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Screening for Resistance to Crown Rust in Oat Genotypes through Morphological and Molecular Parameters

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Abstract

A collection of 20 oat genotypes from different sources were evaluated in small isolated field plots for crown rust severity in natural epidemics with virulent *P. coronata* isolates and further screened with linked molecular markers. Large variation was observed for disease severity under field conditions in spreader plot. Genotypes with partial resistance due to a reduction of disease severity in spite of a compatible interaction (rust score 3) and moderate susceptibility were identified. The twenty genotypes displaying the variable disease severity with visible necrosis were selected for further studies regarding presence or absence of major genes (Pc91 and Pc68) for resistance to crown rust. In field nurseries, on the basis of latency period and disease severity (DS) none of the twenty genotypes fell into the resistant pool (score 1). Most of them showed a prolonged latency period, reduced infection frequency and colony size, and increased percentage of early aborted colonies not associated with host cell necrosis. Result on screening for crown rust resistance in field screening and by linked markers showed the absence of major resistance gene in the target population, several advance lines were identified as moderately resistant to crown rust reaction.

Keywords: Partial resistance; *Puccinia coronate*; Oat; SCAR; NCBI

Introduction

Despite being high fed fodder crop, oat is now gaining importance due to its unique and important quality characteristics, particularly lipid and protein. Oat is predominantly used as green fodder in north India where crown rust has started making its appearance in oat fields. Crown rust, caused by *Puccinia coronata*, is one of the most destructive diseases of oat (*Avena sativa* L.) in major oat growing countries. Over the past 10 years, yield losses of 10 to 20% in oat due to crown rust were reported for various American states from 1991 to 1993 [1]. Crown rust is most important where dews are frequent and temperatures are mild (15-25°C) during the oat growing season, which is a characteristic climate of Pantnagar region. More than 100 race-specific resistance genes to crown rust have been identified out of which 96 were defined as Pc, with the majority considered to be dominant genes [2]. The resistance caused by these genes is typically race-specific, expressed as a hypersensitive reaction, and of limited durability. The non-durability of this resistance has caused breeders to look for more durable types of resistance such as partial resistance (PR). PR has been identified in barley and oat and is expressed as a reduced rate of epidemic development despite a compatible interaction i.e. high infection type [3]. Pc91 is a major crown rust resistance gene effective at all stages of plant development [4] also Pc68, on the other hand, which was introgressed in *A. sativa* from *Avena sterilis* L. [5], is considered to be one of the most effective genes against this disease. The objectives of this work were to screen the oat genotypes for crown rust resistance, to deduce the status of major genes for resistance and to identify useful material for resistance breeding programs.

Materials and Methods

Field studies

The observation on crown/leaf rust reaction was carried out in a spreader plot at the Instructional Dairy Farm of G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India where seeds from Kent and UPO 270 were mixed in equal proportion and then sown as spreader rows on 15th October 2010 in two replications leaving the space for test entries (Figure 1). Row to row distance was kept 20 cm and the experimental test material was sown 20 days after the planting of spreader rows i.e. on 5th November 2010 in between the spreader lines (Figure 2) and separately in an isolated normal plot. Each test entry was represented by 40-45 plants in a 2 m long single row in spreader plot. Artificial inoculation was done at 20 days of planting of spreader rows, by spraying the solution of crown rust spores, cultured from previous year's infected oat leaves, over the

Figure 1: Advance spreader row planting.

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Figure 2: Spreader row along with test entries.

No.	Genotype	Source	No.	Genotype	Source
1.	D. Sel.-1	Pantnagar	11.	UPO-275	Pantnagar
2.	D. Sel.-5	Pantnagar	12.	Kent	Australia
3.	D. Sel.-6	Pantnagar	13.	UPO-212	Pantnagar
4.	Wright	U.S.A	14.	No.-1	Pantnagar
5.	HFO-114	Haryana	15.	OS-6	Haryana
6.	OL-125	Ludhiana	16.	EC-605833	Exotic
7.	UPO-265	Pantnagar	17.	EC-605836	Exotic
8.	EC-246199	Exotic	18.	EC-605838	Exotic
9.	UPO-271	Pantnagar	19.	UPO-260	Pantnagar
10.	UPO-273	Pantnagar	20.	UPO-270	Pantnagar

Table 1: List of oat genotypes evaluated for crown rust resistance.

spreader rows. Appropriate moisture was maintained in the spreader plot, for optimum growth conditions of the pathogen throughout the crop growth period, by partial irrigation (Table 1) [6].

