

# New sources of resistance to *Septoria tritici* blotch in wheat seedlings

María Rosa Simón · Nadia S. Castillo · Cristina A. Cordo

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**Abstract** The resistance of a set of Argentine wheat cultivars, differentials, and foreign lines with known genes of resistance to *Zymoseptoria tritici* was assessed at the seedling stage against a set of molecularly characterized Argentine fungal isolates under three experimental conditions. The experimental design was a split-split-plot with two replications. The environment conditions were the main plots, the fungal isolates were the subplots and the wheat lines with known resistance genes or used as differential lines or the Argentine commercial cultivars were the sub-subplots. Sixteen fungal isolates were inoculated on 23 wheat accessions including 12 foreign lines with known resistance genes, two differential lines, and nine Argentine cultivars that were selected on the basis of

their moderate resistance to natural infection, to identify isolate-specific and partial resistances. The genotypes TE 9111 and Oasis among the foreign lines along with the Argentine cultivars Pro INTA Puntal, Klein Volcán and Buck 75 Aniversario expressed the highest number of specific-resistance interactions. Flame, Tadinia and Kavkaz among the foreign lines showed partial resistances to a higher number of fungal isolates; a comparable resistance pattern was also obtained in the Argentine cultivars Klein Sagitario, Klein Dragón, Don Ernesto and Buck Arriero. Our study demonstrated that the presence of resistance genes in several foreign lines against *Septoria tritici* blotch is effective against some of the Argentine fungal isolates. In addition, the Argentinean wheat cultivars that showed isolate-specific and nonspecific resistances probably carry novel resistance genes against the pathogen.

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## Introduction

*Septoria-tritici* blotch (STB) is caused by the fungus *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (syn. *Mycosphaerella graminicola*, *Septoria tritici*). STB is a major foliar disease of wheat worldwide. Therefore, new sources of resistance along with a more extensive knowledge concerning the genetics of resistance have become necessary in order to improve the breeding for resistance to this disease (Chartrain et al.

2004). *Z. tritici* is a highly genetically diverse pathogen (McDonald et al. 1999); moreover, the fungus may reproduce sexually several times during the growing season of wheat (Kema et al. 1996c). This fecundity increases the risk of adaptation of the pathogen to resistance genes acquired in the host population. The use of fungicides to control STB is expensive as well as not entirely reliable (Chartrain et al. 2004) and may lead to the emergence of fungicide-resistant strains. Although the use of fungicides in some situations is unavoidable, resistant cultivars provide an effective, economical, and environmentally friendly approach to the control of STB epidemics (Eyal 1999). Until the most recent decades, however, little was known about the genetics of resistance to the disease.

Both qualitative and quantitative resistances have now been identified in wheat germplasm. The former typically gives nearly complete resistance that is isolate-specific, and simply inherited, following a gene-for-gene relationship (Brading et al. 2002). In contrast, quantitative or partial resistance is incomplete, polygenic (Jlibene et al. 1994; Simón and Cordo 1998; Zhang et al. 2001), and isolate-nonspecific (Chartrain et al. 2004), and often more durable (Simón and Cordo 1998). Although complete resistance is preferential because of the almost total absence of symptoms in the host, a partial resistance is highly relevant owing to the concomitant durability and expression under a broad spectrum of pathogen isolates. Several authors have reported specific interactions between wheat cultivars and particular isolates of *Z. tritici* (Chartrain et al. 2004; Eriksen et al. 2003; Kema and Van Silfhout 1997) although quantitative resistance has also been found among different genotypes (Brown et al. 2001; Chartrain et al. 2004; Jlibene et al. 1994; Simón et al. 2005). Eighteen major resistance genes have been identified in bread wheat (*Triticum aestivum* L.): *Stb1* to *Stb15* (McIntosh et al. 2008), and *Stb16* to *Stb18* (McIntosh et al. 2012). In addition, several quantitative-trait loci for resistance (QTL) were also found (Eriksen et al. 2003; Kelm et al. 2012; Miedaner et al. 2012; Raman et al. 2009; Risser et al. 2011; Simón et al. 2004; Simón et al. 2007).

Extensive variations in the virulence of the fungus towards different cultivars have been detected during pathogenesis at the seedling stage (Arraiano et al. 2001; Chartrain et al. 2004; Eyal et al. 1985; Kema et al. 1996a, b; Simón et al. 2005). In Argentina, breeders have classified most commercially-grown cultivars in the range of moderate resistance to susceptibility, suggesting the presence of quantitative, nonspecific

resistance in those cultivars, although isolate-specific quantitative resistance may also be present in some cultivars. Although specific interactions between cultivars and isolates have been reported (Perelló et al. 1991; Simón et al. 2005), an accurate characterization of the resistance levels of new cultivars against different fungal isolates is scarce, as the type of resistance has not been investigated previously. Novel sources of resistance and knowledge about the effectiveness of a given resistance against isolates molecularly different are substantially necessary to provide the genetic materials required for improvement of the resistance against STB. In addition, the behavior of germplasm with resistance against the STBs caused by Argentine isolates is still unknown. This knowledge along with the behavior of local cultivars is essential for the planning of crosses with complementary resistances.

The aim of this study was therefore to identify STB resistance at the seedling stage in a set of Argentine wheat cultivars and to compare those characteristics to the properties of foreign wheat lines with known genes and differential lines against 16 molecularly characterized isolates of the pathogen *Z. tritici*.

