[13,22–25] through specific inhibition of PA biosynthesis.

Fungi that cause diseases in higher plants bring colossal loss to food crops, and also bring about destruction of valuable materials. To control these diseases, several synthetic agrochemicals have been used. There is no doubt that agro-chemicals are necessary for profitable production of high quality food. However, most of these compounds are hazardous and can cause environmental pollution and damage to wildlife. Keeping this in mind, several attempts have been made in recent years by many researchers to harness alternate strategies for the protection of crop plants.

A promising substitute for usual pesticide protectants would be a group of chemicals that mimic the action of antimicrobial agents, but selectively inhibits the pathogen through inhibition of an essential biosynthetic pathway. Notable exception is the recent development of an entirely new method for the control of plant diseases by selective inhibition of fungal PA biosynthe-

sis[22,28]. This novel approach has attracted the attention of several scientists, who have also reported the protective efficacy of PA biosynthesis inhibitors in the control of several fungal plant diseases (Table 2).

Fungi that cause diseases in higher plants also need PAs for normal growth and differentiation[28], but unlike plants they have but a single pathway (ODC) for the biogenesis of PAs[2,26]. It should be possible, therefore, to eradicate a fungal pathogen on a crop plant without affecting the growth of host plant by specific inhibition of fungal ODC pathway[22,28]. This is particularly interesting since higher plants have an alternative pathway (ADC) for the production of an essential PUT. In fact, PUT is mostly derived from ADC pathway in most plants[8,39].

Table 2. Range of fungal plant diseases controllable by inhibitors of PA biosynthesis

Disease	Causal organism	Inhibitor	Ref. no.
French bean rust	*Uromyces phaseoli*	DFMO	22
Broad bean rust	*U. viciae-fabae*	DFMO	57
Tomato wilt	*Verticillium dahliae*	DFMO	40
Wheat leaf rust	*Puccinia recondita*	DFMO/ DFMA	54
Wheat stem rust	*P. graminis* f. sp. *tritici*	DFMO	53, 54, 58
Wheat powdery mildew	*Erysiphe graminis*	DFMO	54
Wood decay	*Postia placenta*	DFMO	51, 61
Barley powder mildew	*E. graminis* f. sp.	DFMO/ MGBG	59
Oat stem rust	*P. graminis* f. sp. *avenae*	DFMO	25
Corn common rust	*P. sorghi*	DFMO	25
Bean powdery mildew	*E. polygoni*	DFMO	25
Apple powdery mildew	*Podosphaera leucotricha*	DFMO	25
Corn leaf blight	*Helminthosporium maydis*	DFMO/ DFMA	25

Inhibition of plant pathogenic fungi *in vitro*

The work on *in vitro* inhibition of several phytopathogenic fungi using a variety of PA biosynthesis inhibitors is summarized in Table 3.

Rajam and Galston[28], for the first time, demonstrated that DFMO at 0.1, 0.5 and 1.0 mM strongly inhibits the growth of several phytopathogenic fungi (*Botrytis* sp., *B. cinerea*, *Monilinea fructicola* and *Rhizoctonia solani*) *in vitro*, and that such inhibitions can be totally reversed by exogenously supplied PAs (PUT or SPD). This also indicated the absolute requirement of PAs for normal growth and development of fungal hyphae. DFMA also effectively inhibited mycelial growth of all fungi tested, and they suggested that the inhibition caused by DFMA could be due to the existence of ADC pathway in those fungi tested, or the conversion of DFMA to urea plus DFMO by arginase[40–45].

Table 3. Range of phytopathogenic fungi inhibited in *in vitro* using inhibitors of PA biosynthesis

Pathogen	Inhibitor	Ref. no.
Botrytis sp., *B. cinerea*, *Monilinea fructicola*, and *Rhizoctonia solani*	DFMO/DFMA/ DFMO+DFMA	28
Helminthosporium maydis	DFMO	44
Verticillium dahliae	DFMO/DFMA	40
Pyrenophora teres, *Gaeumannomyces graminis*, *Fusarium culmorum* and *Septoria nodorum*	DFMO/MGBG/ CHA/DFMO+ MGBG	45
Fusarium oxysporum f. sp. *lycopersici*, *Ceratocystis ulmi*, *H. maydis* and *H. carbonum*	DFMO/DFMA	47
Pyricularia penniseti	D-Arginine	46
Pyrenophora avenae	DFMO/MGBG	49
Humicola lanuginosa, *Mucor pusillus* and *Talaromyces emersonii*	DFMO	50
Postia placenta	DFMO/DFMA	51