Laboratorial studies

CTAB procedure was used for isolation of DNA [7]. Further PCR amplification by using the 25 µl reaction mixture containing 1X KCl buffer (Fermentas) containing 0.2 mM dNTPs, 30 ng of each forward and reverse primer, 1.5 mM $MgCl_2$, 0.8 U Taq DNA polymerase (Fermentas) and 100 ng of DNA. Thermal cycler reaction were carried out according to the following temperature profile 4 min initial denaturation at 94°C; 37 cycles of 94°C for 1 min, varying annealing Tm according to primer for 45 s, 72°C for 1 min and final extension of 7 min at 72°C; and final hold 4°C. All amplifications were performed twice and independently to make sure that the results were correct. Electrophoresis was done at 50 V for 4 h in 1 X TBE electrophoresis buffer for SCAR. Gels were documented using Gel Doc system (Bio-Rad) and electrophoresis of amplified product was done sequentially. All the primers used in the study were synthesized by Merck Specialties Pvt. Ltd. In laboratory screening through molecular markers, three primers were used. Primer 1 and primer 2 are SCAR markers linked to Pc91 reported by McCartney et al. [4] while primer 3 was designed by using software primer 3+ available at NCBI website, using the sequence of Pc68 gene submitted by Satheeskumar et al. [8] (Table 2).

Observation and scoring assessment

All the twenty entries were evaluated for crown rust reaction separately. To estimate latency period (LP) the number of days was recorded since the seedling emergence till the first disease symptom manifestation for all entries. Disease severity (DS) was estimated two times during the growing season in terms of the percentage of leaves covered by the lesions (orange-yellow spores exposed by rupture of the leaf epidermis) at 30 days after sowing of test entries and then at 50% heading stage. The observation was taken by counting the number of lesions with its size of 1.0 cm and above. Based on the frequency of lesions and number of infected leafs, the 0 to 9 scale was used and the entries were scored accordingly. At maturity biological yield of all test entries was measured by weighing 5 plants randomly selected from the spreader plot and normal irrigated plot. The genotype with lowest LP, maximum number of infected leaves, lesion number at both stages of observation and maximum decrease in biological yield was given the score 9 (most susceptible), and rest were accordingly scored.

Results

Field reaction of genotypes

Disease severity (DS) ranged from very high to low, and the frequency distribution was markedly skewed towards high DS (Figure 3). The observation on leaf rust reaction was recorded two times in the spreader row plot first at 30 days after planting of the entries and again at 50% heading stage. Rust scoring was done as per ICARDA rust scale (0-9) depending upon the number of infected leaves, the number of lesions and decrease in biological yield in stressed conditions. Four of selected genotypes viz. D. Sel.-1, D. Sel.-5, EC-605838 and UPO-260 showed a significantly longer relative latency period (RLP) than rest of the genotypes evident from the late appearance of rust spores in them. The relative infection frequency (RIF) of these genotypes was significantly lower than the susceptible pool genotypes as visible leaf necrosis in them was less and also showed least decrease in biological yield in stressed conditions (Table 3).

Laboratory results

The three crown rust linked primers did not gave amplification

Sr.		Primer seq 5'- 3'	Phase	%GC	Tm
1	F	GGACTATCTAGTTTATGGAGGAG	Dominant-coupling	43.0	56.6
	R	AGGCAAAACGAGCAGTGTAA		45.0	62.3
2	F	CTTGTATTGTGCGTTGGAA	Dominant-repulsion	45.0	59.2
	R	CTTGTATTGTGCGTTGGAA		42.0	59.7
3	F	CGAGGGCTACATTCAAGAGC	Designed by primer 3+ (NCBI)	55.0	60.0
	R	TCCAAGGGTCTTCTCGGTAA		50.0	59.7

Table 2: Details of rust resistance linked primers used in the study of oat genotypes.

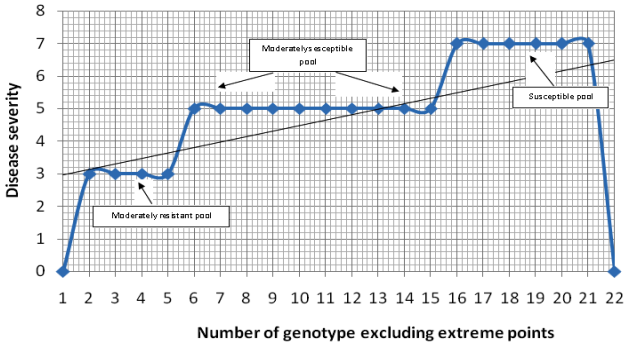

Figure 3: Frequency distribution of test genotypes against disease score/ severity.

