

MELANIN BIOSYNTHESIS INHIBITORS (MBIs) FOR CONTROL OF RICE BLAST

Yoshio Kurahashi, The Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Saitama 351-0198, Japan, discusses DHN melanin biosynthesis as a target for rice blast control agents

Introduction

In the search for new chemicals for the control of rice blast disease, it was noted that several organochlorine compounds and fused polycyclic compounds active against rice blast disease exhibited DHN melanin¹ biosynthesis inhibition. This mechanism now accounts for the action of some highly effective commercial fungicides used specifically to control rice blast. Recently the melanin biosynthesis inhibitors in a new chemical class (amide derivatives with substituted benzyl or phenoxy moieties) have been discovered and developed.

Role of melanin biosynthesis

The biosynthesis pathway of DHN melanin (polymers of 1,8-dihydroxynaphthalene) was first discovered in a study using a melanin-deficient mutant of *Verticillium dahliae* (Bell *et al.*, 1976); the process appeared to be common in *P. oryzae*, *Colletotrichum lagenarium* and various fungi belonging to Ascomycetes and Fungi Imperfecti (Wheeler, 1983) (Figure 1).

In many fungi that produce DHN melanin, the reasons for its accumulation are unclear. However, in a few fungi belonging to *Pyricularia* and *Colletotrichum*, DHN melanin plays an important role in fungal penetration into the host epidermis through an appressorium. The appressoria are formed at the tip of the germ tube and turn dark brown as they mature by accumulation of DHN melanin. An infection peg is formed directly under a matured appressorium and penetrates mainly into the motor cells² of the rice epidermis (Figure 2). A melanin-deficient mutant of *P. oryzae* and the wild-type strain treated with MBIs formed a hyaline appressorium which failed to penetrate the cellophane membrane and the rice epidermis

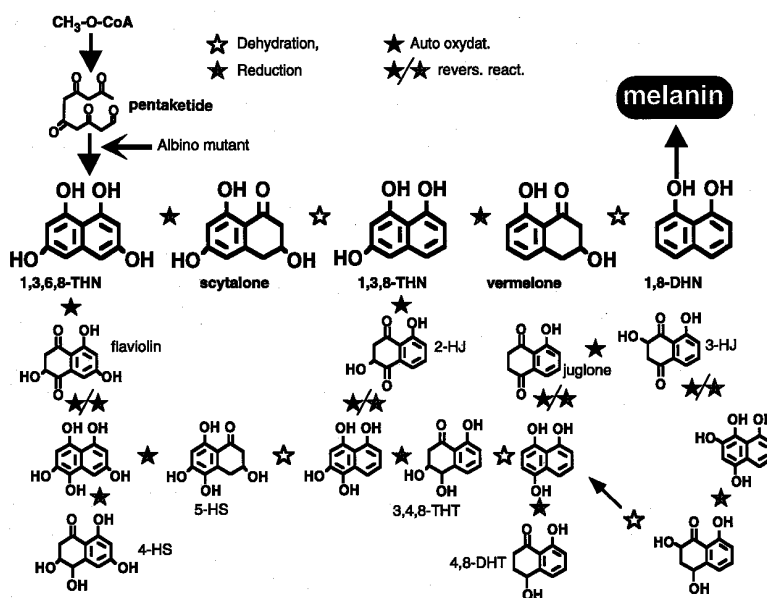


Figure 1. Fungal melanin biosynthesis pathway. Abbreviations for intermediates: 1,3,6,8-THN = 1,3,6,8-tetrahydroxynaphthalene; 1,3,8-THN = 1,3,8-trihydroxynaphthalene; 1,8-DHN = 1,8-dihydroxynaphthalene; 2-HJ = 2-hydroxyjuglone; 3,4,8-THT = *cis*-dihydro-3,4,8-trihydroxy-1-(2*H*)naphthalenone; 4-HS = 4-hydroxyscytalone; 4,8-DHT = 3,4-dihydro-4,8-dihydroxy-1-(2*H*)naphthalenone.

and did not show any pathogenicity (Kurahashi *et al.*, 1997). Therefore, melanin accumulation in the appressorium is essential in their penetration ability into host plants – indispensable in accomplishing the pathogen's life cycle under natural conditions.

