Chapter 3

Race Typing of *Puccinia striiformis* on Wheat

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Abstract

A procedure for virulence phenotyping of isolates of yellow (stripe) rust using spray inoculation of wheat seedlings by spores suspended in an engineered fluid, Novec™ 7100, is presented. Differential sets consisting of near-isogenic Avocet lines, selected lines from the "World" and "European" sets, and additional varieties showing race-specificity facilitate a robust assessment of race, irrespectively of geographical and evolutionary origin of isolates. A simple procedure for purification of samples consisting of multiple races is also presented.

Key words Race, Infection type, Yellow (stripe) rust, Spray inoculation, Genetic interpretation, Purification

1 Introduction

In cereal rust pathology, "race" is often defined by the pattern of compatible and incompatible interactions between host and pathogen. The pathogen phenotype is described as "virulent" in case of a compatible interaction, conferred by "high" infection type scores on host differential lines, and "avirulent" in case of incompatible interactions conferred by "low" infection types ([1], Fig. 1). The genetic interpretation of such race (pathotype) data depend on the extent that R-genes have been identified in the host differential lines, and additional resistance specificities have been resolved by exposure of the lines to a wide array of pathogen isolates of diverse geographical and evolutionary origin.

Historically, the study of race dynamics in *Puccinia striiformis* populations in Europe [2–4], North America [5, 6], China [7], and Australia [8] has often focused on virulence dynamics of national or regional relevance, with limited overlap of host differential lines among laboratories. Significant influences of experimental conditions such as light quality, intensity and duration, temperature regimes, local procedures, interpretation of data, and the presence

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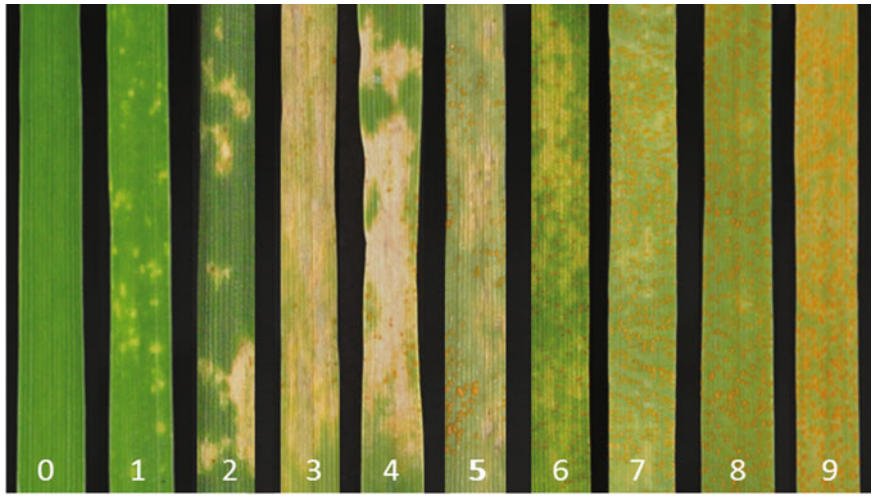

Fig. 1 Disease scoring scale (0–9) of infection types (IT) for *yellow* (*stripe*) rust according to McNeal et al. Distinct appearance of chlorosis and necrosis for individual IT may vary depending on R-genes involved; IT 0–6 generally considered incompatible (avirulent) and IT 7–9 compatible (virulent). IT from left to right, *0*: no visible disease symptoms (immune), *1*: minor chlorotic and necrotic flecks, *2*: chlorotic and necrotic flecks without sporulation, *3–4*: chlorotic and necrotic areas with limited sporulation, *5–6*: chlorotic and necrotic areas with moderate sporulation, *7*: abundant sporulation with moderate chlorosis, *8–9*: abundant and dense sporulation without notable chlorosis and necrosis

of undetected resistance specificities toward particular isolates, have further restricted the comparison of race typing results from different parts of the world. The initiative by Ron W. Stubbs and colleagues in Wageningen, the Netherlands, is one significant exception since they offered yellow rust race typing for many countries worldwide between the 1960s and 1980s [9]. The service was stopped around 1990, and in 2010 the collection of more than 5000 spore samples preserved in liquid nitrogen was transferred to the Global Rust Reference Center, Aarhus University, Denmark [10]. The escalating yellow rust epidemics worldwide in recent years [11], and the spread of epidemics to new areas, where the disease have previously been absent or nonsignificant, have increased the need for understanding spread, establishment, and evolution of yellow rust races at a global scale [12, 13].

