

*IN VITRO* AND FIELD BASED EVALUATION FOR GRAIN MOLD RESISTANCE AND  
ITS IMPACT ON QUALITY TRAITS IN SORGHUM [*Sorghum bicolor* (L.) Moench]

by

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## Abstract

Grain mold (GM) is an important biotic constraint limiting yield and market value of sorghum grains. It results in kernel discoloration and deterioration. Such kernels have reduced seed viability, low food and feed quality. Breeding for grain mold resistance is challenging because of the complex nature of host-pathogen-environment interactions. This complex task could be made simpler by utilizing molecular markers. Utilization of marker resources may help to find genomic regions associated with grain mold resistance. In this study, three sets of field and laboratory based experiments were performed which will help in finding potential grain mold pathogens responsible for kernel deterioration in the studied environment and search for genotypes with better kernel quality and grain mold resistance.

In the first part of the study, *in vitro* screening of 44 grain mold resistant sorghum genotypes developed and released by Texas A & M AgriLife Research. This study was aimed at identifying sources resistance to grain mold infection through laboratory screening. The result revealed that genotypes Tx3371, Tx3373, Tx3374, Tx3376, Tx3407, Tx3400, and Tx3402 were have high level of resistance and were identified as potential sources of grain mold resistance as each showed minimal fungal infection and higher grain quality traits.

The second experiment was performed to optimize surface sterilization protocol for the extraction of fungal pathogens from the kernel surface (pericarp) and to study the effect of bleach percentage and time period on pathogen extraction. Seven treatments using sterilized double distilled water (0 % bleach (v/v)) and different bleach (NaOCl) concentrations (2.5, 5, 7.5, 10, 12.5 and 15 %) were used with a time interval of 2.5, 5, 7.5 and 10 min. Optimized surface sterilization in the range of 7.5 to 15 % bleach (v/v) for 7.5 to 10 min resulted least contamination and fungal genera isolation from the surface of the kernel.

The third study was aimed at characterizing genotypes (sorghum association panel) for grain mold pathogen *F. thapsinum* and by using genome wide association (GWA) tool in order to find genomic regions associated with grain mold resistance. We studied the effect of different

agronomic and panicle architecture traits on grain mold incidence and severity. Effects of grain mold on kernel quality traits were also studied. We reported two loci associated with grain mold resistance. Based on first year field screening results, 46 genotypes having grain mold ratings 1-5 (1 = < 1% panicle kernel molded; 5 = > 50% panicle kernel molded) were selected for a detailed study aimed at understanding grain mold x fungal pathogen interactions to physical and chemical kernel traits. Seed germination test, vigor index, and tetrazolium viability test were performed to study effect of grain mold infection on kernel viability and vigor. *Alternaria*, *Fusarium thapsinum*, *F. verticillioides* and *F. proliferatum* were the main fungal genera isolated from bisected kernels. Based on two year screening, SC623, SC67, SC621, SC947 and SC1494 were most resistant based on both PGMR and TGMR rating while SC370, SC833, SC1484, and SC1077 showed the most susceptible reaction and this was consistent for individual location analysis. SC309, SC213, SC833, SC971 and SC1047 are genotypes having identified loci for grain mold resistance.

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# Chapter 1 - *In vitro* evaluation of sorghum lines to identify traits conditioning grain mold resistance

## Abbreviations

Ash = Ash %; Alt = *Alternaria*; Asp = *Aspergillus*; Cla = *Cladosporium*; Cur = *Curvularia*; Coc = *Cochliobolus*; CV = Coefficient of variation; Deo = 3-Deoxyanthocyanidins; DNA = Deoxyribonucleic acid; FFK = Fungal free kernel; Fus = *Fusarium*; Fat = Fat %; Fib = Fiber %; HI = Hardness index; ITS = Internal Transcribed Spacer; KW = Kernel weight; KD = Kernel diameter; Moi = Moisture %; NIR = Near-infrared reflectance; PCA = Principal component analysis; PCR = Polymerase chain reaction; PDA = Potato dextrose solution; PD = Protein digestibility; Phe = Total phenolic acids; PDP = Protein digestibility per cent; Pen = *Penicillium*; Pho = *Phoma*; QTL = Quantitative trait loci; rDNA = Ribosomal DNA; Rhi = *Rhizopus*; SKCS = Single kernel characterization system; TPP = Total protein per cent; TEF = Translation elongation factor; Tan = Total tannins; SD = Standard deviation; Sta = Starch %; Yea = Yeast

