

EPPO Datasheet: *Xanthomonas oryzae* pv. *oryzae*

Last updated: 2022-09-29

IDENTITY

Preferred name: *Xanthomonas oryzae* pv. *oryzae*

Authority: (Ishiyama) Swings, van den Mooter, Vauterin, Hoste, Gillis, Mew & Kersters

Taxonomic position: Bacteria: Proteobacteria:

Gammaproteobacteria: Lysobacterales: Lysobacteraceae

Other scientific names: *Bacillus oryzae* Hori & Bokura, *Bacterium oryzae* (Uyeda & Ishiyama) Nakata, *Phytomonas oryzae* (Ishiyama) Magrou, *Pseudomonas oryzae* Uyeda & Ishiyama, *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye, *Xanthomonas kresek* Schure

Common names: BLB, bacterial blight of rice, bacterial leaf blight of rice, kresek disease of rice, leaf blight of rice

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EPPO Categorization: A1 list

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EU Categorization: A1 Quarantine pest (Annex II A)

EPPO Code: XANTOR



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Notes on taxonomy and nomenclature

A blight disease of rice, which was first believed to be caused by acidic soils, was already observed in the Fukuoka Prefecture in Japan in 1884 (Ou, 1985). The causal bacterium which was isolated, was named *Bacillus oryzae* (Hori & Bokura) and the disease bacterial leaf blight of rice (Bokura, 1911). The bacterium was reclassified as *Pseudomonas oryzae* (Uyeda & Ishiyama) by Ishiyama in 1922 and Uyeda & Ishiyama (1928) (This is not *Pseudomonas oryzae* (Yu *et al.*, 2013), described by Yu *et al.*, 2013), subsequently as *Bacterium oryzae* (Uyeda & Ishiyama) Nakata by Nakata in 1927, *Phytomonas oryzae* by Magrou in 1937 and later as *Xanthomonas oryzae* (Uyeda & Ishiyama) Dowson by Dowson in 1943.

In 1978, Dye reclassified the pathogen as *Xanthomonas campestris* pv. *oryzae* (Ishiyama) Dye.

Reitsma and Schure (1950) reported a disease called 'kresek' in Indonesia. The causal organism was named *Xanthomonas kresek* (Schure, 1953). This disease later (Mizukami & Wakimoto, 1969, Reddy, 1984) was shown to be a severe form of bacterial leaf blight of rice, caused by *X. oryzae* pv. *oryzae*, which is found in various regions of the tropics in Asia and Africa.

Apart from bacterial leaf blight, caused by the vascular pathogen *X. oryzae* pv. *oryzae*, a bacterial disease with very similar leaf symptoms, but not vascular, named bacterial leaf streak of rice was first observed (but interpreted to be bacterial leaf blight in the Philippines in 1918 (Reinking, 1918). Subsequently it was 'rediscovered' in China in 1957, described as bacterial leaf streak of rice and the causal bacterium named *Xanthomonas oryzicola* (Fang *et al.*, 1957). Bacterial leaf streak is a bacterial spot/streak disease in which the causal organism does not penetrate the vascular system, probably due to its ability to quickly decompose cell walls and kill cells (Cao *et al.*, 2020). *X. oryzicola* was reclassified in the following years as *X. translucens* f. sp. *oryzicola* and as *X. campestris* pv. *oryzicola* (Bradbury, 1971; Reddy & Ou, 1974). For further detailed information on bacterial leaf streak and [X. oryzae](#) pv. [oryzicola](#), see [EPPO Datasheet](#). For its detection and identification see the EPPO Standard PM 7/80 (1) *Xanthomonas oryzae* (EPPO, 2007), which covers both *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*.

On the basis of a polyphasic taxonomical study Swings *et al.* (1990) placed both pathogens as pathogenic varieties in a separate species: *Xanthomonas oryzae*, the bacterial leaf blight pathogen as *X. oryzae* pv. *oryzae* and the bacterial leaf streak pathogen as *X. oryzae* pv. *oryzicola*.

A slightly deviating strain isolated from the (invasive) perennial grass weed species *Leersia hexandra* (southern

cutgrass or rice swamp grass) was described already in 1957 from China by Fang *et al.* as *X. leersiae*. Strains from this host were only weakly pathogenic to rice and were not pathogenic to *Zizania latifolia* (Manchurian wild rice). In a more recent study Lang *et al.* (2019), using comparative genomics (Average Nucleotide Identity, ANI), identification of Type III (T3) secretion-based pathogenicity/host range effectors, TALE (transcription activator-like effectors) determination and disease phenotyping, classified strains of *L. hexandra* from China, Burkina Faso, India, Mali, and Uganda as *X. oryzae* pv. *leersiae*. *X. oryzae* pv. *leersiae* is most closely related to *X. oryzae* pv. *oryzicola*, but it is still also a close relative of *X. oryzae* pv. *oryzae* (Lang *et al.*, 2019).

X. oryzae strains occurring in the United States, and first reported in 1989 (Jones *et al.*, 1989), appear to be (slightly) different from *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola* as well as *X. oryzae* pv. *leersiae*. These strains have low virulence on rice and contain no TALEs and form a separate clade, although taxonomically to date they have not been distinguished as separate pathovars and are referred to as (also used in this document) *X. oryzae* 'USA' (Xu & Gonzales, 1991; Gonzalez *et al.*, 2007; Triplett *et al.*, 2011; Hajri *et al.*, 2012; Poulin *et al.*, 2015; Lang *et al.*, 2019).

X. oryzae is genetically closely related to *X. vasicola* pv. *vasculorum*, causing leaf scald of maize and sugarcane and some other Poaceae and *X. vasicola* pv. *musacearum*, causing banana xanthomonas wilt, but is only distantly related to other *Xanthomonas* species and pathovars pathogenic to Poaceae, such as the host specialized pathovars of *X. translucens* and *X. albilineans* (Rodriguez *et al.*, 2012; Hersemann *et al.*, 2017).