The methodologies presented in this chapter are based on the experiences of virulence phenotyping at the Global Rust Reference Center of more than 1000 isolates from recent years, representing 46 countries and five continents, and linking to results in the past by the recovery of more than 200 spore samples from the Stubbs collection [14]. Procedures for purification of spore samples of mixed races and the rationale for a robust phenotyping by taking into account the results from multiple differential lines with shared R-genes are also presented.

2 Materials

1. Six to eight seeds of each wheat differential line per set (Table 1; *see* **Note 1**).
2. Plastic pots (7 × 7 × 8 cm).

Table 1
Current standard and extended set of wheat differential lines used for race typing of *P. striiformis* isolates at the Global rust Reference Center (GRRC), www.wheatrust.org

Differential set	Differential line	Yellow rust resistance genes (*Yr*)[a]	GRRC standard set	GRRC extended set
World (W)	Chinese 166	*1*	X	X
	Vilmorin 23	*3*, +	X	X
	Heines Kolben	*6*, +	X	X
	Lee	*7*, +	X	X
	Moro	*10*	X	X
	Strubes Dickkopf	*Sd*, *25*, +		X
	Suwon 92/Omar	*Su*		X
European (E)	Hybrid 46	*4*, +	X	X
	Heines Peko	*2*, *6*, *25*, +		X
	Heines VII	*2*, *25*, +		X
	Compair	*8*, +		X
	Carstens V	*32*, *25*, +	X	X
	Spaldings Prolific	*Sp*, *25*, +		X
Avocet near-isogenic lines	Avocet S	*AvS*	X	X
	Avocet/*Yr1*	*1*, *18*[b], *AvS*		X
	Avocet/Yr5	*5*, *18*[b], *AvS*		X
	Avocet/Yr6	*6*, *AvS*	X	X
	Avocet/Yr7	*7*, *AvS*		X
	Avocet/Yr8	*8*	X	X
	Avocet/Yr9	*9*, *AvS*	X	X
	Avocet/Yr10	*10*, *18*[b], *AvS*		X
	Avocet/Yr15	*15*, *18*[b], *AvS*		X
	Avocet/Yr17	*17*, *AvS*	X	X
	Avocet/Yr24	*24*, *AvS*		X

(continued)

Table 1
(continued)

Differential set	Differential line	Yellow rust resistance genes (*Yr*)[a]	GRRC standard set	GRRC extended set
	Avocet/Yr27	*27, AvS*		X
	Avocet/Yr32	*32, AvS*		X
	Avocet/YrSp	*Sp, 18*[b], *AvS*	X	X
Additional	Ambition	*Amb*[c]	X	X
	Anja	*25*, +		X
	Brigadier	*17, 9*, +		X
	Cortez	*15*	X	X
	Kalyansona	*2*, +	X	X
	Opata	*27, 18*[b], +	X	X
	Sleipner	*9*, +		X
	TP 981	*25*, +	X	X
	VPM1	*17*, +	X	X
References	Cartago	*Unknown*[d]	X	X
	Morocco	*Unknown*[e]		X
Number of entries			20	36

[a]According to [8, 15, 16] and the present study.
[b]*Yr18* detected by PCR test at GRRC according to [17]
[c]Resistance specificity of variety Ambition.
[d]Generally susceptible except for particular isolates from Himalayan region.
[e]Resistance specificity toward *Yr2* avirulent isolates.

3. Standard peat-based substrate with slow release nutrients optimized for cereal growth.
4. Plastic trays with transparent lids: Standard set trays of the size 50 × 40 × 8 cm (L:W:H) were used to fit up to 36 pots, one pot for each differential line.
5. Airbrush spray gun, vacuum pump and glass flask.
6. Novec™ 7100, a hydroflourether engineered fluid.
7. Urediniospore sample from a single isolate (10–20 mg dried spores).
8. Hand mist sprayer with distilled water.

