

Review of agronomic and genetic diversity of Moroccan rice varieties, and their resistance to blast disease (*Pyricularia oryzae*)

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Abstract. The study on agronomic and genetic characteristics of rice has given us scope to select varieties with desirable characteristics to mitigate various constraints. Rice (*Oryza sativa*) is the staple food for half of the world's population. However, its production is hampered by a variety of biological constraints. The Blast disease (*Pyricularia oryzae*) is an important rice disease, and one of the most effective control methods is to use resistant varieties. Study areas in Morocco include the Gharb plains. For all methods, cultural practises like soil levelling seem to be important, but biological control is not widely adopted due to cost, efficacy, and climatic conditions. The bibliographic synthesis was carried out in this context with the main goals of contributing to a better understanding of rice cultivation in Morocco; to identify and characterise the structure of the rice blast pathogen (*Pyricularia oryzae*), which will allow us to characterise the effects of rice blast; and to research on the Gharb rice field, which resulted in resistant varieties, which will potentially allow producers to have resistant varieties to overcome the diseases. The introduction and development of new rice varieties with high agronomic and socioeconomic value; the selection of lines with high yield, good grain quality, and precocity that are adapted to Moroccan conditions; as well as the development of new lines from Moroccan rice, are among the specific goals.

Key words: rice's agronomic and genetic, control methods, *pyricularia oryzae*, resistant varieties, morocco.

INTRODUCTION

Rice, being the major staple food and one of the main sources of income and employment, is an important crop all over the world. Almost 90% of the global production and consumption of rice is reported from Asia, where a considerably large part of the world's population resides (www.fao.org; accessed on January 20, 2022).

As a cereal grain, rice (*Oryza sativa* L.) is one of the most widely consumed staple foods globally (FAOSTAT, 2022).

In Morocco, the total area of rice crops reached 7,973 hectares with a production of 64,598 tonnes (FAOSTAT, 2020). Nevertheless, rice domestic consumption is

considered to be one of the lowest in the world (1.2 kg of rice per capita), which represents a major constraint to rice production in the country (Food and Agriculture Organization of the United Nations, 2003).

Furthermore, rice production is affected by biotic and abiotic factors (Acharya et al., 2019). Drought, cold, acidity, and salinity are abiotic factors, while pests, weeds, and diseases are biotic factors (Onyango, 2014). Among the biotic factors, fungal diseases alone are estimated to reduce annual rice production by 14% globally (Agrios, 2005), and among the fungal diseases of rice, rice blast caused by *Magnaporthe oryzae* is of significant economic importance and can cause 70%–80% yield losses of rice (Nasruddin & Amin, 2012; Miah et al., 2013).

Pyricularia oryzae Cavara (teleomorph: *Magnaporthe oryzae*) is one of the most important phytopathogenic fungi because it is the causative agent of rice blast diseases, the most destructive and detrimental disease in rice (Gabriel et al., 2022). *Pyricularia oryzae* affects more than 50 species of grasses, such as wheat, barley, oats, and millet (Dean et al., 2012; Langner et al., 2018; Kurrata et al., 2019).

According to Lage (1997), the presence of *Pyricularia* infection, Helminthosporium disease, and weeds (*Echinochloa crus-galli*, *Panicums spp.*, *Typha spp.*, and *Cyperus spp.*) could slow down rice production (Boulet & Bouhache, 1990). The Food and Agriculture Organization of the United Nations (FAO) stated in 2003 that the most common weed species affecting rice in the Mediterranean region belong to the Poaceae and Cyperaceae. In the Gharb region, the most common weeds are *Panicum* (*P. repens*, *Ligustrum obtusifolium* Del.), *Typha* (*T. latifolia* L., *T. marsii* Bat.), *Scirpus spp.*, *Cyperus spp.*, and *Echinochloa spp.* (Miège, 1951). These species are well adapted to the different agroecosystems where rice is cultivated and can promote the conservation and multiplication of pathogenic species (Pugh & Mulder, 1971; Singh et al., 2008).

Benkirane et al. (2000) observed that Moroccan isolates of *Pyricularia oryzae*, originating from *Stenotaphrum secundatum*, are pathogenic for rice. Likewise, Serghat et al. (2005) found that the fungal pathogen *Pyricularia oryzae*, isolated from *Echinochloa phyllopogon* and *Phragmites australis*, induces leaf lesions and sporulates on the foliage of certain rice varieties.

In Morocco, surveys in the rice-growing area and a study of the mycoflora of rice have revealed several pathogens responsible for the diseases, including foliar diseases. Among these diseases, rice blast (*Pyricularia oryzae*) and rice Helminthosporiose (*Helminthosporium oryzae*) are the most dominant, but their effects on yield are not known in Mediterranean regions such as Morocco (Tajani et al., 2001). In Morocco, most of the cultivated rice varieties are susceptible to several fungal species (Katsura et al., 2007). This shows that in Morocco, rice leaf diseases largely reduce grain weight. The calculated yield losses change depending on the severity of the attack and the efficiency of the fungicide at the treatment stage. Early epidemics were discovered to be more devastating than late epidemics (nearly a 15% yield reduction). To reduce output losses owing to restrictions such as genetic constraints, genetic improvement using biotechnology instruments remains a viable option (Moinina et al., 2018).

The most common approach to combating blast disease is to use resistant strains of rice plants. Initially, the use of resistant strains was effective in controlling blast disease. However, in most cases, host resistance becomes ineffective due to the emergence of recent blast races (Kurrata et al., 2019). High genetic variation, or genomic adaptation, is one of the mechanisms of *P. oryzae* that can overcome host resistance to prevent host

recognition (Longya et al., 2020). In this regard, biological control could be an effective alternative to controlling blast disease. Biocontrol of diseases in plants controls the population of phytopathogens with the aid of living organisms (Heimpel & Mills, 2017; O'Brien, 2017).

In Morocco, pyriculariosis is formerly known, and significant losses have already been reported (Duangporn, 1977; Lakrimi, 1989). During surveys carried out from 1997 to 1999, it was found that this disease causes damage mainly in the Larache region. The absence of fungicides registered for this disease in Morocco does not allow for treatments. As the selection of new rice varieties takes place in the Gharb region, where pyriculariosis pressure is low, pyriculariosis resistance has never been considered in the selection objectives. As a result, the level of resistance of the varieties used by farmers is not known. The major epidemics observed in the Larache region have raised questions about the potential risks of epidemics in the Gharb region and, in particular, about the level of resistance of Moroccan varieties (El Guilli et al., 2000). The objective of this study was therefore to evaluate the resistance to pyriculariosis of several varieties used or newly selected in Morocco.

