

Original Research Article

**Association Mapping for Leaf and Stem Rust Resistance using
Worldwide Spring Wheat collection**

Running Head: Wheat Stem and Leaf rust Resistance Evaluation in Egypt

Keywords: Wheat, GWAS, leaf and stem rust, SNP markers

Abstract:

Wheat leaf rust (caused by *Puccinia triticina* Eriks.) and stem rust (caused by *P. graminis* f. sp. *tritici*) are among the most common, widespread and devastating diseases in Egypt and worldwide. A total of 2111 spring wheat accessions (882 landraces; 493 breeding lines; 419 cultivars and 317 with uncertain classification) were obtained from a single plant selection from wheat (*Triticum aestivum* L.) core collection. The wheat accessions were genotyped through the Triticae Coordinated Agriculture Project using the Illumina iSelect 9K wheat array at the USDA-ARS genotyping laboratory in Fargo, ND, USA. The primary objectives of this study were to: 1. Evaluate the spring wheat collection for stem and leaf rust resistance at adult plant stage under field conditions and detect new sources of resistance, and 2. Identify potential QTLs linked with stem or leaf rust resistance genes. Our results indicated that 6.96% (147 accessions) and 5.87 % (124 accessions) of the tested accessions were found to be resistant to leaf and stem rust, respectively. Genome-wide association mapping (GWAS) was used to identify QTLs associated with leaf and stem rust resistance genes. Overall GWAS results demonstrated that 46 SNP markers were significantly linked with stem rust resistance, while 36 SNP markers found to be significantly linked with leaf rust resistance. Most of the significant SNP markers were co-located with previously identified stem rust resistance genes (*SrIRS_{Amigo}*, *Sr21*, *Sr_{ND643}*, *Sr35*, *Sr52*, and *Sr22*) and leaf rust resistance genes (*trp-1*, *Lr 11*, *Lr 52* and *Lr 47*). Future work will focus on crossing several leaf and stem rust resistant accessions to pyramid genes and to develop nested association mapping populations to incorporate multiple resistance genes into elite breeding wheat lines.

29 **Keywords:** Wheat, GWAS, leaf and stem rust, SNP markers

30 **Introduction**

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32 Wheat leaf rust (caused by *Puccinia triticina* Eriks.) and stem rust (caused by *P. graminis*
 33 f. sp. *tritici*.) are among the most common, widespread and devastating diseases of wheat
 34 (*Triticum aestivum* L.) in Egypt and worldwide. Leaf rust occurs more frequently worldwide
 35 than stem rust. Thus leaf rust results in greater total annual losses than stem rust (Dholakia et al.,
 36 2013; Oelke and Kolmer, 2005). Leaf rust disease leads to yield losses more than 50% in some
 37 susceptible wheat genotypes (Aktar-Uz-Zaman et al., 2017). Stem rust can affect the entire wheat
 38 crop especially during the early growth stages leading to stunted plants or lodging and eventually
 39 death causing yield losses of up to 100 % (Kokhmetova et al., 2011). Host plant resistance is the
 40 most practical, economical and safe approach to control both stem and leaf rusts especially when
 41 farmers' access to fungicides is limited (Khatabi et al., 2013; Macdonald, 2004; Meentemeyer et
 42 al., 2011; Mundt, 2014; Rosewarne et al., 2013). Developing resistant genotypes has been a
 43 primary objective of several plant breeding programs worldwide since the 1900s (Ellis et al.,
 44 2014). Over the past century, traditional wheat breeding efforts resulted in lines with high levels
 45 of resistance to one or more races of leaf and stem rusts (Ellis et al., 2014). Over time, new
 46 virulent stem and leaf rusts races have evolved. Hence, the race-specific resistant lines did not
 47 remain resistant long, and the lines rapidly became susceptible to rusts after few years (Kertho et
 48 al., 2015). Understanding the genetic basis of rust resistance and the gene for gene theory (Flor,
 49 1956) helped wheat breeders in designing and building different pyramiding strategies in which
 50 breeders used several resistance genes to obtain race non-specific, durable resistance (Mundt,
 51 2014).

52 Race non-specific resistance or durable resistance is based on including several minor or
 53 major proven rust resistance genes in a single line (Johnston et al., 2017). Currently, the focus of
 54 many disease resistance breeding programs is to achieve durable resistance which requires
 55 reliable rust screening nurseries, appropriate host genotypes, an efficient crossing strategy, and
 56 effective selection protocols often supported by molecular markers (Khan et al., 2013). Breeding
 57 for durable resistance is challenging and slow due to a limited number of accessions with

confirmed resistance genes (REX Consortium, 2016), continued the evolution of pathogens, and fluctuations of the prevalence of the pathogen and its races due to the yearly variability of temperature and precipitation (Mundt, 2014). One of the potential sources of multiple resistance genes is the national and international collections in various gene banks (Aktar-Uz-Zaman et al., 2017). Hence it is desirable to screen a large number of accessions from the gene banks to identify new sources of resistance (Hoisington et al., 1999; Kumar et al., 2016). In this context, rust characterization should be undertaken at stem and leaf rust hotspots (e.g., regions where highly virulent pathogens naturally occur) (Prins et al., 2016). Such screening may reveal new genes for use as a source of resistance (Yu et al., 2016). Several experiments were conducted in the past, including global initiatives to screen over 200,000 wheat accessions for resistance to the stem rust race(s) Ug99 in Kenya (Huerta-Espino et al., 2011). Furthermore, wheat germplasm was screened in Pakistan for stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) resistance (Bux et al., 2012), and 19460 wheat accessions were tested in India for stripe rust and spot blotch caused by *Cochliobolus sativus* (Kumar et al., 2016). Using traditional breeding methods to screen, identify, select, and develop homozygous resistant entry is a time-consuming, slow process that mostly results in creating inferior phenotypes due to transferring additional genes linked to the resistant genes, the “dragging effect” (Hoisington et al., 1999; Randhawa et al., 2013). Thus, to overcome the previous difficulties plant breeders incorporated DNA molecular marker tools in their efforts to identify and introgress disease resistance genes with minimal linked genes into their elite lines (Kassa et al., 2017).

One of the most successful applications of molecular markers in plant breeding is marker-assisted selection (MAS). Marker-assisted selection uses molecular markers to select for desirable plants based on knowledge of associations between DNA molecular markers and traits of interest (Das et al., 2017; Zhao et al., 2014). ~~Marker-assisted selection (MAS)~~ was used recently to reveal the association between DNA molecular markers and several quantitative and qualitative traits (Bian and Holland, 2017; Contreras-Soto et al., 2017; Qu et al., 2017). The success of GWAS relies on the recombination events that occur throughout the evolutionary history of germplasm. Genome-wide association mapping was applied to a worldwide durum wheat (*T. turgidum* L. var. *durum*) collection, in which several QTLs were identified and found to be linked with leaf rust resistance. Rouse and Anderson (2015) applied association mapping on North American spring wheat breeding germplasm, and their results revealed loci conferring

resistance to Ug99 (Race TTKSK) and other African stem rust races. Li et al. (2016) performed GWAS on a panel of 1596 winter wheat lines. The germplasm accessions were evaluated for leaf rust reaction by testing with a bulk of *P. triticina* Eriks. (Pt) isolates which were collected from Oklahoma during 2013 and 2015. They identified 14 QTLs that were significantly associated with leaf rust resistance. Recently, (Gao et al., 2017) evaluated the global spring wheat collection (2,152 accessions) for four races of stem rust under the seedling and adult plant stages. Their results indicated that 47 SNP markers were significantly linked with stem rust resistance. Moreover, (Kankwatsa et al., 2017) evaluated 159 landraces from the global spring wheat collections plus old cultivars for over 35 stem, leaf and yellow rusts pathotypes in Australia. Their ~~gwas~~ results demonstrated that 79 SNP markers significantly associated with rust resistance, which were mapped on chromosomes 1A, 1B, 1D, 2A, 2B, 3A, 3B, 3D, 4A, 5A, 5B, 6A, 6B, 6D, 7A,7B, and 7D.

In the current study, a collection of 2111 accessions of a global spring wheat collection was evaluated for stem and leaf rust resistance under ~~the~~ Egyptian field conditions. According to the CIMMYT's rust tracker website (http://rusttracker.cimmyt.org/?page_id=1019, verified June 10, 2017), Egypt is considered to be a hotspot for the stem, leaf, and yellow rusts. Furthermore, leaf and stem rust epidemics are frequent in Egypt (Draz et al., 2015; El-Orabey et al., 2015; M Patpour et al., 2016; Prins et al., 2016). Nevertheless, no large-scale adult plant evaluation for stem or leaf rust under field conditions has been conducted in Egypt. The rust evaluations were performed in Egypt either using a limited number of accessions or with accessions from specific geographic region. The wheat accessions used in this study were obtained from several geographic regions, and ~~it~~ consisted of landraces, breeders' materials, and cultivars. However, most of these lines were never ~~been~~ before for leaf or stem rust resistance in Egypt. Testing a large number of accessions in one of the world hotspots for stem and leaf rust, i.e., Egypt, might provide useful sources of resistance for these two diseases while increasing the power of detecting QTLs co-located with the known or novel resistant genes. The primary objectives of this study were to; 1- Evaluate a comprehensive spring wheat collection for resistance to stem and leaf rusts at adult plant stage and identify resistant accessions, 2- Identify potential QTLs associated with stem or leaf rust resistance genes using GWAS.

