Analyzing wheat cultivars grown in Czech Republic for eight stem rust resistance genes

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Abstract Stem rust is a disease of wheat caused by a basidiomycete Puccinia graminis f. sp. tritici (Pgt). Emergence of new populations of the pathogen and their spread over the world calls for growing cultivars with lasting resistance. Knowledge of resistance gene composition in local cultivars in the fields together with knowledge of global movements of pathogen races is crucial. Present study focused on using molecular markers to detect eight resistance genes in 58 wheat cultivars encompassing more than 85% of wheat-growing area of Czech Republic. Presence of genes within the cultivars was compared to disease severity in 2014–2020 field trials. Local samples of Pgt were collected and tested for their virulence profiles using differential lines and two of the accessions were then tested for virulence on the 58 wheat lines. Gene Sr38 was present most frequently (63.79%) and while cultivars with this gene showed lower infection in the non-race specific field trials, pathotypes from 2020 season were mostly virulent to it. Sr31 and Sr24 are present with 10.34% and 13.79% frequencies. The fact that none of the Pgt races collected were virulent to Sr31 and Sr24 suggests that races from the Ug99 lineage are currently not present in Czech Republic. This study showed that wheat cultivars grown in Czech Republic rely heavily on a single resistance gene that is being overcome. Two other resistance genes, while still effective, occur sporadically.

Keywords Puccinia graminis · Pathotypes · Sr genes · Resistance · Wheat cultivars

Introduction

Stem rust is a disease of cereal crops and related Poaceae species caused by a fungal pathogen Puccinia graminis var. tritici (Pgt). During the pathogen’s asexual phase, urediniospores infect cereal crops via plant stomata (Solanki et al., 2019), then nutrient uptake is carried out by rust haustoria inside the infected host tissues (Garnica et al., 2014). The fungus then produces uredinia that rupture the plant surface and large amounts of urediniospores are produced. These urediniospores quickly spread over fields and cause infestations with yield losses of up to 100% (Park, 2007), making Pgt one of the most destructive pathogens in agriculture.

The most effective way to combat stem rust is to grow resistant cultivars. Crop breeders have been developing stem rust resistant cultivars of bread wheat (Triticum aestivum L.) utilizing: (i) race-specific all-stage resistance conferred by major resistance (R) genes, and (ii) adult plant resistance (APR)
that can be inherited monogenically by qualitative R genes or oligo-/polygenically by quantitative trait loci (QTL) (reviewed by Ayliffe et al., 2008). Genes in the first group (R genes) are inherited qualitatively, they confer hypersensitive reaction to specific races of the pathogen to stop the infection from spreading, however their effectiveness can be broken down by emergence of new Pgt races. Their presence can be tested in phenotype tests by inoculating seedlings of distinct races of the pathogen and recording the plants’ response (Stakman et al., 1962). APR genes, on the other hand, slow down the infection in the adult plant stage and usually confer resistance against multiple pathogens. Due to their smaller effect, addition of multiple APR genes is needed to provide near-immune response.

Pgt’s ability to overcome resistance genes and cause damage is best illustrated by the emergence of a new race in Uganda in 1998 (Pretorius et al., 2000). This race was virulent to then widely utilized resistance gene Sr31 and was named Ug99. Its virulence was described as TTKS according to international nomenclature (Roelfs & Martens, 1988). The quick spread of this race over large distances raised a global concern over crop protection against stem rust (Singh et al., 2011). Recent Pgt surveys in Germany (Olivera Firpo et al., 2017), Sicily (Bhattacharya, 2017), and Great Britain (Lewis et al., 2018) show that new populations of Pgt are also spreading over Europe. Moreover, stem rust affects the spring wheat-growing regions in Kazakhstan and Western Siberia (Shamannin et al., 2016).