## Materials and methods

### Wheat genotypes and design of experiments

Disease assays were conducted under three different environments on plants grown in the field, in pots outdoors at different locations, and in pots in a growth chamber through use of a split-split-plot design with two replications. The field experiment was performed at the Estación Experimental J. Hirschhorn, Los Hornos, Argentina (*EE* environment). Experiments on plants grown in pots placed outdoors (*FA* environment) or in growth chamber (*GW* environment) were conducted at the Facultad de Ciencias Agrarias y Forestales, La Plata. The environments were the main plots, the isolates the subplots, and the genotypes (*i. e.*, the lines and cultivars) the sub subplots. Sixteen isolates were inoculated on 23 accessions (12 genotypes with known genes, two differential genotypes, and nine Argentine cultivars; Table 1). The set of Argentine cultivars was selected because they had been found to have acceptable resistance levels against natural infections of the pathogen. Most of the foreign genotypes were chosen because they carried known resistance genes and two of them because

**Table 1** Lines and cultivars of *Triticum aestivum* tested with 16 isolates of *Zymoseptoria tritici* in two environments

Line/ Cultivar	Origin	Resistance genes mapped or reason for selection for this study
Flame	United Kingdom	<i>Stb6</i>
Israel 493	Israel	<i>Stb3</i> and <i>Stb6</i>
Kavkaz -K4500 L.6 A.4	Kenya	<i>Stb6</i> , <i>Stb7</i> , <i>Stb10</i> and <i>Stb12</i>
Oasis	Russia	<i>Stb1</i>
Tadinia	UnitedStates	<i>Stb4</i> , <i>Stb6</i>
TE 9111	Portugal	<i>Stb6</i> , <i>Stb7</i> and <i>Stb11</i>
Tonic	UnitedKingdom	<i>Stb9</i>
Veranópolis	Brazil	<i>Stb2</i> , <i>Stb6</i> and <i>Stb11</i>
W7984 SyntheticHexaploid	UnitedStates	<i>Stb5</i> and <i>Stb8</i>
Capelle	France	differential line
IAS 20	Brazil	differential line
Saratovskaja 19	Russia	susceptible control
Janetzki Probat	Austria	susceptible control
Buck 75 Aniversario	Argentina	Argentine cultivar
Buck Arriero	Argentina	Argentine cultivar
Buck Ranquel	Argentina	Argentine cultivar
Don Ernesto INTA	Argentina	Argentine cultivar
Klein Dragón	Argentina	Argentine cultivar
Klein Sagitario	Argentina	Argentine cultivar
Klein Volcán	Argentina	Argentine cultivar
Klein Zorro	Argentina	Argentine cultivar
Pro INTA Puntal	Argentina	Argentine cultivar

they were differentials against different isolates of *Z. tritici*. Saratovskaja 19 and Janetzki Probat were included as susceptible controls because they had proved to be sensitive to certain fungal isolates in Argentina in natural infections (Simón, M. R., unpublished data). Ten seeds per genotype were sown in each row in the field or into 10-L pots outside or in 1-L pots in the growth chamber. The plants in the pot and growth chamber experiments were watered regularly (every 2–3 days) and provided at sowing with 50 kg.ha<sup>-1</sup> N as urea and 50 kg.ha<sup>-1</sup> of ammonium diphosphate, then at tillering with 50 kg.ha<sup>-1</sup> as urea.

#### Zymoseptoria tritici isolates and inoculation

Sixteen monosporic isolates of the pathogen *Z. tritici* were chosen based on differences in their molecular characteristics determined by ten ISSR molecular markers (Castillo et al. 2010). In this study, the dendrogram generated by the similarity matrix based on Dice's coefficient and the unweight-group method with

arithmetic-mean (UPGMA) clustering algorithm showed that at the 0.20 similarity level, 13 distinct clusters were detected (Table 2). We selected the following isolates belonging to different clusters from the different locations: FALP 00105 (1N), 00205 (2N), 01405 (14N), 02005 (20N), 02405 (24N), 05205 (52TA), 20107 (201NJ), 20507 (P), 21707 (217NJ), 22607 (226NJ), 23007 (230NJ), 24307 (243NJ), 24407 (244NJ), 24707 (247NJ), 24807 (248NJ), and 25405 (254NJ). Table 3 lists the codes, names, and origins of *Z. tritici* isolates used, and all of them were obtained from bread-wheat cultivars growing at different locations (Fig. 1; Castillo et al. 2010): Necochea [N] and Tres Arroyos [TA] (wheat sub region IV, Argentina) or Nueve de Julio [NJ] and Pla [P] (wheat sub region II South, Argentina).

Cluster I was the largest cluster containing 50 isolates. In cluster I, isolates 1N, 2N, 20N, and 24N from Necochea, 52TA from Tres Arroyos, and 226NJ from Nueve de Julio were selected for inoculation in this study.