## Abstract

*In vitro* screening of 44 grain mold-resistant sorghum genotypes along with four grain mold resistant (Sureño and Tx2911) and susceptible (RTx430 and RTx2737) checks was conducted to 1) identify the fungal complex involved in grain mold infection, 2) identify source of grain mold resistant for use in breeding program, 3) to test the hypothesis that total fungal recovery from internal portion of kernel of genotypes with high mold resistance will lower than kernel of genotypes with low mold resistance, and 4) assess the relationship between disease severity and grain quality, and nutritional traits. The results indicated that most of the genotypes had low levels of infection from *Fusarium*, *Curvularia*, *Phoma*, *Aspergillus* and *Penicillium* species. However, genotypes showed variable levels of infection by *Cladosporium* (5.26 to 24.47 %) and *Alternaria* (45.65 to 77.89 %). Non-pathogenic fungi *Rhizopus* (bread mold) and yeast together constituted 19.53 % of total isolated fungal genera. Correlations between grain mold fungi suggested some fungal genera exhibit an interaction or association effect. Correlation analysis between quality traits suggested harder kernels were smaller in size with low kernel weight, high fiber and low 3-Deoxyanthocyanidins content. Total protein per cent was negatively correlated with protein digestibility per cent and starch per cent. Poor digestibility of sorghum proteins during cooking is a nutritional constraint of sorghum as a food/feed. Results suggest high fiber and starch per cent increases protein digestibility although the positive correlation of protein digestibility per cent with starch per cent contradicts previous findings, which is not easily explainable, and further verification is required. Sorghum genotypes Tx3371, Tx3373, Tx3374, Tx3376, Tx3407, Tx3400, and Tx3402 were identified as potential sources of grain mold resistance as each showed minimal fungal infection and higher grain quality traits.

## Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop of arid and semi-arid regions. Apart from food, it is also used as a source of feed, fodder, fuel and in industries for production of starch, alcoholic beverages and biofuels. It is the fifth largest cereal crop produced in the world after maize, rice, wheat and barley, with a harvested value of US\$1.7 billion in 2013 in the United States alone (FAOSTAT, 2014; USDA-NASS, 2014). Sorghum is primarily utilized as source of feed for livestock in the United States. But it is gaining attention as a bioenergy crop for bioethanol and bio-industrial products (Taylor et al., 2006; Wang et al., 2008). The gluten-free characteristic and potential health benefits such as slow digestibility, and cholesterol-lowering, anti-carcinogenic, and anti-inflammatory properties have increased interest in sorghum as a healthy food (Turner et al., 2006; Dykes and Rooney, 2006; Bralley et al., 2008; Yang et al., 2009; Burdette et al., 2010; Moraes et al., 2012; Ganapathy et al., 2015).

Grain mold is a globally important disease of sorghum and is a continuous problem in the semi-arid tropical production regions of Asia, Africa, and the Americas (Navi et al., 2005; Prom et al., 2014). It is a major biotic constraint when wet and warm weather conditions prevail during grain development (Bandyopadhyay et al., 2000; Ambekar et al., 2011; Prom et al., 2014). Grain mold is a condition in which fungal infection and colonization of spikelet tissues occur prior to grain maturity while grain weathering, occurs when fungi colonize the developing grain after physiological maturity but prior to harvest (Forbes et al., 1992; Little, 2000; Das et al., 2011; Little et al., 2012). Colonization of grain mold fungi occurs on the internal developing tissues of the floret and developing grain (i.e., living tissue). Grain weathering fungi primarily colonize non-living tissue, i.e. mostly external surfaces of the grain (Forbes et al., 1992; Bandyopadhyay et al., 2000; Das et al., 2011; Audilakshmi et al., 2011; Little et al., 2012;). Traditional sorghum cultivars often escape grain mold due to their photoperiod-sensitive nature. Losses are between 30 to 100% depending upon cultivar, flowering time, maturity, and soil type (Williams and Rao, 1981; Singh and Bandyopadhyay, 2000). Global annual economic losses of US\$ 130 million have been reported from Asia and Africa (ICRISAT, 1992). Indian Institute of Millet Research

(IIMR), Hyderabad, India estimated that between 2001-2010 on an average 3000 to 5000 million rupees i.e. around US\$ 50 to 83 million (1US\$= 60 Indian Rupees) was lost per year due to grain mold from 5 major sorghum producing states in India (Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu and Gujarat) (Das and Patil, 2013).

Several fungal genera are found (>40 genera of fungi) to be associated with grain mold (Williams and Rao, 1981; Navi et al., 1999; Singh and Bandyopadhyay, 2000; Thakur et al., 2006; Das et al., 2011; Little et al., 2012). *Fusarium*, *Curvularia*, and *Alternaria* are three genera that are principally associated with grain mold (Little et al., 2012). Differences between early infection and post-maturity colonization can be difficult to substantiate in the field. If sorghum grain is harvested after physiological maturity and incubated on non or semi-selective media the above fungi can be isolated. Based on the relative frequency of isolated fungi, deterioration of kernels due to grain mold can be evaluated and comparisons made among germplasm.

