EFFECTORS AND EFFECTOR DELIVERY IN *MAGNAPORTHE ORYZAE*

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ABSTRACT

Rice blast, caused by the fungus *Magnaporthe oryzae*, is one of the most destructive rice diseases. All plant parts can be affected including leaves, leaf collars, necks, panicles, pedicels, and seeds. The disease symptoms are caused in part by the effectors produced by *Magnaporthe oryzae*. *Magnaporthe oryzae* apoplastic effectors are secreted from invasive hyphae into the extracellular compartment through the conventional secretory pathway, Golgi complex to the plasma membrane, and are released into the apoplastic space. The biotrophic interfacial complex (BIC) appears to be the site of transfer of some of the cytoplasmic effectors into the host. Experimental results suggest that effector secretion to the BIC is associated with a unique secretion system involving exocyst components and the Sso1 t-SNARE complex. This manuscript reviews the most recent advances in our understanding of the rice-*Magnaporthe oryzae* interactions based on effectors.

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INTRODUCTION

Rice blast is one of the most devastating diseases of rice. This disease is the outcome of a compatible rice-*Magnaporthe oryzae* interaction. In order to understand this disease, it is important to have good knowledge of both the host and the pathogen, and their interactions. In that direction, chapter one mainly covers the description of various rice species, including their distribution, habitat, morphological characters, and specific traits, to provide the readers a broader understanding of the host system. Whereas chapter two mainly focuses on the pathogen system. Initially, it covers the description of rice blast disease, which is followed by a more in-depth discussion of the fungus, *Magnaporthe oryzae.* Chapter three focuses on the effectors, which are the weapons used by the pathogen to overcome the host defenses, and the transport of the effectors from *Magnaporthe oryzae* to the host.

CHAPTER ONE. DESCRIPTION OF THE HOST (RICE)

Importance of rice

Rice, wheat, and corn are the leading food crops in the world in terms of the area under cultivation and production (FAO, 2004). Rice is the staple food for more than half of the world's population and provides 20% of the world's dietary energy supply, whereas wheat supplies 19% and maize only 5% (FAO, 2004). Of the three major crops, rice by far is the most important in regards to human consumption. For example, in Asian countries where more than 90% of the rice is grown and consumed, 59% of the world's population resides (Muthayya et al., 2014). China and India alone accounts for 50% of the rice grown and consumed worldwide. The International Food Policy Research Institute has assessed that the production of rice and has determined that rice production must rise by 38% by 2030 to satisfy the food demands of the world (IFPRI, 2002).

The Food and Agriculture Organization of the United Nations (FAO) estimates that the total commercial harvest of rice in 2015 was 491.4 million tons (FAO, 2015). Within Asia, China and India were the world's largest rice producers. Indonesia, Bangladesh, Vietnam, Thailand, and Philippines are the next largest rice producers (Table 1). Rice is grown mostly in developing countries where it is consumed. The United States is 10th in rice production (Table 1) but exports nearly half of its total rice production. Whereas China exports only 7% of its total rice production (GRiSP, 2013). Within the United States, Arkansas, California, Louisiana, Mississippi, Missouri, and Texas are the six rice-growing states (FAO, 2010).

Human consumption of rice represents 85% of the total worldwide production, in contrast to wheat where 72% is used for human consumption and only 19% of corn (FAO, 2004). In Asia, yearly rice consumption is 200-400 lb (90–181 kg) per person (FAO, 2004). However, in the

Unites States, rice consumption is only 25 lb (11 kg) per person per year, mostly because of the importance of other crops, such as wheat and maize, in the American diet.

Table 1. Top eleven rice producing countries in 2010

Rank Position	Country	Production (in million tons)
1	China	144.5
2	India	103.0
3	Indonesia	37.0
4	Bangladesh	34.6
5	Vietnam	28.1
6	Thailand	19.3
7	Philippines	12.0
8	Brazil	8.4
9	Japan	7.9
10	United States	7.1
11	Pakistan	6.4

Source: International Rice Research Institute (IRRI), 2011

Table 2 gives the nutritional values for rice. Although rice contains less protein than wheat and corn, the protein in rice is more digestible making it a slightly better protein source than wheat or corn. When compared to wheat and corn, rice has a higher content of four essential amino acids, lysine, methionine, threonine, and tryptophan (FAO, 2004).

In addition to human consumption, rice is a popular source of animal feed and is used in the production of alcohol and drinks such as sake (GRiSP, 2013). Additionally, rice is used to make face washes, liquid shower soaps, and hair products. Rice straw, a byproduct of rice production, is used to make caps, shoes, and tatami mats (GRiSP, 2013). In the United States and Thailand, rice milk is produced for lactose-intolerant individuals. The husk is used as boiler fuel, and the rice bran is used in the production of such things as, vegetable oil, feed, and fertilizers (GRiSP, 2013).

Table 2. Composition of the edible portion of milled rice in a 100g sample

Energy	345.0 kcal
Carbohydrates	78.2 g
Proteins	6.8 g
Fat	0.5 g
Fiber	0.2 g
Calcium	10.0 mg
Iron	0.7 mg
Magnesium	90.0 mg
Folic acid	8.0 g
Phosphorus	160.0 mg
Minerals	0.6 g
Moisture	13.7 g
Amino acids	1.09 mg
Riboflavin	0.06 mg
Thiamine	0.06 mg
Niacin	1.9 mg
Copper	0.14 mg

Source: Gopalan et al., 2007

Rice also has an important role in social, religious, and cultural traditions in some parts of the world. In West Africa, for example, rice bread, rice cake, and rice porridge are used for ceremonies such as funerals, weddings, and traditional religious rituals (GRiSP, 2013). Rice is also used as medicine for the treatment of some illnesses in parts of Africa (GRiSP, 2013).

Species of rice

Rice belongs to the family Poaceae, subfamily Ehrharteae, tribe Oryzeae, and the genus *Oryza* (Nayar, 2014) (Table 3). The genus *Oryza* includes 25 wild species and two cultivated species, *Oryza sativa* and *Oryza glaberrima* (Morishima et al., 1984; Vaughan, 1994). Rice species have been identified and re-identified, classified and reclassified multiple times in the last two centuries (Prodoehl, 1922; Roschevicz, 1931; Chevalier, 1932; Chatterjee, 1948; Sampath, 1962; Tateoka, 1963; Change, 1985; Lu, 1999; Sharma, 2003; Vaughan et al., 2003;

Table 3. Classification of rice

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Poaceae
Subfamily	Ehrharteae
Tribe	Oryzeae
Genus	*Oryza*

Clayton et al., 2010). This difficulty in classifying rice has resulted in some confusion about the number of rice species, especially to those who are not in this field. Several species have been transferred to other genera and others have been renamed using names from the previous species. For example, *Oryza barthii* has been named *Oryza longistaminata* by some authors, and *Oryza breviligulata* has also been named *Oryza barthii* by some authors. There have also been disagreements as to whether two variants are separate species, as is the case of *Oryza meyeriana* and *Oryza granulata*. Nayar (2014) created a list of 39 different species from a survey of 10 authors who have classified rice species. From this list, he created a consensual list of 26 species and subspecies. Tateoka grouped the various species into eight complexes. The members of three of these complexes have subsequently been transferred to other genera, and he revised his list in 1962 (Tateoka, 1962). The initial listing consisted of five species complexes, including *Oryza latifolia* complex, *Oryza sativa* complex, *Oryza glaberrima* complex, *Oryza ridleyi* complex, and *Oryza meyeriana* complex. Nayar (2014) recently added a sixth complex of "Outlier group."

For this discussion of rice complexes and species, we will use the list compiled by Nayar (2014), which is based on the groupings of Tateoka (1962). Given below is a short description of the various rice species, including distribution, habitat, morphological characteristics, and specific traits. The *Oryza latifolia* complex consists of six to eight species depending on the

designation of the species (Tateoka, 1962). In this paper, eight species are listed in the complex, although two of the species have also been designated subspecies, which is indicated in Table 4.

Table 4. *Oryza* species, geographical distribution, and their designated complexes

I. *Oryza latifolia* species complex			
Species	Genome	Geographical Distribution	Comments/alternative classification
Oryza latifolia	CCDD	Central & South America	*Oryza latifolia* ssp. *latifolia*
Oryza alta	CCDD	Central & South America	*Oryza latifolia* ssp. *alta*
Oryza grandiglumis	CCDD	South America	
Oryza punctata	BB	Africa	
Oryza minuta	BBCC	Philippines, Papua New Guinea	
Oryza eichingeri	CC	Central and East Africa & Sri Lanka	The only species found in both Africa and Asia
Oryza officinalis	CC	Tropical Asia to Papua New Guinea	*Oryza officinalis* ssp. *officinalis*
Oryza malampuzhaensis	BBCC	Southern India	
II. *Oryza sativa* species complex			
Species	Genome	Geographical Distribution	Comments/alternative classification
Oryza sativa	AA	Worldwide	
Oryza rufipogon	AA	Tropical Asia to Northern Australia	
Oryza nivara	AA	South Asia	
III. *Oryza glaberrima* species complex			
Species	Genome	Geographical Distribution	Comments/alternative classification
Oryza glaberrima	AA	West Africa	
Oryza barthii	AA	Tropical Africa	Syn. *Oryza breviligulata* A. Chev. et Roehr.
Oryza longistaminata	AA	Sub-Saharan Africa	

Table 4. *Oryza* species, geographical distribution, and their designated complexes (continued)

IV. *Oryza ridleyi* species complex			
Species	Genome	Geographical Distribution	Comments/alternative classification
Oryza longiglumis	HHJJ	Asia, New Guinea	
Oryza ridleyi	HHJJ	Southeast Asia	
V. *Oryza meyeriana* species complex			
Species	Genome	Geographical Distribution	Comments/alternative classification
Oryza granulata	GG	South & Southeast Asia	Variety of *O. meyeriana*
Oryza meyeriana	GG	South & Southeast Asia	
VI. The Outlier group			
Species	Genome	Geographical Distribution	Comments/alternative classification
Oryza australiensis	EE	Northern Australia	
Oryza brachyantha	FF	Africa	
Oryza schlechteri	HHKK	Indonesia, Papua New Guinea	
Oryza coarctata	HHKK	Tropical Asia	Syn. *Porteresia coarctata*
Oryza neocaledonica	GG	New Caledonia	
Oryza rhizomatis	CC	Sri Lanka	
Oryza meridionalis	AA	Australia, Indonesia, and Papua New Guinea	

The species in this complex are *Oryza latifolia*, *Oryza alta*, *Oryza grandiglumis*, *Oryza punctata*, *Oryza minuta*, *Oryza eichingeri*, *Oryza officinalis*, and *Oryza malampuzhaensis*.

The American species of the *Oryza latifolia* complex, *Oryza latifolia* Desv., *Oryza alta* Swallen, and *Oryza grandiglumis* (Doell) Prod., are tetraploids (2n=48). *Oryza latifolia* was first described in 1813 (Desvaux, 1813) and is distributed throughout Central and South America. *Oryza latifolia* is found in low, rainy, and secondary growth forests, open woodlands and swamps, savannas and pastures, cultivated fields, hill slopes, and coastal belts. It grows in or near water, in places such as streams, riverbanks, and the edges of pools, in moist clays, and

sandy seashores. This perennial usually grows to 1-2 m tall with 5 cm broad leaves and spikelets that are 5 to 9.5 mm long and 2 to 2.7 mm wide. *Oryza latifolia* is resistant to brown planthopper and lodging, and produces a large biomass.

Oryza alta, first described by J. R. Swallen in 1936, is found in Central and South America, and has been identified in Belize, Brazil, Colombia, Guyana, and Paraguay. It grows in sunny locations, such as savanna, and sometimes in woodlands. It also may form floating mats if there is sufficient water. It grows up to 4 m tall with broad leaves of about 5 cm wide. The spikelets are 7 to 8 mm long and 2.4 to 3 mm wide. *Oryza alta* is resistant to stem borers, has the potential to provide genes for increased yield, and can be used to contribute to biomass production.

Oryza grandiglumis was described by Prodoehl in 1922, and is a native of South America. It is found in several countries including Bolivia, Brazil, Colombia, Ecuador, French Guiana, Paraguay, and Peru. It grows in wet clay and alluvial soils, and in the water at the edges of rivers. *Oryza grandiglumis* grows up to 4 m tall with broad leaves (3-5 cm), with spikelets 8.2 to 9.3 mm long and 2.3 to 4 mm wide. It produces three spikelets of which the lower two are sterile. It also can contribute to the high production of biomass.