In Czech Republic, stem rust generally occurs less frequently than leaf rust. Epidemics of this plant disease causing yield losses were recorded in 1930s and 1940s, followed by mild epidemics in 1958 and 1962 (Zadoks, 1967). Latest epidemic was recorded in 1972 when Pgt population overcame resistance gene Sr5 (Bartoš, 2010). Genes Sr31 and SrTmp remained effective at that time. After 1972 stem rust has lost importance, occurred rarely, and populations were not monitored. In recent years, however, stem rust has been reappearing and new Pgt samples have been collected from fields and stored in the Collection of Biotrophic Fungi of the Crop Research Institute in Prague (more information about the collection available at https://www.vurv.cz/en/research/genetic-resources/culture-collection-of-microorganisms-crop-research-institute/).

The reappearance of Pgt in Czech fields and spread of new races in neighboring areas warrants the need to achieve broad and durable resistance by combining distinct resistance genes and by introducing new genes in breeding. However, breeding efforts should be supported by studies of the genetic basis of present-day resistance, as it is not clear what specific genes are currently present in most of the locally grown cultivars.

Out of resistance genes relevant to our study, Sr38 seems to be most common in temperate areas around the globe. For instance, in a study of local Chinese cultivars, it was detected in 9 out of 75 varieties (Xu et al., 2017). In a study of North American winter wheat, it was detected in 26 varieties out of 137 (Zhang et al., 2014). The frequency of Sr38 seems to be even higher in Europe, where Flath et al. (2018) postulated it in 29 German cultivars of 97 tested. Sr31 in wheat cultivars is present on a 1BL.1RS translocation that comes from ‘Petkus’ rye. Cultivars with 1BL.1RS have been widely utilized on all five continents (Rabinovich, 1998). It has been postulated in numerous wheat cultivars registered in Czech Republic in the 1980’s and 1990’s (Bartoš et al., 1994, 2002). Sr24 is completely linked to Lr24 and is present in wheat germ-plasm grown around the globe including temperate Europe. It was postulated in 8 German cultivars including ‘Elixer’ and ‘Gordian’ which are also registered in Czech Republic and present in our study (Flath et al., 2018). In our previous study focused on leaf rust resistance, we detected Sr24/Lr24 in 3 Czech cultivars out of 19 tested (Hanzalová et al., 2020). Although Sr6 was reported in cultivar ‘Košútka’ registered in Czechoslovakia in 1981 (McVey, 1992) and Sr36 was present in a 1985 Czechoslovakian cultivar ‘Agra’ (https://old.vurv.cz/wheat/pedigree/krizeni2.asp?id=3710), it is not known whether they were used in further breeding and whether they made their way to cultivars that are currently used.

In our study we used molecular markers to detect eight selected stem rust resistance genes in wheat cultivars registered in Czech Republic. We also collected and identified samples of Pgt from 2020 to establish the virulence of present races. Finally, we performed greenhouse tests with the identified races on tested cultivars to evaluate efficacy of the current resistance gene make-up.
Materials and methods

Germplasm selection

We selected 58 wheat (*Triticum* spp.) cultivars from the 2019 Summary of entered propagation areas of wheat released by Central Institute For Supervising and Testing In Agriculture (http://eagri.cz/public/web/file/628488/Prehled_prihlasenych_mnoziteleckych_ploch_2019__1_cast.pdf) in a way that the selected varieties cover >80% of total wheat propagation area in Czech Republic in 2019. Cultivars reportedly containing the *Sr* alleles to be used as positive controls were selected according to the Wheat Pedigree and Identified Alleles of Genes Online database (http://old.vurv.cz/wheat/pedigree/). All seed material was supplied by the Genbank of Crop Research Institute Prague (https://grinczech.vurv.cz/gringlobal/).

Non-race-specific field resistance tests

Field resistance tests were carried out in the locality Prague – Ruzyně (50.087441 N, 14.298697 E, 376 m above sea level) in an artificially infected field, with a mixture of stem rust isolates. Each cultivar was grown in three-row microplots 0.5 m long and 0.6 m wide in two replicates. Seeds of the cultivars were provided by Central Institute for Supervision and Testing in Agriculture, Czech Republic. The inoculum for the experiments was prepared in the greenhouse conditions from urediniospores on leaves collected during previous growing seasons to simulate a range of races that appears naturally. When plants reached growth phase BBCH 33–36, a water suspension of urediniospores was injected into the stems of the susceptible variety (Michigan Amber), from where the infection spread to the test materials. The density of spores in water suspension was 100–120 mg/l. Symptoms were evaluated on three dates on a scale of 1 to 9 (1 - resistant; 9 - susceptible).

