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Genome wide association mapping of stripe rust resistance in Afghan wheat landraces

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ABSTRACT

Mining of new genetic resources is of paramount importance to combat the alarming spread of stripe rust disease and breakdown of major resistance genes in wheat. We conducted a genome wide association study on 352 un-utilized Afghan wheat landraces against stripe rust resistance in eight locations. High level of disease variation was observed among locations and a core-set of germplasm showed consistence performance. Linkage disequilibrium (LD) decayed rapidly ($R^2 \approx 0.16$ at 0 cM) due to germplasm peerless diversity. The mixed linear model resulted in ten marker-trait associations (MTAs) across all environments representing five QTL. The extensively short LD blocks required us to repeat the analysis with less diverse subset of 220 landraces in which R^2 decayed below 0.2 at 0.3 cM. The subset GWAS resulted in 36 MTAs clustered in nine QTL. The subset analysis validated three QTL previously detected in the full list analysis. Overall, the study revealed that stripe rust epidemics in the geographical origin of this germplasm through time have permitted for selecting novel resistance loci.

1. Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most destructive fungal diseases that can limit wheat production worldwide. Grain yield losses can reach 40% and up to 100% under severe infections [1]. Moreover, the recent breakdown of the race-specific resistance gene *Yr27*, a gene present in several commercial varieties in Asia and Africa, caused yield losses between 10% and 80% in many countries of Central West Asia and North Africa (CWANA) region [2]. Managing stripe rust epidemics is difficult and using resistant cultivars carrying both major and minor resistance genes is the best way to control stripe rust [3]. The continuous evolution of pathogens creates a need to develop new resistance cultivars through time. Two type of wheat resistance genes have been found and deployed in different breeding programs which are 1) race-specific and 2) race-nonspecific genes [4,5]. Race specific genes usually shows major resistance against *Pst* while race nonspecific genes are minor genes that are usually expressed in the adult stage and supposed to be affective against all *Pst* races, theoretically. Due to the rapid evolution of the rust pathogen population, different breeding programs are now focusing on pyramiding both race-specific and race nonspecific resistance genes to improve the resistance durability [6,7]. So far, about 67 designed and 42 temporarily named stripe rust resistance

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genes have been reported [8]. Unfortunately, many of those utilized resistance genes in different breeding programs are no longer effective against different new *Pst* races [9], which has alarmed the need for new sources of resistance deployment including landraces.

Developing new stripe rust resistant wheat varieties requires continuous exploration of new genetic resources. Afghan landraces, untapped genetic resources collected and preserved by Dr. Hitoshi Kihara, are among the germplasms never characterized so far to know their potential for contributing stripe rust resistance. The place of origin and the previous study on genotypic characterization offer the possibility to mine new resistance sources for stripe rust [10,11]. Also the recent report showed the Himalayan and surrounding region as centre of origin of *Pst* virulence races [9]. Zeven [12] well documented the co-evolution of landraces along with stress and their importance to choose as a breeding and/or pre-breeding material for crop improvement. For each site and for each year their composition becomes adapted to the conditions of that site and that year. These adaptations have taken place by changing the frequencies of phenotypes and hence genotypes for self-fertilizing by absorbing new genotypes either introduced from elsewhere or else which have originated by mutation or by low degree of interplant hybridization. It is necessary to consider these special qualities of landraces that have enabled man to obtain sufficient food to survive during some 10,000 years [13].

Genome-wide association studies (GWAS) utilize linkage disequilibrium (LD) in a set of genotypes to identify quantitative trait loci (QTL), thus it exploit historical recombination since the population diversion. Recent domestication of wheat from a narrow selection of wild relatives caused longer LD blocks with an advantage of the need for smaller number of markers to cover the full genome [14]. GWAS has been used successfully in mapping QTL for different traits in wheat. In specific to stripe rust, Zegeye [15], Maccaferri [16], Naruoka [17], Jighly [18] carried out GWAS using different germplasm but not exclusively landraces.

This study utilized 352 Afghan common wheat (*Triticum aestivum* L.) landraces in order to evaluate their adult-plant response to stripe rust infection under field conditions in multiple environments and identify genomic regions associated with stripe rust resistance in order to utilize them for breeding purposes.

