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

Yogesh Ruwali, Lalan Kumar* and JS Verma
G.B. Pant University of agriculture and technology, Pantnagar, U.S. Nagar, Uttarakhand, India

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. coronate 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 coronate, 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 is 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
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 MgCl2, 0.8 U Taq DNA polymerase buffer (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 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 leaves, 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

| No. | Genotype  | Source       | Sr. Primer seq 5'-3' | Phase     | %GC | Tm |
|-----|-----------|--------------|----------------------|-----------|-----|----|
| 1   |            |              | F                    | Dominant-coupling | 43.0 | 56.6 | 62.3 |
| 2   |            |              | R                    | Dominant-reputation | 45.0 | 59.2 | 59.7 |
| 3   |            |              | R                    | Designed by primer 3+ (NCBI) | 55.0 | 60.0 | 59.7 |

Table 1: Details of rust resistance linked primers used in the study of oat genotypes.
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 Pc91 Pc68 linked primer (Table 3) it can be stated that none of the two major gene i.e. Pc91 and Pc68 gives the particular band with all three primers. The infection behaviour of this disease was varied widely amongst the genotypes showing moderate resistance reaction with rust reaction score 3 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 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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Table 3: Details of selected oat genotypes in the field and with SCAR markers.

| S.No | Genotype          | Biological yield/ plant (gm) | Rust scores* | Amplification with linked primer |
|------|-------------------|------------------------------|--------------|---------------------------------|
|      |                   | Normal Plot                  | Spreader Plot| Pc91                           | Pc68                           |
| 1    | 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