For some additional taxonomic and nomenclatorial information also see CABI (2022a and b) and Niño-Liu *et al.* (2006).

HOSTS

The principal host of *X. oryzae* pv. *oryzae* is cultivated rice, *Oryza sativa*. Other hosts also belong to the Poaceae family, including wild *Oryza* species such as *O. australiensis* (Australian wild rice), *O. longistaminata* (African wild rice, long stamen rice or red rice), *O. rufipogon* (brownbeard wild rice), as well as annual and perennial grasses such as *Cenchrus ciliaris* (buffelgrass), *Cynodon dactylon* (Bermuda grass), *Echinochloa crus-galli* (cockspur or barnyard millet), *Leersia hexandra* (southern cutgrass or swamp rice grass), *L. japonica*, *L. oryzoides* (common rice cutgrass), *L. sayanuka*, *Leptochloa chinensis* (red sprangletop), *L. mucronata* (mucronate sprangletop), *Megathyrsus maximus* (Guinea grass or green panic grass), *Paspalum scrobiculatum* (Kodo millet), *Urochloa (Brachiaria) mutica* (para grass), *Zizania aquatica* (southern wild rice), *Z. latifolia* (Manchurian wild rice), *Zizania palustris* (northern wild rice), *Zoysia japonica* (Korean or Japanese lawn grass or zoysia grass) Aldrick *et al.*, 1973; Reddy & Nayak, 1974; Li *et al.*, 1985; Ou, 1985; Gonzalez *et al.*, 1991; Mew *et al.*, 1993; Noda & Yamamoto, 2008; Lang *et al.*, 2019; EFSA, 2018; CABI, 2022a). In particular, *Leersia* spp. may be latently infected and form reservoirs of *X. oryzae* pv. *oryzae* that are pathogenic and cause symptoms in rice upon artificial inoculation (Gonzalez *et al.*, 1991; Lang *et al.*, 2019).

Within the grass-like family of Cyperaceae, there is a single record mentioning *Cyperus difformis* and *C. rotundus* as hosts (Chattopadhyay and Mukherjee, 1968).

Host list: *Cenchrus ciliaris*, *Cynodon dactylon*, *Cyperus difformis*, *Cyperus rotundus*, *Echinochloa crus-galli*, *Leersia hexandra*, *Leersia japonica*, *Leersia oryzoides*, *Leersia sayanuka*, *Leptochloa chinensis*, *Leptochloa mucronata*, *Megathyrsus maximus*, *Oryza australiensis*, *Oryza glaberrima*, *Oryza longistaminata*, *Oryza rufipogon*, *Oryza sativa*, *Paspalum distichum*, *Paspalum scrobiculatum*, *Urochloa mutica*, *Zizania aquatica*, *Zizania latifolia*, *Zizania palustris*, *Zoysia japonica*

GEOGRAPHICAL DISTRIBUTION

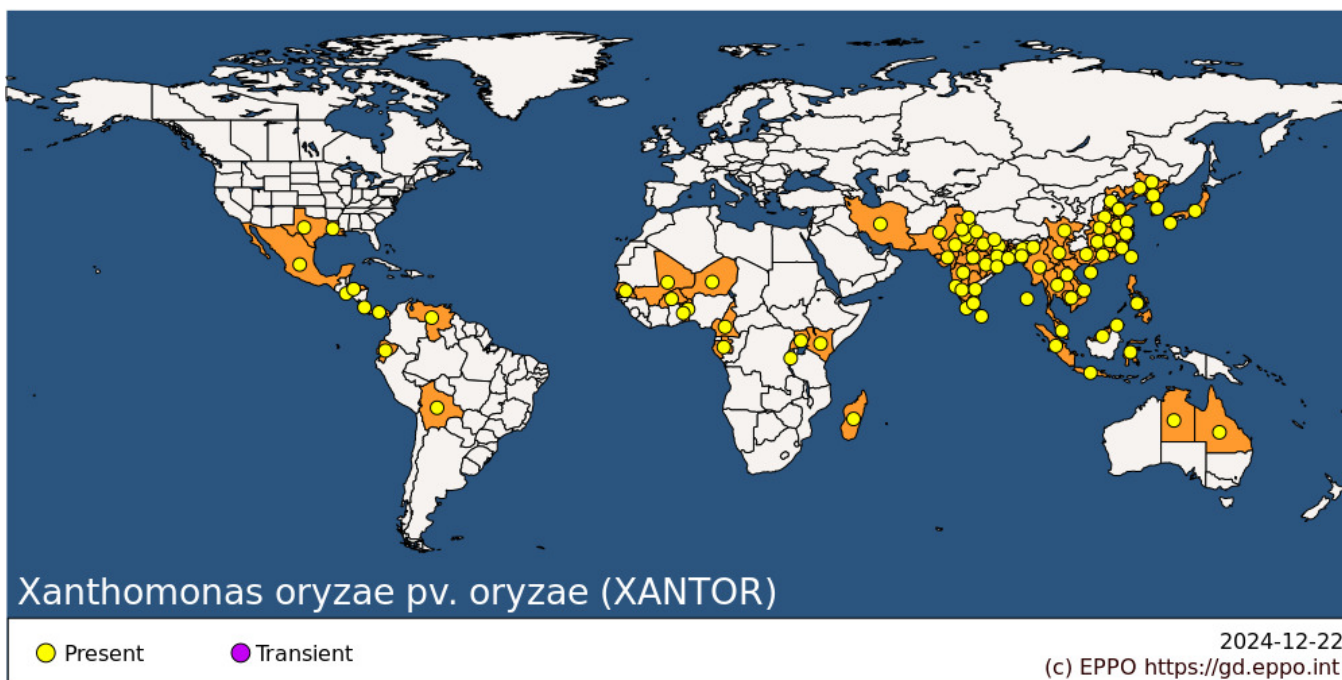
Both *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* are present in the main rice-producing areas of the world. *X. oryzae* 'USA' has only been reported from the USA in two states, Louisiana and Texas, *X. oryzae* pv. *leersiae* has been reported from China, Burkina Faso, India, Mali, and Uganda (Lang *et al.*, 2019).