**Table 2** Cluster analysis and *Zymoseptoria tritici* isolates belonging to each cluster

Cluster	Isolates	Origin
Cluster I	FALP00105, FALP00205, FALP00305, FALP02005, FALP02405, FALP02605, FALP03005, FALP03305, FALP03505, FALP03805, FALP04305, FALP04405, FALP04905, FALP05605, FALP07005, FALP07205, FALP07605, FALP07705, FALP08205, FALP08305, FALP08705, FALP09505, FALP09705, FALP10205, FALP10305, FALP10605, FALP12205, FALP13005, FALP14205, FALP15205, FALP22505, FALP22705, FALP23905, FALP25305 and FALP29305.	Necochea
	FALP05005 and FALP0 5205	Tres Arroyos
	FALP20207, FALP22607, FALP22807, FALP22907, FALP23207, FALP23607, FALP23707, FALP23807, FALP24907, FALP25507, FALP26007 and FALP29207 FALP22407	Nueve de Julio Pla
Cluster II	FALP07405, FALP08505, FALP13705 and FALP14905	Necochea
	FALP21507, FALP24707, FALP25007, FALP26507 and FALP29107 FALP04705 and FALP08905	Nueve de Julio Tres Arroyos
Cluster III	FALP09805 and FALP10005	Necochea
Cluster IV	FALP03905 and FALP07505	Necochea
	FALP20507	Pla
	FALP20807	Nueve de Julio
Cluster V	FALP7805 and FALP12005	Necochea
Cluster VI	FALP24807	Nueve de Julio
Cluster VII	FALP08105 and FALP10505	Necochea
	FALP24407	Nueve de Julio
Cluster VIII	FALP20107 and FALP21407	Nueve de Julio
	FALP20307	Pla
Cluster IX	FALP09305	Tres Arroyos
	FALP25405	Nueve de Julio
Cluster X	FALP21707	Nueve de Julio
Cluster XI	FALP23007	Nueve de Julio
Cluster XII	FALP07905	Necochea
Cluster XIII	FALP09905	Necochea

One isolate, 247NJ, was selected from total 12 isolates of cluster II. No isolates were selected from clusters III and V because each of these clusters contained only two isolates collected from Necochea, a location already well represented by the isolates of other clusters. Likewise, clusters XII and XIII were excluded since they contained only one isolate each. Among the four isolates of cluster IV, 205P was chosen because Pla was not represented in the set of isolates selected. Clusters VI, X, and XI, containing a single isolate each from Nueve de Julio, were all selected for inoculation. Of clusters VII and VIII containing three isolates each from Nueve de Julio, we selected one isolate from each: 244NJ and

201NJ, respectively. Similarly, from the Cluster IX comprising only two isolates, we chose 254NJ.

In addition to these 14 isolates selected from the dendrogram, we chose two more isolates, 14N and 243NJ, neither of which produced PCR amplicons with any of the primers used and therefore were excluded from the resulting dendrogram. These two isolates likely belong to distinct clusters.

The selected fungal isolates were kept in silica gel and transferred to malt-extract agar for 5–6 days before harvesting spores for inoculations. The inoculum was prepared by suspending conidia scraped from sporulating colonies in deionized water at a concentration of

**Table 3** Codes, names, origins, and years of collection of *Zymoseptoria tritici* isolates used in this study

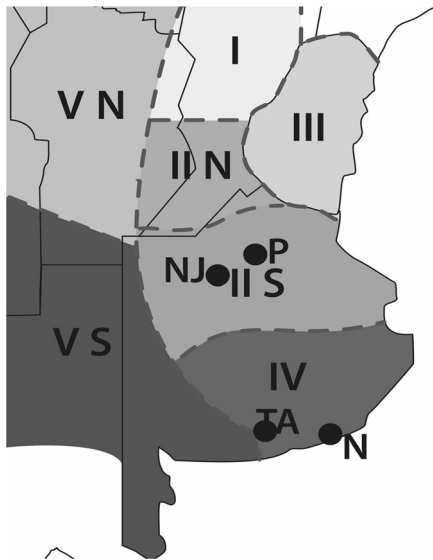
Code of isolate in the text	Name of isolate	Origin	Year of collection	Host
1N	FALP00105	Necochea	2005	Bread wheat
2N	FALP00205	Necochea	2005	Bread wheat
14N	FALP01405	Necochea	2005	Bread wheat
20N	FALP02005	Necochea	2005	Bread wheat
24N	FALP02405	Necochea	2005	Bread wheat
52TA	FALP05205	Tres Arroyos	2005	Bread wheat
205P	FALP20507	Pla	2007	Bread wheat
201NJ	FALP20107	Nueve de Julio	2007	Bread wheat
217NJ	FALP21707	Nueve de Julio	2007	Bread wheat
226NJ	FALP22607	Nueve de Julio	2007	Bread wheat
230NJ	FALP23007	Nueve de Julio	2007	Bread wheat
243NJ	FALP24307	Nueve de Julio	2007	Bread wheat
244NJ	FALP24407	Nueve de Julio	2007	Bread wheat
247NJ	FALP24707	Nueve de Julio	2007	Bread wheat
248NJ	FALP24807	Nueve de Julio	2007	Bread wheat
254NJ	FALP25405	Nueve de Julio	2005	Bread wheat

Letters following each code indicate the origin of each isolate: *N* Necochea, *TA* Tres Arroyos; *P* Pla, *NJ* 9 de Julio

$5 \times 10^6$  spores  $\text{ml}^{-1}$  with Tween 20 ( $0.5 \text{ ml.l}^{-1}$ ) added as a surfactant. The wheat plants were inoculated at the two-leaf stage, GS 12 (Zadoks et al. 1974). For the experiment in pots placed in growth chambers or outdoors, the plants were covered with a transparent plastic wrap for 48 h immediately after inoculation, to maintain

a high level of humidity. For the experiment in the field, the plants were kept moist by spraying water for 15 min with 2 h interval for 3 days after the inoculations.