Later, Birecka *et al.*[44] reported that DFMO at 0.5 to 2.0 mM significantly inhibited the mycelial growth of the southern corn leaf blight fungus, *Helminthosporium maydis* in the dark. PUT at 0.25 mM completely prevented the inhibitory effects of DFMO. They also observed that 2.0 mM DFMA had a weak inhibitory effect of the fungus and there was no detectable ADC activity in the fungus. Inhibition of mycelial growth of *Verticillium dahliae in vitro* by using DFMO was reported by Mussell *et al.*[40]. West and Walters[45] have shown the retardation of growth of several necrotrophs such as *Pyrenophora teres*, *Gaeumannomyces graminis*, *Fusarium culmorum* and *Septaria nodorum in vitro*, using a range of PA biosynthesis inhibitors: DFMO, methylglyoxal *bis*(guanylhydrazone) (MGBG, an inhibitor of S-adenosylmethionine decarboxylase), cyclohexylamine (CHA, an inhibitor of SPD synthase), Δ-(fluoromethyl) dehydroornithine (ΔMFMO), Δ-(fluoromethyl) dehydroornithine methyl ester (Δ-MFMO.CH_3) and (2R, 5R)-6-heptyne-2, 5-diamine (RR-MAP). It was shown that D-arginine inhibits the mycelial growth of millet leaf blast fungus, *Pyricularia penniseti*, and that such inhibitions can be reversed by L-arginine or usual PAs[46]. Khan and Minocha[47] found the inhibition of four phytopathogenic fungi, namely *H. maydis*, *H. carbonum*, *F. oxysporum* f. sp. *lycopersici* and *Ceratocystis ulmi* using DFMO (1 to 5 mM). Similar results were seen with DFMA, except in *H. maydis* which remained unaffected even by the highest concentration (5 mM) of DFMA. They also studied the distribution of two PA biosynthetic enzymes, ODC and ADC in these fungi. Three species (*H. maydis*, *H. carbonum* and *F. oxysporum*) had high level of ODC compared to ADC activity, while in *C. ulmi*, ADC was predominant with very little or no ODC activity. DFMO and DFMA significantly inhibited ODC and

ADC activity respectively in all species. Earlier, they had presented evidence for the existence of a biosynthetic ADC in two fungi *Ceratocystis minor* and *Verticillium dahliae*[48]. In a recent paper, Foster and Walters[49] reported the inhibition of mycelial growth of the oat-infecting fungus *Pyrenophora avenae in vitro* by using DFMO, MGBG and ethylmethylglyoxal *bis*(guanylhydrazone) (EMGBG). DFMO and MGBG singly or in combination, reduced the activity of ODC in the fungus. Significant growth inhibitions of some thermophilic moulds such as *Mucor pusillus, Humicola lanuginosa* and *Talaromyces emersonii* using DFMO were observed[50]. Inhibition of mycelial growth of wood deteriorating fungus *Postia placenta* by using DFMO and DFMA was also reported by Illman[51].

Our recent work has shown that control of several other fungal pathogens can also be achieved using specific inhibitors of PA biosynthesis. Significant inhibitions of growth were obtained against *H. oryzae*, *Curvularia lunata*, *Pythium aphanidermatum* and *Colletotrichum capsici* using DFMO, MGBG and *bis* (cyclohexylammonium) sulphate (BCHA, an inhibitor of SPD synthase). Among the inhibitors used, MGBG was found to be very effective, and this is most likely due to its effect on mitochondria[49].

DFMO and DFMA can cause changes in mycelial morphology and cell size. Cell length was much reduced and cell diameters were increased in inhibitor-treated cultures, while considerable increase in cell length and diameter was observed in many PA-treated cultures[28]. West and Walters[45] showed that the cell size of *G. graminis* barely changed, but some treatments produced a decrease in cell length. *Pyrenophora teres* also showed reductions in lengths on exposure to several inhibitors. By contrast, *S. nodurum* and *F. culmorum* exhibited increased cell lengths as a result of inhibitor treatments; the diameters of *F. culmorum* were also increased. In a recent paper, Foster and Walters[49] reported that neither the PA inhibitors nor exogenous PAs had any significant effect on the cell length of *P. avenae*.

