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Identification of temperature sensitive resistance to *Puccinia striiformis* f. sp. *tritici* in wheat differential hosts and its potential applications in resistance breeding

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Abstract

Temperature affects wheat resistance responses infected by *Puccinia striiformis* f. sp. *tritici (Pst)*. In order to identify if thirty-one entries of Chinese, international and other tester wheat cultivars possess temperature sensitive resistance, the entries were studied in seedling stage at two different day/night temperature regimes (24/18°C and 14/10°C). Four entries, Lutescens 128, Funo, Lee and Carstens V, were confirmed no temperature sensitive resistance genes. Six wheat cultivars, Early Piemium, Fengchan 3, Fulhard, Heines VII, Mentana and Virgilio, have shown temperature sensitive resistance. Comparison with four standard lines (S110, S111, S112, S113) with 0-3 temperature sensitive genes, derived from crosses of Itana/PI 178383, the resistance to *Pst* race 10E162 in Virgilio controlled by two temperature sensitive genes, and Mentana and Fulhard each possessed one temperature sensitive gene. Virgilio, Fulhard and Mentana as the temperature sensitive gene resources are useful in breeding for resistance to stripe rust. As the differential hosts of wheat stripe rust, it is necessary to strictly control the temperature without exceeding 18°C, since infection type may differ due to the different temperature.

Keywords: Differential hosts; *Puccinia striiformis* f. sp. *tritici*; Temperature sensitive resistance; Wheat

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Introduction

Wheat stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici (Pst)*, is a major disease that can lead to the tremendous losses of wheat production worldwide. *Pst* is a low temperature pathogen and occurs serious in cool areas. Temperature affects spore germination and infection, latent period, sporulation, spore survival, and host resistance [1]. Resistance of the host is much influenced by temperature and light, which in turn influence disease assessment of the infected plant [2]. Global warming is continuing without letup; however, wheat stripe rust has a lower optimum temperature for development. CHENG et al. [3], suggested that high wheat canopy temperatures provide unfavourable conditions for the development of stripe rust. It is important to draw attentions to the effects of temperature on wheat stripe rust.

It was first noted in the late 1920s that the temperature effected on the host-pathogen interaction in cereals. Temperature sensitivity was a specific property of particular resistance genes. RAPILLY [4], GERECHTER-AMITAI et al. [5], PARK et al. [6], and LINE [7], reviewed the effects of temperature on stripe rust. Both post-inoculation and pre-inoculation temperature on the interaction of wheat-rust had been recognized [8,9]. PARK et al. [6], found the resistance expressed at higher temperatures to stripe rust in some Australian wheat cultivars was unaffected by pre-inoculation temperature. Temperature sensitive resistance to stripe rust in selected Australian wheat cultivars was found to be most strongly expressed at a constant post-inoculation temperature of 19°C [10]. High temperature adult plant (HTAP) resistance was expressed only at the high post-inoculation temperature [11]. This paper presented that the post-inoculation conditions affect the expression of the resistance.

The expression of some genes in wheat for resistance to stripe rust was influenced by temperature and/or plant developmental stage [12]. *YrCK* was the only known temperature sensitive stripe rust resistance gene, characterized for the seedling stage and located on chromosome arm 2DS in Cook [6,13] and *Yr18* was known to be temperature mediated and became more effective at higher temperatures [6]. It is clear that the relatively high temperature in the late growth stage of wheat will be propitious to the presence of temperature sensitive resistance. Thus, temperature sensitive resistance to stripe rust for host all growth stages might need more attention.

Pst accessions are classified into physiological races based on their virulence on a set of wheat differential lines, whose infection responses may be influenced by environmental temperature. In this research, thirty-one entries of Chinese and international hosts for *Pst* were studied post-inoculation under two temperature regimes in seedling stage to identify if they possess temperature sensitive resistance. Comparison with 0-3 minor genes lines, the numbers of temperature sensitive resistance genes were postulated.

Materials and Methods

Plant material

A total of 36 wheat cultivars, including sets of differential hosts: international and European, Chinese differential hosts, and additional testers with known minor genes for yellow rust resistance. Sixteen entries of Chinese differential hosts for *Pst* : Trigo Eureka, Fulhard, Lutescens 128, Mentana, Virgilio, Abbondanza, Early Piemium, Funo, Danish 1, Jubilejina II, Fengchan 3, Lovrin 13, Kangyin 655, Suwon 11, Lovrin 10, Hybrid 46; Fifteen international and European differential hosts for *Pst* : Chinese 166, Lee, Heines Kolben, Vilmorin 23, Moro, Strubes Dickkopf, Suwon 92/Omar, Clement, Reichersberg 42, Heines

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Peko, Nord Desprez, Compair, Carstens V, Spaldings prolific, Heines VII. Besides, the cultivars such as S110, S111, S112, S113 with zero, one, two or three minor genes respectively, and PI 178383 [14] were used as standard comparison cultivars for temperature sensitive resistance. Mingxian 169 (MX) is used as susceptible control which is highly susceptible to all known Chinese yellow rust races by now.