Rice in Morocco

In Morocco, the rice sector is socio-economically important. It has performed remarkably well in recent years, thanks to a series of measures taken as part of the Green Morocco Plan, which has helped to organise the sector. The cultivation of rice has experienced a remarkable dynamic, allowing the national production to cover more than 72% of the country's consumption needs. The Gharb region contributes to 75% of the national production (MAPM, 2020).

In Morocco, rice consumption is considered to be one of the lowest in the world (1.2 kg of rice per capita), which represents a major constraint for the development of rice production in the country (FAO, 2003). Gharb rice production estimates the average gross yield at 77.7 kg ha⁻¹ for a harvested area of 4,999 ha (ORMVAG 2013). In 2004, a study on Gharb showed that the sector had a turnover of 200 million Dh, considered too low compared to the real potential of 600 million Dh. The Plan Maroc Vert (PMV) aims to exploit 9,000 ha by 2020 (The Economist, 2016).

The Regional Office for the Development of Gharb (ORMVAG, 2013) discovered that some technical, economic, and organisational constraints remain to be overcome in order for this sector to be truly integrated into the Regional Agricultural Development Plan. But since the implementation of the Plan Maroc Vert and given the need to upgrade this important sector at the regional level, the Moroccan government has committed to providing it with the necessary support within the framework of a programme contract linking it to an interprofessional. The overall indicators for the rice industry are shown in Table 1.

Table 1. Overall indicators for the rice industry by 2020 (Source: ORMVAG, 2013)

| Indicators | Initial situation 2010 | Horizon 2020 |
|-------------------------------------|------------------------|--------------|
| Total surface area (ha) | 4,500 | 9,000 |
| Average yield (t ha ⁻¹) | 7,5 | 8,0 |
| Production (t) | 33,750 | 72,000 |
| Added value (MDH) | 54,00 | 112,6 |
| Gross margin (DH ha ⁻¹) | 81,400 | 10,000 |
| Employment (1,000 d.t) | 306 | 585 |
| Number of projects of aggregation | 4 projects | 5 |
| Number of aggregates | - | 100 |

Evolution of the rice sector in Morocco

More than 75% of the seeded fields are owned by the agrarian reform cooperatives, which use nearly 8,100 ha of the 12,000 ha designated for rice production in the Gharb. The remaining 25% are owned by collectivists and Melkists (MAPM, 2020).

In Gharb, the total number of rice farmers is 5,500, of which about 1,500 grow rice regularly. The provision of certified seed subsidies, increased yields at the Gharb level (about 8,108 t ha⁻¹) were made possible by leveling with the complementary, 107 and irrigation control.

In Larache, yields (6.5 t ha⁻¹) are still below target, owing to sparrow attacks and the region's unique environment (cold at the beginning of the season, heat waves during the flowering phase). It's worth noting that the yield achieved in 2018–19 (8.2 t ha⁻¹) was higher than the production culture target of 8 t ha⁻¹. Peaks of more than 11 t ha⁻¹ were detected in 5% of the rice growers who were notified. The evolution and production of rice are illustrated in Fig. 1 and Fig. 2.

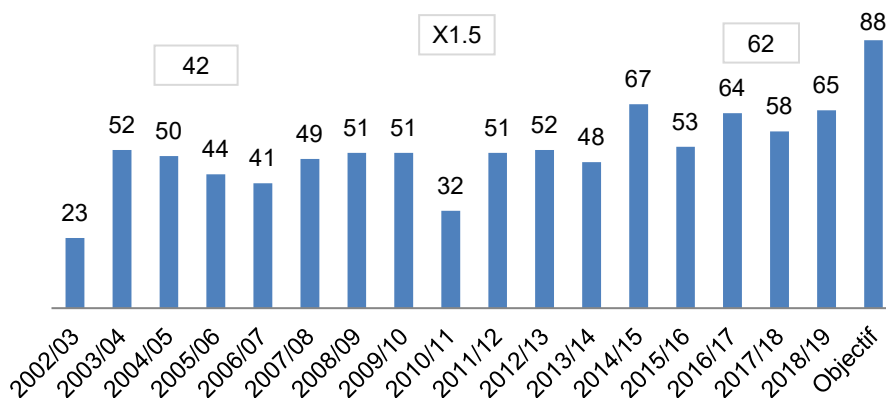


Figure 1. The Evolution of Rice Production (Source: MAPM, 2020).

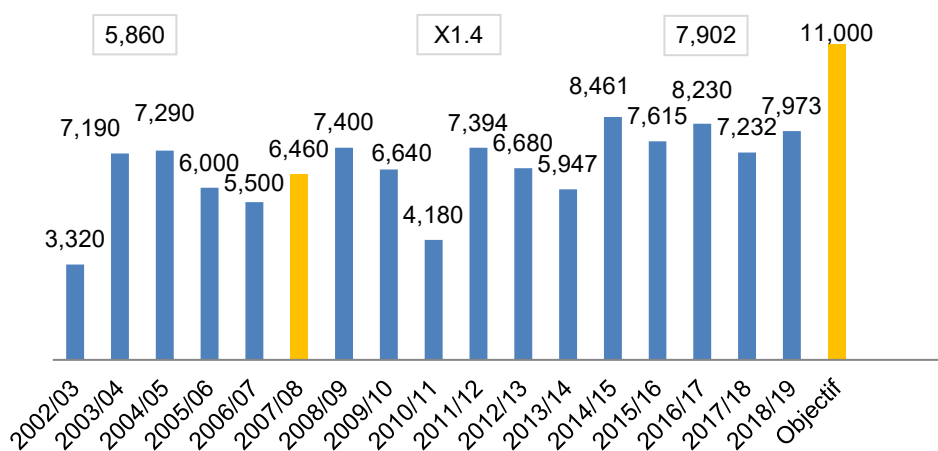


Figure 2. Evolution of the rice area (ha) (Source: MAPM, 2020).

Pedoclimatic characteristics of the Gharb region and their impact on rice cultivation

Western Morocco offers a diverse range of climates, ranging from desert to sub-humid bioclimatic stages. Continentality and latitude are the primary determinants of climate dispersion (Zidane et al., 2010).