Oryza punctata Kotschy ex Steud. was described in 1854 (Steudel, 1854). *Oryza punctata* is morphologically similar to *Oryza sativa* and *Oryza officinalis. Oryza punctata* is widely distributed in tropical Africa, from Cote d'Ivorie to South Sudan, Madagascar and Swaziland. The natural habitat of *Oryza punctata* is open grasslands, wetlands, water holes, streams, and open water. It grows up to 1 m tall, has a basal panicle with widely spreading branches and spikelets that are more than 5.5 mm long and 2.3 mm wide. Its leaves are 15-45 cm long and 0.5-2.5 cm wide. *Oryza punctata* is resistant to bacterial leaf blight (*Xanthomonas*

oryzae pv. o*ryzae*) and rice pests such as the brown planthopper (*Nilaparvata lugens*). This rice has been consumed in the Sudan during times of famine, however the United States Department of Agriculture (USDA) has classified it as a noxious weed in the United States.

Oryza minuta J. S. Presl ex C. B. Presl (Presl, 1830), is distributed throughout the Philippines and Papua New Guinea and is a perennial. It is a tetraploid with the BBCC genome (2n=48). The natural habitat of *Oryza minuta* is near lowland streams and riverbanks, in fertile clay or loamy soils. *Oryza minuta* grows up to 1.5 m tall with spikelets of 4 to 6 mm long and 1 to 2 mm wide. This species also has two basal florets that are sterile and one that is fertile. The glumes are either absent or obscured. The leaves of this plant are 14-28 cm long and 0.6-1.3 cm wide.

Oryza eichingeri A. Peter (Peter, 1930), is distributed in tropical Africa and Sri Lanka. It grows in forest margins, and evergreen and undisturbed forest. *Oryza eichingeri* is also capable of growing in pools of water, marshy places, the banks and beds of streams and rivers, ditches, and sandy or gray loamy clay soils. It usually grows more than 1 m tall with hard and slender culms with spikelets of 4.5 to 6.2 mm long and 1.6 to 2.8 mm wide. It is resistant to *Yellow mottle virus,* and pests such as the brown planthopper and white-backed planthoppers.

Oryza officinalis Wall. ex Watt was first described in 1828 by Wallich and revised by Watt (1891). It grows in forests or forest edges, swampy areas and wetlands, including the banks of streams, and water bodies throughout Southeast Asia to Papua New Guinea. It is considered an endangered species since it is found in only very few areas of the world and only in small populations. *Oryza officinalis* grows to 1.5 m, has basal panicles that are whorled, and spikelets that are 4 to 9 mm long and 2 to 3 mm wide. Tateoka (1963) pointed out that *Oryza officinalis* is morphologically similar to *Oryza minuta, Oryza punctata,* and *Oryza eichingeri.*

Oryza malampuzhaensis Krish. et Chand. (Krishnaswamy and Chandrasekharan, 1958) is a tetraploid species which is found in Southern India. It is a perennial grass that grows on river and stream margins, in shaded and partially shaded areas and on marshy land. *Oryza malampuzhaensis* has been considered a subspecies of *Oryza officinalis* (Tateoka, 1963), a tetraploid race of *Oryza officinalis* (Vaughan, 1994), and as a separate species (Krishnaswamy and Chandrasekharan, 1958). Thomas et al. (2001) investigated 23 physical characteristics and used random amplified polymorphic DNA (RAPD) markers to investigate the relationship between the two species. Based on cluster and principal component analysis of physical and genetic traits they concluded that it represents its own species. The spikelets of this species are 5.4 mm long and 2.3 mm wide, and the flag leaf is 1.4 cm wide.

The *Oryza sativa* complex includes *Oryza sativa, Oryza rufipogon,* and *Oryza nivara* (Nayar, 2014). All are diploids possessing the AA genome and form the primary gene pool for rice improvement. *Oryza sativa* L., described by Linnaeus in 1753, is the most widely grown cultivated species. It is grown worldwide throughout Asia, North and South America, Europe, the Middle East, and Africa. *Oryza sativa* is classified into two major ecological groups (rice races), japonica and indica. Recent genetic evidence demonstrated that both japonica and indica originate from the Pearl River Valley region of China (Wei et al., 2012). Japonica varieties are normally grown in dry fields, in temperate East Asia, upland areas of Southeast Asia, and high elevations in South Asia. Japonica plants are short, and the leaves look narrow in shape, are dark green in color, and have medium-height tillers (Wei et al., 2012). Grains of japonica varieties have low amylose content, making them moist and sticky when cooked. Indica, primarily a lowland rice, is normally grown submerged. Indica is distributed in Sri Lanka, Indonesia, India, Pakistan, Java, Central and Southern China, Philippines, and in some African countries.

Indica plants grow 1-2 m tall, with broad to narrow leaves that look light green in color, and the grain has high amylose content, making them non-sticky when cooked (Wei et al., 2012).

Oryza rufipogon Griff. (Griffith, 1851) is considered to be one of the progenitors to the domesticated rice, *Oryza sativa*. It looks very similar to *Oryza sativa* and so cannot be distinguished in the field. Due to this, and that it has a lower yield, this rice species has been classified as a noxious weed by the USDA in the rice-growing states. *Oryza rufipogon* is believed to have originated in Vietnam but was first domesticated in China. It is a source for aluminum tolerance and has resistance to bacterial blight and rice tungro disease. It has also been used as a source for cytoplasmic male sterility. It is a perennial with a variable plant height from 1-5 m and has open panicles. Its leaves are 20-40 cm long and 0.5-1 cm wide and its spikelets are 7.7 to 12.3 mm long and 2.3 to 3.5 mm wide. It is found in India, China, throughout Southeast Asia, Indonesia, and in Northern Australia.

Oryza nivara Sharma et Shastry (Sharma and Shastry, 1965) is an annual diploid wild rice from India and is found in Southeast Asia. It grows in and around rice fields, ditches, swampy areas, and near stream and ponds. *Oryza nivara*, whose status as a species has been drawn into question (Tateoka, 1963; Oka, 1988; Vaughan et al., 2003), is sometimes called *Oryza sativa* f. *spontanea* or *Oryza rufipogon* sensu stricto (Tateoka, 1963). *Oryza nivara* has spikelets that are 6-8 mm long and 2.3-3 mm wide. *Oryza nivara* is a source of resistance to the brown planthopper (Madurangi et al., 2011) and drought resistance (Thanh et al., 2006).

Oryza glaberrima complex has three species including, *Oryza glaberrima* Steud., *Oryza barthii* A. Chev., and *Oryza longistaminata* Chev. et Roehr. *Oryza glaberrima* (African rice) is one of two cultivated rice species and was first described by Steudel in 1854. *Oryza glaberrima* was domesticated nearly 3000 years ago along the Niger River Delta. It is derived from *Oryza*

barthii. There are two ecotypes, one that grows in deep water and one that will grow in lowlands. This species has small, red, pear-shaped grains with black to olive seed coats. The grains are difficult to mill and shatter easily. It is resistant to water fluctuations, will grow in infertile soils, and tolerates neglect well. Its main drawback, compared to *Oryza sativa*, is that the yield is much lower. *Oryza glaberrima* has resistance to *Rice yellow mottle virus* (Thiémélé et al., 2010), rice blast (Silue and Notteghem, 1991), stem borers (Sauphanor, 1985), and many other pests and diseases.

Oryza barthii (Chevalier, 1910) is found in tropical Africa. Natural habitats of *Oryza barthii* are mopane woodlands, savanna, and savanna woodlands. It grows well in clay or black cotton soils, and usually on seasonally flooded lands. It will grow in stagnant or slowly flowing water, or even deep water. It grows up to 1.5 m tall in tufts, has panicles that rarely have secondary branching, with spikelets 7.7 to 12.3 mm long and 2.3 to 3.5 mm wide. *Oryza barthii* is resistant to bacterial leaf blight (Vikal et al., 2007) and sheath blight (Prasad and Eizenga, 2008).

Oryza longistaminata Chev. et Roehr. is found throughout tropical Africa and South Africa where it forms dense stands that are used for cattle grazing. This perennial grass grows up to 2.5 m tall and has long branching rhizomes. The leaves are dark green, 10-45 cm long and 0.5 to 1.5 cm wide. Its spiklets are 4.5 to 11 mm long, 2-3 mm wide, is found in shallow or deep water in swamps and river banks, and up to an altitude of 1800 m. It is unique from other wild rice species by having long pointed ligules. This species produces few seeds since it is partly self-incompatible and reproduces mainly through rhizomes. It can produce hybrids with cultivated rice and suppresses cultivate rice growth. It is a source of resistance to bacterial leaf blight.

The *Ridleyi* complex has two tetraploid species, *Oryza ridleyi* Hook. and *Oryza longiglumis* Janson. *Oryza ridleyi*, described by Hooker in 1897 (Nayar, 2014), is a perennial, tufted grass that grows in marshes and along stream banks in forests and open spaces in Southeast Asia and New Guinea. *Oryza ridleyi* is 1-2 m tall with smooth leaves, 15-30 cm long and 1.5-2.5 cm wide. The panicles are open with spikelets 7-13 mm long and 2-3 mm wide. The spikelets have one fertile floret and two basal sterile florets. *Oryza ridleyi* has resistance to bacterial blight, rice blast, whorl maggot, and stem borers. The literature about *Oryza longiglumis* is very limited. *Oryza longiglumis* was discovered by Janson in 1953. This species is found in shaded forests near rivers and swamps in Asia and New Guinea. It is a perennial that grows 1-2 m tall, has open panicles with spikelets 7-8 mm long and approximately 2 mm wide. It is a source of rice blast and bacterial blight resistance.

The *Meyeriana* complex has two diploid species, *Oryza meyeriana* and *Oryza granulata* Nees et Arn. ex Watt. *Oryza meyeriana* (Zoll. et Mor. ex Steud) Baill. was described in 1894 (Baillon, 1894) and is distributed throughout Southern and Southeastern Asia. It is found in primary lowland tropical and well-developed secondary forests, in shaded sandy soil by streams or dry riverbanks, but not in standing water. It is a perennial, and a short grass with lanceolate and dark green leaves, has compact panicles without secondary branching, and spikelets of 6.5 to 8.5 mm long and 2 to 2.5 mm wide.

Oryza granulata Nees et Arn. ex Watt (Watt, 1891) is usually found in forests and bamboo thickets on limestone hills, and in mountainous areas near streams and waterfalls. It grows up to 0.8 m in sandy and organic loamy soils in the shade and is not found in standing water. *Oryza granulata* is a perennial grass, with compact panicles, and spikelets which are 5.2 to 6.4 mm long and 2.4 to 2.7 mm wide. It is found in Southeast Asia and Southern Asia

including China, India, Sri Lanka, and Indonesia. *Oryza granulata* is difficult to cross with cultivated rice, but is considered an important germplasm source since it is immune to bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*, is tolerant to drought, and is resistant to brown planthopper.

Taxa that do not fall clearly within any of the above five species complexes are grouped into the Outlier group. The Outlier group proposed by Nayar (2014), consists of *Oryza meridionalis, Oryza brachyantha, Oryza australiensis, Oryza schlechteri, Oryza coarctata, Oryza neocaledonica,* and *Oryza rhizomatis.*

Oryza meridionalis Ng (Ng et al., 1981), is found in Northern Australia, Indonesia, and Papua New Guinea. Its common name is Australian rice and it is found in freshwater, 10-20 cm deep, near freshwater lagoons and seasonal swamps. Interestingly, it is considered both an annual and perennial. *Oryza meridionalis* grows in black, clay soils to a height of 1-2 m, has compact panicles 11-24 cm long, with slender spikelets 6-9 mm long. This rice has two traits that are of interest to breeders: drought resistance and elongation ability.

Chevalier and Roehrich described *Oryza brachyantha* in 1914. It is a tufted annual grass, possibly weakly perennial, and is distributed in the tropical regions of Africa in open wetlands. It grows up to 0.3-0.8 m in height and has compact panicles 4-5 cm long. Spikelets are 7.7 to 10 mm long and 1.4 to 1.8 mm wide. *Oryza brachyantha* is a diploid and the only species containing the F genome. It is a source of resistance to bacterial blight (Djedatin et al., 2011), leaf folder, *Cnaphalocrocis medinalis* (Guenée) (Ramachandran and Khan, 1991), and the yellow stem borer (Panigrahi and Rajamani, 2008).

Oryza australiensis was described by Domin in 1915. It is distributed in Northern Australia. It grows to a height of 1-2 m, with open panicles, and has spikelets that are pear

shaped 6-9 mm long and 2-3 mm wide. This species is a diploid of the E genome and is considered a source of resistance to brown planthopper and may provide drought resistance.

Oryza schlechteri was first described by Pilger in 1914. *Oryza schlechteri* has been found only in Papua New Guinea. It is usually found in shade or partial sun in forests or beside rivers. After its initial collection, the living material was not available again until 1990 (Vaughan and Sitch, 1991). It is described as both a perennial and an annual, depending on the source; it grows up to 0.3-0.9 m with a panicle 3-6 cm long and spikelets up to 2 mm long. This organism has moved between the genera *Oryza* and *Leersia* over the years, but recent scanning electron microscopy has confirmed it to be an *Oryza* species (Naredo et al., 1993). At this time, there are no known resistances provided by this species.