3 Methods

3.1 Inoculation and Incubation

1. Seeds of each wheat differential line are sown in individual pots containing Pindstrup peat-based substrate. One pot of each line is placed in a tray and grown in spore-proof greenhouse cabins (*see* **Note 2**) with 16 h of natural light supplemented with sodium light at 100 $mE/m^2/s$ when daylight is <10,000 lux. Temperature is set to 17–12 °C (day–night) and relative humidity to 70–80%.
2. The seedlings are kept in the greenhouse until inoculation at the time when the second leaf is halfway unfolded (12–14 days).
3. Connect the airbrush spray gun to the vacuum pump. Ten to twenty milligrams of urediniospores of a single isolate (*see* **Note 3**) is suspended in 5 mL Novec™ 7100 in a glass flask and connected to the airbrush spray gun (Fig. 2a).
4. Inoculate seedlings in fume hood using the airbrush spray gun from 10 to 15 cm distance on all sides and from the top by turning the tray (Fig. 2b).
5. Mist the transparent lid and the seedlings with water before covering the tray to ensure dew formation during incubation at 10 °C in the dark (Fig. 2c, d). Transfer the tray to a spore proof greenhouse cabin after 20–24 h of incubation, after which the lid is removed (Fig. 2e).
6. The plants are scored for infection types after 15–18 days (*see* **Note 4**; Fig. 2f).

3.2 Scoring and Interpretation of Infection Types

1. Visual assessment of infection type is carried out according to McNeal et al. [18]. Scores are from 0 to 9 which represent no or less infection to severe infection as detailed in Fig. 1.
2. Infection type is scored individually on the first and second leaf of each plant within a pot. Number of leaves with a particular infection type is noted.
3. Leaves showing no signs of infection (including chlorosis and necrosis) are categorized as escape.
4. The general disease level is assessed based on susceptible lines. The disease level is categorized as low, medium or high where low represent cases with many escapes per pot, in which case the results may not be reliable.
5. If one or few plants within a pot show distinct different responses than the rest this is noted as seed contamination (*see* **Note 5**).