Materials and Methods

Plant Materials and Field Growth Conditions:

A total of 2111 spring wheat accessions (882 landraces; 493 breeding lines; 419 cultivars and 317 with uncertain classification) obtained from a single plant selection from a *T. aestivum* core collection. The seeds of the current accessions were provided by the USDA-ARS National Small Grains Collection (NSGC) located in Aberdeen, ID, USA. The accessions originated from 107 countries, including 35 accessions from Egypt, representing global diversity. The current plant accessions include old and new wheat accessions; i.e., deposited in the collection from 1920 until 2012. The accessions were screened in Egypt during 2015/2016 and 2016/2017 growing seasons for leaf and stem rust in two locations; Elkhazan (31°05'35.2"N, 30°30'10.4"E) and Elbostan (30°45'19.4"N, 30°29'04.8"E), Behira governorate. Each accession was planted in two replicates using a randomized incomplete block design (Giesbrecht and Gumpertz, 2011) in plots of four rows wide with 25 cm between rows and two meters long. The incomplete blocks consisted of 50 accessions in addition to the three check cultivars, i.e., “Sids 13”, Gimmiza 9”, and “Giza 168”. The border surrounding the experimental areas of one meter wide, planted with a spreader cultivar, i.e., “Morocco.” For field inoculation with leaf and stem rusts, the spreader cultivar was sprayed with a mist of water and dusted with mixture of urediniospores of the prevalent and aggressive pathotypes of leaf rust, i.e., NKTSS, PKTTT, PTTPT, STTTK, TTTST and stem rust i.e. FTTTC, TTTTC TKTTC, PTQMC and KTSC mixed with a talcum powder at a ratio of 1 : 20 (v/v) (spores: talcum powder). The plants were dusted in the early evening (at sunset) before dew point formation on the leaves. The inoculation of the spreader plants was conducted at the booting stage according to (Shahin and El-Orabey, 2014). The urediniospores of leaf and stem rusts obtained from Wheat Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Standard agronomic practices including recommended fertilization application and irrigation schedule were followed at each location.

Disease Assessment:

Leaf and stem rust evaluation were conducted using the modified Cobb's scale described by (Peterson et al., 1948). The scoring was based on both the percentage of infected tissue (severity) and the plant response to infection (infection type). The percentage of disease severity for each of the tested accessions was recorded when the flag leaf of the susceptible check cultivar (Morocco) was severely rusted, and its disease severity reached the maximum level. The infection type was expressed in the following types, i.e., Immune=0, R = resistant, small uredinia surrounded by necrosis; MR = Moderately resistant, medium to large uredinia surrounded by necrosis; MS = moderately susceptible, medium to large uredinia surrounded by chlorosis; S = susceptible, large uredinia without necrosis or chlorosis (Roelfs et al., 1992). For statistical association analyses, the disease severity was combined with the infection type in a single value forming the average coefficient of infection (ACI). The average coefficient of infection (ACI) was calculated by multiplying the severity by a constant value for each infection types; Immune=0, Resistant (R) = 0.2, Moderately resistant (MR) = 0.4, Moderately susceptible (MS) = 0.8 and Susceptible (S) =1 (Aoun et al., 2016; Yu et al., 2011)

SNP Genotyping:

The spring wheat accessions included in this study were genotyped through the Triticae Coordinated Agriculture Project using the Illumina iSelect 9K wheat array (Cavanagh et al., 2013) at the USDA-ARS genotyping laboratory in Fargo, ND, USA. High-quality 5090 SNPs markers were used for association mapping. The marker data are available at http://triticeaetoolbox.org/wheat/display_genotype.php?trial_code=NSGCwheat9K_winter_fac. Genotypic data were coded as $x = \{-1, 0, 1\}$, where -1 represents homozygous for the minor allele, 0 represents heterozygotes, and 1 represents homozygous for the major allele. After removing SNP markers with missing values (>10%) and minor allele frequency (MAF < 5%), missing values were imputed using random forest regression (Breiman, 2001). All the filtered SNP markers were plotted in Manhattan plots using "wnsp 2013 consensus map"; available on: <https://triticeaetoolbox.org/wheat/>; as per (Wang et al., 2014).

Statistical Analysis

Analysis of variance (ANOVA) was performed using the average coefficient of infection (ACI) for all locations within and across years. The ANOVA assumptions and homogeneity of variance were tested in SAS9.4 (SAS Institute, Cary NC) using Levene's test (Brown and Forsythe, 1974). Pearson's correlation coefficients were calculated for leaf and stem rust for each year, location and location x year.

The following mixed model was fit using the ACI for leaf and stem rust:

$$Y_{ijkl} = \mu + g_i + e_j + r_{k(j)} + b_{l(kj)} + \varepsilon_{ijkl}$$

Where μ is the overall mean, g_i (Fixed) is the effect of i^{th} wheat accession, e_j (Fixed) is the effect of the j^{th} environment or location, $r_{k(j)}$ (Fixed) is the effect of the k^{th} complete block nested within the j^{th} environment or location, $b_{l(kj)}$ (Random) is the effect of the l^{th} incomplete block nested within the k^{th} complete block, and j^{th} environment or location and ε_{ijkl} (Random) is the residual effect. All random effects were assumed to be independent and normally distributed.

The best linear unbiased estimates (BLUE) for ACI and SNP markers were subjected to association analysis using mixed linear model (MLM) in R package rrBLUP (Endelman, 2011). The association analysis was carried out by performing a linear mixed model with restricted maximum likelihood estimates as follows:

$$Y = \mu + Zu + Wm + e$$

Where Y is a vector of ACI, μ is a vector of intercepts, u is a vector of $n \times 1$ of random polygene background effects, e is a vector of random experimental errors with mean 0 and covariance matrix Var (e), Z is a matrix relating Y to u. Var(u)=2KVg, where K is a known $n \times n$ matrix of realized relationship matrix, Vg is a scalar of the unknown genetic variance. m is a vector of fixed effect due to SNP markers, W is a matrix that relates Y to m. Var (e) =RVR, where R is a $n \times n$ matrix, and VR is scalar with unknown residual variance. P-values estimated from the association model were subjected to false discovery rate (FDR) corrections using Q-value estimates applied in the R package q-value (Storey and Tibshirani, 2003).

Results

Phenotypic Results:

The average coefficient of infection (ACI) of both leaf and stem rusts across years were homogeneous based on Levene's test (Brown and Forsythe, 1974). Thus combined statistical analysis for years and locations was conducted, in which the effect of the two and three-way interactions was highly significant (years x locations, years x genotypes, locations x years and years x location x genotypes). However, the focus of the current study was to assess the response of the tested accessions to leaf and stem rust pathogens under different environmental conditions, which might be a result of the presence of various rust races in the testing locations across years. Hence the analysis of variance was conducted for each year independently. The independent results of the 2016 and 2017 analysis of variance indicated significant statistical effect for locations, genotypes and the interaction between locations x genotypes (Tables 1 and 2). In the same context, Person's correlations were calculated among ACI within and across 2016 and 2017 locations for stem and leaf rusts (Figures 1 and 2). The correlations among accessions across testing locations for leaf rust within and across years were significant (ranged from 0.43 to 0.62, Figure 1). Moreover, similar results for stem rust testing locations within and across years were obtained, in which correlation was also significant (ranged from 0.43 to 0.61, Figure 2). Furthermore, our results indicated a lack of significant correlation between leaf and stem rust across various locations and years.

The percentage of accessions with overall leaf rust resistance across locations and years was 6.96 % (147 accessions), while 42 % (892 accessions) were moderately resistant, 24 % (503 accessions) were moderately susceptible, and 27 % (569 accessions) were susceptible. The percentage of accessions with stem rust resistance was 5.87 % (124 accessions), while 24.77 % (523 accessions) were moderately resistant, 20 % (425 accessions) were moderately susceptible, and 49.6 % (1048 accessions) were susceptible (Figure 1). List of leaf and stem rust resistant accessions along with their improvement status and origin were provided in the supporting information (Tables 1 and 2). Overall, only four accessions (ID.No: 520005, 260896, 313098 and 213593) were found to be resistant to both stem and leaf rust across years and locations.

Table 1: Analysis of variance for the average coefficient of infection (ACI) for leaf rust across locations for 2016 and 2017 growing seasons.

Source	DF	Mean Square	
		2016	2017
Location	1	402.69***	25100.16***
Iblock(Block Location)	84	18.0	15.0210
Genotypes	2113	274.83***	309.79***
Location*Genotypes	2113	48.74***	59.86***
Error	168	15.9512	16.04

***Significant at 0.001 probability level.

Table 2: Analysis of variance for the average coefficient of infection (ACI) for stem rust across locations for 2016 and 2017 growing seasons.

Source	DF	Mean Square	
		2016	2017
Location	1	4369.3***	11377.20***
Iblock(Block Location)	84	24.0642	26.4199
Genotypes	2113	283.54***	269.41***
Location*Genotypes	2113	60.88***	67.84***
Error	168	25.18	22.81

