



Draft report prepared by WP1 partners in RustWatch - May 2022

Executive summary

The aim of expanding pathogen surveys for wheat yellow rust, wheat leaf rust and wheat stem rust beyond countries with national diagnostic laboratories, France, United Kingdom, Germany, Poland and Denmark, to include samples from VCU trial sites, and to integrate results from national diagnostic laboratories based on alignment experiments across laboratories and methodologies was highly successful. These efforts allowed analyses of national results in a European and global context, and to visualize national results using common maps and charts generated by the Wheat Rust Toolbox (www.wheatrust.org). Stem rust was analysed from 15 European countries in 2021, of which more than 10 have no history of stem rust surveillance capacity, yellow rust was received from 11 countries in 2021 without national rust diagnostic labs, and leaf rust was received from 4 countries, in total representing more than 300 isolates that were successfully genotyped from these areas and trial sites in 2021. Additional analyses are ongoing awaiting common scientific publication in the near future.

KEY HIGHLIGHTS IN 2021

STEM RUST

- Additional spread of stem rust in Europe, in particular in France, but also in other countries in Central/West Europe where it was reported on multiple locations and wheat varieties.
- Clade III-B (race TTRTF) was widespread in southern part of Europe, and in 2021 also detected in Switzerland. Since first detection in Sicily in 2016, we have confirmed TTRTF in nine countries in Europe, and 4 countries in Africa/Asia. This race has virulence to Sr13, which is particularly relevant for durum wheat.
- Clade IV-F (race TKKTF) has now been detected in 13 European countries including Austria, Czech Republic, Denmark, France, Germany, Italy, Norway, Poland, Slovakia, Slovenia, Spain, Sweden and Switzerland in 2021. Previously we have also detected TKKTF in Azerbaijan, Egypt, Iran, Iraq and Tajikistan.
- Clade IV-B contains the races TKTTF, TKRTF and TTTTF. Clade IV-B has been detected in nine countries in Europe including Belgium, Denmark, France, Germany, Ireland, Norway, Spain, Sweden and UK in 2021. TKRTF was observed in a single isolate from Spain; TTTTF was observed in East Africa but not in Europe.
- Clade VIII containing two closely related races HFCNC and RFCNC (predominant) was observed in Eastern Europe. In 2021, this group was observed in Czech Republic and Slovakia, but previously detected in Germany, Czech Republic, Slovakia, and Hungary.
- Clade I (Ug99) was not detected in Europe.

YELLOW RUST

- PstS10 is the most prevalent genetic group in Europe based on incoming samples in 2021. At least four races have been detected in PstS10, the original Warrior(-) race, Kalmar, Benchmark and Amboise (named after cultivars where they first caused epidemics). A new variant was confirmed in the UK with virulence on the differential variety 'Ambition', therefore indistinguishable from the Warrior race in seedling tests. PstS10 has also been detected in Australia and South America.
- A single race/genotype was predominant in PstS13, but additional Yr10-virulence was observed in isolates from Poland and Germany. In Europe, PstS13 has severely affected triticale and durum wheat, but this group has also severely affected bread wheat in South America and Australia.
- The group provisionally termed ME2018 was detected in Estonia in 2021 and Latvia in 2020. This was first detected in Egypt in 2018 and Turkey in 2019.
- Genetic group PstS7 (Warrior race) was less prevalent than in previous years, and PstS8 (Kranich race) was not detected in 2021. Up to now, PstS7 has also been detected in northern Africa and South America.
- Additional genetic groups were detected in low frequencies in incoming samples, including PstS14 and PstS15, each consisting of a single race.



- These conclusions were supported by results from national rust diagnostic labs in United Kingdom (NIAB), France (INRAE), Germany (JKI), and Poland (IHAR). After appropriate alignment, both RustWatch and national results have been uploaded to the Wheat Rust Toolbox and available on public websites including www.wheatrust.org.

LEAF RUST

- The main leaf rust group Fam 166 was widely spread and found in all countries from where samples were submitted in 2021.
- Fam 106 314 2 was the second largest leaf rust group, even though the number of samples in 2021 was relatively low.
- Fam 165 vir24 has been consistently detected in Central Europe since 2019.
- A new genetic group and race was detected in Czech Republic from a sample collected on spelt.
- Genetic variation within the Durum group, wider than previously recorded, was observed with the usual SSR marker set used at INRAE.

Introduction

This report comprises results for the wheat yellow/stripe rust (*Puccinia striiformis*), wheat stem rust (*P. graminis* f.sp. *tritici*) and wheat leaf rust (*P. triticina*). It is based on European samples of rust infected wheat (occasionally other cereals) collected in 2021 and with respect to yellow rust, also results from alignment experiments January-March 2022, where samples from national rust diagnostic labs were submitted to Aarhus University, GRRC, Denmark. This allowed upload of results from national labs to the Wheat Rust Toolbox and presentation of results in a European context utilizing the tools, maps and charts provided by GRRC website www.wheatrust.org.

Results from a global rust surveillance program organized by the BGRI (i.e., beyond RustWatch) are available on the GRRC website. The same SSR genotyping and race phenotyping methodologies have been used (annual reports 2008-2021), i.e., the results are directly comparable with RustWatch results. Global summary of SSR genotyping and race phenotyping results are available online (<http://www.wheatrust.org/>), including an updated table showing the [relationship between races and genetic groups](#).

In 2021, a total of 539 samples and derived isolates of wheat stem rust, leaf rust and yellow rust from 19 countries were handled. Each sample generally consisted of multiple rust infected leaves or stems. Representative leaves/stems were selected for alive spore recovery in parallel to genotyping of incoming samples (Table 1, Table 2, and Table 3). More than 400 alive isolates of yellow rust, stem rust, and leaf rust were recovered based on submissions in 2021.

The genotyping of yellow- and stem rust was based on Simple Sequence Repeat (SSR) markers based on incoming samples of rust infected plant material without prior recovery, whereas the race phenotyping was based on bioassays using differential wheat lines inoculated with spore samples of alive, pure isolates grown under strict experimental conditions (Hovmøller et al. 2017; Patpour et al., 2022). Incoming leaf rust samples were recovered and purified, then phenotyped from bioassays on a set of differential wheat lines (Goyeau et al., 2006), and genotyped based on a set of 19 SSR markers (Goyeau et al., 2012).

Nomenclature of races and genetic groups

In yellow rust, we have generally observed a strong correlation between genetic groups and race phenotypes in clonal populations, similarly, most races of wheat stem rust and leaf rust were associated with specific genetic groups ('clades' or "families")

Genetic groups of yellow rust are named Pst followed by a digit. Race variants are designated by the additional virulence observed or (-) in case a new variant had fewer virulences than the first defined race within the considered lineage. For details, see the rationale for the naming of significant *P. striiformis* races and genetic



groups (Ali et al. 2017) and an updated summary of genetic groups and races on the GRRRC website.

The genetic grouping of stem rust is based on Szabo et al. (2022) and detailed alignment of the SSR and SNP genotyping results between the Cereal Disease Lab (USA) and GRRRC; the race grouping is based on 20 internationally agreed wheat lines differentiating virulence in stem rust (Jin et al., 2008). A more comprehensive documentation of the nomenclature of stem rust races and genotypes, and the underlying experimental procedures are available on the GRRRC website. However, in sexual populations we observed unique and diverse genotypes and races. These and other infrequent groups are termed “other” in tables and online maps (www.wheatrust.org).

For leaf rust, genetic groups are named “families”, noted “Fam”, followed by a 3 to 7 digits number coding the virulence phenotype, based on an octal code adapted from Gilmour (1973). Thus, a family consists of individuals sharing the same genotype according to the 19 SSR markers used at INRAE, and a similar combination of virulence.