Oryza coarctata was described by Roxburgh in 1832 (Roxburgh and Carey, 1832). It is distributed in tropical Asia, the Indian subcontinent, and is found in river estuaries. Roschevicz (1931) noted certain unique features that differed from other *Oryza* species, including hard leathery leaves and non-flattened spikelets. Later Tateoka (1964; 1965) moved it to its own genus, *Porteresia*, since it had an unusually large embryo with features distinct from other rice species. Recently, based on genetic studies, Lu and Ge (2003) returned it to the genus *Oryza*.

Oryza neocaledonica is one of the newest *Oryza* species identified and was described by Morat et al. in 1994. It has been found in only four locations on New Caledonia Island in the understory of tropical dry forests in temporarily flooded black clay soils. It appears to be a diminutive version of *Oryza granulata* (Vaughan et al., 2003). Due to the clearing of these forests, *Oryza neocaledonica* has become an endangered species. It is a perennial and short grass, which grows up to 0.6-0.8 m with spikelets 6 to 7 mm long and 1 to 1.5 mm wide.

Oryza rhizomatis was described by Vaughan in 1990. This perennial is only found in the seasonally dry/wet portions of the tropical forests in Sri Lanka. *Oryza rhizomatis* grows in partial shade or full sun to a height of 1-3 m tall with open panicles and spikelets around 7 mm long and 2 mm wide. It is considered to be a source of drought resistance since it grows in drier areas than most rice species.

Rice genome

The genome of rice is small (approximately 430 Mbp) when compared with other grain crops, such as sorghum (approximately 1000 Mbp), maize (2400 Mbp), barley (4900 Mbp), and wheat (16000 Mbp) (Bennetzen, 2002; Sasaki and Sedoroff, 2003). Rice consists of diploid and tetraploid species.

There are six different diploid genome sets, AA, BB, CC, EE, FF, and GG, and four tetraploid combinations, BBCC, CCDD, HHJJ, and HHKK (Vaughan, 1989). Draft sequences of *Oryza sativa* ssp. *indica* was published for the first time in 2002 and was obtained by a whole-genome shotgun sequencing approach at relatively low coverage (Yu et al., 2002). The International Rice Genome Sequencing Project (IRGSP) completed the map-based sequence of the rice genome using the cultivar Nipponbare of *Oryza sativa* ssp. *japonica* in 2005 (IRGSP, 2005) (Table 5).

The draft genome sequences of *Oryza glaberrima* (AA), *Oryza barthii* (AA), *Oryza longistaminata* (AA), *Oryza punctata* (BB), and *Oryza brachyantha* (FF), were completed within the past few years. For both *Oryza nivara* (AA) and *Oryza glumaepatula* (AA), assembly is currently in progress (Table 5).

Table 5. Status of rice species genome sequencing projects

Species	Genome	Genome size (Approx.)	Sequencing method	Sequencing Status
Oryza sativa ssp. *indica*	AA	400 Mb	WGSGS[1]	2002 (Draft)
Oryza sativa ssp. *japonica*	AA	400 Mb	Clone-by-clone/ PMI[2]	2004
Oryza glaberrima	AA	354 Mb	BAC pool	2010
Oryza barthii	AA	411 Mb	WGSGS / PMI	2012
Oryza brachyantha	FF	260 Mb	WGSGS/ PMI	2011
Oryza longistaminata	AA	352 Mb	WGSGS	2011 (Draft)
Oryza nivara	AA	448 Mb	BAC pool / PMI / WGSGS	Assembly in progress
Oryza rufipogon	AA	445 Mb	WGSGS	2013 (Draft)
Oryza glumaepatula	AA	464 Mb	WGSGS/ PMI	Assembly in progress
Oryza punctata	BB	423 Mb	BAC pool / PMI / WGSGS	2012
Oryza meridionalis	AA	435 Mb	WGSGS/ PMI	2013 (Draft)
Oryza australiensis	EE	960 Mb	WGSGS/ PMI	In progress
Oryza officinalis	CC	653 Mb	WGSGS/ PMI	In progress
Oryza eichingeri	CC	650 Mb	WGSGS	In progress
Oryza rhizomatis	CC	650 Mb	WGSGS	In progress
Oryza granulata	GG	862 Mb	WGSGS/ PMI	In progress

[1]Whole Genome Shot Gun Sequencing
[2]Physical Mapping Integration

Moreover, sequencing is in progress for *Oryza granulata* (GG), *Oryza rhizomatis* (CC), *Oryza eichingeri* (CC), *Oryza officinalis* (CC), and *Oryza australiensis* (EE) (Jacquemin et al., 2013). Rice shares collinearity with other major cereal crops including maize, sorghum, and wheat (Moore et al., 1995). Moreover, rice has a well-established transformation system. These elements have formed rice into a model system to study host-pathogen interactions and evolutionary relationships.

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CHAPTER TWO. DESCRIPTION OF RICE DISEASES

Introduction to rice diseases

Rice diseases are one of the limiting factors of rice production. Although bacteria, viruses, fungi, and parasitic nematodes can cause diseases in rice, the majority are caused by fungi. Table 6 lists many of the disease found in rice. Among the diseases listed, rice blast, bacterial blight, sheath blight, and *Rice yellow mottle virus* cause the greatest yield losses worldwide. The most important of these diseases is rice blast, which is the topic of this document. Given below is a short description of the other diseases which is followed by a more in-depth discussion of rice blast.

Bacterial blight is caused by *Xanthomonas oryzae* pv. *oryzae* (Mew et al., 1993). Bacterial blight is usually found in irrigated fields and rainy lowland areas of tropical and temperate environments. It is most prevalent in Asia during the monsoon season. Symptoms of this disease are wilt in seedlings, and drying and discoloration (yellowing) of leaves (Mew et al., 1993). Successful infections occur under favorable conditions, a relative humidity above 70% and temperatures between 25 and 34^{o}C (Mew et al., 1993). Typically, this disease results in 10-20% yield reduction but can be as high as 50% and, if it occurs during early tillering, may lead to complete yield loss. If roots are damaged during transplanting, and the pathogen is present, a form of the disease called kresek can occur and will result in complete loss of the crop (Mew et al., 1993). Growing resistant varieties is the most efficient and least expensive method to control this disease. Over 38 different resistance genes have been identified in rice for this pathogen (Cheema et al., 2008), which has over 30 different races worldwide (Lore et al., 2011).

Rice yellow mottle virus is caused by a *Sobemovirus*, a single-stranded RNA virus, with five open reading frames (Truve and Fargette, 2012). It is transmitted by over 30 different insect

Table 6. Common name, causal agent, and distribution of rice diseases

Bacterial diseases		
Name of the disease	Causal agent	Distribution
Bacterial blight	*Xanthomonas oryzae* pv. *oryzae*	Latin America, West Africa, Asia, Australia
Bacterial leaf streak	*Xanthomonas oryzae* pv. *oryzicola*	Southern Asia, Central Africa
Foot rot	*Erwinia chrysanthemi*	East Asia
Grain rot	*Pseudomonas glumae*	Asia
Sheath brown rot	*Pseudomonas fuscovaginae*	Asia, South America, Africa
Fungal diseases		
Name of the disease	Causal agent	Distribution
Blast	*Magnaporthe oryzae*	Worldwide
Sheath blight	*Rhizoctonia solani*	Worldwide
Sheath rot	*Sarocladium oryzae*	Worldwide
Sheath spot	*Rhizoctonia oryzae*	Worldwide
Crown sheath rot	*Gaeumannomyces graminis*	America
Alternaria leaf spot	*Alternaria padwickii*	South Asia
Stem rot	*Magnaporthe salvinii*	Central America
Narrow brown leaf spot	*Cercospora janseana*	Asia, Africa, Australia, America
Leaf scald	*Microdochium oryzae*	Asia, Africa, Central and South America
Leaf smut	*Entyloma oryzae*	Asia, America, Africa
Kernel smut	*Tilletia barclayana*	America
False smut	*Ustilaginoidea virens*	Worldwide
Eyespot	*Drechslera gigantea*	South America
Downy mildew	*Sclerophthora macrospora*	America
Brown spot	*Helminthosporium oryzae*	South and Southeast Asia
Aggregate sheath spot	*Ceratobasidium oryzae-sativae*	Worldwide
Black kernel	*Curvularia lunata*	Worldwide
Root rots	*Fusarium* spp., *Pythium* spp.	Worldwide
Seedling blight	*Cochliobolus miyabeanus*, *Curvularia* spp., *Fusarium* spp., *Sclerotium rolfsii*	Worldwide

Source: Hollier et al., 1993

Table 6. Common name, causal agent, and distribution of rice diseases (continued)

Virus diseases		
Name of the disease	Causal agent	Distribution
Giallume	*Barley yellow dwarf virus*	Worldwide
Rice black streak dwarf	*Rice black streak dwarf virus* (RGSDV)	East Asia
Rice dwarf	*Rice dwarf virus* (RDV)	East Asia
Rice grassy stunt	*Rice grassy stunt virus* (RGSV)	South, Southeast, and East Asia
Rice hoja blanca	*Rice hoja blanca virus* (RHBV)	South and Central American
Rice necrotic mosaic	*Rice necrotic mosaic virus* (RNMV)	East Asia
Rice rugged stunt	*Rice rugged stunt virus* (RRSV)	Asia
Rice stripe	*Rice stripe virus* (RStV)	East Asia
Rice transitory yellowing	*Rice transitory yellowing virus* (RTYV)	Asia
Rice yellow mottle	*Rice yellow mottle virus* (RYMV)	Africa
Tungro (rice tungro disease) (RTD)	*Rice tungro spherical virus* (RTSV), *Rice tungro bacilliform virus* (RTBV)	South and Southeast Asia

Source: Hollier et al., 1993

vectors, beetles appearing to be the most important (Koudamiloro et al., 2015). Over 54 different sequences have been reported for this virus indicating a large diversity (Sire et al., 2008). This disease is most often found in irrigated rice in Africa (Ventelon-Debout et al., 2008). Symptoms of this disease are discoloration (browning) of leaves, stunting, and spikelet sterility (Ventelon-Debout et al., 2008). Two recessive resistance genes, *rymv1* (Albar et al., 2006) and *rymv2* (Thiémélé et al., 2010), have been identified in rice. The protein encoded by *rymv1* is a translational initiator eIF(iso)4G1 (Poulicard et al., 2010; Traoré et al., 2010) and has four alleles (Thiémélé et al., 2010). The gene *rymv2* has been proposed to be the rice analogue to the *Arabidopsis thaliana* constitutive expresser of pathogenesis-related genes-5 (*CPR5*) (Orjuela et al., 2013). As with most diseases, growing resistant varieties of *Oryza sativa* and *Oryza glaberrima* is the most efficient method to control this disease (Ventelon-Debout et al., 2008).

Sheath blight is caused by the fungus *Rhizoctonia solani* (Lee and Rush, 1983). The pathogen overwinters in the soil as sclerotia which are the primary source of infection. The sclerotia become buoyant as they mature, and will accumulate around rice plants in flooded paddies, where the initial infection takes place (Lee and Rush, 1983). The first symptoms are elliptical, green, water-soaked spots that enlarge, and the centers of the lesions bleach to a gray color with brown boarders as they dry (Lee and Rush, 1983). Environmental conditions that favor disease progression include warm temperatures of 28 to 32°C with high humidity. Also, excess nitrogen increases the number of tillers and canopy density, which subsequently increases the humidity near the stems (Lee and Rush, 1983), and makes the plants more susceptible. Currently, cultural practices such as wide plant spacing and fungicides, are being used to control this disease (Lee and Rush, 1983).

Significance of rice blast

Rice blast is undoubtedly one of the most destructive rice diseases throughout the world. The first report of rice blast was in China in 1637. Over the course of the next 300 years it moved from country to country, appearing in Japan in 1704, Italy in 1828, the United States in 1876, and lastly in India in 1919 (Ou et al., 1971). Rice blast has been identified in all rice growing regions, including over 85 countries (Greer and Webster, 2001). Rice blast can produce yield losses of up to 100% under favorable conditions (IRRI, 2010). There have been many reports of rice blast causing yield losses ranging from 75% in India (Padmanabhan, 1965), 40 % in Nigeria (Awodera and Esuruoso, 1975), and 50% in the Philippines (Ou, 1985). Annually, rice blast infections result in yield losses estimated at 30%, which is enough to fulfill the annual food requirement of 60 million people (Pennisi, 2010). Baker et al. (1997) estimated that during the period from 1975 to 1990, 1.6 billion dollars were lost because of rice blast. Extensive research

funds and time have focused on understanding this pathogen and breeding resistance to this disease.