Temperature and humidity

Depending on the phenological stage (25 to 31 °C for tillering and 30 to 33 °C for heading), the best temperature for rice development and growth is 25 to 35 °C.

According to Regional Office for the Development of Gharb (ORMVAG, 2013), rice cannot be grown at temperatures below 10 °C or beyond 45 °C. High temperatures in late spring and early summer in Gharb are ideal for this crop.

According to Tajani et al. (1997), the amount of annual rainfall is highly variable. The annual rainfall averages between 450 and 600 mm, with a 90 percent concentration between October and April. In the winter, the average daily temperature is 11 °C, while in the summer it is 27 °C. From mid-April through the end of September, the weather is ideal for rice growing. Rice, on the other hand, is normally planted from June to July due to a scarcity of water before this date.

Soils

In the Gharb, the *tirs* (vertisols) and *merjas* soils (vertisols hydromorphic) are well adapted to rice farming, except for those that are too draining (permeable) or too compact (FAO, 2003). Their proportion of total limestone ranges from 0% to 49% (Miège, 1951). Their organic matter concentration ranges between 0.74 and 2.88 percent, and their pH (6.75–8.57) is normally basic, rarely plainly basic, and only rarely mildly acidic (Zidane et al., 2010). Furthermore, surface water NaCl concentrations ranged from 0.2 to 1.7 g L⁻¹, while pore water NaCl concentrations ranged from 0.25 to 3 g L⁻¹ (El Bildi et al., 2006). Rice is intensively grown and automated in Morocco. It is grown in enclosures that are built to allow submersion watering. The hydromorphic soils of the Gharb region have benefited from this crop (Lage, 1997).

Rice production techniques in Gharb

Tillage and seeding season

Soil cultivation starts in May and is dependent on the availability of materials as well as weather conditions. The soil must be slightly dry. ORMVAG is in charge of this project, which is sponsored by Plan Maroc Vert (PMV). A rice farmer submits a specification to the ORMVAG before the work begins. The head of the Rice Growers' Cooperative confirms these specifications (ORMVAG, 2013).

The Society of Moroccan Agricultural Works is in charge of soil tillage (STAM). The ploughing depth is 14 to 15 cm, and it is done in one pass with the stubble plough. The cover crop is then passed twice to begin the levelling and planning of the soil. Because the levelling operation is critical for all subsequent processes, this last one is accomplished with a resurfacing or board. Many farmers have dry fields; many farmers plant rice after soaking it in water, on submerged fields, and in submerged water. The soil is ploughed 1 to 2 times. It is planted at 140–200 kg ha⁻¹ (Tajani et al., 1997).

Principal varieties

The most prevalent round rice types on the Gharb plain are Elio, Megassa, and Thaiparla, while the most common long rice varieties are Thaibonet, Lido, Arba, and Puntal. The most extensively farmed variety, according to Chataigner (1997), is Elio, which accounts for 80 per cent of all rice-growing acreage. According to FAO (2003), short-grain rice genotypes are the most extensively farmed in Morocco due to their disease resistance compared to long-grain genotypes. The latter are early-maturing and have high production potential, but they require careful watering and soil levelling.

Nutrient requirements

In this area, usual practice is application of fertiliser right after tillage operation. DAP (diammonium phosphate) is administered at a rate of 3 kilogrammes per hectare, either manually (broadcast) or by fertiliser spreader. In terms of cover crop application, urea at a rate of 10 kg ha⁻¹ is applied 2 or 3 days after planting, during the growth of the crop (jas). The yield of grains is determined by nitrogen. It is suggested that at least 15 days pass between applications (Tajani et al., 1997). However, excessive nitrogen fertilization often leads to environmental pollution, lodging, and diseases, especially rice diseases, especially rice blast (*Pyricularia oryzae*), so a judicious distribution of this fertilizer is considered necessary.

Constraints related to rice production

In rice production, weeds, pests, and pathogens, especially rice blast (*Pyricularia oryzae*) and rice *Helminthosporium oryzae*, are of great economic importance. Rice blast (*Pyricularia oryzae*) and rice helminthosporium (*Helminthosporium oryzae*) are of great economic importance. Oerke (2006) estimated the potential losses from these pests to be 37, 25, and 13%, respectively. Surveys in rice fields in Morocco (Tajani et al., 2001) have identified these dominant fungal diseases, but their effects on yield are not known.

Rice blast disease

The rice blast is distributed in about 85 countries on all continents where rice is grown, both in paddy and upland conditions. In both rice fields and upland conditions, it is one of the most devastating diseases of rice (*Oryza sativa* L.) under favourable conditions (Ou, 1985; Miah et al., 2017). In addition to rice, *Pyricularia oryzae* also infects other agronomically important crops, such as barley, wheat, and millet (Valent et al., 1991).

The pathogen of rice blast was first known as *Pyricularia oryzae* Cavara in 1892, but it is indistinguishable from *Pyricularia grisea*, which causes greasy spots on other grasses (Agrios, 2005). The genus *Pyricularia*, first described in 1880, was named after *Pyricularia grisea* (Cooke) Sacc., the name given to the anamorph of crabgrass isolates. According to Chauhan et al. (2017), *Magnaporthe grisea* (Hebert Barr) is the teleomorph of crabgrass, a flaming ascomycete fungus, and belongs to the family Magnaporthaceae family. The fungus produces several toxins, e.g., *Pyricularin* and *-Picolinic*, which appear to contribute to the development of rice blast (Agrios, 2005).

The rice blast disease cycle

The asexual cycle is the only mode of reproduction observed in nature (Zeigler, 1998). When conditions are favourable, from the mycelium, conidia are produced (Saleh,

2011). There is also a sexual cycle (Fig. 3), which has never been directly observed in nature but is produced in vitro.

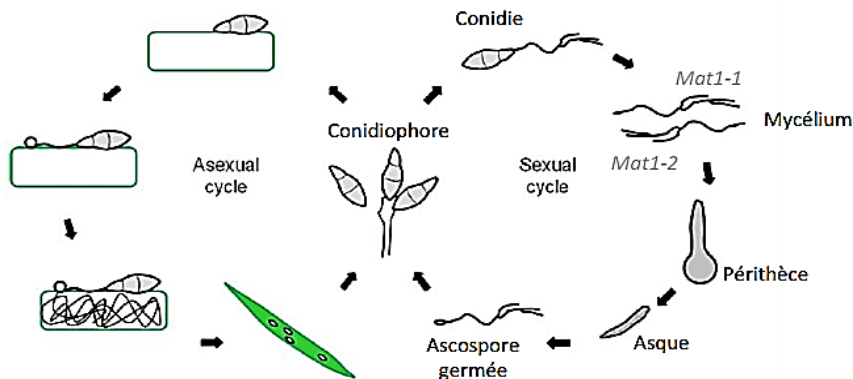


Figure 3. Sexual and asexual reproductive cycles of *Pyricularia oryzae* Source (Saleh, 2011).