***Significant at 0.001 probability level.

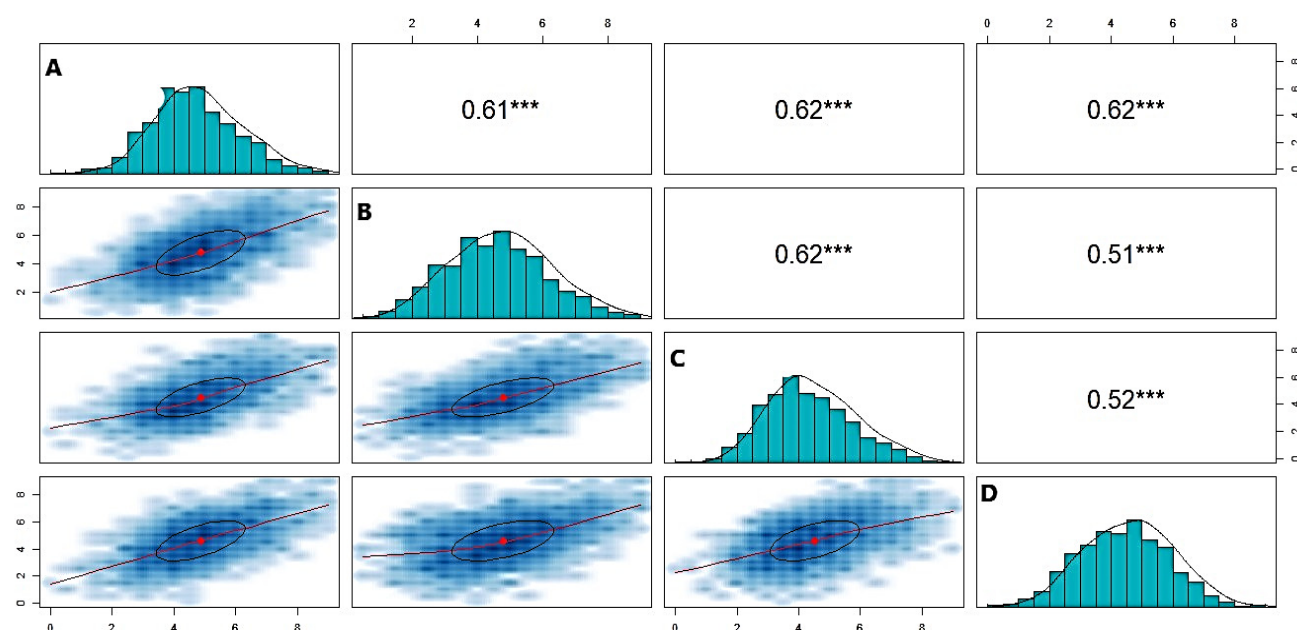
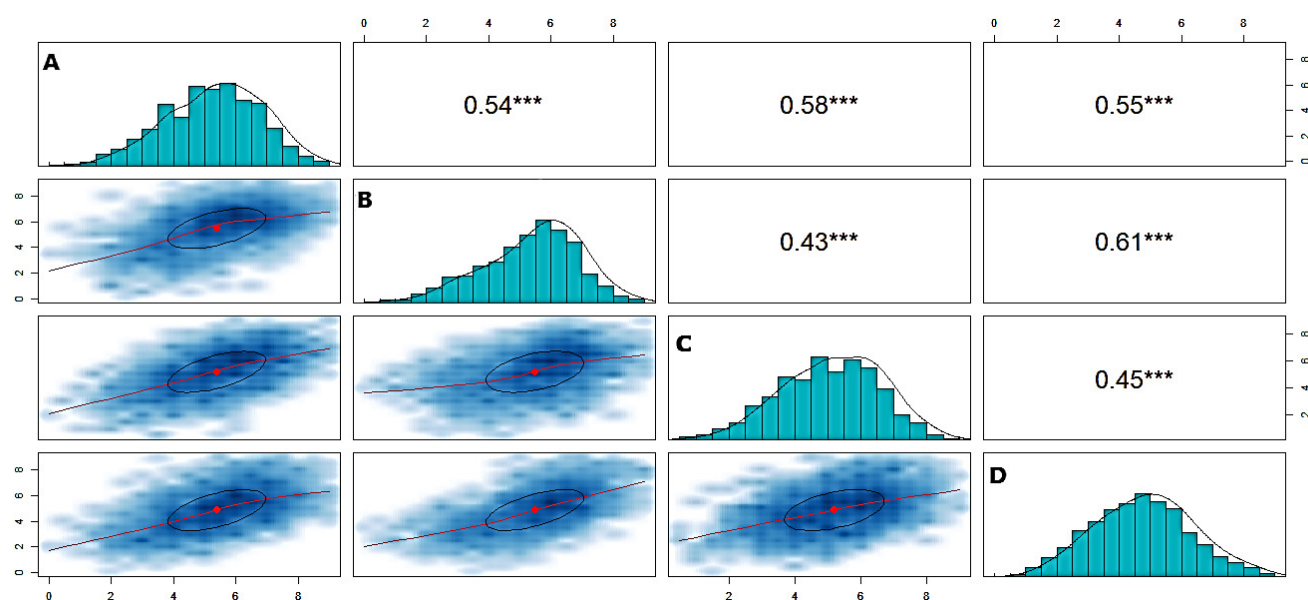


Figure 1: Histogram, scatterplot and correlation matrix for the average coefficient of infection (ACI) for leaf rust among 2016 locations; Elbostan (A), and Elkhazan (B); and 2017 Locations; Elbostan (D) and Elkhazan (C).

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278 **Figure 2:** Histogram, scatterplot and correlation matrix for the average coefficient of infection
 279 (ACI) for stem rust among 2016 locations; Elkhazan (A), and Elbostan(C); and 2017
 280 Locations; Elbostan (D) and Elkhazan (B).

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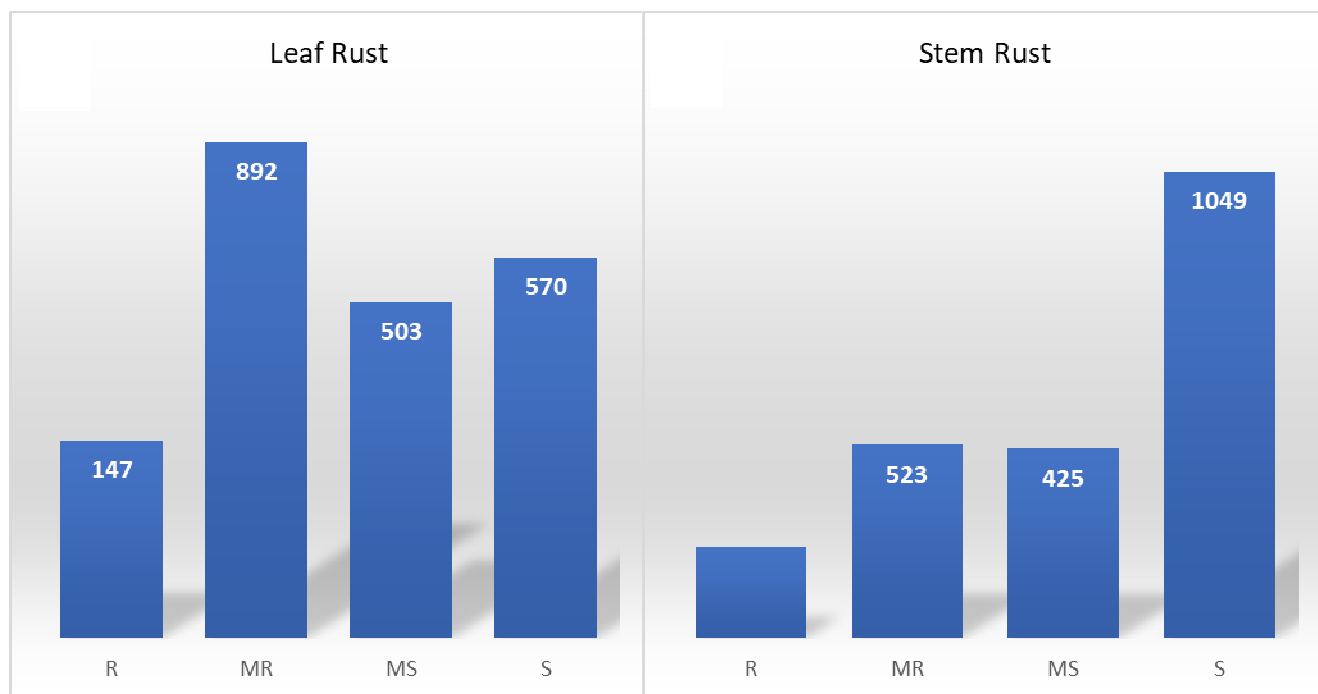


Figure3: Distribution of the overall ~~all~~ performance of the tested wheat accessions to the four classes of infection types i.e. resistant (R), moderately resistance (MR), moderately susceptible (MS) and Susceptible (S) to leaf and stem rust under the field conditions across locations and seasons.

Association mapping for leaf and stem rust response:

Eigenvector decomposition of the kinship matrix (K) was used to investigate the population structure among the studied accessions (Figure 4). Figure 4 indicated that the first principal component (PCA) accounts for less than 1% of the total variance. Furthermore, GWAS models with K matrix plus 0, 1, 2 or three PCA were compared using Bayesian information criterion (BIC). The results indicated noticeable difference between the four models, in addition the first model, i.e., with 0 PCA produced the largest BIC values, giving that the largest is the best. Therefore, we reported the results of association mapping using only the K matrix which accounted for most of the stratification on the studied materials (Figure 5).

