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Characterization of wheat germplasm for stripe rust (*Puccini striiformis* f. sp. *tritici*) resistance

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Abstract

Stripe rust or yellow rust caused by *Puccinia striiformis* f. sp. *tritici* is an important disease of wheat causing considerable yield losses in wheat growing areas worldwide. The pathogen is one of the very important yield limiting factors in Pakistan. Present study was carried out to identify wheat genetic resources for stripe rust resistance to enhance cultivar improvement efforts. Wheat germplasm consisting of 20 Chinese cultivars, 95 synthetic hexaploids and 85 advanced breeding lines were evaluated under field conditions at two hot spot locations (Pirsabak and Islamabad) in Pakistan during 2007-08 and 2008-09 wheat growing seasons. Same germplasm were also evaluated at seedling stage under controlled greenhouse conditions. Seedling testing revealed that synthetic hexaploids have seedling resistance with likely presence of stripe rust resistance genes; *Yr3, Yr5, Yr10, Yr15, YrSP* and *YrCV*. Advanced lines and Chinese cultivars showed adult plant resistance under field conditions wherein most of the genotypes were susceptible at seedling stage. Both types of seedling and adult plant resistance identified in wheat germplasm offer promising genetic stocks for accumulating both resistances to acquire durable resistance and long lasting control against stripe rust pathogen in Pakistan.

Key words: Chinese cultivars, yellow rust, seedling resistance, adult plant resistance, durable resistance.
Abbreviations: advanced line (AL), Crop disease research programme (CDRP), international wheat and maize improvement center (CIMMYT), infection type (IT), khyber pakhtunkhwa (KPK), *puccinia striiformis* f. s. *tritici* (PST), quantitative trait loci (QTL), relative area under disease progress curve (rAUDPC), synthetic hexaploids (SH).

Introduction

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici*, is an important foliar disease of wheat. It has been reported to be prevailing at higher altitudes and cool and temperate regions where wheat is grown (Johnson, 1992; McIntosh et al., 1995; Boyd, 2005). All continents except Antarctica host this disease of wheat. Its wider prevalence therefore has been a global threat to wheat production inflicting about 30 to 100% grain losses. The safeguard measures against the destructive pathogen are therefore paramount in the context of food security (Chakravarty, 2011). Having disseminating ability, wheat rusts are scattered all around and travelling the continents (Kolmer, 2005). Their plasticity and adaptability to changing climatic conditions made them fit in most of the places around the world. Such characteristics include mutation, migration, somatic and sexual hybridization of wheat rusts (Stubbs, 1985; Johnson, 1992; Kolmer, 2005; Jin et al., 2010). Although all three diseases; leaf rust, stem rust and stripe rust are economically important cereal diseases with varying losses especially in terms of grain damage. Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* is widely distributed and dangerous (Chen, 2005). It affects wheat crop through damaging its respiratory system, kills foliar parts, makes growth of plant stunted, most importantly reduces grain yield by shriveling grain, reducing weight and affecting its quality (Line, 2002; Chen, 2005). Grain losses caused by this devastating pathogen have been reported from 10-70 percent. In severe disease epidemics, the grain damage scales up to 100 percent (Chen, 2005). To combat with stripe rust, various strategies are being applied worldwide. Use of chemicals and agronomic practices has proved to be fruitful to reduce the losses to some extent. Although chemical method is in vogue all around the world, this remedial measure is not affordable by the farmers in developing countries. Most feasible method otherwise is host genetic resistance to control stripe rust. Utilization of genetic resistance is economical, and carries no health and environmental hazards (Chen, 2007; Farrokhi et al., 2011). Furthermore, resistant varieties fight with the disease for a longer time ensuring crop sustainability. Two types of genetic resistance, race specific and race non-specific are well-recognized. Former type of resistance works according to gene for gene model first proposed by Flor et al. (1942). After the evolution of new stripe rust strains, race specific genes become ineffective approximately within three to five years (Line and Qayoum, 1992). Race non-specific resistance is controlled by minor genes and is long lasting.