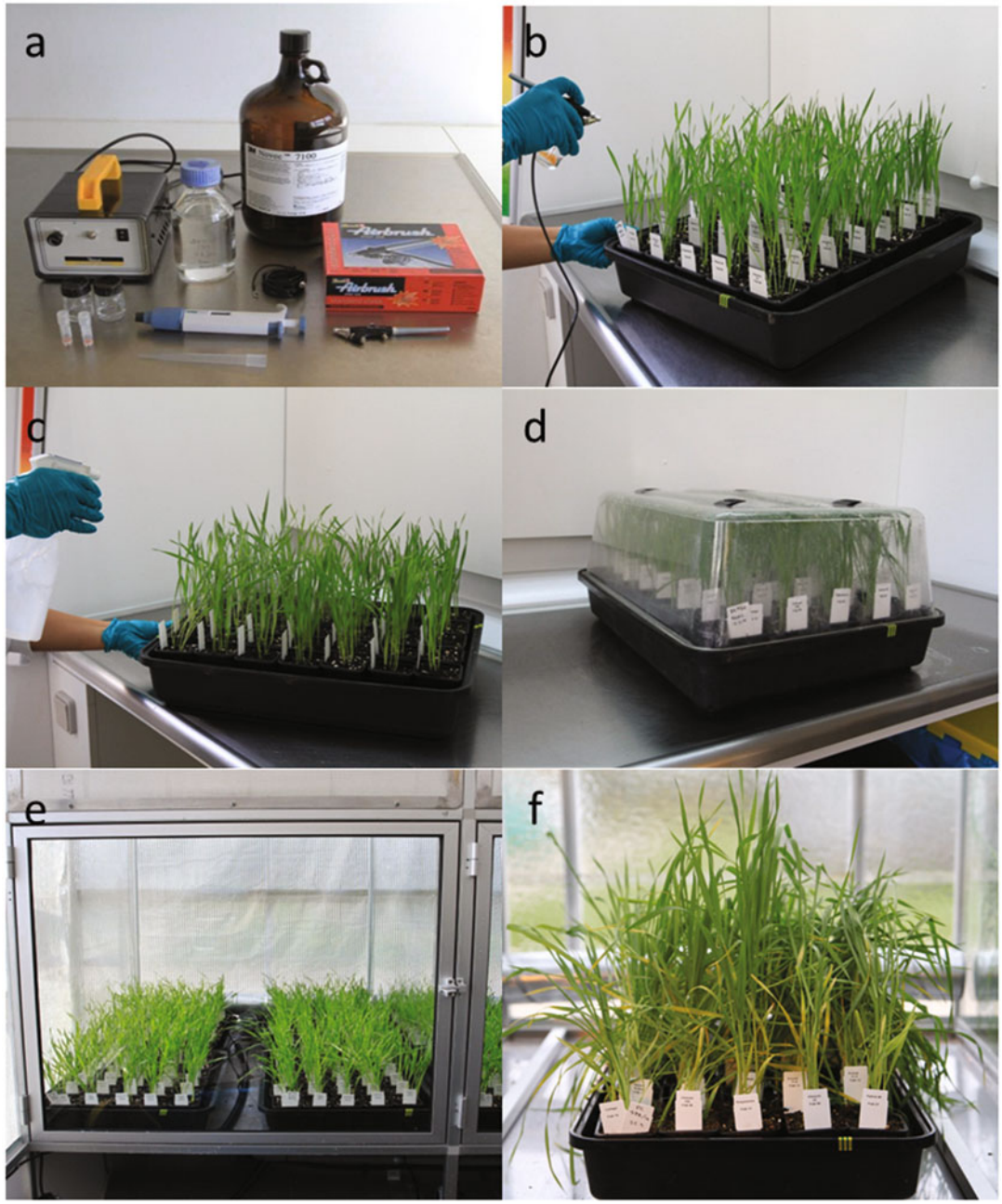

Fig. 2 Procedure for inoculation of differential sets for virulence phenotyping. (**a**) Pipette, glass flask, spore sample, airbrush spray gun, vacuum pump, and Novec™ 7100 engineered fluid, (**b**) 12–14-day-old seedlings inoculated using an airbrush spray gun in a fume hood, (**c**) seedlings misted with water before incubation to ensure dew formation, (**d**) seedlings covered and ready for incubation, 20–24 h at 10 °C in darkness, (**e**) inoculated plants transferred to spore-proof greenhouse cabins with automatic watering, (**f**) virulence phenotyping 15–18 days after inoculation when infection types are well developed

Fig. 3 Signs of multiple races revealed by contrasting infections types (IT) of compatible and incompatible interactions on the second leave of the near-isogenic lines Avocet *S* and Avocet/*Yr*9. (**a**) Avocet *S* displaying IT 1 (*lower* to *middle part* of the leaf) and IT 6–7 (*upper part* of the leaf), (**b**) Avocet/*Yr*9 displaying IT 1–2 (*lower* to *middle part* of the leaf) and IT 7 (*upper part* of the leaf)

6. Contrasting infection types conferred by clearly compatible and incompatible interactions within a leaf may indicate the presence of more than one race (Fig. 3; *see* **Note 6**). Contamination involving intermediate infection types (IT 4–6) of individual races may be difficult to resolve.
7. The virulence phenotype of individual isolates is generally inferred based on infection types across multiple wheat differential lines carrying shared host R-genes (Table 2). The complementarity of Avocet near-isogenic lines and additional differential lines is illustrated by three *Yr6*-virulent isolates of different origin (Fig. 4) and three *Yr17*-virulent isolates and one avirulent isolate (Fig. 5).
8. Avirulence to resistance genes present in multiple differential lines, e.g., the resistance specificity in Avocet S (present in all Avocet lines considered except Avocet/*Yr8*) and *Yr25* present in seven differential lines, generally restrict the genetic resolution of results (Tables 1 and 2).

Table 2
Rationale for the assessment of virulence phenotype of isolates of *P. striiformis* of diverse origin based on infection type scores on wheat differential lines