Results

In 2021, 182 stem rust, 308 yellow rust, and 49 leaf rust samples and derived isolates were handled (Table 1, 2 and 3). Each sample generally consisted of multiple rust infected leaves or stems. Representative leaves/stems were selected for alive spore recovery, and parallel genotyping based on the incoming sample, resulting in live recovery of 129 (stem rust), 205 (yellow rust) and 42 (leaf rust) isolates based on submissions from European countries. Special efforts have been made to study genetic diversity in leaf rust populations in France in 2019-2020, where this pathogen has persisted for many years, explaining a higher number of leaf rust samples than originally scheduled in RustWatch. Phenotyping and genotyping results of isolates collected in France 2021 as part of national surveillance activities is ongoing.

Geographi	Country	2021		Total
		D	L	
Europe	Austria		3	3
	Belgium	2	1	3
	Czech Republic	4	5	9
	Denmark		5	5
	France	17	26	43
	Germany		1	1
	Ireland		4	4
	Italy	19	36	55
	Norway		6	6
	Slovakia	2	8	10
	Slovenia	5	1	6
	Spain	4	3	7
	Sweden		16	16
	Switzerland		10	10
	United Kingdom		4	4
Europe Total		53	129	182

Table 1. Number of stem rust isolates (*P. graminis* f.sp. *tritici*) derived from samples collected in Europe and handled by GRRRC. D: dead, L: Live (recovered) isolates.



Geographic group	Country	Testing year		Grand Total
		2021 & Q1 2022		
		D	L	
Europe	Czech Republic	11	3	14
	Denmark	11	27	38
	Estonia	1	2	3
	France	3	12	15
	Germany	8	21	29
	Hungary		2	2
	Italy	6	10	16
	Latvia		6	6
	Netherlands		5	5
	Norway	10	27	37
	Poland	10	25	35
	Portugal	1	1	2
	Slovakia	3		3
	Spain	10		10
	Sweden	18	18	36
	United Kingdom	11	46	57
Europe Total		103	205	308

Table 2. Number of yellow rust isolates (*P. striiformis*) derived from samples from Europe, and tested at GRRC in 2021 and first quarter of 2022. D: dead, L: Live (recovered) isolates.

In 2021, 49 (leaf rust) samples and derived isolates were handled (Table 3). Representative leaves were selected for alive spore recovery, and parallel genotyping based on the incoming sample, resulting in alive recovery of 42 (leaf rust) samples based on submissions from European countries.

Leaf rust samples 2021	Recovery state		
	Dead	Recovered	Total
Czech Republic		10	10
Bread wheat		9	9
Spelt		1	1
Italy		21	21
Bread wheat		8	8
Durum		13	13
Slovakia		5	5
Bread wheat		5	5
Slovenia	7	3	10
Bread wheat	7	3	10
United Kingdom		3	3
Bread wheat		3	3
Total	7	42	49

Table 3. Number of leaf rust samples and derived isolates (*P. triticina*) collected in Europe in 2021 and handled by INRAE.



WHEAT STEM RUST 2021

A total of 123 isolates from 15 countries in 2021 were successfully genotyped, which is a significant increase compared to 2020 (Table 4); 72 of these were also race typed using a standard set of 20 wheat lines used to differentiate stem rust races (Table 5 and 6; Figure 1).

Country	SSR_name	Year		Total
		2020	2021	
Austria	Clade III-B	2	2	4
	Clade IV-F	1	1	2
Belgium	Clade IV-B		3	3
Czech Republic	Clade III-B	2	2	4
	Clade IV-F		2	2
	Clade VIII	4	1	5
Denmark	Clade IV-B		3	3
	Clade IV-F		1	1
	Other	2		2
France	Clade IV-B	6	21	27
	Clade IV-F		16	16
Germany	Clade IV-B		3	3
	Clade IV-F		1	1
	Clade VIII	1		1
Hungary	Clade III-B	1		1
	Clade VIII	1		1
Ireland	Clade IV-B	4		4
Italy	Clade III-B	13	21	34
	Clade IV-F	11	10	21
Norway	Clade IV-B		1	1
	Clade IV-F	1	1	2
	Other	1	3	4
Poland	Clade IV-F		5	5
Slovakia	Clade III-B	6	3	9
	Clade IV-F	1	1	2
	Clade VIII	3		3
Slovenia	Clade III-B	16		16
	Clade IV-F	2	1	3
Spain	Clade IV-B	14	1	15
	Clade IV-F		4	4
Sweden	Clade IV-B		1	1
	Clade IV-F		2	2
	Other		1	1
Switzerland	Clade III-B		4	4
	Clade IV-F		6	6
United Kingdom	Clade IV-B		2	2
Grand Total		92	123	215

Table 4. SSR genotyping of samples of *P. graminis* f.sp. *tritici* collected in 2020 and 2021. Results are shown as number of isolates within genetic group (clade). Significant races within clades are shown in Table 5 and virulences in Table 6. Graphical presentation of results available on www.wheatrust.org.



SSR_name	Race_name	Total
Clade III-B	TTRTF	16
	n.a.	56
Clade III-B Total		72
Clade IV-B	TKRTF	1
	TKTTF	24
	n.a.	34
Clade IV-B Total		59
Clade IV-F	TKKTF	18
	n.a.	49
Clade IV-F Total		67
Other	4 races	4
	n.a.	3
Other Total		7
Clade VIII	HFCNC	2
	RFCNC	7
	n.a.	1
Clade VIII Total		10
Grand Total		215

Table 5. Relationship between genetic groups and prevalent races detected within these in Europe (2020 and 2021), n.a.: not race tested.

Table 6. Correspondence between race name and virulence on common stem rust differential lines

Race_name	Virulence corresponding to NA differentials 1-20 (main R-gene indicated)																			
	Sr5	Sr21	Sr9e	Sr7b	Sr11	Sr6	Sr8a	Sr9g	Sr36	Sr9b	Sr30	Sr17	Sr9a	Sr9d	Sr10	SrTmp	Sr24	Sr31	Sr38	SrMcN
RFCNC	5	21	-	7b	-	-	8a	9g	-	-	-	17	9a	-	10	-	-	-	-	McN
TKKTF	5	21	9e	7b	-	6	8a	9g	-	9b	30	17	9a	9d	10	Tmp	-	-	38	McN
TKTTF	5	21	9e	7b	-	6	8a	9g	36	9b	30	17	9a	9d	10	Tmp	-	-	38	McN
TTKSK	5	21	9e	7b	11	6	8a	9g	-	9b	30	17	9a	9d	10	-	-	31	38	McN
TTKST	5	21	9e	7b	11	6	8a	9g	-	9b	30	17	9a	9d	10	-	24	31	38	McN
TTKTK	5	21	9e	7b	11	6	8a	9g	-	9b	30	17	9a	9d	10	Tmp	-	31	38	McN
TTKTT	5	21	9e	7b	11	6	8a	9g	-	9b	30	17	9a	9d	10	Tmp	24	31	38	McN
PTKTK	5	-	9e	7b	11	6	8a	9g	-	9b	30	17	9a	9d	10	Tmp	-	31	38	McN
TTRTF	5	21	9e	7b	11	6	8a	9g	36	9b	-	17	9a	9d	10	Tmp	-	-	38	McN
TTTTF	5	21	9e	7b	11	6	8a	9g	36	9b	30	17	9a	9d	10	Tmp	-	-	38	McN

Clade III-B (TTRTF) has since the first detection in Sicily in 2016 been confirmed in nine European countries and four countries in Africa/Asia (Table 4, Figure 2). In 2019, first detected in Spain, Tunisia and Iran, and in 2020 first detected in Austria, Czech Republic, Slovakia, Slovenia and in 2021 also in Switzerland. This is the only common race, which has virulence to Sr13, which is particularly relevant for durum wheat.

