Virulence of *Blumeria graminis* f. sp. *tritici* in Brazil, South Africa, Turkey, Russia, and Australia

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The globally distributed causal agent of powdery mildew on wheat, *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most rapidly adapting plant pathogens and requires monitoring for shifts in virulence to wheat resistance (*Pm*) genes. Virulence frequencies were assessed in a total of 346 *Bgt* isolates from several countries that had either lately recorded increasing powdery mildew epidemics (Brazil, South Africa, and Australia) or not recently been surveyed (Turkey and Russia). The results were compared to previously published surveys of United States and Egyptian *Bgt* (390 isolates). Many of the *Pm* genes that have potentially been employed longer (*Pm1a–Pm17*) were shown to have lost effectiveness, and the complexity of virulence to those genes was higher among Brazilian isolates than those from any other country. Some cases of high virulence frequency could be linked to specific *Pm* gene deployments, such as the widespread planting of cultivar Wyalkatchem (*Pm1a*) in Australia. Virulence was also assessed to a set of *Pm* genes recently introgressed from diploid and tetraploid wheat relatives into a hexaploid winter wheat background and not yet commercially deployed. The isolate collections from Fertile Crescent countries (Egypt and Turkey) stood out for their generally moderate frequencies of virulence to both the older and newer *Pm* genes, consistent with that region's status as the center of origin for both host and pathogen. It appeared that the recently introgressed *Pm* genes could be the useful sources of resistance in wheat breeding for other surveyed regions.

Keywords: common wheat, powdery mildew, resistance genes, virulence frequency, virulence complexity, genetic diversity, *Blumeria graminis* f. sp. *tritici*, durability

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important global staple crops in area (218 million hectares), as primary calorie source (18%), and as protein source (19%) (FAOSTAT, 2017), and the diseases of wheat have become a global concern, causing ∼13% annual losses in yield (Oerke, 2005; Savary et al., 2019). While the area of wheat cultivation has

not increased significantly over the last 50 years, global wheat production tripled during that time (FAOSTAT, 2017). This increase was due to the widespread application of "Green Revolution" practices, such as synthetic fertilizers, pesticides, and irrigation (Tilman et al., 2001; Zhu and Chen, 2002; Chen et al., 2007; Bradley and Thomas, 2019), as well as genetic gains made with modern wheat cultivars (Lantican et al., 2005; Reynolds and Borlaug, 2006). Such changes have resulted in large-scale, dense plantings of genetically uniform, semi-dwarf wheat cultivars, and increasing risk of epidemics caused by several pathogens (Bennett, 1984; Imani et al., 2002; Van de Wouw et al., 2009; McDonald and Stukenbrock, 2016).

Powdery mildew, caused by the ascomycete *Blumeria graminis* f. sp. *tritici* (*Bgt*), is among the top seven pathogens that affect global wheat (*Triticum* spp.) production (Savary et al., 2019). Depending on the climate zone, *Bgt* epidemics commence with the release of conidia from mycelium on infected host plants or ascospores from sexual fruiting bodies (chasmothecia) that allow the pathogen to perennate during hot dry seasons or cold winters (Glawe, 2008; Zou et al., 2018b). With the start of milder weather in autumn or spring, primary *Bgt* inoculum is dispersed to newly sown wheat crops. An increase in humidity provides optimal conditions for the development of asexually produced conidia and secondary infections that enable polycyclic disease development and larger epidemics (Te Beest et al., 2008).

Whereas powdery mildew has long been common in temperate regions and areas with humid, mild climates, it has recently become increasingly important in drier and hotter areas due to intensified agricultural production and in areas with shifting climates that favor earlier disease development (Tilman et al., 2001; Lantican et al., 2005; Oerke, 2005; Morgounov et al., 2011; Abdelrhim et al., 2018; Tadesse et al., 2019). The recent reports of more frequent powdery mildew epidemics in South Africa, Brazil, and parts of Turkey, Russia, Kazakhstan, and Australia exemplify this trend (D. Lesch, Z. Pretorius, W. Boshoff, P. Kuhnem, A. Morgounov, personal observation; Costamilan, 2005; Morgounov et al., 2011; Golzar et al., 2016).

The obligate-biotrophic lifestyle of *Bgt* is characterized by the infection of epidermal leaf cells *via* a specialized infection structure, the appressorium, and retrieval of nutrients employing a specialized feeding structure, a haustorium (Zhang et al., 2005). This highly specialized form of parasitism requires biotrophic pathogens such as *Blumeria* to employ a set of secreted effector proteins to interfere with the host's immune system and facilitate colonization (Wicker et al., 2013; Mueller et al., 2019). Although the exact function of these effectors is not known, genetic mapping and characterization of a subset of these genes, such as *AvrPm1a*, *AvrPm2*, and *AvrPm3*, has shown that they are the triggers of plant immune reactions following their recognition by cognate host resistance genes (Bourras et al., 2015, 2019; Praz et al., 2017; Hewitt et al., 2021).

Compared to the highly effective, durable powdery mildew resistance conferred by the *mlo* gene in barley (Acevedo-Garcia et al., 2017; Kusch and Panstruga, 2017), powdery mildew resistance in wheat is limited to partial, race-non-specific resistance and race-specific, nucleotide-binding leucine-rich repeat-type resistance genes, referred to as *Pm* (powdery mildew) genes (Johnson et al., 2003; Krattinger et al., 2009; Alam et al., 2011; Cowger et al., 2012; Moore et al., 2015; Kang et al., 2020). Although over 100 *Pm* alleles have been mapped to 63 different loci in wheat (Lu et al., 2020), a much smaller number of *Pm* genes have been deployed commercially, likely due to fungicide availability, a breeding emphasis on yield, and in some cases linkage drag.

Due to the high capacity of *B. graminis* to overcome host resistance (McDonald and Linde, 2002; Spanu, 2012; Mueller et al., 2019), many *Pm* genes have lost effectiveness against the pathogen a few years after their deployment; e.g., *Pm3a* (Niewoehner and Leath, 1998) and *Pm4a* (Parks et al., 2008) in the mid-Atlantic region of the United States. In that same region, another example was a shift in the *Bgt* population to *Pm17* virulence that occurred within 5 years of widespread planting of cultivars bearing that gene (Cowger et al., 2009, 2018).

Pm genes remain a key component of sustainable disease control in wheat. Thus, management strategies to counter the appearance of virulent *Bgt* strains have relied on the introgression of novel *Pm* genes from wild relatives into the germplasm of breeding programs, and the replacement of cultivars containing ineffective *Pm* genes with others bearing previously unused resistance genes (Xu et al., 2015; Wiersma et al., 2017; Zou et al., 2018a; Li et al., 2019b; Tan et al., 2019; Alemu et al., 2021).

The goal of this study was to assess the virulence of *Bgt* in several major wheat-growing countries, including parts of Brazil, South Africa, and Australia, where the disease has become an emerging problem. Also included were regions of Turkey, Russia, and Kazakhstan where the disease has long been endemic, but the pathogen population had not been thoroughly surveyed in the past decade. *Bgt* populations were tested for virulence against a differential set of 19 wheat lines, each carrying a single *Pm* gene, including *Pm* genes that had potentially been in commercial use for a relatively longer time and *Pm* genes that were recently introgressed into hexaploid wheat from diploid and tetraploid wild relatives. The goals were to compare the efficacy of *Pm* genes among geographic regions and to determine whether and where the new genes could be useful sources of resistance for breeding programs.

MATERIALS AND METHODS

Sampling of Isolates

Samples of infected wheat bearing chasmothecia of *Bgt* were collected from 33 locations in Brazil, South Africa, Turkey, Russia, Kazakhstan, and Australia from 2016 to 2019 (**Table 1**; **Supplementary Table 1**; **Figure 1**). At each location, field samples were collected at several places within a field, with each field sample consisting of between 8 and 15 wheat leaves. Exceptions to this occurred at Omsk, Russia, where each sample was collected from a plot of a breeding line within a larger breeding nursery, and in Australia, where only one or two single-pustuled isolates were derived from conidial samples per region (**Table 1** and **Supplementary Table 1**).