As stated before, bacterial leaf blight was first reported from the Fukuoka Prefecture, Japan, in 1884. This disease subsequently was observed in other continents. Since the early 1960s, bacterial leaf blight has been reported from virtually all South East Asian countries where it is widespread, and affects rice crops in its severe form (Ou, 1985;

Goto, 1992). It has also been reported from several (mainly West-) African countries, from Australia and North America (Louisiana and Texas, USA), and from Central and South America (CABI, 2022a).

There are only several dated (and poorly substantiated) reports on the occurrence of *X. oryzae* pv. *oryzae* in Mexico and parts of Central and South America, indicating that bacterial leaf blight is not of importance in those areas. It cannot be excluded, however, that in those countries strains similar to the *X. oryzae* 'USA' occur, since infestations reported were low to moderate. In whatever cases, climate could also play a role in moderation of the infection (Lozano, 1977; Ou, 1985; Bradbury, 1986; Guevara & Maselli, 1999; USDA, 2013).

At present bacterial leaf blight is not known to be present in the EPPO region. Iran, where bacterial leaf blight spread rapidly since 2004, is the nearest country to Europe where *X. oryzae* pv. *oryzae* has been reported (Ghasemie *et al.*, 2008; Khoshkdaman *et al.*, 2009 and 2012).



Africa: Benin, Burkina Faso, Burundi, Cameroon, Gabon, Kenya, Madagascar, Mali, Niger, Senegal, Togo, Uganda

Asia: Bangladesh, Cambodia, China (Anhui, Fujian, Guangdong, Guangxi, Hainan, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Shandong, Sichuan, Yunnan, Zhejiang), India (Andaman and Nicobar Islands, Andhra Pradesh, Assam, Bihar, Chhattisgarh, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Jharkand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Nagaland, Odisha, Punjab, Rajasthan, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal), Indonesia (Java, Sulawesi, Sumatra), Iran, Japan (Honshu, Kyushu), Korea Dem. People's Republic, Korea, Republic, Laos, Malaysia (Sabah, Sarawak, West), Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam

North America: Mexico, United States of America (Louisiana, Texas)

Central America and Caribbean: Costa Rica, El Salvador, Honduras, Panama

South America: Bolivia, Ecuador, Venezuela

Oceania: Australia (Northern Territory, Queensland)

BIOLOGY

X. oryzae pv. *oryzae* is a vascular pathogen, colonizing mainly vascular tissues and causing a leaf blight disease, as opposed to *X. oryzae* pv. *oryzicola* which invades mainly the mesophilic parenchymal tissues, causing a leaf streak disease. Bacterial leaf blight occurs in both temperate and tropical rice-growing climate zones, with temperatures between 25°- 34°C and over 70% relative humidity. Conditions of strong wind and frequent, heavy rains (e.g., typhoons) are conducive for disease development (Mew *et al.*, 1993). Spread is principally via flood and irrigation water (Dath & Devadath, 1983; Ou, 1985; Niño-Liu *et al.*, 2006).

The bacterium enters mainly via water pores (hydathodes) at the leaf tip and margin, and also via stomata and wounds on leaves, stems or roots. When a hydathode is infected, subsequently bacterial multiplication takes place in the epithem (parenchymatic cells without chlorophyll, lining the cavity under the hydathode), and then the bacteria move to the xylem vessels causing the typical bacterial blight symptoms on the rice leaves (Tabei, 1977; Mew *et al.*, 1984; Guo & Leach, 1989; Mew *et al.*, 1993). After multiplication bacteria may exude in slime droplets and re-enter the plant through hydathodes. Inside the vascular system, bacteria multiply and move in both directions (Ou, 1985; Huang & De Cleene, 1989; Leach *et al.*, 1989; Noda & Kaku, 1999).

Inoculum sources include infected planting material (including seed), volunteer rice plants, contaminated water, infected straw, stubble or chaff, and infected weed hosts (with or without symptoms), although the exact role of these sources in nature is still poorly understood and dependent on the crop system. In rice monoculture areas, for example, most if not all infected material such as stubble, straw, and other plant material are drastically diminished when the land is prepared for the next crop and therefore there is less risk of maintaining the pathogen in the environment (Reddy & Nayak, 1974; Durgapal, 1985; Ou, 1985; Devadath & Dath, 1985; Reddy & Yin, 1989; Vera Cruz *et al.*, 2017).

In temperate regions, *X. oryzae* pv. *oryzae* survives the winter in the rhizosphere of weeds of the genera *Leersia* and *Zizania* and in roots and stem bases of rice stubble (Mizukami and Wakimoto, 1969, Hsieh & Buddenhagen, 1974; Reddy and Nayak, 1974; Reddy & Yin, 1989).

The disease occurrence and development are favoured in areas with insufficient weed and stubble control in both tropical and temperate climates. High levels of nitrogen fertilization can induce severe outbreaks of bacterial leaf blight (Noda & Kaku, 1999; Yu *et al.*, 2015; IRRI, 2021).