New *P. graminis* races phenotyping and greenhouse tests

Samples of *Pgt* were collected in Czech Republic and Slovakia in 2020 mostly from variety trials located across the countries and organized by the Central Institute for Supervising and Testing in Agriculture. The pathogen samples were propagated on a susceptible cultivar and single pustules were isolated and tested on the differential Thatcher near-isogenic lines (NILs) with genes *Sr5, Sr11, Sr36, Sr9a, Sr24, Sr21, Sr6, Sr9b, Sr9d, Sr31, Sr9e, Sr8a, Sr30, Sr10, Sr38, Sr7b, Sr9g, Sr17, SrTmP, SrMcN*. The differentials were kindly provided by Dr. Brande Wulff from The John Innes Centre in Norwich, UK. Seedlings were inoculated by rubbing leaves with a urediniospore water suspension and then sprayed with water and placed in glass cylinders for 24–48 h to maintain humidity. Thereafter plants were kept in the greenhouse at 18–22 °C. Infection types based on Stakman et al. (1962) were scored after 10–14 days. Two distinct races with newly identified virulence profiles were then selected and used to test our set of 58 wheat cultivars using the same procedure.

Screening with molecular markers

Seeds were sown in pots and total plant DNA was isolated from the two first leaves using DNeasy Plant Mini Kit according to the manufacturer’s instructions (Qiagen, Germany). Molecular markers were amplified using PCR with reaction mixtures and cycles taken from the original sources of the individual markers (Table 1). We used a *Taq* polymerase and a PCR buffer manufactured by Qiagen, Germany. Oligonucleotides used as primers were supplied by Generi Biotech, Czech Republic. In case of the CAPS marker *csSr2*, the PCR product was digested by a restriction enzyme FastDigest *BspHI* according to manufacturer’s instructions (Fermentas, Lithuania). All PCR products were visualized on agarose gels stained with ethidium bromide, using a GeneRuler 100 bp DNA Ladder as a reference for nucleotide lengths (Thermo Scientific, USA), and scored according to the corresponding studies.

Results

DNA samples of 58 varieties were tested for the presence of 8 genes. All of the markers showed clear and replicable results except marker for *Sr22* which only amplified a product indicating a negative result, even in five additional varieties with previously reported *Sr22* allele presence: ‘Aroona’, ‘Marquis’, ‘
\begin{table}
\centering
\begin{tabular}{lllll}
\hline
Gene & Marker & Primer sequences & Positive allele product & Source \\
\hline
Sr2 & csSr2 & F-5’ CAAGGTTGCTAGGATTGGAA AAC & 172, 112, 53 bp* & Mago et al., 2011 \\
& & R-5’ AGATAACTCTTATGTCTTACATT TTTCTG & & \\
Sr6 & Xcdf43 & F-5’ AACAAAAGTCGGAGCTGCC & 215 bp & Tsilo et al., 2009 \\
& & R-5’ CCAAAAAACCATGTTAAAAAGGGG & & \\
Sr22 & XcsIH81-BM & F-5’ TCTCATAAGTTCTACTACATG & 257 bp & Periyannan et al., 2011 \\
& & R-5’ TAGACAAACAAAGATTTAGCAC & & \\
& & XcsIH81-AG & ** & Periyannan et al., 2011 \\
Sr24 & SCS1326 & F-5’ GCGATGTCAGCTTAGTTCTG & 613 bp & Gupta, Charpe, Koul, et al., 2006a \\
& & R-5’ AGGACATGTTAAAAAGAGAACA & & \\
Sr25 & SCS265 & F-5’ GCAGGATAAGAGCAAGAG & 512 bp & Gupta, Charpe, Prabhu, & Haque, 2006b \\
& & R-5’ GGCAGATAGTTCTGTAAG & & \\
Sr31 & iag95 & F-5’ CTCTGTAGTGAATTCTTGAC & 1100 bp & Mago et al., 2002 \\
& & R-5’ CTCTTGAGACATGCTG & & \\
Sr36 & Xwmc477 & F-5’ CGTGCAAAAAAGCGATACCTCC & 187 bp & Tsilo et al., 2008 \\
& & R-5’ GCGAAAGAAATAGCCCTGATG & & \\
Sr38 & VENTRIUP & F-5’ AGGGGCTACTGACCAAGGCT & 259 bp & Helguera et al., 2003 \\
& LN2 & R-5’ TGCGACTACAGCTATG & & \\
\hline
\end{tabular}
\caption{Molecular markers used in PCR assays to detect resistance genes. Reaction mixes and conditions are available in respective sources.}
\end{table}