TABLE 1 | Origins of 346 *Blumeria graminis* f. sp. *tritici* isolates evaluated for virulence, including closest town and province, region, country of the collected field samples, and number of isolates recovered.[a]

Location number (see Figure 1)	Town, province	Production region	Country	Number of isolates
1	Santa Isabel do Ivaí, Paraná State	South Brazil	Brazil	3
2	Campo Mourâo, Paraná State	South Brazil	Brazil	14
3	Cascavel, Paraná State	South Brazil	Brazil	4
4	Santa Izabel do Oeste, Paraná State	South Brazil	Brazil	9
5	Santa Rosa, Rio Grande do Sul	South Brazil	Brazil	12
6	Condor, Rio Grande do Sul	South Brazil	Brazil	6
7	Passo Fundo, Rio Grande do Sul	South Brazil	Brazil	3
8	Coxilha, Rio Grande do Sul	South Brazil	Brazil	4
9	Wellington, Western Cape Province	Swartland	South Africa	8
10	Caledon, Western Cape Province	Rûens	South Africa	13
11	Napier, Western Cape Province	Rûens	South Africa	38
12	Geraldton, Western Australia	Western Australia	Australia	3
13	Woorree, Western Australia	Western Australia	Australia	1
14	Moora, Western Australia	Western Australia	Australia	1
15	Goomalling, Western Australia	Western Australia	Australia	2
16	Kellerberrin, Western Australia	Western Australia	Australia	2
17	Perth, Western Australia	Western Australia	Australia	1
18	Bentley, Western Australia	Western Australia	Australia	1
19	Bibra Lake, Western Australia	Western Australia	Australia	1
20	Williams, Western Australia	Western Australia	Australia	1
21	Narrogin, Western Australia	Western Australia	Australia	1
22	Arthur River, Western Australia	Western Australia	Australia	1
23	Edirne, Edirne	West Turkey	Turkey	32
24	Menemen, Izmir	West Turkey	Turkey	13
25	Adapazari, Sakarya	Northwest Turkey	Turkey	12
26	Eskisehir, Eskisehir	Northwest Turkey	Turkey	40
27	Adana, Adana	South Turkey	Turkey	26
28	Krasnodar, Krasnodar Krai	Black Sea	Russia[b]	1
29	Rostov on Don, Rostov Oblast	Black Sea	Russia[b]	8
30	Timiryazevskaya, Chelyabinsk Oblast	Western Siberia	Russia	18
31	Kurgan, Kurgan Oblast	Western Siberia	Russia	4
32	Karabalyk, Kostanay Oblast	Western Siberia	Kazakhstan[c]	11
33	Omsk, Omsk Oblast	Western Siberia	Russia	52
total				346

[a]*Collection year of isolates: Brazil, 2019; South Africa, 2017; Australia, 2016; Turkey, 2017/2018; Russia, 2017/2018.*
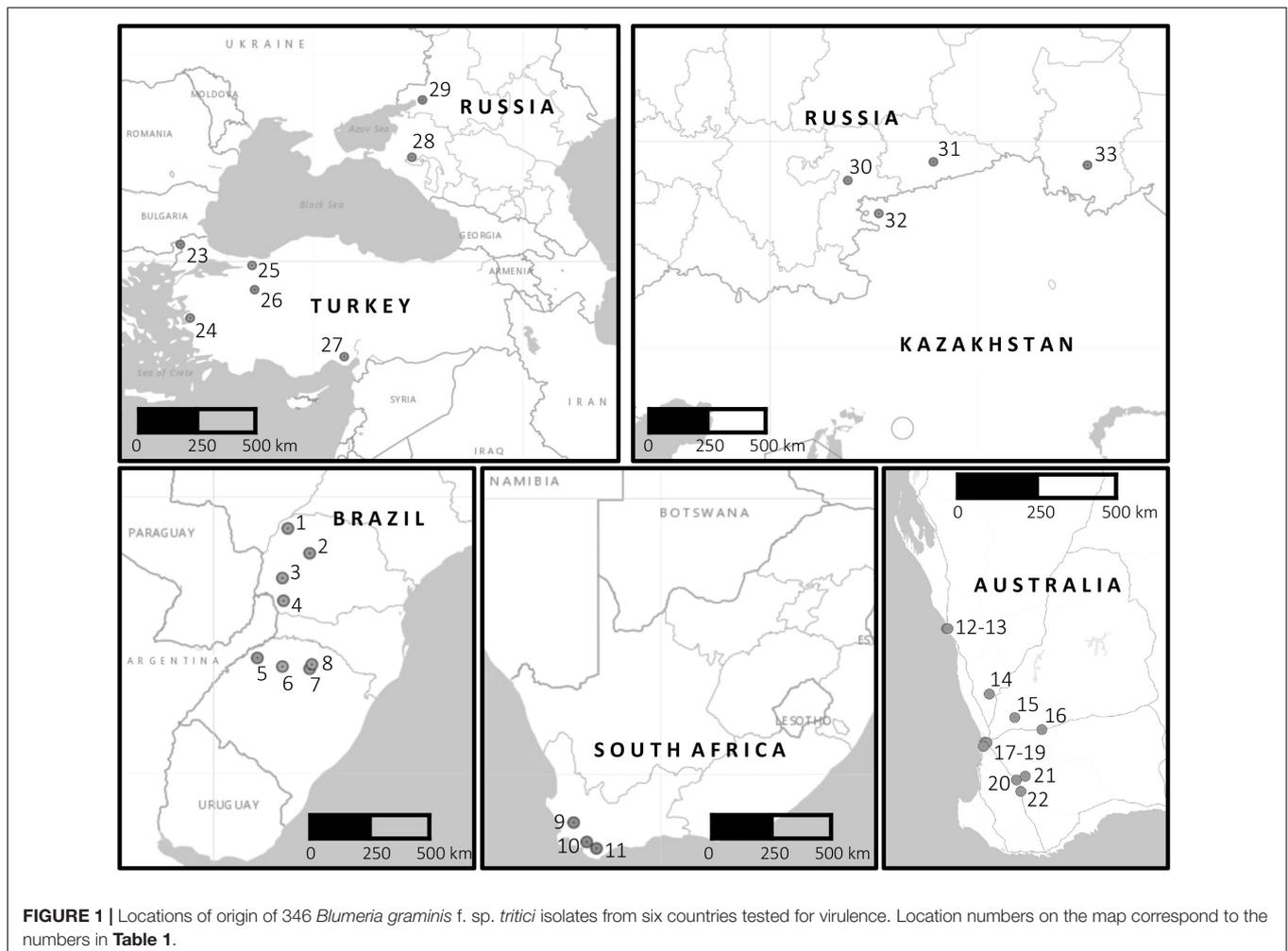[b]*Isolates from Krasnodar and Rostov were grouped with those from Turkey (refer to* **Figure 1***).*
[c]*Isolates from Karabalyk were grouped with those from Russia (refer to* **Figure 1***).*

Recovery of Isolates

Field samples were shipped in paper envelopes and after receipt were stored at 18°C for 30 days to facilitate maturation of chasmothecia (Legler et al., 2012). For the derivation of pure cultures, the protocol of Abdelrhim et al. (2018) was followed: infected leaf samples were cut into 5-cm-long segments, which were then placed in a moist chamber for differentiation of chasmothecia, followed by the ejection of ascospores onto detached segments of 10-day-old primary leaves of susceptible cultivar Jagalene floated on 0.5 w/w% water-agar containing 0.0053 mg L^{-1} benzimidazole in a 60 mm x 15 mm petri dish. Plates with infected Jagalene leaf segments were incubated in a growth chamber for 10 days at 17°C with a 12-h photoperiod, using fluorescent lamps.

