Research Article

Field Based Variability in Oat Crown Rust (*Puccinia coronata* f. sp. *Avenae*) Resistance in Exotic Oat Germplasm

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Abstract | Rust pathogens represent a major threat to cereal crops, though little is known about the status of oat crown rust in Pakistan. This study was designed to assess crown rust status and field based partial resistance of 16 exotic oat lines introduced from Europe along with local check, under natural field conditions at District Mansehra, Pakistan. Data on crown rust severity and host reaction was taken at three scoring dates to assess partial resistance using area under rust progress curve, infection rate, co-efficient of infection and final rust severity. A moderate crown rust pressure was observed during oat season 2015-16, with relatively late crown rust onset after mid of April. Based on the crown rust resistance response, the tested germplasm was clustered into four groups i.e., G1 contained eight, G2 contained three, G3 contained two and G4 contained three genotypes. Group 1 could be regarded as the most resistant genotypes group, and contained genotypes O-SA-1, O-SA-5, O-SA-15, O-SA-10, O-SA-14, O-SA-4, O-SA-6 and O-SA-11. Genotype O-SA-9, O-SA-7 and O-SA-12 had relatively high crown rust susceptibility. Further studies on across location and over-years crown rust resistance response of these genotypes must enable their exploitation in oat genetic improvement and crown rust control.

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Introduction

Oat (*Avena sativa*) is one of important member of family gramminea. The native land of oat is Asia and modern oat is originated from Asian wild oat which is mainly considered as a weed (Coffman, 1977). It is mainly used as fodder crop but in many countries it is also used as food crop, e.g., in Scotlant, Germany and Scandivian countries (Welch, 1995). The crop has a wide utility as medicinal plant, with reported protection against cancer and cardiac diseases through increasing body immunity and stabilizing blood glucose level (Welch, 1995; Menon et al., 2016). Along with medicinal usage of this crop, oat is also utilized in manufacturing whisky, coffee substitute, cosmetics, fibers paper, pillow filling etc. The oat crop waste is also used in lubricant oils refinery, in manufacturing herbicide, fungicide, and soil fumigants and also in production of nylon (Welch, 1995).

In Pakistan it is mostly used as fodder for animals. It is a favorite feed of all animals because its straw is soft as compared to other fodder crops. Nutritionally this fodder crop is very rich and capable of fulfilling nutritional requirement of animals such as it has high total digestible nutrients (TDN), digestible crude protein, fat, vitamin and minerals such as phosphorus and iron (Sterna et al., 2016). Similarly, oat grains are the most enjoyable feed for horses, dairy cows and poultry (Menon et al., 2016). Despite the importance of the crop as food and fodder, its production and yield in Pakistan is very limited.

The low production and yield is due to limited efforts made for genetic improvement of oat in Pakistan to cope with biotic and abiotic factors adversely affecting crop growth and development (Khalil, 2008). Among various biotic factors, oat diseases are the most important including rusts, powdery mildew, barley yellow dwarf virus (BYDV), scab and ergot. Among rusts, crown rust is an important disease, which frequently prevails in oat crop under humid and cold climate (Simon, 1985).

Crown rust is caused by the fungus *Puccinia coronata* f.sp. *avenae* and is present most parts of the world where oat is cultivated (Agrios, 2004). Spores of the disease appear as brown-orange rust on the leaf in clusters called pustules (Figure 1). These urediospores could be transported by wind over large geographical areas resulting in long distances dispersal of the pathogen. The disease is prevalent in mild weather within a temperature range of 10-25°C while at temperature of 30°C, growth of the pathogen is inhibited (Simon, 1985). The urediospores can reproduce asexually on oat crop completing few to several cycles (Zhao et al., 2016), producing a large number of spores and causing reduction in yield. The main source of primary infection is urediospore, while little is known about the role of alternate host in its epidemiology. This pathogen can attack almost all species of oat along with other grasses. The pathogen occurs on oat in telial and uredial phase, while the spermagonial and aecial phases occur on *Rhamnus* bushes, which is the alternate host of pathogen (Zhao et al., 2016).