Judicious use of genetic resistance has been proposed as gene pyramiding of major (race specific) and minor (race non-specific) genes. Resulting varieties equipped with conglomeration of both type of resistances will sustain longer against the pathogen (Singh et al., 2004). Therefore to control the pathogen adequately, wheat genetic resources with diverse resistances are needed (Bux et al., 2011). In this regard, characterization of wheat germplasm for identification of such diverse resistances is paramount. Present study was carried out to identify wheat genetic resources with different types of resistance to enhance cultivar improvement efforts in Pakistan.

Results

Chinese Cultivars

At seedling stage, 4 (20%) Chinese cultivars were resistant (IT 0-3) and16 (80%) were susceptible (IT 7-9) (Figure 1). None of the cultivar demonstrated intermediate type of resistance. Field data revealed that during 2007-08, at Islamabad location, 16 (79%) Chinese cultivars were resistant (RAUDPC 0-10), while 1(5%) was intermediate (RAUDPC 11-30), and 3(16%) were susceptible (RAUDPC >30). At Pirsabak, 13(58%) were resistant (RAUDPC 0-10), 2(16%) intermediate (RAUDPC 11-30), and 5 (26%) were susceptible (RAUDPC >30). During 2008-09, 11(53%) cultivars were resistant (RAUDPC 0-10), 6(32%) were intermediate (RAUDPC 11-30), and 3 (15%) were susceptible (RAUDPC >30) at Islamabad. While at Pirsabak, 12(58%) cultivars were resistant (RAUDPC 0-10), 4(21%) were intermediate (RAUDPC 11-30), and 4(21%) were susceptible (RAUDPC >30) (Figure 2).

Advanced lines

At seedling stage, 4 (5%) advanced lines were resistant (IT 0-3), 5 (6%) were intermediate (IT 4-6) and 74 (89%) were susceptible (IT 7-9) (Figure 1). Disease data at adult stage revealed that during 2007-08, at Islamabad, 61(71%) lines were resistant (RAUDPC 0-10), 21(25%) were intermediate (RAUDPC 11-30) and 8 (4%) were susceptible (RAUDPC >30). While at Pirsabak, 46 (54%) Advanced lines were resistant (RAUDPC 0-10), 32 (37%) were intermediate (RAUDPC 11-30) and 11(9%) were susceptible (RAUDPC >30). During 2008-09, at Islamabad, 60 (70%) lines were resistant (RAUDPC 0-10), 22 (26%) were intermediate (RAUDPC 11-30) and 7 (4%) were susceptible (RAUDPC >30). While at Pirsabak, 41(48%) were resistant (RAUDPC 0-10), 37(43%) were intermediate (RAUDPC 11-30), and 11(9%) were susceptible (RAUDPC >30)(Figure 2).

Synthetic hexaploids

Based on seedling data, 46 (63%) synthetic hexaploids were resistant (IT 0-3), 12 (16%) were intermediate (IT 4-6) and 15 (21%) were susceptible (IT 7-9) at seedling stage (Figure 1). Adult testing under field conditions revealed that during 2007-08, at Islamabad, 75 (80%) synthetics were resistant (RAUDPC 0-10), 6 (6%) were intermediate (RAUDPC 11-30) and 14 (14%) were susceptible (RAUDPC >30). While at Pirsabak, 69 (72%) were resistant (RAUDPC 0-10), 14 (13%) were intermediate (RAUDPC 11-30) and 12 (15%) were susceptible (RAUDPC >30). During 2008-09, at Islamabad, 68 (78%) were resistant (RAUDPC 0-10), 12 (7%) were intermediate (RAUDPC 11-30) and 15 (15%) were susceptible (RAUDPC >30). While at Pirsabak, 69 (72%) were resistant (RAUDPC 0-10), 13 (13%) were intermediate (RAUDPC 11-30) and 13 (15%) were susceptible (RAUDPC >30) (Figure 2).