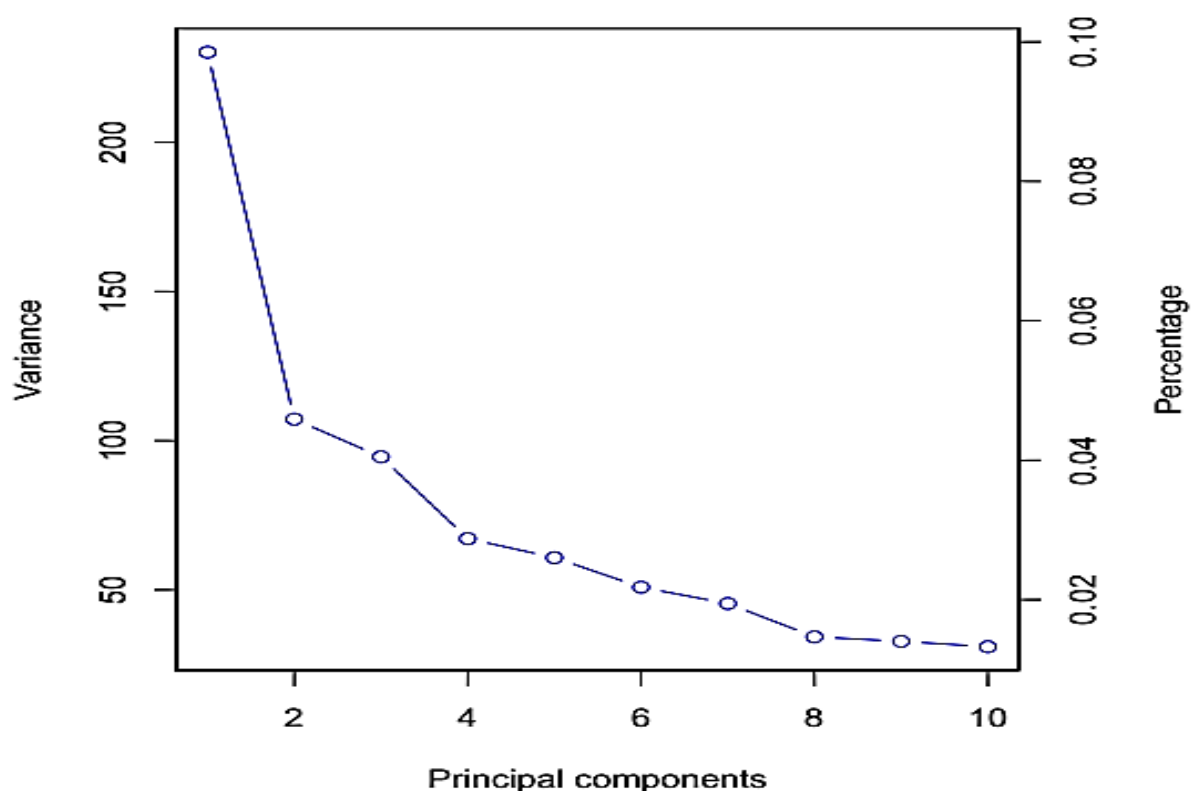
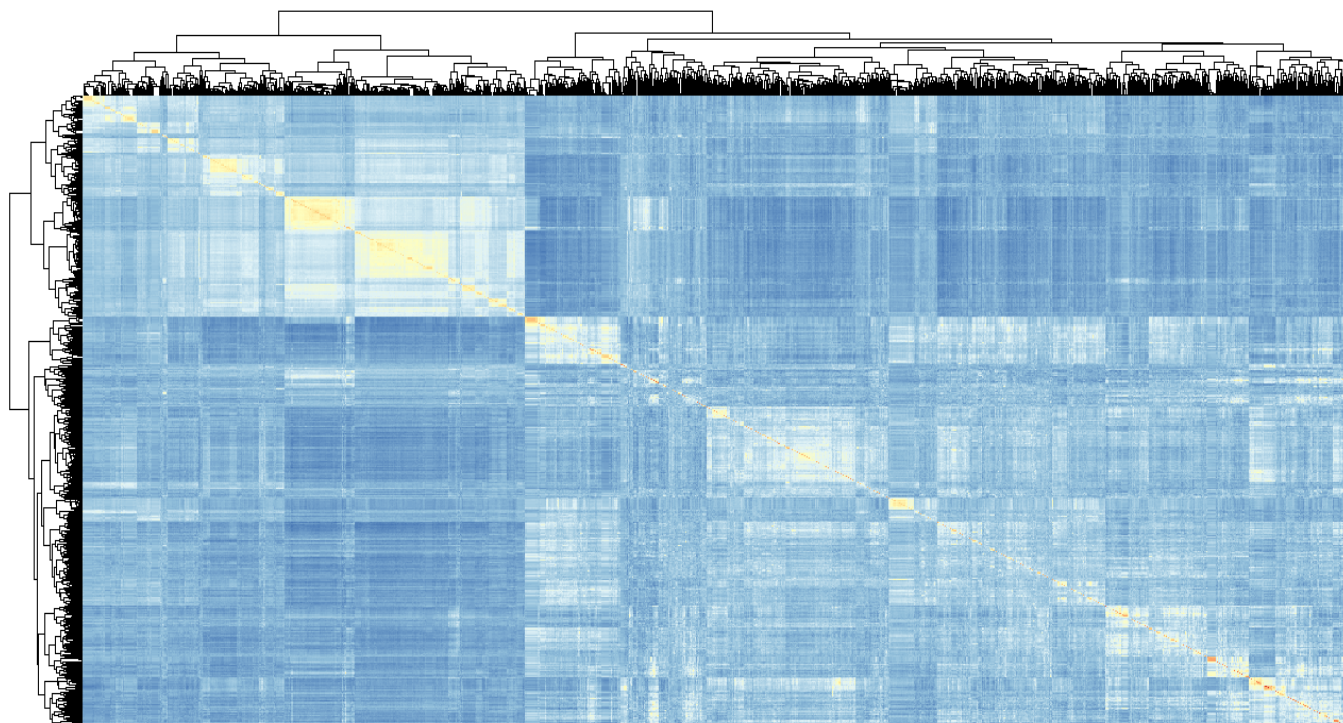


Figure (4): The percentage of variance explained by principal component (PCA)

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343 Figure (5): Heatmap and dendrogram of a kinship matrix estimated using the A.mat function
 344 (rrBLUP package) based on 5090 SNPs among 2111 wheat accessions.

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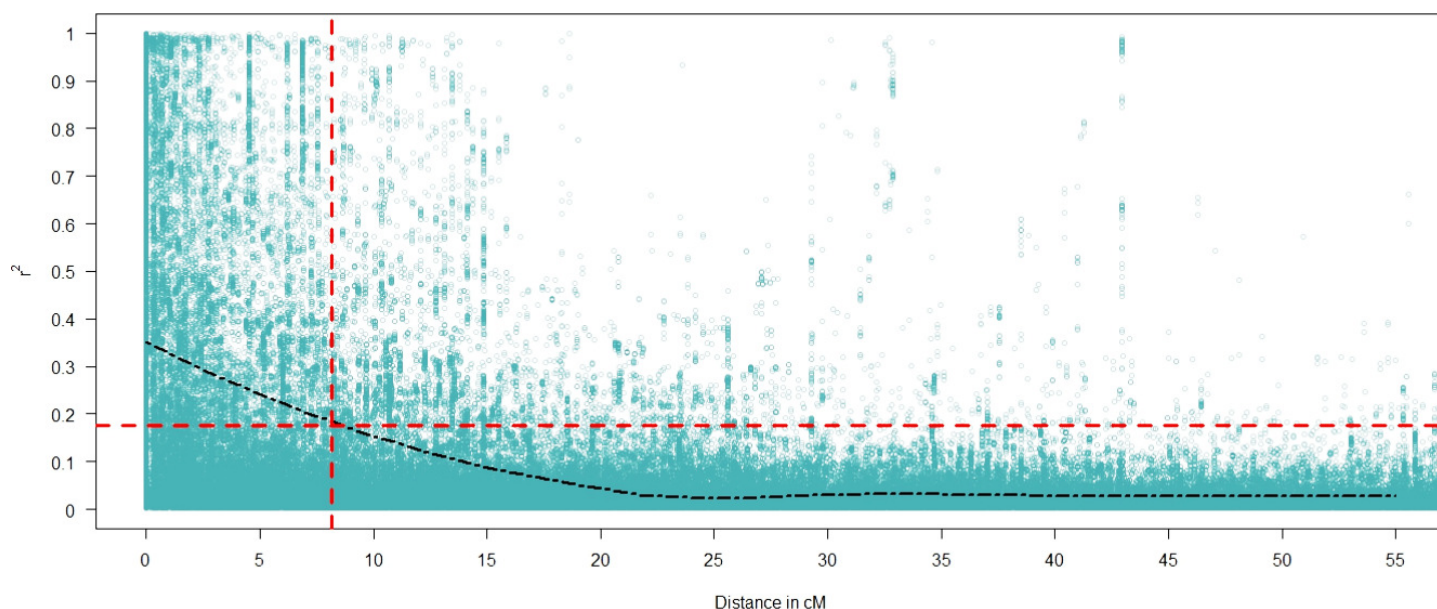


Figure (6): Genome-wide linkage disequilibrium (LD) decays for 2111 accessions and 3215 SNP markers. The horizontal and vertical dotted lines represent LD value (0.175) and distance (8 cM), respectively, in which LD decays to 50% of its initial value.

A total of 3215 mapped SNPs were used for estimating the extent of linkage disequilibrium (LD) in the 2111 spring wheat accessions. Only SNP loci having $MAF \geq 0.05$ and missing values $\leq 10\%$ were used to estimate r^2 across all SNPs. The estimates of r^2 for all pairs of SNPs loci were used to determine the rate of LD decay with genetic distance. Across the three wheat genomes, i.e., A, B and C and using only markers with significant r^2 (p -value=0.001), the LD ranged from 0 to 0.35. Overall, LD declined to 50% of its initial value at about eight cM (Figure 6).

Genome-wide association mapping for stem rust based on Average Coefficient of Infection (ACI) indicated that 46 SNP markers were significantly associated with stem rust. The stem rust associated SNP markers were located on chromosomes 1A (2 SNPs), 1B (3 SNPs), 2A (1 SNP), 2D (1 SNP), 3A (2 SNPs), 3B (1 SNPs), 4A (8 SNPs), 5A (1 SNP), 5B(6 SNPs), 6A (2 SNPs), 6B (1 SNP), 6D (1 SNP), 7A (4 SNPs), 7B (12 SNPs) and 7D (1 SNP) (Figure 6). Out of the 46 significant SNP markers, 15 SNPs found to be significantly linked with stem rust across the four trials (ELKhazan and Elbostan in 2016 and 2017) (Table 3). Only a single

marker (IWA5123) ~~found to be~~ linked with stem rust in two trials (ElKhazan 2016 and 2017), thirty SNPs marker were only significant in a single trial (Table 3).

In the same context, 36 SNP markers found to be significantly linked with leaf rust. The significant SNP markers were located on chromosomes 1A (9 SNPs), 2A (4 SNPs), 2B (3 SNP), 2D (4 SNPs), 3A (6 SNPs), 5A (2 SNPs), 5B (5 SNPs), 6 A(1 SNP) and 7A (2 SNPs) (Figure 7). Out of the 36 significant SNPs, fourteen SNP markers were consistently significantly associated with leaf rust in the four trials. While three markers (IWA5786, IWA8155 and IWA3190) were significant in two trials (ElBostan 2016 and 2017). However, nineteen markers found to be significant only in a single trial (Table 4).