Clade IV-F, containing the race TKKTF, has by 2021 been detected in 13 European countries, including Austria, Czech Republic, Denmark, France, Germany, Italy, Norway, Poland, Slovakia, Slovenia, Spain, Sweden and Switzerland in 2021 (Table 4). Clade IV-F has also been detected in Ethiopia, Kenya, Tanzania, Tunisia, Azerbaijan, Egypt, Iran, Iraq and Tajikistan in our studies (Figure 2).

Clade IV-B contained TKTTF in Europe, except for one single isolate from Spain, which was TKRTF. Clade IV-B has been detected in nine European countries including Belgium, Denmark, France, Germany, Ireland, Norway, Spain, Sweden and UK in 2021. Clade IV-B also contains the race TTTTF, which has been detected in several countries in Africa and West/Central Asia (Figure 2).

Clade VIII contains two closely related races, HFCNC and RFCNC (predominant), which was observed in Eastern Europe. In contrast to prevalent races, these two races were avirulent to Sr6, Sr9b, Sr9d, Sr9e and Sr38

Clade I (Ug99) and associated races have not been detected in Europe. However, multiple Sr31-virulent races, which were not related to clade I, have previously been detected in recombining populations in Spain and Russia.

In Figure 2, the results are compared with results from 2019-2020 in Europe and results from Africa and Asia. The latter results are presented in annual GRRC reports in more detail, see www.wheatrust.org, Patpour et al. (2022).

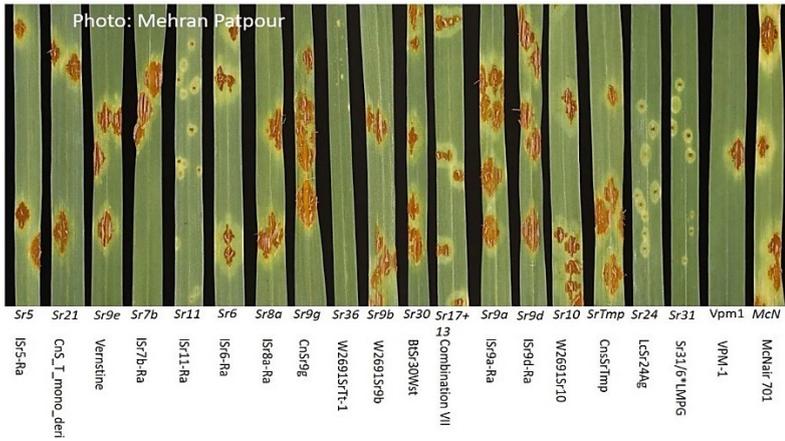


Figure 1. Infection type responses on wheat differential lines of stem rust race TKKF (Clade IV-F), which was widespread in Europe 2021. Main Sr-genes in differential lines indicated at the bottom.

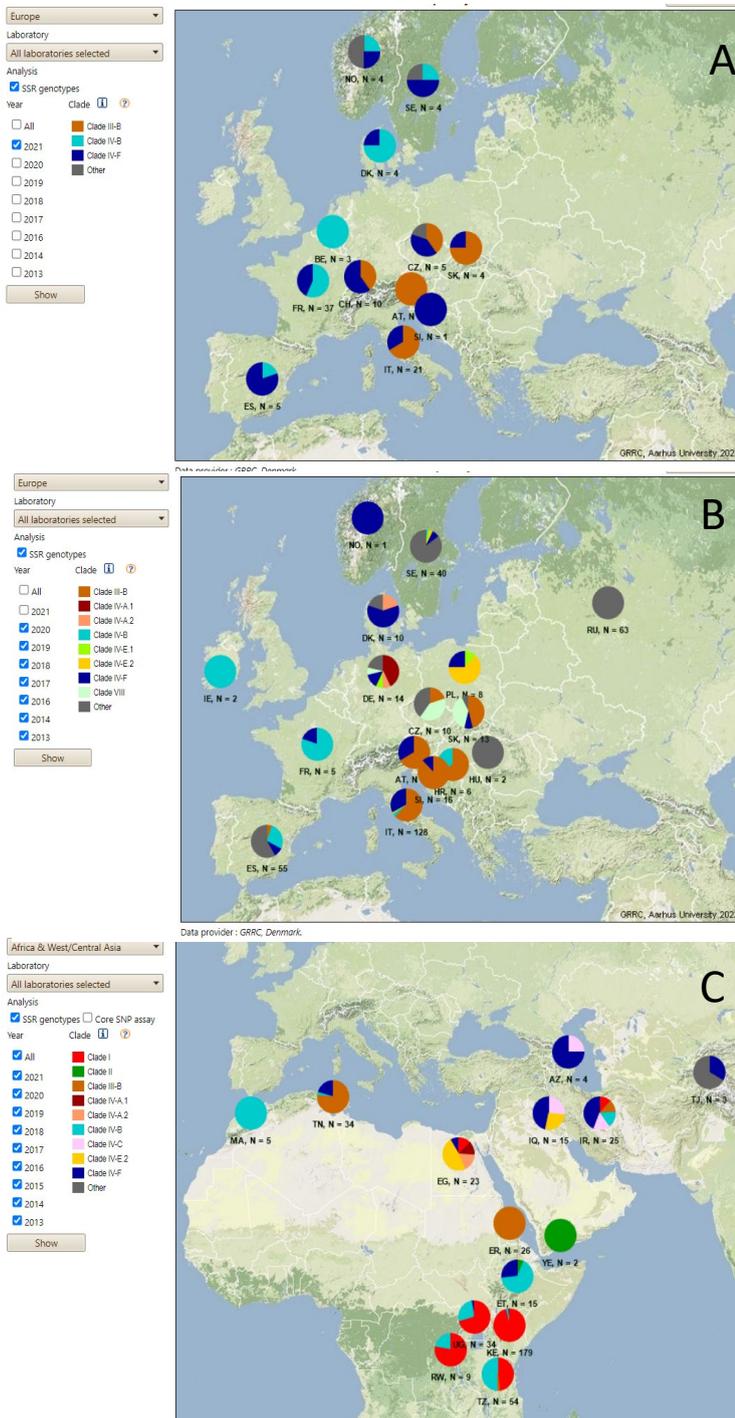


Figure 2. Stem rust genetic group frequency maps: **A.** Europe 2021, **B.** Europe 2013-2020 and **C.** Africa & West/Central Asia 2013-2021. Genetic groups outside Europe are based on samples submitted to GRR and CDL. European results from before 2018 in B and results shown in C are not part of RUSTWATCH activities



WHEAT YELLOW RUST 2021

Yellow rust samples 2021 were received via the VCU network (WP3), alignment exchanges as well as requests from collaborators and throughout Europe, including non-member states and organisations from outside the RustWatch consortium. The alignment efforts allowed upload of results from national rust diagnostic labs into the Wheat Rust Toolbox (milestone reports M1.6, M1.14, M1.22) allowed inclusion of results from national diagnostic labs to be included in maps and charts visualized on www.wheatrust.org.

Access to samples and results from non-European countries, from where samples were submitted to GRRC, Denmark, facilitated an interpretation of results in a wider perspective. These results are also available on wheatrust.org.