The data on seed transmission show that it is possible, though probably infrequent under most natural conditions (e.g., Mew *et al.*, 1989; Reddy & Yin, 1989; Sakthivel *et al.*, 2001; Vera Cruz *et al.*, 2017). *X. oryzae* pv. *oryzae* has been isolated from the glumes (leaf-like structures below the spikelet) and a few times from within the endosperm of seeds originating from heavily infected fields (Fang *et al.*, 1956; Srivastava & Rao, 1964; Hsieh & Buddenhagen, 1974). Murty & Devadath (1984) found that the bacterium could survive for 120-180 days in rice seeds, but had difficulty in demonstrating that this seed infection gave rise to infected plants in the field. Singh (1971) found that the bacterium cannot survive in unsterilized soil, but survived 15-38 days in field and pond water and >12 months in tap and distilled water. Hsieh & Buddenhagen (1974) found that in wet, warm (flooded) soil or in leaves the pathogen survived up to 40 days, and under colder dryer conditions up to almost a year. Reddy (1972) stated that *X. oryzae* pv. *oryzae* survives for 7-8 months in rice seed, but for only 3-4 months in straw and stubble; Kauffman & Reddy (1975) reported that, although glumes were readily infected, viable bacteria could not be detected on seed stored for 2 months. Sakthivel *et al.* (2001) using bio-PCR could recover *X. oryzae* pv. *oryzae* from naturally infected seeds after storage at 4°C for up to 9 months. The bacterium was also detected in seedlings, mature plants and seeds from plants raised from naturally infected seeds. Singh *et al.* (2015) could detect *X. oryzae* pv. *oryzae* on seed, obtained after artificial inoculation of rice plants at the flowering stage for up to 8 months using bio-PCR. Hassankiadeh *et al.* (2011) using bio-PCR could detect the bacterium in seed washes from naturally infected seeds and found up to 10 months survival in seeds. They also obtained infected seedlings from naturally infected seeds.

Leaf clipping in young plants, when transplanting rice, is an effective means of spread and can lead to the severe so-called 'kresek' form of the disease in these transplants. These young plants show pale yellow leaves, severe wilting, and frequent plant death.

Isolates of *X. oryzae* pv. *oryzae* from different regions of the world show a high genetic diversity, which is to some extent geographically determined, generally necessitating tailored breeding programmes to obtain resistance against local pathotypes (also called races), see e.g., Leach *et al.* (1992). For example, Chen *et al.* (2012) detected that pathotype (race) variation can be altitude dependent. Low virulence strains have been reported from the United States and India (Jones *et al.*, 1989; Gnanamanickam *et al.*, 1993).

Moreover, geographically distinct lineages occurring in Asia and Africa, and the USA have been characterized, where South American strains were congruent with the Asian lineage (Gonzalez *et al.*, 2007; Hajri *et al.*, 2012; Poulin *et al.*, 2015). Three new races were recently characterized in African strains of *X. oryzae* pv. *oryzae*, although these strains are generally less variable than the Asian ones (Verdier *et al.*, 2012; Poulin *et al.*, 2015; Djedatin *et al.*,

2016; Tran *et al.*, 2018).

The pathogenicity of *X. oryzae* pv. *oryzae* is based on a type-3 secretion system, that injects a range of type-3 effectors into rice cells (Niño-Liu *et al.*, 2006; Jiang *et al.*, 2020). This includes members of the Transcription Activator-like Effector family (TALEs), major virulence factors, activating susceptibility genes of the host (Hutin *et al.*, 2015).

For further information also see Ezuka, 2000; EFSA 2018, IRRI (2021).

DETECTION AND IDENTIFICATION

Symptoms

Bacterial leaf blight appears on leaves of young plants, after planting, as pale-green to grey-green water-soaked streaks near the leaf tip and margins. In the early morning bacterial ooze may be observed on these water-soaked streaks. On panicles grey to light brown lesions may be observed on glumes, which may result in infertility or impaired quality of the grains. In later stages of the disease development lesions coalesce and become yellowish-white with wavy edges. Eventually, the whole leaf is affected and, becomes whitish or greyish and then dies. Leaf sheaths and culms of the more susceptible cultivars may be attacked. Systemic infection, known as 'kresek' (Reitsma & Schure, 1950; Mizukami & Wakimoto, 1969, Reddy, 1984), on young plants or during the tillering stage of older plants of very susceptible cultivars results in desiccation and wilting of leaves and death, particularly of young, transplanted plants. In older plants, the leaves become yellow, wither and may die. Surviving plants appear yellowish and stunted. In later stages, the disease may be difficult to distinguish from bacterial leaf streak (BLS) caused by *X. oryzae* pv. *oryzicola*. Bacterial leaf blight in temperate regions is usually observed in the later part of the seed bed stage (Ou, 1985). For more information see Ou, 1985; Goto 1992; Mew *et al.*, 1993; Niño-Liu *et al.*, 2006; EPPO, 2007; EFSA, 2018.

A rapid, preliminary test on symptomatic or asymptomatic plants can be performed using classical (bio-) PCR according to Sakthivel *et al.* (2001), see also EPPO (2007).

Morphology

X. oryzae pv. *oryzae* is an aerobic, motile, Gram-negative, non-spore-forming, capsulated rod, occurring singly or in pairs, 1.1-2.0 x 0.4-0.6 µm in size, with one polar flagellum.

Isolation of *Xanthomonas* from symptomatic material is preferably performed using Peptone sucrose agar (PSA), Nutrient Broth Yeast Extract agar medium (NBY), Growth Factor (GF) agar or otherwise using semi-selective media (Agarwal *et al.*, 1989; Sakthivel *et al.*, 2001; EPPO, 2007). On nutrient agar (NA), after 3-7 days of growth, colonies of *X. oryzae* pv. *oryzae* are circular, entire, smooth, convex, opaque, and pale to straw yellow, 1-2 mm in size. Optimum growth temperature is between 25 and 30°C. Survival of *X. oryzae* pv. *oryzae* on solid media is short. For growth on other media, see EPPO (2007).

