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SNP markers linked to leaf rust and grain mold resistance in sorghum

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Abstract Grain mold and rust are diseases that can significantly reduce sorghum grain yield. Breeding for resistance to these diseases is hindered by inefficient disease screening. A viable option to greatly improve breeding efficiency is to identify molecular markers or genes linked to the host resistance. In this study, we applied 14,739 single nucleotide polymorphism markers to the sorghum mini core of 242 accessions that had been evaluated for rust resistance in both greenhouse and field and for grain mold in the field for 2 years. Through association mapping we have identified two loci linked to grain mold resistance and five loci linked to rust resistance. Among the two loci linked to grain mold resistance, one contained a homolog of the maize nonhost resistance gene *Rxo1*. Two of rust-linked loci each contained the rust resistance gene homologous to the maize rust resistance gene *Rp1-D* which is the B locus (the A locus containing *Pu* was not linked in this study) and to the wheat rust resistance gene *Lr1*. The remaining loci contained genes important in other steps of the defense response, such as cyclophilins that mediate resistance response preceding hypersensitive response (HR) and *Hin1* directly involved in producing HR. The results from this study will facilitate marker-assisted selection of host resistance to grain mold and rust in sorghum.

Keywords Sorghum · Association mapping · Mini core collection · Grain mold · Rust · Resistance genes

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Introduction

Fungal diseases are perennial threats to plant productivity, jeopardizing food security (Fisher et al. 2012). Two of the fungal diseases causing significant crop damage in sorghum are leaf rust caused by *Puccinia purpurea* and grain mold caused by a complex of fungal pathogens that may vary among locations. This complex consists of over 40 fungal pathogens that infect the developing caryopsis from anthesis through grain development (grain mold) or after grain maturity (grain weathering). The most important of these are *Fusarium* spp. (*F. proliferatum*, *F. thapsinum*, *F. equiseti*, *F. andiyazi*, and *F. sacchari*), *Curvularia lunata*, *Alternaria alternata*, *Phoma sorghina*, and *Colletotrichum sublineolum* (Thakur et al. 2007; Sharma et al. 2011). The importance of these species in grain mold development can vary from location to location (Rodriguez-Herrera et al. 2000), but *Fusarium*, *Curvularia* and *Alternaria* are the main pathogens that cause the disease (Little et al. 2012). Grain

mold is favored by conditions of high humidity and temperature during grain development. Yield loss in susceptible varieties is due to caryopsis abortion (Little and Magill 2009), reduced seed filling, and low grain density (Little et al. 2012). Sorghum seed is called "caryopsis" because the endosperm is fused directly with the pericarp. Among the two types of endosperm, soft (chalky) and hard (corneous), larger proportions of corneous endosperm are correlated with greater grain mold resistance (Jambunathan et al. 1992; Audilakshmi et al. 1999), and the latter is associated with pigmented testa (Menkir et al. 1996). Klein et al. (2001) presented genetic evidence that one of the two mapped grain hardness quantitative trait loci (QTLs) colocalizes with one grain mold resistance QTL. Because multiple pathogens cause the disease, it is possible that host resistance is also controlled by multiple genes. It has been estimated that four to ten genes may control the resistance and because the causal pathogen may vary from location to location, resistance should be evaluated in target environments (Rodriguez-Herrera et al. 2000; Audilakshmi et al. 2005). Polygenic resistance has been demonstrated in a mapping study by Klein et al. (2001).

Fewer studies have focused on sorghum leaf rust. Loss of grain yield from leaf rust can be up to 50 % in susceptible varieties, mostly due to reduced seed weight (Hepperly 1990), although lower figures have been reported (White et al. 2012). In addition, severe rust infection also reduces the sugar content of the juice in sweet sorghum (Coleman and Dean 1961). It has also been reported that rust is conducive to the occurrence of other diseases, such as anthracnose (Murali Mohan et al. 2010). An initial study on the inheritance of resistance to rust identified resistance as dominant (*Pu*) in sweet sorghum crosses between the susceptible cultivar Planter and the resistant cultivar MN 960 (Coleman and Dean 1961). The *Pu* allele from Rio or PI267474 is dominant in Puerto Rico throughout the season when the opposite allele is pu^{r} (from Combine Shallu), but it is recessive late in the season when the opposite allele is *pu* (from B406). Furthermore, *Pupu* plants are resistant only during the early part of the season while *PuPu* plants are resistant throughout the season (Miller and Cruzado 1969). Later studies found that resistance to sorghum rust is governed by three major genes with susceptibility being dominant (Rana et al. 1976; Indira et al. 1982). More recent studies have confirmed the polygenic inheritance nature of the rust resistance in sorghum (Tao et al. 1998; McIntyre et al. 2005; Murali Mohan et al. 2010).