Meteorological data were obtained from the Agrometeorological Station located at the Experimental Station, J. Hirschhorn, Los Hornos or at the Astronomical Observatory in La Plata.



**Fig. 1** Map of Argentine Wheat Region showing the locations where the *Zymoseptoria tritici* isolates were collected within the Province of Buenos Aires. *N* Necochea, *NJ* Nueve de Julio, *P* Pla; *TA* Tres Arroyos

#### Evaluation of disease reaction and data analysis

To estimate seedling resistance, the second leaf of each plant was visually assessed for the percent pycnidial coverage at 28 to 30 days after inoculation in the field or pots outside, whereas pycnidial coverage was estimated at 21–22 days after inoculation in the growth chamber. For the statistical analysis, the Genstat 12th edition 2009, was used. Data normalization was performed by arcsine–square-root transformation (Sokal and Rohlf 2012). Environments, pathogen isolates, host accessions, and the interactions between these factors were fitted as fixed effects; whereas blocks were fitted at random within a split–split-plot design. For testing specific interactions between the genotypes and the fungal isolates, we calculated the least significant differences (LSDs) of the means of genotype by isolate–interaction values (Cherif et al. 2007).

The lowest mean of transformed disease severity (0 % for pycnidial coverage) was used as the resistance control: therefore, the means of interactions that were not greater than the LSD values at a  $P \leq 0.01$  level were considered as resistant. In addition, values that were significantly different from the lowest value, but also different from the highest value, indicated a certain level of partial resistance. According to this, only those values up to 35 % in the EE environment, up to 7 % in the FA environment and up to 43 % in the GC environment were considered as carrying partial resistance. To evaluate the general resistance of the wheat genotypes to the isolates tested and the aggressiveness of the latter to the wheat genotypes, the mean disease severities, were calculated by omitting the data for specific interactions (Brown et al. 2001; Chartrain et al. 2004; Ghaneie et al. 2012).

## Results

During the period between the inoculation and the evaluation of disease reaction, in the field experiments (EE environment) the mean temperature was 12 °C and the mean relative humidity was 77.0 %; while in the experiments where plants grew in pots outdoor (FA environment), though the mean temperature was similar, the relative humidity was slightly lower at an average of 74 %. For the experiments where plants grew in chambers (GC environment), temperature was set at 20–22 °C and humidity was at 80–90 %. The GC and EE environments evidenced a tendency to be more conducive to the formation of pycnidia. The mean value of the percent pycnidial coverage fluctuated between 0 and 60.8 % for the EE environment, 0 and 17.7 % for the FA environment and 0 and 71 % for the GC environment.

Analysis of variance in disease reactions indicated significant differences between the genotypes and the isolates with respect to the percent pycnidial coverage. All double and triple interactions were also significant (Table 4). Back transformed means are presented in Table 5. Omitting specific-resistance interactions, the isolates 254NJ, 248NJ, 244NJ and 20N showed the least aggressiveness, whereas 2N, 14N and 24N were the most aggressive isolates (Table 5).

Among all foreign lines, genotypes IAS 20, TE 9111, Oasis, Israel 493 and Kavkaz, were the most resistant, whereas Saratovskaja 19 and Janetzki Probat were the most susceptible. Within the Argentine cultivars, Klein

**Table 4** ANOVA of percent leaf area covered by pycnidia on the second leaf (GS 12) of 23 lines and wheat cultivars inoculated with 16 isolates of *Zymoseptoria tritici*, in two environments

Source of variation	<i>d.f.</i>	<i>m.s.</i>	<i>F pr.</i>
Environment	2	16.417	0.059
Residual	2	1.022	
Isolate	15	0.525	<0.001
Environment × Isolate	15	0.037	<0.001
Residual	45	0.006	
Line-Cultivars	22	0.690	<0.001
Environment × Line-Cultivar	44	0.050	<0.001
Isolate × Line-Cultivar	330	0.076	<0.001
Environment × Isolate × Line-Cultivar	660	0.013	<0.001
Residual	1056	0.006	

Volcán and Buck 75 Aniversario were the most resistant; whereas Klein Zorro and ProINTA Puntal were the most susceptible (Table 5).

Pycnidial coverage values not significantly different from zero in the three environments were considered as indicative of specific resistance, whereas those values that were significantly different from the lowest value but also different from the highest in each environment were considered as indicative of partial non-specific resistance. In addition, we considered as carrying partial resistances, those genotypes that manifested specific resistance in some environments and partial resistance in the others (Table 5).

Both susceptible controls (Janetzki Probat and Saratovskaya 19) did not exhibit any specific resistance interaction. Furthermore, Janetzki Probat carried partial resistance to 8 isolates, being susceptible to isolates 14 and 247 in the three environments, whereas Saratovskaya 19 manifested susceptibility to isolates 1; 14 and 226 in all the environments, carrying partial resistance to 7 isolates. ProINTA Puntal, Klein Dragón, Klein Sagitario, Klein Zorro, Don Ernesto INTA and Buck Arriero were susceptible to isolate 24 and W 7984 and Buck Ranquel were susceptible to isolate 2 and 52 respectively. Most foreign accessions did not show any susceptibility when the three environments were considered, while the majority of Argentine cultivars were susceptible to isolate 24 (Table 5).