Race Material

The races of *Pst* 10E162 and 82E22 were provided by IPP, CAAS and were increased on MX. The race 82E22 can overcome the major gene *Yr10* from PI 178383 and was used to postulate the temperature sensitive gene.

Experimental Design

The evaluation of the test material in the seedling stage was carried out in growth chambers, which were programmed at different temperature regimes. Prior to inoculation, wheat seeds were planted in pots 10 cm in diameter and seedlings were raised at normal temperatures conditions in growth chambers. Ten-fifteenth seeds were sown of each entry and two similar sets of seedlings were grown simultaneously. The seedlings were inoculated with the race 10E162 of *Pst* when the second leaf appeared. Following inoculation, plants were misted with deionized water and placed in a dew simulation chamber maintained at 10°C for 24 h in the dark. After incubation, one set of each isolate was kept at a high temperature profile of a maximum of 24°C daytime and a minimum of 18°C night. Following the high temperature treatment two days, the cultivars were separated into two groups with constant temperature (18/24°C, night/day) and normal temperature (10/18°C, night/day) to determine the effect of constant temperature. The other set was kept at a low temperature profile of a maximum of 18°C daytime and a minimum of 10°C night. In both profiles, light conditions in both growth chambers were 23000 Lux for 14 h, a dark period for 10 h.

Identification of Resistance

When the susceptible check MX was heavily infected, infection type (IT) on the seedlings was recorded using a 0 - 4 scale [15]. Plus (+) and minus (-) were added to the digit where reactions were slightly higher or lower, respectively, than the mean infection type for the class. The number of days from inoculation to the rupture of the first pustule (latent period: one of the resistance components) was carefully examined and recorded. The variation of the number of days under different conditions was determined that if the temperature sensitive resistance can delay the development of the disease.

Data analysis

In order to facilitate the use of statistical software for calculation and analysis of the original data, infection type was transformed into infection index. PC SAS (SAS, Version 8. 0) was used for statistical analysis. The data were processed through the analysis of variance and the Duncan mean separation test was used.

Results and Discussion

Identification of temperature sensitive resistance

Infection type was recorded and showed in Table 1. Infection type in Lutescens 128, Funo, Lee and Carstens V, was similarly susceptible at both temperature regimes and did not show a shift toward resistance, indicating there was no temperature sensitive resistance. Six cultivars (Fulhard, Mentana, Virligo, Early Piemium, Heines VII and Fengchan 3) were susceptible at normal temperature, while at a high temperature regime there appeared to be an adverse effect on the pathogen, displaying a change toward resistance. Heines VII is used as differential host with *Yr2* and *YrHVII* [16]. In this study its temperature sensitive resistance was also confirmed. Heines Peko has strong resistance with many resistance genes *Yr2*, *Yr6*, *Yr25* and *Yrhp* [17]. In this experiment, it cannot be

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determined whether Heines Peko contains temperature sensitive genes. Virgilio expressed the most resistance at higher temperatures (IT 0;) than at normal temperatures (IT 4). Except these, other cultivars were resistant at both temperature regimes and cannot be determined with or without temperature sensitive resistance. The expression of resistance may be affected by the race of pathogen used or not [6].

For temperature affecting the expression of resistance, in order to obtain the accuracy and reliability of results the resistance evaluation for seedling wheat must be strictly maintained standard temperature condition within 1°C of the set temperature. The evaluations in the field also should considerate the environment effects, for high temperature adult plant resistance maybe occur. As wheat differential hosts for wheat stripe rust must be able to clearly identify, but those differential hosts whose temperature sensitive resistance genes are easily affected by temperature, lead to the variation of infection types of *Pst* races. Efficient identification is most important for comparison the results of various research groups or different regional samples. So, it is important to strictly control the experimental temperature for the identification resistance of cultivars or new *Pst* races.

Effects of constant temperature on sporulation and infection type

In order to observe the effects of post-inoculation temperature, two treatments (high temperature for 2 days than normal temperature treatment and constant high temperature treatment) were carried out. The cultivars which had been shown to possess temperature sensitive resistance in previous experiments were used. The sporulation of *Pst* on cultivars at two different regimes are presented in Table 2. The sporulation in Virligo, Early Piemium and Heines VII was delayed about 9 d, 4 d, and 3 d, respectively, and that in Fulhard, Metana and Fengchan 3 was 1 d, with average 3.4 d, indicating that temperature resistance can restrict development of rust to produce spores. The representative pictures of symptom were showed (Figure 1). The longest latent periods occurred at the cultivar Virgilio about 20 days.