The function of DHN melanin in *P. oryzae* has been gradually elucidated in studies over recent decades. Sisler and coworkers proposed that melanin accumulation in the appressorium increase cell wall rigidity and contributes to cell capacity to resist turgor pressure (Woloshuk *et al.*, 1980, 1983).

Moreover, DHN melanin, which accumulates at the inner layer in the appressorial cell wall, changes the porous condition of the wall. As a result, an efflux of any large molecules from the cell is hindered and an osmotic gradient arises due to an increase in intracellular glycerol concentration. Because of the osmotic gradient, influx of water molecules into the cell is accelerated and a turgor pressure (>8.0 MPa) is generated, enabling mechanical piercing of such a hard barrier as the rice epidermis (Howard *et al.*, 1991) enzymes excreted from the germinating spores in the dewdrops may contribute to perforation by softening the plant barrier.

¹ There are various kinds of melanins, classified into eumelanins, phaeomelanins and allomelanins. DOPA melanin is the most common eumelanin, produced in many animals including humans from tyrosine through DOPA (3,4-dimethylphenylalanine). DHN melanin is one of the eumelanins observed in several genera of fungi and produced by polymerisation of 1,8-DHN (1,8-dihydroxynaphthalene).

² motor cells are observed between vascular bundles of the epidermis in rice leaf blades. The rice blast fungus penetrates into the motor cells via the penetration peg at the beginning of infection.

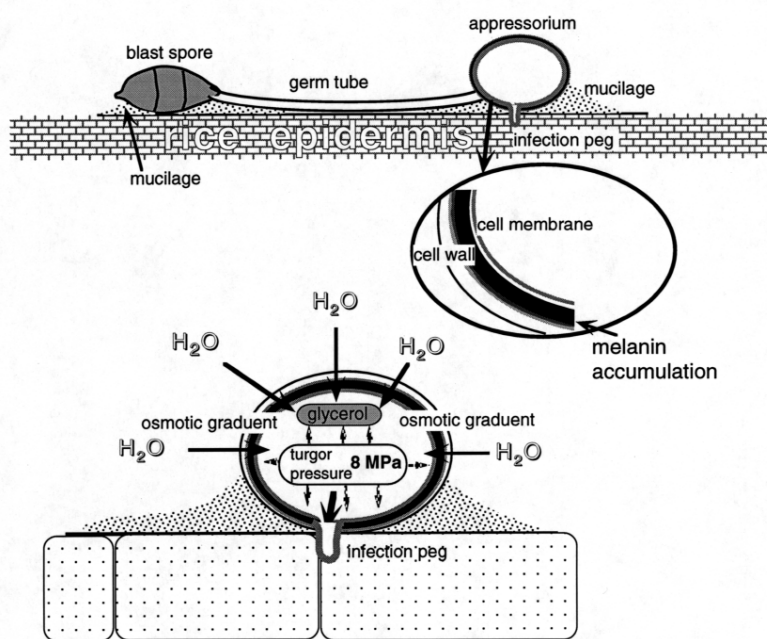


Figure 2. Appressorial penetration mechanism of *Pyricularia oryzae* into rice epidermis.

Germinating spores, germ tubes and appressoria of *P. oryzae* excrete adhesive materials called mucilage, which enable the fungal body to tightly adhere to the rice epidermis within 2 hours after the spores landed on the rice epidermis in the infection drops (Xao *et al.*, 1994). By these ingenious mechanisms the rice blast fungus can physically breach the defence barrier of rice and achieve infection (Figure 2).

Mode of action of MBIs

The action sites of the main inhibitors in the melanin biosynthesis pathway are summarized in Figure 3. Studies involving such melanin biosynthesis inhibitors as TCZ (tricyclazole), PRQ (pyroquilon), CAR (carpropamid) and BFS in *P. oryzae* and *C. legerianum* showed that the main reactions in DHN

Dehydratase inhibitors

MBIs *via* dehydratase inhibition are shown in Table 2. Dehydratase inhibitors were not discovered until a long time after reductatase inhibitors had been used for rice blast control. In the 1990s, a few dehydratase inhibitors were developed; e.g. carpropamid and diclocymet. Fenoxanil, a phenoxycarboxamide derivative is also under development for rice blast control. Recently, another potent inhibitor of scytalone dehydratase, cyclobutane carboxamide, was found through *in-vitro* screening of combinatorial chemistry of existing scytalone dehydratase (SDH) inhibitors (Jennings *et al.*, 1999).