Seventy one fungal-isolate-specific-resistance interactions were detected among a total of 388 combinations (23 accessions × 16 isolates). Within the foreign accessions, TE9111 and Oasis showed the highest number of specific interactions (7), followed by Tonic, Veranópolis and Israel 493 (6) and IAS 20 (5). Within



**Table 5** Means of percent pycnidial coverage on the second leaf (GS12) for the interaction Isolate × Line/Cultivar on 23 lines and wheat cultivars inoculated with 16 isolates of *Zymoseptoria tritici* in two environments

Line-Cultivar/Isolate	IN	2N	14N	20N	24N	52TA	201NJ	205P	217NJ	226NJ	230NJ	243NJ	244NJ	247NJ	248NJ	254NJ	ALE	ALTE	
Foreign																			
Capelle	EE	5.00	32.00	27.19	20.06	8.14	8.56	11.35	15.72	25.00	28.00	0.67	15.09	5.10	10.42	15.83	25.50	16.84	15.27
	FA	1.50	14.50	15.43	6.17	7.09	0.84	10.00	2.84	4.00	4.67	0.42	5.83	0.00	6.59	6.00	3.38	5.92	6.00
Saratovskaja 19	GC	7.5	46.00	49.00	26.00	10.00	9.50	23.50	22.50	26.00	36.50	2.00	22.00	6.00	12.00	21.00	28.00	23.03	23.00
	EE	48.75	23.34	54.37	22.06	16.26	39.62	25.25	30.80	30.83	43.93	4.88	31.15	25.84	23.00	0.00	11.13	28.42	26.95
TE 9111	FA	10.75	8.50	25.42	4.66	0.84	11.58	6.75	16.66	13.93	10.84	1.25	17.70	5.71	9.16	9.00	3.57	10.34	9.00
	GC	65.00	30.50	67.50	29.00	27.50	25.50	33.50	52.00	62.50	66.50	7.00	65.00	35.50	39.00	15.00	17.50	42.1	39.00
Flame	EE	7.75	1.25	11.25	8.34	5.16	4.24	18.75	0.00	15.00	6.88	3.50	7.09	2.84	1.00	0.75	1.84	9.38	8.47
	FA	0.50	0.00	5.00	1.67	0.00	0.00	0.59	0.84	10.00	1.00	0.59	0.00	4.00	0.00	0.00	0.00	2.08	0.00
W 7984 S. H.	GC	10.5	3.5	19.5	10.00	6.00	6.50	18.00	1.00	35.00	10.50	5.00	9.50	5.00	2.00	4.00	4.00	13.94	2.00
	EE	1.50	6.25	22.50	15.43	15.21	19.69	21.46	25.84	20.16	3.75	8.44	21.67	9.34	6.66	11.34	10.13	15.29	6.66
Janetzkiis Probat	FA	0.50	2.50	3.75	6.00	2.50	1.54	1.75	6.67	3.00	2.09	3.09	5.13	1.67	0.38	0.00	0.71	2.76	0.38
	GC	2.00	7.50	23.50	23.50	10.00	20.50	23.50	36.00	27.50	5.00	10.00	27.50	12.00	9.00	17.00	2.00	17.82	9.00
Tonic	EE	20.00	36.25	15.00	14.80	15.00	2.00	7.00	20.00	20.25	0.00	17.50	2.00	0.00	12.50	1.67	0.71	15.08	12.50
	FA	10.00	10.00	6.67	0.00	1.60	2.00	0.00	6.26	4.25	0.00	2.25	4.70	0.63	5.50	0.00	1.00	4.13	5.50
Oasis	GC	42.50	50.50	25.50	18.00	13.50	18.50	8.00	30.00	26.00	0.00	23.50	5.50	1.50	13.50	2.00	16.50	23.83	13.50
	EE	28.13	33.75	54.37	30.94	33.06	24.23	14.11	24.02	37.50	12.50	13.94	33.34	31.67	60.83	16.25	17.92	29.16	60.83
