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Identification of Resistant Sources to Crown Rust (*Puccinia Coronata*) in Oat

تحديد مصادر مقاومة لمرض الأوراق في محصول الشوفان

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Abstract

This study was conducted to search for new resistant sources to oat leaf rust as well as to characterize the macroscopic components of resistance to crown rust under controlled conditions. One hundred twenty different accessions of oats obtained from the National Small Grains Collection, Idaho (NSGC), USA, in addition to a local susceptible wild accession were used in this study during the growing season of 2008-2009. Disease severity (DS) was calculated three times during the growing season at two week intervals. DS values were used to calculate Area under Disease Progress Curve (AUDPC). The results of this study revealed that the local susceptible (control) accession had 48% DS (100% AUDPC) of leaf rust, and DS for the tested accessions ranged from very high to very low, and the distribution was markedly shifted towards low DS. 50% of the collection showed AUDPC < 50%, while thirteen cultivars. (10.8% of the collection) with AUDPC ≤ 20%, were selected to study their reaction to artificial inoculation with uredio spores of *Puccinia coronata* at seedling stage. All the tested accessions showed significantly higher relative latency period (RLP) and lower relative infection frequency (RIF) in comparison to the susceptible control. These resistant cultivars were planted in the field to produce grains for further studies.

Key words: Latency period, oat, partial resistance, *Puccinia coronate*.

ملخص

أجريت هذه التجربة للبحث عن مصادر مقاومة لمرض صدأ الأوراق في محصول الشوفان؛ وكذلك لدراسة المكونات الدقيقة لمقاومة هذا المرض تحت الظروف المخبرية. استخدم في الدراسة ١٢٠ صنفاً من أصناف الشوفان تم الحصول عليها من NSGC، إضافة الى صنف بري محلي قابل للإصابة بمرض الصدأ، وذلك في الموسم الزراعي ٢٠٠٨-٢٠٠٩. وفي فترة النمو تم تقييم نسبة إصابة النباتات بهذا المرض ثلاث مرات بواقع مرة كل أسبوعين، واستخدمت النتائج في تحديد منحنى تطور المرض (AUDPC). أظهرت النتائج تبايناً بين أصناف المجموعة في شدة الإصابة حيث بلغت على الشاهد ٤٨%. بشكل عام كان هنالك ميل واضح نحو قلة شدة الحساسية للمرض، بينما أظهر عدد قليل من الأصناف درجة عالية من المقاومة الجزئية للمرض (تقريباً ١١% من النباتات) وترافقت المقاومة العالية لبعض الأصناف مع زيادة ملحوظة في فترة الحضانة لمسبب المرض تراوحت بين ٢٧.٣-٤٦.٧% أطول من الشاهد. تم انتخاب الأصناف التي اظهرت مقاومة عالية للمرض لإجراء المزيد من الدراسات عليها.

Introduction

Oat is one of the important winter cereal crops in cold climatic areas of the northern hemisphere (López-Bellido, 1991). Oats were among the top five cereal crops produced worldwide. Today oat production decreased to the sixth place found in cereal crops (FAO, 2009). The common oat cultivars grown in the world have been bred mainly for: high yields, high protein contents and with feasible palatable qualities for both feed and human consumptions. Although the yield of cultivated oat has increased by 40% through plant breeding (Peltonen-Sainio & Karjalainen, 1991). Different diseases were reported to attack oat of which: crown rust or leaf rust caused by the fungus *Puccinia coronata* f.sp. *avenea* is the most wide spread and destructive disease of oat, the disease drastically affecting oat resulting in 30% reduction in yield (Long *et al.,* 2006). Resistant oat cultivars are the most economical and environmentally safe measures to control crown rust (Hsam *et al.,* 1998). Two types of resistance against crown rust are distinguished; hypersensitive reaction (HR) monogenic or race specific resistance, and the partial resistance (PR) controlled by few minor genes and generally lasting more than HR (Parlevliet, 1978). PR results from reduced infection frequency, a longer latent period, and a reduced spore production (Jacobs &d Kiriswa, 1993). However during the last few years of cultivation, race specific resistant oat cultivars, new virulent phenotypes or strains of the pathogen have developed and overcome commonly used resistant genotypes. Subsequently, none of the known sources of crown rust resistant present in cultivated oat could provide adequate protection against the disease. This study was conducted in order to search for new resistant sources against oat leaf rust (*Puccinia coronata* f.sp. *avenae*) and to study the partial resistant components at macroscopic level.