Discussion

Genetic base of Pakistan wheat cultivars has been reported as narrow for major biotic stresses. Most of the national wheat varieties in Pakistan has been established around few seedling resistance genes against stripe rust, therefore are vulnerable to its new pathotypes emerging from time to time (Bux et al., 2011). Such an alarming scenario demands widening of narrow genetic composition of local wheat cultivars through incorporating genetically diversified germplasm in breeding programmes. We are reporting in the present study, stripe rust resistance both at seedling and adult stage of wheat germplasm having diverse genetic background. In order to identify seedling resistance sources, germplasm was screened at the seedling stage using bulk of urediniospores under controlled greenhouse conditions. Seedling testing revealed that, 20%(4), 5%(4) and 63%(46) genotypes were resistant from Chinese cultivars, advanced lines and synthetic hexaploids, respectively. Remaining genotypes from three germplasm categories were susceptible showing lack of seedling resistance. Based on virulence/avirulence profile of the pathogen, seedling resistant genotypes may possibly have genes *Yr3, Yr5, Yr10, Yr15, YrSP* and *YrCV*. These seedling resistance genes have been reported to carry resistance under field conditions in different wheat growing regions in Pakistan (Ali et al., 2007; Rizwan et al., 2010). However, validation of these genes in studied germplasm still needed through molecular diagnostic markers, multipathotype testing and genetic studies. For testing the credibility of field resistance, same set of germplasm was screened at two locations, Pirsabak and Islamabad. Pirsabak (KPK) is well known as a hot spot for stripe rust (Bux et al., 2011). Heavy stripe rust epidemics have been reported at this station making it a suitable site for wheat germplasm screening. At adult stage, disease data was used to calculate RAUDPC of each genotype comparing with the severity of susceptible check Morocco. This measure denotes the progress of the disease at adult stage in given time and gives a clue of level of resistance against the disease in host germplasm. Therefore, this measure has been widely used to identify slow rusting or durable resistance in test germplasm. In similar studies conducted previously, RAUDPC have been used by various wheat pathologists for stripe rust data analysis (Ma et al., 1995; Rizwan et al., 2007; Shah et al., 2010). Field observations revealed that at the adult stage, 53% Chinese cultivars were found resistant showing RAUDPC 0-10 at both locations. While 5% Chinese cultivars showed intermediate type of resistance with RAUDPC 11-30. These Cultivars showing resistance to moderate resistance under field conditions were largely susceptible at seedling stage indicating presence of adult plant resistance. Present study has identified Chinese cultivars as a potential source of adult plant resistance against stripe rust. These cultivars developed originally in China, a neighboring country of Pakistan, may possibly be useful genetic resource for both, China and Pakistan. Because both countries have similar virulence pattern of stripe rust. In China, genes*; Yr5*, *Yr10*, *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr15*, *Yr24*, *Yr26*, *YrZH84* and some other genes have been documented as effective against local stripe rust fungus (Wan et al., 2007; Zhang et al., 2010). Furthermore, these lines found resistant can be introduced in Pakistani local conditions as resistant cultivars after corroborating their agronomic potential

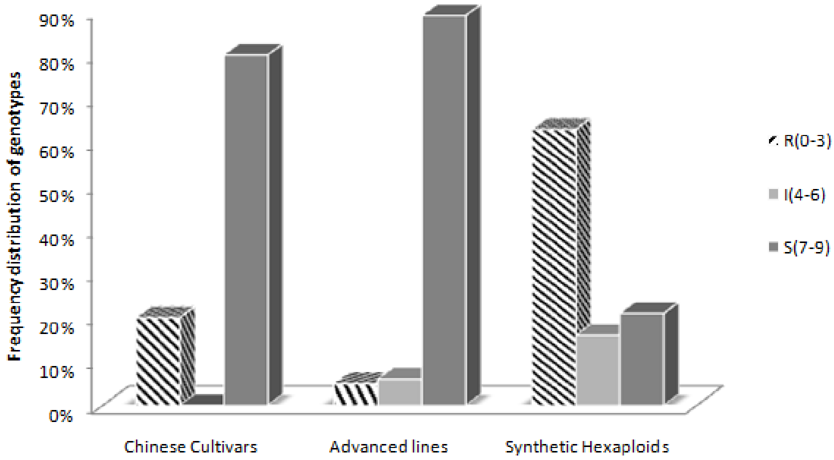

Fig 1. Frequency of wheat germplasm resistant, intermediate and susceptible at seedling stage against stripe rust.