Country	SSR_name	Total
Czech Republic	PstS10	10
	PstS13	1
	PstS7	1
Denmark	PstS10	24
	PstS13	3
	PstS7	1
	Other	2
Estonia	ME2018	3
France	Other	1
	PstS10	7
	PstS15	1
Germany	PstS7	1
	PstS10	7
	PstS13	5
Hungary	PstS7	1
Italy	PstS10	6
	PstS13	7
	PstS7	1
Latvia	ME2018	1
	PstS15	1
Netherlands	PstS10	4
Norway	Other	2
	PstS10	29
	PstS7	1
Poland	Other	2
	PstS10	2
	PstS13	8
	PstS7	1
Portugal	PstS10	2
Slovakia	PstS10	1
	PstS13	1
	PstS14	1
Spain	PstS10	5
	PstS13	1
	PstS14	1
Sweden	PstS10	25
	PstS13	6
United Kingdom	PstS10	27
	PstS7	3
	PstS8	2
Europe Total		208

Table 7. SSR genotyping of samples of *P. striiformis*, GRRC 2021 and first quarter of 2022 (alignment samples). Results are shown by number of isolates within genetic groups in each country. Other: Genetic groups which have not yet been named. Significant races and virulences within each group are shown in Table 8.

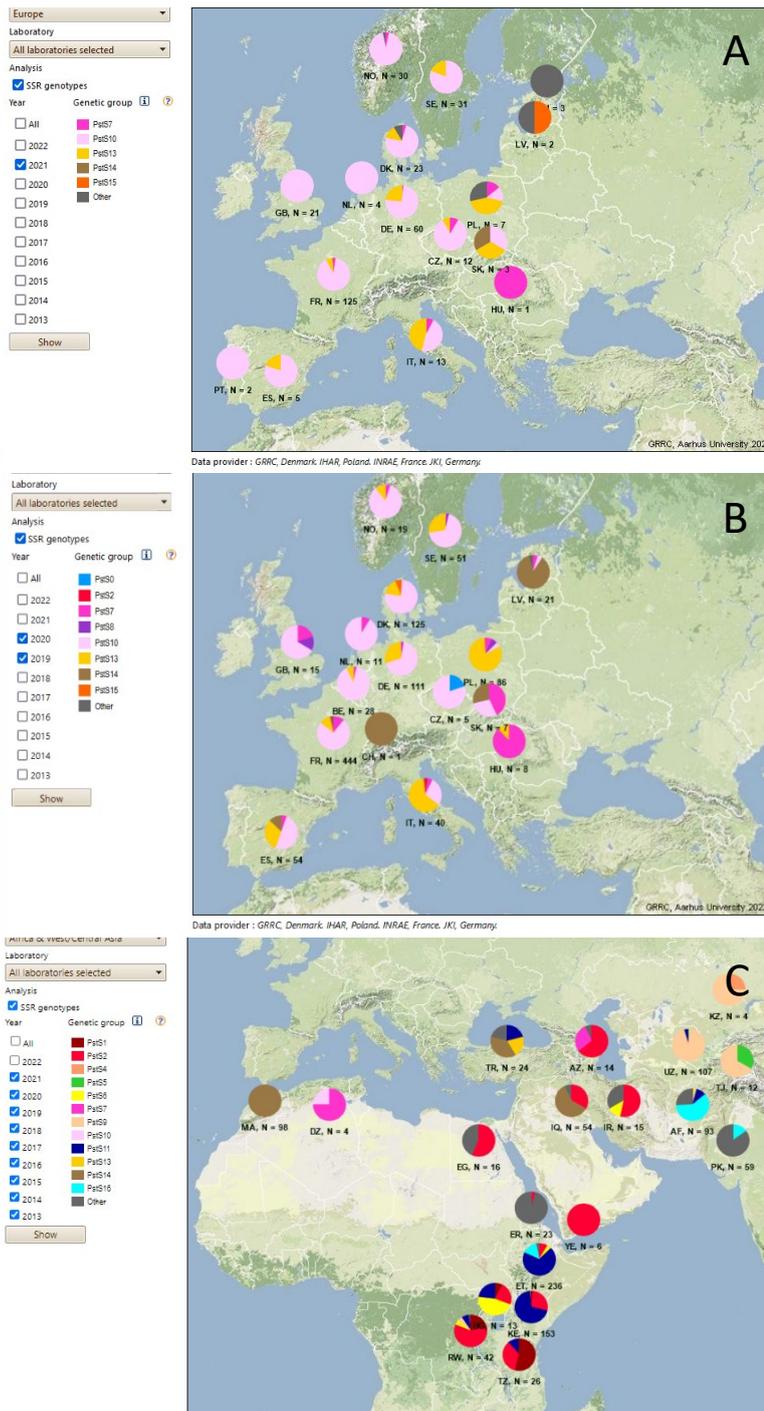


Figure 3. Yellow rust genetic group frequency map **A.** Europe 2021, **B.** Europe 2019-2020. Distribution of genetic groups of yellow rust in 2021 is based on samples submitted to Aarhus University, INRAE, JKI, and IHAR. **C.** Africa & West/Central Asia 2019-2021 (GRRC only). Detected races within groups are shown in Table 8. Results shown in C are not part of RUSTWATCH activities (downloaded 28.04.2022).

SSR genotyping results 2019-2021, including those from European national rust diagnostic labs, are shown on maps in Figure 3, data retrieved from the Wheat Rust Toolbox. Figure 3C shows similar results for Central-West Asia and Africa. Race typing was done for 55 isolates for alignment and confirmation of genetic grouping and emergence of new races within genetic groups.

PstS10 was the most prevalent group on bread wheat in areas covered by RustWatch sampling, up to 2015-2016 dominated by a single race termed Warrior(-), virulence phenotype: 1,2,3,4,-,6,7,-,9,-,-,17,-,25,-,32,Sp,AvS,-, Table 5. Since 2017, new races adapted to local wheat varieties have emerged within PstS10, resulting in quantitative shifts in rust susceptibility of such varieties, e.g., from ‘resistant’ to ‘susceptible’ or from ‘susceptible’ to ‘extreme susceptible’. At least four races have been detected in PstS10: The original Warrior(-) race, Kalmar, Benchmark and Amboise (named after cultivars where they first caused epidemics). PstS10 has also been detected in Australia (Ding et al. 2021) and South America (data not shown). So far, it has not been possible to diagnose the new races within PstS10 by molecular techniques nor by standard wheat differential lines.



In terms of virulence, the races within PstS10 are similar to the Warrior race (PstS7), except for virulence towards the resistance specificity in the varieties Warrior (UK) and Ambition (DK). For this reason, a new variant with virulence on the differential variety ‘Ambition’, which was detected in the UK, is therefore indistinguishable from the Warrior race in seedling tests. However, the two races were easily separated by SSR markers.

The “Warrior” race is still present in Europe, but often in low frequency and mainly in Eastern Europe. Likewise, the PstS8 group (containing the “Kranich” race) was rarely observed. A new genotype in the Middle East (provisionally termed ME2018, ‘Other’ on the map), was detected in both Estonia and Latvia in 2021. This observation was confirmed by independent assays of incoming infected leaves, race typing of recovered isolates, and re-submission of additional samples from the sampling area. Two races differentiated by Yr10-virulence have been detected within ME2018.

PstS13 has been detected in multiple countries and continents in recent years. In 2019, a new Yr10-virulent variant was detected in Poland, and in 2020 also in Germany. PstS13 isolates are highly aggressive on several crop types, e.g., multiple triticale varieties (northern Europe and South America), durum wheat (southern Europe) and multiple spring wheat varieties (South America) resulting in significant yield losses (Carmona et al., 2019). Alignment experiments in 2021, based on DNA samples supplied by the University of Sydney, Australia, confirmed PstS13 in Australia (Ding et al., 2021). At present, PstS13 is widespread across three continents, Europe, South America and Australia.