Because markers linked to resistance gene can be used to facilitate the breeding of resistant varieties, genetic mapping of grain mold and rust has been conducted by a number of groups. Wang et al. (2006) genotyped 96 core accessions from the USDA-GRIN collection evaluated for rust and grain mold. Klein et al. (2001) mapped five grain mold resistance QTLs distributed on sorghum chromosomes 4, 6, 7, 9, and 10 (SBI-04, -06, -07, -09, and -10, respectively). Interestingly, while the QTL on SBI-07 was detected in four of the six environments in three locations, QTLs on SBI-06 and SBI-10 were only detected in one of the two remaining environments. In addition, two different QTLs were detected in the same location in different years (Klein et al. 2001). These findings suggest the possibility of shifting grain mold pathogen populations among different environments, as discussed by Little et al. (2012). Two rust resistance QTLs have also been mapped to SBI-06 by Murali Mohan et al. (2010); one is clustered with QTLs conferring resistance to other diseases, and the other rust QTL has been mapped to SBI-08 (Tao et al. 1998; McIntyre et al. 2005). This QTL is believed to be the *Pu* gene (Coleman and Dean 1961; Miller and Cruzado 1969) that is homologous to the maize *Rp1-D* (Collins et al. 1999; Sun et al. 2001; Ramakrishna et al. 2002; McIntyre et al. 2004) and is located between 2,487,742 and 2,514,226 bp (Mace and Jordan 2010).

In this study, we applied 14,739 single nucleotide polymorphism (SNP) markers to a sorghum mini-core collection of 242 accessions (Upadhyaya et al. 2009) that had been evaluated for grain mold and rust each in two environments and identified two regions for grain mold and five regions for rust resistance. Annotated genes related to plant disease resistance are also listed. The identified genes and mapped markers may be developed into tools for use in the molecular breeding of sorghum for resistance to rust and grain mold.

Materials and methods

Disease phenotyping

Field evaluation of the mini core for reaction to grain mold and rust was as described previously (Sharma

et al. 2010, 2012). For grain mold resistance, 140 mini-core accessions were evaluated in the sorghum grain mold nursery in the 2007 and 2008 rainy seasons at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Screening was done with natural grain mold infection because sufficient inocula of mold fungi were present during the rainy season in the sorghum fields at ICRISAT, India for natural field epiphytotic conditions. High humidity [>90 % relative humidity (RH)] was also provided through sprinkler irrigation on rain-free days from the flowering stage to physiological maturity. The visual panicle grain mold rating (PGMR) was recorded on ten flowering plants of each accession at physiological maturity, using a scale of 1–9, where 1 = no mold infection and 9 = 76–100 % molded grains on a panicle. For rust evaluation, urediniospores collected from infected foliage in the previous season were used as inoculum after being suspended in water containing a few drops of a surfactant (Tween 20). In the greenhouse rust evaluation, ten seedlings from each of the 242 accessions were sprayed at the three- to five-leaf stage with the suspension (concentration 1 × 105 urediniospores/l). Inoculated seedlings were kept in a moist chamber (>95 % RH, 25 ± 2 °C) for about 18 h and then transferred to a greenhouse at 25 ± 2 °C for disease development. Rust severity was recorded 15 days after inoculation using the modified Cobb's scale (Thakur et al. 2007). Field evaluation for rust resistance was carried out during the 2010 rainy season for all 242 accessions. Rust severity was recorded at the dough stage using the same scale as in the greenhouse evaluation. Plant height, maturity, peduncle length, panicle length, and width were also recorded.

Genotyping

Genotyping of the mini core was as described by Wang et al. (2013). Five hundred ng of genomic DNA was digested with the CpG methylation-sensitive restriction endonuclease *Fse*I (New England Biolabs, Ipswich, MA, USA) ligated with adapters containing 4 bp identifier tags and sequenced on an Illumina GAIIx (Illumina, San Diego, CA, USA). Raw sequences were aligned to the sorghum genome sequence and analyzed for SNPs using the CLC Bio Genomics Workbench software (CLC Bio, Cambridge, MA, USA). SNPs were named based on the chromosome on which they mapped followed by the physical location in bp (e.g., SNP chr1_46978664, resides on SBI-01 at position 46,978,664 bp). Only markers scored in at least 50 % of the 242 accessions were retained. A total of 14,739 SNPs were developed and used in this study.

Data analysis

Marker-trait associations were identified using two mixed linear models (MLM; Yu et al. 2006): the kinship matrix (K model) and the K with PCA (principal component analysis) matrix (PK model); both have been shown to be efficient in eliminating false-positive associations (Casa et al. 2008; Huang et al. 2010; Setter et al. 2011; Yang et al. 2011; Pasam et al. 2011). These models were implemented in TASSEL 3.0 (Bradbury et al. 2007) available from http://www.maizegenetics.net/. The PCA matrix was generated in R (ver. 2.15.1, 64 bit; available at www.r-project.org/). SNP data were transformed manually, and the PCA was performed using the prcomp() procedure in R. The K matrix was generated in TASSEL with 168 evenly distributed unlinked SNP markers. The significance of associations between markers and traits was based on two different threshold p values. The first was $p < 6.78 \times 10^{-6}$, a stringent Bonferroni correction calculated by dividing 0.10 (Setter et al. 2011) by 14,739, the total number of markers used in this study. The second was the p value (10^{-4}), as used in similar studies with the 44K SNP markers (Famoso et al. 2011).