The sexual cycle of *Pyricularia oryzae* is suspected to have existed within populations attacking rice in many localised areas of Asia (Zeigler 1998; Tharreau et al., 2009; Saleh et al., 2012; Gladieux et al., 2018; Thierry et al., 2020).

Rice blast is a polycyclic disease that occurs regularly. The infection process of *P. oryzae* can be summarised in five basic steps: (1) conidia generation and dissemination; (2) conidia attachment to a host surface; (3) appressorium creation; (4) penetration of the initial host cell; and (5) invasive hyphae growth (Hamer et al., 1988). During periods of high relative humidity (90 percent or higher), the fungus develops and releases conidia (Kato, 2001; Miah et al., 2017).

When a conidia lands on the surface of a rice leaf, the champignon begins its infection process (Ou, 1985; Wilson & Talbot, 2009). When there is free water, the conidies grow, and the germinating tube becomes an appressorium in which the champignon feeds on the plants (Wilson & Talbot, 2009; Miah et al., 2017). The symptoms begin to appear 4 to 5 days after the infection (Kato, 2001). These sporulent lesions emancipate conidies, which are dispersed by the wind. A single lesion can cause up to 6,000 conidies in one night, and an infected rice spikelet can cause up to 20,000 conidies in one night (Ou, 1981).

The conditions for the development of the disease

Rice blast expression is very variable and is influenced by both environmental and plant-specific variables. Moisture, temperature, fertilizer, and light are the most important elements. Moisture is required for the growth of *Pyricularia oryzae*, particularly for germination and the generation of conidia. Furthermore, elevated nitrogen levels encourage infection. *Pyricularia oryzae* grows best at temperatures between 24 and 28 degrees Celsius. At these temperatures, the fungus can penetrate the rice plant in 6–8 hours if there is enough moisture, although, at 34 °C, it appears impossible (Traoré, 2000).

Symptoms of the disease

The initial symptoms appear as white to grey-green lesions or white to grey-green spots, with dark green borders and green borders. Rice blast can infect most rice organs except the root system. Organs of rice except the root system (Lanoiselet, 2008). Infected seeds are a source of the primary inoculum. Dead infected seeds could serve as the primary inoculum when placed on the field during seedling development (Hubert et al., 2015; Long et al., 2000). If panicle infection occurs early, the grains do not fill and the panicle remains erect. If the panicle is infected later, the seeds become partially filled and, due to the weight of the grain weight of the seeds, the base of the panicle breaks and the panicle (Agrios, 2005).

Pyricularia grisea can infect and develop on different aerial parts of the rice plant. Thus, one distinguishes different symptoms, according to the attacked organ. Foliar blast on the leaf blade (Fig. 4, a), small greyish spots 1 to 2 mm in diameter appear first (Andrianarisoa, 1970). These small spots each correspond to a conidial infection point from which the developing parasite will form spindle-shaped or oval lesions (DPV and GTZ, 1990).

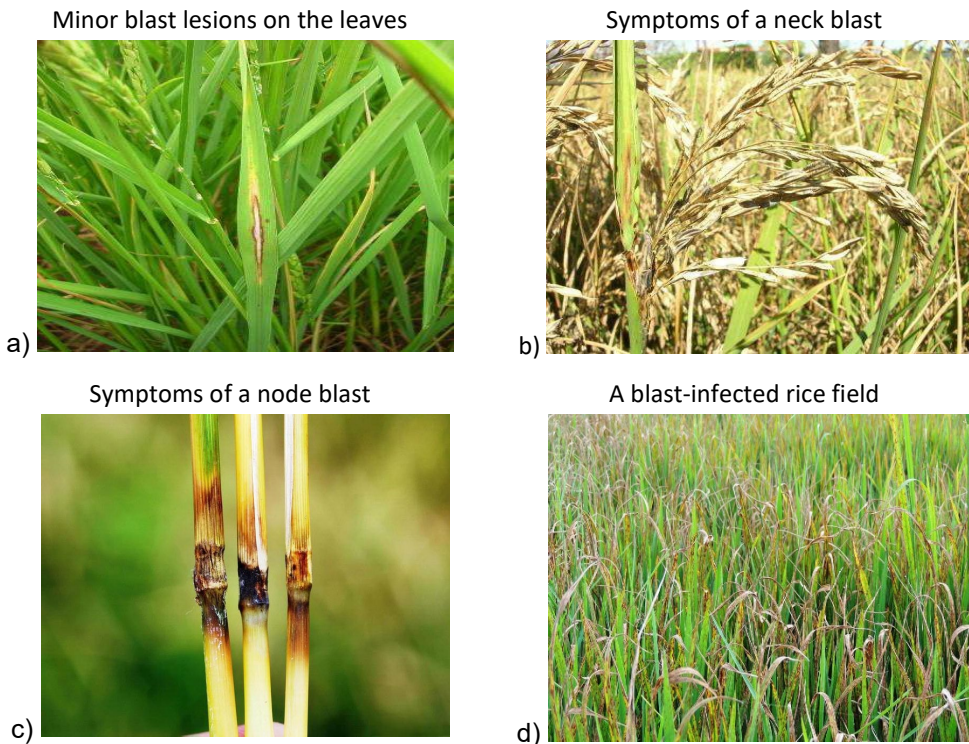


Figure 4. Symptoms of a rice blast (Chauhan et al., 2017): a) minor blast lesions on leaves; b) neck symptoms; c) node blast symptoms; d) blast-infected rice field Spread of blast disease.

At maturity, a typical lesion is characterised by a pale grey or greyish-white central area surrounded by a fairly well-defined brownish area. This is an area of necrosis. At the periphery of this central zone appears a zone of destruction of the chloroplasts. It is light yellow. Following a severe attack on a leaf, the blades can be completely dried,

taking on a burnt aspect. At this stage, the disruption of photosynthetic activity considerably affects the growth of the plant (Andrianarisoa, 1970).