S.No	Genotype	Biological yield/ plant (gm)		Rust scores*	Amplification with linked primer	
		Normal Plot	Spreader Plot		Pc91	Pc68
	D. Sel.-1	76	71.8	3	nil	nil
2	D. Sel.-5	55.7	55.1	3	nil	nil
3	D. Sel.-6	53.9	40.8	7	nil	nil
4	Wright	52	48.4	5	nil	nil
5	HFO-114	54	53.3	5	nil	nil
6	OL-125	60.1	49.6	7	nil	nil
7	UPO-265	54	48.4	7	nil	nil
8	EC- 246199	61	56.7	5	nil	nil
9	UPO-271	55.7	51	5	nil	nil
10	UPO-273	46	42.5	5	nil	nil
11	UPO-275	60.3	56.3	5	nil	nil
12	KENT	51.7	43.1	7	nil	nil
13	UPO-212	46.7	44	5	nil	nil
14	No.- 1	62.2	57.1	5	nil	nil
15	OS- 6	45.1	36.4	7	nil	nil
16	EC-605833	54.7	51.6	5	nil	nil
17	EC-605836	54.6	49	5	nil	nil
18	EC-605838	50.1	50.5	3	nil	nil
19	UPO-260	52.7	51.8	3	nil	nil
20	UPO-270	62.7	39.8	9	nil	nil

*1=resistant, 3=moderately resistant, 5=moderately susceptible, 7=susceptible, 9=highly susceptible

Table 3: Details of selected oat genotypes in the field and with SCAR markers.

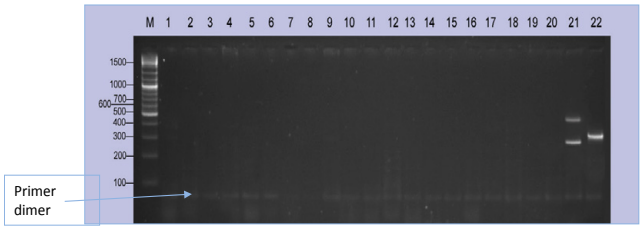

Figure 4: PCR based amplification of all 20 genotypes along with control genotype by SCAR Pc68 and Pc91 crown resistance gene marker.

bands with any of the test genotype even after three precautious reaction procedures. Only primer-dimer were visible in the gel-doc visualization; however the control crown rust resistance genotype Amalgam and starter which were donor of Pc91 and Pc68 gives the particular band with all three primers (Figure 4) thus its confirmed that no complimentary sequence was available in the target genotype DNA. Based on the results obtained by screening of the oat genotypes with linked primer (Table 3) it can be stated that none of the two major gene viz., Pc91 and Pc68 responsible for crown rust resistance, were present in the oat genotype evaluated. It was further supported by the field scoring data where none of the genotypes has fallen in the resistant pool (rust score 1).

Discussion

Puccinia coronata, is one of the most destructive diseases causing agent of Crown rust in (*Avena sativa* L.) in major oat growing countries. The infection behaviour of this disease was varied widely amongst the susceptible and resistance genotype as well as stressed conditions. This may be due to unfavourable growth behaviour of *Puccinia coronata*. It was further supported by the field scoring data where none of the genotypes has fallen in the resistant pool (rust score 1). Thus, the resistance difference among the 20 test genotypes can be generalized by acknowledging the absence of the two major resistance genes among them and other minor resistance genes at play, i.e. for the genotypes showing moderate resistance reaction with rust reaction score 3 it can be generalized that this type of partial resistance could be by virtue of genes with minor effect for crown rust resistance [9] and for genotypes with moderate susceptibility and susceptibility reactions for crown rust it can be generalized that the pathogen has evolved with matching virulent genes.

Absence of the two major gene i.e. Pc91 and Pc68 for resistance to crown rust in the experimental material of present study may be compensated by the fact that several advance generation improved lines (D. Sel.-1 and D. Sel.-5, UPO-260), and exotic material (EC-605838) have been found partially resistant to crown rust reaction, thus can be used against crown rust in more affected regions. The observation in the present investigation for crown rust reaction may have been confounded by presence of other pathogens in the field, causing induced resistance/susceptibility reaction [10,11], but the authenticity been provided by SCAR markers for absence of major resistance gene in the test genotypes. As the population of suitable genotype (matching races) of pathogen, increases in the field location where the cropping of single oat genotype may prove fatal because of the rapid buildup of infection [12]. Thus there is an urgent requirement for the incorporation of major genes for resistance in different oat genotypes and subsequently finding new genes for crown rust resistance, so that growing oats in field remains economical in future.

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