Virulence inferred	Differential lines	Resistance genes	Refinement comment
v1	Chinese 166 Avocet/*Yr1*	*Yr1* *Yr1, Yr18, YrAvS*	*Avr*AvS: consider Chinese 166
v2	Heines VII Kalyansona Heines Peko	*Yr2, Yr25,* + *Yr2,* + *Yr2, Yr6, Yr25,* +	*Avr25* and *Avr6*: consider Kalyansona
v3	Vilmorin 23	*Yr3,* +	High and intermediate IT (5–6) imply *v3*
v4	Hybrid 46	*Yr4,* +	High and intermediate IT (4–6) imply *v4*, often associated with high IT on Suwon/Omar
v5	Avocet/*Yr5* *Triticum spelta album*	*Yr5, Yr18, YrAvS* *Yr5*	*Avr*AvS: consider *Triticum spelata album*; *v5* rarely observed
v6	Avocet/*Yr6* Heines Kolben Heines Peko	*Yr6, AvS* *Yr6,* + *Yr2, Yr6, Yr25,* +	*Avr*AvS: consider Heines Kolben and Heines Peko. Low IT (1–3) on Heines Kolben and Heines Peko: consider Avocet/Yr6
v7	Lee Avocet/*Yr7*	*Yr7,* + *Yr7, AvS*	*Avr*AvS: consider Lee
v8	Compair Avocet/*Yr8*	*Yr8,* + *Yr8*	Intermediate IT (4–6) on Compair: consider Avocet/*Yr8*,
v9	Sleipner	*Yr9,* +	Low IT on Sleipner: consider Avocet/*Yr9*
	Avocet/*Yr9*	*Yr9, AvS*	*Avr*AvS: consider Sleipner
v10	Moro Avocet/*Yr10*	*Yr10* *Yr10, Yr18, YrAvS*	*Avr*AvS: consider Moro
v15	Cortez Avocet/*Yr15*	*Yr15* *Yr15, Yr18, AvS*	*Avr*AvS: consider Cortez; *v15* rarely observed
v17	VPM1 Avocet/*Yr17*	*Yr17,* + *Yr17, AvS*	*Avr*AvS: consider VPM1 Low IT (1–3) on VPM1: consider Avocet/*Yr17*
v24	Avocet/*Yr24*	*Yr24, AvS*	*Avr*AvS: *v24/Avr24* not accessible
v25	TP 981 Anja	*Yr25,* + *Yr25,* +	Intermediate IT (5–6) on TP981: consider Anja Intermediate IT (3–5): consider TP981
v27	Opata Avocet/*Yr27*	*Yr27, Yr18,* + *Yr27, YrAvS*	*Avr*AvS: consider Opata Intermediate IT (5–6) on Opata: consider Avocet/Yr27
v32	Carstens V Avocet/*Yr32*	*Yr32, Yr25,* + *Yr32, AvS*	*Avr*AvS: consider Carstens V Low IT (0-2) on Carstens V: consider Avocet/*Yr32*

(continued)

Table 2
(continued)

Virulence inferred	Differential lines	Resistance genes	Refinement comment
vSp	Spaldings Prolific Avocet/*YrSp*	*YrSp*, *Yr25*, + *YrSp*, *YrAvS*	*Avr*AvS: consider Spaldings Prolific Low and intermediate IT on Spaldings Prolific: consider Avocet/*YrSp*
vAvs	Avocet S	*YrAvS*	*Avr*AvS conferred by IT 0–1
vAmb	Ambition	*YrAmb*	Resistance component(s) in Ambition conferred by IT 1–5
Reference	Cartago	None	Susceptible check, avirulence only observed in particular isolates from Pakistan. IT 6–7 observed for isolates of Warrior race

Virulence is generally inferred by the highest infection type within groups of two or more differential lines sharing a considered resistance gene