The pathogen

Magnaporthe oryzae is a hemi-biotrophic filamentous ascomycete fungus. Originally, Rossman et al. (1990) considered *Magnaporthe oryzae* and *Magnaporthe grisea* as one ascomycete species based on the phenotypic similarities. However, Couch and Kohn (2002) separated *Magnaporthe oryzae* from *Magnaporthe grisea* based on the study of mating, host specificity, and genetic analysis. *Magnaporthe* isolates can be separated into two distinct clades. The first clade is capable of infecting crabgrass (*Digitaria* species), which are associated with *Magnaporthe grisea*. The second clade is capable of infecting rice, wheat, finger millets, and other grasses, which are associated with *Magnaporthe oryzae* (Couch and Kohn, 2002). *Magnaporthe oryzae* is a heterothallic, haploid fungus with two mating type loci, *MAT1-1* and *MAT1-2* (Kang et al., 1994). During sexual reproduction, two opposite mating types pair together and form fruiting bodies (perithecia) that are dark pigmented, flask-shaped globular structures, which contain asci. Each ascus carries eight ascospores (Hebert, 1971). *Pyricularia oryzae* is the asexual stage (anamorph) of *Magnaporthe oryzae* and is the most common form found in the United States.

Life cycle of *Magnaporthe oryzae*

The asexual life cycle of *Magnaporthe oryzae* begins with the attachment of a three-celled conidium on the surface of the rice tissue via the spore tip mucilage, which requires standing water for its function (Hamer et al., 1988; Howard and Valent, 1996; Talbot, 2003; Wilson and Talbot, 2009). Spore adhesion is a passive process and does not require active fungal metabolisms. By producing a thin viscous pad, spore tip mucilage helps the conidium to attach

tightly to the wax-covered hydrophobic rice surface. Once the spore is attached to the surface, the fungus forms a single polarized germ tube from the conidium within 2 hours (Talbot, 2003; Ribot et al., 2008). During the germ tube development, an extracellular matrix (ECM) is produced by the germ tube. ECM is made of a variety of proteins and carbohydrates, such as hydrophobin, collagen, vitronectin, fibronectin, laminin, and integrin (Inoue et al., 2007). ECM aids the adhesion ability of *Magnaporthe oryzae* on the plant surface (Inoue et al., 2007; Ikeda et al., 2012). Once the germ tube extends to about 15–30 μm, the tip of the germ tube forms a terminal hook which initiates the formation of dome-shaped appressoria. Contact with a hard, wax-covered, hydrophobic rice surface triggers appressoria formation (Howard and Valent, 1996; Talbot, 2003; Galhano and Talbot, 2011). Appressoria formation can also be triggered by nutrient starvation and chemical stimuli, such as plant cutin monomers (cis-9, 10-epoxy-18-hydroxyoctadecanoic acid), or lipid monomers (1, 16-hexadecanediol) (Howard and Valent, 1996; Talbot, 2003; Galhano and Talbot, 2011).

Cell cycle control is essential for appressoria development (Hamer et al., 1988; Wilson and Talbot, 2009). The three-celled conidium contains a single nucleus in each section of the cells. A single round of nuclear division happens in the cell from which the germ tube emerges (Hamer et al., 1988; Wilson and Talbot, 2009). After mitosis, a daughter nucleus enters into the developing appressorium, while the other goes back to the conidium. Autophagy, which is the main method crucial for the degradation of organelles and cytosolic macromolecules in the vacuole (Yoshimoto et al., 2010), becomes activated within the conidium. The three nuclei in the conidium are then degraded together with the rest of the spore contents, leaving a single nucleus in the mature appressorium (Hamer et al., 1988; Wilson and Talbot, 2009).

Once the appressorium has matured, the cell wall becomes chitin-rich, with the inner side composed of a darkly pigmented melanin layer (Howard and Valent, 1996; Talbot, 2003). Melanin is classified into four groups based on biosynthesis; 3, 4-dihydroxyphenylalanine (DOPA) melanins, γ-glutaminyl-3-4-dihydroxybenzene (GDHB) melanins, catechol melanins, and 1, 8-dihydroxynaphthalene (DHN) melanins (Bell and Wheeler, 1986; Butler and Day, 1998; Henson et al., 1999). In *Magnaporthe oryzae*, melanin is a DHN type, and functions to maintain a high internal solute concentration by lowering the permeability of the appressorium wall. It also supports a high hydrostatic pressure, as high as 8.0 Mpa (80 bars = 1000 psi), which is comparable to 40 times the pressure in a car tire (Howard and Ferrari, 1989; Money and Howard, 1996; Talbot, 2003). Glycerol is the major solute that is used to generate the turgor pressure to break through the rice cuticle (Howard and Valent, 1996; Talbot, 2003).

Genes and the signaling pathways involved in appressoria development

Numerous genes play an important part in the control of appressoria formation and development. The *MPG1* gene, coding for a hydrophobin, is expressed during spore adhesion, appressorium formation, and colonization (Talbot et al., 1993; Ebbole, 2007; Wilson and Talbot, 2009). It has been shown that isolates with *mpg1* mutations have a reduction in disease symptoms due to the reduced number of appressoria produced. Moreover, *mpg1* mutants also produce fewer conidia (Talbot et al., 1993; Ebbole, 2007; Wilson and Talbot, 2009).

The *PTH11* gene, coding for a transmembrane G-protein coupled receptor, is important for appressorium differentiation in response to surfaces signals (DeZwaan et al., 1999; Wilson and Talbot, 2009). On highly inductive surfaces, it activates appressorium formation, whereas on poorly inductive surfaces it suppresses morphological differentiation (DeZwaan et al., 1999;

Wilson and Talbot, 2009). It has been shown that *pth11* mutants fail to make appressoria efficiently on hydrophobic surfaces (DeZwaan et al., 1999; Wilson and Talbot, 2009).

Plant stimuli are transformed into morphological differentiation of the fungus via classical signal transduction pathways (Xu, 2000). Development of an appressorium is an active process that involves many signal transduction pathways. The mitogen-activated protein kinase (MAPK) pathway, involving PMK1 (Zhao et al., 2007), and the cyclic AMP-dependent pathway (cAMP) are the two independent signal transduction pathways that regulate the formation and development of appressoria.

The *MGB1* gene, coding the β-subunit of a G-protein, is one of the first upstream components of MAPK signaling. MGB1 protein has been shown to influence various cellular processes such as conidiation, appressoria formation, penetration, and invasion (Nishimura et al., 2003).

In the MAPK signaling pathway, MST11 and MST7 kinases are the two downstream components. Mutants of *MST11* and *MST7* do not form appressoria on hydrophobic surfaces. In a *Δmst11* background, *MST7* dominant active alleles can form appressoria on hydrophobic surfaces. Therefore, MST7 kinase is downstream of MST11 in the MST11-MST7-PMK1 cascade.

MST50 appeared to be associated with MST11-MST7-PMK1 cascade. Mutants of *MST50* fail to produce appressoria and are nonpathogenic. Yeast two-hybrid analysis proposed that MST50 and MST11 have a strong interaction with each other, whereas MST50 and MST7 have a weaker interaction between each other (Park et al., 2006). Yet, no interaction between MST50 and PMK1 has been identified. Therefore, MST50 might associate with upstream

components of the MST11-MST7-PMK1 cascade and also act as an adaptor protein to stabilize the interaction between MST11 and MST7.

The *PMK1* gene is involved in several processes such as germ-tube tip growth, formation of appressoria, generation of turgor pressure, and blockage of invasive growth (Xu and Hamer, 1996). Mutants of *pmk1* are unable to develop appressoria and cannot grow invasively in plants, even when spores are inoculated directly into wounded leaf tissue (Xu and Hamer, 1996). MST12 is a transcription factor and acts downstream of PMK1. Mutants without MST12 expression can form appressorium but cannot penetrate the plant cells (Park et al., 2002).

The cAMP pathway is an alternative pathway that the fungus can use to facilitate appressoria morphogenesis (Lee and Dean, 1993). The *MAC1* gene, coding a membrane-associated protein, is an adenylate cyclase involved in production of cAMP from ATP (Choi and Dean, 1997). Mutants of *mac1* completely lose the ability to form appressoria and are non-pathogenic. These mutants are also unable to produce perithecia. However, with the addition of exogenous cAMP to Δ*mac1* deletion mutants, they can form appressoria and become pathogenic. Moreover, the addition of exogenous cAMP to wild-type *Magnaporthe oryzae* strains can also induce appressoria formation on hydrophilic surfaces.

The binding of cAMP to the regulatory subunit of the cAMP-dependent protein kinase A (PKA), causes the dissociation of the catalytic subunit from the protein kinase and results in the activation of the catalytic subunit. The regulatory subunit is encoded by a cytoplasmic component protein kinase A gene, *CPKA* (Mitchell and Dean, 1995; Xu et al., 1997). Mutations in the PKA regulatory subunit results in the activation of PKA. Deletion of *CPKA* leads to a small non-functional appressoria. The evidence suggests that PKA activation is required for the differentiation of pathogenic appressoria.

Compatible interaction

The mature appressorium develops a specialized hyphae called the penetration peg. This penetration peg uses the physical pressure produced by the appressorium to penetrate the plant cuticle. Once the penetration peg reaches the epidermal cell lumen, it enlarges to form a filamentous primary invasive hypha (IH), which is enclosed by a plant-derived membrane called the extra invasive hyphal membrane (EIHM) (Kankanala et al., 2007). The peg then becomes a channel for transferring the nucleus and cytoplasmic substances from the appressorium into the growing primary invasive hypha.

IH differentiates into a thicker bulbous invasive hypha that grows inside this first-invaded cell for 8-12 hours. Once the invasive bulbous hypha partially or completely fills the host plant cell, it switches again to filamentous IH, which then cross into neighbor cells. The invasion of neighboring cells appears to be via the plasmodesmata (Kankanala et al., 2007). Plasmodesmata are the plasma membrane-lined channels that cross plant cell walls and join the cytoplasm of plant cells into a symplastic system. It takes approximately 2-3 hours for the fungus to fill the neighboring cell, after the initial 8-12 hours necessary to fill the primary infected cell. Initially, the fungus uses a biotrophic invasion process, then switches to a necrotrophic phase, resulting in the appearance of lesions 7-8 days after infection (Heath et al., 1990; Kankanala et al., 2007; Veses and Gow, 2009; Giraldo et al., 2013).

The fungus can also infect the roots of rice (Sesma and Osbourn, 2004; Marcel et al., 2010). Unlike the leaf infection, a unique process called the tissue-adapted fungal infection strategy is used to infect the rice root tissue. Marcel et al. (2010) showed that the invasive hypha (IH) grow intracellularly in roots and move to a new cell without causing the primary cell to die. Marcel et al. (2010) also revealed the absence of expression of the necrotrophy-associated genes

during the initial penetration of fungus into root tissue. These results confirmed that *Magnaporthe oryzae* acts as a biotrophic fungus to cause root infection in rice.

Incompatible interaction

During an incompatible interaction, infection is stopped at the penetration stage or during early colonization. During tissue invasion, *Magnaporthe oryzae* IH secrete many novel biotrophy-associated secreted (BAS) proteins, especially avirulence (AVR) effectors, into the rice cytoplasm via EIHM. *AVR* genes encode AVR effector proteins that are recognized by the host's resistance (*R*) gene products, and this recognition induces the hypersensitive response (HR), preventing fungal colonization (Flor, 1956; Jia et al., 2000; Hulbert et al., 2001). Effector proteins and host resistance genes will be discussed in detail in the next chapter.

Symptoms of rice blast

Rice blast can affect most parts of the rice plant including the leaves, leaf collars, necks, panicles, pedicels, root, and seeds. Leaves are the most commonly affected tissue in the United States, and leaf blast symptoms occur during the vegetative stage of growth (Bonman, 1992). At first, the lesions on the leaves appear gray-green and water-soaked with a darker green border. In time, the lesions on the leaves enlarge in length and typically become diamond-shaped with gray or white centers and necrotic borders (Webster, 2000). The centers of mature lesions appear cottony due to conidia production. On susceptible cultivars, symptoms are light tan colored lesions with necrotic borders, whereas on resistant cultivars the lesions often remain small in size, around 1-2 mm, and are brown to dark brown in color (Wang et al., 2014).

Although leaf blast is most common, symptoms can appear on all other parts of the plant. The following is a short description of the symptoms on other plant parts. Collar rot is the rice blast symptom that occurs at the junction of the leaf blade and leaf sheath. If the disease is severe

at the leaf collar, it can result in the entire leaf being killed (Hajime, 2001). Disease lesions on the leaf collar appear brown in color. The portion of the stem that supports the seed head or panicle is called the neck and can also be infected by this pathogen. Severe disease in this part of the plant can lead to a failure in seed production, which is called blanking (Ou, 1985). Lesions on stem nodes appear dark purple to blue-gray, due to conidia production, and can result in stem breakage (Webster, 2000). *Magnaporthe oyzae* can also infect the panicles as the seeds form, causing lesions on the panicle branches, spikes, and spikelets. The lesions are often seen as gray-brown discolorations of the branches of the panicle. Panicle branches may break at the lesion site over time (Webster, 2000). On seeds, rice blast symptoms appear as brown spots or blotches, and are rarely the classic diamond-shaped lesions (Webster, 2000).