The effect on mycelial growth of inhibitors of PA biosynthesis is dependent upon the particular fungus[28,44,45,47,49]. This may be because of differences between genera in the inhibitor uptake and distribution within the cell, sensitivity of the corresponding enzymes or differences in the required cellular concentration of PAs for growth and morphogenesis[44,45,49]

Inhibition of fungal spore germination and sporulation

The work on the inhibition of fungal spore germination and sporulation is summarized in Table 4.

Rajam *et al.*[22] noticed that DFMO totally inhibited rust uredospore germination on infected bean leaf surfaces, and this effect was partially reversed with the application of PUT or SPD to DFMO-treated leaves. They also determined that per cent germination and pathogenicity of rust uredospores collected two weeks after infection from bean plants treated with DFMO; spores collected from plants treated with 0.01 and 0.05 mM DFMO showed no significant differences, while 0.1 mM DFMO caused significant decrease in both germination and pathogenicity[52] (spores could not be collected from plants treated with 0.25 mM DFMO or higher as the plants protected from infection). DFMO and DFMA at 0.01, 0.1 and 1.0 mM concentrations produced successively greater inhibition of uredospore germination *in vitro*. They also delayed the timing of spore germination and suppressed the germ tube elongation[52]. In our recent study, marked inhibitions of spore germination *in vitro* in five plant pathogenic fungi, *F. oxysporum*, *C. lunata*, *Trichothecium roseum*, *Lasioidiplodia theobromae* and *Drechslera spicifer* have been recorded following spore treatment with MGBG and BCHA. We also found significant inhibitions of hydrolytic enzymes (amylase, cellulase and protease) in *F. oxysporum* and *L. theobromae* following methylglyoxal *bis*(guanyl hydrazone) (MGBG) and *bis*(cyclohexyl ammonium) sulphate (BCHA) treatments.

Inhibition of sporulation of *H. maydis* in the dark using DFMO was reported by Birecka *et al.*[44]. The inhibitory effect on sporulation was greatly enhanced under light conditions. Gaur *et al.*[46] showed that D-arginine inhibited the sporulation of *P. penniseti*. DFMO when injected into primary leaves of infected susceptible plants 24 h after inoculation reduced the production of uredospores; the effect being especially marked in the tip area of the leaves[53].

Table 4. Inhibition of spore germination/sporulation of plant pathogenic fungi using PA inhibitors

Pathogen	Inhibitor	Ref. no
Inhibition of spore germination		
Uromyces phaseoli	DFMO	22, 52
Inhibition of sporulation		
Helminthosporium maydis	DFMO	44
Puccinia recondita	DFMO	53
Pyricularia penniseti	D-Arginine	46

Control of fungal plant diseases

The range of fungal plant diseases controllable by PA inhibitors is presented in Table 2.

Rajam *et al.*[22] demonstrated, for the first time, the remarkable efficacy of DFMO (0.5 mM or higher) as a protectant against the bean rust fungus, *Uromyces phaseoli*, race 0 (Figure 4). The inhibitor (~ 400 µl) was applied as single spray to unifoliolate leaves of pinto beans