Further action of MBIs

In addition to the penetration inhibition of pathogenic fungi, MBIs seem to affect the rice blast fungus and host plants at a later stage. Reduction in the secondary infection to other rice plants from rice blast lesions treated with MBIs was observed and has been ascribed to the decrease in spore formation and the low virulence of the spores formed on the treated lesions. These factors, however, did not satisfactorily explain the effect. MBIs clearly hindered spore release from blast sporophores. Melanin accumulation in the spore hilum and the pedicel is probably related to spore liberation (Kurahashi *et al.*, 1999b).

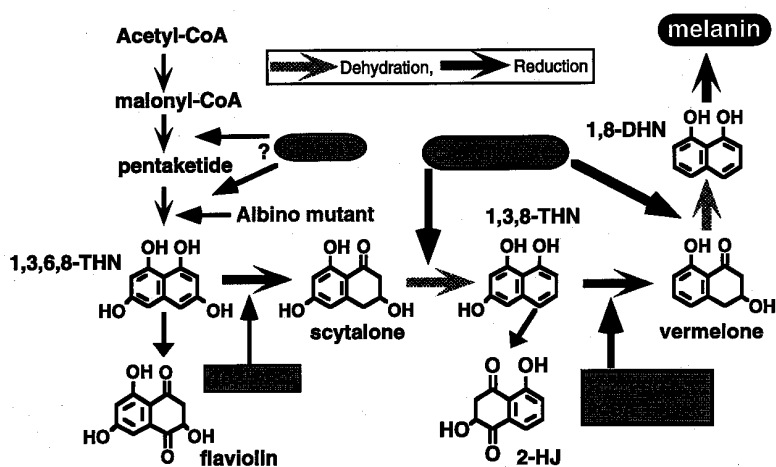


Figure 3. Inhibition sites of MBIs in fungal melanin biosynthesis pathway. Abbreviations for inhibitors: CAR = carpropamid; DCM = diclocymet; AC = fenoxanil; TCZ = tricyclazole; PRQ = pyroquilon; FTL = fthalide; PCBA = pentachlorobenzyl alcohol.

melanin synthesis are enzymatic reduction and dehydration. Reductase and dehydratase are therefore considered to be the target enzymes for developing new melanin biosynthesis inhibitors.

MBIs as rice blast control agents

Reductase inhibitors

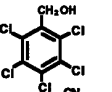

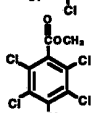
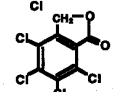
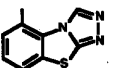
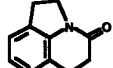
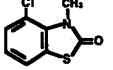
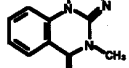
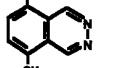
MBIs *via* reductase inhibition are shown in Table 1. All reductase inhibitors have a planar structure of fused bicyclic or tricyclic rings and competitively interfere with the binding of the planar bicyclic substrates. Tricyclazole (TCZ) and pyroquilon (PRQ) were registered in Japan in 1981 and 1985, respectively, as granule formulations by nursery box treatment or by submerged application in irrigated paddy fields. From the successful use of these compounds, various fused bicyclic or tricyclic chemicals, thought to be related to the melanin intermediates and TCZ and PRQ, were studied.

Present situation of MBIs in rice blast control

Various MBIs, e.g. tricyclazole and pyroquilon, have been used in practice for rice blast control for more than 30 rice-growing seasons; FTL, TCZ, PRQ and CAR are the principal MBIs in current use. DCM and AC will be launched in the

RICE BLAST CONTROL

Table 1. Melanin biosynthesis inhibitors: reductase inhibitors.