Disease transmission

Rice blast is a polycyclic disease with many cycles in a single crop growing season. In temperate ecosystems, the fungus takes one week to complete its first lifecycle, and 8-11 days to complete the secondary cycle (Kyu, 1994). *Magnaporthe oryzae* over seasons as mycelium and conidia on rice straw and seeds (Agrios, 1997). Sporulation occurs under favorable conditions, such as high moisture for at least a 12-hour period, moderate temperature (~24°C), and high relative humidity (90-92%). Spores produced at the disease lesions (Wang et al., 2014) initiate the secondary disease cycles. Lesions on a leaf can produce up to 20,000 spores, and on one spikelet up to 60,000 spores in one night. Spores of *Magnaporthe oryzae* are commonly dispersed by the wind and usually infect young leaves. The infection then spreads to collars, nodes, and panicles of rice plants. Rice blast is also a seed-borne disease (Manandhar et al., 1998). Seed transmission requires favorable conditions, like being lightly covered with soil and moisture. Unfavorable conditions, like water seeding, prevents seed transmission by creating an

anaerobic environment for the fungus (Manandhar et al., 1998). Spores, crop residue or secondary hosts are also sources of inoculum (Teng, 1994; Greer and Webster, 2001).

Rice blast management

The following four categories are the core of many rice blast control strategies; cultural practices, chemical applications, biological control, and the use of resistant cultivars. Although fungicides are the most commonly used chemical control method, cultural practices such as fertilizer management, water management, and planting time are also used to cope with the disease.

Fertilizing nutrients, such as nitrogen and silicon, can affect disease development. For example, an excess nitrogen supply decreases the number of silicated epidermal cells, thus encouraging disease development (Miyake and Ikeda, 1932). Excess nitrogen also increases the number of leaves and canopy density leading to increased humidity. On the other hand, excess silica increases the number of silicated epidermal cells and increases rice blast resistance (Kawashima, 1927). The application of silica slag (calcium silicate slag) gave results similar to the application of the fungicide Benomyl (Datnoff et al., 1997).

Water availability can also affect blast disease development. Rice grown in flooded soil is more resistant than rice grown under dryer upland conditions (Kahn and Libby, 1958). Thus, it is important to maintain a stable >4-inch flood in fields to manage the disease. Disease transmission between seeds and seedlings can be avoided by sowing into the water. However, in fields with residues, the water will cause the sclerotia to float, moving to the stems, and resulting in infection. Although disease transmission decreases if seeded into the water, once the disease has developed, water increases the disease incidence. Shade can also increase disease development by lengthening the time of wet conditions (Pooja and Katoch, 2014).

Time of planting is another important factor in the development of rice blast. Early planting in tropical upland rice is more resistant to blast infection (Prabhu and Morais, 1986). During the rainy season, tropical upland rice crops sown early are more resistant to blast infection, whereas late-sown crops are often blasted severely.

Fungicides are the commonly used chemical control method to manage rice blast disease. In Japan, copper fungicides were used effectively to control rice blast until the 1950s. The disadvantage of copper fungicides was their high phytotoxicity to many plants. To manage this problem, copper fungicides were mixed with phenylmercuric acetate (PMA), to reduce the harmfulness. Later, Japan adopted the use of a combination of PMA and slaked lime since it was less toxic, cheaper, and added active control of rice blast (Ogawa, 1953). Ultimately, these fungicides were banned by the Japanese government in mid-1968 (Ou, 1985) as they were found to be severe environmental pollutants.

In the early 1970s, kasugamycin (an antibiotic) was a commonly used fungicide to manage rice blast disease. Application of this fungicide occurred four to five times per growing season (Miura et al., 1975). However, *Magnaporthe oryzae* developed resistance to this fungicide. In the late 1970s, organophosphorus fungicides were introduced to Japan to control rice blast. The resistance of *Magnaporthe oryzae* to organophosphorus fungicides was observed by 1976 (Katagiri et al., 1978; Yaoita et al., 1978). However, rotating the fungicide used in applications and mixing them reduced the development of highly resistant populations (Uesugi, 1978).

Systemic fungicides were also introduced to control rice blast (Siddiq, 1996). Melanin biosynthesis inhibitor fungicides, targeting scytalone dehydratase (MBI-D), were introduced in Japan in 1998. Scytalone dehydratase (SDH) is a key enzyme in the biosynthesis of melanin.

MBI-D fungicides, such as carpropamid, diclocymet, and fenoxanil, inhibit scytalone dehydratase in fungal melanin biosynthesis. However, *Magnaporthe oryzae* soon developed resistance to these fungicides and their use was stopped. Melanin biosynthesis inhibitors targeting polyhydroxynaphthalene reductase (MBI-R), another key enzyme in the biosynthesis of melanin, were also introduced in Japan to control rice blast. MBI-R fungicides, such as tricyclazole, pyroquilon, and phthalide, have been broadly used to control rice blast.

In the late 1990s, quinone outside inhibitors (QoI) were introduced to control rice blast. QoI fungicides, such as azoxystrobin, methominostrobin, and orysastrobin, have high control efficacy against rice blast. It was recommended to use QoIs only once per year on rice, if necessary, and rotate with other fungicides, such as MBI-R fungicides. However, *Magnaporthe oryzae* started to develop resistance to these fungicides as well.

Models that forecast the probability of the disease occurring can be used to determine when to spray fungicides. The favorable conditions for blast infection are RH of 90% or above and a minimum temperature of 24°C (Padmanabhan, 1963). There are numerous computer simulation centered forecast models available, including; (1) LEAFBLST (Choi et al., 1988), (2) EPIBLAST (Kim and Kim, 1993), and (3) EPIBLA (Manibhushanrao and Krishnan, 1991). Kaundal et al. (2006) states that forecasting through machine learning techniques, based on online support vector machines (SVM), is more effective than the existing machine learning techniques and conventional multiple regression (REG) approach currently used in forecasting plant diseases (Kaundal et al., 2006).

Biological control is also being used to control rice blast. *Chaetomium cochliodes* has antagonistic activity against *Magnaporthe oryzae*. Rice seeds coated with a spore suspension of *Chaetomium cochlioides* can reduce blast incidence and produce healthy seedlings (Pooja and

Katoc, 2014). According to the University of Madras in India, three strains of *Pseudomonas fluorescens,* five of *Bacillus* spp., and one of *Enterobacter* spp., among 400 bacterial isolates collected from rice fields, were found to be inhibitory under *in vitro* conditions (Pooja and Katoc, 2014). Microbes have also been engineered to help control rice blast. For example, *Erwinia ananas* has been transformed with the chitinolytic enzyme gene (*Chi A*) from an antagonistic bacterium, *Serratia marcescens* strain B2, which is an epiphytic tomato bacterium (Someya et al., 2004). *Bacillus subtilis* strains B-332 (Mu et al., 2007), 1Pe2, 2R37, and 1Re14 (Yang et al., 2008), and *Streptomyces sindenius* isolate 263 also exhibit good antagonistic activity against rice blast.

Host resistance is an environment-friendly method to control diseases. However, in the case of *Magnaporthe oryzae*, growing resistant varieties has been successful for only a brief time due to the presence of isolates that can overcome the host's resistance (IRRI, 2010). Combining major blast *R* genes by traditional breeding methods and marker-assisted selection (MAS) of important traits at the early developmental stages are some of the methods that have been used to select for resistant varieties to control this disease in the field and greenhouse (Moose and Mumm, 2008; Wang et al., 2014). Many blast *R* genes have been used in practical rice cultivation to improve resistance. For example, in Japan, rice multilines have been effectively used to control blast epidemics (Koizumi et al., 2004). Rice multilines are "sets of near isogenic lines containing different *R* genes." To date, 99 blast *R* genes have been mapped with closely linked DNA markers (Wang et al., 2014). Blast *R* genes will be discussed in detail in the next chapter.

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CHAPTER THREE. EFFECTORS AND EFFECTOR DELIVERY

Plant immunity

Plants have a two layer innate immunity system composed of PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). The plant cell wall is the first barrier pathogens encounter once they penetrate the plant cuticle. Pathogens use different strategies, from chemical degradation to physical force, to penetrate the plant cell wall and enter the plasma membrane. Once inside the cell wall, pathogens encounter pattern recognition receptors (PRRs), which are transmembrane receptor-like kinases (RLKs), and transmembrane receptor-like proteins (RLPs) (Jones and Dangl, 2006; Zipfel and Robatzek, 2010; Spoel and Dong, 2012; Liu et al., 2013; Zipfel, 2014; Walters, 2015). The PRRs recognize pathogen-associated molecular patterns (PAMPs), molecules that are released by pathogens as they grow. PAMPs are defined as "conserved molecules present in whole classes of microbes (nonself) with a crucial role in the lifestyle of these microbes" (Medzhitov and Janeway, 1997; Zipfel, 2014). PRRs bind PAMPs and form the host-pathogen interface. This binding activates the first layer of plant innate immunity known as PAMP-triggered immunity (PTI) (Jones and Dangl, 2006; Zipfel and Robatzek, 2010; Liu et al., 2013; Zipfel, 2014; Walters, 2015). After the activation of PTI, any of the following events may occur: reactive oxygen intermediates accumulate, mitogen-activated protein kinase cascades (MAPKs) activate, accumulation of antimicrobial compounds, and plant cell wall reinforcement by callose deposition (De Wit et al., 2009; Zipfel and Robatzek, 2010; Walters, 2015).

Over time, pathogens have developed mechanisms to counteract the PTI system. In this circumstance, plants use the second plant immunity system known as effector-triggered immunity (ETI). Effectors are small proteins that are produced by the pathogen to manipulate the

function and structure of the host. In the ETI system, the hosts have developed resistance (R) proteins that recognize the specific avirulence (AVR) effector proteins produced by the pathogen. The detection of the AVR protein by the resistance protein then activates the hypersensitive response (Jones and Dangl, 2006; Liu et al., 2013; Zipfel, 2014; Walters, 2015). AVR effectors are defined as "pathogen effectors that trigger resistance via activation of specific cognate host R proteins" (Van der Hoorn and Kamoun, 2008). The hypersensitive response (HR) is a localized programmed cell death (De Wit et al., 2009) which results in the pathogen being unable to obtain nutrients.

This selection pressure on the pathogen provided by the plant's immunity system, results in the selection of natural mutations in the pathogen's AVR proteins, such that they are no longer recognized by the host, thus suppressing ETI. The subsequent selection pressure on the host plant results in the development of new R proteins facilitating recognition of the new effectors (Walters, 2015). This constant arms race between the pathogens and the plants known as the zigzag model of plant immunity (Jones and Dangl, 2006).

Fungal effectors and resistance (R) proteins

Fungal effector proteins can be divided into two types based on localization; cytoplasmic effectors and apoplastic effectors. Cytoplasmic effectors are translocated into the host cytoplasm, whereas apoplastic effectors are secreted into the apoplastic area of the host's cells. Additionally, some effectors have an avirulence function that is commonly defined as the inability of a pathogen to cause disease on a resistant cultivar. AVR effectors, encoded by avirulence genes, are typically small proteins with a signal peptide and may be cysteine-rich.

The first bacterial *AVR* gene was cloned in 1984, but it wasn't until 1991 that the first fungal *AVR* gene was cloned. AVR effector proteins activate host defenses via recognition by

specific host resistance R proteins. The largest class of *R* genes encodes the nucleotide-binding domain leucine-rich repeat (NB-LRR) protein family. NB-LRR proteins act as intracellular receptors which recognize effector proteins directly or indirectly (Qi and Innes, 2013). NB-LRR proteins contain a nucleotide binding (NB) domain in the central region, and a leucine-rich repeat (LRR) protein interaction domain at the C-terminus. NB-LRR proteins can be further subdivided into two major subclasses based on their amino-terminal sequence (Meyers et al., 1999; Pan et al., 2000). One is the TIR-NB-LRR class of proteins that harbor an N-terminal Toll/interleukin-1 receptor (TIR) domain and the second is the CC-NB-LRR class of proteins which possess a structured coiled-coil (CC) domain (Meyers et al., 1999; Pan et al., 2000).