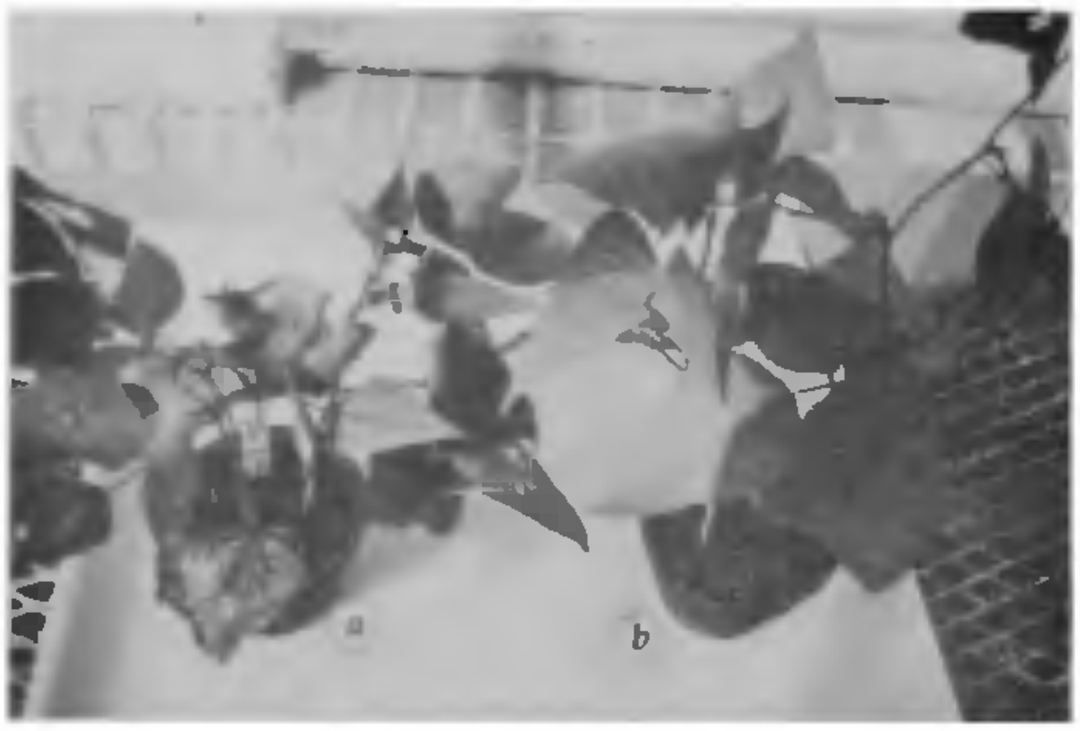

Figure 4. Control of bean rust by DFMO. Unprotected control bean plant (3-week-old) (*a*), and protected plant with 0.5 mM DFMO spray (*b*).

(*Phaseolus vulgaris* L., var. Pinto) after and before inoculation with pathogen. Postinoculation treatments with DFMO were generally more effective than preinoculation; the possible reason for this is that the DFMO that penetrates the leaf during preinoculation treatment reacts with and inhibits some portion of the endogenous ODC of the plant cell, reducing the effective titer of DFMO prior to inoculation of the plant with fungal spores[54]. DFMO also conferred protection against infection even in unsprayed regions of the bean plant, suggesting translocation of the compound or some other inhibitory substance as naturally occurring antimicrobial agent (e.g. phytoalexins) produced as a result of DFMO treatment in the host plant[22,55]. α-Difluoromethyl arginine (DFMA) even at 5 mM, had no protective effect on bean rust infection[22]. A time course analysis of bean rust inhibition by DFMO indicated that foliar applications of DFMO up to 3 days postinoculation still completely inhibited fungal growth, while 4 to 5 day postinoculation applications significantly reduced the severity of the infection[56]. PAs alone did not affect the rust infection of beans, however, when SPD was supplied 1 h after DFMO, the inhibition conferred by DFMO was substantially reduced[56]. This suggests that the effect of DFMO was related to inhibition of PA biosynthesis.

This work was confirmed by recent results from various laboratories which showed quite dramatic effects of DFMO on the control of several fungal plant diseases (Table 2). Walters[57] showed the control of rust infection in broad bean (*Vicia faba* L.) caused by *U. viciae-fabae* by DFMO. DFMO and DFMA, applied as foliar spray, gave good control of soil-borne *Verticillium* wilt of tomato (*Verticillium dahliae*), a fungus disease refractory to chemical control[40]. These inhibitors also delayed the appearance of symptoms of *Verticillium* wilt as in bean rust disease[22]. Weinstein *et al.*[54] obtained the effective control of wheat leaf and stem rust (caused by *Puccinia recondita* and *P. graminis* f. sp. *tritici*, respectively) with sprays of DFMO (0.005–0.2 mM). Further, they also reported the control of powdery mildew disease of wheat (caused by *Erysiphe graminis*) by this compound. Control of wheat rust was also reported by Balass and Cohen[58]. The mildew infection of barley leaves caused by *E. graminis* f. sp. *hordei* was effectively controlled by using a variety of PA inhibitors such as DFMO, Δ-MFMO, Δ-MFMO-CH_3, RR–MAP, DFMA, CHA and MGBG[59,60]. More recently, the control of several other fungal plant diseases such as the common rust of corn (caused by *P. sorghi*), southern corn leaf blight (*H. maydis*), oat stem rust (*P. graminis* f. sp. *avenae*), apple powdery mildew (*Podosphaera leucotricha*) and bean powdery mildew (*E. polygoni*) has been achieved using DFMO and/or DFMA[25]. Complete control of wood decay fungus (*Postia placenta*) in wood samples was achieved with low concentrations of DFMO by Illman[61], and she suggested the use of PA inhibitors as wood preservatives.

