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Sources of Partial Resistance to Leaf Rust in Hard Wheat Landraces Cultivated in Palestine

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Abstract

A collection of 7 landraces of Palestinian durum wheat was screened for resistance to leaf rust (*Puccinia triticina*) under controlled conditions. Latent period was more pronounced in adult plants than in seedlings and within adult plants the latency period was higher in flag leaf. In sixth leaf stage, all lines except White Dibeyah had a relative latency period greater than Meridiano and similar to Akabozu. Black Dibeyah had a latency period superior to Akabozu (partially resistant check). Black Dibeyah, Kahatat and Akabozu had a higher percentage of early abortion without host cell abortions (EA-) than the susceptible control in seedling stage. In the sixth leaf stage, only line Black Dibeyah had a higher % EA- than the rest. In flag leaf stage lines, Black Hiteyah, Yellow Hiteyah, Black Dibeyah, Kahatat and Akabozu had a higher percentage of early aborted colonies than Meridiano. These lines may be useful additional sources of partial resistance to leaf rust and they might be used in breeding programs.

Keywords: Landraces, partial resistance, *Puccinia triticina*, *Triticum turgidum*, wheat

Introduction

Wheat leaf rust, caused by *Puccinia triticina*, is an important disease worldwide. When a severe epidemic occurs, yield losses can reach 30 % or more [1]. Wheat breeding for leaf rust resistance in modern agriculture has traditionally been based on hypersensitive resistance governed by major genes and is race-specific [2]. This type of resistance is associated with plant cell necrosis around the infection site resulting on low infection type. Hypersensitive resistance yields quick and significant results in terms of control of the disease, but pathogen populations commonly are able to overcome the resistance genes resulting in new virulent races. There is an increasing concern in the durability of the resistance (partial resistance) [3]. Several strategies can be adopted to prolong the durability of the hypersensitive resistance, such as gene pyramiding, diversification and cultivar-mixtures [4].

Partial Resistance has been described in many pathosystems including wheat/wheat and barley/barley leaf rust. It is generally polygenic, but not necessarily. *Lr*34 is an example of a single gene that confers an incomplete resistance not based in hypersensitivity that can be considered partial resistance [5]. *Lr*34 resistance comes from Terenzio wheat and is present in many wheat cultivars with known durable resistance to leaf rust [6]. Lr 46 might be another example [7]. Partial resistance is defined as a resistance that reduces the epidemic build-up despite a high infection type [8,9]. It is reported that landraces of different crops usually build up levels of nonhypersensitive resistance to rust rather than high frequencies of hypersensitivity resistance. One of the most interesting aspects of partial resistance is its high stability in different environments and its apparent durability [9]. Landraces may have fair levels of partial resistance. Farmers have made an unconscious selection against extreme susceptibility generation after generation.

In this paper, a collection of seven wheat landraces grown in Palestine was screened for resistance to leaf rust. This study aims to determine the level and type of resistance in the collection as well as to study the mechanisms of resistance of the lines with the highest level of partial resistance.

Material and methods

Plant material

A collection of 7 wheat landraces commonly grown in Palestine (Black Hiteyah, Yellow Hiteyah, Black Dibeyah, White Dibeyah, Kahatat, Nab Al-jamal, Kahlah) and two checks (Akabozu with a high level of partial resistance and Meridiano, very susceptible to wheat leaf rust) were used in the study

Seedling studies

Plants were grown in soil in plastic trays (35×20×10 cm) in a complete randomized design (CRD) with three replicates. In each tray, 7 accessions plus Akabozu (high level of partial resistance) and Meridiano (very susceptible) checks were included (3 leaves per line). Eleven days after sowing, first leaf of each seedling was fixed in a horizontal position with the adaxial side upward. Urediospores (3 mg per tray) of a local isolate collected from the field was mixed with talcum powder (1:10, vol/vol) and applied using special dusting equipment. The inoculum density was about 200 spores/cm^2 [21]. After inoculation the plant boxes were incubated 12 h in darkness at 100 % relative humidity, and then transferred to a compartment at continuous 20 - 22 °C and 12/12 h of white florescence light.

Infection type (IT), latency period (LP), and infection frequency (IF) were determined. Infection type was recorded 12 days after inoculation according to a 0 to 9 scale [10]. The latency period was determined by counting daily the number of uredia visible in a marked area on the leaves till the number of uredia no longer increased. The latency period was taken as the time period from the beginning of incubation to the time at which 50 % of the uredia have appeared. Infection frequency was determined on the marked areas of the leaves. The final number of uredia was used to calculate the number of uredia per cm^2.

Adult plant experiments

The experiments were done in 2 adult plant stages, 6th leaf (DC 30) and flag leaf (DC 48-59) [11]. Three series were performed, of three boxes each. Inoculation was performed by dusting urediospores mixed with talcum powder over the plants. 0.5 mg of urediospores was used per plant. Components of resistance were measured as described above both in seedlings and mature plants after inoculation with the same isolate used at the seedling stage.