After pustules appeared on infected Jagalene, pure cultures were obtained in two consecutive rounds of single-spore isolation, using an autoclaved paintbrush and subculturing on fresh Jagalene leaves under the conditions described above. Up to three single-spored isolates were obtained from each of the 5-cm leaf segments if the colonies that arose from the ascospore infections were at least 2 cm apart. This was done because the viability of chasmothecia in some samples was low, and only a fraction of the 5-cm leaf samples yielded successful primary ascospore infections. The pure isolates were cultured and increased every 10 days for several rounds on Jagalene by dispersing conidiospores from an infected leaf onto a fresh set of leaves with the aid of a spore-settling plastic cup.

FIGURE 1 | Locations of origin of 346 *Blumeria graminis* f. sp. *tritici* isolates from six countries tested for virulence. Location numbers on the map correspond to the numbers in **Table 1**.

Virulence Assay

The virulence profile of each isolate was determined by inoculating leaf segments of 19 differential plant lines, each bearing a single *Pm* gene. The plant lines represented two sets of *Pm* genes. The first was a set of genes that were introgressed and/or identified in common wheat earlier (1960s–2008) and may have been in commercial use relatively longer; these will hereafter be referred to as "older *Pm* genes" (*Pm1a–Pm17*, **Table 2**). The second was a set of genes exclusively sampled from wild, diploid, and tetraploid wheat relatives, originating in the Fertile Crescent, and more recently introgressed into North Carolina soft red winter wheat germplasm in the program of Dr. Paul Murphy, North Carolina State University. These genes will be referred to here as "newer *Pm* genes" (*Pm25–Pm53*, **Table 2**).

Two 2.5-cm primary leaf segments of each 10-day-old differential line and four segments of Jagalene were placed alongside each other on large water-agar petri dishes. Jagalene leaf segments near the perimeter of the dish served as a susceptible control. Two dishes were prepared for each isolate, such that there were four replicate leaf segments for each combination of isolate and differential line. After 10 days of incubation under the conditions described above, the differential lines were scored for

their disease reaction on a 0–9 scale adapted from Moseman et al. (1984): 0 = no symptoms, 1 = faint discoloration, 2 = necrotic lesions, 3 = necrotic lesions and traces of mycelium, 4 = chlorotic lesions with mycelium, 5 = one to two pustules of conidia, 6 = less than 20% of a leaf area covered with pustules, 7 = 20–50% coverage with pustules, 8 = medium-sized pustules and 50–90% coverage, 9 = large pustules and 100% coverage. Based on an average over the four leaf segments, each isolate was rated as avirulent for average scores of 0–4 (differential line was resistant), intermediately virulent for scores of 5–6, and fully virulent for scores of 7–9 (differential line was fully susceptible).

Assessment of Clonality

For the purpose of this study, over-representation of virulence haplotypes due to clonality from polycyclic increase in individual genotypes was undesirable. Isolate clonality was possible because sampling resolution was in some cases as high as two isolates being derived from the same 5-cm leaf segment of an infected wheat leaf sample. Potential clonality was assessed by comparing isolates from a given location for an exact match in their virulence profiles to the 19 *Pm* genes (**Supplementary Table 1**). If one or more isolates originated from the same plant sample and matched

TABLE 2 | *Pm* genes and differential cultivars used in virulence tests of 346 *Blumeria graminis* f. sp. *tritici* isolates from six countries.

Gene set[a]	Gene	Cultivar	Resistance donor species	Chromosome	References
old	*Pm1a*	C.I. 14114	*Triticum aestivum*	7AL	Sears and Briggle, 1969
old	*Pm1b*	MoczZlatka	*T. monococcum*	7AL	Hsam et al., 1998
old	*Pm2*	C.I. 14118	*Aegilops tauschii*	5DS	McIntosh and Baker, 1970
old	*Pm3a*	C.I. 14120	*T. aestivum*	1A	Briggle and Sears, 1966
old	*Pm3b*	C.I. 14121	*T. aestivum*	1A	Briggle and Sears, 1966
old	*Pm4a*	C.I. 14123	*T. dicoccum*	2AL	Ma et al., 2004
old	*Pm4b*	Ronos	*T. carthlicum*	2AL	Yi et al., 2008
old	*Pm6*	Coker 747	*T. timopheevii*	2B	Helmsjørgensen and Jensen, 1973
old	*Pm8*	Kavkaz	*Secale cereale*	T1BL.1RS	Hsam and Zeller, 1997
old	*Pm17*	Amigo	*S. cereale*	T1AL.1RS	Heun et al., 1990
new	*Pm25*	NC96BGTA5	*T. monococcum* subsp. *aegilopoides*	1A	Shi et al., 1998
new	*Pm34*	NC97BGTD7	*A. tauschii*	5DL	Miranda et al., 2006
new	*Pm35*	NC96BGTD3	*A. tauschii* subsp. *strangulata*	5DL	Miranda et al., 2007a
new	*NCA6*	NC96BGTA6	*T. monococcum* subsp. *aegilopoides*	7AL	Miranda et al., 2007b
new	*NCAG13*	NC06BGTAG13	*T. timopheevii* subsp. *armeniacum*	unknown	Murphy et al., 2007
new	*Pm37*	NC99BGTAG11	*T. timopheevii* subsp. *armeniacum*	7AL	Perugini et al., 2008; Baik and Sturbaum, 2014; Green et al., 2014
new	*MIAG12*	NC06BGTAG12	*T. timopheevii* subsp. *armeniacum*	7AL	Maxwell et al., 2009
new	*MIUM15*	NC09BGTUM15	*A. neglecta*	7AL	Worthington et al., 2014
new	*Pm53*	NC09BGTS16	*A. speltoides*	5BL	Petersen et al., 2015

[a]*Pm1a to Pm17 were introgressed and/or identified in common wheat earlier (1960s–2008) and may have been in commercial use relatively longer, compared to Pm25 to Pm53, introgressed more recently from wild wheat relatives into a hexaploid wheat background in North Carolina.*

in their virulence profile, all but one of those isolates were excluded. A total of 47 isolates were matched in virulence profiles with at least one other isolate derived from the same location. Of them, 11 isolates were derived from the same plant as other isolates, and thus, two isolates from Brazil, six from South Africa, two from Turkey, and one from Australia were excluded.

Data Analysis

To make geographically broader inferences about the effectiveness of *Pm* genes, additional data matching the present *Pm* gene set were taken from recently published surveys in Egypt and the United States, which were conducted in the same laboratory as the current one, using the same methods. Data from Egypt were included to represent a wheat-growing region near the Fertile Crescent (Abdelrhim et al., 2018). Data from the United States consisted of virulence profiles of isolates of the Southeast, Mid-Atlantic, and Great Lakes regions, the United States wheat-growing regions most conducive to *Bgt* epidemics; these were thus a subset of the isolates in the previous report (Cowger et al., 2018). Because the sampled location Karabalyk, Kazakhstan was close to the two Russian locations Timiryazevskaya and Kurgan (**Figure 1**), all of which are in a spring wheat region, data from these three locations were grouped together. Isolates from Russian locations Krasnodar and Rostov on Don were grouped with those from Turkey, because these two locations were representative of the winter wheat region close to Turkey and remote from the sampled locations in the Russian spring wheat region.

For a given country (as defined above and shown in **Table 1**), frequencies of virulence to each *Pm* gene were calculated

as the percentage of isolates virulent to each *Pm*-gene line. Global frequencies of virulence to *Pm* genes were estimated as unweighted means using countries' mean virulence frequencies, recognizing the limitations imposed by unsampled geographic regions and varying sample sizes. Virulence complexity was calculated as the percentage of *Pm* genes to which an isolate was virulent. To test whether mean virulence complexity was significantly higher for the older set of *Pm* genes than for the newer set, mean virulence complexity of each country was calculated separately for older and newer genes and then compared in one-tailed Student's *t*-test (α = 0.01), using the function "t.test" in R. Virulence complexity values allowed a within-country comparison of older and newer sets of *Pm* genes for their effectiveness, as well as an overall comparison between countries. For the comparison of countries' mean virulence complexity, residual analysis was applied to test for a linear relationship between the country as the independent variable and virulence complexity as the dependent variable. Arcsine-transformed data fitted a linear regression model best and were chosen for testing whether means differed significantly. The data were fitted to a linear model using the modeling function "lm" in R. For statistical separation of countries' virulence complexity means, a Dunnett–Tukey–Kramer pairwise multiple comparison test, implemented in the DTK package in R, was used with a familywise error rate of α = 0.05.