‘Steinwedel’, ‘Waldron’, and ‘Warigal’. Therefore, no results are included for Sr22.

Markers for Sr2, Sr25, and Sr36 were successfully optimized but showed only negative results in our selection, suggesting that these resistance genes are present in none of the 58 cultivars. Additionally, Sr36 marker Xwmc477 amplified both the negative and positive allele in our positive control ‘Agra’, suggesting heterozygosity in the tested sample. Those genes are not represented in Table 2 for redundancy.

Genes Sr6, Sr24, Sr31, and Sr38 were detected with various frequencies (Table 2). Marker for Sr6 was only present in one cultivar – ‘Viriato’, Sr24 was detected with a 13.79% frequency, Sr31 with a 10.34% frequency. The positive allele for Sr38 was amplified most frequently, in 63.79% of the samples. Six cultivars were tested positive for two resistance genes at once. None of the samples were positive for more than two resistance genes at once.

According to these results it was possible to divide the cultivars into seven groups according to their resistance gene composition. All stem rust resistance field tests for all cultivars in the study from years 2014–2020 were grouped by R gene composition and their variance was analysed by ANOVA. Groups showed statistically significant differences (F = 32.8; p < 0.001). For cultivars with no Sr genes detected in this study, the mean of resistance trial results was 7.118. For cultivars with 1 Sr gene detected the mean was 3.309, for cultivars with 2 Sr genes the mean was 2.094.

Considering the high frequency and importance of Sr38 in local germplasm, we also compared field trial results of cultivars based on the presence of this gene to evaluate its effectiveness. The mean of field trial resistance score for cultivars with Sr38 was 3.163 compared to 7.118 for cultivars without any Sr gene detected. Lowest infections were recorded for cultivars where Sr38 was detected in combination with one more Sr gene. The mean score was 2.094.

Nine Pgt samples from six localities in Czech Republic and Slovakia were collected in 2020.
Table 2 Results of PCR assays used to determine presence of resistance genes in a selection of wheat cultivars and results of greenhouse tests with two *Pgt* races scored according to Stakman et al. (1962)

