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Review Article

A Review of the Studies and Interactions of *Pseudomonas syringae* Pathovars on Wheat

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Wheat is affected by some pathovars of *Pseudomonas syringae* and by other *Pseudomonas* species. Of these, *P. syringae* pv. *syringae* is the major one responsible for reduction. Recent studies have been made to characterize and identify the pathogen and to determine its aggressiveness and the pattern of colonization in seed and its effects on seed yield, yield components, and source-sink relationships during postanthesis. It was found that the reduction in the aerial biomass production is the best way to evaluate the aggressiveness of this bacterium, and the spray inoculation is good tool to make evaluations at seedling stage. The characterization of bacteria fingerprintings with molecular markers such as RAPD-PCR, ERIC, and REP-PCR is available. Genomic evolution has been elucidated with next-generation genome sequencing. Also, the colonization pattern shows that, early on, microcolonies are frequently detected in the aleurone layer, later in the endosperm and finally close to the crease and even in some cells of the embryo itself. In the wheat cultivars Seri M82 and Rebeca F2000 seed yield and its components are negatively affected. In general, *P. syringae* pv. *syringae* reduces the plant height, seed yield, and yield components, as well as the growth of most organs. When this bacterium attacks, the stems are the predominant sink organs and the leaf laminae and panicles are the predominant source organs.

1. Introduction

1.1. Global Importance of Wheat. Wheat (*Triticum* spp.) is one of the four major staple foods for human consumption [1, 2], ranking the first above the others for cultivated land area and yield and also providing the highest percentage of protein and carbohydrate [3–5]. While considered primarily a human food and much valued for its baking characteristics, wheat is also grown for animal feed and for industrial processing [4]. International demand for wheat is estimated to be growing at about 2% per year [4, 6]. World production exceeds 689 million tons from 25 high-producer countries [4, 7].

1.2. Importance of Wheat in Mexico. Mexico produces about 3.7 million tonnes of wheat on 0.71 million ha situated mainly in the north, southwest, central, and southeast regions. The major part (85%) of Mexico production is in the states of Sonora (35%), Guanajuato (17.5%), Baja California (11.5%), Sinaloa (9.2%), Michoacán (6.4%), and Jalisco (4.4%) where it is the fourth most important crop in terms of land area, being exceeded only by maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), and sorghum (*Sorghum bicolor* [Linn.] Moench) [5]. National wheat production in Mexico in terms of the area planted and the yield per unit area has increased over the 60-year period 1925–1985 but there have been small annual declines since 1985. This has made it necessary to import 1.35 million tonnes per year to meet domestic demand with a *per capita* annual consumption of 54 kg [4, 8].

2. Importance of Wheat Diseases Caused by *Pseudomonas Syringae* and Other Pseudomonads

2.1. Generalities. There are about 50 pathovars described for *P. syringae* and at least nine different species or

genomospecies based on DNA homology [9, 10]. The *Pseudomonas* strains causing the wheat disease "basal glume blotch" area are classified as *Pseudomonas syringae* pv. *atrofaciens* (McCulloch) [11] while those causing leaf blight are grouped under *Pseudomonas syringae* pv. *syringae* van Hall 1902. In many countries including Argentina, Australia, New Zealand, Italy, USA, Canada, South Africa, and Pakistan the occurrence of *P. syringae* pv. *atrofaciens* and/or pv. *syringae* has been reported only once [12, 13]. Diseases caused by *P. syringae* pv. *syringae* on wheat are known as dieback or leaf blight. This bacterium induces water-soaked spots on expanding leaves which become necrotic and turn from grey-green to tan-white. The entire leaves may become necrotic. During periods of high rainfall, white droplets containing cells of *P. syringae* pv. *atrofaciens* (McCulloch) may be visible causing basal glume blotch [13]. *P. syringae* pv. *japonica* (McCulloch) can cause dull, brownish-black discolored areas at the base of each glume covering the kernel, as well as blight or striated areas on the nodes. At last, *Pseudomonas cichorii* causes stem or shank melanosis and *Pseudomonas fuscovaginae* induces a black rot in the wheat sheath. In wheat, although major emphasis has been placed towards the study of diseases caused by fungus, studies of bacterial diseases such as *P. syringae* pv. *syringae* are scarce. This has delayed the development of specific information on the impact of this group of pathogens. These bacteria are considered important in cereals for their broad host range and because some of them are transmitted in the seed. The incidence of *Pseudomonas syringae* pv. *syringae* tends to be sporadic and their geographical distribution is limited [14–16]. Studies on the bacterial diseases of wheat are scarce, so only limited quantitative information on, for example, yield loss and disease epidemiology is available, especially under field conditions. *P. syringae* subp. *syringae* is considered unique among *P. syringae* pathovars for its ability to cause disease in at least 180 plant species from several unrelated genera [17]. Diseases caused by this bacterium, although classified as of low importance [15], have decreased yield by over 50% in Germany [18]. Maximum damage is usually associated with conditions of high humidity and low temperature [15]. The bacterium first infects the glumes, lemma, palea, and caryopsis and then invades and multiplies in the intercellular spaces of the seed tissues [19].