Results

Phenotypic evaluation

In both years, grain mold resistance was consistent among the 140 accessions, with a Pearson's correlation coefficient of 0.96. As expected, severity of the disease was negatively correlated with panicle length, with correlation coefficients (r) of −0.45 and −0.47 for 2007 and 2008, respectively. To a much less degree, disease severity was also negatively correlated with panicle width, with $r = -0.03$ and −0.23 for 2007 and 2008, respectively. These findings suggest that loose panicles showed less severe molds than more compact panicles. Grain mold severity was also

negatively correlated with peduncle length ($r = -0.32$ and -0.23 for 2007 and 2008, respectively), i.e., a longer peduncle was associated with less severe grain mold. In contrast to grain mold, greenhouse and field evaluations for rust were only marginally correlated, with $r = 0.15$. Greenhouse rust severity was not correlated with height, maturity, and panicle phenotypes, but field rust severity was negatively correlated with plant height ($r = -0.44$) and maturity ($r = -0.59$) and positively correlated with peduncle length ($r = 0.32$). This result suggests that taller plants had less severe foliar rust symptoms.

Comparing the K and PK association models

To compare the models, p values for each trait evaluated in a particular environment were ranked for each model and plotted in a cumulative way for both leaf rust and grain mold (Fig. 1) as described by Kang et al. (2008). A uniform p value distribution, as represented by a straight line, indicates an ideal model. Using this criterion, both PK and K were effective in producing trait-marker associations. This finding suggests that models controlling for either kinship alone or structure as measured by the principal components and kinship minimize the chance of spurious association. Because of this, only association results from K model are presented in this study.

Markers linked to grain mold resistance

To identify loci linked to resistance, we looked for clusters of SNP markers located within 200 bp of each other. If there was a positive association with a resistance phenotype, there should be at least two markers that show a strong association. This association mapping criterion has been used previously with high-density markers (Hiltunen et al. 2001; Beló et al. 2008). Each cluster is referred as the SNP locus. For all loci presented in this paper, the two flanking markers were also included to mark the genomic region for the convenience of identifying disease resistance-related genes. Ideally, all markers from a locus truly associated with a resistance phenotype will show a low p value, whereas the two flanking markers some distance from the locus will have much higher p values. Based on the preset p value of 10^{-4}, two SNP loci [grain mold (GM) Loci 1 and 2] were found associated with grain mold resistance in both 2007 and 2008 but only GM Locus 1 was linked to grain mold resistance with a p value lower than the Bonferroni threshold of 6.78×10^{-6} (Fig. 2).

For GM Locus 1, the association was stronger in 2008 when the p values for all three markers associated with resistance were lower than the Bonferroni threshold. The association was also strong in 2007, with all the markers in the locus having association

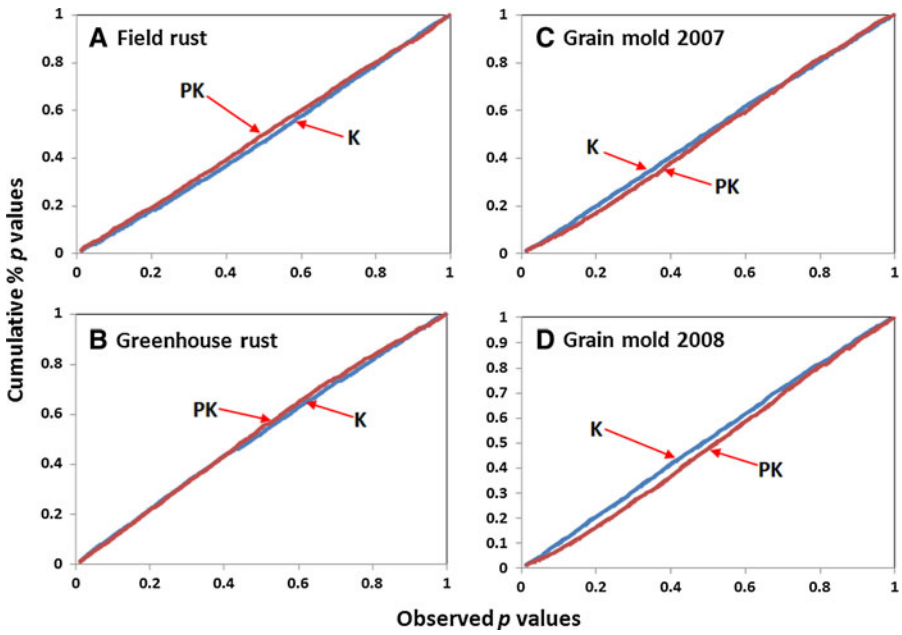


Fig. 1 Cumulative distribution of p values computed from 14,739 single nucleotide polymorphisms (SNP) for the K (kinship), and PK (principal component and kinship) association models with sorghum leaf rust and grain mold (*GM*). A more uniform p value distribution indicates a better model. The *lines* of K, and PK partially overlap in both traits