| Gene   | Sr6 | Sr24 (Lr24) | Sr31 (Lr26) | Sr38 (Lr37) | Greenhouse tests* |
|--------|-----|-------------|-------------|-------------|-------------------|
| Cultivar | Xcfd43 | SCS1326 | iag95 | Ventriuip / LN2 | Genes detected | G 0103** | G 0106*** |
| Advokat  | (185 bp) | - (null) | + (1100 bp) | - (null) | Sr31 | ;1 | ; |
| Angelus  | (185 bp) | - (null) | - (null) | - (null) | none | ;2 | 3 |
| Annie    | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3- | 4 |
| Apostel  | (185 bp) | - (null) | - (null) | - (null) | none | 3- | 3 |
| Arkeos   | (185 bp) | - (null) | - (null) | - (null) | none | 0 | 0 |
| Askaban  | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 4 |
| Asory    | (185 bp) | + (607 bp) | - (two amplicons) | + (259 bp) | Sr24, Sr38 | 0 | ;1 |
| Athlon   | (185 bp) | + (607 bp) | - (null) | - (null) | Sr24 | ;1-2 | 0; |
| Avenue   | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 4 |
| Balitus  | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Bernstein| (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 4 |
| Bodyček | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Bohemia  | (185 bp) | - (null) | - (null) | - (null) | none | ;2 | 3 |
| Bonanza  | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 2-3 | 3- |
| Brilliant| (185 bp) | - (null) | + (1100 bp) | - (null) | Sr31 | ;1 | 0; |
| Butterfly| (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Campesino| (185 bp) | + (607 bp) | - (two amplicons) | + (259 bp) | Sr24, Sr38 | ; | 0 |
| Chiron   | (185 bp) | - (null) | - (null) | - (null) | none | 0 | 0; |
| Dagmar   | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Elixer   | (185 bp) | + (607 bp) | - (null) | - (null) | Sr24 | ;1 | ;2 |
| Elly     | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Evina    | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | ; | 3 |
| Fakir    | (185 bp) | - (null) | - (null) | - (null) | none | 4 | 3 |
| Fenomen  | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 2-3 | 4 |
| Frisky   | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Futurum  | (185 bp) | + (607 bp) | - (null) | - (null) | Sr24 | ; | ; |
| Gaudio   | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3- |
| Genius   | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Golem    | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Gordian  | (185 bp) | + (607 bp) | - (null) | - (null) | Sr24 | 3- | ;2 |
| Grizzly  | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 3 |
| Hyfi     | (185 bp) | + (607 bp) | - (null) | - (null) | Sr24 | 3 | ; |
| Illusion | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3- | 3 |
| Johnson  | (185 bp) | - (null) | + (1100 bp) | - (null) | Sr31 | ; | ; |
| Julie    | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | ;2 | 3 |
| Lear     | (185 bp) | - (null) | - (null) | - (null) | none | 3 | 4 |
| LG Imposanto | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 3 | 4 |
| LG Mocca | (185 bp) | - (null) | - (null) | - (null) | none | 3 | 4 |
| Matchball| (185 bp) | - (null) | + (1100 bp) | + (259 bp) | Sr31, Sr38 | 0 | ;1 |
| Norin    | (185 bp) | - (null) | - (null) | + (259 bp) | Sr38 | 4 | 4 |
| Patras   | (185 bp) | - (null) | - (null) | - (null) | none | 2-3 | 3 |
Seven distinct pathotypes were identified among the samples. All the pathotypes were avirulent to \( Sr31 \) and \( Sr24 \) and some were virulent to \( Sr38 \). The races can be described by the North American stem rust nomenclature (Jin et al., 2008; Roelfs & Martens, 1988) as MFTPC, RFHPC, TKTTC, RFKPC, TTKRC, PHRTF, and TKRPF.

The 58 varieties were then tested for virulence to two of the 2020 pathotypes, TKRPF and PHRTF, to evaluate the effect of resistance genes that were postulated with molecular markers (Table 2). Those two races were chosen for the tests because their virulence profiles have been detected in Czech Republic before, and were saved as accessions G0103 and G0106, respectively. The greenhouse tests confirmed that all cultivars with \( Sr31 \) were resistant to both \( Pgt \) pathotypes. Cultivars with \( Sr6 \) and \( Sr24 \) were mostly resistant to the two pathotypes with some rare exceptions.

Out of the 37 cultivars with \( Sr38 \), 17 were completely susceptible to the 2020 races, other eight cultivars were resistant only to race G0103 which suggests presence of additional resistance in the cultivars.

