



A review on *Pyricularia oryzae*; biological and taxonomical finding in Iran

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Abstract: The review provides an overview of the biological and taxonomical findings of the blast disease causal agent, *Pyricularia oryzae*, in Iran. Comprehensive and comparative symptomology and geographical distribution of the *P. oryzae* strains are described. The taxonomic history of the *P. oryzae* compares to close species is illustrated and the host specificity and the population genetics of the strains obtained from weeds and crops are elucidated. Sexual status and mating type information of *P. oryzae* are also discussed.

Keywords: blast disease, geographical distribution, host, taxonomic criteria

Disease symptoms

Pyricularia oryzae, the causal agent of blast disease on crop plants, destroys plenty of food supply to sustain millions of people (Pennisi 2010; Liu et al. 2014; Milazzo et al. 2019). *P. oryzae* is a highly destructive pathogen on rice (*Oryza sativa*), wheat (*Triticum aestivum*), and turfgrass (*Lolium perenne*), causing up 100 % yield loss on rice in some areas worldwide, 40% to 100% on wheat in Brazil, and over 90% destruction on turfgrass (*Lolium perenne*) in several golf courses and stadiums (Uddin et al. 1999; Skamnioti & Gurr 2009; Pennisi 2010; Milazzo et al. 2019). Blast disease is the most important rice disease in two major rice-growing areas of Iran including Guilan, and Mazandaran provinces (north of Iran). The loss caused by this fungus on rice in Guilan has been estimated at around 15 to 45% (Javan-Nikkhah & Hedjaroude 2000).

Under field conditions, the fungus can infect all aerial parts of rice, resulting in leaf, node, neck, and panicle blast (Behdad 1979; Wilson & Talbot 2009; Pordel et al. 2015) (Fig. 1, a). Blast symptoms on rice gradually enlarge, and the center of the spots is usually gray or white, and the margins are reddish-brown (Behdad 1979; Wilson & Talbot 2009; Male 2011; Pordel et al. 2016; Cruz & Valent 2017) (Fig 1, b). Neck blast causes direct crop damage, leading to weak filling of seeds and collapse of the rice head (Fig. 1, c). The pathogen infects maize (*Zea mays*), and blast symptoms initially appear as grey lesions with a light margin, expand rapidly to several centimeters in length, and become lighter in color with a distinct brown margin (Pordel et al. 2021). Leaf spots with a brown margin and yellow center appear on barnyard grass (*Echinochloa crus-galli*) (Fig. 1 f-g) (Pordel et al. 2021). On wild foxtail millet (*Setaria viridis*), leaf spots appear in the form of elongated, spindle-shaped that completely cover the entire leaf, and on the head, dark brown spots (Fig. 1, h-i). Brown spots on heads and lesions on leaves appear like a diamond shape with a light margin on foxtail millet (*Setaria italica*) (Pordel et al. 2018) (Fig. 1, j).

Disease severity and plant mortality are often higher on seedlings and young plants, especially in crop plants, including maize, rice, and foxtail millet. On the crop plants including rice and foxtail millet, the pathogen causes heads to collapse after seven days of infection (Pordel et al. 2018, 2021).

Geographical distribution

First-time, rice blast disease (*P. oryzae*) was reported in 1966 in Guilan province (Sharif 1966) and now is distributed all over the south of the Caspian Sea and northeast of Iran (Fig. 2) (Bargnil 2012; Motallebi et al. 2009, 2013; Niknam-Galejugi et al. 2014; Pordel et al. 2015). Maize blast disease was observed in three provinces of Iran, including Mazandaran, Guilan, and Golestan (Pordel et al. 2016). Blast epidemics on barnyard grass growing as weeds in the blasted maize fields were observed in Guilan and Mazandaran provinces. The major incidence blast disease on barnyard grass was occurred in Golestan province near maize fields (Pordel et al. 2021). Blast disease on foxtail millet was reported first time in Mazandaran

provinces (Pordel et al., 2018). The disease on wild foxtail millet was reported several times in north and northeast provinces, including Guilan, Mazandaran, Golestan, Razavi Khorasan provinces (Bargnil 2012;

Motallebi et al. 2009, 2013; Niknam-Galejugi et al. 2014; Pordel et al. 2016). Recently, *P. oryzae* was isolated from *Sorghum halepense* (Johnson grass) in Guilan province (Pordel et al. 2020).