Discussion

Egypt is one of the hotspots for wheat stem and leaf rust virulent races and may play a role as a green-bridge among the wheat belts in East and North Africa, the Middle East, and Mediterranean regions for *P. triticina* Eriks. and *P. graminis* f. sp. *tritici*. Thus, stem and leaf rust screening efforts should be intensified in Egypt. The geographical distribution of leaf rust pathotypes in Egypt was studied by (El-Orabey et al., 2015), and they found that a total of 243 leaf rust pathotypes were present during different seasons and locations including the testing locations of the current study. Moreover, previous reports identified several wheat stem rust pathotypes in Egypt and found that race groups TT--- and TK--- were the most frequent during 2008/2009 growing season. While, only race group TT--- was the most common during 2009/2010 growing season (Abou-Zeid et al., 2013). Patpour et al. (2016) identified TTKTT in Egypt and DNA from isolates were analyzed using a diagnostic qPCR assay, in which it confirmed that these samples belonged to the highly virulent and mutable Ug99 lineage. In Egypt Ug 99 was reported for the first time in 2014 (M Patpour et al., 2016). Thus, new sources of resistance to wheat stem rust are needed as an effective tool to control this disease.

The response of the tested accessions, for both stem and leaf rust, among same locations across years (i.e., ELkhazan 2016 and ELkhazan 2016) were highly significant. However, the correlations between different locations within the same year (i.e., ELkazan 2016 and Elbostan 2016) were higher than the correlations between locations across different years (i.e., Elkhazan 2016 and Elbostan 2017). The previous correlation results might be due to the adaptability of

various leaf and stem rust races to specific environmental conditions in a particular location or year (Ellis et al., 2014). Overall our phenotypic data indicated that the fluctuation in the environmental conditions from year to year was greater than that from location to location. Tsilo et al., (2014) found a significant and higher correlation (ranged from 0.63 to 0.83) among artificially inoculated accessions with a known mixture of leaf rust races in five environments than the correlation they found among naturally infected locations which indicated that higher correlation was due to having similar virulences in the inoculum. Similar results was reported by (Gupta and Saini, 1993) in which they found that the expression of resistance genes depends upon the genotype and environment, whereby the pathogen is part of that environment.

This study found that 147 (6.96%) of the tested accessions were resistant to stem rust and 124 (5.92%) were resistant to leaf rust. However, only four accessions were resistant to both pathogens. Stem rust resistance accessions included 38 accessions (30.6%) from the breeding materials, 21 cultivars (16.9%), and 48 landraces (38.7%) and 17 with an unknown degree of improvement (13.7%). Leaf rust resistance accessions contained 46 accessions (31.29%) from the breeding materials, 25 cultivars (17%), and 53 landraces (36%) and 23 with an unknown degree of improvement (15.6%). The distribution of leaf and stem rust resistant accessions among different improvement categories (i.e., cultivars, breeding materials and landraces) agrees with previous literature, in which wheat landraces were useful as a source for ~~will know~~ 41 rust resistance genes (Wulff and Moscou, 2014). Furthermore, stem rust resistance gene *Sr2* was transferred to hexaploid wheat from emmer wheat by (Stakman and Harrar, 1958). Overall, during the last century plant breeders performed thousands of crosses between breeders' elite lines and landraces. The key motivation for such crosses was to recapitulate disease resistance (Wulff and Moscou, 2014). Recently, ~~(Kankwatsa et al., 2017)~~ identified several resistance genes in spring wheat landraces. In this study, a mixture of stem and leaf rust races was used rather than a single-race analysis at the adult plant stage, which limited our ability to draw conclusive ~~discussion~~ about the nature of resistance observed. However, this study has significant implications for wheat breeders. ~~Deploying in breeding programs~~ the resistant accessions identified in this study may increase the probability of obtaining crossing parents with race non-specific durable resistance. Durable rust resistance is complex and based on the interaction of several genes with intermediate or minor effect. Rust resistance genes were categorized in the literature into two major categories; seedling resistance genes (assumed to be major genes) and

adult plant resistance genes (expected to be minor genes). Minor genes tend to show intermediate resistance and to attain an acceptable level of resistance, breeders need to combine at least three minor genes for a useful level of resistance (Bajgain et al., 2015). Plant breeders were successful in achieving resistance lines by combining several genes of partial resistance into the same line. Nevertheless, the fluctuations in the environmental conditions coupled with the dynamic nature of leaf and stem rust pathogens lead to continuous evolution and emergence of new rust races, that can rapidly increase in frequency and overcome major resistance genes in wheat cultivars. Therefore, new sources of rust resistance are continuously required, and it is necessary to undertake the evaluation of leaf and stem rusts under hotspot field conditions in which pathogens are present to provide wheat breeders with new sources of rust resistance while determining those resistance genes have that been defeated (Mujeeb-Kazi et al., 2013).

Genome-wide linkage disequilibrium (LD) declined to 50% of its initial value at about eight cM. The estimated LD value in this study is similar to the LD values reported by (Bajgain et al., 2015; Chao et al., 2010). Furthermore, our results showed lack of population stratification which suggested that fitting the K matrix only in the association mapping model is adequate for correcting for the relationships among the accessions (Endelman, 2012). These results agree with those of (Zhao et al., 2007), in which they found that correcting for population structure using the kinship matrix was as effective in reducing the false-positive discovery rate as using the Q + K model. Association mapping for stem rust identified several markers on chromosomes 1A, 2A, 3A, 4A, 6A, and 7A. These markers are most likely for the widely used *Sr1RS_{Amigo}*, *Sr21*, *Sr_{ND643}*, *Sr35*, *Sr52*, *Sr22* genes, respectively. For example, IWA7699, IWA3191, IWA4699 and IWA5123 markers were located in the same region on chromosome 4A (72.1cM – 73.8cM) where the temporarily designated gene, *Sr_{ND643}*, was mapped. *Sr_{ND643}* found to be effective against the Ug99 group races at both seedling and adult growth stages. (Yu et al., 2017). Recently, (Gao et al., 2017) identified QTL (IWA8495) linked with spring wheat resistance to stem rust at 63.29cM in chromosome 4A. Markers identified on chromosomes 1B, 5B and 7B might be for *Sr58*, *Sr56*, *Sr17* genes or alleles, respectively. Cluster of significant markers (IWA151, IWA7450, IWA4191, and IWA655) was located in chromosome 7B at (65.6 cM) in which *Sr17* was mapped (Yu et al., 2014). In the same context, markers identified in chromosome 2D and 6D were close to *Sr46* and *Sr22* genes, respectively.

Out of the nine chromosomes that contained markers associated with leaf rust, chromosomes 3A and 5A do not include any previously identified leaf rust resistance genes (Kertho et al., 2015). Therefore the six markers identified in genomic regions in 3A (68.77 cM, 69.47 cM, 70.39 cM and 72.50 cM), and 5A (11.22cM) appear to be associated with novel sources of resistance could be useful in breeding programs for resistance to leaf rust. Markers identified on chromosomes 1A, 2A, 6A, and 7A are most likely for alleles at the *trp-1*, *lr1* and *lr47* loci, respectively. Markers identified in chromosomes 2B and 5B are probably for alleles at the *lr16* and *lr52* loci, respectively. Moreover, markers identified in chromosome 2D were close to *lr2a-c* and *lr15* loci.

The large number of QTLs identified in this study for stem, and leaf rust highlights the complexity of the genetics that controls resistance to both pathogens during the adult plant stage (BOLTON et al., 2008; Kassa et al., 2017; McCallum et al., 2008; Oliver, 2014). Consistent with phenotypic results, the trials with the strongest correlation had more common QTLs, which indicated that pathogen diversity and environmental conditions in these trials led to similar plant responses to the artificial and natural infection for leaf and stem rust. Furthermore, no common QTLs between stem and leaf rust was detected, which was also consistent with the phenotypic results in which there was no significant correlation detected between leaf and stem rust resistance. Our findings agree with previous results reported by (Oliver, 2014; Olivera et al., 2008) in which they indicated that the genes responsible for stem and leaf rusts were often located in different linkage groups.

The current results identified new sources of adult plant resistance to leaf and stem rust which might enhance spring wheat disease resistance to both pathogens. For example, several markers (IWA4151, IWA3812, IWA7699 and IWA5391) linked with stem rust QTLs and several markers (IWA7191, IWA8215 and IWA7910) linked with leaf rust QTLs were not reported before and found to be consistently significant on all the studied trials. In addition, the results revealed that several markers linked to QTLs identified in this study co-located with previously reported major *Sr* and *Lr* loci as well as with recently identified QTLs in winter wheat landraces for leaf rust (Kertho et al., 2015), thus validating our approach. One strength of this approach is that by coupling phenotypic and genomic information, we could quickly identify lines with new resistance loci, lines with different resistance loci, and lines with similar resistance loci. This information allows researchers to focus resources on new gene deployment and pyramiding

different known genes for more durable resistance. The segregation patterns from the progeny of gene pyramiding efforts will further confirm these genetic results from association mapping on which genes are unlinked or linked.