Genotyping

To test for the presence of certain *Pm* genes in cultivars from which isolates were sampled and/or that have been popular in Brazil and Australia, a genetic evaluation was conducted on 10

Brazilian spring wheat cultivars, provided by Biotrigo Genetica, and two Australian winter wheat cultivars provided by InterGrain through the Australian Grains GeneBank (**Supplementary Table 2**). DNA was extracted from three primary leaves per cultivar as biological replicates, using the EZNA HP Plant DNA Mini Kit D2485-02 (Omega Bio-Tek, Norcross, GA, United States), following the manufacturer's protocol for dried specimens. DNA samples were tested by endpoint genotyping using the PCR Allelic Competitive Extension (PACE) assay (3cr bioscience) and kompetitive allele-specific PCR (KASP) markers to detect polymorphisms linked to *Pm1a*, *Pm6*, and *Pm8* (**Supplementary Table 3A**; Sarinelli, 2017; Sarinelli et al., 2019). Gene-specific markers were unavailable for the other *Pm* genes used in this study.

To provide positive controls, DNA was extracted from the following genotypes: C.I. 14114 for *Pm1a* (Sears and Briggle, 1969), Coker 747 for *Pm6* (HelmsjØrgensen and Jensen, 1973), and Kavkaz for *Pm8* (Hsam and Zeller, 1997). The cultivar Shirley was included as a control representing a modern United States cultivar and for testing the KASP markers for *Pm1a* and *Pm8*, which that cultivar is believed to possess (Hsam and Zeller, 1997; Baik and Sturbaum, 2014; Green et al., 2014). DNA from universally susceptible cultivar Jagalene was used as a negative control for all genes.

For endpoint genotyping, each DNA sample was analyzed in two independent reactions amplified on a QuantStudio 3 real-time PCR cycler (Thermo Fischer Scientific, Waltham, MA, United States), using thermal cycling conditions recommended by the manufacturer in a reaction volume of 10 µl (**Supplementary Table 3B**). Each reaction was composed of 10 ng of sample DNA, 0.138 µl PACE assay mix, 1.5 µM ROX as a reference dye, 30 µM of a common reverse primer, and each 12 µM of two allele-specific primers. The allele-specific primers contained unique 5′ tail sequences that corresponded to either the HEX (resistance allele) or FAM (susceptible allele) fluorescent cassettes present in the master mix. The excitation maxima (FAM = 493 nm and HEX = 538 nm) and emission maxima (FAM = 517 nm and HEX = 554 nm) of the dyes deviated by similar amounts from the wavelength spectrum of the QuantStudio 3 machine for excitation [channel x1 = 470 nm (FAM), channel x2 = 520 (HEX)] and emission [channel m1 = 520 nm (FAM), channel m2 = 558 (HEX)]. Real-time PCR reporter fluorescence, measured at 33–35 cycles and normalized to ROX, was used for allele discrimination using the "analyze" function in Design and Analysis Software 2.6.0 (Thermo Fischer Scientific, Waltham, MA, United States). Assays for *Pm6* and *Pm8* were rerun on additional DNA samples and new reagents to exclude any source of contamination. The two independent assays showed similar allele discrimination patterns for the tested samples.

RESULTS

In total, 346 isolates were derived from samples originating in six countries: 55 from Brazil, 59 from South Africa, 123 from Turkey, 83 from Russia, 11 from Kazakhstan, and 15 from Australia

(**Table 1** and **Figure 1**). As described in the methods, previously published data on virulence to the same set of 19 *Pm* genes from 70 Egyptian isolates and 320 United States isolates (Abdelrhim et al., 2018; Cowger et al., 2018) were compared with the data obtained in this survey. Virulence to *Pm* genes varied from complete fixation (all isolates were virulent) to total absence of virulence across countries and between *Pm* genes (**Table 3**).

Older *Pm* Genes (*Pm1a–Pm17*)

Among the older *Pm* genes, generally high frequencies of virulence were observed, although this varied considerably by country (**Table 3A**). Across all countries, the highest virulence frequencies were recorded for *Pm6*, *Pm8*, and *Pm17*, with most isolates virulent to at least one if not all of those genes. Beginning around the center of origin of *Bgt*, isolates from Turkey and the southern Russian Black Sea region (locations 28 and 29, **Figure 1**) exhibited comparatively moderate frequencies of virulence to the older *Pm* genes (35–65%), except for *Pm1b*, *Pm6*, *Pm8*, and *Pm17* (**Table 3A**). Virulence to *Pm1b* was low in all surveyed populations, but the frequency was highest in Turkish isolates at 18%, in line with the generally intermediate levels of virulence among these isolates. Also in the Fertile Crescent, a pattern of mostly moderate virulence frequencies had been observed among Egyptian isolates previously tested on these older *Pm* genes (Abdelrhim et al., 2018).

Moving farther afield, isolates from the spring wheat region of Russia and Kazakhstan (Western Siberia) exhibited a different pattern than that found for Turkey and the Black Sea winter wheat region. Isolates from these Central Asian regions possessed near-complete virulence to *Pm2*, *Pm4a*, and *Pm4b*, but their virulence to *Pm1a*, *Pm1b*, *Pm3a*, and *Pm3b* was low (<30%).

In the diaspora of countries most remote from the Fertile Crescent, frequencies of virulence to the older *Pm* genes tended to be either high or low. For example, *Pm1a* was either virtually ineffective (Brazil, Australia) or fully effective (United States, South Africa). Among Brazilian isolates, virulence frequencies to the older genes were all ≥75% except for *Pm1b*, and for six of those ten genes (*Pm2*, *Pm3a*, *Pm4a*, *Pm4b*, *Pm8*, and *Pm17*), virulence was ≥95%, indicating a likely complete loss of effectiveness when confronted with natural *Bgt* populations in the field.

In South Africa, virulence to the majority of older *Pm* genes was absent or low (0–13%); however, as mentioned above, *Pm3b*, *Pm6*, *Pm8*, and *Pm17* showed moderate to high virulence frequencies (58–75%). A distinctive virulence pattern was seen in the Australian sample, with *Pm1a* and *Pm6* being completely ineffective, whereas virulence to *Pm8* and *Pm17* was relatively low (14 and 29%, respectively). Overall, virulence to seven of the ten older *Pm* genes was absent or <30% in Australia. However, these results should be treated with caution as the Australian sample was small.

For the older *Pm* genes, average virulence complexity (the percentage of *Pm* genes to which isolates of a country were virulent) was significantly higher in Brazil (85%) than in any other country (**Figure 2A**). South Africa and Australia had the lowest mean complexity of virulence to the older genes, whereas

TABLE 3 | Virulence frequencies of *Blumeria graminis* f. sp. *tritici* isolates collected from six countries, compared to previously published frequencies in the United States and Egypt, for **(A)** ten older *Pm* genes, identified earlier and potentially in use internationally, and **(B)** nine newer *Pm* genes, more recently introgressed into hexaploid wheat in North Carolina.[a]

(A)

Country/region	No. of isolates	*Pm1a*	*Pm1b*	*Pm2*	*Pm3a*	*Pm3b*	*Pm4a*	*Pm4b*	*Pm6*	*Pm8*	*Pm17*	Mean
Egypt[b]	70	61	7	9	41	23	44	21	100	93	93	49
Turkey[c]	130	65	18	47	44	35	63	52	88	99	98	61
Russia/Kazakhstan[d]	85	9	5	98	28	24	98	88	100	99	94	64
United States[e]	320	0	0	55	99	89	78	11	99	92	96	62
Brazil	53	92	0	100	100	83	100	100	74	100	96	85
Australia	14	100	0	0	0	57	0	0	100	29	14	30
South Africa	53	2	0	0	13	74	0	0	75	72	58	29
Mean[g]	725	47	4	44	47	55	55	39	91	83	79	