Detection and identification methods

Like the genus as a whole, *X. oryzae* is catalase-positive, unable to reduce nitrate and a weak producer of acids from carbohydrates. Pathovars *oryzae* and *oryzicola* can be differentiated by (a) acetoin production (*X. oryzae* pv. *oryzae*–, *X. oryzae* pv. *oryzicola*+), (b) growth on l-alanine as sole carbon source (*X. oryzae* pv. *oryzae*–, *X. oryzae* pv. *oryzicola*+), (c) growth on 0.2% vitamin-free casamino acids (*X. oryzae* pv. *oryzae*–, *X. oryzae* pv. *oryzicola*+), and (d) resistance to 0.001% Cu (NO₃)₂ (*X. oryzae* pv. *oryzae*+ , *X. oryzae* pv. *oryzicola*–) (Dye & Lelliott, 1974; Reddy & Ou, 1976; Gossele *et al.*, 1985; Mew & Misra, 1994; Niño-Liu *et al.*, 2006; EPPO, 2007).

Direct isolation from seeds or plants, seedling tests, detection via use of bacteriophages, (semi-selective) media, immuno-fluorescence (IF), the enzyme-linked immuno-sorbent assay (ELISA), where both polyclonal and monoclonal antibodies can be used (Zhu *et al.*, 1988; Benedict *et al.*, 1989; Cottyn *et al.*, 1994; Wu *et al.*, 2015; EPPO, 2007) and (real-time) PCR are available for screening of symptomatic or asymptomatic plant samples and

seed extracts. For detection in seeds a bio-PCR, which includes an enrichment step in semi-selective medium, can also be used. Moreover, diagnostic kits for ELISA and PCR are available on the (European) commercial market (Mew & Misra, 1994; Alvarez *et al.*, 1997; EPPO, 2007; Vera Cruz *et al.*, 2017).

A specific TaqMan probe for detection in seed was developed by Zhao *et al.* (2007). A specific TaqMan-based multiplex PCR for detection and discrimination of *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* was developed and validated by Lang *et al.* (2010), Noh *et al.* (2012) and Lee & Vera Cruz (2014). A SYBR green-based multiplex PCR for this purpose and also including *Burkholderia glumae* (causing bacterial grain rot of rice), was developed by Lu *et al.* (2014). Another, SYBR-green based test is the bio-PCR for *X. oryzae* pv. *oryzae* of Cho *et al.* (2011). Song *et al.* (2012 and 2014) developed a race-specific PCR (for a new race K3a emerging in Korea), based on an AFLP-derived marker. A padlock probe (PLP)-based PCR with dot blot hybridisation was developed for simultaneous detection of *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* by Tian *et al.*, 2014. Lang *et al.* (2014) developed a sensitive and rapid loop-mediated isothermal amplification (LAMP) test, using primer sets to distinguish not only between *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*, but also between Asian and African lines within the species *X. oryzae* pv. *oryzae*.

Kang *et al.* (2016) developed a multiplex PCR for the detection of *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola* and *Burkholderia glumae*, the causal agent of rice grain rot. Cui *et al.* (2016) developed a multiplex conventional and real-time PCR for the simultaneous detection of six bacterial pathogens of rice, *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, *Pseudomonas fuscovaginae* causing rice sheath brown rot, *Burkholderia glumae*, *B. gladioli* causing bacterial panicle blight of rice and *Acidovorax avenae* subsp. *avenae* causing bacterial brown stripe of rice. A validated multiplex PCR to detect *P. fuscovaginae*, *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*, *Burkholderia* (both *B. glumae* and *B. gladioli*) as well as *Sphingomonas* and *Pantoea* spp. was published by Bangratz *et al.* (2020).

Apart from recent molecular tests mentioned above, there are seed testing methods based on seedlings grow out tests (Cottyn *et al.*, 1994) or seed soaking followed by plating on semi-selective media (Gnanamanickam *et al.*, 1994) or serological detection (Benedict *et al.*, 1989). The seedling and plating methods have the advantage over serology and PCR methods that they are selective for live cells of the pathogen.

Pathogenicity tests include needle-pricking, spraying and leaf clipping, or dipping of non-leaf parts of rice in a bacterial suspension (Akhtar *et al.*, 2008; EPPO, 2007). The leaf clipping method was originally developed by Kauffman *et al.* (1973), where crosscut veins are exposed to a suspension of *X. oryzae* pv. *oryzae* by cutting off leaf tips with *X. oryzae* pv. *oryzae* suspension contaminated scissors. The latter method has been perfected, detailed and validated by Ke *et al.* (2017).

Details about presumptive diagnosis with rapid tests, detection and identification methods (including methods for extraction of bacterial cells and DNA), biochemical, serological and molecular and pathogenicity tests for latent and symptomatic infected material, including seeds, flow chart, culture media, chemicals and reference material) are provided in Vera Cruz *et al.* (2017) and EPPO Standard PM 7 on *Xanthomonas oryzae* pv. *oryzae* and pv. *oryzicola* (EPPO, 2007).

PATHWAYS FOR MOVEMENT

X. oryzae pv. *oryzae* can only move short distances in infected crops, mainly via contaminated (flood and irrigation) water and wind-driven rain (Devadath & Dath, 1970; Dath & Devadath, 1983). The only means of long-distance dispersal is via infected rice (or other hosts) seeds. The bacteria are usually found in the glumes, but may also penetrate the endosperm. Seed material used in breeding programmes is therefore a possible means of spreading the pathogen. For example, using a genetically heritable sequence, clustered regularly interspaced short palindromic repeats (CRISPR) and genomic (SNP) sequencing showed that some strains in Taiwan were related to Japanese strains of *X. oryzae* pv. *oryzae* and others to those from the Philippines, which could be related to earlier imports of rice breeding material from those countries (Chien *et al.*, 2019).