Conclusion:

Based on upon literature and our results, we concluded that testing 2111 of spring wheat accessions, previously genotyped with the 9 K SNP marker in multiple environments in one of the global hotspots for stem and leaf rust at the adult plant stage, i.e., Egypt; resulted in identifying several accessions with resistance to stem and leaf rust. Only four accessions were resistant to both stem and leaf rust. Furthermore, several QTLs identified in this study co-located with previously reported major *Sr* and *Lr* genes. However, novel QTLs also were found to be consistent and significantly linked with stem and leaf rust across all trials. Allelism tests on the resistant lines that have QTLs on the same chromosomal region may be needed to identify whether they carry the same or different resistant alleles. The stem and leaf rust-resistant lines identified in this study will be included in various crossing blocks to enhance leaf and stem rust resistance in elite lines. Our future research will focus on developing several nested association mapping (NAM) populations using the resistant accessions to combine several leave and stem rust resistance genes. Furthermore, Kompetitive Allele Specific PCR (KASP) markers will be developed to accelerate the incorporation of resistance genes into the elite breeding wheat lines.

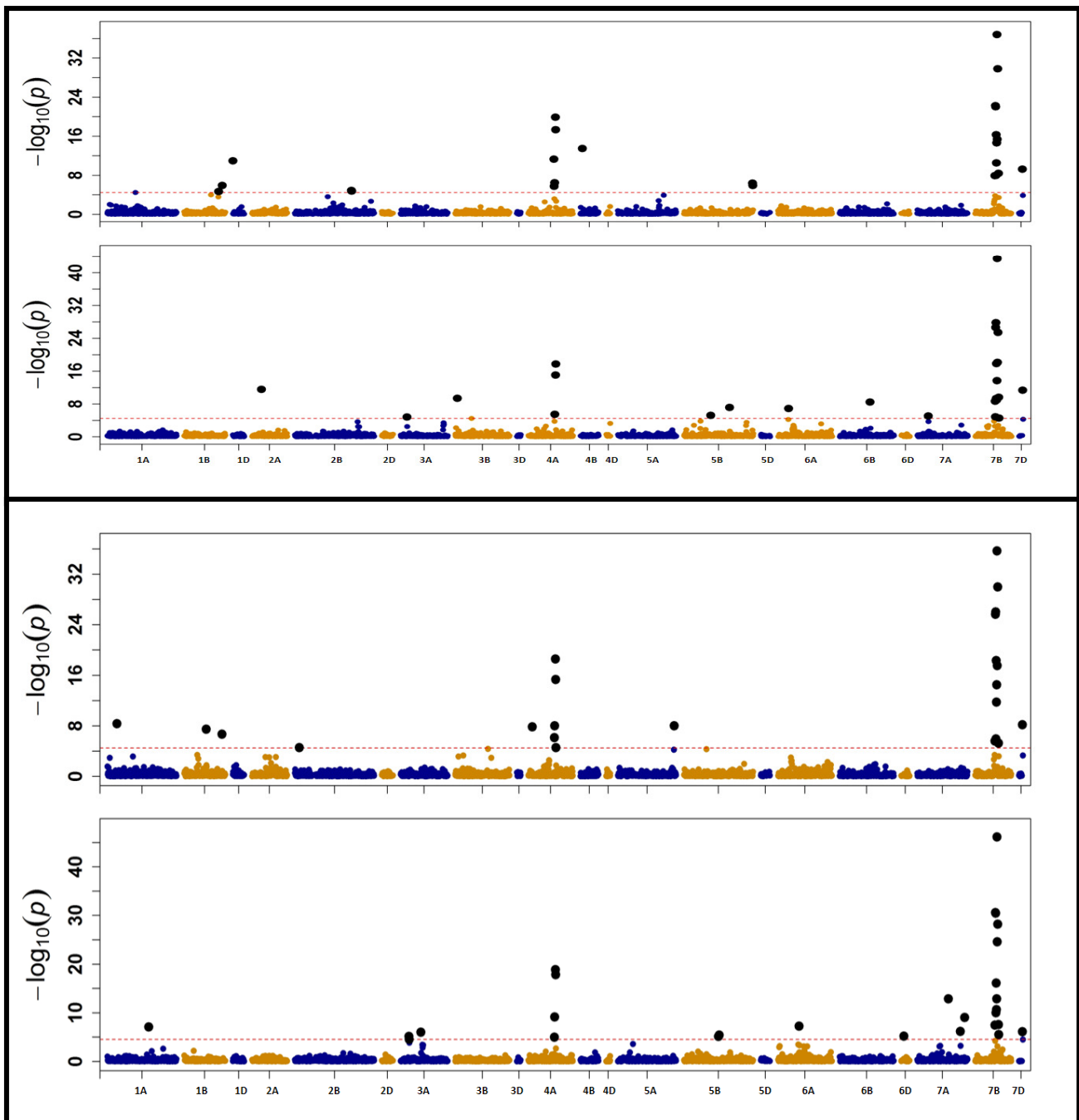


Figure (7): Manhattan plot for stem rust results obtained from genome-wide association mapping across years and locations.



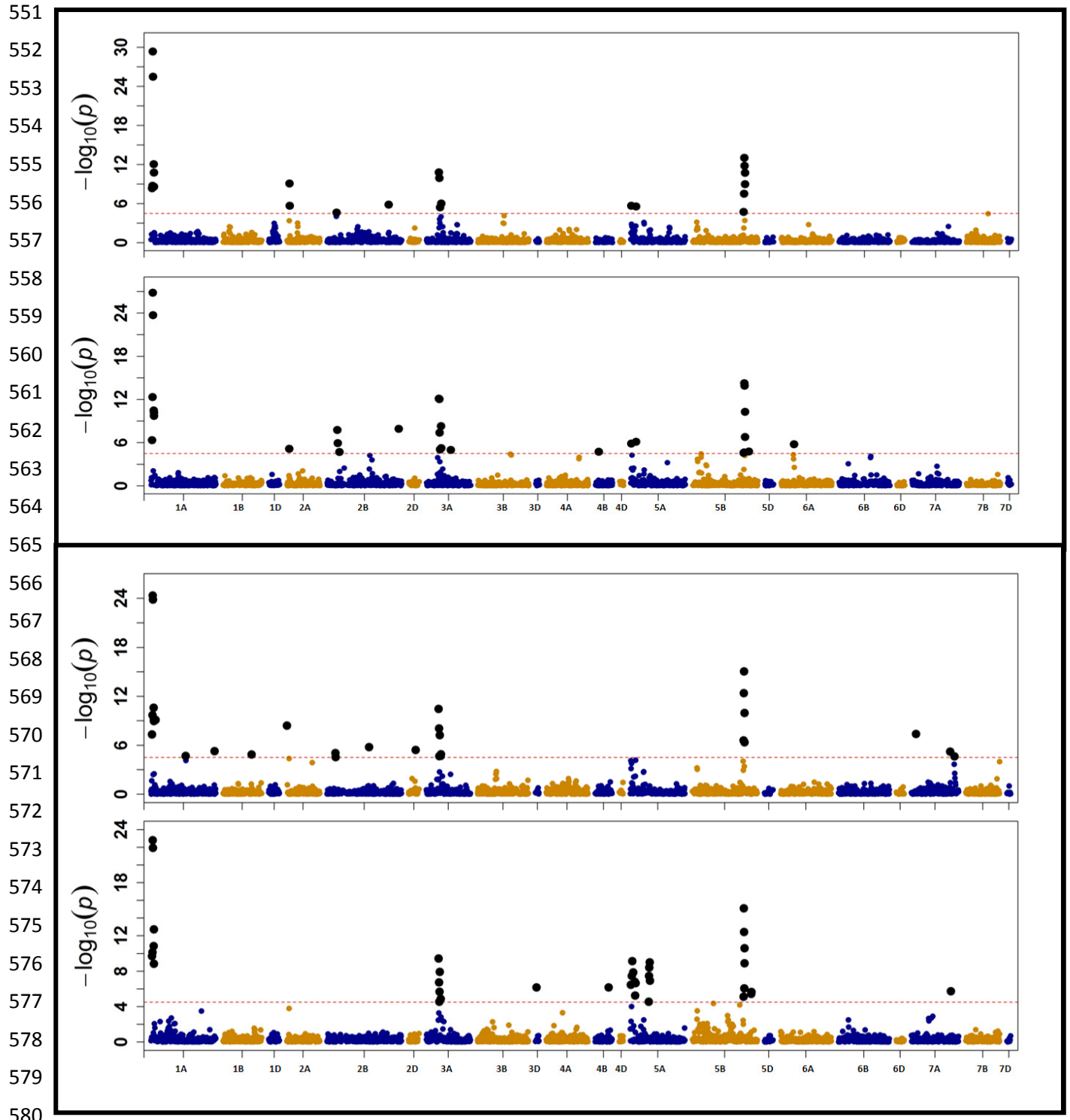


Figure (8): Manhattan plot for leaf rust results obtained from genome-wide association mapping across years and locations.

Table (3): Significant markers associated with resistance stem rust in 2016 and 2017 in ElBostan and Elkhazan location

Marker	Chrom	Position	ElBostan		Elkhazan		Maf	Additive Effect	R ²
			2016	2017	2016	2017			
IWA7699	1A	57.6	+	+	+	+	0.46	0.33	4.4
IWA3191	1A	85.0	+	+	+	+	0.46	0.12	2.6
IWA4699	1B	61.4	+	+	+	+	0.41	0.24	2.1
IWA5123	1B	111.2	-	-	+	+	0.49	0.44	2.2
IWA4151	1B	119.9	+	+	+	+	0.29	-0.46	6.5
IWA3438	2A	78.1	+	+	+	+	0.44	-0.16	5.3
IWA3437	2D	3.9	+	+	+	+	0.44	-0.07	0.8
IWA7450	3A	55.0	+	+	+	+	0.24	0.07	0.93
IWA4250	3A	78.2	+	+	+	+	0.23	0.62	4.9
IWA3691	3B	14.5	+	+	+	+	0.32	0.33	3.8
IWA3812	4A	20.6	+	+	+	+	0.34	0.50	3.4
IWA4190	4A	39.3	+	+	+	+	0.26	0.23	4.8
IWA3655	4A	70.2	+	+	+	+	0.26	0.15	4.3
IWA3810	4A	71.1	+	+	+	+	0.18	-0.40	4.0
IWA4191	4A	72.1	+	+	+	+	0.26	0.19	4.7
IWA5391	4A	72.5	+	+	+	+	0.26	0.11	3.0
IWA3998	4A	73.8	+	-	-	-	0.16	0.06	1.2
IWA5996	4A	73.8	+	-	-	-	0.22	0.22	2.6
IWA4319	5A	183.0	+	-	-	-	0.30	-0.40	3.2
IWA7604	5B	63.7	+	-	-	-	0.28	-0.43	4.5
IWA4569	5B	98.6	+	-	-	-	0.43	-0.15	4.1
IWA6580	5B	98.6	+	-	-	-	0.38	0.12	3.0
IWA6579	5B	117.0	+	-	-	-	0.38	0.24	1.8
IWA4126	5B	226.5	-	+	-	-	0.20	0.07	0.5
IWA3836	5B	226.5	-	+	-	-	0.17	0.09	0.8
IWA7662	6A	63.2	-	+	-	-	0.24	-0.21	2.4

585

586 “+”: Significant association

587 “-”: No significant association

588 **Maf:** minor allele frequency

589 **Additive effect:** Calculated as the average of the additive effect across all trials.