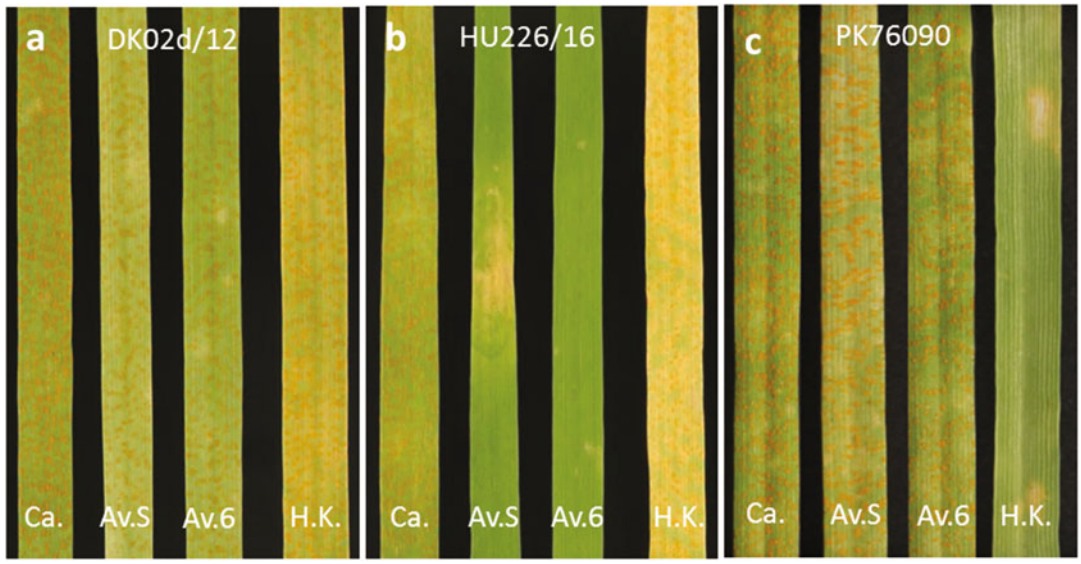

Fig. 4 Infection types (IT) on a 0–9 scale for the isolates DK02d/12, HU226/16, and PK7690 inoculated on wheat lines carrying *Yr6*, i.e., Avocet/*Yr*6 (Av.6) and Heines Kolben (H.K.). Cartago (Ca.) and Avocet S (Av.*S*) are included as susceptible controls. (**a**) DK02d/12 displaying a compatible interaction (IT 7–8), (**b**) HU226/16 displaying IT 8 on Ca., IT 2 on Av.S, IT 1 on Av.6, and IT 9 on H.K., (**c**) PK76090 displaying IT 7-8 on Ca., Av.S, and Av.6 and IT 2 on H.K

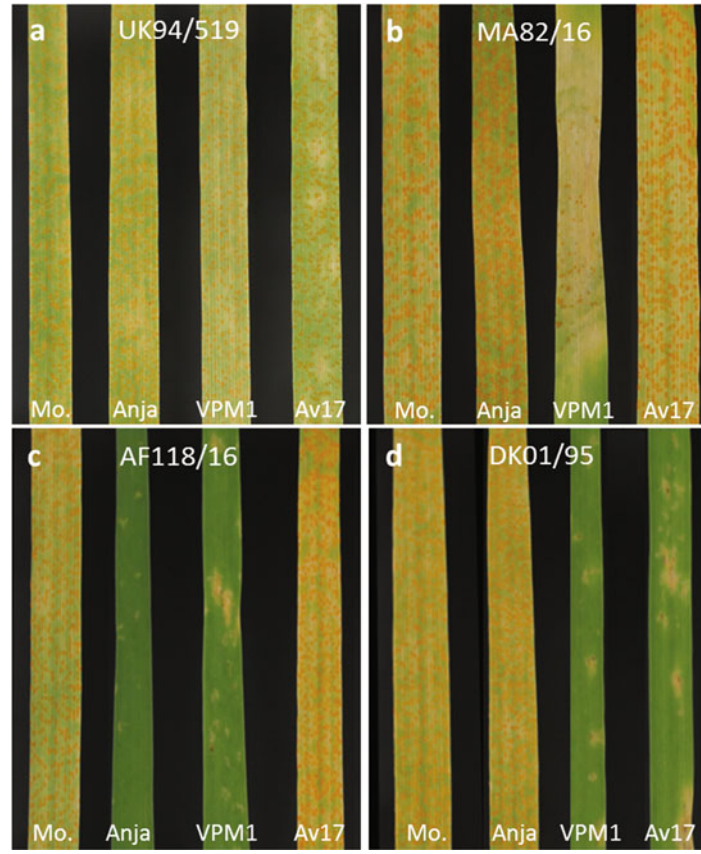

Fig. 5 Infection types (IT) on a 0–9 scale for the isolates AF118/16, DK01/95, UK94/519, and MA82/16 on *Yr17* resistant wheat varieties, i.e., VPM1, Avocet/*Yr17* (Av.17). Morocco (Mo.) and Anja are included as susceptible controls. (**a**) UK94/519 displaying a compatible interaction (IT 7–8); note chlorotic flecks on Avocet/*Yr17* which is consistent for *Yr17*-virulent isolates from the NW-European *P. striiformis* population, (**b**) MA82/16 displaying IT 8-9 on Mo., Anja and Av.17, and IT 4 on VPM1, (**c**) AF118/16 displaying IT 7-8 on Mo. and Av.17 and IT 1-2 on Anja, and VPM1, (**d**) DK01/95 displaying IT 8-9 on Mo. and Anja, and IT 1-2 on VPM1 and Av.17

4 Notes

1. Commercial local varieties or other lines of special interest may be included in the set. New races are often identified because they overcome resistance in widely grown varieties.
2. It is important that the plants are grown in disease-free environment prior to inoculation to avoid unintentional contamination.