Table 8. Correspondence between genetic groups and prevalent races in *P. striiformis* since 2000.

Common names of prevalent races and genetic groups in yellow rust - GRRC, March 2022			
Genetic group	Race	Virulence phenotype ^b	Prevalence in geographical region
PstS0	Brigadier	1,2,3,9,17,25,-,AvS,-	Europe
	Brigadier,v4	1,2,3,4,9,17,25,-,AvS,-	Europe
	Madrigal_Lynx	1,2,3,6,9,17,25,-,AvS,-	Europe
	Madrigal_Lynx,v4	1,2,3,4,6,9,17,25,-,AvS,-	Europe
	Robigus	1,2,3,4,9,17,25,-,32,-,AvS,-	Europe
	Solstice_Oakley	1,2,3,4,6,9,17,25,-,32,-,AvS,-	Europe
	Tulsa	-,2,3,4,6,9,17,25,-,32,-,AvS,-	Europe
PstS1	PstS1/2 ^a	-,2,6,7,8,9,25,-,AvS,-	North America, Australia
	PstS1/2,v1	1,2,6,7,8,9,25,-,AvS,-	East Africa
	PstS1/2,v1,v27	1,2,6,7,8,9,25,-,AvS,-	East Africa
PstS2	PstS1/2	-,2,6,7,8,9,25,-,AvS,-	East Africa, West Asia, South Asia
	PstS1/2,v1	1,2,6,7,8,9,25,-,AvS,-	East Africa
	PstS1/2,v3	-,2,3,6,7,8,9,25,-,AvS,-	East Africa
	PstS1/2,v27	-,2,6,7,8,9,25,27,-,AvS,-	East Africa, West Asia, North Africa
	PstS1/2,v1,v27	1,2,6,7,8,9,25,27,-,AvS,-	East Africa, West Asia
	PstS1/2,v3,v27	-,2,3,6,7,8,9,25,27,-,AvS,-	East Africa
	PstS1/2,v10	-,2,6,7,8,9,10,-,24,25,-,AvS,-	East Africa, West Asia
	PstS1/2,v10,v27	-,2,6,7,8,9,10,-,24,25,27,-,AvS,-	West Asia
	PstS1/2,v3,v10,v27	-,2,3,6,7,8,9,10,-,24,25,27,-,AvS,-	East Africa
PstS3	PstS3	-(2),6,7,8,9,25,-,AvS,-	Europe, Asia
	PstS3,v1	1,(2),6,7,8,9,25,-,AvS,-	Asia
PstS4	Triticale2006	-,2,6,7,8,9,10,-,24,-,AvS,-	Europe
PstS5	PstS5	1,2,3,4,6,9,25,-,32,-,AvS,Amb	Central Asia
	PstS5,v17	1,2,3,4,6,9,17,25,-,32,-,AvS,Amb	Central Asia, South Asia
PstS6	PstS6	1,2,6,7,9,17,25,-,27,-,AvS,-	East Africa, Central Asia, South Asia
PstS7	Warrior	1,2,3,4,6,7,9,17,25,-,32,Sp,AvS,Amb	Europe, North Africa
PstS8	Kranich	1,2,3,6,7,8,9,17,25,-,32,-,AvS,Amb	Europe
PstS9	PstS9	1,2,3,4,6,9,25,27,32,-,AvS,Amb	Central Asia, South Asia
	PstS9,v17	1,2,3,4,6,9,17,25,27,32,-,AvS,Amb	Central Asia
PstS10	Warrior(-)	1,2,3,4,6,7,9,17,25,-,32,Sp,AvS,-	Europe, North Africa
	Kalmar	1,2,3,4,6,7,9,17,25,-,32,Sp,AvS,-	Europe
	Benchmark	1,2,3,4,6,7,9,17,25,-,32,Sp,AvS,-	Europe
	Amboise	1,2,3,4,6,7,9,17,25,-,32,Sp,AvS,-	Europe
PstS11	PstS11	-,2,-,(4),6,7,8,9,17,25,-,27,32,-,AvS,-	Central and West Asia, East Africa
PstS12	Hereford	-,2,3,6,7,8,9,17,25,-,32,-,AvS,-	Europe
PstS13	Triticale2015	-,2,6,7,8,9,-,-,-,-,AvS,-	Europe, South America, West Asia
	Triticale2015,v10	-,2,6,7,8,9,10,-,24,-,-,-,AvS,-	Europe
PstS14	PstS14	-,2,3,6,7,8,9,17,25,-,32,(Sp),AvS,-	Europe, North Africa, West Asia
PstS15	PstS15	1,2,3,6,7,9,17,25,-,32,-,AvS,Amb	Europe

a) PstS1 and PstS2 not distinguishable by race and SSR (but SCAR). b) Figures and symbols designate virulence and avirulence (-) corresponding to yellow rust resistance genes: Yr1, Yr2, Yr3, Yr4, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr24, Yr25, Yr27, Yr32, and the resistance specificity of Spalding Prolific (Sp), Avocet S (AvS) and Ambition (Amb), respectively. Kalmar, Benchmark and Amboise are races in PstS10, which can only be distinguished by these varieties.



WHEAT LEAF RUST RESULTS 2021

In 2021, a total of 49 samples were received from five European countries. They were collected from at least 24 different cultivars (15 bread wheat cv., 8 durum cv. and a single sample from spelt). Seven samples were not recovered and were probably dead upon arrival. A single-pustule isolation was made for the 42 recovered samples. Purified leaf rust samples collected on bread wheat were race typed according to Goyeau *et al.* (2006). All of the samples, including those that could not be recovered, were SSR genotyped.

The 11 live samples collected from durum wheat were genotyped, assessing 9 of them were part of the known genetic group “durum”. Previous work conducted on recent durum wheat leaf rust populations in France revealed very little diversity on the durum differential set (Goyeau *et al.*, 2012), and on the set of 19 SSR used for bread wheat. However, European samples tested in 2021 presented genetic variations on 4 loci (RB8, PtSSR164, PtSSR61 and PtSSR68). Out of the 9 samples from Italy identified as Durum wheat race, 5 carried a variation on RB8, 4 carried a variation on PtSSR164, 4 on PtSSR61, and 3 different variations were identified on PtSSR68. The identified alleles on PtSSR68, unknown in France, had already been observed on Italian durum wheat samples in previous years. In 2021, the level of diversity observed, especially when considering the low number of samples, is surprisingly high for leaf rust originating from durum wheat.

Race typing from bread wheat samples allowed us to postulate five families across Europe according to their profile of virulence, which was confirmed by the genotyping results (Table 10, Fig. 4). Two well-known families were still observed this season: Fam 106 314 2 and Fam 166, already identified in France and across Europe as being the two main leaf rust groups (Fig. 5). Both were less represented than in the previous years (60% of the samples) which could be a result of the reduced number of participating countries and number of samples analyzed. Other less prevalent families were found, such as Fam 165 vir24, which seems to be establishing in Central Europe as it has been found in several participating countries since 2019 (even though its frequency did not significantly increase), and a rare family close to Fam 167. An original genotype with its associated pathotype was also identified in Czech Republic, from a sample originating from spelt.

Fam 106 314 2 is virulent on gene *Lr28*, introduced in British cultivars in 2006, and then used in the European germplasm, with cultivars registered from 2011 for example in France. Fam 166 combines 12 virulences against the 23 *Lr* genes (table 9) included in the differential set.

The decrease in number of samples could once again in 2021 be a result of low incidence of leaf rust epidemics, or the global travel restrictions that made sampling more difficult for the European collaborators.