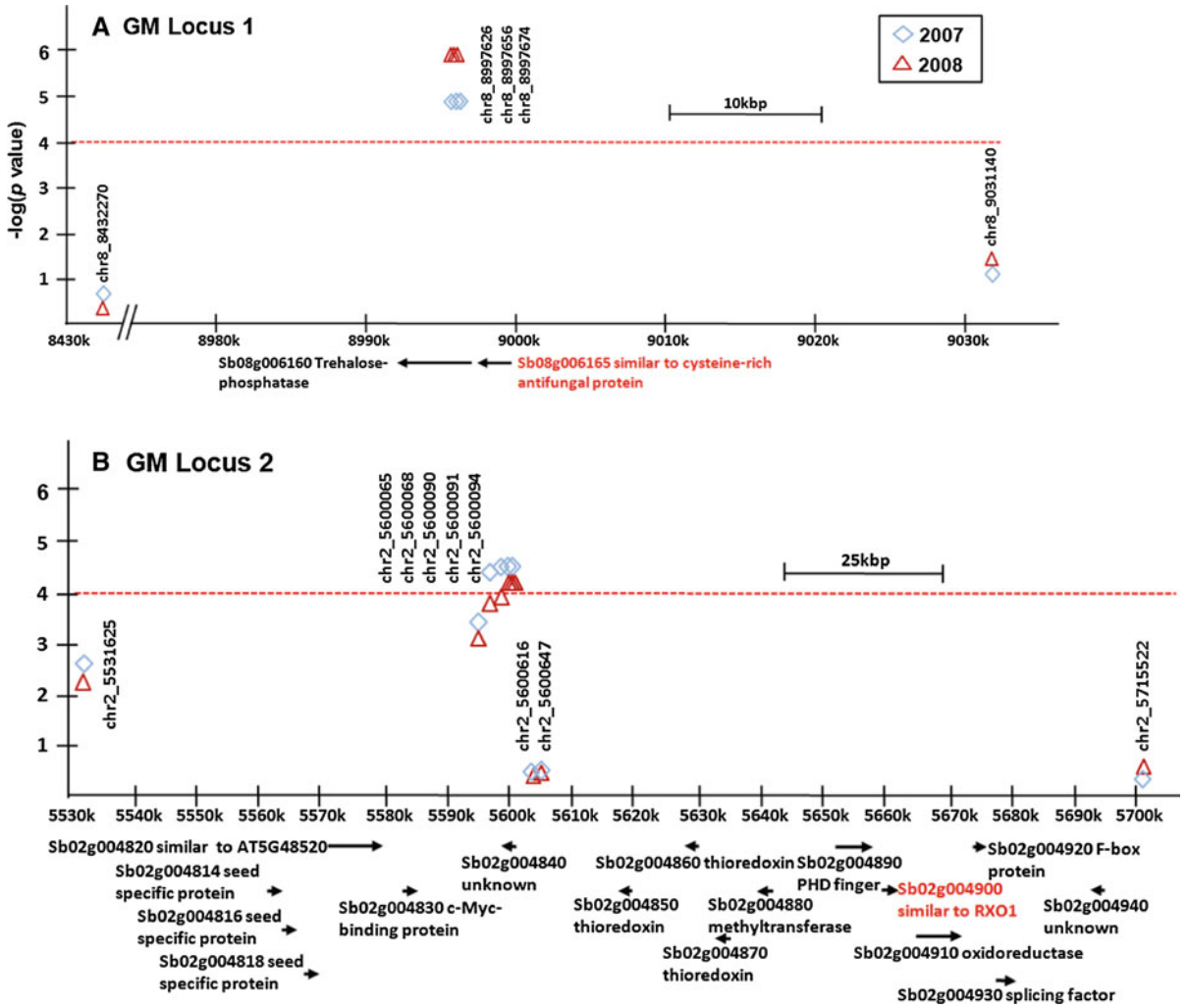


Fig. 2 Two clusters (loci) of SNP markers (*diamond* or *triangle*) associated with sorghum grain mold. **a** GM Locus 1, **b** GM Locus 2. *Dashed lines* represent the preset association p value of 10^{-4}. *Y*-axis is the $-\log(p)$, a measure of p value, *X*-axis is the physical location along the chromosome in base pairs. *Below* the *X*-axis are all annotated genes in the region shown around the loci. *Arrow* and *length* represent gene direction and size, respectively. All markers in each locus were shown and were spread out to allow better view. Otherwise, all positions were drawn to the scale shown. Gene annotation and physical location were based on data from www.phytozome.net/sorghum

p values of $<10^{-4}$. This locus represents the ideal case of clustered SNP markers associated with the phenotypic trait: all markers in the locus were associated with similarly low p values, while the two flanking markers which were 33 and 564 kb away, respectively, were not associated with grain mold resistance (Fig. 2). Association of GM Locus 2 with grain mold resistance was less strong because some markers in the locus showed much lower p values than others: the five markers close to the flanking marker chr2_5531625, namely, chr2_5600065, chr2_5600068, chr2_5600090, chr2_5600091, and chr2_5600094, were more strongly associated with grain mold than the two markers on the other side, chr2_5600616 and chr2_5600647. These two loci were associated with the resistance in both years, suggesting robust and true associations.

Markers linked to rust resistance

As described above, the results from field and greenhouse rust evaluations were not highly correlated. Based on our criteria for identifying markers strongly associated with phenotypes as described above (association of at least two markers in the same locus with

p values of $<10^{-4}$), no associations with rust resistance in the greenhouse evaluation were found. Disease scores from the field evaluation showed more phenotypic variation, with a variance of 482.18 and a mean of 25.79 compared to 364.03 and 47.29, respectively, from the greenhouse evaluation. In contrast, the association analysis based on the field evaluation identified five SNP loci linked to rust resistance (Rust Loci 1–5; Fig. 3). Among the five, Loci 1, 3, and 5 represented ideal linked loci: all markers in a locus truly associated with a resistance phenotype all showed a low p value, whereas the two flanking markers some distance from the locus had much higher p values. For Loci 2 and 4, the association strength as measured by p values was less uniform because among adjacent markers one may be linked but another may not be linked to resistance. For example, in Locus 2, chr3_64341071 was associated with rust resistance with a p value of 0.0054. However, its two flanking markers, chr3_64341029 and chr3_64341099, were associated with p values of 9.34×10^{-6} and 5.87×10^{-4}, respectively. Based on p values of all markers in a locus, Locus 5 was most strongly associated with field rust resistance (Fig. 3).