Fig. 1. Blast symptoms in field condition, a-c: on rice, d-e: on maize, and Leaf spot f-g: on barnyard grass, h-i: on wild foxtail millet, j: on foxtail millet.



Fig. 2. Geographic distribution of blast disease in four provinces of Iran.

Taxonomic history of the pathogen

In 1880, Saccardo created the generic name *Pyricularia* based on the asexual form, *P. grisea*, isolated from the *Digitaria sanguinalis*. Isolates from rice were introduced as *P. oryzae* by Cavara in 1892, now known as the rice blast causal agent. Afterward, two species, *P. oryzae* and *P. grisea*, considered synonymous (Rosman et al. 1990), because there were not enough morphological criteria to separate the two species, and the cross of isolates from rice and non-rice hosts leads to the production of sexual form, which indicates a genetic relationship between the two groups of isolates. The survey of type samples in herbarium was confirmed the morphological similarity of the two species. Therefore, *P. grisea* (Cooke) Sacc. was introduced as the correct name for the asexual stage of both groups of isolates (rice and non-rice isolates) due to the name's priority. In 2002, phylogenetic analysis based on three gene regions, including Actin, Beta-tubulin, and Calmodulin, resulted in separating *P. oryzae* from *P. grisea* as new species (Couch & Kohn 2002).

In Iran, based on the study on morphology criteria of 150 isolates from weeds and crop plants, *P. oryzae* differ from *P. grisea* in morphology and host plant (Pordel et al. 2016). Conidiophores size are 100-250 μm in *P. oryzae* and 70-175 μm in *P. grisea*, and conidia size are 16-25 \times 7-10 μm in *P. oryzae*; and 26-31 \times 6-8 μm in *P. grisea* (Fig 3) (Pordel et al. 2016). Phylogenetic analyses have been done by partial sequences of the internal described spacer regions with the 5.8S nuclear ribosomal RNA gene (ITS), the large subunit of the nuclear ribosomal RNA gene cluster (LSU), and several protein-encoding genes, including the partial RNA polymerase II largest subunit (*RPB1*), actin (*ACT*), calmodulin (*CAL*), and DNA replication licensing factor (*MCM7*), proved the name of *Pyricularia oryzae* for isolates on maize (*Zea mays*), rice (*Oryza sativa*), Indian goosegrass (*Eleusine indica*), barnyard grass (*Echinochloa crus-galli*), bermuda grass (*Cynodon dactylon*), knotgrass (*Paspalum distichum*), wild foxtail millet (*Setaria* sp.), foxtail millet (*Setaria italica*) and banana (*Musa acuminata*) and the *P. grisea* name is approved only for isolates on *Digitaria* sp. (Pordel et al. 2015, 2016).

Host-specificity of the pathogen

Multilocus sequence typing and whole-genome sequence showed *P. oryzae* is subdivided into multiple clades; each could infect the limited number of host species (Couch et al. 2005; Gladieux et al. 2018). Host specificity is, therefore, a probable driver of genetic divergence (Couch et al. 2005; Gladieux et al. 2018). Mostly, pathogenicity test was confirmed this species' host-specificity, except for barley, which seems to be susceptible to strains from all clades. Strains from rice have a limited host range and were reported to infect only oat and *Lolium* in addition to rice (Tosa & Chuma 2014; Klaubauf et al. 2014). *Pyricularia oryzae* strains from other host plants such as *Eleusine*, *Setaria*, and *Triticum* are also host-specific and unable to infect rice (Kato et al. 2000; Couch et al. 2005; Murata et al. 2014; Tosa & Chuma 2014; Castroagudin et al. 2016).