Panicular blast disease

Panicular blast (Fig. 4, b), or neck blast forms the most characteristic symptom of this disease. Brown to black spots is observed on the inflorescence reaches or the spikelet (Hari et al., 1997). A large lesion may form at the base of the panicle which becomes white. In the most extreme cases, the stem eventually breaks. At this stage, the disease prevents grain filling (Sere, 1981). The fungus causes spots on the leaves, nodes, and various parts of panicles and grains, but rarely on the sheath in nature. The spots are elliptical with more or less elongated tips. In general, the centre of the spots is grey or whitish, and the periphery is brown or reddish-brown (Wopereis et al., 2008).

Nodal blast disease

This is an attack of the disease on the nodes of the culms (Fig. 4, c). A brown ring can be seen on these nodes at the beginning of the infection. This colour turns greyish as the cellulose tissue is destroyed. The stem becomes brittle and can easily break at the nodes (Dpv and Gtz, 1990).

Climate factors such as rain and wind play an important role in spore dispersal, while humidity and temperature caused by dew and fog are involved in the development of the fungus and increase its ability to infect. The source of inoculum can be infested rice or crop residues (straw) since the mycelium can survive for up to three years at temperatures between 18 °C and 32 °C and can survive changes in the environment. Conidia can live for one year at a temperature of 8 °C and a relative humidity of 20% (Zeigler et al., 1994).

Global genetic structure of *Pyricularia oryzae*

Rice *Pyricularia Oryzae* populations have been extensively studied to understand the evolution of the pathogen and to adapt control techniques and breeding programs. These researchers used a variety of molecular markers to characterize global populations of *P. oryzae*, including simple sequence repeats (SSR), sequence-characterised amplified region (SCAR), single nucleotide polymorphism (SNP), and others (Adreit et al., 2007; Tharreau, 2008; Gladieux et al., 2018; Zhong et al., 2018; Thierry, 2019; Thierry et al., 2020). By comparing the number of clonal lineages reported in different investigations, Zeigler (1998) concluded that the genetic diversity of *Pyricularia oryzae* was greater in the area spanning South, East, and Southeast Asia than in other regions of the world. The most in-depth investigations into the genetic organisation of *P. oryzae* populations around the world found three or four distinct groups. This research revealed that Asia will be the core of rice pathogenic population diversity and origin for all world populations (Tharreau et al., 2009; Saleh et al., 2014; Gladieux et al., 2018; Zhong et al., 2018; Thierry et al., 2020).

The control of rice blast disease

Rice blast is controlled using a variety of strategies, some of which are employed in conjunction (Ghazanfar et al., 2009): cultural practises (Biological control), chemical control, and the adoption of resistant varieties (genetic control). Biological control is not used in the field, as far as we know.

Chemical Controls

To control blast pathogen infestations, farmers depend heavily on chemical fungicides because they are readily available and quick-acting. Research conducted in Chitwan, Nepal, found that applying Tricyclazole 22% + Hexaconazole 3% SC three times at weekly intervals from the booting stage resulted in the best disease control (87.03% and 79.62% in leaf and neck blast, respectively), the highest grain yield (4.23 t ha⁻¹), and a 56.09% improvement in yield over the control one (Magar et al., 2015). Experiments performed in Pakistan by Hajano et al. (2012) discovered that using the fungicide mancozeb at 1,000 and 10,000 ppm fully inhibits the mycelial growth of *Magnaporthe grisea*, making it the most effective fungicide. Similarly, experiments conducted in Thailand by Kongcharoen et al. (2020) found that mancozeb exhibited the highest level of fungicidal activity against the blast pathogen *Pyricularia oryzae* with an EC₅₀ value of 0.25 parts per million (ppm). Furthermore, experiments conducted in Nigeria concluded that two systemic fungicides, benomyl and tricyclazole, were found to be effective and significantly increased grain yield over the control one by 18.14% and 42.17%, respectively (Enyinnia, 1996). In an experiment conducted by Padmanabhan et al. (1971), it was found that spraying copper and organic mercury-based fungicides in a schedule covering 5–6 sprays - one spray at the seed bed (on 21-day-old seedlings), two to three sprays at the post-tillering phase at an interval of 10–15 days, and two sprays at ear emergence - one spray before emergence and another 5 days later - were also effective in controlling neck blast infections on local indica varieties. The use of chemicals is non-environmentally friendly (Thapa et al., 2019), and overuse of chemicals for a successive year develops a resistance in the fungus and poses serious threats in the future. Moreover, pesticide exposure leads to acute pesticide poisoning that has adverse health effects on vital body systems such as the digestive, respiratory, and nervous systems, and farmers are the most at risk of pesticide poisoning because of their prolonged exposure during the production season (Pingali & Roger, 2012). The residue of chemicals persists in the grain, straw, and soil, which may cause adverse effects on farm labour (Pingali & Roger, 2012).

Biological control

The indiscriminate use of various plant protection chemicals has resulted in environmental hazards, so finding alternative sources is of immense importance and also preferable (Thapa et al., 2019; Ahamad et al., 2020).

Biological control of plant diseases is typically inexpensive, long-lasting, and safe towards the environment and living organisms, however, biological control can be a slow process, and the search for suitable biocontrol agents requires considerable time and effort (Law et al., 2017).

The first report of a biological agent found effective against *Pyricularia oryzae* was *Chaetomium cochliodes* (Pooja & Katoch, 2014). When the rice seeds were coated with the spore suspension of *C. cochliodes*, the early infection by blast was controlled, and the seedlings were healthy and taller than the control (Pooja & Katoch, 2014). Experiments conducted by Bhusal et al. (2018) showed that seed treatment with *Trichoderma viridi* in 5 mL L⁻¹ of water was found to be effective against leaf blast. Furthermore, Hajano et al. (2012) discovered that, of the six bio-control agents tested against *M. oryzae*, *P. lilacinus* inhibited the most, followed by *T. pseudokoningii*, *T. polysporum*, and *T. harzianum*. According to greenhouse studies conducted by Law

et al. (2017), infected rice seedlings treated with *Streptomyces* resulted in an up to 88.3% reduction in rice blast disease. Furthermore, recent studies on the biocontrol of rice blast showed that *Bacillus subtilis* strain B-332, 1Pe2, 2R37, and 1Re14 were found to be more effective (Changqing et al., 2007; Jin-Hyoung et al., 2008). Rice blast biocontrol experiments revealed that a powder formulation of *Pseudomonas fluorescens* strain Pfl at 10 g kg⁻¹ inhibits rice blast growth (Vidhyasekaran et al., 1997).