Resistance-related genes in linked loci

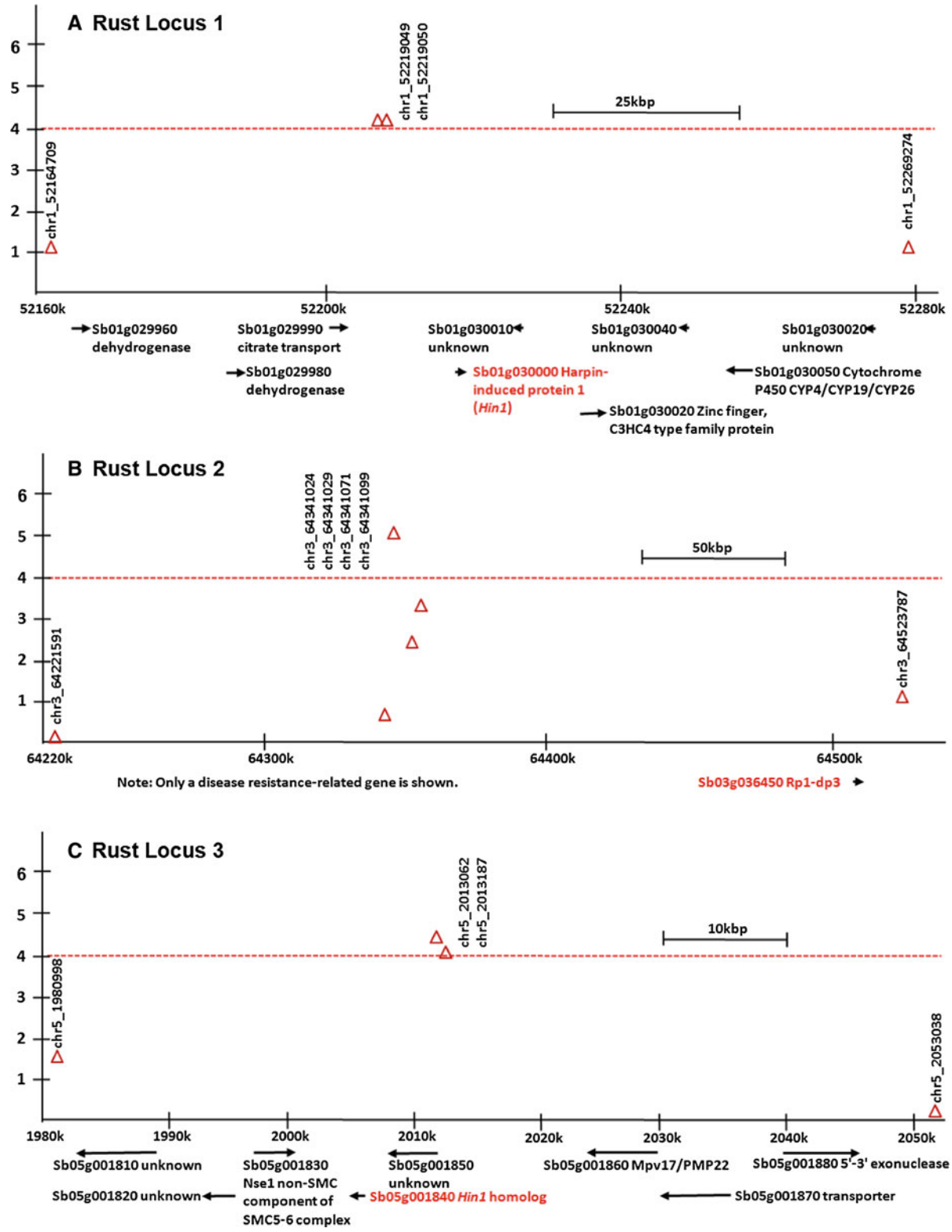
As described above, among the five loci linked to rust resistance, Loci 2 and 4 showed a mixed association strength among markers in the same locus. Among the two grain mold resistance-linked loci, GM Locus 2 showed a mixed association strength. Coincidentally, resistance (*R*) gene homologs were found only around these three loci –and not in GM Locus 1 nor in Rust Loci 1, 3, and 5. And all belonged to the NB-ARC class of *R* genes. GM Locus 2 contained a NB-ARC-LRR class of R gene (Sb02g004900) that shares 37 % identity and 57 % similarity to the maize nonhost resistance protein Rxo1 (accession AAX31149). Rust Locus 2 housed one of the two rust resistance loci (*Rp1*) (Luo et al. 2011), Sb03g036450 (*Rp1-dp3*), which is 59 % identical and 70 % similar to the maize rust resistance protein *Rp1-D* (accession AAD47197). Rust Locus 4 had five *R* genes, namely, Sb08g022200, Sb08g022190, Sb08g022180, Sb08g022170, and Sb08g022150, located 98–138 kb from the locus. Sb08g022180, Sb08g022170, and Sb08g022150 are not shown in Fig. 3. Sb08g022200, Sb08g022190, and Sb08g022180 were 33 % identical and 50 % similar to the wheat rust resistance protein Lr1 (accession ABS29034), and the other two share a lower homology. For the remaining loci mapped, Rust Loci 1 and 3 were each close to the Harpin-induced 1 (*Hin1*) gene (Sb01g030000 and Sb05g001840, respectively); Rust Locus 5 contained two cyclophilin genes (Sb10g026950 and Sb10g026940) and GM Locus 1 contained a gene (Sb08g006165) similar to Arabidopsis cysteine-rich receptor-like protein kinase (CRK) 27 and 13 (Figs. 1, 2).