Approximately 500 NB-LRR coding *R* genes have been predicted in the rice genome (Monosi et al., 2004; Zhou et al., 2004; Cesari et al., 2013). There are around 100 major rice blast *R* genes that have been characterized genetically (Wang et al., 2014). Among them, 45% were found in cultivars of japonica, while 51% were found in the cultivars of indica. The remaining 4% were found in wild rice species: the *Pi9* gene was domesticated from *Oryza minuta*, the *Pi54rh* gene was domesticated from *Oryza rhizomatis*, the *Pi40(t)* gene was domesticated from *Oryza australiensis*, and the *Pirf2-1(t)* gene was domesticated from *Oryza rufipogon*. To date, only 22 rice blast associated *R* genes have been cloned (Table 7) (Wang et al., 2014). Kiyosawa identified the first rice blast *R* gene, *Pia* from the japonica variety Aichi Asahi in 1967 (Wang et al., 2014). Most of the cloned rice blast *R* genes are in the CC-NB-LRR class of proteins, however the *Pid-2* rice blast *R* gene encodes a receptor-like kinase (Chen et al., 2006; Cesari et al., 2013) (Table 7). Typically, individual NB-LRR proteins recognize AVR effectors and promote resistance, but in rare cases, pairs of NB-LRR proteins are required for resistance (Ashikawa et al., 2008; Lee et al., 2009b; Okuyama et al., 2011; Brotman et al., 2013;

Cesari et al., 2013; Wang et al., 2013). For example, Pik-1 and Pik-2 are a pair of NB-LRR proteins that are required to recognize *Magnaporthe oryzae* effector AVR-Pik and promote resistance (Zhai et al., 2011; Kanzaki et al., 2012). Sometimes more than one effector can be recognized by a single *R* gene. For example, two *Magnaporthe oryzae* effectors, AVR-Pia and AVR1-CO39, are recognized by RGA4 and RGA5, a pair of NB-LRR proteins (Cesari et al., 2013; Wang et al., 2013).

Table 7. Summary of the cloned blast resistance genes

Resistance gene	Chromosome	Protein type	References
Pit	1	CC-NBS-LRR	Hayashi and Yoshida, 2009
Pi37	1	NBS-LRR	Liu et al., 2007b
Pish	1	CC-NBS-LRR	Takahashi et al., 2010
Pib	2	NBS-LRR	Wang et al., 1999
pi21	4	NBS-LRR	Hua et al., 2012
Pid2	6	Receptor kinase	Chen et al., 2006
Pi9	6	NBS-LRR	Qu et al., 2006
Pi2	6	NBS-LRR	Zhou et al., 2006
Piz-t	6	NBS-LRR	Zhou et al., 2006
Pid3	6	NBS-LRR	Shang et al., 2009
Pi25	6	CC-NBS-LRR	Chen et al., 2011
Pi36	8	NBS-LRR	Liu et al., 2007a
Pi5	9	CC-NBS-LRR	Lee et al., 2009a
Pi1	11	NBS-LRR	Hua et al., 2012
Pik	11	CC-NBS-LRR	Zhai et al., 2011
Pikm	11	NBS-LRR	Ashikawa et al., 2008
Pikp	11	CC-NBS-LRR	Yuan et al., 2011
Pikh	11	NBS-LRR	Sharma et al., 2005
Pi54rh	11	CC-NBS-LRR	Das et al., 2012
Pia	11	NBS-LRR	Okuyama et al., 2011
Pb1	11	CC-NBS-LRR	Hayashi et al., 2010
Pita	12	NBS-LRR	Bryan et al., 2000

R gene proteins can interact with AVR effector proteins directly or indirectly (Dangl and Jones, 2001; Van der Hoorn and Kamoun, 2008; Collier and Moffett, 2009). Typically, R gene proteins bind directly with AVR effector proteins following a gene-for-gene relationship as described by Dr. Harold H. Flor (1971) from the Department of Plant Pathology, NDSU. The gene-for-gene concept states, "For each gene that conditions resistance in the host there is a corresponding gene in the parasite that conditions pathogenicity" (Flor, 1971). The interaction of the AVR protein and the R gene protein results in a hypersensitive response (HR). HR is associated with programmed cell death of the infected and nearby cells. R gene proteins can also interact with AVR proteins indirectly following guard or decoy models. The guard model states, "R proteins act by monitoring (guarding) the effector target and that modification of this target by the effector results in the activation of the R protein, which triggers disease resistance in the host" (Van der Biezen and Jones, 1998; Dangl and Jones, 2001; Van der Hoorn and Kamoun, 2008). The decoy model states that "the effector target monitored by the R protein is a decoy that mimics the operative effector target" (Van der Hoorn and Kamoun, 2008).

Effectors of *Magnaporthe oryzae*

More than twenty different effectors, including AVR effectors, have been identified in *Magnaporthe oryzae* (Table 8). Most AVR effectors are relatively small proteins of 70-150 amino acids. For example, the AVR effectors PWL1 (Kang et al., 1995), PWL2 (Sweigard et al., 1995), AVR-Pita (Orbach et al., 2000), and AVR Pii (Yoshida et al., 2009) are all less than 150 amino acids. The AVR effector ACE1 is the largest protein and most likely synthesizes the molecule that is sensed by the plant. Of the non-AVR effectors, most are smaller, like the AVR effectors, with the exception of BAS113 which is over 600 amino acids long. (Mosquera et al., 2009).

Table 8. Characteristics of *Magnaporthe oryzae* effectors

Effector Protein	# AAs	Resistance Protein	Properties	Localization
PWL1	145	Unknown	Glycin-rich hydrophilic protein	Cytoplasmic/BIC
PWL2	145	Unknown	Glycin-rich hydrophilic protein	Cytoplasmic/BIC
PWL3	137	Unknown	Glycin-rich hydrophilic protein	Apoplastic
PWL4	138	Unknown	Glycin-rich hydrophilic protein	Apoplastic
AVR1-CO39	Not cloned	PiCO39	Expressed in IH, triggers HR reaction	Apoplastic
AVR-Pita	233	Pita	Zn metalloprotease	Cytoplasmic/BIC
ACE1	4035	Pi33	Hybrid polyketide synthase/ non-ribosomal peptide synthase	Not secreted
AVR-Piz-t	-	Piz-t	Targets rice ubiquitin ligase APIP6	Cytoplasmic/BIC
AVR-Pia	85	Pia	Triggers HR reaction	Cytoplasmic
AVR-Pii	70	Pii	Triggers HR reaction	Cytoplasmic
AVR-Pik/km/kp	113	Pik/km/kp	Triggers HR reaction	Cytoplasmic
IUG6	72	Unknown	Targets both salicylic acid (SA) and ethylene (ET) pathways	Cytoplasmic/BIC
IUG9	80	Unknown	Targets both SA and ET pathways	Cytoplasmic/ BIC
SLP1	162	Unknown	LysM domain protein; suppresses chitin-induced immunity in rice	Apoplastic
BAS1	115	Unknown	Biotrophy-associated secreted protein	Cytoplasmic/BIC
BAS2	102	Unknown	Biotrophy-associated secreted protein	Cytoplasmic/BIC

Table 8. Characteristics of *Magnaporthe oryzae* effectors (continued)

Effector Protein	# AAs	Resistance Protein	Properties	Localization
BAS3	113	Unknown	Biotrophy-associated secreted protein	Apoplastic
BAS4	102	Unknown	Biotrophy-associated secreted protein	Apoplastic
BAS107	132	Unknown	Biotrophy-associated secreted protein	Unknown
BAS113	659	Unknown	Biotrophy-associated secreted protein	Unknown
MC69	54	Unknown	Important for virulence in both monocot and dicot hosts	Unknown
MoCDIP1	-	Unknown	Induces plant cell death in rice and *Nicotiana benthamiana*	Unknown

Identification of effectors

Most of the AVR effectors were identified via genetic and biochemical approaches based on map-based cloning and the protein's ability to trigger a host hypersensitive response (HR) (Sweigard et al., 1995; Farman and Leong, 1998; Bryan et al., 2000; Orbach et al., 2000; Jia et al., 2004; Li et al., 2009). The effector protein AVR-Pita was cloned using map-based cloning from Chinese field isolate O-137 (Orbach et al., 2000). AVR-Pia was identified by map-based cloning, as well as resequencing and association genetics (Miki et al., 2009; Yoshida et al., 2009). The AVR1-CO39 effector protein was cloned from a weeping lovegrass isolate using map-based cloning, while AVR effectors, such as AVR-Pii and AVR-Pik/km/kp, were identified by genome sequencing and association genetics, using two isolates of *Magnaporthe oryzae* (Ballini et al., 2008; Yoshida et al., 2009). The ACE1 effector was cloned from *Magnaporthe oryzae* isolates pathogenic on rice (Böhnert et al., 2004). The PWL1 effector was identified from a cross between the finger millet pathogen WGG-FA40 and the weeping lovegrass pathogen

K76-79 (Valent et al., 1986; Kang et al., 1995). The PWL2 effector protein was also identified in a genetic cross, in this case it was between two laboratory strains that infected rice (Valent and Chumley, 1991; Kang et al., 1995). Biotrophy-associated secreted **(**BAS) proteins were identified based on their infection-specific expression and identification of expressed sequence tags (ESTs) (Mosquera et al., 2009). Novel effectors, such as IUG6 and IUG9, were identified using genome and transcriptome analyses (Dong et al., 2015).

Common features of the effectors

The presence of cysteine residues that form disulfide bridges is a common feature of most of the apoplastic effectors, as well as some of the cytoplasmic effectors. The disulfide-bridge is often crucial for protein stability in the harsh protease-rich environment of the host apoplast.

Effectors are typically secreted proteins with less than 250 amino acids. Most do not have homology to any currrently known proteins. The *AVR-Pita* gene encodes a secreted preprotein of 223 amino acids (AVR-Pita$_{223}$) with homology to fungal zinc-dependent metalloproteases. The mature protein is processed into an active 176 amino acid protein (AVR-Pita$_{176}$) and promotes avirulence activity by binding to the cognate Pita resistance protein in rice (Bryan et al., 2000; Orbach et al., 2000). The *AVR-Pia* gene encodes a secreted protein of 85 amino acids that does not have any known protein domains. *AVR-CO39* encodes a secreted protein of 89 amino acids with no homology to any other proteins in the databases (Ribot et al., 2013). The *AVR-Pik* gene encodes a 113 amino acid protein with an N-terminal 21 amino acid signal peptide. *AVR-Piz-t* encodes a protein of 108 amino acids with a secretion signal at the N-terminus (Li et al., 2009). This protein has no sequence homology to any known protein in fungi. *AVR-Pii* encodes a 70 amino acid secreted protein also with no similarity to any known protein (Yoshida et al., 2009). The *PWL* gene family encodes four glycine-rich secreted proteins. *PWL1* encodes a protein of

147 amino acids, *PWL2* encodes a protein of 145 amino acids, *PWL3* encodes a protein of 137 amino acids, and *PWL4* encodes a protein of 137 amino acids (Kang et al., 1995). The *MC69* gene encodes a putative novel secreted protein of 54 amino acids. The *SLP1* gene encodes a putative secreted protein of 162 amino acids, which contains two LysM domains (Mentlak et al., 2012). Most LysM domains are from 44-65 amino acids long and appear to bind to molecules like chitin through the N-acetyl-glucosamine (Buist et al., 2008).

Not all blast effectors are secreted proteins, for example, the ACE1 effector is an enzyme involved in the biosynthesis of secondary metabolites (Böhnert et al., 2004; Chen et al., 2007; Collemare et al., 2008). There are 22 polyketide synthases (PKSs) (synthesize polyketides), 8 non-ribosomal peptide synthases (nRPSs) (synthesize non-ribosomal peptides), and 10 PKS–nRPS hybrid secondary metabolite producing enzymes found in *Magnaporthe oryzae*. PKSs are divided into three types. One is an iterative type I PKS, containing numerous enzymatic domains, including ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP) domains. Type II PKS enzymes are only found in bacteria. Type III PKS enzymes contain homodimeric KS domains. The *ACEl* gene encodes a PKS–nRPS hybrid enzyme of 4035 amino acids. The PKS portion of the hybrid enzyme is an iterative type I PKS enzyme, consisting of KS, AT, and ACP domains, in addition to several modifying domains. The nRPS portion of the hybrid enzyme consists of adenylation (A), thiolation (T), condensation (C), and terminal release or cyclization domains (Böhnert et al., 2004; Collemare et al., 2008; Wilson and Talbot, 2009; Yun et al., 2015).

Role of the effectors in suppressing the host immune systems

Apoplastic effectors suppress the host immunity, by preventing PAMP recognition and PRR activation, while cytoplasmic effectors suppress host immunity by interfering with cellular

signaling, secretion, or by controlling gene expression. Cytoplasmic effectors manipulate the structures and functions of the host cells by deactivating ubiquitination systems, vesicle trafficking systems, transcription systems, hormone signaling, and secondary metabolism.