Yield losses caused by crown rust may vary, depending on crop genotype and the prevalent climate. In severe cases the disease can cause yield losses of up to 50% in susceptible cultivars (Simon, 1985). It causes serious structural, physiological and biochemical changes in host plant in favor of the fungus resulting in adverse effect on the crop. It mainly attacks leaf area and after inoculation gaseous exchange within leaf area is disturbed, but symptoms of diseases take about five days to appear. The photosynthesis is reduced only in infected leaf area in the start, which expands to the whole leaf in about eight days during sporulation, while fully inhibiting photosynthesis in whole leaf surface and even in areas not attacked by pathogen becomes very low (Scholes and Roelfs, 1996). Thus, a high oat yield could not be achieved without proper disease management.

Figure 1: *Typical disease symptoms of oat crown rust (caused by Puccinia coronata f. sp. avenae) on oat plant. The upper left corner shows the zoom-in represents the symptom of a heavily infected leaf.*

Various measures could be adopted for crown rust management, including the use of fungicide and genetic resistance (Carson, 2011). However, the genetic resistance has been the most economical and environment friendly way to control the disease, particularly in developing countries (Ali et al., 2007; Ali et al., 2009c). To exploit the potential of genetic resistance, improved varieties must be developed expressing resistance against crown rust. Crown rust could be controlled through chemical methods but it is very expensive, environment unfriendly and sometime not available (Ali et al., 2007). Control through the use of genetically resistance varieties against the pathogen population has been proven to be the most economical and environment friendly measure (Singh et al., 2016). Introduction of resistance gene to oat is successful method to fight against crown rust disease. The frequency and extent of epidemics has been reduced due to genetic improvement efforts, ensuring high oat yields (Fetch et al., 2005).

Long term benefits from genetic resistance could only be achieved if these are deployed in a diversified way to combat the pathogen ability to evolve new races, virulent to the prevailing resistant genes (McDonald and Linde, 2002). This will avoid the loss of effectiveness of resistance due to its large scale deployment imposing a strong selective pressure against the avirulent types and in favor of the virulent type (McDonald and Linde, 2002; de Vallavieille-Pope et al., 2012). This acquisition of virulence by the pathogen population is dependent at least partly, on the deployment of resistance genes (McDonald and Linde, 2002). Thus, the resistant varieties must be deployed while considering the genetic basis of its resistance, attempting to adopt a durable deployment of resistance genes.

Despite the reduction in oat yield due to crown rust, little is known about the disease status of crown rust in various genotypes grown in Pakistan, while no effort is made to exploit resistance sources in exotic sources of oat germplasm. This necessitates identifying, characterizing and deploying resistance in oat germplasm. The present research work was thus designed to assess the field based resistance reaction and rust severity in oat germplasm introduced from Europe along with local check. The specific objectives of this study were (i): to evaluate the prevalence of oat crown rust in exotic oat germplasm and (ii): to assess the field resistance response of this exotic oat germplasm.

Materials and Methods

Germplasm and location selection

The present study was designed to assess the crown rust status in 16 exotic oat lines introduced from Europe through Global Rust Reference Centre, Aarhus University, Denmark. A local oat line, provided by the Animal Farm of the University of Agriculture, Peshawar, Pakistan was also included as a reference check. The selected lines sown in the farmer field at Labarkoat, Mansehra, Pakistan, which lies in the Himalayan region with cold climate favorable for rust diseases (Ali et al., 2015).

Field layout, sowing and crop management

The field layout was designed using a randomized complete block design with two replications. Each replication included 17 oat lines. Each entry was sown in two rows with each row of 1 m length and with 0.3 m row-to-row distance and 0.6 m plot-to-plot distance. The field sowing was carried out on 24th November 2015, after proper preparation of the soil. The field was maintained as per recommended crop husbandry practices. It should be noticed that an unexpected snowfall occurred on 10th February which covered the field for at least one day.

Disease scoring and analysis

Considering the climatic conditions of Mansehra, natural infestation was relied upon for crown rust infection. Inspection of the field for crown rust was started in the first week of April and disease scoring was started when rust appeared. Disease scoring was carried out three times in the season to enable estimation of partial resistance parameters, as explained previously (Ali et al., 2009a). Disease scoring included an overall severity, considering percent of leaf infected and incidence in the field, and host reaction. The host reaction was categorized as: **I:** Immune; when no visible infection could be observed; **R:** Resistant; when necrosis could be noticed without sporulation; **MR:** Moderately Resistant; when small pustules could be seen with surrounded necrotic area; **MS:** Moderately Susceptible; when medium-sized pustules could be seen without necrosis, but with some chloroses; **M:** Moderately Resistant-Moderately Susceptible; it is a combination of both MR and MS; **S:** Susceptible; when large pustules are observed without any necrosis or chlorosis.