Table (3): (Continue)

Marker	Chrom	Position	ElBostan		Elkhazan		Maf	Additive Effect	R ²
			2016	2017	2016	2017			
IWA6867	6A	89.9	-	+	-	-	0.26	0.31	6.2
IWA3985	6B	80.9	-	+	-	-	0.26	0.58	5.0
IWA4371	6D	17.3	-	+	-	-	0.31	-0.90	3.6
IWA3624	7A	69.8	-	+	-	-	0.41	0.07	3.8
IWA4601	7A	99.9	-	+	-	-	0.08	0.11	4.3
IWA4124	7A	145.1	-	+	-	-	0.23	-0.19	4.6
IWA4187	7A	173.3	-	+	-	-	0.40	0.09	4.2
IWA5071	7B	65.2	-	+	-	-	0.26	-0.47	4.4
IWA8424	7B	65.6	-	-	-	+	0.48	-0.06	2.5
IWA5299	7B	65.6	-	-	-	+	0.14	0.00	2.7
IWA4004	7B	65.6	-	-	-	+	0.18	-0.16	2.2
IWA7372	7B	65.6	-	-	-	+	0.16	-0.11	2.5
IWA6806	7B	65.6	-	-	-	+	0.47	0.12	4.3
IWA3797	7B	65.6	-	-	-	+	0.45	-0.40	4.7
IWA3318	7B	65.6	-	-	-	+	0.48	-0.04	2.4
IWA3398	7B	65.6	-	-	+	-	0.37	-0.22	1.7
IWA7491	7B	65.6	-	-	+	-	0.29	0.12	6.6
IWA8543	7B	83.9	-	-	+	-	0.44	-0.39	5.5
IWA7836	7B	83.9	-	-	+	-	0.47	-0.21	5.3
IWA3604	7D	125.8	-	-	+	-	0.36	0.16	6.2

“+”: Significant association

“-”: No significant association

Maf: minor allele frequency

Additive effect: Calculated as the average of the additive effect across all trials.

Table (4): Significant markers associated with resistance leaf rust in 2016 and 2017 in ElBostan and Elkhazan locations.

Marker	Chrom	position	ElBostan		ELKhazan		Maf	Additive effect	R ²
			2016	2017	2016	2017			
IWA3182	1A	9.45	+	+	+	+	0.35	1.82	3.8
IWA5150	1A	9.87	+	+	+	+	0.19	2.24	3.4
IWA4351	1A	11.59	+	+	+	+	0.35	3.87	4.8
IWA6649	1A	11.59	+	+	+	+	0.35	3.78	4.3
IWA4643	1A	20.96	+	+	+	+	0.28	1.96	4.0
IWA4753	1A	21.74	+	+	+	+	0.10	2.45	4.7
IWA7191	1A	21.74	+	+	+	+	0.13	2.85	3.0
IWA4163	1A	32.8	-	-	+	-	0.39	-0.13	2.2
IWA6916	1A	183.5	-	-	+	-	0.35	0.43	2.6
IWA6391	2A	0.0	-	-	+	-	0.45	0.11	3.2
IWA5108	2A	13.6	-	-	-	+	0.34	-1.63	4.5
IWA4441	2A	18.7	+	-	-	-	0.29	0.03	4.1
IWA5762	2A	19.1	+	-	-	-	0.30	0.42	3.0
IWA5392	2B	71.8	-	-	+	-	0.19	0.06	1.8
IWA4189	2B	149.4	-	-	+	-	0.10	1.31	6.5
IWA4956	2B	185.7	+	-	-	-	0.47	0.32	5.8
IWA5753	2D	76.0	-	-	-	+	0.16	-0.55	5.4
IWA7567	2D	76.0	-	-	-	+	0.16	-0.40	6.2
IWA5896	2D	167.3	-	-	+	-	0.13	-0.01	5.0
IWA3596	2D	252.7	-	-	-	+	0.46	0.28	3.6
IWA5006	3A	68.77	+	+	+	+	0.30	3.52	3.8
IWA5005	3A	69.47	+	+	+	+	0.30	3.14	4.3
IWA8577	3A	69.8	-	-	-	+	0.36	1.93	4.6
IWA8215	3A	70.39	+	+	+	+	0.24	1.96	4.2
IWA3156	3A	72.2	-	-	-	+	0.37	2.05	4.4
IWA5786	3A	72.5	+	+	-	-	0.32	-1.88	2.5

“+”: Significant association

“-”: No significant association

Maf: minor allele frequency

Additive effect: Calculated as the average of the additive effect across all trials.

Table (4) : (Continue)

Marker	Chrom	Position	ElBostan		Elkhazan		Maf	Additive	R ²
			2016	2017	2016	2017			
IWA8155	5A	11.22	+	+	-	-	0.28	2.30	5.6
IWA3190	5A	11.22	+	+	-	-	0.28	2.22	5.6
IWA6718	5B	154.8	-	-	+	-	0.45	1.49	3.6
IWA7732	5B	155.79	+	+	+	+	0.44	2.47	3.9
IWA7733	5B	155.79	+	+	+	+	0.19	2.60	4.4
IWA7989	5B	156.74	+	+	+	+	0.22	2.10	4.3
IWA7910	5B	156.74	+	+	+	+	0.41	2.06	3.9
IWA4147	6A	79.1	-	-	-	+	0.27	-0.07	4.0
IWA7192	7A	46.6	-	-	+	-	0.21	-0.35	2.4
IWA4620	7A	135.2	-	-	+	-	0.44	-0.01	2.4

“+”: Significant association

“-”: No significant association

Maf: minor allele frequency

Additive effect: Calculated as the average of the additive effect across all trials.

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870 Appendix_1

Genotypes	LR (IT score)	IMPROVEMENT	COUNTRY
237650	1.25	LANDRACE	Cyprus
262623	1.5	LANDRACE	Ukraine
384414	1.625	UNCERTAIN	Nigeria
352124	1.625	BREEDING	Switzerland
181387	1.75	LANDRACE	Afghanistan
574331	1.75	BREEDING	Czech Republic
94563	1.75	LANDRACE	Israel
254124	1.75	BREEDING	Kenya
315837	1.875	CULTIVAR	Germany
351904	1.875	CULTIVAR	Germany
254134	1.875	BREEDING	Kenya
181244	2	LANDRACE	Afghanistan
499971	2	LANDRACE	Georgia
525440	2	UNCERTAIN	Morocco
7641	2	LANDRACE	Russian Federation
351903	2	BREEDING	Switzerland
639387	2	LANDRACE	Tajikistan
314915	2	CULTIVAR	Zimbabwe
366795	2.125	LANDRACE	Afghanistan
542675	2.125	LANDRACE	Algeria
351504	2.125	CULTIVAR	Argentina
153780	2.125	CULTIVAR	Egypt
438966	2.125	CULTIVAR	Kazakhstan
534372	2.125	LANDRACE	Tunisia
234234	2.125	BREEDING	Zambia
181251	2.25	LANDRACE	Afghanistan
262624	2.25	LANDRACE	Armenia
565209	2.25	LANDRACE	Bolivia
4936	2.25	UNCERTAIN	Canada
249819	2.25	BREEDING	Israel
525282	2.25	LANDRACE	Morocco
205714	2.25	BREEDING	Peru
634774	2.25	BREEDING	South Africa
55884	2.25	LANDRACE	Tajikistan
366831	2.375	LANDRACE	Afghanistan

367088	2.375	LANDRACE	Afghanistan
534421	2.375	LANDRACE	Algeria
192071	2.375	UNCERTAIN	Germany
193732	2.375	BREEDING	Ireland
Genotypes	LR (IT score)	IMPROVEMENT	COUNTRY
254133	2.375	BREEDING	Kenya
525361	2.375	LANDRACE	Morocco
410581	2.375	LANDRACE	Pakistan
631516	2.375	BREEDING	United States
234237	2.375	BREEDING	Zambia
470824	2.5	LANDRACE	Algeria
577786	2.5	LANDRACE	Algeria
57182	2.5	LANDRACE	Georgia
525434	2.5	LANDRACE	Morocco
192131	2.5	UNCERTAIN	Mozambique
184994	2.5	CULTIVAR	Norway
7635	2.5	LANDRACE	Russian Federation
574350	2.5	UNCERTAIN	Saudi Arabia
534352	2.5	LANDRACE	Tunisia
182751	2.5	LANDRACE	Turkey
502627	2.5	CULTIVAR	Uzbekistan
234235	2.5	BREEDING	Zambia
326330	2.5	CULTIVAR	Zimbabwe
345938	2.5	BREEDING	Zimbabwe
481718	2.625	LANDRACE	Bhutan
185355	2.625	UNCERTAIN	Brazil
184634	2.625	CULTIVAR	Germany
272508	2.625	BREEDING	Hungary
623445	2.625	LANDRACE	Iran
623507	2.625	LANDRACE	Iran
313098	2.625	CULTIVAR	Ireland
508387	2.625	BREEDING	Israel
238413	2.625	BREEDING	Kenya
262620	2.625	LANDRACE	Kyrgyzstan
520381	2.625	BREEDING	Mexico
613296	2.625	BREEDING	Mexico
191996	2.625	CULTIVAR	Mozambique
192294	2.625	CULTIVAR	Norway
205722	2.625	BREEDING	Peru
191261	2.625	BREEDING	Spain
262631	2.625	LANDRACE	Tajikistan