2.2. Losses in Grain Yield. In the field, losses in grain yield caused by *Pseudomonas syringae* depend on many factors including the incidence and severity of the disease, the aggressiveness of the pathogen, environmental conditions (especially temperature and humidity), the resistance or susceptibility of the host, and the phenological stage in which the infection occurs. *P. syringae* pv. *syringae* populations are almost always present epiphytically on the surfaces of wheat plants and other hosts, which indicates that weather conditions are more relevant to disease outbreaks than the presence of the inocula [13]. Internationally, yield losses range from 5 to 50% [15, 20] and, in Mexico, from 5 to 20% [21]. Moreover, it has been shown that infestation of the seed is very important in the epidemiology of the disease [12, 22]. For example, seeds inoculated with *P. syringae* pv. *atrofaciens* and *P. syringae* pv. *syringae* have been sown and these bacteria have subsequently been found in the leaf blades of the resulting seedlings, which suggests transmission by seed [23]. This demonstrates the need to ensure that wheat seed is free of plant pathogenic bacteria because, although it is unusual for all the conditions needed for extreme damage to occur in the field, the shipment of contaminated seed could spread the disease to regions where it has not previously been reported. Many seedling inoculation methods have been evaluated, and several indicators have been developed to estimate the aggressiveness of the strains of *P. syringae* pv. *syringae*. The production of aerial biomass (dry weight) at 34 days after emergence seems to be the best indicator to detect differences among the *Pseudomonas* strains, inoculation methods and their interactions [22], so this would seem to be the alternative method for evaluating aggressiveness when the seed is sprayed or vacuum infiltrated with the bacterium. In general, *P. syringae* pv. *syringae* reduces the wheat plant height, seed yield, and yield components, as well as the growth of most organs in wheat cultivars Seri M82 and Rebeca F2000. When this bacterium attacks, the stems are the predominant sink organs and the leaf laminae and panicles are the predominant source organs. In conclusion, it was suggested that wheat disease records caused by this bacterium should complement crop physiological variables to evaluate and to explain bacterial disease effects [24].

2.3. Study of the Pattern of Spikelet and Seed Colonization. The studies conducted by Fukuda et al. [19] on the histology of wheat seeds invasion by *Pseudomonas syringae* pv. *japonica* indicate that the bacteria colonize first the lemma and palea, then continue to invade the funiculus caryopsis, and then multiply in the intercellular spaces. The sequence and the timing with which the seed tissues are infected have not been explained in detail. Reporter genes can be used to detect changes in plant tissues. The principle of their use is that the amount of protein quantified is a measure of gene expression. The reporter gene for the enzyme β-glucuronidase (GUS) has been used in various plant species but its use has not been reported for determining patterns of colonization of seeds by pathogenic bacteria [25]. Experiments with the GUS gene require destructive sampling, making it impossible to carry out multiple analyses on the same piece of tissue [26]. The use of green fluorescent protein (GFP) overcomes some of the disadvantages of GUS gene detection. The GFP was first discovered in 1962 [27] and was isolated in its natural form from the jellyfish (*Aequorea victoria*). It is characterized as a polypeptide of 27 kDa, which converts the blue chemiluminescence of the Ca^{2+} plus aequorin (a naturally luminescent protein) into blue light [28]. The main advantage in using GFP over GUS is that it can be detected nondestructively in living tissues and even in individual cells, whereas the GUS enzyme activity is often expressed as patches around the tissue under observation, as well as in transformed cells [29]. In plant pathology, the use of the *gfp* gene is increasing. For example, Sexton and Howlett [30] determined that the fungus *Leptosphaeria maculans* can colonize and cause symptoms specifically in the cotyledons and stems of *Brassica* spp., and Du et al. [31] were able to

identify and quantify the accumulation of aflatoxins in corn caused by *Aspergillus flavus*.

In wheat seeds inoculated with *Streptomyces* sp. transformed with the *gfp* gene, Coombs and Franco [32] determined that pathogen causes infection in the tissue of the embryo, endosperm, and radicle. Similarly, studies with pathogens marked with the *gfp* gene in yeast have allowed visualization of expression and confirm the usefulness of the *gfp* gene as a reporter in the study of plant diseases [33, 34]. We have used the *gfp* reporter genes to detect microorganisms and to study biological properties [23]. The pattern of colonization by *P. syringae* pv. *syringae* in wheat was recently elucidated [23]. One strain of this bacterium was transformed to express the GFP in wheat. The *gfp*-tagged bacteria showed strong GFP expression when visualized under green light. After 6 h it was detected in the aleurone layer and, 24 h later, in the endosperm cells. By 36 h it was close to the crease and by 48 h it appeared as patches in some cells of the embryo tissue.