In *Magnaporthe oryzae*, secreted LysM protein 1 (SLP1) is an apoplastic effector protein that suppresses chitin-induced immunity in rice by preventing chitin recognition and the activation of the rice chitin elicitor binding protein (CEBiP). The cell wall of the fungus contains chitin, thus many chitin immune receptors have been found in plants (Kaku et al., 2006; Shimizu et al., 2010; Zeng et al., 2012), and these chitin immune receptors recognize chitin fragments and trigger defense responses. The rice plasma membrane glycoprotein, chitin elicitor binding protein (CEBiP), is an RLP that contains a transmembrane portion and two LysM binding domains, however, it does not have an intracellular kinase domain for signal transduction (Kaku et al., 2006; Liu et al., 2013). CEBiP exhibits high-affinity chitin-binding activity. Knockdown of CEBiP expression suppresses the chitin-triggered immunity response and leads to increased susceptibility to rice blast fungal infection (Kaku et al., 2006; Liu et al., 2013). Therefore, CEBiP plays an essential role in the perception of chitin oligosaccharides as well as defense signal transduction (Kaku et al., 2006; Liu et al., 2013). Chitin elicitor receptor kinase 1 (CERK1) is a plasma membrane protein that contains three LysM motifs in the extracellular domain. It also contains an intracellular kinase domain with autophosphorylation/myelin basic protein (MBP) kinase activity. CERK1 cooperates with CEBiP to control chitin-triggered immunity in rice (Miya et al., 2007; Liu et al., 2013).

SLP1 directly binds to chitin oligosaccharides released from the fungal cell wall to avoid recognition by the rice chitin elicitor receptor protein, CEBiP. SLP1 is a virulence determinant in *Magnaporthe oryzae* because deletion of SLP1 compromises fungal pathogenicity. Targeted

gene silencing of *CEBiP* in rice fully restores the capability of the *slp1* mutant to cause disease (Mentlak et al., 2012; Liu et al., 2013). These results indicate that SLP1 has an essential role in the disease process by competing directly with CEBiP to bind chitin and suppress chitin-induced plant immune responses.

AVR-Piz-t is a cytoplasmic effector protein that targets the ubiquitin-proteasome degradation system to suppress PTI in rice. Ubiquitination is a critical protein post-translational modification occuring in eukaryotic cells. The ubiquitin-ligation system is composed of three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) (Vierstra, 2003; Liu et al., 2013). Ubiquitination occurs in numerous biological processes in plants, including the cell cycle, circadian rhythm control, hormone signaling, growth, and development. In the ubiquitin-proteasome system, the proteasome is a complex of proteases with at least 22 peptides. AVR-Piz-t targets the host ubiquitin-proteasome degradation system which leads to suppression of PTI in plants. AVR-Piz-t interacts with the E3 ubiquitin ligase and suppresses its E3 ligase activity *in vitro*. The E3 ubiquitin ligase has also been labeled AVR-Piz-t interacting protein 6 (APIP6). APIP6 also ubiquitinates AVR-Piz-t *in vitro* and promotes degradation of the AVR-Piz-t protein *in vivo* (Park et al., 2012).

The AVR-Pii effector targets the vesicle trafficking system in rice. Exocyst component proteins, Exo70s, are found at the site of polarized exocytosis. They are important for the fusion of the vesicles to the plasma membrane at this site (Munson and Novick, 2006). Plants have multiple *EXO70* genes while yeast and mammals have only one. In the rice genome, there are 47 *EXO70* genes that have been identified (Fujisaki et al., 2015). Two rice Exo70 proteins, OsExo70-F2 and OsExo70-F3, form a complex with AVR-Pii. OsExo70-F3 is required for the Pii-mediated response to the AVR-Pii effector (Fujisaki et al., 2015).

The isolate unique gene 6 (IUG6) and isolate unique gene 9 (IUG9) were identified in *Magnaporthe oryzae* field isolate 98-06. Both target the salicylic acid (SA) and the ethylene (ET) signaling pathways in rice and have a role in fungal propagation and pathogenicity (Dong et al., 2015). In rice, overexpression of these effectors results in suppression of defense-related gene expression. Also, a *Δiug*6 mutant did not develop the penetration peg, and *Δiug*9 mutants were less able to penetrate the host. These results suggest that the effectors may play a part in biotrophy by interfering with the host's SA and ET signaling pathways (Dong et al., 2015).

AVR proteins versus R proteins

Plant *R* gene protein products recognize AVR effectors directly or indirectly to induce HR and prevent disease. *Magnaporthe oryzae* effectors typically follow the gene-for-gene relationship. AVR effectors, such as AVR-Pita, AVR-Pik/km/kp, AVR1-C039, and AVR-Pia, show direct interaction between AVR effector proteins and R gene proteins, whereas the ACE1 effector has an indirect interaction (Kanzaki et al., 2012).

The AVR-Pita and Pita interaction was the first example of direct binding of an AVR effector protein and R gene protein. AVR-Pita containing *Magnaporthe oryzae* isolates are not capable of infecting rice cultivars containing the *Pi-ta* resistance gene. The *Pi-ta* gene encodes a putative cytoplasmic receptor with a centrally localized nucleotide-binding site and leucine-rich domain (LRD) at the C-terminus. Yeast two-hybrid and *in vitro* binding assays revealed direct binding of AVR-Pita$_{176}$ to the LRD domain of Pita (Bryan et al., 2000).

AVR-Pik also has a direct interaction with Pik-1 in rice. The CC domain of Pik-1 physically binds to AVR-Pik and induces the HR (Kanzaki et al., 2012; Liu et al., 2013). AVR-Pia was shown to be recognized within rice cells by expressing AVR-Pia which was lacking the signal peptide in rice cells that contained the cognate *R* gene. Coexpression of the proteins

resulted in cell death (Yoshida et al., 2009). RGA4 and RGA5 are a pair of NB-LRR proteins located next to each other at the *Pia* locus. These proteins are important in the recognition of AVR-Pia as well as in mediating *Pia* based resistance (Cesari et al., 2013; Okuyama et al., 2011). RGA4 and RGA5 interact with AVR-Pia through their coiled-coil domains. Both RGA4 and RGA5 form homodimer and heterodimer complexes. RGA4 mediates cell death activation, whereas RGA5 acts as an AVR receptor in both rice protoplasts and in *Nicotiana benthamiana*. Additionally, RGA5 acts as a repressor of RGA4 (Cesari et al., 2013; Okuyama et al., 2011).

AVR1-CO39 binds directly to the resistance protein Pi-CO39 and triggers the hypersensitive response (HR) (Ribot et al., 2013). The RGA5 receptor can also bind to AVR1-CO39 through a small non-LRR C-terminal domain (Cesari et al., 2013). AVR-Pii binds to the cognate rice resistance protein Pii. *Pii* encodes a pair of CC-NB-LRR-type NLR proteins (Takagi et al., 2013). AVR-Piz-t effector protein binds directly to the NBS-LRR resistance protein Piz-t (Li et al., 2009), and is highly similar to Pi2 and Pi9 (Zhou et al., 2006). Pi2 differs from Piz-t by only eight amino acid changes, that are restricted to three consecutive LRR repeats, which regulate resistance specificity (Zhou et al., 2006).

ACE1 and its cognizant R protein, Pi33, have an indirect interaction because the secondary metabolites produced by ACE1 are necessary for avirulence activity (Böhnert et al., 2004; Fudal et al., 2005). ACE1 containing *Magnaporthe oryzae* isolates are not capable of infecting rice cultivars containing the *Pi33* resistance gene. However, isolates or mutants defective in ACE1 are able to infect *Pi33* containing cultivars, and deletion mutants are not compromised in their virulence (Böhnert et al., 2004; Fudal et al., 2005).

Effector evolution

Transposon insertions, gene deletions, and other genetic rearrangements are the main mechanisms for gain of virulence for *Magnaporthe oryzae* that express effector genes. For example, the transposable element Pot3, which is situated at the *AVR-Pita* promoter region, is linked to the virulence of AVR-Pita isolates. This transposable element has been shown to upset the protease motif of the *AVR-Pita* allele making it ineffective (Zhou et al., 2007; Dai et al., 2010; Singh et al., 2014). Moreover the insertion of a MINE (1.9 kb) retrotransposon in the last exon of *ACE1* increases the virulence of strain 2/0/3 (Fudal et al., 2005). Additionally, gene deletion of *AVR-Pita* can lead to a gain of virulence. Effector genes that are located at the chromosome end tend to evolve at higher rates than the rest of the genome. For example, the location of *AVR-Pita* is tightly linked to a telomere on chromosome 3 in the genome of *Magnaporthe oryzae* (Jia et al., 2000; Orbach et al., 2000). Novel effector *IUG6* is located on the subtelomeric regions of chromosome 2 and *IUG9* is located on the subtelomeric regions of chromosome 1.

Knockout mutants of the *MC69* gene do not develop any disease symptoms in rice. Moreover, deletion of the *MC69* orthologous gene in *Colletotrichum orbiculare* (cucumber anthracnose fungus) also reduces its pathogenicity on both cucumber and *Nicotiana benthamiana* leaves (Liu et al., 2013). This suggests that MC69 is a secreted pathogenicity protein that is required for infection by *Magnaporthe oryzae* on monocot plants and by *Colletotrichum orbiculare* on dicot plants.

Instability of *AVR-Pita* alleles is another mechanism that can allow the fungus to avoid the plant's defenses (Zhou et al., 2007; Khang et al., 2008; Dai et al., 2010; Liu et al., 2013). Khang et al. (2008) showed that *AVR-Pita*, which has been renamed *AVR- Pita1*, fits into a gene

family with at least two more members: *AVR-Pita2* and *AVR-Pita3*. AVR-Pita2 functions as an elicitor of Pita-mediated defense responses, however, AVR-Pita3 does not. *AVR-Pita1* and *AVR-Pita3* are the result of a gene duplication event that happened after separation of *Magnaporthe oryzae* from *Magnaporthe grisea*. This is suggested by the fact that *AVR-Pita3* is present only in *Magnaporthe oryzae* isolates, but *AVR-Pita2* and *AVR-Pita1* are found in both *Magnaporthe oryzae* and *Magnaporthe grisea* isolates (Khang et al., 2008).

Five alleles of *AVR-Pik*, *AVR-Pik-A*, *AVR-Pik-B*, *AVR-Pik-C*, *AVR-Pik-D*, and *AVR-Pik-E*, were identified from 21 *Magnaporthe oryzae* isolates from Japan (Kanzaki et al., 2012). Phylogenetic analysis demonstrated that the *AVR-Pik-D* allele is most likely the ancestral allele of the five *AVR-Pik* alleles (Kanzaki et al., 2012). The rice resistance gene *Pik* also has five alleles, including *Pik*, *Pikp*, *Pikm*, *Piks*, and *PikhI*, that are highly polymorphic at two positions in the N-terminal coiled-coil (CC) domain (amino acids 229 and 252) (Costanzo and Jia, 2010). The proteins from the *AVR-Pik* alleles have different interaction specificities with the different proteins produced from the *Pik* alleles. For example, Pikp from variety K60 identifies AVR-Pik-D but does not recognize AVR-Pik-A, AVR-Pik-C, or AVR-Pik-E (Kanzaki et al., 2012). Likewise, Pik from Kanto51 recognizes AVR-Pik-D and AVR-Pik-E, but does not recognize AVR-Pik-A or AVR-Pik-C. Lastly, Pikm from the variety Tsuyuake recognizes AVR-Pik-A, AVR-Pik-D, and AVR-Pik-E, but not AVR-Pik-C (Kanzaki et al., 2012). Taken together, sequence diversification or knockdown of effector genes were correlated with gain of virulence.

Identification of biotrophic interfacial complex (BIC)

In 2007, Kankanala and his research group performed live-cell imaging experiments to investigate the development of invasive hyphae (IH), and the plant's response, inside successively invaded rice cells. They used the endocytotic dye FM4-64 to study the development

of invasive hyphae inside the rice cells. This experiment was used to study the dynamics of the organelles and, in particular, the endocytotic pathway in eukaryotic cells.

There are two pathways, exocytosis and endocytosis, involved in vesicular trafficking in eukaryotic cells. The exocytosis pathway refers to the pathway where traffic moves from the endoplasmic reticulum (ER), through the Golgi cisternae- cis, medial, and trans- to the plasma membrane (PM). In the endocytosis pathway, traffic moves from the plasma membrane to the vacuole in fungi and plants.