Grain quality is influenced by biochemical and physical characteristics of the sorghum kernel. Important quality traits that are closely correlated with grain mold resistance are kernel hardness (Menkir et al., 1996; Reddy et al., 2000; Audilakshmi et al., 1999; Das et al., 2012), kernel density and integrity (Waniska et al., 1992; Klein et al., 2001), red pericarp (Jambunathan et al., 1992; Reddy et al., 2000; Esele et al., 1993), endosperm texture (grain with a larger proportion of corneous to floury endosperm usually exhibits less weathering (Glueck and Rooney, 1980), phenolic compounds (e.g. phenolic acids) (Waniska et al., 1992; Waniska et al., 2001; Rodriguez-Ballesteros et al., 2008), tannins (Harris and Burns 1973; Menkir et al., 1996; Melake-Berhan et al., 1996), and flavonoids (Jambunathan et al., 1991; Mukuru, 1992; Martizen et al., 1994). Anti-fungal proteins inhibiting fungal growth have been identified from sorghum and are more abundant in grain with hard endosperm (Kumari et al., 1992; Kumari and Chandrashekar, 1994; Waniska et al., 2001).

Very little tannin sorghum is produced in the USA since tannin grain is discounted in the grain market. Phenol and tannin compounds cause dark colors, astringency and decrease nutritional value of food and feeds. The condensed tannins reduce feed efficiency by slowing

and decreasing digestion of the grain component. It is estimated that digestibility and efficiency of absorbed nutrients of consumed sorghum reduced by 3 to 15 % in presence of Tannins. But tannin sorghum is used for making food products such as porridges and alcoholic beverages in Africa (Awika and Rooney, 2004). Tannin sorghums are used in production of beers and alcoholic beverages due to their dark color (Rooney and Awika, 2004). 3-Deoxyanthocyanidins (Deo) is a common studied flavan-4-ols, which have potential use in natural food colorants. Deo is produced as phytoalexins in plants as a response to mold invasion or other stress factors in sorghum (Lo *et al.*, 1999; Seitz, 2004; Waniska and Rooney, 2000). However, selecting sorghums for concentration of flavan-4-ols has been ineffective in creating resistance to molds (Dykes and Rooney, 2006).

Three primary sources of resistance to grain mold are: (i) morphological or physical characters of the seed, glume, and panicle which block fungal penetration of conidial hyphae into host tissues and cells, (ii) secondary metabolites, antifungal proteins and seed storage proteins which usually have significant impact against early infection, and (iii) host plant resistance, which is the result of cumulative effects of many genes affecting several plant characters subjected to the host-pathogen-environment interaction. This interaction is highly complex and variable due to pathogen and environmental variability, and modulates the level of grain mold resistance. Breeding resistance for grain mold in sorghum cultivars is quite challenging. This is because of complex interaction of host-pathogen-environment and multiple mechanism of resistance (major or minor genes and epistatic gene interactions). Due to these hurdles, breeding for increased grain mold resistance has had limited success (Williams and Rao, 1981; Audilakshmi *et al.*, 1999; Chandrashekar *et al.*, 2000; Audilakshmi *et al.*, 2000; Little, 2000; Bandyopadhyay *et al.*, 2000; Waniska and Rooney, 2000; Rodriguez *et al.*, 2000; Rooney and Klein, 2000; Audilakshmi *et al.*, 2005; Thakur *et al.*, 2006; Klein *et al.*, 2001; Das *et al.*, 2011; Audilakshmi *et al.*, 2011).

The main objectives/purpose for undertaking this research study were to: (i) identify the fungal complex involved in grain mold infection, ii) identify resistant sources, iii) to test the hypothesis that total fungal recovery from internal portion of kernel of lines with high mold resistance are lower than kernel of lines with low mold resistance, and (iv) assess the

relationship within and between grain mold fungi and quality traits.

## **Materials and Methods**

**Genetic materials:** Forty-four grain mold-resistant sorghum genotypes along with four-grain mold checks (resistant: Sureño and RTx2911; susceptible: RTx430 and RTx2737) were planted in three replications in a field trial at Lubbock, Texas in 2013. The forty-four genotypes were developed and released by Texas A&M AgriLife Research as sources of resistance to grain mold and grain weathering in improved agronomic backgrounds (Rosenow et al., 2014). These genotypes were evaluated for grain-mold ratings conducted at College Station, TX (2007, 2008, 2009, and 2012) and Weslaco, TX (2012). Grain mold resistance was based on a visual evaluation scale for the grain in the field (Frederiksen et al., 1991; Thakur et al., 2006). In addition to grain weathering rating, these genotypes were evaluated for lodging on a scale of 1 to 9 (1 = 0-10% lodging; 9 = 81-100% lodging) at Lubbock, TX, in 2009 and Corpus Christi, in 2012 (Rosenow et al., 2014). The checks were selected based on knowledge of their response to grain mold. Agronomic practices followed were common for limited irrigation sorghum production in the region. After maturity three randomly selected individual open pollinated panicles per genotype per replication were harvested. For grain mold screening and quality trait analysis, bulked seeds per genotype per replication were used.