Veranópolis	FA	3.00	2.25	13.33	10.33	5.00	10.25	6.00	2.17	1.25	4.66	0.84	6.25	2.50	17.10	0.00	3.17	5.51	17.10
	GC	36.50	44.00	69.00	41.50	45.50	40.00	23.50	36.50	42.50	22.50	17.50	51.00	42.50	71.00	17.50	2.00	37.68	71.00
Israel 493	EE	4.25	17.50	14.37	9.29	5.84	11.85	14.38	4.50	7.41	3.13	2.50	22.91	19.50	3.50	2.50	3.79	12.68	3.50
	FA	1.17	6.75	10.00	1.53	0.00	0.67	0.00	0.66	2.17	0.00	0.00	10.62	2.50	1.63	5.50	0.00	3.42	1.63
Estanzuela Federal	GC	4.75	26.00	23.50	17.50	7.00	7.00	16.00	5.00	8.00	4.50	4.00	33.50	22.50	5.00	4.00	26.00	18.7	5.00
	EE	3.00	13.75	15.56	12.50	6.07	4.27	1.43	2.67	6.25	1.44	3.38	6.67	3.17	3.75	20.00	1.25	9.79	3.75
Kavkaz	FA	5.50	2.75	2.00	1.88	0.00	1.42	4.50	0.50	5.63	0.00	0.80	0.00	0.00	0.00	5.00	0.00	2.69	0.00
	GC	7.50	20.00	18.00	21.00	7.00	6.50	5.50	5.00	8.00	3.50	6.50	6.50	4.00	25.00	3.50	3.50	13.28	4.00
Tadmia	EE	5.00	27.50	21.31	12.15	4.13	3.67	20.00	29.59	1.75	1.25	9.50	14.17	1.00	13.50	19.91	2.75	17.26	13.50
	FA	9.00	1.75	0.00	1.75	0.00	2.50	0.00	1.25	0.42	0.00	0.42	0.00	0.00	0.63	0.00	0.00	1.48	0.63
Estanzuela Federal	GC	10.50	25.00	22.00	10.00	5.00	4.50	19.00	30.00	2.00	2.00	14.00	19.00	1.00	19.00	23.00	2.00	19.15	1.00
	EE	15.00	22.50	10.00	6.00	10.00	6.42	3.57	2.00	2.00	0.00	5.63	5.67	12.33	2.75	1.25	0.00	10.39	2.75
Kavkaz	FA	6.75	8.25	0.00	1.00	0.00	0.00	0.88	0.00	0.88	2.50	0.00	0.00	2.50	0.00	0.00	0.00	1.94	2.50
	GC	26.50	33.50	13.50	7.50	13.50	9.00	4.50	2.00	14.50	2.50	7.00	7.00	17.50	4.50	2.00	4.00	14.95	4.50
Tadmia	EE	4.33	23.75	25.31	15.94	32.81	20.10	22.61	26.79	15.00	18.60	25.84	1.00	10.42	8.50	6.00	6.00	19.01	8.50
	FA	0.25	3.34	10.00	5.25	10.00	4.25	7.50	2.50	5.00	2.09	12.50	0.00	1.67	10.83	0.00	0.00	5.35	10.83
Kavkaz	GC	4.00	34.00	44.00	27.50	52.50	31.00	29.50	27.00	22.50	22.50	28.50	1.50	15.00	8.50	0.00	0.00	26.53	8.50
	EE	11.25	5.00	17.98	17.85	3.50	13.75	7.09	2.15	19.16	7.88	15.00	7.17	3.00	19.00	4.45	6.77	11.13	7.17
Tadmia	FA	3.00	1.00	0.83	5.96	2.79	0.00	3.63	2.50	5.50	2.21	0.84	1.20	1.25	8.50	2.50	1.67	2.83	8.50
	GC	18.50	6.50	21.50	30.00	6.00	16.00	7.50	4.50	20.00	10.00	22.50	7.50	4.00	26.00	6.00	7.50	14.68	26.00
Tadmia	EE	12.50	10.00	30.00	25.93	22.45	14.17	15.84	12.09	6.66	11.06	2.50	3.67	5.50	13.75	6.75	6.75	14.42	13.75
	FA	0.00	2.00	11.66	4.25	0.50	7.00	1.67	2.25	2.50	2.55	0.47	0.25	0.00	0.00	0.00	2.57	0.00	0.47