Only limited investigations into dispersal via machinery, humans and animals and water have been carried out and this is poorly understood. In particular, there are no substantiated data on spread or transmission via insects or other animal (Ou, 1985; Niño-Liu *et al.*, 2006; EFSA, 2018).

Persistence and continuation of infection in the field can be due to colonization (epiphytic and endophytic) of symptomless-host plants, especially *Leersia* and *Zizania* spp. (Dath & Devadath, 1983; Gonzalez *et al.*, 1991).

Therefore contaminated/infected seeds are the most probable, although poorly proven, way to spread the pathogen to other areas in the world. Symptomless weed and cultivated hosts and surface water play a local role.

PEST SIGNIFICANCE

Economic impact

Bacterial leaf blight is the most serious disease of rice in South-East Asia, particularly since the widespread cultivation of dwarf high-yielding cultivars in the 1960s (Reddy *et al.*, 1979; Ou, 1985; Mew, 1987). In Japan over the years up to 400 000 ha have been reported to be infested annually with losses of 20-50% and when kresiek form occurs, even 70% and more (Mizukami and Wakimoto, 1969; Reddy *et al.*, 1979; Ou, 1985). In Africa, losses of 2.7-41% in grain yield were reported (Awoderv *et al.*, 1991). Severe infection leads to degradation of seed quality, i.e., nutritional composition and broken and less developed, sterile grains (Reddy, 1979; Ou, 1985; Adhikari *et al.*, 1994a and b). The disease was first reported in India in 1951, but it was not until 1963 that a major outbreak occurred. In the Philippines, in the 1970s losses were in the order of 22% during the wet season and 7% during the dry season in susceptible crops and 9 and 2%, respectively, in resistant crops (Exconde, 1973). In China epidemics were recorded in the 1970s. Then, after a quiet period of approximately 20 years (starting in 1980), disease incidence and yield losses increased since the 2000s (Zhang, 2009), although very little information on exact damage and losses are known from this country. In the Republic of Korea, from 2002 to 2005 the infested acreage increased more than 10-fold to reach approximately 27 000 ha (Noh *et al.*, 2007). In this country, the disease spread especially in rice-cultivating areas of the southwestern coastal plain, and the epidemic in 2003 caused substantial yield loss with the emergence of a new race (K3a) of *X. oryzae* pv. *oryzae* (Jeung *et al.*, 2006). Rajarajeswari & Muralidharan (2006) in an extensive survey in India recorded 17-44% crop losses. In Pakistan, Ahsan *et al.*, 2021 reported losses of 30-100% and year after year increasing incidence and severity of bacterial leaf blight. Losses are generally lower in the less fertile soils and in summer-grown crops (December-April). However, crops that are transplanted in autumn (May-September) and winter (July-December) suffer considerable losses. Epidemics that start before panicle initiation are especially vulnerable to substantial damage and losses (Reddy *et al.*, 1979) due to significantly reduced, panicle fertility, kernel weight and ultimately grain yield.

Control

The most effective measures to prevent the entry, establishment and spread of *X. oryzae* pv. *oryzae* are the use of resistant varieties, the application of appropriate cultural control measures and the use of healthy seeds. Seed transmission is not common for *X. oryzae* pv. *oryzae*, (compared to *X. oryzae* pv. *oryzicola*) so this is a measure is not as important as for *X. oryzae* pv. *oryzicola*.

Chemical control

Chemical treatments, including sodium hypochlorite (NaOCl), mercury and copper compounds and chemical compounds such as probenazole, L-chloramphenicol, nickel-dimethyldithiocarbamate, dithianon, fentiazon, tecloftalam, phenazine oxide, nickel dimethyldithiocarbamate and antibiotics, either applied to seeds or sprayed on plants have not been found very effective against *X. oryzae* pv. *oryzae* and their use in many cases has led to severe phytotoxicity (Mizukami & Wakimoto, 1969; Ou, 1985; Chand *et al.*, 1979; Devadath, 1989; Niño-Liu *et al.*, 2006; Shekhar *et al.*, 2020). The use of mercury compounds has been practically banned worldwide, and use of antibiotics against plant pathogens is not permitted in many EPPo countries, although in Asia their use is still ongoing and resistance of *X. oryzae* pv. *oryzae* has already been established (Xu *et al.*, 2010; Xu *et al.*, 2013; Niño-Liu *et al.*, 2006). Fubianezuofeng (FBEZF), a sulfone bactericide with an oxadiazole moiety, viz 2-(4-fluorobenzyl)-5-(methylsulfonyl)-1,3,4-oxadiazole, applied in China, has a good control effect on bacterial leaf blight, but resistance against the compound by strains of the pathogen was recently reported (Yi *et al.*, 2020). Bacterial leaf blight is effectively controlled by niclosamide, an oral anthelmintic drug and molluscicide. This compound also has a direct control effect on *X. oryzae* pv. *oryzae* (cell membrane disruption, interfering with biofilm regulating genes/proteins and some enhancement of systemic resistance by inducing some defence-related genes. This compound is plant- and environmentally friendly (Kim *et al.*, 2016; Sahu *et al.*, 2018)

Heat treatment

Hot water treatment of rice seeds at 52-54°C for 30 min, preceded by 8-10 hour of pre-soaking at room temperature in water, has been recommended and successfully used to treat seeds against *X. oryzae* pv. *oryzae* (Jain, 1970; Reddy, 1983). However, it never became a general practice, probably due to the fact that seed contamination and transmission for this bacterium, unlike *X. oryzae* pv. *oryzae* is not common.