In order to achieve successful biological control, the biocontrol agents should be isolated from and applied to locations with similar environmental conditions (Suprapta, 2012).

Genetic control

The use of varieties that are resistant to rice blast disease offers better control strategies. It is less expensive and not as laborious as other methods. Although developing a rice blast disease resistance variety is time-consuming and difficult for plant breeders because the fungus can evolve and mutate to overcome resistance genes (Zhou et al., 2007). Blast-resistant rice genotypes have been developed with the use of marker-assisted backcrossing (Miah et al., 2017).

Cultivation of the host-resistant plants is the most efficient way to manage the disease because it is a convenient, cost-effective, environment-friendly, long-term, reliable, and realistic approach to plant protection for resource-constrained farmers (Ou, 1985; Bonman et al., 1992). Studies show that the degree of resistance increases with an increase in the proportion of silica applied and also with the amount of silicon accumulated in the plant (Pooja et al., 2014).

Generally, horizontal and vertical resistance are used in developing disease resistant cultivars (Rijal & Devkota, 2020). Due to the high genetic variability of the fungus, resistance to infection by *Pyricularia oryzae* can be short-lived (Khemmuk, 2017).

The breakdown of resistance to *Pyricularia oryzae* results from the evolution of genetic variants (races) in the pathogen populations (Liu et al., 2011).

The genetic diversity of rice

The *Oryza sativa* genome comprises more than 150,000 varieties cultivated around the world and about 107,000 accessions in the IRRI gene bank, including 5,000 of which 5,000 are wild species (Courtois, 2007). This diversity comes from natural crosses of *O. sativa* with wild or weedy forms of or weedy forms of *O. rufipogon* or from intra-sativa crosses combined with natural and human selection since domestication (Khush, 2005). To evaluate the genetic diversity of accessions and better exploit its potential, the exploitation of its potential, the use of markers remains indispensable. A good marker should be single-inherited, multi-allelic, and co-dominant. The rice genome has been completely sequenced since 2005, and nearly 400 million DNA 'letters' have been identified and positioned. We used the microsatellite molecular markers of the 'Core Map' of Orjuela et al. (2009). The development of molecular markers during the last decade has offered the possibility of establishing new approaches to improve breeding strategies (Najimi et al., 2003). They have become an essential tool in breeding programmes for new rice varieties (*Oryzaspp.*) for resistance to biotic and abiotic stresses and offer alternatives to the use of traditional phenotypic markers. Molecular genetic markers are of different types (RAPD, RFLP, AFLP, SSR, and SNP). In genetic

diversity studies, microsatellite markers have been the most widely used in rice in recent years (Semon et al., 2005).

The genotyping characterization was conducted using SSR (Simple Sequence Repeats) markers (22 microsatellites), and continued with genetic diversity and polymorphism information content (PIC) analysis (Puspito et al., 2022).

Among the studied set of microsatellite markers, two of the most informative SSR-markers - RM 7481 and PrC3 - showed high efficiency in detecting intraspecific polymorphism of rice varieties. About 400 backcrossed self-pollinated rice lines with introgressed and pyramided resistance genes *Pi-1*, *Pi-2*, *Pi-33*, *Pi-ta*, *Pi-b* to *Pyricularia oryzae Cav.* were obtained within the frameworks of program to develop genetic rice sources resistant to blast. The conducted testing for resistance to blast and the assessment by economically valuable traits have allowed to select the prospective rice samples. The plant samples of F2 and BC1F1 generations with combination of resistance to blast genes (*Pi*) and submergence tolerance gene (*Sub1A*) in homozygous and heterozygous state that is confirmed by the results of analysis of their DNA have been obtained. The obtained hybrid plants are being tested in breeding nurseries for a complex of economically valuable traits. The best plants will be selected and sent to State Variety Testing system. Their involving in rice industry will reduce the use of plant protection chemicals against diseases and weeds, thereby increasing the ecology status of the rice industry (Dubina et al., 2022).

Three SSR markers (introduced by SBS Genetech Co., Ltd., China) linked to rice blast resistance genes; *Pi* genes (Akagi et al., 1996; Temnykh et al., 2001; Hassan et al., 2017) were screened on DNA templates. The details of the used markers and the primer sequences are presented in Table 2.

Table 2. The three used SSR molecular markers, their primers, nucleotide sequences, and essential information

| Primer | F/R Primer 5'→3' | CL | Linked <i>Pi</i> gene | Repet motif | Annealing temperature | References |
|--------|--|----|-----------------------|-------------|-----------------------|---|
| RM155 | F-GAGATGGCCCCCTCCGTGATGG R-TGCCCTCAATCGGCCACACCTC | 12 | <i>Pita-2</i> | (CTT) 7 | 68 | Akagi et al. (1996); Hassan et al. (2017) |
| RM512 | F-CTGCCTTTCTTACCCCCTTC R-AACCCCTCGCTGGATTCTAG | 12 | <i>Pi-12</i> | (TTTA) 5 | 60.5 | Temnykh et al. (2001); Hassan et al. (2017) |
| RM541 | F-TATAACCGACCTCAGTGCCC R-CCTTACTCCCATGCCATGAG | 6 | <i>Pi-9</i> | (TC) 16 | 60.5 | Temnykh et al. (2001); Hassan et al. (2017) |

F/R Primer: forward/reverse primer, CL: chromosomal location.

Currently, approximately 100 genes of resistance (*R*) to rice blast are known; of these, 51% are from *indica* genotypes, 45% from *japonica* genotypes, and 4% from wild species of rice (Sharma et al., 2012) (Table 3). The identified *R* genes have broad nomenclature, and, often, the same resistance gene can have different names (Koide et al., 2009).