3. Taxonomy of Wheat Pathogenic *Pseudomonas*

The genus *Pseudomonas sensu lato* has been subdivided based on rRNA-DNA hybridization, 16S rRNA sequence comparisons, and multilocus sequence typing [35–38]. Phytopathogenic fluorescent *Pseudomonas* representatives are grouped in the genus *Pseudomonas sensu stricto*, within rRNA similarity group I for γ-Proteobacteria subclass [39]. Most members of this group are saprophytic *Pseudomonas* and are metabolically and physiologically versatile [40]. Revisions of the genus *Pseudomonas* differentiation is provided in detail by Kersters et al., Silva-Rojas, and Anzai et al. who reported five rRNA homology groups, highlighting their importance in phytopathogenic groups I, II, III, and V [35, 41, 42]. *Pseudomonas syringae* is genetically diverse and is subclassified into approximately 50 pathovars and at least nine genomospecies based on pathogenicity and host range and DNA homology [10, 43, 44]. It is a bacillary bacterium, negative, mobile with a polar flagella, and strictly aerobic. In solid King B medium it produces a green fluorescent pigment under UV irradiation resulting in a former case of grouping [45]. The characterization and identification of this species, in addition to biochemical tests, can be accomplished with commercial systems such as API-50CH, 50AO, and 50AA, BIOLOG (Biolog Inc., Hayward, USA), and Biotype 100 (bioMérieux, La Balme Les Grottes, France) has been used to determine the ability of isolates to assimilate or oxidize a wide range of organic compounds in the presence of tetrazolium salt. However, with the application of such tools it has still not been possible to distinguish between *P. syringae* pv. *syringae* and *P. syringae* pv. *japonica*, making it necessary to rely on results of molecular tests.

In this sense, to identify pathogenic bacteria, particularly of the genus *Xanthomonas* and, the technique of repeated sequences (REP-PCR) has been used successfully to identify *X. translucens* [46] and other species of this genus [47, 48]. Fatty acid methyl ester (FAME) analysis has been applied to distinguish *Pseudomonas syringae* but it was not conclusive for this genus [45]. At this moment, no research using repeated sequences like ERIC and REP or FAME analysis has been used to distinguish *Pseudomonas syringae* pathovars affecting wheat worldwide. Other molecular tools, like DNA genomic restriction fragment length polymorphism (RFLP) analysis of DNA by pulsed field gel electrophoresis and ELISA-PCR, have also been used for genomic characterization of bacteria on wheat [15]. Direct sequencing of the amplification product of the small subunit ribosomal 16S rRNA is a discriminative method that identifies strains of prokaryotes rapidly [49, 50]. This subunit is a characteristic universally distributed among all prokaryotic species [51]. The respective sequence is compared with the GenBank database (National Center for Biotechnology Information, NCBI) which establishes phylogenetic relationships and results in a prompt identification. Our understanding of the evolution of plant pathogenesis in *Pseudomonas syringae* strains has further improved by next-generation genome sequencing [52]. It is possible to identify *P. syringae* pv. *syringae* on the basis of amplifying and sequencing the small 16 rRNA subunit. However, results are unavailable for the application of molecular markers like RAPD-PCR to characterize and identify plant pathogenic bacterial strains in wheat [53].

4. Conclusion

The main control measures for plant diseases caused by pathogens are crop rotations with nonhost species, inoculation with antagonistic bacteria (e.g., fluorescent *Pseudomonas* genus), seed production in areas free of the disease, and plant breeding for resistance. In Mexico there are not yet any effective treatments for the control of basal glume blotch in wheat caused by *Pseudomonas syringae* pv. *syringae.* In experiments in Egypt and Russia, which assess the resistance of wheat genotypes to diseases caused by *P. syringae* pv. *syringae* and *P. syringae* pv. *atrofaciens*, sources of genetic resistance have been found in the varieties Sakha 69, Len, Marchal, Nowesta, Red River 68, Bounty 208, Bonanza and Alex as well as in species of the *Aegilops* grasses. The evaluation of alternative controls using physical and chemical measures has been shown not to be feasible for large amounts of seed and is anyway considered likely to impair its viability or not to have sufficient efficacy to be worthwhile. Therefore, at this stage it is important to use healthy seed, from an uninfected crop, grown in an upland area.

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