The control of some infections by DFMA is striking. In few cases, DFMA was as effective or more effective than DFMO[25]. This could be due to the conversion of DFMA to urea plus DFMO by arginase[41,42]. However, this does not explain why DFMA is more effective than DFMO in controlling certain pathogens. A possible explanation is that a mechanism to convert DFMO to DFMA exists in bean plants and in certain fungi that was found to be more active than the arginase-mediated conversion of DFMA to DFMO[25]. The very effective control of certain infections at very low concentrations of DFMO suggests that it is not a single blockage of one pathway that kills the fungus but not the plant, but it may also activate defence system and induce naturally occurring fungal inhibitors like phytoalexins in the host plant[22,25]. Although DFMO has been reported to possess some systemic activity[22], very little is known about its movement within the plant and subsequent effects on fungal infection[55]. DFMO can be taken up by roots of intact barley seedlings and transported to the shoots[62]. DFMO uptake was reduced in darkness and was markedly influenced by transpiration rate. The inhibitor was taken up from host cells by the powdery mildew fungus, *Erysiphe graminis* f. sp. *hordei* Marchal; DFMO fed to roots, or to excised leaves at different times after inoculation, gave substantial control of mildew infection[62]. By contrast, Slocum *et al.*[55] observed the poor translocation of DFMO in wheat plants, this suggests that control of fungal pathogen through inhibition of PA biosynthesis alone is unlikely.

The precise mechanism of metabolism of DFMO and

DFMA in living organisms is not well known. However, it has been suggested that ^{3}H-DFMO may be largely metabolized to ^{3}H-DFMA, or a related metabolite, while ^{3}H-DFMA is also metabolized, to a much smaller extent (8% of total), to ^{3}H-DFMO in bean plants[55]. Earlier, Slocum and Galston[41] had shown that DFMA is largely metabolized to DFMO in tobacco ovary tissues exhibiting high arginase activities. DFMO metabolism has also been noticed in animal cells. Erwin and Pegg[63] have shown that after 12 h incubation in [5-^{14}C]-DFMO, only 50% of the radioactivity in mouse SV-3T3 cells was recovered as ^{14}C-DFMO, the rest residing primarily in two unknown metabolites.

Effect of PA biosynthesis inhibitors on growth and PA biosynthesis of plants

Bean plants protected from infection by DFMO application did not exhibit any morphological alterations or reduction in growth rate compared to unsprayed, uninfected controls. By contrast, unprotected, infected plants showed a marked reduction in height (Figure 4)[22]. The foliar spray with DFMO or DFMA (0.01, 0.1 and 1.0 mM) produced no significant growth effects or changes in endogenous PA levels in any of the inhibitor-treated bean plants; in fact, PUT and SPD titers were significantly increased by the highest concentration of DFMO or DFMA (1 mM)[56,64]. This seems to be due to the paradoxical stimulation of ADC activity by DFMO[41]. Walters[57] observed that DFMO at 0.4 mM had no effect on either growth or total intracellular PA concentrations in *Vicia faba* plants. Weinstein *et al.*[54] also observed that PA inhibitors (DFMO and DFMA) had no apparent effect on the growth of wheat plants. Mussell *et al.*[40] showed that DFMO, even at 20 mM had no apparent effects on the growth and development of the tomato plants for the duration of the 28-day-observation period. A single injection of 10–30 μl of a 1 mM solution of DFMO into primary wheat leaves did not influence growth and PA contents of the plant[53].

Conclusions and future prospects

The sensitivity of various broad host-range plant fungal pathogens to DFMO and other PA inhibitors suggests the potential use of these inhibitors in controlling fungal infections of crop plants. In general, biotrophic fungi like rusts and powdery mildews seem to be more amenable to control using PA inhibitors[13,25].

It is evident from the foregoing discussion that most fungal plant diseases can be controlled by low concentrations of DFMO or DFMA (<1 mM)[22,25,38,60]. Interestingly, in at least some cases 50% effective dose values of the DFMO concentration were as low as 0.008 mM (in case of wheat rust) and 0.025 mM (bean and oat rust). Since these inhibitors had no apparent effect at concentrations which were used for disease control (mostly <0.5 mM) on growth[22,25,40,54] and PA biosynthesis[59,64] of the host plant, I strongly believe that in the coming years the PA biosynthesis inhibitors, singly or in conjunction may prove to be useful target specific fungicides (based on inhibition of an essential biochemical pathway in fungi) for use in plant disease control.