(B)

Country/region	No. of isolates	*Pm25*	*Pm34*	*Pm35*	*NCA6*	*NCAG13*	*Pm37*	*MlAG12*	*MlUM15*	*Pm53*	Mean
Egypt[b]	70	24	16	66	16	26	9	30	63	6	28
Turkey[c]	130	32	28	23	22	18	10	32	48	20	26
Russia/Kazakhstan[d]	85	85	19	48	14	2	2	6	7	12	22
United States[e]	320	83 [f]	80 [f]	89 [f]	96 [f]	0	1	0	0	1	39
Brazil	53	0	100	6	72	0	0	2	23	36	26
Australia	14	0	0	43	0	0	0	0	100	7	17
South Africa	53	0	6	13	0	0	0	0	0	0	2
Mean[g]	725	32	35	41	31	7	3	10	34	12	

[a]*For each country or region, virulence frequency was calculated as the percentage of isolates virulent to a Pm gene. Color gradient indicates low (white) and high (red) virulence frequency.*
[b]*Abdelrhim et al. (2018).*
[c]*Turkey/Black Sea region: Isolates from Russian locations Krasnodar and Rostov were grouped with those from Turkey (refer to Table 1).*
[d]*Western Siberia region: Isolates from Karabalyk, Kazakhstan were grouped with those from Russia (refer to Table 1).*
[e]*Cowger et al. (2018).*
[f]*Genes Pm25–NCA6 were effective in the field against natural United States Bgt populations (Cowger et al., 2018).*
[g]*Unweighted global mean.*

complexity in Egyptian, Turkish, Russian, and United States collections was intermediate.

Because isolates from Brazil had comparatively high frequencies of virulence to the older *Pm* genes, available KASP markers were employed to test whether cultivars from which isolates were derived possessed *Pm1a*, *Pm6*, or *Pm8*. Likewise, the fixation of virulence to *Pm1a* and *Pm6* observed in the Australian *Bgt* collection was investigated by assessing whether two popular Australian cultivars (Wyalkatchem and Mace) contained *Pm1a* or *Pm6*. Endpoint genotyping for *Pm1a* using three diagnostic KASP markers confirmed the presence of a *Pm1a* allele conferring resistance to powdery mildew in C.I. 14114 (*Pm1a* control), Wyalkatchem and Shirley, whereas all other cultivars possessed the alternate allele, indicating the absence of *Pm1a* (**Supplementary Table 2** and **Supplementary Figure 1**). Tests for *Pm6* and *Pm8* showed that only Shirley and the positive controls (cultivars Coker 747 and Kavkaz, respectively) had the resistance allele, whereas all other cultivars possessed the alternate allele.

Newer *Pm* Genes (*Pm25–Pm53*)

Overall, virulence to the recently introgressed set of *Pm* genes was low relative to the older genes (**Table 3B**). Across all countries,

unweighted mean virulence frequencies were 31–41% for five *Pm* genes (*Pm25*, *Pm35*, *Pm34*, *NCA6*, and *MlUM15*) and ≤12% for an additional four genes (*NCAG13*, *Pm37*, *MlAG12*, and *Pm53*).

Isolates from the Fertile Crescent countries Egypt and Turkey (along with the adjacent Russian winter wheat locations 28 and 29) tended to exhibit low to intermediate frequencies of virulence to the newer *Pm* genes. For example, among Turkish isolates, some virulence was evident to all newer *Pm* genes, with virulence frequencies of 10–48% (**Table 3B**). The consistently moderate virulence in Turkish isolates was comparable to the virulence profiles of Egyptian isolates, although in the latter virulence to *Pm35* and *MlUM15* exceeded 60%.

Moving northeast to the Russian spring wheat region and Kazakhstan, virulence frequencies were still in the low to moderate range for *Pm34*, *Pm35*, *NCA6*, and *Pm53*, but high for *Pm25* and very low for the four remaining genes (*NCAG13*, *Pm37*, *MlAG12*, and *MlUM15*). Virulence to the newer genes was generally low in isolates from countries where wheat powdery mildew epidemics have only recently increased. In Australia, virulence to the newer *Pm* genes was virtually absent, with the exceptions of virulence to *Pm35* (43%) and *MlUM15* (100%). Similarly, in South Africa, isolates were mostly avirulent to the newer *Pm* genes, except for *Pm35* with 13%. Whereas Brazilian isolates displayed virulence frequencies ≤6% to five new *Pm*

genes (*Pm25*, *Pm35*, *NCAG13*, *Pm37*, and *MlAG12*), these isolates were fixed for virulence to *Pm34*, and 72% were also virulent to *NCA6*.

Across countries, mean virulence complexity was significantly lower for the newer set of *Pm* genes than for the older set, as would be expected ($p < 0.05$; **Figure 2B**). The mean virulence complexity to the newer set was similar for Egypt, Turkey, Russia, Brazil, and Australia and ranged from 17 to 28%, whereas South Africa could be statistically separated from all countries with a virulence complexity of 2%. In the eastern US, the mean virulence complexity of 39% to the newer *Pm* genes was nearly exclusively due to the virulence frequencies ≥83% for *Pm25*, *Pm34*, *Pm35*, and *NCA6* (**Table 3B** and **Supplementary Figure 2A**), although as previously reported (Cowger et al., 2018), those genes were fully effective against field populations in North Carolina and remain effective (C. Cowger, personal observations). The reason for the difference between the results of the detached leaf assay and field performance of these genes remains unknown. In contrast, there was a virtual absence of virulence (≤11%) among the United States isolates to the remaining five newer genes (**Table 3B** and **Supplementary Figure 2B**).

Of the isolates that were found to be virulent to newer *Pm* genes in each country, more than half showed intermediate levels of virulence, except for Brazil and Australia (**Supplementary Figure 3B**). Particularly, a high proportion of intermediate virulence was recorded for virulent South African isolates to older and newer *Pm genes* (65 and 100%, respectively), suggesting there was some level of resistance conferred by these *Pm* genes that had not been completely overcome.

It was noteworthy that in isolates of most countries (Egypt, Australia, United States, and South Africa), there was a near-perfect co-segregation of virulence/avirulence to *Pm1a* and *MlUM15* (**Supplementary Table 4**). In contrast, however, more frequent recombination between these two virulence traits was observed in populations from Brazil and Turkey, with 70 and 19% recombinants, respectively; i.e., some isolates were virulent to *Pm1a* but not to *MlUM15*, and vice versa. *MlUM15* mapped close to the multi-allelic *Pm1* locus on wheat chromosome 7AL (Worthington et al., 2014).

DISCUSSION

Frequencies of virulence to powdery mildew resistance genes were investigated and compared for several global wheat production regions across the world where the disease was either relatively long endemic or recently emergent. The results indicated a widespread loss of effectiveness of many older *Pm* genes, likely in at least some cases because of greater exposure of pathogen populations to these *Pm* genes, or their genetic linkage to other resistance genes that have been deliberately used in commercial cultivars to control other diseases, such as stem rust and leaf rust. Fortunately, judging from the present results, a number of newer *Pm* genes more recently introgressed from wild relatives of wheat would likely be effective across most surveyed countries. However, many of

these newly introgressed genes were already associated with moderate frequencies of *Bgt* virulence in countries closer to the Fertile Crescent landscapes where accessions contributing the genes were collected. This is likely due to standing variation for virulence in the *Bgt* populations that have co-evolved with those wild wheat relatives.

While it is nearly impossible to determine the relative area and duration of use of a *Pm* gene, widespread use of particular *Pm* genes appears to have driven the selection of virulent strains in local *Bgt* populations, such as virulence to *Pm3a*, *Pm4a*, *Pm6*, and *Pm17* in the United States (Bennett, 1984; Niewoehner and Leath, 1998; Cowger et al., 2009, 2018). Moreover, the gene pool giving rise to commercial wheat cultivars has become smaller and is nowadays extensively shared among countries (Balfourier et al., 2019).