Table 9. Pathotypes found in the genetic group Fam 166.

Genetic group	Pathotype Code	Virulence phenotype																			
Fam 166	166 316	1	-	-	-	3	3bg	-	10	13	14a	15	-	17	-	-	-	17b	37	-	-
	166 317	1	-	-	-	3	3bg	-	10	13	14a	15	-	17	-	-	26	17b	37	-	-
	166 336	1	-	-	-	3	3bg	-	10	13	14a	15	-	17	20	-	-	17b	37	-	-
	166 337	1	-	-	-	3	3bg	-	10	13	14a	15	-	17	20	-	26	17b	37	-	-
	166 357	1	-	-	-	3	3bg	-	10	13	14a	15	-	17	-	23	26	17b	37	-	-
	166 376	1	-	-	-	3	3bg	-	10	13	14a	15	-	17	20	23	-	17b	37	-	-
	166 377	1	-	-	-	3	3bg	-	10	13	14a	15	-	17	20	23	26	17b	37	-	-

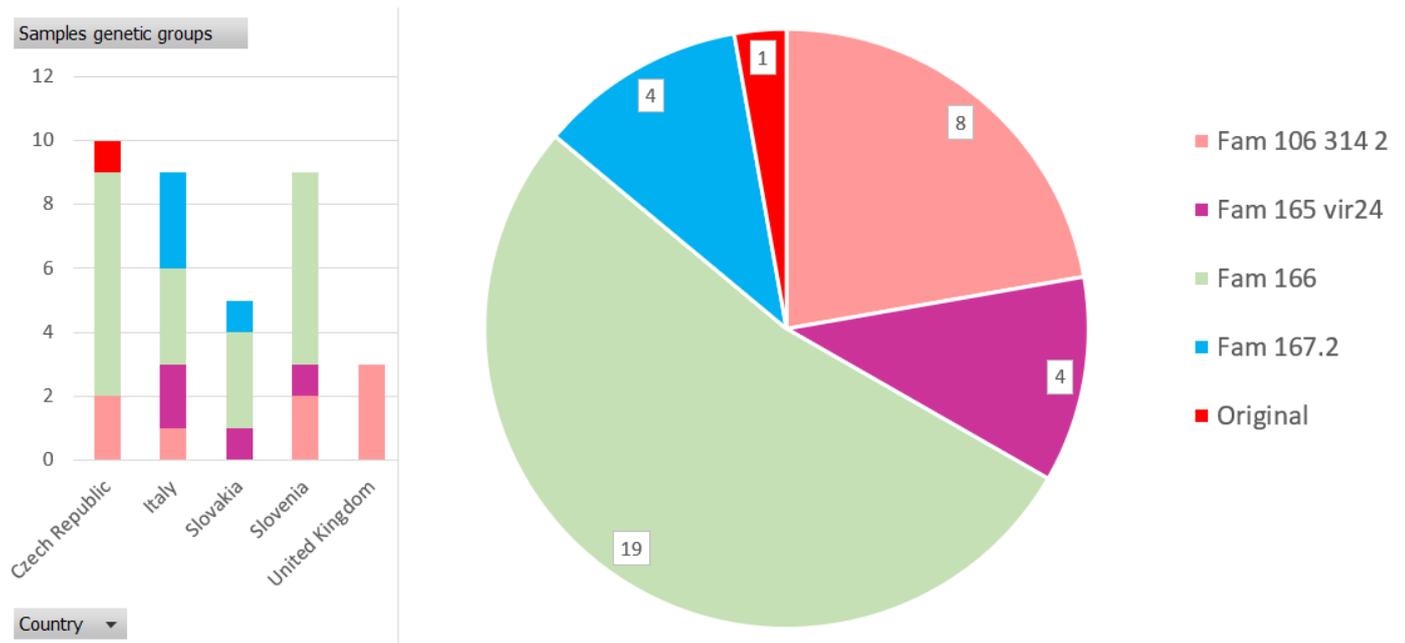


Figure 4. Leaf rust genetic groups frequency for Europe in 2021

Table 10. Leaf rust virulence phenotypes of 2021 samples

Postulated genetic group	Pathotype Code	Virulence phenotype
Fam 106 314 2	106 314 2	1 - - - - - 10 13 14a 15 - 17 - - - - 37 - 28
	106 314	1 - - - - - 10 13 14a 15 - 17 - - - - 37 - -
	106 334 2	1 - - - - - 10 13 14a 15 - 17 20 - - - - 37 - 28
	106 334	1 - - - - - 10 13 14a 15 - 17 20 - - - - 37 - -
Fam 166	166 317	1 - - - 3 3bg - 10 13 14a 15 - 17 - - 26 17b 37 - -
	166 357	1 - - - 3 3bg - 10 13 14a 15 - 17 - 23 26 17b 37 - -
	166 376	1 - - - 3 3bg - 10 13 14a 15 - 17 20 23 - 17b 37 - -
	166 377	1 - - - 3 3bg - 10 13 14a 15 - 17 20 23 26 17b 37 - -
Fam 167.2	167 314	1 - - 3 3bg 3ka 10 13 14a 15 - 17 - - - 37 - -
	167 315	1 - - 3 3bg 3ka 10 13 14a 15 - 17 - - 26 - 37 - -
	167 335	1 - - 3 3bg 3ka 10 13 14a 15 - 17 20 - 26 - 37 - -
Fam 165 vir24	165 356 1	1 - - - 3 3bg 3ka - 13 14a 15 - 17 - 23 - 17b 37 24 -
Original type (CZ)	714 237	1 2a 2b 2c - - - - 13 - 15 - 17 20 - 26 17b 37 - -