### Discussion

Our results demonstrate that wheat cultivars in Czech Republic rely heavily on \( Sr38 \), its frequency is higher than that reported by Flath et al. (2018) in cultivars grown in Germany. Cultivars with this gene still showed higher average resistance in yearly non-race-specific field trials (Table 3). However, greenhouse tests with two pathotypes collected in 2020 show that resistance conferred by \( Sr38 \) is largely overcome by new \( Pgt \) races. This dependency on a single R gene can cause problems when

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**Table 2 (continued)**

| Cultivar  | Genes detected | Greenhouse tests* |
|-----------|----------------|------------------|
| Penelope  | Sr38           | G 0103**         |
| Pirueta   | Sr38           | G 0106**         |
| Ponticus  | Sr38           |                  |
| Rebell    | Sr31, Sr38     |                  |
| RGT Premiant |          |                  |
| RGT Reform |                |                  |
| RGT Sacramento |       |                  |
| Rivero    | Sr38           |                  |
| Sally     | Sr38           |                  |
| Sheriff   | Sr24           |                  |
| Sofru     | Sr38           |                  |
| Steffi    | Sr38           |                  |
| Tobak     | Sr38           |                  |
| Turandot  | Sr38           |                  |
| Vanessa   | Sr31, Sr38     |                  |
| Viki      | Sr38           |                  |
| Viriato   | Sr6, Sr38      |                  |

*Infections types in seedling test: − chloroses, 0, 1, 1–2, 2 – resistant, 3, 4 – susceptible

**Avirulence/virulence on testing lines: \( Sr11 \), \( Sr30 \), \( Sr9d \), \( Sr24 \), \( Sr31 \) / \( Sr5 \), \( Sr21 \), \( Sr9e \), \( Sr7b \), \( Sr6 \), \( Sr8a \), \( Sr9g \), \( Sr36 \), \( Sr9b \), \( Sr17 \), \( Sr9a \), \( Sr10 \), \( SrTmp \), \( Sr38 \), \( SrMcN \)

***Avirulence/virulence on testing lines: \( Sr21 \), \( Sr11 \), \( Sr8a \), \( Sr30 \), \( Sr24 \), \( Sr31 \) / \( Sr5 \), \( Sr9e \), \( Sr7b \), \( Sr6 \), \( Sr9g \), \( Sr36 \), \( Sr9b \), \( Sr17 \), \( Sr9a \), \( Sr9d \), \( Sr10 \), \( SrTmp \), \( Sr38 \), \( SrMcN \)
Table 3  Disease severity in studied wheat varieties in yearly field trials under artificial infection with *Pgt*, on a scale of 1 to 9 (1: resistant; 9: susceptible). Second column lists genes detected using molecular markers. Least-squares means (LS-means) were calculated across years to compare cultivars with missing and unbalanced data (Confidence level used: 0.95)