It is conceivable that some older *Pm* genes were introduced to the surveyed countries in common wheat lines distributed through efforts of the International Maize and Wheat Improvement Center (CIMMYT) and collaborating national agricultural research institutions (Evenson and Gollin, 2003; Lantican et al., 2005; Joukhadar et al., 2017; Kirsten and Nhemachena, 2017). Cultivars sampled in Turkish locations Menemen, Adapazari, and Adana represented mostly CIMMYT material (A. Morgounov, personal observation). Most of those wheat lines were developed for rust resistance, which may have led to an indirect selection of *Pm* genes in linkage (Lillemo et al., 2010). For example, selection for the rust resistance gene complex *Sr31/Lr26/Yr9,* translocated from rye into the B genome of hexaploid wheat, has been common in CIMMYT material and likely led to a co-selection of *Pm8* (Hsam and Zeller, 1997; Mago et al., 2005).

However, the influence of CIMMYT germplasm is not the only factor contributing to the high levels of virulence to the older *Pm* genes. Winter wheat in Russian locations Krasnodar and Rostov often utilizes the Bezostaya-1 background, which contains the *Lr34/Yr18/Sr57/Pm38* complex of genes (Morgounov et al., 2011; Rinaldo et al., 2017), as well as *Pm8* in cultivars Kavkaz and Aurora derived from Bezostaya-1. This common background has probably helped to select for virulence at least to *Pm8* (Bennett, 1984; Singrün et al., 2004). CIMMYT germplasm has not been important in cultivar development in the Russian-Kazakh spring wheat region (A. Morgounov, personal observation), yet virulence frequencies to genes such as *Pm6*, *Pm8*, and *Pm17* were as high as those in Turkey. This indicated that these *Pm* genes may have also been present in germplasm used for breeding in this spring wheat region.

Historically, South African wheat germplasm has contained a low proportion of CIMMYT material (Byerlee and Moya, 1993; Trethowan et al., 2005). However, it is possible that high virulence frequencies to certain older genes, such as *Pm8* and *Pm17*, were driven by the few cultivars derived from CIMMYT germplasm and released in that country. For example, cultivar Kavkaz (*Pm8*) and Amigo (*Pm17*) were used in breeding South African cultivars released in the 1980s and 1990s, such as Gamtoos (Kavkaz/Buho'S'//Kalyansona/Bluebird), Letaba (Warrior5*/Agent//Kavkaz), and Riemland (Flamink/Amigo)

(Heisey et al., 2002; Smit et al., 2010; Kirsten and Nhemachena, 2017).

The development of Australian cultivars has drawn heavily from CIMMYT material since 1960 (Joukhadar et al., 2017), again with a focus on introgression of rust resistance genes, some of which are linked to *Pm* genes (Cuddy et al., 2016). For example, the *Lr20-Sr15-Pm1* locus has tightly linked alleles for resistance to leaf rust and powdery mildew (Neu et al., 2002). A succession of popular wheat cultivars that has dominated the Western Australian wheat market since 2008 was thought to have this resistance gene complex, although responses to powdery mildew or the presence of resistance alleles were heterogeneous (Cuddy et al., 2016; Park et al., 2020; GRIS, 2021). First released in 2003, Wyalkatchem dominated over 30% of the area sown to wheat until 2010, when it was replaced by its descendant Mace out of the cross Wyalkatchem/Stylet//Wyalkatchem. Mace occupied over 50% of wheat plantings from 2013 to 2016 (Zaicou-Kunesch et al., 2017). Third in this succession was Scepter, a cultivar related to Mace (A. Milgate, personal communication), which was released in 2015 and overtook Mace by market share in 2018 (Shackley et al., 2019).

In Western Australia, wheat powdery mildew has been recognized as a disease that requires annual control and monitoring for virulence to *Pm* genes (R. Lopez and A. Milgate, personal communication). Surveys from 2011 to 2014 detected high virulence to *Pm1a*, *Pm6*, *Pm8*, and *Pm17*, whereas virulence was low for *Pm2*, *Pm3a*, and *Pm4a* (Golzar et al., 2016). Although Golzar et al. (2016) rated mean disease scores on single-gene differential seedlings using a composite inoculum from 40 plant samples originating in various locations across Western Australia, their study and the present one found similar levels of virulence to *Pm1a*, *Pm2*, *Pm3a*, and *Pm4a*, whereas virulence was higher to *Pm6* and lower to *Pm8* and *Pm17* in the current collection. Discrepancies between the two studies could be due to the lower sample size in the present one or to a virulence shift in the population. Although Golzar et al. (2016) proposed that most Australian cultivars lack *Pm* genes, *Pm1a* was identified in Wyalkatchem in this study using KASP markers. At the time of those authors' survey in 2011–2014, when they observed high *Bgt* susceptibility in both Wyalkatchem and the *Pm1a* differential line Thew, the Australian *Bgt* population had probably already been selected for virulence to *Pm1a* due to widespread planting of Wyalkatchem. The cultivar Mace, the dominant wheat cultivar in Western Australia in 2013–2016, was found in this study to not possess *Pm1a*. Interestingly, the apparent fixation of *Pm1a* virulence in the present sample of Australian isolates from 2016 was apparently maintained despite reduced exposure to *Pm1a* after Wyalkatchem declined in market share starting in 2010.

While certain *Pm* genes are apparently ineffective in Australia and South Africa, the generally low virulence frequencies among *Bgt* populations in those countries to other *Pm* genes are likely due to a combination of three factors. First, as the relatively hot and dry climate of those regions is less conducive to cereal powdery mildew epidemics, *Bgt* populations have historically been small, leading to a lower probability of the appearance and establishment of virulent isolates (Tadesse et al., 2019). Second, most commercial wheat cultivars in South Africa and
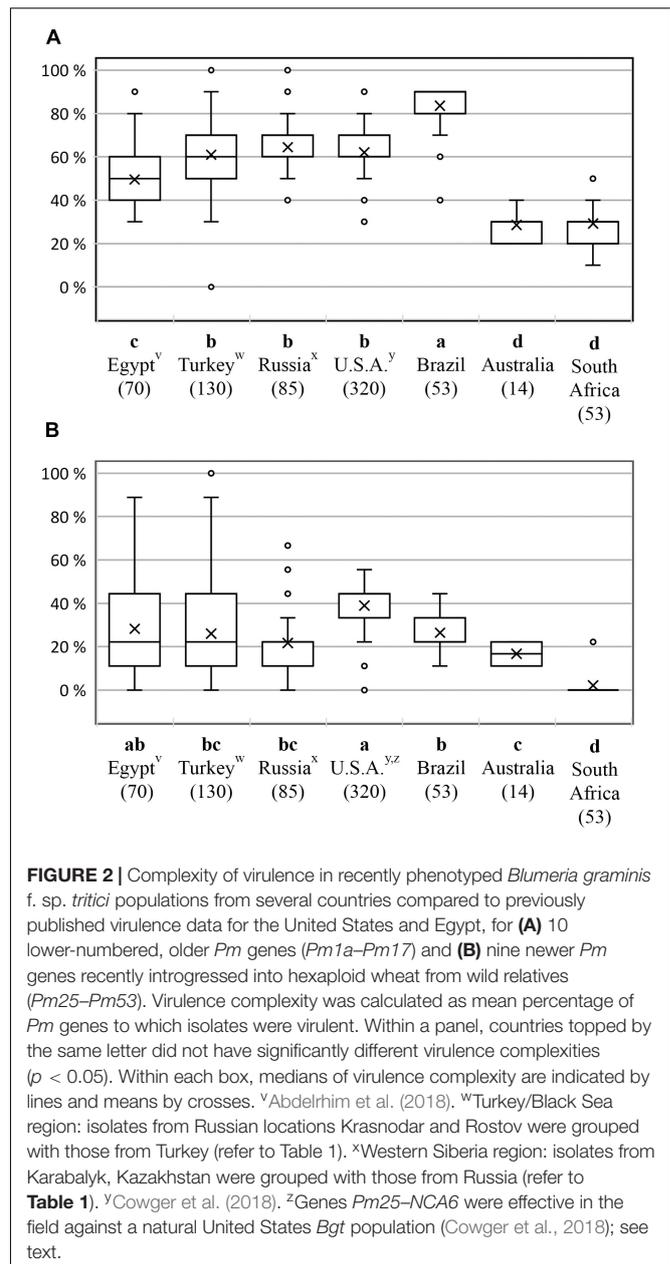
FIGURE 2 | Complexity of virulence in recently phenotyped *Blumeria graminis* f. sp. *tritici* populations from several countries compared to previously published virulence data for the United States and Egypt, for **(A)** 10 lower-numbered, older *Pm* genes (*Pm1a–Pm17*) and **(B)** nine newer *Pm* genes recently introgressed into hexaploid wheat from wild relatives (*Pm25–Pm53*). Virulence complexity was calculated as mean percentage of *Pm* genes to which isolates were virulent. Within a panel, countries topped by the same letter did not have significantly different virulence complexities (*p* < 0.05). Within each box, medians of virulence complexity are indicated by lines and means by crosses. [v]Abdelrhim et al. (2018). [w]Turkey/Black Sea region: isolates from Russian locations Krasnodar and Rostov were grouped with those from Turkey (refer to Table 1). [x]Western Siberia region: isolates from Karabalyk, Kazakhstan were grouped with those from Russia (refer to **Table 1**). [y]Cowger et al. (2018). [z]Genes *Pm25–NCA6* were effective in the field against a natural United States *Bgt* population (Cowger et al., 2018); see text.