Table 3. List of genes manipulated for rice blast resistance

| Gene name | Function | Manipulation transgenic | Effects | Reference |
|-----------------|--|-------------------------|-----------------------------------|--------------------------|
| <i>OsPi-d2</i> | <i>R</i> gene | Overexpression | Resistance to neckblast incidence | Chen et al. (2010) |
| <i>MoHrip1</i> | Elicitor gene | Overexpression | High resistance against blast | Wang et al. (2017) |
| <i>OsWRKY53</i> | <i>R</i> gene | Overexpression | High resistance against blast | Chujo et al. (2014) |
| <i>OsGF14b</i> | Induces expression of jasmonic acid (JA) | Overexpression | Resistance to neckblast incidence | Liu et al. (2016) |
| <i>WRKY45</i> | Induces expression of salicylic acid (SA) | Overexpression | High resistance against blast | Shimono et al. (2007) |
| <i>CYP71Z18</i> | - | Overexpression | High resistance against blast | Shen et al. (2019) |
| <i>MoSDT1</i> | Effector protein | Overexpression | High resistance against blast | Wang et al. (2019) |
| <i>Pi54</i> | <i>R</i> gene | Overexpression | High resistance against blast | Singh et al. (2020) |
| <i>OsCPK4</i> | Calcium-dependent | Overexpression | High resistance against blast | Bundó & Coca (2016) |
| <i>RACK1A</i> | Receptor for activated C-kinase 1A | Overexpression | High resistance against blast | Nakashima et al. (2008) |
| <i>OsCDR1</i> | <i>R</i> gene | Overexpression | High resistance against blast | Prasad et al. (2009) |
| <i>OsWRKY13</i> | Regulating defense-related genes in salicylate-and jasmonate-dependent signaling | Overexpression | High resistance against blast | Qiu et al. (2007) |
| <i>GH3-2</i> | - | Overexpression | High resistance against blast | Fu et al. (2011) |
| <i>OsGH3.1</i> | Component of the hormonal mechanism regulating | Overexpression | High resistance against blast | Domingo et al. (2009) |
| <i>OsNAC6</i> | Transcription factor | Overexpression | High resistance against blast | Nakashima et al. (2007) |
| <i>OsSBP</i> | Homologue of mammalian Selenium-binding proteins | Overexpression | High resistance against blast | Sawada et al. (2004) |
| <i>OsRacB</i> | Allene oxide synthase gene | Overexpression | High resistance against blast | Jung et al. (2006) |
| <i>OsAOS2</i> | Allene oxide synthase gene increases the endogenous jasmonic acid level | Overexpression | High resistance against blast | Mei et al. (2006) |
| <i>OsSERK1</i> | Regulates somatic embryogenesis | Overexpression | High resistance against blast | Hu et al. (2005) |
| <i>OsOxi1</i> | Regulates basal disease resistance | Overexpression | High resistance against blast | Matsui et al. (2010) |
| <i>Gns1</i> | Stress-inducible β -glucanase | Overexpression | High resistance against blast | Nishizawa et al. (2003) |
| <i>Rir1b</i> | Defense-related | Overexpression | High resistance against blast | Schaffrath et al. (2000) |
| <i>OsWAK1</i> | Wall-associated receptor-like protein kinase gene | Overexpression | High resistance against blast | Li & Li (2009) |
| <i>OsSYP71</i> | Oxidative stress and rice blast response gene | Overexpression | High resistance against blast | Bao et al. (2012) |
| <i>BSR1</i> | Putative receptor-like cytoplasmic kinase gene | Overexpression | High resistance against blast | Dubouzet et al. (2011) |
| <i>OsACS2</i> | Key enzyme of ethylene biosynthesis | Overexpression | High resistance against blast | Helliwell et al. (2013) |

The resistance of Moroccan varieties

Several varieties were tested for resistance. The two most widely grown varieties in Morocco are Elio and Thaibonnet. Other varieties include Hayat, Dinar, and Kenz varieties. Nachat, Maghreb, and Bahja are varieties newly registered in the official catalogue by INRA. Farah, INRAM 6, and INRAM 11 are new INRA varieties proposed for the official catalogue. Two other varieties were used as references. Ariete is a French variety whose resistance is acceptable in the Camargue (South of France), and Maratelli is an Italian variety used here as a sensitive control (El Guilli et al., 2000). Sowing seeds in rows was done in trays (45×29×7 cm) containing potting soil that was kept moist after sowing. The trays were then placed in a greenhouse and the seeds were planted (El Guilli et al., 2000).

A collection of Moroccan isolates were collected in 1997 and 1998 from lesions on leaves or panicular stems. The selection of isolates for inoculation was based on the preliminary results of a study of the diversity of the Moroccan population of *M. grisea* using molecular markers and pathogenicity tests (El Guilli et al., 2000).

Eleven isolates, representative of the different clonal lines and existing breeds in the Moroccan population of *Pyricularia oryzae* were used. For the characterization of the resistance of Farah, 10 additional isolates from different countries (China, Cameroon, Ivory Coast, and Thailand) known for their broad virulence spectrum (13 specific resistance genes mounted on 13 tested) have been inoculated on this variety (El Guilli et al., 2000).

Inoculum preparation and inoculation

Identifying sources of inoculum can help to reduce the disease's occurrence and severity (Raveloson et al., 2011). Residues of infected rice and disease-affected seed are the main sources of primary inoculum for the blast (Long et al., 2001; Guerber & TeBeest, 2006; Raveloson et al., 2013).

The isolated and purified *Pyricularia oryzae* inoculum, stored at 5 °C, was re-cultured in PDA medium (Miura et al., 2005). Procedure was adopted in the preparation of conidial suspension. The inoculated plates were incubated in the dark for 12–14 days at 26 °C. For the inducement of heavy sporulation, the culture was scraped aseptically with a sterile toothbrush, and the plates were exposed to near-ultraviolet light at 25 °C for 10 days. Conidia were dislodged by gently rubbing the incubated plates with a small, sterile toothbrush in sterilized, distilled water. The conidial suspension was well filtered through layers of gauze mesh (aperture 300 μ m), and the concentration was adjusted to a final concentration of 1×10^6 spores per ml using a haemocytometer. Tween 20 was added to the prepared suspension (0.02% Tween 20 in 0.25% gelatin) to enhance the proper adherence of conidia to the rice aerial parts (Jia et al., 2003).

The rice plant leaves were inoculated 20 days after planting by spraying the prepared 1×10^6 spores per ml of conidial suspension containing 0.02% Tween 20 in 0.25% gelatin per plot using a knapsack sprayer. Spraying was done slowly and carefully to achieve uniformity on the plant's aerial parts until runoff. The inoculum was sprayed around 18:00 hours of the day and ensured that the entire rice plant surface became wet with conidial suspension, and 20 cm was adopted at three stands per hill and later thinned to two stands per hill two weeks after planting (Azgar et al., 2018).