FM4-64 endocytotic dye follows the endocytotic pathway. After addition of FM4-64 inside the infected rice cells, the dye internalized inside the plant plasma membrane and small organelles, and then integrated into the plant cell's vacuoles (Fischer-Parton et al., 2000; Atkinson et al., 2002; Bolte et al., 2004). However, rice blast IH inside rice cells failed to internalize FM4-64, even after increased dye exposure times of up to 6 hours. They observed that the FM4-64 dye was outlining the IH. They hypothesized that there was a plant plasma membrane outlining the IH of the fungus which blocked the dye from entering the fungal plasma membrane. They named this plant plasma membrane the extra invasive hyphal membrane (EIHM). To confirm the presence of EIHM surrounding the IH, a transmission electron microscope (TEM) image of growing primary hyphae inside the rice cell was obtained. The TEM image confirmed that the IH was enclosed in an EIHM outside the fungal cell wall. Also, when looking at the tip of the hyphae, they observed a cap-like structure in the space between the EIHM and the IH cell wall. In a follow-up study, in 2010, Khang and his group did a detailed analysis of this cap-like structure and named it the biotrophic interfacial complex (BIC).

Giraldo and his group (2013) showed that the BIC is a plant-derived structure. They labeled the cytoplasmic effector PWL2 with monomeric red fluorescent protein (PWL2:mRFP),

and made the fungal plasma membrane visible by labeling the plasma membrane ATPase with a green fluorescent protein (GFP). The PWL2:mRFP appeared to be outside of the pathogen's plasma membrane suggesting BIC was not from the pathogen. By measuring fluorescence intensity in a line crossing the BIC they showed that for the most part the PWL2:mRFP signal was outside of the GFP signal and overlapped only slightly. The overlap was due to the rounded nature of the infection hyphae. This experiment clearly indicated that the BIC was not formed from the pathogen. Using similar experiments, they found that BIC is composed of both the plant plasma membrane and endoplasmic reticulum, confirming that the BIC is a plant-derived structure. The BIC-associated invasive hyphae cell is enhanced in secretion machinery components for cytoplasmic effectors, as a result, BIC is involved in mediating the delivery of pathogen effectors into the rice host cytoplasm (Mosquera et al., 2009; Khang et al., 2010; Giraldo et al., 2013).

Localization and movement of effectors

Khang and his group produced fungal transformants that expressed fluorescently tagged blast effector proteins (AVR-Pita1, PWL1, and PWL2) under the control of their native promoters. Using live-cell imaging and epifluorescence microscopy, they showed that the fluorescent proteins were secreted into the BIC soon after appressorial penetration of the first-invaded epidermal rice cell. The BIC was left behind beside the first IH cell when the fungus switched to pseudohyphal growth. Repeatedly, fluorescent BIC development was also observed for hyphae that had invaded neighboring cells.

Fluorescence recovery after photobleaching experiments revealed that fluorescently tagged PWL2 and BAS1 (another BIC-localized protein), but not BAS4 (a non-BIC-localized protein), were translocated into the cytoplasm of invaded rice cells. The nuclear localization

signal (NLS) improved the sensitivity of detecting the effector in host cells by concentrating it in the cell nucleus. Khang et al. (2010), hypothesized that cytoplasmic effectors labeled with fluorescent proteins that gather in BICs were translocated into the cytoplasm of living rice cells (Yi et al., 2009; Giraldo et al., 2013). This result confirmed that cytoplasmic effectors are translocated into the host cytoplasm. Interestingly, fluorescently tagged PWL2 effector proteins were also identified in the neighboring cells, up to four cell layers away from the infected cell. This evidence revealed the cell-to-cell movement of the blast effectors inside the rice cells, and that the blast effectors can be translocated into un-invaded rice cells, possibly to prepare them for subsequent fungal entry. Khang et al. (2010) demonstrated that when PWL2 was linked to a tandem dimer tomato fluorescent protein (PWL2:tdTomato), mw 68.3 kD, the PWL2:tdTomato protein was not able to move from the penetrated cell to surrounding cells. When PWL2 was linked to the red fluorescent protein mCherry (PWL2:mCherry), the 39.3 kD PWL2:mCherry fusion protein moved from the penetrated cells to surrounding cells. This evidence demonstrated that the cell-to-cell movement of effector proteins most likely occurs through the plasmodesmata and is dependent on the size of effector fusion proteins.

Live cell-imaging analysis revealed the distinct localization of biotrophy-associated secreted (BAS) proteins (Mosquera et al., 2009). BAS3 accumulated near crossing points of the cell wall, whereas BAS4 outlined the IH. BAS1 and BAS2, preferentially accumulated in the BIC, were translocated into infected rice cells, and also entered un-invaded neighboring cells (Mosquera et al., 2009; Khang et al., 2010). This distinct localization of these effectors raised the question as to how they are secreted into the plant cells.

Effector secretion systems

In eukaryotic systems, such as fungi, proteins that are to be secreted have a secretion signal peptide (SP) at the N-terminus and are able to follow the conventional secretion pathway. However, fungal proteins that are secreted without an SP follow a distinct secretion pathway. In the conventional secretion pathway, secreted and membrane-bound ribosomally-synthesized proteins are initially directed to the rough endoplasmic reticulum (RER) by a signal peptide (Sweigard et al., 1995). The signal peptide is a sequence of 20 to 30 amino acids at the amino terminus of the protein, which associates with the signal recognition particle (SRP) in the cytoplasm, and directs the ribosome to the endoplasmic reticulum where it docks at a protein complex in the ER membrane. The signal peptide contains a net positive charge at the N-terminus, a highly hydrophobic core, and a hydrophilic C-terminus. The signal sequence also has alpha helical characteristics and has a small amino acid, such as glycine or alanine, within two amino acids of the cleavage site. Cleavage of the signal peptide at the C-terminus allows the protein to either enter into the lumen of the ER to be secreted, or to become incorporated into the membrane components of the cell (Sweigard et al., 1995).

When the SRP binds to the signal sequence, it halts translation. Once the ribosome-mRNA-SRP docks at the ER protein, the SRP is released and translation then continues. The signal peptide is then cleaved by a protease present in the lumen of the ER. Once inside the ER, the protein folds. Next, the protein moves through the ER and is transported in vesicles to the cis-Golgi (Sweigard et al., 1995). By cisternal relocation, cis-Golgi vesicles with their luminal protein cargo move through the Golgi complex to the trans-Golgi reticulum. The primary function of the Golgi is to sort proteins and modify them by attachment of sugar molecules and other moieties. However, most effectors are not modified by the Golgi but pass through on their

way to secretion. After passing through the Golgi, proteins are transported from the trans-Golgi reticulum to the plasma membrane. When the membrane vesicle is fused with the plasma membrane, the proteins are released into the extracellular space (Sweigard et al., 1995).

In *Magnaporthe oryzae*, apoplastic effectors are secreted into the extracellular space and follow the conventional secretion pathway. However, the cytoplasmic effectors, which preferentially accumulate in the BIC and are translocated into the host cytoplasm, follow an unconventional pathway. This was proven by Brefeldin A (BFA) experiments performed by Giraldo and his research group (2013). BFA is a fungal metabolite that upsets protein secretion in eukaryotic cells, by disturbing the Golgi apparatus, but does not have an effect on protein synthesis (Giraldo et al., 2013). BFA inhibits the conventional secretion pathway indirectly by preventing the formation of coat protein or COPI-mediated transport vesicles (Giraldo et al., 2013). The COPI protein complex is involved in transporting vesicles from the ER to the Golgi. BFA inhibits protein secretion by inhibiting the movement of proteins from the ER to the Golgi and transport through the Golgi.

The cytoplasmic effector PWL2 was labeled with red fluorescent protein (RFP) along with a nuclear localization signal (NLS). Apoplastic effector BAS4 was labeled with green fluorescent protein (GFP). After secretion, the cytoplasmic effector, PWL2:RFP, was not only detected in the BIC, but also inside the cytoplasm, and was translocated into the nucleus of the host. Whereas the apoplastic effector BAS4:GFP showed apoplastic localization outlining the IH. In the presence of BFA, PWL2:RFP remained BIC-localized and translocated into the nucleus, indicating the complete transport of the effector into the cytoplasm of the host cell. However, BAS4:GFP was retained in the fungal ER. This demonstrated that BFA blocked secretion of apoplastic effectors, however, not BIC associated cytoplasmic effectors. This experiment was

repeated with two additional cytoplasmic effectors (AVR-Pita and BAS1), and the same results were obtained. These results confirmed that the Brefeldin A was unable to block the secretion of the cytoplasmic effectors that are transported to the BIC. Therefore, cytoplasmic effectors have a unique mechanism for their secretions.

Giraldo et al. (2013) also performed a fluorescence recovery after photobleaching (FRAP) experiment in order to demonstrate the continuous secretion of cytoplasmic effector PWL2:GFP into the BIC in the presence of BFA. Rice tissue was infected with a fungal strain expressing PWL2:GFP and BAS4:mRFP and was incubated in BFA for three hours before photobleaching. As previously observed, in the presence of BFA, PWL2 remained BIC-localized and translocated into the nucleus, but the secretion of apoplastic effector BAS4 was blocked. After photobleaching, the green fluorescent protein labeled cytoplasmic effector PWL2 disappeared, however it was detected again a few hours later (Giraldo et al., 2013). This result also supported the conclusion that cytoplasmic effectors have a Brefeldin-independent, unique, secretion mechanism.

Exocyst-mediated pathway

The exocytic pathway plays a key role in morphonogenesis and pathogenicity in *Magnaporthe oryzae* and is involved in several processes, including generation of cell polarity and effector delivery. Additionally, the exocyst system is crucial for membrane trafficking in response to diverse signals. The exocyst complex is composed of eight proteins, including Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84. These proteins are involved in docking and tethering of exocytic vesicles to the targeted plasma membrane sites. The exocyst components Exo70 and Sec5 are involved in the secretion of cytoplasmic effector proteins.

In a wild type strain of *Magnaporthe oryzae,* cytoplasmic effector PWL2:mCherry:NLS was detected in the BIC, as well as in the rice cell nucleus, with no fluorescence observed in the BIC-associated IH cell. Apoplastic effector BAS4:GFP was localized to the EIHM compartment (Giraldo et al., 2013). However, an Δ*exo70* mutant strain showed partial retention of PWL2:mCherry:NLS, mainly in the BIC-associated IH cell, but the secretion of apoplastic effector BAS4 was not blocked. Similarly, an exocyst component Δ*sec5* mutant strain also showed partial retention of PWL2:mCherry:NLS inside the BIC-associated IH cell, but secretion of apoplastic effector BAS4 appeared to be normal (Giraldo et al., 2013). Results of this experiment confirmed that secretion of cytoplasmic effectors follows the exocyst-mediated pathway.

BIC development involves SNAREs component

Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) direct the fusion of secretory vesicles to the plasma membrane. SNARE proteins are found in vesicles, v-SNAREs, and in the plasma membrane, t-SNAREs. The exocyst directs the precise docking and tethering of secretory vesicles to the target plasma membrane for the final SNARE-mediated membrane fusion event (Giraldo et al., 2013). Effector secretion through the BIC is associated with the Sso1 t-SNARE protein. In an experiment, mutation of the Sso1 protein led to the inappropriate secretion of cytoplasmic effector PWL2:mCherry:NLS, as it was not only located in the BIC, but also in a secondary point halfway down the primary infection hyphae. Giraldo et al. also observed another infection site, and they again observed the inappropriate secretion of PWL2:mCherry:NLS. Likewise, an additional cytoplasmic effector BAS1:mRFP expressed by an Δ*sso1* mutant also showed inappropriate secretion. These results confirmed that the Sso1 t-

SNARE protein plays an important role in the unique secretion system through the BIC (Giraldo et al., 2013).

Conclusion

Magnaporthe oryzae secretes multiple effectors during biotrophy tissue invasion. These effectors can manipulate the structure and function of the host. By an unknown mechanism, the pathogen manipulates the host to form a unique plant-derived structure known as the biotrophic interfacial complex (BIC). Effectors can be divided into two types based on cellular localization. Cytoplasmic effectors initially accumulate in the BIC and are translocated into the host cells, while apoplastic effectors are secreted into the space between the IH and EIHM. BIC is involved in mediating the delivery of some cytoplasmic effectors into the host cytoplasm.

Magnaporthe oryzae has two distinct secretion mechanisms. Apoplastic effectors are secreted through the conventional ER-Golgi pathway, and some cytoplasmic effectors are secreted by an exocyst-mediated pathway. Brefeldin A (BFA) is a fungal metabolite which can inhibit the conventional ER-Golgi pathway. After exposure to BFA, apoplastic effectors were retained in the fungal ER, while cytoplasmic effectors were accumulated in the BIC and were translocated into the host cell. Exocyst components, Exo70 and Sec5, regulate the secretion of cytoplasmic effectors. Mutation of either *SEC5* or *EXO70* genes resulted in impaired secretion of cytoplasmic effectors. However, the secretion of apoplastic effectors was not blocked in these mutants. SNARE component Sso1 t-SNARE plays an important role in mediating the delivery of cytoplasmic effectors through the BIC. Key questions remaining to be addressed are; how is the BIC structure formed and what is the exact role of this structure? Moreover, the mechanism defining which secretion system will be used for a particular effector needs to be explored.

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