Host specificity of strains from different hosts have been assessed by spray inoculation of spore suspensions on important crops and weeds susceptible to *P. oryzae* (Pordel et al. 2019; Pordel et al. 2021). Strains from rice, maize, barnyard grass, wild foxtail millet, and foxtail millet showed severe symptoms on their isolated host (Table 1). Rice strains do not produce symptoms on five tested hosts and made different types of lesions on Iranian rice varieties (Javan-Nikkhah et al. 2003). Eight strains from barnyard grass produced susceptible-type lesions on maize and two maize strains out of ten produced susceptible-type lesions on barnyard grass (Table 1). The strains of wild foxtail millet produced severe lesions on foxtail millet, and the strains of foxtail millet made severe lesions on wild foxtail millet plants (Table 1) (Pordel et al. 2016, 2019, 2021).

To survey the reaction of the different cultivars of rice to *P. oryzae*, fifty isolates were checked by Javan-Nikkhah et al. (2003). They studied differential varieties from international standard cultivars set, CO39 and five near-isogenic lines (NILs) and twelve selected Iranian cultivars. In this study, the differentiating ability of NILs was evaluated by comparing those international differentials and Iranian cultivars. The set of five NILs was able to differentiate 50 isolates into two pathotypes (races) in which one isolate represented pathotype "A" and 49 were grouped into pathotype "B". Fifty isolates were classified into seven pathogenicity groups by their reaction to twelve Iranian cultivars. Their results showed the *P. oryzae* isolates had high specificity on different cultivars (Javan-Nikkhah et al. 2003). Many studies confirmed that *P. oryzae* has various types of lesions on the different rice cultivars (Moradi et al. 2010, Mousanejad et al. 2010).

Population structure and genetic diversity

DNA fingerprinting has been successfully used to assess genetic diversity and population genetics of pathogens by inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), and