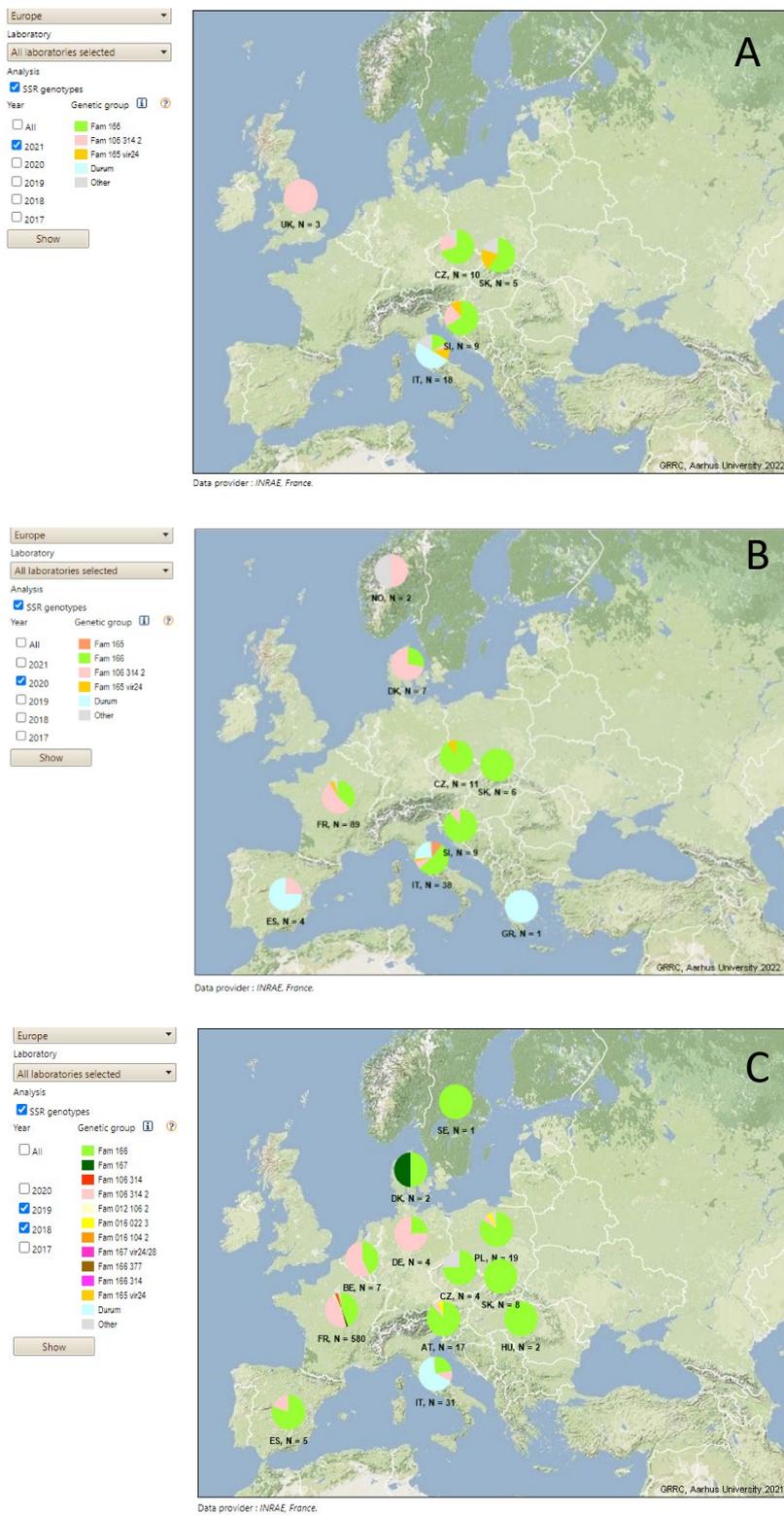


Figure 5. Leaf rust genetic group frequency map for Europe A. 2021 and B. 2020 and C. 2018-2019)

**ALIGNMENT of results from national rust diagnostic labs**

Alignment experiments for yellow rust were carried out in Denmark, January-March 2022, based on isolates from UK, Germany, Poland, and France (Table 11).

Country	Crop_season_year	Samples submitted	No. isolates	
			Race typed	SSR genotyped
France	2018	1	2	4
Germany	2021	5	- *	11
Poland	2021	6	6	13
United Kingdom	2021	7	5	14
Total		19	13	42

Table 11. Number of yellow rust isolates investigated by GRRC in alignment experiments in 2022

Overall, the experiments were successful and proved highly consistent SSR genotyping results from participating laboratories and across replications. By far most rust samples appeared pure, two samples of yellow rust showed too low level of infection for race scoring, and two samples were contaminated by leaf/stem rust. The follow-up genotyping results, generally based on two independent leaf segments from two contrasting differential lines, were conclusive in all cases, supporting the race typing results, including cases with indication of a low level of mixture (multiple races). The experiments also resolved cases, where samples consisted of more than a single genotype, which was observed in few cases.

The results confirmed that the most widely prevalent genetic group in yellow rust, PstS10, contain multiple races, e.g., Warrior(-), Kalmar, Benchmark, and Amboise, and that these are present in multiple countries. In one case, three genotypes were detected within a single sample, confirming that multiple races may co-exist on individual plants under field conditions.

The virulence phenotyping experiments confirmed the influence of environment and assessment practice on the final result, in particular for incompatible 'resistance'/'avirulence' combinations conferring intermediate infection types (e.g., IT5-6 on a 0-9 scale), i.e., relatively minor quantitative differences between 'virulent/avirulent' isolates. It is therefore recommended to include standard isolates (if available) for which 'virulence'/'avirulence' to critical resistance genes has been confirmed in repeated tests.

Country	Crop_season_year	Samples submitted	No. isolates	
			Race typed	Genotyped
Austria	2021	2	1	-
Germany	2021	8	3	8
Poland	2021	5	-	5
United Kingdom	2019	1	1	1
	2021	1	1	1
Grand Total		17	6	15

Table 12. Number of stem rust isolates investigated by GRRC in alignment experiments in 2022

The stem rust experiments confirmed a complete agreement between race typing and genotyping results, as well as the general results from the respective countries and years (Table 12). The UK isolates were part of a research paper about stem rust in the United Kingdom and Ireland (Tsushima et al. 2022), i.e., these alignment experiments allowed us to connect the results from the latter paper with results from continental Europe (Patpour et al., 2022).

Alignment experiments for leaf rust have been carried out in France in spring 2021 based on isolates from the UK, Poland, Czech Republic and Slovakia. All the samples were successfully recovered, phenotyped, and genotyped.

Most of the samples were identified as being part of leaf rust groups already recorded at INRAE, using our genotyping and phenotyping tools (table 10). Two samples from the UK consisted of two races from known genetic groups. A single isolate from Poland was from an unknown group associated with a triticale race-like phenotype, and two Czech and one Polish isolate carried a variation at one locus. The new Polish genotype, from an isolate collected on triticale, revealed potentially interesting loci from our usual set that could help identify new triticale groups.



Genotyping in 2021 has been made on a set completed with the SSR markers proposed by Pawel Czembor from IHAR based on his recent publication (Czajowski et al., 2021), in an effort to build a common European SSR set. Among the 34 SSR markers proposed for leaf rust, 21 have been chosen for their polymorphism and compatibility with the current set and method, leading to a set of 42 markers. 11 of them revealed a deeper layer of polymorphism when compared with the usual 19 SSR set used at INRAE and showed a particular advantage in the identification of triticale groups. They are considered for routine use in a side-project for analyses of *Puccinia triticina* samples collected on triticale.

As for race typing, differences were observed between the participating countries and the French virulence assessment on the same lines. Such differences can be explained on some differentials (Lr3ka, Lr16, Lr23 and Lr26) by high intermediate infection types due to the partial resistance of these genes, making the scoring open to interpretation on these lines, supporting the need for an in-person workshop to adjust the rating between labs. The detailed feedback of the alignment experiment on leaf rust is available in milestone report M1.22.

Leaf rust samples were received from the UK, Czech Republic and Slovakia as part of the alignment experiment of spring 2022 (Table 13). They are still under analyses; however, first results are showing consistent observations, with isolates from the groups Fam 166, Fam 106 314 2 and Fam 165 vir24. Two isolates from Czech Republic were of an unusual phenotype, and part of original genetic groups, one of them having been identified in the 2021 survey.

Country	Number of isolates	Race and genotype record at INRAE	
		Recorded genotype/pathotype	New genotype/pathotype
UK	5	5	0
Poland	10	8	1 (+1)
Czech Republic	6	4	0 (+2)
Slovakia	4	4	0

Table 13. Leaf rust isolates under investigation in alignment experiments in 2021 (+1) indicates the identified genotype has already been observed at INRAE, but carries a variation on a single locus on the tested isolate.

Alignment experiments for wheat stem rust was ongoing at time of reporting the present report; these results will be reported in M1.26.

In conclusion, the set-up and procedures in these alignment experiments for all three wheat rusts have ensured a common European “language” for analyses and interpretation of spread and evolution of rust races in Europe, including a presentation of results from national rust diagnostic on common maps and charts without creating bias in results arising from ‘technical/experimental’ differences among labs and environments. The visualization of results was supported by an interactive information system hosted by the Wheat Rust Toolbox However, steps to ensure continued efforts for trans-national alignment of results of national rust diagnostic labs are required.