Assessment for partial resistance

Temporal disease severity and host reaction data was further analyzed to assess the partial resistance in oat germplasm by estimating area under rust progress curve (AURPC), infection rate (IR), Co-efficient of infection (CI) and final rust severity (FRS) as explained previously (Ali et al., 2009a).

Statistical and multivariate analysis

The data generated on crown rust parameters were subjected to statistical analysis, including analyses of variance (ANOVA) appropriate for RCBD using R-software, multivariate analyses using MULTIBASE add-in for Excel. Cluster analyses were made using the Ward method of hierarchal clustering (Ward, 1963) to identify overall grouping of genotypes and infer on their partial resistance as described earlier (Ali et al., 2009a), based on disease severity over three scoring dates, CI over three scoring dates and AURPC.

Results and Discussion

Field based assessment of 16 exotic oat germplasm introduced from Europe along with one local oat line, revealed a moderate crown rust pressure under the field conditions. Variations were observed for different genotypes tested with many exposing good level of resistance, including the local check. Highly significant differences (<0.01) were obtained among the genotypes and over time for oat severity response in the tested germplasm. These results are detailed below:

Overall rust prevalence and progression over time

The crown rust pressure observed could be considered as a moderate one (Figure 2). The overall disease status increased overtime from the maximum of 20% at the first scoring date to the maximum of 40% at the last scoring date. This progression when analyzed over individual genotypes, revealed a variable trend for oat rust severity progression over time (Figure 3). Most of the genotypes depicted an increasing trend in crown rust severity, except the local check with a stable low severity (3%) and two exotic lines with a stable moderate severity (O-SA-3 = 15% and O-SA-16 = 20%). None of the line had a decreasing crown rust severity from the previous scoring date and nine genotypes (O-SA-4, O-SA-5, O-SA-7, O-SA-8, O-SA-10, O-SA-12, O-SA-13, O-SA-14 and O-SA-15) had a stable severity after the second score.

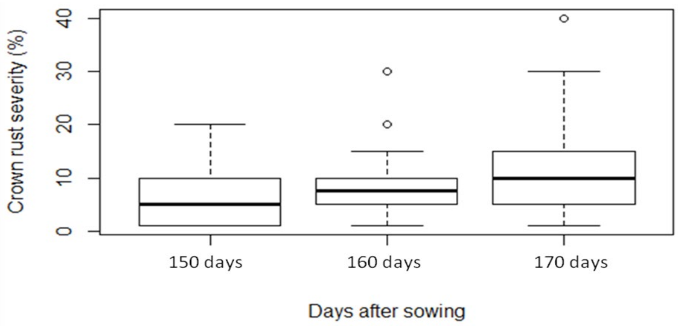

Figure 2: *Boxplots showing the minimum, maximum and average of crown rust severity as exhibited by exotic oat germplasm during oat season 2015-16 at District Mansehra, Pakistan.*

Slow crown rusting parameters

Variability was observed for slow crown rusting behavior of the tested lines was assessed using final rust severity, final co-efficient of rust infection and area under disease progress curve. The maximum value for final rust severity was recorded for O-SA-9 (28%) while the minimum values of 3% was recorded for the genotypes O-SA-8 and O-SA-13 which was similar to local check (Table 1). Seven genotypes (O-SA-2, O-SA-1, O-SA-4, O-SA-5, O-SA-10, O-SA-14 and O-SA-15) exhibited final disease severity of between 5 to 10%, while six genotypes (O-SA-7, O-SA-3, O-SA-6, O-SA-11, O-SA-12 and O-SA-16) depicted relatively higher disease severity from 11 to 20%.

Final coefficient of infection (FCI) ranged from the minimum value of 1 observed for O-SA-2, O-SA-8 and the local check to the maximum value of 19 recorded for O-SA-9 followed by O-SA-7 (15). Three genotypes O-SA-3, O-SA-6 and O-SA-12 showed a final CI value of 5-10, while the rest had an FCI value below 5 (Table 1).