Material and Methods

A: Field experiment

One hundred twenty different accessions of oats obtained from the National Small Grains Collection, Idaho (NSGC), USA, in addition to a wild local susceptible cultivar were tested in the field against natural infection with crown or leaf rust of oat.

The experiment was conducted at the experimental farm of the Faculty of Agriculture in Tulkarm, Palestine during the growing season 2008–2009. Accessions were sown in November 2008. The layout of the experiment was CRBD, where 25–30 seeds of each line were sown in a single row of 1 m long. Every five accessions were separated by a single row planted with local susceptible cultivar. Each tested accession was replicated three times.

Inoculation method

No artificial inoculation was performed since crown rust is a common disease and wide spread.

Scoring assessment

When rust development started, disease severity was assessed at two-week intervals by visual estimation of the leaf area covered with rust pustules. These data were used to calculate the area under the disease progress curve (AUDPC), using the formula:

$$\text{AUDPC} = \sum_{i=1}^{k} \tfrac{1}{2}\,[(S_i+E_{i+1})\,(t_{i+1}-t_i)]$$

Where Si is rust severity at evaluation date i, ti is the number of days after the first observation on assessment date i and k is the number of successive observations. The means of the observed AUDPC values of each tested accession were converted into relative values and expressed as a percentage of the susceptible local line (Sillero *et al.,* 2000).

B: Growth chamber experiment

Seeds of the selected accessions, those that showed a AUDPC ≤25% in the field, were sown in soil in plastic trays (35 x 20 x 8 cm) with three replicates in each tray and three plants in each replicate. In each tray, eight to ten accessions were grown. Eleven days after sowing, the first leaf of each plant was placed in a horizontal position by the aid of metal staples for homogenous inoculation with uredial spores of TU-09 strain of *Puccinia coronata* f.sp. *avenae* collected from the local wild susceptible cultivar.

The inoculation was carried out by dusting a mixture of freshly collected spores and wheat flour (1:10, v/v). Each tray was inoculated with 3 mg of uredial spores that resulted in about 200 spores/cm^2 deposition (Niks & Rubiales, 1994). The artificially inoculated plants were kept in an inoculation chamber for 12 h in dark at 20 ºC with a relative humidity of about 100%. Plants were then moved to a growth chamber at a temperature of 18–22 ºC and with white fluorescent light (12 h light/12 h dark).

Disease scoring: LP, IF and IT were determined for the different tested artificially inoculated accessions as macroscopic PR parameters. LP was determined by daily counting the number of uredia pustules visible in a marked area (2–3 cm^2), using a 6x pocket lens. The LP was calculated as the time from the inoculation to the time at which 50% of the uredia had appeared (Parlevliet, 1975). The final number of uredia pustules was used to determine the IF calculated as the number of uredia pustules per unit area. The infection type (IT) was recorded 12 days after inoculation using the 0–9 scale of McNeal et al. (1971) where: 0, no uredinia or other macroscopic sign of infection; 1, few faint hypersensitive flecks; 2, no uredinia, but clear hypersensitive necrotic flecks present; 3, flecks with small uredinia surrounded by necrosis; 4, small to medium uredinia often surrounded by necrosis and chlorosis, small sporulation; 5, medium uredinia often surrounded by necrosis and chlorosis, reasonable sporulation; 6, medium-sized to large uredinia surrounded by necrosis and chlorosis, reasonable sporulation; 7, medium-sized to large uredinia surrounded by chlorosis but not necrosis, good sporulation; 8, medium-sized to large uredinia surrounded by a little chlorosis but not necrosis, good sporulation; and 9, large uredinia without chlorosis or necrosis, very good sporulation.