Microscopic observations

Five days after inoculation middle segments of 1 to 3 cm^2 were collected from the first or flag leaves (seedling and adult plant experiments, respectively). Three leaves were sampled per series. Leaves were fixed and cleared by boiling for 1.5 min in lactophenol/ethanol (1:2, v/v) and stored overnight in this mixture at room temperature. Segments were then washed once with 50 % ethanol for 30 min, once with 0.05 M NaOH for 30 min, rinsed three times in water (10 min each), then were soaked in 0.1 M Tris/HCl buffer (pH 8.5) for 30 min. Then they were stained with a 0.1 % solution of Uvitex 2B in the same buffer. This was followed by rinsing four times with water. Segments were then immersed in a solution of 25 % glycerol for a minimum of 30 min (a few drops of lactophenol were added to the solution to prevent deterioration by fungi) and stored until observed. Leaf segments were examined at 100× with Nikon Eclepci 50i epifluorescence equipment [12]. At least 100 sporelings per leaf segments were scored and classified according to their stage of development [13]. Sporelings that developed a germ tube and not an appressorium over a stoma were ignored. Early aborted sporelings were defined as individuals that formed primary infection hyphae and not more than six haustorial mother cells [13]. Sporelings that had developed more haustorial mother cells were classified as established. The length (L), and width (W) of ten arbitrarily chosen established colonies per leaf were measured with an eyepiece micrometer. Colony size (CS) was calculated as the geometric mean of L and W, $CS = \sqrt{(\frac{1}{4} \times L \times W)}$ [14].

Data analysis

Analysis of variance (ANOVA) was calculated by using PROC GLM in an SAS program [15]. Comparisons between lines were made by the Duncan-test.

Results

Macroscopic components

The results of the macroscopic observations are shown in the **Table 1**. Latency period was more pronounced in adult plants than in seedlings and within adult plants the latency period was higher in flag leaf (203 vs 171 h). In seedlings, Black Dibeyah and White Dibeyah lines showed a higher latency period than the susceptible check Meridiano and were similar to Akabozu. In sixth leaf stage all lines except White Dibeyah had a relative latency period greater than Meridiano and similar to Akabozu. Black Dibeyah had a latency period superior to Akabozu (partially resistant check). In the flag leaf stage the trend resembled that of sixth leaf. In this case all lines have a relative latency period higher than the susceptible Meridiano and similar to Akabozu except lines Black Dibeyah and Kahatat with a relative latency period superior to Akabozu.

Table 1 Macroscopic components of resistance (Latency Period, Infection Frequency and Infection Type) of seven Palestinian wheat landraces.

Genotype	Seedling stage			Sixth leaf			Flag leaf		
	RLP[1]	RIF[1]	IT[2]	RLP[1]	RIF[1]	IT[2]	RLP[1]	RIF[1]	IT[2]
Meridiano	100d[3] (148 h)	100a[3] (57 cm^2)	9	100c[3] (171 h)	100a[3] (35/cm^2)	9	100c[3] (203 h)	100a[3] (43/cm^2)	9
Black Hiteyah	107d	104a	9	115b	70ab	9	114ab	94a	9
Yellow Hiteyah	106d	105a	8	114b	44b	5	-	-	1
Black Dibeyah	114bc	67bc	9	136a	47ab	8	148a	27b	9
White Dibeyah	118b	104a	8	110bc	75ab	8	115ab	62ab	5
Kahatat	106d	89ab	8	114b	75ab	8	144a	43a	9
Nab Al-jamal	110cd	90ab	9	117b	42b	8	118ab	63a	8
Kahlah	104d	91ab	8	113b	74ab	8	120b	55a	9
Akabozu	133a	61c	9	118b	54ab	8	122b	83a	9

[1]Relative latency period (RLP) and relative infection frequency (RIF) referred to Meridiano = 100 %. The actual values for Meridiano are presented between brackets.
[2]IT on a scale of 0 to 9 [10].
[3]Data with the same letter per column are not statistically different (Duncan, $p < 0.05$).

The infection frequency in the susceptible check was always higher in seedlings (57 p/cm^2) than in adult plants (35 and 43 p/cm^2). In the seedling stage line Black Dibeyah had a clear reduction in the relative infection frequency together with Akabozu. Lines Yellow Hiteyah and Nab Al-jamal displayed a lower relative infection frequency in the sixth leaf stage with respect to Meridiano although the value of Black Dibeyah is also low. Black Dibeyah showed a drop in the relative infection frequency of flag leaf with respect to the susceptible check.

IT was high (susceptible IT) in the overall of lines in the seedling stage. In sixth leaf all lines showed high infection types except the line Yellow Hiteyah that displayed an IT = 5 (poor sporulation but associated to some necrosis). In flag leaf the IT of this line was reduced to 1 (only necrotic flecks). The IT of the line White Dibeyah was also lower in flag leaf (IT = 5).