2. Materials and methods

2.1. Plant materials

The study included 352 Afghan wheat landraces that well-germinated from the total collection of 446 preserved at the Kihara Institute for Biological Research (here after mentioned as Kihara Afghan Wheat Landraces – KAWLR) (Table S1 in the online version at DOI: http://dx.doi.org/10.1016/j.plantsci.2016.07.018). These landraces were collected from 17 provinces and 160 collection sites. The accessions were grouped as 65% spring, 14% facultative and 21% winter wheat. The harvested seeds from 2011 crop season of Japan were distributed for all evaluations.

2.2. Stripe rust adult-plant field evaluation

The KAWLR set was evaluated under natural disease epidemics in nine field trials performed at six locations from 2011 to 2014 (Table 1). In 2011, the material was planted in winter cycle at Toluca research station of CIMMYT in Mexico and fall sowing of Afghan along with susceptible checks (cvs. Morocco and Avocet). The spreader lines were planted at each location helped uniform disease spread. Natural heavy incidence occurred on both places and the susceptible checks produced large quantities of urediniospores to the spread the disease on the KAWLR set. The seed multiplied at each location was used to expand the evaluation at four locations in Afghanistan and at Toluca, Mexico during 2012. In Afghanistan, the disease severity varied among locations and time of sowing where the late sowing in Badambagh research station showed heavy incidence, however the disease incidence was low in Herat. In Mexico 2012 trial, materials were planted in Toluca under artificial inoculation resulting in excellent yellow rust development providing good data. The spreaders and hills were inoculated with a mixture of *Pst* isolates Mex96.11 and Mex08.13 approximately one month after sowing. In 2013, only one trial was conducted at Herat. In all field trials in Afghanistan, germplasm were evaluated in non-replicated single row plots whereas in Toluca two rows were sown. Field plots consisted of 0.7-m paired rows with approximately 60 plants of each line. A mixture of six susceptible wheat lines derived from an Avocet/Attila cross, Morocco and Avocet near-isoline for gene *Yr31* was used as the spreader in field trials. In order to get uniform and enough inoculum, the spreaders were planted around the experimental area and as hill plots in the middle of a 0.3-m pathway on one side of each experimental plot.

Stripe rust reactions were rated using the following two metrics: 1) The 0–4 scale for reaction type (DR), as described by Line and Qayoum [19], and 2) Disease severity (DS), rated as a percentage of leaf area in the infected row according to the modified Cobb Scale [20]. Data collection was initiated when stripe rust severity on the susceptible parent Avocet reached 80–90% in Toluca. The first note was recorded when the susceptible check Avocet displayed approximately 80% severity and repeated about a week later when it reached 90–100%. To calculate the coefficient of infection (CI), DS score were multiplied by 0.2, 0.4, 0.6, 0.8, or 1.0 which stand for IT of resistance (R, 0), moderately resistance (MR, 1), intermediate (M, 2), moderately susceptible (MS, 3) or susceptible (S, 4), respectively [59].

2.3. Genotyping-by-sequencing (GBS) genotyping

Genomic DNA was extracted from the leaves of individual accession and genotyped with GBS 1.0 V array containing both SNP and

Table 1
Details of Field screening of Stripe rust in Afghan Wheat Landraces. DS: disease severity, DR: disease reaction, CI: coefficient of infection, T: Toluca, K: Kabul, BB: Badambagh, E: early, L: late, H: Herat, SBES: Jalalabad station.

No.	Trial Name (Year)	Location	No. of accessions	Rust Scoring
1	T11 (2011–12)	Toluca Experiment Station, CIMMYT, Mexico	352	DR
2	K11 (2011–12)	Darulaman Research Station, Kabul, Afghanistan	352	DR, DS, CI
3	BB-E12 (2012–13)	Badambagh Research Station, Kabul, Afghanistan	321	DR, DS, CI
4	BB-L12 (2012–13)	Badambagh Research Station, Kabul, Afghanistan	321	DR, DS, CI
5	H12 (2012–13)	Urdu Khan Regional Agricultural Research Station, Herat, Afghanistan	313	DR, DS, CI
6	SBES12 (2012–13)	Agricultural Research Station, Jalalabad, Afghanistan	313	DR, DS, CI
7	T12 (2012–13)	Toluca Research Station, CIMMYT, Mexico	339	DS
8	H13 (2013–14)	Urdu Khan Regional Agricultural Research Station, Herat, Afghanistan	319	DR, DS, CI