As a general rule, the *R* gene homologs were located farther away from the linked loci than the non-*R* related disease resistance genes. For example, the homologs of RXO1, Rp1-D, and Lr1 were 61, 168 and 98–138 kb from their respectively linked loci, while the homologs of the Hin1, cyclophilins, and AT3G22060 in Rust Loci 1, 3, and 5 and GM Locus 1 were 1.8, 8.2, 18.7, and 0.7 kb from their respectively linked loci (Figs. 1, 2). The size of the linkage disequilibrium block in sorghum can range from 10 to 100 kb (Hamblin et al. 2005; Bouchet et al. 2012; Wang et al. 2013).

Fig. 3 Five (**a**–**e**) SNP loci (Rust Loci 1–5) associated with sorghum rust resistance evaluated under field conditions. *Triangle* SNP marker, *dashed lines* the preset association p value of 10^{-4}. The *Y-axis* is the $-\log(p)$, a measure of p value, the *X-axis* is the physical location along chromosome in base pairs. Below the *X*-axis are all annotated genes in the region shown around the loci unless noted otherwise. *Arrow* and *length* represent gene direction and size, respectively. All markers in each locus were shown and were spread out to allow better view. Otherwise, all positions were drawn to the scale shown. Gene annotation and physical location were based on data from www.phytozome.net/sorghum

Discussion

Disease resistance can be variable depending on the plant growth stages evaluated. In sorghum rust, we did not identify any markers linked to rust resistance at the seedling stage (greenhouse evaluation). We noticed that while accessions evaluated as resistant at the seedling stage were resistant in the field at the dough stage, such as IS473, IS23521, and IS23684 (one exception was IS26737), those scored as susceptible at the seedling stage were also resistant at the dough stage, such as IS1004, IS7305, IS7679, and at least 23 other accessions. This finding is in agreement with a previous study by Johnston and Mains (1931) who


A Rust Locus 1
chr1_52219049
chr1_52219050
25kbp
chr1_52164709
chr1_52269274
52160k
52200k
52240k
52280k
Sb01g029960 dehydrogenase
Sb01g029990 citrate transport
Sb01g030010 unknown
Sb01g030040 unknown
Sb01g030020 unknown
Sb01g029980 dehydrogenase
Sb01g030000 Harpin-induced protein 1 (Hin1)
Sb01g030050 Cytochrome P450 CYP4/CYP19/CYP26
Sb01g030020 Zinc finger, C3HC4 type family protein
B Rust Locus 2
chr3_64341024
chr3_64341029
chr3_64341071
chr3_64341099
50kbp
chr3_64221591
chr3_64523787
64220k
64300k
64400k
64500k
Note: Only a disease resistance-related gene is shown.
Sb03g036450 Rp1-dp3
C Rust Locus 3
chr5_2013062
chr5_2013187
10kbp
chr5_1980998
chr5_2053038
1980k
1990k
2000k
2010k
2020k
2030k
2040k
2050k
Sb05g001810 unknown
Sb05g001820 unknown
Sb05g001830 Nse1 non-SMC component of SMC5-6 complex
Sb05g001840 Hin1 homolog
Sb05g001850 unknown
Sb05g001860 Mpv17/PMP22
Sb05g001880 5'-3' exonuclease
Sb05g001870 transporter