Table 1: *Final crown rust severity, final co-efficient of infection and relative area under rust progress curve (r-AURPC) for 16 exotic oat germplasm and one local line, as observed at district Mansehra during oat season 2015-16.*

Genotype	Final rust severity	Final co-efficient of infection	r-AURPC	Clustering group
O-SA-1	6	4	140	G1
O-SA-2	5	1	123	G2
O-SA-3	15	5	1923	G3
O-SA-4	6	4	338	G1
O-SA-5	6	3	244	G1
O-SA-6	15	6	674	G1
O-SA-7	11	15	6158	G4
O-SA-8	3	1	100	G2
O-SA-9	28	19	4103	G4
O-SA-10	8	4	337	G1
O-SA-11	15	4	855	G1
O-SA-12	20	9	2804	G4
O-SA-13	3	2	1581	G2
O-SA-14	8	4	283	G1
O-SA-15	8	3	283	G1
O-SA-16	20	3	1542	G3
Local line	3	1	100	G2

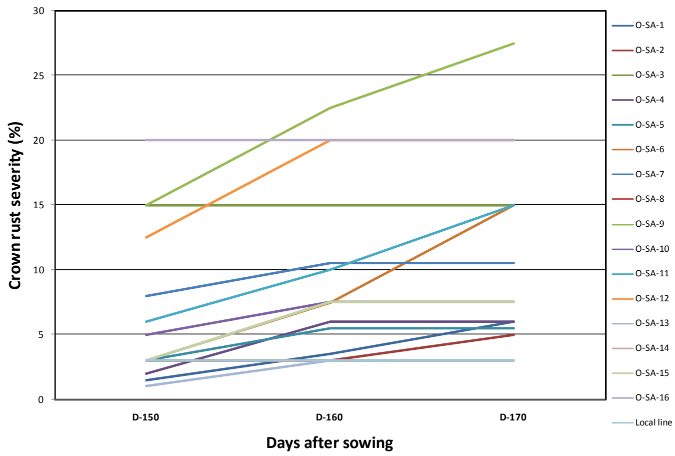

Figure 3: *Crown rust (Puccinia coronata f.sp. avenae) progression over time in exotic oat (Avena sativa) germplasm at District Mansehra during oat season 2015-16.*

Data on area under rust progress curve (AURPC) was converted to relative (r-) AURC through comparison with AURC value of local control. The maximum value of rAURPC was recorded for the genotype O-SA-7 (6158) followed by O-SA-9 (4103) and O-SA-12 (2804), while the minimum value of rAURPC was obtained for O-SA-8 (100) which is similar to local check, followed by O-SA-2 (123) and O-SA-1 (140). All other genotypes exhibited rAURPC values of 244-1923 (Table 1).

Based on the above three parameters, most of the introduced genotypes as well as the local check had a high crown rust resistance, while the genotype O-SA-9, O-SA-7 and O-SA-12 had relatively low crown rust resistance, though still in partial resistance category.

Overall clustering of oat germplasm based on crown rust resistance

The cluster analyses based on disease severity over three scoring dates, CI over three scoring dates and AURPC grouped the 16 exotic oat genotypes and the local control into four major groups (Figure 4 and Table 1). Group 1 contained eight genotypes (O-SA-1, O-SA-5, O-SA-15, O-SA-10, O-SA-14, O-SA-4, O-SA-6 and O-SA-11), which could be considered as the lines showing high level of partial resistance. Group 2 contained three exotic genotypes (O-SA-2, O-SA-8 and O-SA-13) along with the local check, and could be considered as very highly resistant/immune lines. Group 3 was comprised of two genotypes (O-SA-3 and O-SA-16), and were considered as exhibiting moderate level of partial resistance. Group 4 contained the three low partially resistant genotypes (O-SA-7, O-SA-9 and O-SA-12).

Figure 4: *Cluster analyses of exotic oat germplasm based on parameters revealing their response to crown rust infection, observed during 2015-16 in District Mansehra.*

Our study is one of the few attempts to assess the crown rust status in exotic germplasm in Pakistan. A moderate level of crown rust pressure was evident along with the diversity in crown rust resistance in exotic germplasm. Information generated in the current study has implication for both oat genetic improvement and crown rust disease management.