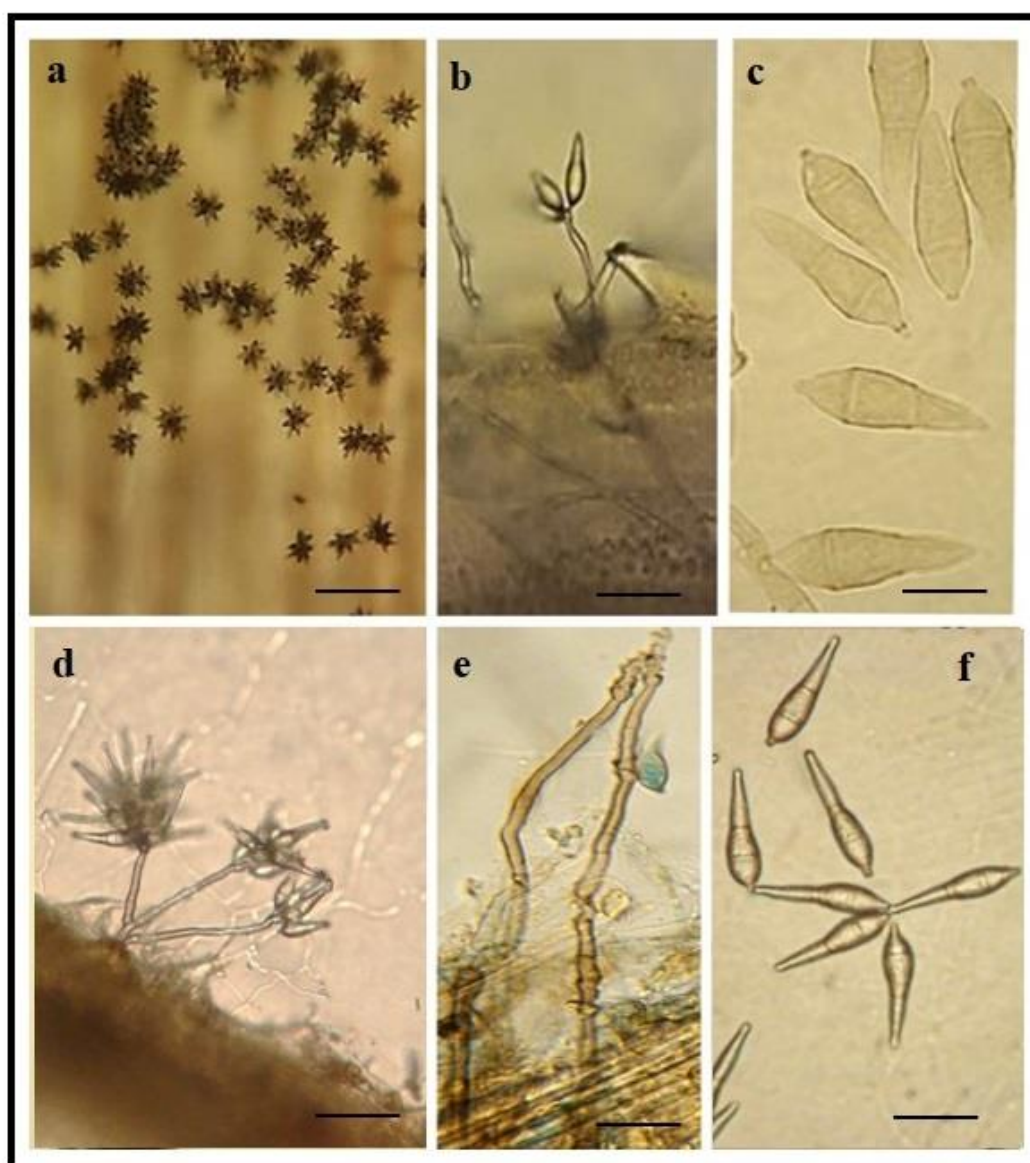


Fig. 3. Morphological comparison of the *Pyricularia oryzae* (a-c) with *P. grisea* (d-f).

amplified fragment length polymorphism (AFLP) techniques (Dubina et al. 2020). Genetic analysis of *P. oryzae* population structure was performed for the first time by Javan-Nikkhah et al. in Iran (2004). His studies based on DNA fingerprinting using a molecular rep-PCR technique using Pot2 primer on 221 monoconidial isolates divided the isolates into six clonal lineages. The results showed the low genetic diversity among the populations, and a specific relationship was observed between rice cultivars. DNA fingerprinting using RAPD-PCR markers on *P. oryzae* population isolated from weeds of *Echinochloa* sp., *Digitaria* sp., *Setaria* sp., unknown weed and rice, showed that isolates from wild foxtail millet and rice are similar to each other and separate from *Digitaria* sp. isolates (Bargnil et al. 2012). In recent years, interesting studies have been conducted in genetic diversity, adaptive vegetative groups, and mating types in Iran (Musanejad et al. 2005; Salimi 2018).

Sexual status and mating type information

Mousanejad et al. (2005) studied the fertility status and distribution of causal agents of the blast disease in Iran. They investigated 158 rice blast pathogen isolates, including 94 isolates from different regions of Guilan province and 64 isolates from the experimental fields of the Rice Research Institute of Iran, in Amol, Mazandaran province. The isolates crossed with standard hermaphrodite and fertile isolates (KA3 and TH12 (representatives of *Mat1-1* mating type) and KA9 and TH16 (representatives of *Mat1-2* mating type)). These crosses' results showed that 62.76% of the isolates from different parts of Guilan province, and 32.81% of the isolates of the Rice Research Institute's experimental field were fertile. The rest of the isolates were infertile in both cases. All fertile isolates were male fertile and of mating-type *Mat1-1*. Salimi et al. (2018) examined 142 isolates of *P. oryzae* obtained

Table 1. Pathogenicity results of *Pyricularia oryzae* strains on maize, barnyard grass, rice, foxtail millet, and wild foxtail millet in Iran (Pordel et al., 2016, 2019, 2021).