Microscopic components in the selected lines

The results of the microscopic observations are shown in the **Table 2**. The differences in the percentage of early abortion without necrosis (EA-) was higher in flag leaf stage followed by sixth leaf stage and seedling stage which was the lowest. Black Dibeyah, Kahatat, Nab Al-jamal and Akabozu had a higher percentage of EA- than the susceptible check in the seedling stage. In the sixth leaf stage, Black Dibeyah had a higher % EA- than the rest. In the flag leaf stage, lines Black Hiteyah, Yellow Hiteyah, Black Dibeyah, Kahatat and Akabozu had a higher percentage of early aborted colonies than Meridiano. It is remarkable the huge percentage of colonies of this type in both Kahatat and Black Dibeyah lines.

Regarding the percentage of colonies associated with necrotic plant cells (%EA+), Yellow Hiteyah displayed the highest percentage of colonies surrounded by necrotic cells in all stages (37, 76 and 64 %). Also EA+ was high in lines Black Dibeyah (29 %) and Kahatat (19 %) in the sixth leaf stage and in flag leaf stage, White Dibeyah showed the highest percentage (62 %) compared to the Meridiano (9 %).

Table 2 Microscopic components of the resistance of seven Palestinian wheat landraces.

Genotype	Seedling Stage		Sixth leaf		Flag leaf	
	%EA-[1]	%EA+[1]	%EA-[1]	%EA+[1]	%EA-[1]	%EA+[1]
Meridiano	4cd[2]	5d[2]	7bc[2]	5ef[2]	9e[2]	9b[2]
Black Hiteyah	5bcd	5cd	13abc	14c	29cd	13b
Yellow Hiteyah	4d	37a	15ab	76a	34bc	64a
Black Dibeyah	10a	7bc	23a	29b	67a	7 b
White Dibeyah	5bcd	8b	5c	6de	14de	62a
Kahatat	8ab	5bcd	11abc	19c	53ab	11b
Nab Al-jamal	7abc	6bcd	16ab	10cd	19cde	7b
Kahlah	5bcd	5bcd	8bc	5ef	16cde	6b
Akabozu	8ab	4d	16ab	3f	28cd	7b

[1]Expressed are percentage of early aborted colonies without host cell necrosis (%EA-), percentage of early aborted colonies associated with host cell necrosis (EA+).
[2]Data with the same letter per column are not statistically different (Duncan, $P < 0.05$)

Discussion

In this study, a collection of tetraploid wheat was screened for resistance to wheat leaf rust *(Puccinia triticina)* and in almost all lines a high IT was found, and in most of them moderate levels of partial resistance which is common in tetraploid wheat [16]. A near-absence of race-specific, major gene resistance and a relatively high frequency of moderate levels of partial resistance to leaf rust was found in a collection of Ethiopian barley landraces [17]. Lines Black Hiteyah, Nab Al-jamal and Kahlah show high levels of partial resistance only slightly inferior to Akabozu. They show a high latency period that is correlated very well with partial resistance, especially in adult plants [14,18]. At a microscopic level they display high levels of EA- and small colonies especially in adult plants. These parameters are also associated with partial resistance [19]. Black Hiteyah and Kahlah express partial resistance as adult plants while partial resistance of Nab Al-jamal is expressed in all stages. Lines Black Dibeyah and Kahatat have a level of partial resistance higher even than Akabozu. The latency period, the EA- is very high and the colony size is very low especially in line Black Dibeyah.

In Yellow Hiteyah the infection type decreases to 5 in sixth leaf and to 1 in flag leaf which may increase the evidence of the presence of adult plant resistance genes in lines Yellow Hiteyah and White Dibeyah. Also the percentage of cell necrosis is high in this stage. It is a gene with an incomplete expression of the resistance in sixth leaf and complete in flag leaf. However, White Dibeyah shows an infection type 5 in flag leaf. It is an infection type where there are pustules but is quite often associated with necrosis. In the literature, there are abundant examples of hypersensitive adult plant resistance to *Puccinia triticina* in wheat [14]. It is difficult to know the level of partial resistance in lines with adult plant resistance genes because hypersensitive resistance is usually stronger than partial resistance. However there are different ways to observe it. In the first place, we could see the level of partial resistance in seedling stage where the adult plant genes are inactive. On the other hand, partial resistance has a prehaustorial nature (prior to haustorium formation) in opposition with hypersensitive resistance that is posthaustorial [13,9]. The level of colonies arrested early with any association of necrosis could be a good indicator of partial resistance even in lines with hypersensitive resistance. White Dibeyah has a high latency period in the seedling stage that doesn't correlate with the percentage of early abortion in adult plants. It seems only a partial resistance is expressed at the seedling stage. The presence of partial resistance or QTLs in the seedling stage has been reported in the pathosystem barley- barley leaf rust [20]. Yellow Hiteyah does not show high levels of partial resistance in seedlings but it might have higher levels of partial resistance in adult plants because it has a high level of early aborted colonies not associated with plant cell necrosis (similar to the positive check Akabozu). The partial resistance of landraces is usually durable [21], therefore these lines may be useful additional sources of partial resistance to leaf rust and they might be used in breeding programs.

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