Chemical group	Common name	Chemical structure	Physicochemical property	Application method	rate(kg/ha)	Registration date in Japan
Poly chlorinated aromatic compounds	PCBA		w.s.=2.0 µg/ml mp=197-198 °C	foliar	0.5-2.0 x3*	1966
	PCMN		w.s.=insoluble mp=163-164 °C	foliar	0.5-1.6 x3*	1966
	CPA		w.s.= µg/ml mp= no data	foliar	0.5-1.2 x3*	1968
	Fthalide** (FTL)		w.s.=2.5 µg/ml log Pow=3.01 mp=209-210 °C	foliar	0.5-1.0 X3*	1970
Fused heterocyclic compounds	Tricyclazole (TCZ)		w.s.=700 µg/ml log Pow=1.40 mp=183-189 °C	foliar systemic	0.2-0.4 x3* 0.8 x1*	1980
	Pyroquilon (PRQ)		w.s.=4000 µg/ml log Pow=1.57 mp=112 °C	systemic	1.5 x2*	1985
	Chlobenthiazone		-	foliar systemic	-	undeveloped
	PP 389		-	foliar systemic	-	undeveloped
	Phthaladine (FTL)		-	foliar systemic	-	undeveloped

Abbreviation used : w.s.=water solubility, mp=melting point, *ordinary application frequency per one growth season, ** fthalide is a bicyclic compound

market in the near future. Some of these MBIs are formulated as special granule formulations for slow release into paddy water. Such slow release of active ingredient is an important property for nursery box treatment to keep the residual effect.

Resistant strains of the blast fungus to MBIs used for more than 30 years have not emerged to date. Since MBIs do not exhibit direct activity on fungal growth there should be a low risk of emergence of tolerant strains. MBIs should continue to occupy an important position in the chemical control of rice blast disease.

References

- Bell, A. A.; Puhalla, J. E.; Tolmsoff, W. J.; Stipanovic, R. D. (1976) Use of mutant to establish (+)-scytalone as an intermediate in melanin biosynthesis by *Verticillium dahliae*. *Canadian Journal of Microbiology*, **22**, 787-799.
- Chida, T.; Uekita, T.; Stake, K.; Hirano, K.; Aoki, K.; Noguchi, T. (1982) Influence of fthalide in penetration process of *Pyricularia oryzae*. *Annals of the Phytopathology Society of Japan*, **48**, 58-63.
- Howard, R. J.; Ferrari, M. A.; Roach, H. D.; Money, J. P. (1991). Penetration of hard substrate by a fungus employing enormous turgor pressure. *Proceedings of the National Academy of Sciences, USA*, **88**, 11281-11284.

IN FUTURE ISSUES

The following are a few of the articles to be published in future issues of *Pesticide Outlook*:

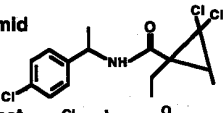
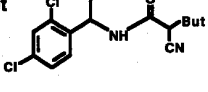
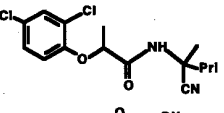
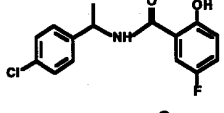
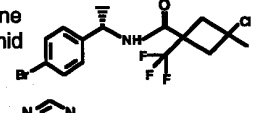
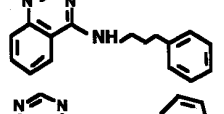
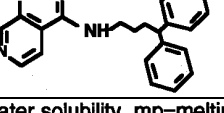
Diagnostics in crop production – by Derek Hollomon and Sheila Kendall (IACR Long Ashton)

Principles and practice in organic crop production – by Tamsin Brown and Rob Haward (Soil Association)

Addressing pest and disease problems for the UK potato industry – by Martin Hims and Steve Hill (Central Science Laboratory)

Rat control in oil palms and ricefields – by Brian Woods (consultant)

Table 2. Melanin biosynthesis inhibitors: dehydratase inhibitors.

Chemical group	Common name	Chemical structure	Physicochemical property	Application method	rate(kg/ha)	Registration date in Japan
Carboxamide derivatives	Carpropamid (CAR)		w.s.=3.6 µg/ml log Pow=4.23 mp=147-149 °C	systemic	0.4 x1*	1997
	Dichlocymet (DCM)		w.s.= 6.4 µg/ml log Pow= mp=154.4-156.6 °C	foliar systemic	0.75-1.2 x3* 0.3 x1*	1999
	Fenoxanil AC 382042 (AC)		w.s.=31 µg/ml log Pow=3.53 mp=69-71.5 °C	foliar systemic	0.2-0.3 x3* 2.7 x2*	under development
	BFS		-	-	-	undeveloped
	cyclobutane carboxamid		-	-	-	found in combinatorial chemistry of existing MBIs
4-aminoquinazolin derivatives			-	-	-	based on the molecular modeling study of scytalone dehydratase
			-	-	-	based on the molecular modeling study of scytalone dehydratase