619369	2.625	BREEDING	United States
574363	2.625	UNCERTAIN	Yemen
234236	2.625	BREEDING	Zambia
125387	2.75	LANDRACE	Afghanistan
Genotypes	LR (IT score)	IMPROVEMENT	COUNTRY
347110	2.75	LANDRACE	Afghanistan
189794	2.75	BREEDING	Argentina
213593	2.75	BREEDING	Argentina
131273	2.75	BREEDING	Australia
565213	2.75	LANDRACE	Bolivia
366072	2.75	LANDRACE	Egypt
331245	2.75	LANDRACE	Eritrea
272348	2.75	CULTIVAR	Hungary
430067	2.75	LANDRACE	India
342641	2.75	CULTIVAR	Lebanon
520005	2.75	BREEDING	Mexico
278389	2.75	UNCERTAIN	Morocco
384346	2.75	UNCERTAIN	Nigeria
184992	2.75	CULTIVAR	Norway
306529	2.75	UNCERTAIN	Romania
410908	2.75	CULTIVAR	Spain
639339	2.75	LANDRACE	Tajikistan
41033	2.75	UNCERTAIN	Tunisia
189776	2.75	BREEDING	Tunisia
247922	2.875	CULTIVAR	Chile
94570	2.875	LANDRACE	Greece
189737	2.875	CULTIVAR	Japan
235221	2.875	BREEDING	Japan
329233	2.875	CULTIVAR	Lebanon
185909	2.875	BREEDING	Mexico
613289	2.875	BREEDING	Mexico
613317	2.875	BREEDING	Mexico
192150	2.875	UNCERTAIN	Mozambique
532286	2.875	LANDRACE	Oman
572795	2.875	LANDRACE	Pakistan
134863	2.875	LANDRACE	Portugal
519908	2.875	BREEDING	South Africa
260896	2.875	CULTIVAR	Sweden
241595	2.875	CULTIVAR	Taiwan
15517	2.875	LANDRACE	Tunisia

534355	2.875	LANDRACE	Tunisia
14087	2.875	UNCERTAIN	Unknown
574364	2.875	UNCERTAIN	Yemen
192474	3	UNCERTAIN	Angola
14350	3	BREEDING	Argentina
Genotypes	LR (IT score)	IMPROVEMENT	COUNTRY
185298	3	BREEDING	Argentina
499967	3	LANDRACE	Armenia
140958	3	BREEDING	Australia
519503	3	BREEDING	Egypt
181458	3	CULTIVAR	Finland
4203	3	UNCERTAIN	Honduras
623201	3	LANDRACE	Iran
572631	3	CULTIVAR	Kazakhstan
186061	3	BREEDING	Mexico
520376	3	BREEDING	Mexico
152445	3	LANDRACE	Morocco
525280	3	LANDRACE	Morocco
384379	3	UNCERTAIN	Nigeria
532275	3	LANDRACE	Oman
270045	3	UNCERTAIN	Pakistan
572794	3	LANDRACE	Pakistan
203083	3	UNCERTAIN	Paraguay
48097	3	UNCERTAIN	South Africa
58560	3	BREEDING	Sweden
574249	3	BREEDING	Syria
15511	3	LANDRACE	Tunisia
13953	3	BREEDING	United States
532891	3	BREEDING	United States
14251	3	BREEDING	Unknown
184598	3	UNCERTAIN	Uruguay
9131	3	CULTIVAR	Uzbekistan
574359	3	UNCERTAIN	Yemen
345936	3	BREEDING	Zimbabwe

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875 Appendix 2

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Genotypes	SR (IT type)	IMPROVEMENT	COUNTRY
203116	1.5	CULTIVAR	Finland
382150	1.625	LANDRACE	Japan
219750	1.75	LANDRACE	Pakistan
520005	1.875	BREEDING	Mexico
593658	1.875	CULTIVAR	Canada
202782	1.875	BREEDING	Peru
469014	2	LANDRACE	Greece
168480	2	LANDRACE	India
213572	2	UNCERTAIN	Argentina
260896	2.125	CULTIVAR	Sweden
326304	2.25	CULTIVAR	Russian Federation
429627	2.25	LANDRACE	Nepal
520346	2.25	BREEDING	Canada
552987	2.25	BREEDING	Canada
445778	2.25	LANDRACE	Nepal
220127	2.25	LANDRACE	Afghanistan
14284	2.25	BREEDING	Mexico
481928	2.375	LANDRACE	Sudan
429669	2.375	LANDRACE	Nepal
481931	2.375	LANDRACE	Sudan
13775	2.375	CULTIVAR	Canada
429670	2.375	LANDRACE	Nepal
520147	2.375	BREEDING	South Africa
254064	2.375	UNCERTAIN	Europe
445688	2.375	LANDRACE	Nepal
15412	2.375	LANDRACE	Tunisia
445696	2.375	LANDRACE	Nepal
481925	2.5	LANDRACE	Sudan
429667	2.5	LANDRACE	Nepal
12814	2.5	BREEDING	United States
429657	2.5	LANDRACE	Nepal
70704	2.5	LANDRACE	Iraq
577770	2.5	LANDRACE	Algeria
445694	2.5	LANDRACE	Nepal
525320	2.5	UNCERTAIN	Morocco
323392	2.5	CULTIVAR	Kenya
313098	2.625	CULTIVAR	Ireland

17750	2.625	BREEDING	Canada
406486	2.625	LANDRACE	Nepal
481927	2.625	LANDRACE	Sudan
406487	2.625	LANDRACE	Nepal
221371	2.625	UNCERTAIN	Serbia
429696	2.625	LANDRACE	Nepal
351736	2.625	CULTIVAR	Romania
641754	2.625	BREEDING	United States
479700	2.625	CULTIVAR	South Africa
254125	2.625	BREEDING	Kenya
462158	2.625	UNCERTAIN	China
372139	2.75	CULTIVAR	Russian Federation
34126	2.75	UNCERTAIN	Spain
185891	2.75	BREEDING	Mexico
532123	2.75	UNCERTAIN	Egypt
126822	2.75	BREEDING	Kenya
344148	2.75	CULTIVAR	Brazil
388038	2.75	BREEDING	Israel
572692	2.75	LANDRACE	Georgia
191864	2.75	UNCERTAIN	Portugal
178752	2.75	LANDRACE	Iraq
106154	2.75	BREEDING	Australia
15377	2.75	UNCERTAIN	Lebanon
15135	2.75	UNCERTAIN	Sudan
428421	2.75	UNCERTAIN	Egypt
630982	2.75	BREEDING	Canada
352185	2.75	BREEDING	Mexico
279454	2.75	CULTIVAR	United Kingdom
15200	2.875	UNCERTAIN	Kenya
352135	2.875	BREEDING	Sweden
264732	2.875	BREEDING	Greece
323388	2.875	CULTIVAR	Kenya
429641	2.875	LANDRACE	Nepal
192522	2.875	UNCERTAIN	Portugal
15863	2.875	BREEDING	Mexico
574501	2.875	BREEDING	Canada
12156	2.875	BREEDING	Canada
317693	2.875	BREEDING	Croatia
193384	2.875	LANDRACE	Pakistan
299425	2.875	CULTIVAR	Kenya
15035	2.875	LANDRACE	Afghanistan

57989	2.875	LANDRACE	India
14821	2.875	LANDRACE	Eritrea
70702	2.875	LANDRACE	Iraq
429659	2.875	LANDRACE	Nepal
519484	2.875	BREEDING	Colombia
14478	2.875	BREEDING	Mexico
62366	2.875	LANDRACE	Venezuela
322071	2.875	UNCERTAIN	India
519842	2.875	BREEDING	Mexico
70714	2.875	LANDRACE	Iraq
518648	2.875	CULTIVAR	Canada
15560	2.875	BREEDING	United States
429699	2.875	LANDRACE	Nepal
340684	2.875	CULTIVAR	Netherlands
213593	3	BREEDING	Argentina
414993	3	BREEDING	Mexico
583681	3	CULTIVAR	United States
17751	3	BREEDING	Canada
625562	3	LANDRACE	Iran
181470	3	UNCERTAIN	Finland
347732	3	CULTIVAR	Japan
481929	3	LANDRACE	Sudan
83481	3	LANDRACE	China
14392	3	BREEDING	Ecuador
17756	3	BREEDING	Canada
278297	3	LANDRACE	Greece
14083	3	UNCERTAIN	Unknown
623335	3	LANDRACE	Iran
278536	3	LANDRACE	Syria
625563	3	LANDRACE	Iran
89188	3	CULTIVAR	Australia
191180	3	LANDRACE	Spain
117621	3	BREEDING	Kenya
322168	3	UNCERTAIN	India
231116	3	BREEDING	Guatemala
384025	3	CULTIVAR	Israel
15471	3	LANDRACE	Tunisia
94571	3	LANDRACE	Greece
170910	3	BREEDING	South Africa
630979	3	BREEDING	Canada
341364	3	LANDRACE	Turkey

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406517	3	LANDRACE	Nepal
520265	3	BREEDING	United States
170902	3	BREEDING	South Africa
202778	3	BREEDING	Peru
278311	3	CULTIVAR	Netherlands

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