LP and IF were expressed as relative latency period (RLP) and relative infection frequency (RIF) by converting actual values to a percentage of the local wild susceptible cultivar's values.

Data analysis

Data on RLP, RIF and DS were statistically analyzed and ANOVA was conducted using PROC GLM in SAS program (SAS Institute, 1988). Mean separation was performed using Duncan multiple range test ($p \leq 0.05$)

Results

A: Field experiment

The distribution of the tested oat cultivars according to their relative AUDPC of crown rust are illustrated in Fig.1, however the susceptible local cultivar showed 48% DS (100% AUDPC), and the DS of leaf rust ranged from high (72%) to low (1.5%) and the majority were shifted to low values of DS. However the Graph showed that 50% of the tested accessions showed AUDPC < 50%, while thirteen cultivars (10.8% of the tested cultivars) showed AUDPC of ≤ 20%. These accessions were selected to study the partial resistance component to oat leaf rust at seedling stage in growth chamber.

B: Growth chamber experiment:

Results of macroscopic component for oat crown rust partial resistance including RLP, RIF, IT and DS in the field for the artificially inoculated seedlings, of the 13 selected cultivars, with uredial spores of *Puccinia coronata* f.sp. *avenae* are listed in Table 1. The results indicated that all the tested cultivars showed infection type of 9 with RLP values significantly longer than the susceptible control ranged from 27.3-46.6%. The accessions (PI 295954, PI 293344 and CIav 4788) showed longer RLPs than the control with 47.6, 45.5 and 44 for the different cultivars respectively. All the tested accessions showed lower RIF lower than that of the control where Clav5234 and PI293347 with 51.6% had 54.3% of the susceptible control.

Discussion

For a long time, the genetic heritage of landrace oat species in the Mediterranean region was investigated by many oat researchers. Zillinsky & Murphy (1967) reported that some landrace oat accessions collected in Tunisia possess a high oat crown rust resistance level. Loskutov, (2002) reported after evaluation of many landrace oat accessions collected from different regions in the world, that resistance to oat crown rust came mostly from North Africa such as Tunisia, Algeria and Morocco.

The results of our study revealed that several accessions had acceptable partial resistance levels. A long latency period (LP) is an indicator for partial resistance to leaf rust of oats (*Puccinia coronate* f.sp. *avenae*) (Brake & Irwin, 1992), barley (Parlevliet, 1979) and wheat (Ohm & Shaner, 1976). The mean latent period of the thirteen selected accessions with partial resistance ranged from 6.3-7.3, (1.3–2.3 days longer than the latent period of control).

Our results are in agreement with the results of Brière & Kushalappa (1995) and Luke *et al.,* (1984) who reported that a 7-day difference in latent period between inoculated adult plants of the slow-rusting cultivar Red Rustproof longer than the susceptible cultivar of Fulghum.

Differences in latent period between barley lines with high partial resistance and susceptible lines varied between 3.7 and 7.8 days (Johnson & Wilcoxson, 1979), and ranged from 1.5 to 4.6 days for wheat slow-rusting lines (Kuhn *et al.,* 1978).

The results obtained on the infection frequency (IF) showed that the same lines that had submitted a higher LP had an IF lower than the susceptible control. Something similar occurs in plants of other species such as in legumes (Sillero *et al.,* 2000) and barley (Shtaya *et al.,* 2006). The observed resistance to rust in oats is incomplete, and is expressed as an increase in LP and a decrease in the number of uredial rust pustules. This is shown as a reduction in final disease severity (Parlevliet, 1979). This is in full agreement with our results because the lines had a higher LP and lower IF.

A study of resistance to oat rust resistance levels were obtained in part in a series of experimental oat lines showed a reduction in the severity of rust after 30 years exposure to diverse populations of the pathogen (Leonard, 2002). Measurments of latency period and infection frequency were performed at the first expanded leaf, according to Brake & Irwin (1992), the reaction of the 1st and 4th leaf reflects the reaction of plants to natural infection with rust under field conditions.