Figure 1 Infection type of response to *Pst* under high temperature (18/24℃, night/day) (left) and normal temperature (10/15℃, night/day) (right) regimes

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Table 1: Effect of temperature on infection type for differential hosts of wheat stripe rust in normal and high temperature regimes to *Pst* race 10E162

Cultivars	Normal temperature (15 ℃/10 ℃)		High temperature (24 ℃/18 ℃)		Temperature sensitive resistance
	First survey	Second survey	First survey	Second survey	
Trigo Eureka	1,2^-	2^-	1,2^-	2^-	?
Fulhard	4	4	2	2^-～2	+
Lutescens	4	4	4	4	-
Mentana	4	4	0;～2	2^-～2	+
Virgilio	4	4	0;	0;	+
Abbonolanza	0;	2^-～2	0;	2^-	?
Early Piemium	4	4	0;～$0;^+$	2^-～2	+
Funo	4	4	4	4	-
Danish 1	0;	0;～2	0;	$0;^+$	?
Jubilejina	0;～2	0 ; ～2	0 ;	0;	?
Fengchan 3	4	4	0～0;	0;～2^-	+
Lovrin 13	0;	0;	0;	0;	?
Kangyin 655	0;	0;	0;	0;	?
Suwon 11	$0;^+$	$0;^+$	0;	0;	?
Lovrin 10	0;	0;	0;	0;	?
Hybrid 46	0;	0;	0;	0;	?
Moro	0;～2^-	0;,4	0;～2^-	0;,2,4	?
Chinese166	$0;^+$～1	$0;^+$～1	$0;^+$～1	$0;^+$～1	?
Lee	4	4	3～4	4	-
Heines Kolben	$0;^+$～1	$0;^+$～1	0;	0;～1	?
Vilmorin 23	2～3	2^+～3	0;～2	2^-	?
Strubes Dickkopf	$0;^+$	$0;^+$,1^+	0;	$0;^+$, 2^-	?
Suwon 92/Omar	$0;^+$	$0;^+$	0;	0;	?
Clement	0;	0;	0;	0;	?
Reichersberg 42	2～3	3^+～4	2^-	3～4	?
Heines Peko	$0;^+$	$0;^+$	0;	0;	?
Nord Despre 2	$0;^+$	2～2^+	0;	2～2^+	?
Compair	$0;^+$	$0;^+$～1	0;～$0;^+$	$0;^+$～1	?
Carstens V	4	4	3	4	-
Spaldings prolific	0;	0;	0;	0;	?

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Heines VII	4	4	0;	0;$^{+}$～2	+
Mingxian 169	4	4	4	4	-

Note: +, means it has temperature sensitive resistance; -, no temperature sensitive resistance;?, undetermined.

Table 2: Sporulation and resistance response of tested cultivars with possible temperature sensitive resistance

Cultivars	Sporulation time					IT	
	Normal temperature	Ⅰ	Time difference	Ⅱ	Time difference	Ⅰ	Ⅱ
Fulhard	10	11	1	11	1	2^{-}～2	3^{-}
Mentana	13	14	1	11	-2	2^{-}～2	3^{-}
Virgilio	11	20	9	13	2	0 ;	1
Early Piemium	10	14	4	10	0	2^{-}	2^{+}～3^{-}
Fengchan 3	11	12	1	10	-1	0;～2^{-}	3
Heines VII	15	18	3	10	-5	1^{+}	2^{+}
Mingxian 169	10	10	0	10	0	4	4

Note Ⅰ: two days at high temperature then to normal temperature; Ⅱ: Continuous high temperature.

Constant high temperature can affect the growth of the pathogen and the expression of resistance in wheat. Compared to sporulation at normal temperature, constant high temperature treatment for MX and Early Piemium had no change. The sporulation time increased one day and two days for Fulhard and Virgilio, respectively. Temperature-sensitive resistance still existed, but the effect was not better than high temperature for 2 days than normal temperature. Under high temperature condition, higher proportion of new incremental biomass was invested on stems rather than on leaves [18]. Long time of lack of nutrition can affect the expression of temperature sensitive resistance. Consequently, continuous high temperature may not be propitious to sustained expressions of high temperature resistance. Conversely, the sporulation time in Mentana, Fengchang 3 and Heines VII was shorter. The growth of *Pst* in wheat was faster under high temperature condition. DENNIS [19], also reported that temperatures above 25°C were detrimental to the development of stripe rust. Infection type on the cultivars was a little increased at constant high temperature than two days high temperature then normal temperature, indicating irreversible temperature had little effect on IT of the cultivars. The resistance was not constant increased with the continuous high temperature. When transferred from high to normal temperature infection types changed from low to high [11]. Both two treatments results provided direct evidence of temperature sensitive resistance to stripe rust in Fulhard and Virgilio, whose expression of resistance was stable. The mechanism of temperature sensitive resistance is not well understood. The temperature sensitive resistance can be race-specific or non-race specific. GOUSSEAU et al. [20], have suggested that changes in plant cell membrane properties which occur in response