| Cultivar/Year | Detected genes | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | LS-means |
|---------------|----------------|------|------|------|------|------|------|------|----------|
| Angelus       | none           | 3.5  | –    | –    | –    | –    | –    | –    | 3.9      |
| Annie         | *Sr38*         | 1.5  | 1.5  | 1.5  | 3.0  | 3.0  | 2.5  | 1.0  | 2.0      |
| Apostel       | none           | –    | –    | –    | –    | 9.0  | 9.0  | –    | 8.4      |
| Arkeos        | none           | –    | –    | –    | –    | 6.0  | –    | –    | 5.5      |
| Askaban       | *Sr38*         | –    | –    | –    | –    | 3.5  | 3.0  | 3.0  | 2.8      |
| Asory         | *Sr24, Sr38*   | –    | –    | –    | –    | –    | 2.5  | 1.0  | 1.5      |
| Athlon        | *Sr24*         | 4.0  | 4.0  | –    | –    | –    | –    | –    | 4.5      |
| Balitus       | *Sr38*         | 2.0  | 3.0  | 3.0  | 3.0  | 4.0  | 4.0  | 1.0  | 2.9      |
| Bernstein     | *Sr38*         | –    | 4.0  | 4.5  | 4.0  | 4.0  | 6.0  | –    | 4.4      |
| Bohemia       | none           | 4.5  | 4.5  | 5.0  | 5.5  | 6.0  | 4.0  | 3.0  | 4.6      |
| Bonanza       | *Sr38*         | –    | 3.0  | 3.0  | 3.0  | –    | –    | –    | 3.2      |
| Butterfly     | *Sr38*         | –    | –    | –    | –    | 4.0  | 3.0  | 4.0  | 3.3      |
| Campesino     | *Sr24, Sr38*   | –    | –    | –    | –    | –    | 1.0  | 1.0  | 0.7      |
| Dagmar        | *Sr38*         | 4.5  | 4.0  | 4.0  | 5.5  | 4.5  | 4.5  | 4.0  | 4.4      |
| Elly          | *Sr38*         | 4.0  | 3.0  | 3.5  | 4.0  | –    | 4.0  | 4.0  | 3.8      |
| Evina         | *Sr38*         | 1.5  | 2.0  | –    | –    | –    | –    | –    | 2.2      |
| Fakir         | none           | 4.0  | 4.0  | 6.0  | 8.0  | 8.0  | 7.5  | 3.5  | 5.9      |
| Frisky        | *Sr38*         | –    | 2.0  | 2.0  | 2.5  | 3.0  | 3.5  | 1.0  | 2.3      |
| Futurum       | *Sr24*         | –    | –    | 1.0  | 2.0  | 2.5  | 4.0  | 2.0  | 2.1      |
| Gaudio        | *Sr38*         | –    | –    | 2.5  | 3.0  | 4.0  | 4.0  | 2.0  | 2.9      |
| Genius        | *Sr38*         | 4.0  | 2.5  | 3.5  | 4.0  | 3.0  | 3.0  | 2.5  | 3.2      |
| Gordian       | *Sr24*         | –    | 7.5  | 8.0  | 7.5  | 7.5  | 8.0  | 8.0  | 7.7      |
| Hyfi          | *Sr24*         | –    | 1.0  | 1.0  | 1.0  | 1.0  | 2.0  | –    | 1.1      |
| Illusion      | *Sr38*         | –    | –    | –    | –    | 4.0  | 4.0  | 4.0  | 3.6      |
| Johnson       | *Sr31*         | –    | –    | –    | –    | 4.0  | 5.0  | 6.0  | 4.6      |
| Julie         | *Sr38*         | 2.0  | –    | 2.0  | 3.5  | 3.0  | 4.0  | 3.5  | 2.9      |
| LG Imposanto  | *Sr38*         | –    | –    | –    | 3.5  | 4.0  | 4.0  | 1.0  | 2.8      |
| LG Mocca      | none           | –    | –    | –    | –    | 9.0  | 9.0  | 9.0  | 8.6      |
| Matchball     | *Sr31, Sr38*   | 3.0  | 1.0  | 1.0  | –    | –    | –    | –    | 2.1      |
| Patras        | none           | 8.0  | 8.0  | 9.0  | 9.0  | 9.0  | 9.0  | 9.0  | 8.7      |
| Penelope      | *Sr38*         | –    | 1.0  | –    | 3.0  | 3.0  | –    | –    | 2.2      |
| Pirueta       | *Sr38*         | –    | –    | –    | –    | 3.5  | 3.5  | 3.5  | 3.1      |
| Rebell        | *Sr31, Sr38*   | –    | –    | –    | –    | 3.0  | –    | –    | 2.5      |
| RGT Reform    | *Sr38*         | –    | –    | –    | –    | 4.0  | –    | –    | 3.5      |
| RGT Sacramento| *Sr38*         | –    | –    | 3.0  | 3.5  | 4.0  | 3.0  | 3.0  | 3.0      |
| Rivero        | *Sr38*         | –    | –    | 3.0  | 3.5  | 4.0  | –    | –    | 3.4      |
| Sally         | *Sr38*         | –    | –    | –    | –    | 4.0  | 4.0  | 4.0  | 3.7      |
| Sheriff       | *Sr24*         | –    | –    | 2.0  | 2.0  | 3.0  | 3.0  | 2.0  | 2.2      |
| Steffi        | *Sr38*         | –    | 1.0  | 1.5  | 3.0  | 3.0  | 3.0  | 4.0  | 2.5      |
| Tobak         | none           | 9.0  | 9.0  | 9.0  | 9.0  | –    | –    | –    | 9.3      |
| Turandot      | none           | 7.0  | 8.5  | 5.0  | 9.0  | 8.0  | 8.0  | 9.0  | 7.8      |
| Vanessa       | *Sr31, Sr38*   | 2.0  | 1.0  | 1.0  | 1.0  | 4.0  | 3.5  | 4.0  | 2.4      |
| Viki          | *Sr38*         | –    | –    | 2.5  | 4.0  | –    | –    | –    | 3.3      |
| Virioto       | *Sr6, Sr38*    | –    | –    | –    | 3.5  | –    | –    | –    | 3.0      |
such new pathotypes spread, because virulent races have evolutionary advantage and can therefore spread quicker. Several races of *Pgt* occurred in Germany’s outbreak in 2013. They were later described (Olivera Firpo et al., 2017) and all of them were virulent to *Sr38*. Similarly, a Rust Tracker tool created by Global Rust Reference Center (https://rusttracker.cimmyt.org/?page_id=6647) reports races TTRTF and TTKTF in recent samples from Slovakia and Austria that are both virulent to *Sr38*.