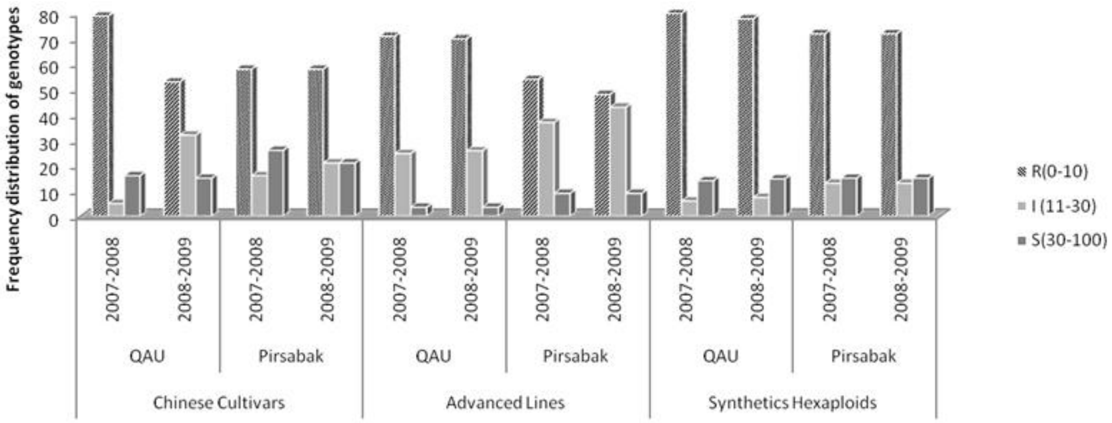

Fig 2. Frequency of wheat germplasm resistant, intermediate and susceptible at adult stage under field conditions against stripe rust.

particularly yield and other important traits through conventional trials. For genetic improvement of elite Pakistan wheat cultivars, their hybridization with diverse exotic germplasm sources has been carried out. To test their resistance status for stripe rust, the resulting advanced lines were tested under field conditions. Field disease data showed that at the adult stage, 53% advanced lines were found resistant showing RAUDPC 0-10, while 25% lines displayed intermediate type of resistance with RAUDPC between 11 to 30 at both locations. Frequency distribution of advanced lines was highest showing adult plant resistance under field conditions and susceptibility in seedling stage. Based on disease response it can be concluded that advanced lines have durable resistance against stripe rust. Besides introducing exotic wheat germplasm as cultivars, these advanced lines which are genetically improved elite cultivars, can better adapt in our local conditions as successful varieties. In a similar study, Afzal et al. (2009) reported field based durable resistance against stripe rust among 188 wheat breeding lines in Pakistan. Genetic erosion of wheat demands diverse genetic resources to broaden the genetic base and attain diversification that ensures high through put resistance against biotic stresses including wheat rusts (El Bouhssini et al., 2011). To achieve this, wheat progenitors; *Aegilops tauschii* and *Triticum turgidum* were crossed and resulting hexaploid has been reported to carry novel allelic diversity for wheat improvement in terms of agronomic traits as well as biotic/abiotic resistance (Schachtman et al., 1992; Reynolds et al., 1999; Pritchard et al., 2001; Villareal et al., 2001). For identification of such novel genetic resources for future utilization, synthetic hexaploids were tested at adult stage under heavy disease pressure of stripe rust besides seedling test. Results revealed that at the adult stage majority of synthetic wheat were resistant showing RAUDPC 0-10 or showed intermediate type of resistance with RAUDPC 11-30 at both test locations. High frequency distribution of synthetic hexaploids showing resistance at seedling and adult stages shows presence of seedling resistance (or All-stage resistance) genes. Apart from possible resistant genes such as *Yr3*, *Yr5*, *Yr10*, *Yr15*, *YrSP* and *YrCV*. These synthetics, being derivatives of wild progenitor; *Ae. tauscii*, may have novel genes for stripe rust resistance. Previous studies have reported stripe rust resistance gene *Yr28* that has been transferred from *Aegilops tauschii*, one of the parents of synthetic hexaploids (Singh et al., 2000). Globally, pathologists recognize that life-span of major genes for rust resistance is short-lived. To sustain genetic resistance, minor genes or QTLs are preferred over major genes. Although identification and utilization of durable sources of resistance is cumbersome, wheat germplasm with durable resistance are well recognized and documented (Singh et al., 2004). Alternative is accumulation of major and minor resistance sources in single background via established protocols of conventional breeding cum marker-assisted selection. Using such established methodologies, seedling resistance sources with possible major genes such as *Yr3, Yr5, Yr10, Yr15* and *YrSP* and adult plant resistance in reported wheat germplasm can be utilized for gene pyramiding to attain long lasting resistance against devastating pathogen-stripe rust in Pakistan.