Australia seem to lack known *Pm* genes, other than *Pm1a*, so that selective pressure for virulence has likely been generally low (Sensako, 2022; A. Milgate, personal communication). And third, a founder effect may have occurred when a subsample of the ancestral *Bgt* population (Troch et al., 2014) was disseminated to those countries, probably with wheat when it was introduced in Australia by colonists (Joukhadar et al., 2017). This is supported by findings on *B. graminis* f. sp. *hordei*, the *forma specialis* that pathogenizes barley (*Hordeum vulgare*), for which genetic variability was low in Australian subpopulations (Kominkova et al., 2016).

On the other hand, it is possible that high levels of virulence to particular *Pm* genes in countries where wheat powdery mildew is

emergent, such as Australia and South Africa, can be attributed to standing variation; i.e., the presence of particular virulence alleles in the founding *Bgt* populations. The *Bgt* gene pool has maintained a high degree of genetic diversity, even through the shift from wild to domesticated wheat genotypes (Wicker et al., 2013), and this may include virulence alleles. For example, virulence alleles of *AvrPm17* were present in *Bgt* populations prior to commercial deployment of *Pm17*, which led to a rapid selection of virulent isolates and loss of effectiveness of the resistance gene in wheat (Mueller et al., 2021).

In contrast to other older *Pm* genes, globally low frequencies of virulence to *Pm1b* indicate that this gene has likely not been widely used or has only been used in combination with quantitative resistance or other *Pm* genes, which would render it more durable. *Pm1b* originated from a *Triticum monococcum* accession collected in the Suweida province of southern Syria (Valkoun and Mamluk, 1993). Exposure to *T. monococcum* (einkorn wheat) or its wild progenitor *T. boeoticum* (Harlan and Zohary, 1966; Heun et al., 1997) likely explains the finding of some *Pm1b* virulence (7–18%) in *Bgt* populations from Turkey and Egypt, Syria's neighbors in the Fertile Crescent.

Interestingly, isolate collections from Turkey and Egypt stood out for their generally moderate levels of virulence to both the older and the newer *Pm* genes, even though the newer *Pm* genes have likely never been commercially deployed there. The newer genes were introgressed mainly in the past two decades from accessions of wild wheat relatives, e.g., *NCA6* and *Pm25* from wild einkorn wheat *T. monococcum* subsp. *aegilopoides* collected in the Karacadag Mountain range of Iraq (Shi et al., 1998; Miranda et al., 2007b), *MlUM15* from *Aegilops neglecta* collected in Karamusa, Central Turkey (Worthington et al., 2014), and *Pm34* from *Aegilops tauschii* collected in Iran (Murphy et al., 1999). *NCAG13* and *MlAG12* were detected in *T. timopheevii* subsp. *armeniacum* accessions, which are native to the Transcaucasian region, including Azerbaijan and Iraq (Murphy et al., 2007; Maxwell et al., 2009). These *Pm* genes are likely not yet widely present outside their regions of origin and the southeastern US, where they were introgressed into regionally adapted winter wheat germplasm. Thus, finding low to moderate frequencies of virulence in Turkey and Egypt suggests previous exposure of *B. graminis* strains to these *Pm* genes in these geographic regions.

Moderate frequencies are the signatures of a stabilizing or balancing selection for virulence, often found in natural ecosystems where wild host plants and pathogen populations have coevolved in a reciprocal genotype frequency-dependent manner (Leonard et al., 2004; Brown and Tellier, 2011). Our findings suggest that the isolates from Turkey and Egypt make up a genetically diverse population and resemble part of the ancestral, natural population of *Bgt* in the Fertile Crescent (Troch et al., 2014), the center of genetic diversity of wheat and its wild progenitors, which *Bgt* can infect (Dubcovsky and Dvorak, 2007; Ben-David et al., 2016). Even though wild wheat relatives comprise a small proportion of the wheat population in the Fertile Crescent nowadays (Özkan et al., 2010), intermixing occurs occasionally between *B. graminis* from wild grasses and *Bgt* from commercial wheat fields, enhancing allelic diversity in the *Bgt* population (Parks et al., 2009; Ben-David et al.,

2016). This contrasts to subpopulations on other continents that may have experienced genetic drift and a loss of virulence diversity after migration to locations remote from their center of genetic diversity.

Estimates of virulence to *Pm* genes based on laboratory detached leaf assays should be considered carefully, since in a previous study, *Pm25*, *Pm34*, *Pm35*, and *NCA6* were rated susceptible to *Bgt* in the laboratory assay although they were and remain effective in the field against North Carolina *Bgt* populations (Cowger et al., 2018; C. Cowger, personal observation). In addition, large proportions of isolates with intermediate virulence to these four newer *Pm* genes were recorded for many countries in this study, such as Turkey (**Supplementary Figure 3B**). In cases where this study indicated high virulence frequencies to some of the newer genes, these genes may still be effective in the field and should be tested there (e.g., *Pm34* in Brazil). Also, intermediate reactions may indicate that virulence has been developing; i.e., to *Pm* genes that have been deployed widely in the sampling area but relatively recently, or genes that have only been deployed in some parts of the sampling area (**Supplementary Table 5**). In a similar vein, resistance to the Australian *Bgt* population conferred by *Pm6* and *Pm8* was confined to the adult-plant stage and was not observed at the seedling stage (Golzar et al., 2016). This also illustrates the importance of phenotyping in the field to complement laboratory studies with seedling leaf tissue.

Surprisingly, Brazilian isolates registered significantly higher complexity of virulence to the older *Pm* genes than those from any other country sampled, despite the relatively recent emergence of serious powdery mildew epidemics in Brazil. Sampling locations for this study targeted the main wheat growing states in the south of Brazil, Parana and Rio Grande do Sul, where powdery mildew has been reported as a common disease (Costamilan, 2005). Surveys across those two states from 1995 to 2003 determined virulence levels to some older *Pm* genes, including a low frequency of virulence to *Pm1a* and *Pm2*, moderate levels to *Pm4a* and *Pm6*, and high frequencies to *Pm3a*, *Pm4b*, and *Pm8* (Costamilan and Linhares, 2002; Costamilan, 2005). In general, wheat powdery mildew was not a major problem in Brazil until 2015 (P. Kuhnem, personal observation). However, in the past 6 years, it became the most prevalent wheat disease in southern Brazil (states of Parana, Santa Catarina, and Rio Grande do Sul). This was likely due to mild, humid winters conducive to *Bgt* epidemics, and the widespread use of susceptible cultivars (P. Kuhnem, personal observation).