Rajam *et al.*[22] estimated that at a spray rate of 100 gallons to an acre (940 l ha^{-1}), about 25 g per acre would be adequate for protection of a bean crop in the field[43]. In view of the non-toxicity of DFMO to the higher plants, this level of application should pose minimal or no problems for animals and humans. The DFMO may not affect the germination and growth of beneficial fungi in the soil as its concentration may get diluted significantly in the soil water. Moreover, the inhibitor is used as foliar spray even in case of the control of soil-borne fungi, *Verticillium dahliae*[40], and therefore a small quantity of inhibitor may enter the soil. Similarly, the inhibitor may not affect the useful soil bacteria like *Rhizobium* as they possess two biosynthetic pathways (ADC and ODC) for PA biogenesis. Furthermore, the ODC inhibitors like DFMO are non-toxic to human beings and animals[17]; hence they may be environmentally safe fungicides and present an interesting alternative to traditional fungicides.

Obviously this work is in its infancy and much basic work is needed to provide the foundation for future developments[13]. In particular, the environmental risks associated with application of PA inhibitors in agriculture for the protection of crops have to be reckoned with. These are now better understood[22,40,54,59,64]. However, appropriate safeguards should be strictly adhered to so that the hazards associated with the testing and release of PA biosynthesis inhibitors in agriculture can be avoided. If this is done, no doubt this novel method can become a valuable tool in agriculture to control phytopathogens in the near future.

This work has been extended to inhibit the growth and ODC enzyme in zoophilic yeast (*Candida* sp.)[65,66] and fungi (*Microsporum*, *Trichophyton* and *Aspergillus*)[67,68]. As animal systems also possess a single pathway (ODC) for PA biogenesis, liposomes may be useful for targetting the PA inhibitor to infected tissue and/or organ and to minimize the side effects, if any. These studies would provide an additional approach to antifungal chemotherapy in human beings[68]. This approach would be promising against skin diseases caused by *Trichophyton*, *Microsporum* and other dermatophytes because of easy application of DFMO or other PA inhibitors at the site of infection.

Attempts were also made to inhibit bacteria using specific inhibitors of PA biosynthesis. Bitonti *et al.*[69] have used a combination of PA biosynthesis inhibitors (DFMO, DFMA and CHA) to inhibit the growth of *Escherichia coli* and *Pseudomonas aeruginosa in vitro*. Therefore, it appears worthwhile to examine the effect of PA biosynthesis inhibitors, singly or in combination, on the control of bacterial infections of plants. In bacteria, ODC pathway is normally the major route for PUT biosynthesis[69], while in most higher plants PUT is mostly derived from ADC pathway[8]. It should be possible, therefore, to eradicate a bacterial pathogen on a crop plant without affecting the growth of host plant by taking a high concentration of ODC inhibitor (like DFMO) and low concentration of ADC inhibitor (like DFMA) or inhibitors of ODC, ADC and SAMDC/SPD synthase enzymes. Added to this, the effect of DFMO, MGBG and BCHA on viral infection of sweet potato has been examined in our laboratory, but these inhibitors failed to control or reduce severity of the infection.

Since insects have only ODC pathway like fungi[70,71], it should also be possible to control insect pests by selective inhibition of PA biosynthesis in insects without affecting the host plant. Interestingly, PA biosynthesis inhibitors such as DFMO, MGBG and BCHA have shown insecticidal activity against tobacco caterpillar (*Spodoptera litura* Fb.)[72]. These PA inhibitors also strongly retarded the growth and development of mosquito larvae with high mortality[72].

There is no doubt that the use of PA biosynthesis inhibitors in the prevention of many more fungal plant diseases based on the selective inhibition of fungal PA biosynthesis would be one of the major interests of plant science research in the coming years and this is because of added excitement and fervour to much of the work in progress in this area of research from several laboratories. This field would also be a lively field for investigation by industry. Furthermore, this approach may also be a viable approach for the protection of crops from insect attack and bacterial pathogens, and towards the control of human diseases of fungal origin.

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