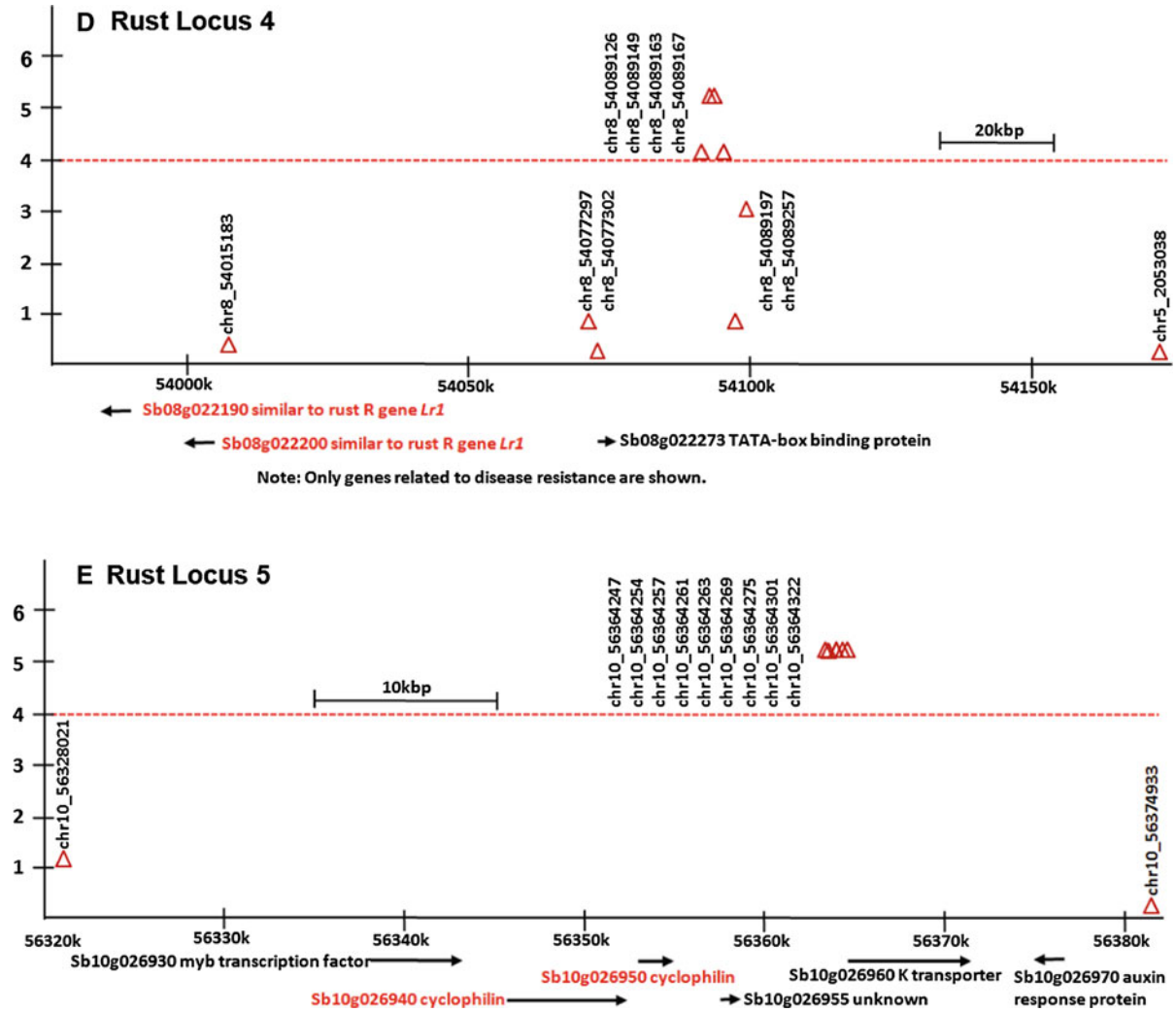


Fig. 3 continued

reported that plants highly resistant at the seedling stage are also resistant at later growth stages in the field, but plants susceptible at seedling stage may be resistant or susceptible in the field. Thakur et al. (2007) suggested that field screening of adult plants be used to identify resistance against the more variable pathogen populations.

In this study, we identified SNP loci linked to grain mold and rust resistance using two association models, K and PK. In agreement with the results of studies in maize (Yang et al. 2011) and barley (Pasam et al. 2011), we also found that the K and PK models produced similar results. We identified five SNP loci linked to rust resistance but only two linked to resistance to grain mold, probably because grain mold resistance was only evaluated at one location. As suggested by Little et al. (2012), grain mold pathogen populations may be different among different environments, causing differences in resistance expression. This has also been demonstrated by Klein et al. (2001) who found a total of five loci linked to grain mold resistance among three locations: College Station, Corpus Christi, and Beeville. Breaking down the results by location, these authors found three resistance loci each in College Station and Beeville but only two in Corpus Christi. This finding may also explain why the two loci mapped in this study did not overlap with those mapped by Klein et al. (2001). However, if we relax the p value requirement, we find that two other loci, chr9_2981112 and chr10_4192844, are close to

two resistance loci mapped by Klein et al. (2001). Chr9_2981112 located at 2.98 Mb on chromosome 9 is close to a grain mold resistance locus around Xtxp67 located at 5.16 Mb by Klein et al. (2001). Chr10_4192844 located at 41.92 Mb on chromosome 10 is either on the same side with the resistance locus close to Xtxp130 at 47.13 Mb or on the other side, as the map could not be oriented based on Xtxp130 (others could not be physically placed) (Klein et al. 2001). Similarly, if we relax the *p* value requirement, we have one marker associated with rust resistance at the seedling stage, chr6_61258880 located at 61.25 Mb on chromosome 6 (SBI-06), which is very close to a rust locus mapped by Murali Mohan et al. (2010) distal to Xtxp17 at 58.25 Mb.