3. Safe handling of spore samples to minimize the risk of unintentional spread is vital.
4. Optimal time of scoring may vary depending on the season and isolate.
5. Seed contamination is based on plant morphology and infection type. Off-type plants are not considered in the analyses.
6. Samples containing more than one race can be purified as follows: single lesions of high infection types are collected from at least two different differential lines, rinsed with water, transferred to petri dish with moist filter paper, and incubated for 2–3 days at 12–14 °C under light to allow germination of detached spores of the off-type and production of new spores from the lesion. The single lesions are subsequently used as basis for new spore multiplications using susceptible lines. Purity of the new isolates can be confirmed on new differential sets.

References

1. McIntosh RA, Welling CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO, Melbourne, VIC. 200 pp
2. Hovmøller MS (2001) Disease severity and pathotype dynamics of *Puccinia striiformis* f. sp. *tritici* in Denmark. Plant Pathol 50:181–189
3. de Vallavieille-Pope C, Ali S, Leconte M, Enjalbert J, Delos M, Rouzet J (2012) Virulence dynamics and regional structuring of *Puccinia striiformis* f. sp. *tritici* in France between 1984 and 2009. Plant Dis 96:131–140
4. Johnson R (1992) Reflection of a plant pathologist on breeding for disease resistance, with emphasis on yellow rust and eyespot of wheat. Plant Pathol 41:239–254
5. Chen X, Penman L (2005) Stripe rust epidemic and races of *Puccinia striiformis* in the United States in 2004. Phytopathology 95(6): S19–S19
6. Line RF, Qayoum A (1992) Virulence, aggressiveness, evolution and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968-1987. US Dep Agric Tech Bull 1788:44
7. Wan A, Zhao Z, Chen X, He Z, Jin S, Jia Q, Yao G, Yang J, Wang B, Li G, BI Y, Yuan Z (2004) Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *tritici* in China in 2002. Plant Dis 88:896–904
8. Wellings CR (2007) *Puccinia striiformis* in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period 1979-2006. Aust J Agr Res 58:567–575
9. Stubbs R (1988) Pathogenicity analysis of yellow (stripe) rust of wheat and its significance in a global context. In: Simmonds NW, Rajaram S (eds) Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico, DF, pp 23–38
10. Hovmøller MS, Yahyaoui A, Singh RP (2009) A global reference centre for wheat yellow rust: pathogen variability, evolution and dispersal pathways at regional and global levels. BGRI Technical Workshop, Cd. Obregón, Sonora, Mexico, March 17–20, 2009
11. Hovmøller MS, Walter S, Justesen AF (2010) Escalating Threat of Wheat Rusts. Science 329 (5990):369–369
12. Hovmøller MS, Sørensen CK, Walter S, Justesen AF (2011) Diversity of *Puccinia striiformis* on cereals and grasses. Annu Rev Phytopathol 49:197–217
13. Walter S, Ali S, Kemen E, Nazari K, Bahri BA, Enjalbert J, Hansen JG, Brown JKM, Sicheritz-Pontén T, Jones J, de Vallavieille-Pope C, Hovmøller MS, Justesen AF (2016) Molecular markers for tracking the origin and worldwide distribution of invasive strains of *Puccinia striiformis*. Ecol Evol 6(9):2790–2804
14. Thach T, Ali S, Justesen AF, Rodriguez-Algaba J, Hovmøller MS (2015) Recovery and virulence phenotyping of the historic 'Stubbs collection' of the yellow rust fungus *Puccinia striiformis* from wheat. Ann Appl Biol 167 (3):314–326
15. Hovmøller MS, Justesen AF (2007) Appearance of atypical *Puccinia striiformis* f. sp. *tritici*

phenotypes in north-western Europe. Aust J Agr Res 58:518–524

16. Nazari K, El Amil R (2013) First report of resistance of wheat line Avocet 'S' to stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst) in Syria. Plant Dis 97(7):996–996
17. Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor Appl Genet 119:889–898
18. McNeal FM, Konzak CF, Smith EP, Tate WS, Russell TS (1971) A system for recording and processing cereal research data. US Agric Res Serv 42:34–121