Fungal strain	Host of origin	Locality (Province/City)	Year	<i>Zea mays</i>	<i>Echinochloa. crus-galli</i>	<i>Oryza sativa</i>	<i>Setaria italica</i>	<i>Setaria viridis</i>
IR0156	<i>Oryza sativa</i>	Mazandaran/Tonekabon	2017	1	1	6	1	1
IR0065	<i>O. sativa</i>	Guilan/Rasht	2015	1	1	6	1	1
IR0244	<i>O. sativa</i>	Guilan/Sangar	2017	1	1	6	1	1
IR0246	<i>O. sativa</i>	Guilan/Someh sara	2017	1	1	6	1	1
IR0261	<i>O. sativa</i>	Mazandaran/Amol	2017	1	1	6	1	1
IR0106	<i>Setaria italica</i>	Mazandaran/Gharakhil	2016	1	1	1	6	5
IR0103	<i>S. italica</i>	Mazandaran/Gharakhil	2016	1	1	1	6	5
IR0104	<i>S. italica</i>	Mazandaran/Gharakhil	2016	1	1	1	6	5
IR0233	<i>S. viridis</i>	Guilan/Lasht-e-Nesha	2017	1	1	1	5	6
IR0253	<i>S. viridis</i>	Mazandaran/Shirgah	2017	2	1	1	5	6
IR0018	<i>S. viridis</i>	Golestan/Gorgan	2012	2	1	1	5	6
IR0001	<i>Zea. mays</i>	Mazandaran/ Gharakhil	2012	6	-	1	1	1
IR0012	<i>Z. mays</i>	Mazandaran/ Gharakhil	2012	6	1	1	3	1
IR0013	<i>Z. mays</i>	Mazandaran/ Gharakhil	2012	6	6	1	1	1
IR0014	<i>Z. mays</i>	Mazandaran/ Gharakhil	2012	6	6	1	2	2
IR0015	<i>Z. mays</i>	Mazandaran/ Gharakhil	2012	6	-	1	1	1
IR0016	<i>Z. mays</i>	Mazandaran/ Gharakhil	2012	6	-	1	3	2
IR0093	<i>Z. mays</i>	Mazandaran/ Gharakhil	2016	6	1	1	1	1
IR0094	<i>Z. mays</i>	Golestan/ Agh-Ghala	2016	6	-	1	1	1
IR0095	<i>Z. mays</i>	Golestan/ Agh-Ghala	2016	6	1	1	1	1
IR0114	<i>Z. mays</i>	Golestan/ Agh-Ghala	2016	6	-	1	1	1
IR0084	<i>Echinochloa. crus-galli</i>	Golestan/ Agh-Ghala	2016	6	6	1	1	-
IR0083	<i>E. crus-galli</i>	Golestan/ Agh-Ghala	2016	6	6	1	1	-
IR0102	<i>E. crus-galli</i>	Golestan/ Agh-Ghala	2016	6	6	1	1	-
IR0088	<i>E. crus-galli</i>	Golestan/ Agh-Ghala	2016	5	1	1	1	-
IR0050	<i>E. crus-galli</i>	Golestan/ Agh-Ghala	2015	5	6	1	1	1
IR0142	<i>E. crus-galli</i>	Golestan/Azadshahr	2016	5	6	1	-	-
IR0030	<i>E. crus-galli</i>	Golestan/Kordkuy	2012	5	6	-	-	-
IR0184	<i>E. crus-galli</i>	Golestan/Toskestan	2017	1	4	1	1	1
IR0205	<i>E. crus-galli</i>	Guilan/Sharafshadeh	2017	5	5			