The collection used in the present study is an important source for partial resistance to oat leaf rust. The selected accessions could be used as a valuable source for partial resistance against oat crown rust. Further studies are needed to identify the number of QTLs present in each selected accessions and the relation between these genes and other known genes for resistance against oat leaf rust

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Table (1): Macroscopic components of partial resistant to crown rust (*Puccinia coronata* f.sp. *avenae*) in selected oat accessions.

Accession	Seedlings in growth chamber			AUDPC	Adult plants in the field
	IT[1]	RLP[2]	RIF[2]		(DS%)[3]
PI 295954	9	147.6^{a,4}	60.3^{def4}	4	1.5^{b4}
PI 293344	9	145.3^{ab}	78^{c}	15	5.3^{b}
CIav 4788	9	144^{abc}	67.6^{cd}	14	5.3^{b}
PI 293348	9	141.6^{bcd}	61^{def}	14	3.6^{b}
PI 341007	9	141.6^{bcd}	75.6^{c}	18	7.1^{b}
PI 293347	9	140.6^{cd}	54.3^{ef}	15	3.7^{b}
PI 298126	9	140.3^{cd}	88^{b}	16	4.2^{b}
PI 295953	9	139.6^{d}	69.6^{cd}	13	3.7^{b}
PI 293345	9	139.3^{d}	63.6^{de}	11	4.5^{b}
CIav 5235	9	133.3^{e}	75.3^{c}	19	4.8^{b}
PI 295955	9	131^{ef}	74.6^{c}	9	2.8^{b}
CIav 5234	9	129.3^{f}	51.6^{f}	19	4.5^{b}
PI 177837	9	127.3^{f}	71.3^{cd}	10	2.5^{b}
Local (wild)	9	100^{g}	100^{a}	100	48^{a}

(1) Infection type (IT) according to the 0-9 scale of McNeal et al. (1971)

(2) Relative latency period (RLP) and relative infection frequency (RIF) referred to Local (wild) = 100 %. The actual values for local (wild) were 120 h (latency period) and 66 pustules per cm^2 (infection frequency).

(3) Estimated as the percentage of leaf area covered by uredia.

(4) Means in the same column with similar superscripts are not statistically different (Duncan, $P \leq 0.05$).

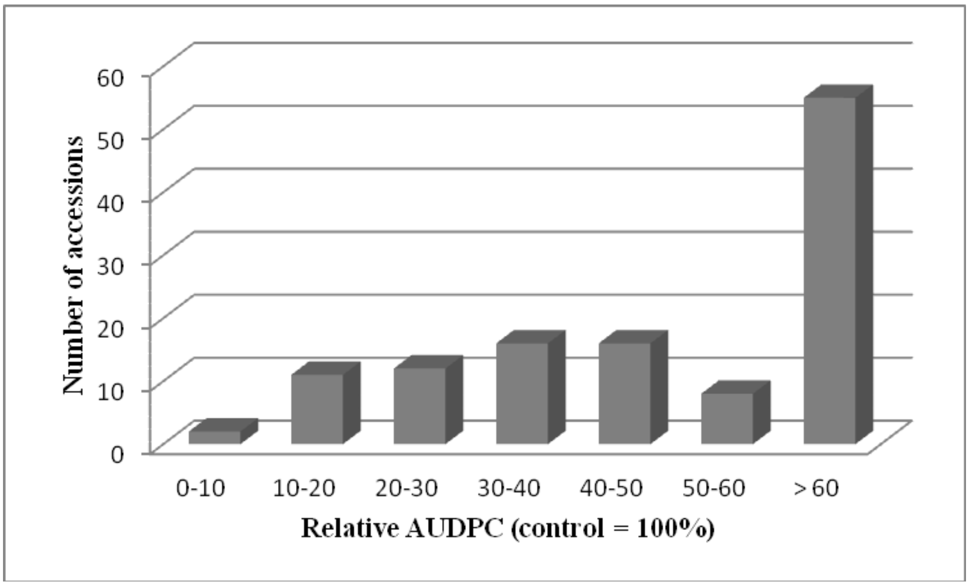

Figure (1): Distribution of the 121 oat accessions according to the relative AUDPC of *Puccinia coronata* f.sp. *avenae*.