Abbreviation used : w.s.=water solubility, mp=melting point, *ordinary application frequency per one growth season

- Ishida, M. (1972) Phytotoxic metabolites of pentachlorobenzyl alcohol. *Environmental Toxicology of Pesticide*, ed. F. Matsumura, G. M. Boush and T. Misato, Academic Press., New York 281-299.
- Jennings, L. D.; Wawrzak, D.; Amorose, D.; Schwartz, R. S.; Jordan (1999) A new potent inhibitor of fungal melanin biosynthesis identified through combinatorial chemistry. *Bioorganic and Medicinal Chemistry Letters*, **9**, 2509-2514.
- Kurahashi, Y.; Sakawa, S.; Kinbara, T.; Tanaka, K.; Kagabu, S. (1997) Biological activity of carpropamid (KTU 3616): A new fungicide for rice blast disease. *Journal of Pesticide Science*, **22**, 108-112.
- Kurahashi, Y.; Araki, Y.; Kinbara, T.; Pontzen, R.; Yamaguchi, I. (1998a) Intermediate accumulation and inhibition sites of carpropamid in the melanin biosynthesis pathway of *Pyricularia oryzae*. *Journal of Pesticide Science*, **23**, 22-28.
- Kurahashi, Y.; Sakawa, S.; Sakuma, H.; Tanaka, K.; Hänbler, G.; Yamaguchi, I. (1999b) Effect of carpropamid on secondary infection by rice blast fungus. *Pesticide Science*, **55**, 31-37.
- Tokuda, T.; Nishiki, M.; Hoshi, H.; Shinoda, K.; Ishida, M.; Misato, T. (1976) Metabolic fate of fthalide (4,5,6,7-tetrachlorophthalide) in compost. *Journal of Pesticide Science*, **1**, 283-294.
- Wheeler, M. H. (1983) Comparisons of fungal melanin biosynthesis in ascomycetous, imperfect and basidiomycetous fungi. *Transactions of the British Mycological Society*, **81**, 29-36.
- Woloshuk, C. P., Sisler, H. D., Tokousbalides, M. C.; Dutky, S. R., (1980) Melanin biosynthesis in *Pyricularia oryzae*: Site of tricy-

- clazole inhibition and pathogenicity of melanin-deficient mutants. *Pesticide Biochemistry and Physiology*, **14**, 256-264.
- Woloshuk, C. P.; Sisler, H. D.; Vigil, E. L. (1983) Action of the antipenetrant, tricyclazole, on appressoria of *Pyricularia oryzae*. *Physiology and Plant Pathology*, **22**, 245-259.
- Xao, J. -Z.; Oshima, A.; Kamakura, T.; Ishiyama, T.; Yamaguchi, I. (1994) Glycoprotein(s) associated with cellular differentiation in *Magnaporthe grisea*. *Molecular Plant-Microbe Interaction*, **7**, 639-664.

Yoshio Kurahashi worked for most of his career at the Institute of Nihon Bayer Agrochem (Nihon Tokushu Noyaku Seizou Co. Ltd.) where he was engaged in fungicide research and development. He was the inventor of two fungicides, carpropamid and pencycuron, which were developed and launched on the market. Dr Kurahashi is now studying the biological activity of carpropamid on host plants as a collaborative researcher in Riken (Physical Chemistry Institute). In November 1998 he received the Otto Bayer medal from Bayer AG, and in March 1999 the Award of the Pesticide Science Society of Japan.

PREVIOUS PESTICIDE OUTLOOK ARTICLES ON RICE

- Chemical control of rice diseases in Japan (Inoue) – *Pesticide Outlook* 1990, 1(4), 31
- Rice Gene Project (Sasaki) – *Pesticide Outlook* 1999, 10(3), 114
- The potential for genetically engineered rice – *Pesticide Outlook* 2000, 11(4), 157

RICE ON THE WEB

- <http://www.riceweb.org/>
- <http://www.planetrice.net/>
- <http://www.riceworld.org/>
- <http://www.cigr.org/irri>
- <http://rice-research.org>