Out of the seven *Pgt* races we identified, TKTTC is virulent to most of the 20 near-isogenic lines. It is a race that frequently appears on African continent and in the Middle East and was found to be the most frequent race in Egypt and Turkey in recent surveys (El-Naggar et al., 2020; Mert et al., 2012). Race TKRPF, on the other hand, is commonly found in Kazakhstan (Rsaliyev et al., 2020) and Western Siberia (Skolotneva et al., 2020). We presume that those two races spread to Central Europe from the regions where they are abundant.

We demonstrated that genes *Sr31* and *Sr24* are effective against local *Pgt* races collected in 2020, and that those pathotypes are not descendants of *Ug99* races, as those are defined by their virulence to *Sr31* (Pretorius et al., 2000) and later *Sr24* (Jin et al., 2008). However, these two genes appear in low frequencies and rarely in combination. To our knowledge, virulence to *Sr31* has not yet been detected locally or in neighboring countries, but virulence to *Sr24* has been detected in Czech Republic in samples from the Collection of Biotrophic Fungi and also in neighboring Germany in race TKKTP (Olivera Firpo et al., 2017). Ideally, those R genes would be present in combinations to achieve durable resistance (Ayliffe et al., 2008).

We discovered resistance to both *Pgt* isolates in cultivars ‘Arkeon’ and ‘Chiron’, which is not conferred by any of the resistance genes we tested for. Further testing will have to be done to determine the source of this resistance. The variability in virulence is surely caused by other genes present in the materials that we have not tested for. For instance, genes *Sr5, Sr11, Sr29, Sr37* and *SrTmp* were all previously reported in local cultivars (Bartoš et al., 2002) and were not tested in our study either due to absence of molecular markers or because it is known that their resistance was overcome.

*Sr2* was the only APR gene that we tested for. Its complete absence from our wheat selection corresponds with Pathan and Park (2007) who found that there were low levels of APR in six European cultivars they tested, but assumed the resistance was not caused by *Sr2* based on the lack of the linked pseudo-black chaff and seedling chlorosis traits. More APR genes that were described recently such as *Lr34/Yr18/Sr57/Pm38* (Krattinger et al., 2009), *Lr46/Yr29/Sr58/Pm39* (Kolmer et al., 2015), *Lr67/Yr46/Sr55/Pm46/Ltn3* (Herrera-Foessel et al., 2014) could account for slight quantitative resistance changes in local cultivars at the adult plant stage but their frequency has not been studied yet.

Our study described the genetic background of wheat stem rust resistance in Czech Republic by the use of molecular markers and phenotype tests. We conclude that only a low number of genes are being deployed and that newly occurring races tend to overcome the resistance. Therefore we recommend breeders to enhance the diversity of *Sr* genes in future cultivars to prevent new pathotypes from spreading.

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**Declarations**

**Ethics approval** Not applicable.

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Consent to participate** Not applicable.

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