Conclusion

In the present study, seedling resistance genes; *Yr3, Yr5, Yr10, Yr15* and *YrSP* have been postulated in synthetic hexaploids. Majority of Chinese cultivars and advanced lines lacked seedling resistance. Field based stripe rust evaluation showed adult plant resistance (durable resistance) in Chinese cultivars and advanced lines. This germplasm with diverse sources of resistance are useful in accumulation of both types of resistances through gene pyramiding for durable resistance against stripe rust to develop resistant and high yielding wheat cultivars in Pakistan.

Materials and methods

Plant materials

The set of synthetic hexaploids (total 95) was obtained from International Wheat and Maize Improvement Centre (CIMMYT) Mexico. Advance lines (total 85) and Chinese varieties (total 20) were received from the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

Seedling test

Approximately 5-7 seeds of each genotype were planted in 7x7 cm pots under greenhouse conditions at Crop and Disease Research Programme (CDRP) Murree. Four to six week old seedlings were inoculated with suspension of urediospore suspended in mixture of 30:70 mineral oil and petroleum ether (Rizwan et al., 2010). *Puccinia striiformis* f. sp. *tritici* (PST) bulk of urediniospores had virulence for genes*Yr1, Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, Yr24, Yr26, Yr28, Yr29,* and *Yr31* and avirulent to *Yr3, Yr5, Yr10, Yr15, YrSP and YrC*. Inoculated plants were placed in open air for two hours to evaporate the oil. Plants were then transferred to a dew chamber set at 10^oC with 16h light and 8h dark photoperiod for 48 hrs. The plants were then transferred to the greenhouse set at 6- 10^oC temperature (Rizwan et al., 2010). Infection types were recorded three weeks after inoculation using a 0-9 scale (Line and Qayum, 1992) when susceptible the check "Morocco" was showing maximum infection. Plants having infection types 0-3 were considered as resistant, those having infection types 4-6 as intermediate resistance, while those having infection types 7-9 were rated as highly susceptible (Rizwan et al., 2010).

Field testing

Same set of 200 wheat genotypes (95 synthetic hexaploids, 85 advanced breeding lines, 20 Chinese cultivars) was evaluated for adult plant resistance. The wheat germplasm was planted at two locations; Quaid-i-Azam University Islamabad and Cereal Crops Research Institute (CCRI), Pirsabak, Nowshara. Approximately 15 to 20 seeds of each genotype were sown in single- row plots of 1m length with 30 cm row spacing at each location. One row of the susceptible variety Morocco was also planted at every 20^{th} entry and along the border as disease spreader rows. Disease infection types at adult stage were recorded according to 0-9 scale as described by Line et al. (1974) thrice at 10-day intervals. The disease severity was recorded based on percentage of leaf area infected on plants (Peterson et al., 1948). The first disease notes were taken when the susceptible check (Morocco) had reached 60-80% severity. The disease severity data were used to calculate the area under the disease progress curve (AUDPC) using AUDPC computer programme developed at CIMMYT. The relative percentage of area under the disease progress curve for each entry was calculated by setting AUDPC of Morocco as 100 percent (Ma et al., 1995).To determine the different categories of resistance, the germplasm having RAUDPC value ranging from 0-10 was categorized as resistant; 11-30 as intermediate and above 30 were considered as highly susceptible.

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