The pathogenicity test was carried out by inoculating *P. oryzae* isolates into the leaves of healthy rice plants. Fungal colonies were harvested using a brush by adding 10 mL of sterile distilled water (dH₂O), including 0.02% Tween 20. The *P. oryzae* inoculum was sprayed on rice plants aged 18–21 days after planting (Kurrata et al., 2019).

Disease assessment, data collection, and analysis

Disease scoring of the inoculated rice plants was done 10 days after inoculation (Challagulla et al., 2015). The severity of the disease was estimated and recorded by using the disease rating scale of the Standard Evaluation System of the International Rice Research Institute, Philippines, based on the level of severity of the infection on each entry (International Rice Research Institute [IRRI], 2013). Based on leaf blast scores assessment, the accession was categorized as highly resistant (0), Resistance (1), moderately resistant (2–3), moderately susceptible (4–5), susceptible (6–7), and highly susceptible (8–9) (Standard Evaluation System of IRRI, 2013) as shown in Table 4.

Table 4. Disease rating scale 0–9 by International Rice Research Institute, Phillipines (IRRI, 2013)

| Grade | Disease severity | Host response |
|-------|--|------------------------|
| 0 | No lesion observed | Highly resistant |
| 1 | Small brown specks of pin point size | Resistant moderately |
| 2 | Small roundish to slightly elongated, necrotic gray spots, about 1–2 mm in diameter, with adistinct brown margin. Lesions are mostly found on the lower leaves | Resistant moderately |
| 3 | Lesion type same as in 2, but significant number of lesions on the upper leaves | Resistant moderately |
| 4 | Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area | Moderately susceptible |
| 5 | Typical susceptible blast lesions of 3mm or longer infecting 4–10% of the leaf area | Moderately susceptible |
| 6 | Typical susceptible blast lesions of 3 mm or longer infecting 11–25% of the leaf area | Susceptible |
| 7 | Typical susceptible blast lesions of 3 mm or longer infecting 26–50% of the leaf area | Susceptible |
| 8 | Typical susceptible blast lesions of 3 mm or longer infecting 51–75% of the leaf area many leaves are dead | Highly susceptible |
| 9 | Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected | Highly susceptible |

The severity of leaf blast disease was assessed on three leaves from each of the three plants at 7 days after inoculation and every 7 days until severity stability or leaf senescence using visual quantification based on a diagrammatic scale of 0–9 developed by the International Rice Research Institute, Philippines (IRRI, 2013) (Fig. 5).

The difference between the diseased and control leaf areas (reduction in the number or size of lesions) was used to measure partial resistance (susceptible plants, lesions types 4 to 6). Using partial resistance to compare varieties (El Guilli et al., 2000). Most of the varieties newly registered or proposed for registration (INRAM11, INRAM6, Maghreb, Nachat, and Bahja) are compatible (susceptible) to Moroccan strains, and their partial resistance is lower than that of currently cultivated varieties. It is recommended

that these crops not be planted under conditions favourable to the development of blast disease (for example, too much nitrogen fertilization). In terms of partial resistance, varieties Kenz and Bahja are comparable to varieties Ariete. Under the conditions of rice cultivation in France (Camargue), the level of field resistance of these varieties is acceptable to farmers, and the level of resistance of these varieties would likely be sufficient in the epidemiological context of Morocco. At the foliar level, despite their susceptibility to all or most Moroccan strains, Elio and Thaibonnet varieties have a good level of partial resistance. This characteristic suggests that the varieties should be resistant under normal growing conditions (especially without excessive amounts of nitrogen fertilizer). These varieties should be field-resistant in the Gharb and Larache regions (El Guilli et al., 2000).

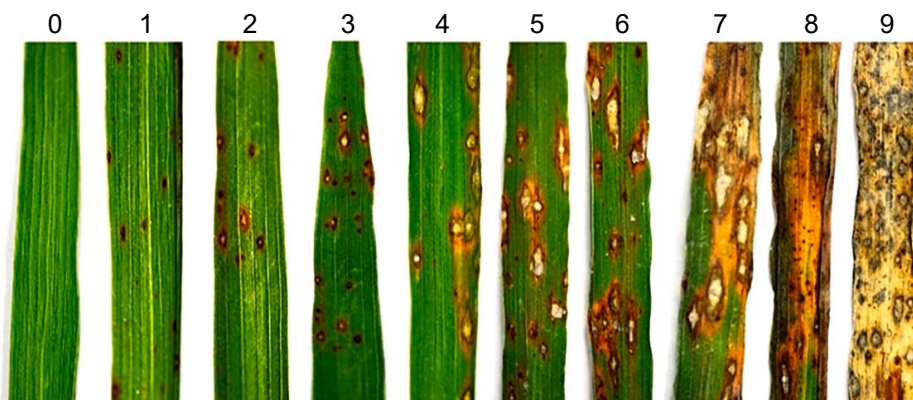


Figure 5. Schematic diagram of the mechanism with the index value for scoring rice blast disease on foliage, from Shrestha et al. (2017).

Trapping experiments with sensitive plants in the Gharb region (results not shown) have shown the presence of *Magnaporthe grisea* strains. The inoculum is therefore present. The absence of a major epidemic in this region could be explained by the cultivation of a variety with a good level of partial resistance (Elio) under conditions not conducive to the development of the disease. On the other hand, epidemics of Thai Bonnet observed at Larache following excessive nitrogen fertilisation appear to show that this partial resistance can be rendered ineffective by inappropriate cultivation practices.

Blast resistance was evaluated at the leaf level in this study. This study allows eliminating the most sensitive varieties at the vegetative stage, but studies of panicle resistance carried out in the field would be a useful complement to this work (El Guilli et al., 2000).

CONCLUSIONS

Rice is a crop of concern since it is a staple food for roughly half of the world's population. Its production is hampered by several biological restrictions. Rice blast (*Piricularia oryzae*), for example, hurts yield. A rice blast is a fungus that attacks rice plants. It is one of the most serious diseases to affect the rice crop, as it can result in

significant yield reductions and possibly crop failure. In the absence of prevention techniques, the annual loss caused by this disease ranges from 10% to 30% of total production, with crop losses reaching 100% in extreme situations for very sensitive types. For this, a characterization of the genetic resources' resistance to pyriculariosis is required, which will allow growers to have resistant varieties at their disposal to alleviate the concerns of harvest loss caused by this disease.

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