Table 5 (continued)

Line-Cultivar/Isolate	IN	2N	14N	20N	24N	52TA	201NJ	205P	217NJ	226NJ	230NJ	243NJ	244NJ	247NJ	248NJ	254NJ	ALE	ALTE	
GC	10.50	14.50	40.00	26.50	27.50	26.50	23.50	13.50	7.00	15.00	3.00	5.00	6.00	20.00	17.50	8.00	18.29		
IAS 20	EE	7.50	5.88	8.93	8.39	6.25	2.59	0.50	11.75	0.00	4.67	4.34	3.00	7.04	1.17	0.84	6.57	5.67	
	FA	2.54	4.00	0.00	3.13	0.00	2.84	0.00	0.00	0.00	0.25	0.00	0.50	0.34	0.00	0.00	1.43		
	GC	10.00	8.00	10.00	12.50	4.50	6.50	0.00	15.00	0.00	7.00	7.00	5.00	7.00	2.00	7.50	9		
Argentinean																			
Pro INTA Puntal	EE	40.84	18.75	25.31	14.29	47.50	8.31	22.32	3.75	9.66	28.34	27.50	4.57	1.67	2.25	0.75	21.28	17.58	
	FA	6.25	3.25	2.92	0.34	11.19	0.50	5.00	4.63	0.40	0.50	1.50	0.00	0.42	0.36	0.00	3.07		
	GC	67.50	28.50	31.50	16.50	67.50	9.50	28.50	5.00	12.00	35.00	29.00	5.00	2.00	2.00	2.00	28.37		
Klein Volcán	EE	8.75	18.75	19.17	5.63	8.00	2.88	12.00	2.00	16.39	17.50	26.67	3.67	4.40	6.17	2.25	12.41	8.93	
	FA	0.63	0.00	0.00	0.59	2.25	2.50	3.00	0.16	2.55	0.58	1.40	0.00	0.17	2.00	0.00	1.47		
	GC	9.00	18.00	17.50	7.50	9.00	4.00	14.50	2.00	20.00	19.00	22.50	5.00	5.00	5.50	2.00	12.92		
Klein Dragón	EE	30.00	1.75	28.75	11.38	38.75	10.41	15.05	6.25	13.34	13.75	32.50	10.08	24.59	10.79	16.25	19.08	14.34	
	FA	5.38	0.40	5.50	0.25	17.18	0.00	4.50	1.67	1.43	6.88	6.75	4.12	1.00	2.57	0.00	3.94		
	GC	27.50	2.00	27.50	10.50	50.00	12.00	18.50	6.50	15.00	14.50	33.50	15.00	15.00	13.50	16.00	20		
Klein Sagitario	EE	28.13	30.00	32.50	13.88	37.81	28.50	8.75	20.83	31.50	17.50	19.21	18.09	11.63	13.88	11.23	20.63	16.00	
	FA	1.84	1.75	4.50	2.13	10.36	0.20	0.34	1.50	1.88	7.25	2.75	0.84	8.13	5.00	0.63	3.14		
	GC	28.50	30.50	33.50	14.50	47.50	24.50	9.50	26.00	40.50	24.50	25.50	25.50	16.50	18.00	15.00	24.22		
Buck Ranquel	EE	28.34	20.00	23.13	15.00	19.06	36.56	24.29	26.75	1.79	18.81	26.67	12.04	13.50	12.65	20.38	20.47	16.65	
	FA	0.96	3.50	1.50	0.00	6.25	10.25	1.88	2.36	0.75	6.00	10.66	1.25	3.13	8.33	2.86	4.03		
	GC	31.00	34.50	26.50	17.00	21.00	48.00	28.00	31.00	2.00	25.00	36.50	15.00	16.50	17.50	26.50	25.47		
Klein Zorro	EE	18.57	51.25	35.94	11.41	44.38	9.50	20.92	16.79	20.00	10.63	4.63	5.34	2.50	4.25	4.00	22.06	18.18	
	FA	2.50	6.00	12.09	0.00	13.81	0.50	1.00	3.45	4.29	6.17	0.88	0.42	1.63	3.75	0.00	4.56		
	GC	26.00	67.50	50.50	10.50	56.50	10.00	23.50	19.00	25.50	15.00	5.00	6.00	5.00	5.00	4.50	27.92		
Buck 75 Aniversario	EE	6.25	16.17	14.69	4.73	16.67	15.00	9.71	1.60	39.00	9.63	6.67	24.41	8.96	1.75	1.84	14.32	11.03	
	FA	1.17	8.75	1.67	1.30	1.00	0.84	8.75	0.41	2.95	3.34	2.50	10.00	0.42	0.00	2.14	3.56		
	GC	7.00	20.00	15.50	6.00	15.00	16.50	11.00	2.00	45.00	10.00	7.00	27.50	2.00	2.00	2.00	15.21		
Don Ernesto INTA	EE	21.25	14.59	27.41	11.63	46.16	19.59	15.67	23.75	18.50	25.00	29.25	7.50	13.98	10.66	14.38	19.95	15.49	
	FA	1.50	4.25	6.25	0.84	8.50	0.25	0.00	0.00	10.71	0.84	1.67	5.00	3.27	0.80	0.00	2.95		
	GC	28.50	19.50	42.50	15.00	71.00	18.00	25.50	32.50	4.50	15.50	15.00	2.00	18.00	24.00	22.50	23.6		
Buck Arriero	EE	26.25	26.25	32.50	11.88	54.65	16.88	21.97	30.63	4.25	13.33	13.57	1.96	17.50	12.50	19.17	21.63	15.59	
	FA	0.50	3.20	10.50	0.88	11.15	0.00	2.58	1.97	0.97	1.32	1.17	1.00	0.30	6.71	4.67	3.3		
	GC	24.5	21.00	33.00	15.00	56.00	18.00	16.50	26.50	25.00	25.50	29.50	8.00	17.50	17.50	13.00	21.84		
Average isolates		15.68	18.05	20.88	11.48	18.62	11.52	13.24	16.97	15.57	11.94	13.15	16.09	11.43	10.47	8.90			

Letters following each isolate code indicate the isolate's origin: N Necochea, TA Tres Arroyos, P Pla, NJ Nieve de Julio

ALE average of each line in each environment, ALTE average of each line in the three environments, IA isolate average. Values indicating specific resistance in both environments were not considered in the averages

EE Estación Experimental J. Hirschhorn, FA Facultad de Ciencias Agrarias y Forestales, GC growth chamber

Values in bold are statistically equal to 0 within each environment (LSD test:  $P=0.01$ )

Values in bold italics are significantly different from the lowest and the highest value in each environment (LSD test:  $P=0.01$ )



the Argentine cultivars, ProINTA Puntal and Klein Volcán achieved the highest number of isolate-specific resistance (5) followed by Buck 75 Aniversario (4). Flame showed high values of partial resistance against 14 isolates, apart from some specific-resistance interactions. Other foreign accessions with high partial-resistance values were Tadinia (13), Kavkaz (13), Capelle (11) and IAS 20 (11) whereas the Argentine cultivars manifesting partial resistances against a higher number of isolates were Klein Sagitario (15) Don Ernesto, Klein Dragón and Buck Arriero (14), Buck Ranquel (12) and Klein Volcán (11). All Argentine cultivars showed partial resistance to more than half of the isolates.