LITERATURE

- Ali et al., 2017. Yellow Rust Epidemics Worldwide Were Caused by Pathogen Races from Divergent Genetic Lineages. *Frontiers in Plant Science*, Vol. 8, 1057, 06.2017.
- Carmona et al., 2019. Rapid emergency response to yellow rust epidemics caused by newly introduced lineages of *Puccinia striiformis* f.sp. *tritici* in Argentina. *Trop. Plant Pathol.* 44, 385–39. <https://doi.org/10.1007/s40858-019-00295-y>
- Czajowski, Grzegorz & Kosman, Evsey & Słowacki, Piotr & Park, Robert & Czembor, P. (2021). Assessing new SSR markers for utility and informativeness in genetic studies of brown rust fungi on wheat, triticale and rye. *Plant Pathology*. doi.org/10.1111/ppa.13347
- Ding Y et al. 2021. Incursions of divergent genotypes, evolution of virulence and host jumps shape a continental clonal population of the stripe rust pathogen *Puccinia striiformis*. *Molecular Ecology*, 20.09.2021.
- Gilmour, J. 1973. Octal notation for designating physiologic races of plant pathogens. *Nature* 242:620.
- Goyeau H, Park R, Schaeffer B, Lannou C. Distribution of pathotypes with regard to host cultivars in French wheat leaf rust populations. *Phytopathology*. 2006 Mar;96(3):264-73. doi: 10.1094/PHYTO-96-0264. PMID: 18944441
- Goyeau, H et al., 2012. Low diversity and fast evolution in the population of *Puccinia triticina* causing durum wheat leaf rust in France from 1999 to 2009, as revealed by an adapted differential set. *Plant Pathology* 61, 761-72. <https://doi.org/10.1111/j.1365-3059.2011.02554.x>.
- Hovmøller et al. 2017: Race Typing of *Puccinia striiformis* on Wheat. In: *Wheat Rust Diseases: Methods and Protocols*, ed. S. Periyannan. Springer, p. 29-40. [Downloading](#) accepted for non-commercial and educational purposes.
- Jin et al. 2008. [Detection of Virulence to Resistance Gene Sr24 Within Race TTKS of Puccinia graminis f. sp. tritici](#) *Plant Disease* 92:6, 923-926 <https://doi.org/10.1094/PDIS-92-6-0923>
- Patpour M., M. S. Hovmøller, J. Rodríguez-Algaba, B. Randazzo, D. Villegas, V. P. Shamanin, A. Berlin, K. Flath, P. Czembor, A. Hanzalova, S. Sliková, E. S. Skolotneva, Y. Jin, L. Szabo, K.J.G. Meyer, R. Valade, T. Thach, J. G. Hansen, A. F. Justesen (2022). Wheat stem rust back in Europe: Diversity, prevalence and impact on host resistance. *Frontiers in Plant Science* (accepted for publication in *Frontiers in Plant Science*, May 2022)
- Szabo, L., Olivera Firpo, P.D., Wanyera, R., Visser, B., and Jin, Y. 2022. Development of a diagnostic assay for differentiation between genetic groups in clades I, II, III and IV of *Puccinia graminis* f. sp. *tritici*. *Plant Disease* (in press).
- Tsushima, A., Lewis, C.M., Flath, K., Kildea, S., and Saunders, D.G.O. (2022). Wheat stem rust recorded for the first time in decades in Ireland. *Plant Pathology* 71(4), 890-900. doi: <https://doi.org/10.1111/ppa.13532>.


Table 14. Sample collectors in 2021

	Country	Collector	Yellow rust	Stem rust	Leaf rust
Europe	Austria	Michael Oberforster		x	
		Monika Opalo		x	
	Belgium	Pierre Hellin		x	
	Czech Republic	Alena Hanzalová		x	x
	Denmark	Anne Ladegaard	x		
		Ellen Jørgensen	x		
		Finn Borum	x		
		Lise Nistrup Jørgensen	x	x	
		Martin Clausen	x		
		Ole Harild	x		
		Peter Spies		x	
		Rikke B. Heinfelt	x		
		Susanne Sindberg	x	x	
	Estonia	Max Kurg	x		
	France	Alexandre Klein		x	
		Anne-Lise Boixel		x	
		Auberi Avalor		x	
		Audrey Remont-warin		x	
		Cédric Picard		x	
		Christophe Jeudi		x	
		Christophe Michelet		x	
		Clément Dusserre		x	
		Corentin Picard		x	
		Courbet		x	
		Damien Coulon		x	
		Emmanuel Heumez		x	
		Fanny Savaete		x	
		Franck Gaudichau		x	
		Jean-Charles Talbourdet		x	
		Jean-Philippe Maigniel	x		
		Jérôme Enjalbert		x	
		Joelle Daucourt		x	
		Lefevre		x	
		Marine Henry		x	
		Pascal Girardeau		x	
		Romain Bandini		x	
		Romain Valade		x	
		Sabine Snyder		x	
		Sandrine Longuet		x	
		Solène Barrais	x		
		Stéphane Porrez		x	
		Sylvain Chombart		x	
		Sylvain Gubrie		x	
		Thierry Demarquet		x	
		Valerie Dufayet		x	



	Germany	Andreas Jacobi		x	
		Bärbel Liebherr	x		
		Claudia Meyer		x	
		Kathlen Spät		x	
		Monika Spiler		x	
		S. Hagen	x		
		Thomas Zschaeckel	x		
		Uta Liesenberg	x	x	
		Victoria Hasler		x	
		Volker Heiden	x		
	Hungary	Bernat Poos	x		
	Italy	Alessandro Infantino		x	
		Andreina Belocchi		x	
		Angela Iori		x	
		Anna Maria Mastrangelo		x	x
		Biagio Randazzo	x	x	x
		Chiara Lanzaova			x
		Daniela Marone	x	x	x
		Eder Grolì		x	
		Elisabetta Mazzucotelli		x	x
		Fabrizio Quaranta		x	
		Francesca Nocente			x
		Giuseppina Goddi		x	
		Lucio Salafia		x	
		Marco Martelli		x	
		Marco Nocentini	x	x	
		Mauro Fornara		x	
		Paolo De Franceschi		x	
		Stefania Licciardello		x	
		Stefania Mascheroni			x
		Virgilio Balmas		x	
	Latvia	Līga Feodorova-Fedotova	x		
	Netherlands	L. van den Brink	x		
	Norway	Andrea Ficke	x		
		Chloé Grieu	x		
		Inga Holt	x		
		Ingvild Evju	x		
		Min Lin	x	x	
		Morten Lillemo	x	x	
		Rune Karlsen	x		
	Poland	Pawel Czembor	x	x	
	Portugal	Asmae Jllat	x		
		Cláudia Rato da Silva	x		
	Slovakia	Svetlana Šliková	x	x	x
	Slovenia	Ales Kolmanic		x	x
	Spain	Dolors Villegas	x		
		Fernando Martinez-Moreno	x	x	
		Roser Sayeras		x	
	Sweden	Alf Djurberg	x		



		Anna von Heideken	x		
		Annika Sohlman	x		
		Anton Hampl	x		
		Ebba Hellstrand	x		
		Elin Almén	x		
		Eva mellqvist	x		
		Gunilla Berg	x		
		Lars Johansson	x		
		Lina Norrlund	x		
		Linda Geijerstam	x		
		Louise Aldén	x		
		Lovisa Eriksson	x		
		Mahboobeh Yazdani		x	
		Mahubjon Rahmatov		x	
		Oskar Gustafsson	x		
		Petter Gustafsson	x		
		Robert Dinwiddie	x		
		Therese Christerson	x		
	Switzerland	Nicole Togni		x	
	United Kingdom	Amelia Hubbard	x		
		Clare Lewis		x	
		Sarah Wilderspin	x		x

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