Candidate genes close to the mapped SNP loci were also identified, such as rust *R* genes homologous to the maize rust resistance gene *Rp1-D* and the wheat rust resistance gene *Lr1*, respectively. The sorghum genome was found to have the ancestral rust resistance *Rp1* haplotype because it has two *Rp1* loci (A locus on SBI-08 and B locus on SBI-03), while the genomes of rice, *Brachypodium distachyon*, and maize maintain only one of these. Specifically, maize harbors locus A, whereas rice and *B. distachyon* harbor locus B, and only sorghum has both; it is therefore possible that the two loci have experienced constant duplications and/or deletions in different lineages of the Poaceae family (Luo et al. 2011). The A locus on SBI-08 contains *Pu* (Coleman and Dean 1961; Miller and Cruzado 1969) located between 2,487,742 and 2,514,226 bp. This locus contains a cluster of six putative rust resistance gene candidates: Sb08g002340, Sb08g002345, Sb08g002350, Sb08g002380, Sb08g002390, and Sb08g002410 (Mace and Jordan 2010). This A or *Pu* locus was not identified in our study, but we did identify the B locus which is Sb03g036450 (*Rp1-dp3*) (Luo et al. 2011). Unlike the A locus, there is only one copy of the gene in the B locus. *Rp1-dp3* is homologous to the maize *Rp1-D* (Collins et al. 1999) as described above and is 56 % identical and 68 % similar to the cloned rice blast resistance gene *Pi37* (Lin et al. 2007). Compared to *Rp1-D* and *Pi37*, *Rp1-dp3* contains two deletions of 22 and 26 amino acids at two amino acids apart in the NB-ARC domain outside the conserved kinase 1a (P-loop), kinase 2, and kinase 3a consensus motifs. Six additional deletions of 8–20 amino acids were also found in the beginning of the LRR (leucine-rich repeats) domain (data not shown).

Rust Locus 4 harbors five homologs of the wheat rust resistance protein Lr1 tandemly arranged in the locus. There are ten copies of this gene in wheat, and three are in one locus, the *Lr1* locus on chromosome 5D (Cloutier et al. 2007). Multiple copies of this gene were also found in the sorghum genome. Based on a protein sequence homology search, in addition to five copies in Rust Locus 4, four copies were each clustered on SBI-06 (Sb06g029220, Sb06g029250, Sb06g029230, and Sb06g028930) and SBI-08 (Sb08g021230, Sb08g021243, Sb08g021280, and Sb08g021290). The latter is about 1 Mb from Rust Locus 4.

Another NB-ARC-LRR class of *R* gene homologous to the maize nonhost resistance protein Rxo1 (Zhao et al. 2005) was found in GM Locus 2. Grain mold is not caused by just one pathogen but may be caused by as many as 42 different pathogens, although at a particular location some pathogens may be predominant (Little et al. 2012). Because of this, deploying pathogen-specific *R* gene system may not be effective. It is sensible that a nonhost resistance gene be used instead. Knockout of *Rxo1* in maize abolishes its resistance to the rice pathogen *Xanthomonas oryzae* pv. *oryzicola*, which causes bacterial streak disease, while overexpression confers resistance to both *X. oryzae* pv. *oryzicola* and the unrelated pathogen *Burkholderia andropogonis*, which causes bacterial stripe in sorghum and maize (Zhao et al. 2005). This finding suggests that *Rxo1* plays an important role in both host and nonhost resistance which is relevant in defense against multiple pathogens as in grain mold.

In the initial stage of infection, the pathogen effector (the Avr protein) is recognized by the R protein. This activates effector-triggered immunity leading to the hypersensitive response (HR) (Jones and Dangl 2006). In Arabidopsis, *Pseudomonas syringae* AvrRpt2 protein is delivered to inside the plant cell in the inactive state. ROC1 cyclophilin then folds AvrRpt2 into an active form. The activated AvrRpt2 then cleaves RIN4 which then serves as a signal to activate RPS2-mediated resistance (Coaker et al. 2005). Two cyclophilin genes were found in Rust Locus 5. The gene in GM Locus 1, Sb08g006165, is partially homologous to the Arabidopsis cysteine-rich receptor-like protein kinase (CRK) 27 and 13. CRKs are induced by pathogen infection, and overexpression can trigger HR in transgenic plants not challenged by pathogens (Chen et al. 2004). The induced HR in turn

restricts the growth of infecting pathogen and limits disease spread in Arabidopsis (Acharya et al. 2007). Overexpression of a cotton cyclophilin gene (*GhCyp1*) in tobacco plants confers resistance to *P. syringae* pv. *tabaci* (Zhu et al. 2011). Homologs of *Hin1* were found in Rust Loci 1 and 3. One tobacco homolog of Sb01g030000 and Sb05g001840 (BAD22533), *Hin9*, is up-regulated during the HR due to tobacco mosaic virus infection (Takahashi et al. 2004). Another homolog, *NgCDM1*, is induced by the HR due to inoculation of *P. syringae* pv. *syringae* 61 and during necrotic cell death caused by infiltration of *P. s.* pv. *Tabaci*; NgCDM1 protein was localized in the vicinity of the HR lesion (Suh et al. 2003).

In conclusion, we have identified two marker loci linked to grain mold resistance and five loci linked to rust resistance using the sorghum mini core collection consisting of 242 accessions with 14,739 SNP markers. For a complex disease like grain mold that can be caused by over 40 different pathogens, we identified a locus that contains a nonhost resistance gene *Rxo1* originally discovered in maize. We also identified loci that harbor two different rust resistance genes. The first is similar to the rust resistance locus B that is also homologous to the maize *Rp1-D*. The second is the homolog of wheat rust resistance gene *Lr1*, and five copies of this gene were found to be tandemly repeated in Rust Locus 4. Additionally, genes involved in hypersensitive response were also found in the loci linked to the two diseases. Results from this study should pave way for a more efficient selection of phenotypic resistance to grain mold and rust in sorghum as these markers/candidate genes can be used in marker-assisted selection.

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