From the present survey, virulence in Parana and Rio Grande appears to have increased dramatically in the past 15 years, such that now all the older *Pm* genes (except *Pm1b*) evidently have lost effectiveness against *Bgt*. Over 70% of the registered cultivars in Brazil had CIMMYT material as background in 2002 (Lantican et al., 2005), but the dominance of that background in current Brazilian cultivars has declined (P. Kuhnem, personal observation). Genes used in Brazilian breeding nurseries included *Pm4a* from cultivar Khapli (Reis et al., 1979; Bennett, 1984) and *Pm17* in the 1A.1R wheat-rye translocation (Byerlee and Moya, 1993). Another factor that could have contributed to the high

complexity of *Bgt* virulence in Brazil is the use of *Pm* genes in neighboring countries, such as Paraguay, where widespread planting of cultivar Timgalen in the 1970s caused an upsurge in virulence to *Pm6* (Scott et al., 1980). Genotyping several wheat cultivars recently popular in South Brazil with KASP markers for *Pm1a*, *Pm6*, and *Pm8* indicated that none of the cultivars had any of those genes.

A generally low level of virulence to the newer *Pm* genes in isolates from countries with recently escalating wheat powdery mildew epidemics suggested that these newer *Pm* genes would likely be effective there. An exception was *MlUM15*, which is tightly linked or allelic to *Pm1* or another *Pm* gene on chromosome 7AL (Hsam et al., 1998; Perugini et al., 2008; Maxwell et al., 2009; Worthington et al., 2014). In this study, virulence to *Pm1a* and to *MlUM15* was linked in most isolates except those from Brazil and Turkey. If *Pm1a* and *MlUM15* were tightly linked, they would be expected to place simultaneous selection pressure on both *AvrPm1a* (Hewitt et al., 2021) and the Avr gene corresponding to *MlUM15*. In that case, *Bgt* isolates would require mutations in both *Avr* genes to become virulent to both *Pm* genes. If instead *Pm1a* and *MlUM15* are allelic to one another, as Worthington et al. (2014) proposed, how could co-segregation of virulence/avirulence to the two alleles be explained? The wheat alleles *Pm3a* and *Pm3f* were shown to have different but overlapping recognition specificities for fungal effector alleles of *AvrPm3a* (Stirnweis et al., 2014; Bourras et al., 2015). It is possible that similarly, *Pm1a* and *MlUM15* have different but overlapping recognition spectra for alleles of *AvrPm1a* and *AvrMlUM15*, such that in most *Bgt* populations, both *Pm* genes recognize both *AvrPm1a* and AvrMlUM15. However, perhaps, Brazilian and Turkish *Bgt* populations have private, unique forms of *AvrPm1a* and *AvrMlUM15* that are recognized by *MlUM15* but not *Pm1a*. This would explain the larger fractions of isolates virulent to *Pm1a* but avirulent to *MlUM15* in *Bgt* populations of those two countries. Nonetheless, virulence to this gene appears to be linked to *Pm1a* virulence in most populations, and thus, the new gene would likely not be useful in those countries. In Brazil, where *MlUM15* might have value, further testing of this gene in the field is advisable. Mapping and characterization of the *Avr* gene that is recognized by *MlUM15* would also be useful.

As new *Pm* genes are made available, it is important to exercise responsible stewardship when they are deployed to help to prevent a rapid appearance of virulent isolates and limit the development of a larger, virulent population of *Bgt* (Li et al., 2019a; Tan et al., 2019; Simeone et al., 2020). Besides cultivar and crop rotations, it is recommended to introgress effective *Pm* genes in combinations of two or more into breeding lines with a quantitative resistance background (Brown, 2015; Rimbaud et al., 2018; Aravindh et al., 2020). Ideally, good stewardship should also include the stacking of resistance genes with complementary function, which can be elucidated by their molecular characterization (Hafeez et al., 2021). A coordinated, international effort to track deployed resistance genes in current and future cultivars with molecular markers and the periodic monitoring of pathogen populations for virulence frequencies would allow the timely identification of breakdowns in resistance

gene stacks in the field and illuminate which genes need to be replaced. Seed of germplasm lines used in this study is freely available and can be obtained from the authors.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

TK helped design the study, cultured, phenotyped, and genetically analyzed isolates and cultivars, conducted statistical analyses, and drafted the manuscript. WB and ZP collected samples in South Africa. BA, AM, and VS collected samples in Turkey, Russia, and Kazakhstan. PK collected the samples in Brazil. PM created germplasm lines containing introgressed genes *Pm25–Pm53*. CC conceptualized and supervised the study and helped to prepare the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.954958/full#supplementary-material

Supplementary Figure 1 | Allele discrimination plots of kompetitive allele-specific PCR (KASP) markers linked to *Pm* gene resistance loci. KASP markers are diagnostic of alleles linked to the presence (red) or the absence (blue) of **(A)** *Pm1a*, **(B)** *Pm6*[z], and **(C)** *Pm8*. Fluorescence values of reporter cassettes measured at 44 cycles were normalized to readings of the ROX reference dye. Gray lines indicate the real-time fluorescence signal of cycles 1–50. [z]Despite lower confidence in allele discrimination for *Pm6* and *Pm8* with medium FAM fluorescence signal (>60,000 relative fluorescence units) detected in all samples (including the no-template control, yellow), positive controls for both alleles separated into distinct clusters, so that all tested cultivar samples could be distinguished from samples with a resistance allele.

Supplementary Figure 2 | Complexity of virulence to wheat resistance genes among *Blumeria graminis* f. sp. *tritici* populations from several countries compared to previously published virulence data for the United States and Egypt, for recently introgressed genes subsetted into **(A)** four *Pm* genes that conferred susceptibility to *Bgt* in detached leaf assays, but were effective against United States *Bgt* populations in the field[d] (*Pm25–NCA6*), and **(B)** five *Pm* genes which were effective both in lab assays and in the field (*NCAG13–Pm53*). Virulence complexity was calculated as mean percentage of *Pm* genes to which isolates were virulent. Within a panel, countries topped by the same letter did not have significantly different virulence complexities (*p* < 0.05). Within each box, medians are indicated by lines and means of virulence complexity by crosses. [v]Abdelrhim et al. (2018). [w]Turkey/Black Sea region: Isolates from Russian locations Krasnodar and Rostov were grouped with those from Turkey (refer to **Table 1**). [x]Western Siberia region: Isolates from Karabalyk, Kazakhstan were grouped with those from Russia (refer to **Table 1**). [y]Cowger et al. (2018). [z]Genes *Pm25–NCA6* were effective in the field against a natural United States *Bgt* population (Cowger et al., 2018).

Supplementary Figure 3 | Complexity of virulence to wheat resistance genes among *Blumeria graminis* f. sp. *tritici* populations from five global regions compared to previously published data from the United States and Egypt, for **(A)** ten older *Pm* genes, in commercial use for a longer time (*Pm1a–Pm17*) and **(B)** nine newer *Pm* genes, more recently introgressed into hexaploid wheat from wild relatives (*Pm25–Pm53*). Virulence complexity was calculated as mean percentage of *Pm* genes to which isolates were intermediately virulent (yellow) and fully virulent (red). Within a panel, countries topped by the same letter did not have significantly different virulence complexities (*P* < 0.05); numbers below countries indicate the number of isolates analyzed. Error bars indicate standard error of the means. [v]Abdelrhim et al. (2018). [w]Turkey/Black Sea region: Isolates from Russian locations Krasnodar and Rostov were grouped with those from Turkey (refer to **Table 1**). [x]Western Siberia region: Isolates from Karabalyk, Kazakhstan were grouped with those from Russia (refer to **Table 1**). [y]Cowger et al. (2018). [z]Newer *Pm* genes *Pm25–NCA6* were effective in the field against natural United States *Bgt* population (Cowger et al., 2018).

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