Lesion types are as follows: -, not tested; 1, no signs of infection; 2, small brown lesions; 3, small lesions with yellow centers and brown margins; 6, large diamond-shaped lesions (Silue, et al., 1992). * ND, no data.

from leaf and neck blast to identify idiomorphs of mating-type using Multiplex PCR. In this study, 555 bp fragment was amplified for all isolates, so the mating type of all isolates was determined to be *Mat 1-1*. Pordel et al. (2019) identified idiomorphs of mating-type 141 isolates from rice, barnyard grass, maize, wild foxtail millet, and foxtail millet. The results showed *Mat 1-1* mating type is the dominant type in isolates from rice and foxtail millet, and *Mat 1-2* mating type is the predominant type in isolates from barnyard grass, maize, and wild foxtail millet. They crossed the weeds and crops isolates with tester strains from CIRAD institute, France. The sexual state's induction between isolates from rice, wild foxtail millet, and foxtail millet crossed with tester isolates was successful under controlled laboratory conditions. Long typical ascocarps of *Pyricularia* sp. were observed over the media after 21 days incubation. Typical ascospore having four cells, spindle-shaped, 17-25 × 4-8 μm. All strains from weeds and crops did not produce perithecia when crossed with each other (Pordel et al. 2019).

Conclusion and Outlook

Many studies have been conducted on *P. oryzae*, blast causal disease agent in Iran, which leads to the understanding of the morphology, molecular criteria, and biology of the blast disease pathogen. Taxonomic studies on Pyriculariaceae showed that conidia of the *P. oryzae* is entirely different from the close species, *P. grisea*, in addition to phylogenetic analysis by gene regions. Host range study confirmed the pathogen

could infect the strict host and infect the original host aggressively. *Pyricularia oryzae* is a heterothallic fungus whose sexual compatibility is controlled by genes at the *Mat1* locus, with sexual reproduction being possible only between strains of the opposite mating type (*Mat1-1* and *Mat1-2*). All studies showed all rice strains belong to *Mat1-1*, but both of *Mat1* locus are found in the weed plants. The sexual stage was conducted with isolates from crops and weeds on rice flour- agar medium opposite each other and testers under laboratory conditions. None of the weeds and crop isolates produced perithecia when crossed with the opposite mating type of the pathogen collected from Guilan, Mazandaran, Golestan and Razavi Khorasan provinces in Iran. Although these mating types and the fertility experiments showed these populations are asexual, they produced the mature perithecia when crossed by tester strains. Cross-fertility tests were confirmed sterility and possible early post-mating genetic incompatibilities acted as substantial barriers to gene flow among the populations in nature. We will integrate discoveries into a comprehensive understanding of the genomic, ecological, and virulence repertoires of *P. oryzae*, as well as molecular mechanisms underlying disease development in the host plant by high-throughput DNA sequencing. The first complete genome sequence of the maize and barnyard grass strains has recently been released in Iran. Whole-genome sequencing allowed us to study the emergence of the maize blast in the north of Iran; we found the barnyard grass strains expand their host and infect the maize.

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مروری بر یافته‌های زیست‌شناسی و آرایه‌بندی در ایران

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چکیده: این بررسی مروری بر یافته‌های زیست‌شناسی و آرایه‌بندی عامل بیماری بلاست، *Pyricularia oryzae*، در ایران را فراهم آورده است. علائم شناسی جامع و مقایسه‌ای و پراکنش جغرافیایی استرین‌های *P. oryzae* شرح داده شده است. تاریخچه آرایه‌بندی *P. oryzae* در مقایسه با گونه نزدیک شرح و اختصاصیت میزبانی و ژنتیک جمعیت استرین‌های بدست آمده از علف‌های هرز و گیاهان زراعی مشخص شده است. وضعیت سازگاری جنسی و تیپ آمیزشی *P. oryzae* نیز مورد بحث قرار گرفته است.

کلمات کلیدی: بیماری بلاست، پراکنش جغرافیایی، میزبان، معیارهای آرایه‌بندی