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to changes in environmental temperature may alter the tertiary protein structure of membrane-associated resistance gene products. Post-inoculation temperatures can alter the sensitivity of membrane-located recognition which restrict signal transduction or the recognition function of resistance gene products [21]. BRYANT et al. [22], suggested that the temperature-responsive resistance does not involve a mechanism dependent on the initial recognition of the pathogen. Fulhard and Virgilio were well experimental materials for study sensitive temperature, which appear to influence the growth of *Pst*.

Postulation of temperature sensitive resistance genes

The temperature sensitive resistance was given for average of infection index (Table 3). Since some varieties resist to *Pst* race 10E162, their temperature sensitive resistance was possibly masked. *Pst* race 82E22 is highly virulent to the major resistance gene from PI 178383 and was used to postulate the numbers of the sensitive resistance gene. S110 possessing no major and no minor genes conditioned index of infection 6 as MX. Fulhard and Mentana contain one temperature sensitive gene, which have shown similar infection type as that of S111 with one temperature sensitive minor gene. Virgilio contained two temperature sensitive genes controlling infection type as S112 with 2 minor ones. Figure 1 illustrated the infection types obtained on Fulhard, Virgilio and Mentana.

Table 3: Average of infection index in wheat cultivars in normal and high temperature regimes to *Pst* race 82E22.

Cultivars	A	B	D	A	C	D
Virgilio	3.93	1.06	2.87[c]	4.57	1.64	2.93[c]
Mentana	5.52	4.76	0.76[b]	6	5.3	0.7[b]
Fulhard	6	5.5	0.5[b]	6	4.9	1.1[b]
S110	6	6	0[a]	6	6	0[a]
S111	6	5.58	0.42[b]	6	5.11	0.89[b]
S112	5.04	2.38	2.66[c]	6	3.20	2.80[c]
S113	5.25	2.23	3.02[d]	5.9	2.87	3.03[d]
Mingxian 169	6	6	0[a]	6	6	0[a]

Note: A: normal temperature, B: high temperature fortwo days then to normal temperature, C: continuous high temperature and D: difference of infection index between the normal and high temperature.
a, b, c, d: The letters indicated difference between treatments at 0.05 according to Duncan mean separation test.

Wheat resource with temperature sensitive minor gene is useful in breeding for resistance to stripe rust. Evidence suggested that Moro contains only the major resistance gene from PI 178383, with no temperature sensitive resistance gene and remained resistant for only 3 years. To utilize the temperature sensitive resistance reasonably it can avoid that happened. The major gene can be effective against a specific race and temperature sensitive genes may affect against the other races. Temperature sensitive resistance is of common

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occurrence in wheat. Wheat cultivars Malakof, Norka, and Democrat were resistant at 18.7°C but became susceptible at a lower temperature. PARK *et al.* [6], reported the presence of temperature sensitive stripe rust resistance in cultivar Cook and its derivatives. It has also been reported that temperature sensitive resistance was found in wild emmer wheat [23]. The temperature sensitive resistance in wheat and wild emmer are advantageous for wheat breeding.

The climate system is becoming globally warming. The temperature during the wheat growing season is always higher in May to July. The adaptation of the rust pathogen to higher temperature is increasing. MILUS & SEYRAN [24], suggested that wheat stripe rust caused by the new isolates developed faster than the old ones at relatively high temperatures. In these cases, the expression of the temperature resistance may be important, which could reduce the epidemic. SHARP & VOLIN [2], demonstrated when temperature sensitive genes were accumulated, they showed additive effects. Also, QAYOUM & LINE [11], showed the temperature sensitive resistance has durable effects. As already mentioned, the race specific resistance is not very durable, there seems to be a better chance for stability. When the temperature resistance combined with other resistances genes to *Pst*, the pathogen will need to overcome them step by step. If the fungus does mutate to overcome minor gene resistance, assuming stepwise mutation of the pathogen, there would be a stepwise but gradual change toward susceptibility. The resistance was in additive combinations and was not typically initiating violent hypersensitive reactions. It means that using minor temperature sensitive resistance genes in conjunction with other types of resistance are helpful and provide a long term and promising strategy of control of wheat stripe rust.

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