Crown rust pressure and its application for rust management

A moderate crown rust pressure was observed in District Mansehra, which lies in the Himalayan region of Pakistan, recently reported to harbor various rust species on the alternate host *Berberis* spp., including crown rust (Ali et al., 2015). The disease increased over time reaching up to 40% at the last scoring date. The climatic condition of Mansehra lies in temperate ad sub-humid zone, with occasional snowfall in winter season and extended cold during spring (Khalil and Jan, 2002). This provides a favorable condition for the growth of *P. coronata* f.sp. *avenae*, which requires a moderate to low climate condition and high humidity (Carson, 2011). The disease was started after mid April, relatively latter than wheat rusts in the region, and increased slowly till mid of May with no further increase, despite the green oat crop. This would be due to increasing temperature after mid of May. Indeed, high temperature could reduce crown rust pressure through elicitation of plant resistance and retarding of pathogen growth (Wahl et al., 1984). Alternatively, the moderate rust severity observed could be resulted from the host resistance, as no known susceptible check was included in the study, mainly due to lack of any study on oat crown rust in Pakistan. Information on the rust pressure and rust progress over time must be useful for crown rust control in district Mansehra and areas with similar climatic conditions.

Diversity in crown rust resistance among oat genotypes and its application

The field based assessment of resistance in oat germplasm against crown rust revealed substantial level of diversity in the introduced germplasm. Our results revealed that most of the introduced genotypes as well as the local check had a high crown rust resistance, while the genotype O-SA-9, O-SA-7 and O-SA-12 had relatively low crown rust resistance, though still in partial resistance category (Pathan and Park, 2007; Ali et al., 2009b). The overall parameters result was jointly reflected into cluster analyses, based on which the tested exotic germplasm and the local control were grouped into four groups, representing different level of immune and partial resistance (Ali et al., 2009a). Group 1 contained eight genotypes showing high level of partial resistance. Group 2 contained three exotic genotypes along with the local check representing the very highly resistant/immune lines. Group 3 was comprised of two genotypes and it could be considered as exhibiting moderate level of partial resistance. Group 4 contained the three low partially resistant genotypes. The resistance level, however, needs to be tested across locations and under greenhouse conditions (Ali et al., 2009a; Ali et al., 2009c), especially in the current case, where the overall natural crown rust pressure was not very high. Field based variation has been reported in oat germplasm in previous studies (Ruwali et al., 2013). Crossing within and among the groups could be made to further diversify the germplasm, which is the pre-requisite of any crop improvement programme (Mondal et al., 2016). The observed diversity in the resistance could also be exploited for diversification of resistance genes in field conditions through cultivar mixture and multiline approach (Mundt and Browning, 1985; Garrette and Mundt, 1999). The partially resistance lines could also be exploited to attain a durable resistance strategy (Pathan and Park, 2007; Ali et al., 2009b).

The resistance lines need to be assessed for their yield potential in future and could be included in oat breeding programme for development of improved resistant oat varieties, as carried out in other crops like wheat against wheat rusts (Swati and Ali, 2015; Singh et al., 2016). Diversity in resistance level could also be applied in rust control strategy based on varietal mixture, where a more durable disease control could be achieved with maintaining variation in the host population (Garrette and Mundt, 1999; McDonald and Linde, 2002). Varietal mixture has been suggested to reduce crown rust severity, especially when the disease initiation is focal i.e., starting from small no. of plants in the field (Mundt and Browning, 1985).

Conclusions and Recommendations

The present study is among the few to assess the crown rust status in exotic oat germplasm in Pakistan, which suggested the overall moderate crown rust pressure during the oat cropping season 2015-16. The crown rust appeared relatively late in the season and could be managed through early sowing if further confirmed through multilocation and over-year trails. The local check had a high level of crown rust resistance and could be recommended for cultivation, along with consideration of other traits. Based on the crown rust resistance response, the tested germplasm was grouped into four groups, out of which G1 contained eight, G2 contained three, G3 contained two and G4 contained three genotypes. Crossing within and among the groups could be made to further diversify the germplasm. The information on rust resistance levels of the germplasm and rust pressure during the season must be exploited in oat genetic improvement and crown rust disease management.

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Author's Contribution

Manzoor Hussain, Farhatullah and Sajid Ali designed the study; Muhammad Awais, Saiqa Bibi and Afifa Khalid conducted field experimentation; Muhammad Awais, Muhammad Rameez Khan and Sajid Ali conducted analyses of the data; Manzoor Hussain, Farhatullah and Sajid Ali provided final interpretation of the data; Muhammad Awais, Saiqa Bibi, Afifa Khalid and Sajid Ali wrote the manuscript; Manzoor Hussain, Farhatullah and Sajid Ali provided resources for the study.

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