Biological control

Biological control has been tried, but has only been applied in a limited manner (Niño-Liu *et al.*, 2006). Fluorescent pseudomonads (Anuratha & Gnanamanickam, 1987), bacteriocinogenic strains of *X. oryzae* pv. *oryzae* (Sakthivel & Mew, 1991) and plant extracts (Wonni *et al.*, 2016) were tried.

Native strains of the rice-associated rhizobacteria, occurring also as endophytes, such as *Pseudomonas fluorescens* and *P. putida* strain V14i (the latter also used in biocontrol of the rice sheath blight pathogen *Rhizoctonia solani*) significantly reduced bacterial leaf blight severity when they were sprayed on leaves. (Sivamani *et al.*, 1987; Gnanamanickam *et al.*, 1999; Johri *et al.*, 2003). *Bacillus* spp. have been used as seed treatment before sowing, as a root dip before transplanting and also as foliar sprays. Vasudevan *et al.* (2002), using *Bacillus* spp. reported 60% disease reduction and two-fold increase in plant height and grain yield. These authors suspected a systemic resistance response to be involved in this successful application. *Pantoea* spp. were also found to be potential biocontrol candidates in a rice microbiome environment infested with *X. oryzae* pv. *oryzae* (Yang *et al.*, 2020). Also see Niño-Liu *et al.* (2006) and Gnanamanickam (2009).

A mixture of Myoviridae bacteriophages reduced disease severity by approximately 50%, in a two-year experiment under field conditions, which was, however, still substantially less effective than the treatment with the standard control chemical tecloftalam (1 g/L), which was used as a control (Chae *et al.*, 2014). Also see Shekhar *et al.* (2020).

Plant resistance

To date breeding for resistance has been the most effective way to control bacterial leaf blight. It must be noted, however, that it can also become a bottleneck when resistance, which is based on one gene only, is widely used and the pathogen breaks this resistance. A clear example is the general use over millions of hectares in South East Asia of the IRRI variety IR20 carrying the Xa4 resistance gene, located at chromosome 11 that occurred during the green revolution of the 1960-2000s. This massive use of this variety (> 80% of the acreage) created a strong selection pressure towards a Xa4 breaking bacterial strain adaptation and subsequent epidemics and yield reduction (Quibod *et al.*, 2020).

To date, more than 50 resistance (R) genes have been identified, originating primarily from *O. sativa* subsp. *indica* cultivars, a few from subsp. *japonica* and wild rice species such as *O. longistaminata*, *O. minuta*, *O. officinalis* and *O. rufipogon* (Brar and Khush, 1997; Lee *et al.*, 2003; Chukwu *et al.*, 2019; Oryzabase, 2022). Some resistance genes or alleles were obtained by mutation, using N-methyl-N-nitrosourea, thermal neutron irradiation, or somaclonal mutagenesis (Gao *et al.*, 2001; Lee *et al.*, 2003; Nakai *et al.*, 1988, Taura *et al.*, 1991; Busungu *et al.*, 2016; Niño-Liu *et al.*, 2006)

Most resistance genes have been introgressed into the susceptible indica cultivar IR24 in order to obtain near isogenic lines (NILs), and several of those genes have been combined in a single new line. To this end classical breeding and (mainly DNA based) marker-assisted selection, but also genetic engineering has been applied (Narayanan *et al.*, 2002; Singh *et al.*, 2012, Chukwu *et al.*, 2019, Kesh & Kaushik (2020). Pyramid lines have the advantage of a higher level and/or wider spectrum of resistance than the parental NILs with single resistance genes, implying that there is synergism and complementation among resistance genes (Huang *et al.*, 1997; Adhikari *et al.*, 1999; Narayanan *et al.*, 2002). The pyramid lines now available, yielding a broader resistance (as it is based on more than one gene) provide a more durable form of resistance (Hsu *et al.*, 2020).

However, recently a broad-spectrum resistance gene (Xa23) obtained from wild rice (*Oryza rufipogon*) was described by Wang *et al.* (2015). Furthermore Chen *et al.* (2021), after a decade of research, reported the isolation and characterisation of a new executor resistance gene, Xa7, that confers extremely durable, broad-spectrum, and heat-tolerant resistance to *X. oryzae* pv. *oryzae*. This gene may become important in durable resistance breeding in the (near) future. Highly resistant (often, but not always, immune) populations of the wild rice species *Oryza meyeriana*

were discovered in Yunnan province, China. Their resistance genes were characterized, and they are evaluated to be further used in breeding programs (A *et al.*, 2021)

Recent genome-editing methodology, via zinc-finger nucleases (ZFNs), TAL effector nucleases (TALEs) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (CRISPR-associated protein-9 nuclease), has also been applied to obtain targeted modifications, leading to improved and broad-spectrum resistance in the varieties modified (Ji *et al.*, 2018; Li *et al.*, 2019; Kim *et al.*, 2019; Jiang *et al.*, 2020; Zeng *et al.*, 2020; Tao *et al.*, 2021). A curated TALE database (daTALbase - <http://bioinfo-web.mpl.ird.fr/cgi-bin2/datalbase/index.cgi>) has been created and include TALE-related data for rice bacteria (Pérez-Quintero *et al.*, 2018). Reviews on the availability of resistance genes/varieties and their interaction with *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* are available (e.g., Vikal & Bathia, 2017; Jiang *et al.*, 2020).

An excellent infrastructure and body of resources is available for rice, including for example an expanding, well-characterized germplasm collection, completed genome sequence, whole genome microarrays and a growing collection of mutant libraries. Large collections of documented geographically distinct isolates and pathotypes are also available for *X. oryzae* pv. *oryzae* (see Quibod *et al.*, 2020 - <https://mhn1.shinyapps.io/PathoTracer/>).