## Discussion

The temperature and humidity values for growing wheat under field conditions or in pots in the present study were within the normal range during growing period when wheat is at the vegetative growth stage in the principal wheat-growing areas in Argentina. Although the temperature was rather low compared to the optimum temperature for the development of *Z. tritici*, it represents naturally occurring conditions at seedling stages of wheat growth in most of the temperate regions of Argentina.

Pycnidial coverage on leaf surface was assessed as a measure of the level of infection and/or resistance. Although other scoring methods have been proposed for assessing the disease severity of STB in wheat (McCartney et al. 2002; Adhikari et al. 2003), the scoring of disease severity based on a visual estimation of the percent leaf area covered by lesions bearing pycnidia still remains as the most common approach (Kema et al. 1996a; Brown et al. 2001; Chartrain et al. 2004). Pycnidial coverage is considered to be more accurate because symptoms cannot be confounded with natural senescence or other pathogens. Notwithstanding, in several experiments host tissue necrosis and pycnidia coverage were found to be highly correlated (Brown et al. 2001; Simón et al. 2005). In our experiments, pycnidial production was more pronounced in the GC and the EE experiments compared to the FF experiment. This difference in pycnidial production could be attributed to the optimum environmental conditions in GC associated with somewhat high level of humidity in the EE environment compared to the FA environment. In field

conditions higher density of the canopy might enhance the atmospheric moisture. High humidity is necessary in all stages of the pathogen development during infection processes-*i.e.*, conidial germination, penetration, mycelial invasion within the host tissues, and eventual formation of pycnidia (Browning 1979; Hookes 1957; Shaner and Finney 1976).

This study has identified new sources of resistance in Argentine wheat cultivars at the seedling stage of wheat growth against several molecularly characterized Argentine isolates of *Z. tritici*. This study also provides informative results in determining the behavior of foreign genotypes with known genes against those of same fungal isolates.

The 16 isolates were genetically diverse (Castillo et al. 2010). In addition, those isolates varied in virulence towards the 23 genotypes. The isolates from Nueve de Julio (showing 12.77 % of pycnidial coverage) were on the average less aggressive than the isolates from Necochea (16.94 %). Isolates 244NJ, 226NJ and 205P displayed the highest number of incompatible interactions (with eight occurrences in the three environments) towards the various wheat accessions, thus indicating that these isolates may possess the greatest number of avirulence genes. Although, so far, no avirulence genes have been cloned from *Z. tritici*, all classical genetic approaches indicate that the *Z. tritici*-wheat pathosystem follows the gene-for-gene model (Kema et al. 2000; Brading et al. 2002). We therefore expected that many of the resistant genotypes possessed common or different resistance genes such that their products could recognize the corresponding avirulence gene products. We determined that the genes for resistance in certain of the foreign genotypes were effective against several of the Argentine isolates. In addition, both the foreign and the Argentine cultivars showed specific-resistance interactions in addition to exhibiting partial resistances within the same genotypes in this pathosystem. Similar results had been found by other researchers dealing with different germplasms (Simón et al. 2005; Ghaneie et al. 2012).

Within the foreign lines, Oasis, together with TE9111, displayed the highest number of specific interactions with several isolates. Tonic, Veranopolis, Israel 493 (6) and IAS 20 (5) also showed a high number of specific interactions. Gilchrist et al. (1999) found that the high resistance of IAS 20 appeared in different countries through disease testing carried by the International Septoria Observation Nursery

(ISEPTON). Rosielle and Brown (1979) demonstrated that the resistance in IAS 20 and Veranópolis could be controlled by the same genes because of the similarity regarding to their inheritance patterns, a common ancestor, and comparable levels of disease resistance between these two Brazilian lines. Although our results also indicated a specific resistance against two common isolates (226NJ and 244NJ), IAS 20 and Veranópolis were likewise resistant to certain additional isolates, suggesting that these genotypes could harbor other unknown genes that are effective against Argentine isolates.

Lines Oasis and TE 9111, which carried two known resistance genes, respectively (*Stb1* and *Stb6* in Oasis; *Stb7* and *Stb11* in TE 9111) showed a broad spectrum of resistance against several Argentine isolates, indicating that either all fungal isolates carry the avirulence factors recognized by those resistant genes or both cultivars may harbor additional unknown resistance genes. It was also interestingly noticed that the Portuguese breeding line TE 9111 showed specific resistance interactions with several Argentine isolates. This line is also the most resistant to STB described and has long been used as a major source of resistance breeding in Europe (Brown et al. 2001).

The Argentine cultivars manifested different resistance patterns from those of the foreign lines which indicates possible presence of novel resistance genes or different resistance gene combinations, thus making these local cultivars of interest in the effort to broaden the spectrum of resistance to this ubiquitous pathogen. These cultivars are attractive in resistance selection because they showed moderate resistances against natural infections in the field. For this reason, we expect that they would exhibit resistances against several local isolates even though their resistance mechanisms against STB are still unknown.

The present study has characterized the resistance levels of several genotypes of Argentine and foreign wheat cultivars with known resistance genes against a selected set of molecularly characterized Argentine isolates of *Z. tritici*. In addition, the results have enabled a deeper understanding concerning possible novel sources of resistance within existing Argentine cultivars.

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