Cultural control

Prophylactic measures (such as use of healthy seeds, adequate fertilization and irrigation, destruction or ploughing under of crop residues, disinfection of machinery and equipment, production of seedlings in boxes and removal of diseased plants and weed hosts from fields and along canals) have all been found useful in the control of bacterial leaf blight. Regular monitoring for disease symptoms in rice fields, including in weed hosts is advised.

Forecasting of bacterial leaf blight and bacterial leaf streak has been practised, but appeared to be difficult, due to variations in climatic regions, cultivars and cultural practices and the limited possibilities for chemical or biological control. Methods used were scouting for early disease development, including in weed hosts (since they may show the disease earlier) and correlation to weather conditions (Mizukami & Wakimoto, 1969; Devadath, 1989). Presence of *X. oryzae* pv. *oryzae*-specific bacteriophages in flood/irrigation water has also been used to forecast bacterial leaf blight in temperate regions (Murty & Devadath, 1982; Wakimoto & Mew, 1979).

For further information see also Goto (1992); Mizukami & Wakimoto (1969); Ou (1985); Niño-Liu *et al.* (2006); USDA (2013), COSAVE (2018) and CABI Plantwise Knowledge Bank (2022).

Phytosanitary risk

Bacterial leaf blight is a severe disease, causing extensive crop losses in the Far East, but is not known to occur in the European rice-growing areas. Its existing distribution suggests that it could survive in Mediterranean countries, and it clearly presents a serious risk for the EPPO region (EFSA, 2018).

Rice cultivation (mainly *O. sativa* subsp. *japonica*) in the EPPO region occurs in Bulgaria, France, Greece, Hungary, Italy Portugal, Romania, the Russian Federation, Spain, Turkey and Ukraine. In the EU, about 80% of the rice production takes place in Italy (>220 000 ha) and Spain (>115 000 ha), another 12% in Greece and Portugal (some 20-25 000 ha each). The remainder is cultivated in Bulgaria, France, Hungary and Romania, (10-20 000 ha each). Outside the EU, rice is also grown in the Russian Federation (120 000 ha in the Krasnodar region) as well as in Ukraine (25 000 ha). In those countries, all rice fields are under irrigation, planted in spring and harvested in autumn (Ferrero & Nguyen, 2004; <https://ricepedia.org/rice-around-the-world/europe>). Resistance of European rice varieties against *X. oryzae* pv. *oryzae* is unknown. Non-European varieties are only introduced, in small quantities, for breeding (Cai *et al.*, 2013; Kraehmer *et al.*, 2017).

The main weeds in European rice cultivation are *Cyperus*, *Echinochloa* and *Heteranthera* spp., some of which have been reported as hosts of *X. oryzae* pv. *oryzae* (Kraehmer *et al.*, 2017). Other *Oryza* species reported as hosts may become or are already invasive weed hosts and may introduce *X. oryzae* pv. *oryzae* to the EPPO region. These are *O. barthii*, *O. longistaminata*, *O. rufipogon* and *O. australiensis* (Aldrick *et al.*, 1973). Some related weed species which have also already become unwanted invasive species (e.g., *Leersia* spp. in the USA) or occur in the EPPO region (e.g., *Leersia hexandra* in the southern Mediterranean basin) may also introduce the disease and potentially contribute to its establishment.

Climatic conditions for *X. oryzae* pv. *oryzae* in the southern parts of Europe could allow establishment of *X. oryzae* pv. *oryzae* when compared with the temperate climatic conditions of Iran, Japan and parts of China and the Republic of Korea where bacterial leaf blight is widespread and from time to time a major problem in rice cultivation. This is contradictory to the outcome of a study using a NAPFAST prediction model for *X. oryzae* pv. *oryzae* (Magarey *et al.*, 2011) where the model only used temperature over 30°C and high humidity growing conditions. Europe seemed not to be vulnerable, however the model predicted the same for Japan, where the disease is widespread and where climatic conditions are very similar to those in Southern Europe. Also see EFSA (2018).

No interceptions of *X. oryzae* pv. *oryzae* were reported in the EU from 1995 to May 2022 https://ec.europa.eu/food/plants/plant-health-and-biosecurity/european-union-notification-system-plant-health-interceptions-europhyt/interceptions_en. The main risk of introduction is via imported rice seed used for breeding purposes (germplasm) and therefore direct sowing. Milled rice poses negligible risk, because hulls are removed, and endosperm infection is very rare. Moreover, milled rice has its main destination outside rice-growing areas.

PHYTOSANITARY MEASURES

Phytosanitary (quarantine) measures can be implemented to reduce the risk of long-distance dissemination of the pathogen. It can be recommended that consignments of rice seeds should have been produced from pest-free areas, or from pest-free places of production.

General inspection and sampling procedures for imported rice, which include *X. oryzae* pv. *oryzae* are described in EPPO Standard PM 3/78(2) 'Consignment inspection of seed and grain of cereals'. Seed inspections of rice intended for breeding purposes in international trade may assist in preventing spread of the pathogen to areas with no history of the disease. However, visual inspection of imported seeds is not very reliable due to the occurrence of latent infections and therefore, when material is imported from areas where the disease is known to occur, certification for disease freedom via field inspections and laboratory testing are necessary. For diagnostic testing methods see EPPO (2007).

In other parts of the world, COSAVE (2019), via the Inter-American Institute for Cooperation on Agriculture developed a surveillance program for the detection of bacterial leaf blight and *X. oryzae* pv. *oryzae* in rice and weed hosts in South America. In the USA a contingency plan, to prepare for possible introductions of *X. oryzae* pv. *oryzae*, was developed by the USDA (USDA, 2013).

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