**Fungi isolation:** Kernels were incubated in a 10% bleach solution (NaOCl) for 10 min followed by repeated washing with autoclaved double distilled water and dried. Bisected kernels were treated again with 10% bleach for 10 min followed by repeated washing with autoclaved double distilled water. Bisected kernels were transferred to filter paper for drying. Individual bisected kernels (10 bisected kernels per plate) were plated endosperm-side down on half-strength potato dextrose agar (PDA) plates in order to expose interior caryopsis tissues directly to the fungal isolation medium. The media was treated with streptomycin (20 µg per L), tetracycline (10 µg per L), and penicillin (10 µg per L) to prevent bacterial contamination. Plates were incubated at 26°C approximately for 84 to 96 hours. Fungal colonies growing from kernels were counted and identified for species as described below.

**Fungi identification:** To obtain deoxyribonucleic acid (DNA), single-spore fungal isolates were grown in PDA plates for 72 hours at 26°C. Genomic DNA was extracted from the resultant mycelium using Epicentric- an Illumina company - MasterPure™ DNA extraction Kit. Extracted fungal DNA was quantified using a ND-1000 Nanodropper spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). Using Internal Transcribed Spacer (ITS) primers [ITS4 (5' TCCTCCGCTTATTGATATGC- 3' and ITS5 (5'GGAAGTAAAAGTCGTAACAAGG- 3')] morphological groups were identified to genus via sequencing (White et al., 1990). 1.0 µl (0.25 pmoles per µl) of ITS PCR primers (ITS 4 and ITS 5) were used for amplifying fungal DNA (1-20 ng per µl). Standard PCR reaction mixture (25 µl) consisted of 12.5 µl PCR Mastermix (Promega), 2 µl each of forward and reverse primers, 2 µl DNA template and 6.5 µl nuclease free water. PCR cycling conditions for the amplification profile were: initial denaturation of the template DNA at 94°C for 1 min, it was followed by 34 cycles (Denaturation) at 94°C for 30 sec, 50 °C for 45 sec, 72 °C for 1 min (Annealing) and followed by a cycle at 72 °C for 7 min (Extension). *Fusarium* colonies were classified up to species level using *ef1* forward (5'-ATGGGTAAGGA(A/G)GACAAGAC - 3') and *ef2* reverse (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') primers (O' Donnell et al., 1998). In case of translation *ef* PCR primers, 2.0 µl (2.5 pmoles per µl) of each primer (*ef1* and *ef2*) (O' Donnell et al., 1998) were used for amplification of *Fusarium* template DNA (1-20 ng/µl). Standard PCR reaction mixture for *ef* was identical as for ITS. PCR cycling conditions for *ef* were: a one min initial denaturation at 94°C; 35 cycles of amplification of 30 s at 94°C, 45 sec at 56°C and a 1 min elongation at 72°C and final elongation of 7 min at 72°C. All PCR reactions were performed on an Applied Biosystems Veriti® 96-Well fast thermal cycler (Life Technologies, NY, USA). PCR products were cleaned using a MIDSCI RapidTip purification kit. Cleaned PCR products were used for sequencing at Department of Plant Pathology, Kansas State University sequencing facility. Using BioEdit (v. 7.2.5; Hall, 1999) sequences were edited manually. Edited sequences were aligned with sequences available from the BLAST database ([www.ncbi.nlm.nih.gov/Blast.cgi](http://www.ncbi.nlm.nih.gov/Blast.cgi); Altschul et al., 1990). For *Fusarium* samples in addition to BLAST database, *Fusarium* ID (Geiser et al., 2004) database developed by Penn State University (<http://isolate.fusariumdb.org/index.php>) was

used.

**Quality traits analysis:** The genotypes were analyzed using the single kernel characterization system (SKCS) for hardness index (HI) and physical grain traits including kernel weight (KW) and kernel diameter (KD) (Bean et al., 2006; Pedersen et al., 1996). Additionally, total protein per cent (TPP) at dry basis and protein digestibility per cent (PDP) were determined at the USDA Grain Marketing and Research Laboratory, Manhattan, Kansas. Eight quality traits were analyzed (phenols, tannins, moisture, fat, fiber, ash, starch and Deo) using the near-infrared reflectance (NIR) spectroscopy technique at Texas A&M University, College Station, Texas (Dykes et al., 2014). For each trait, measurements were replicated two times. For accurate prediction of total phenol, condensed tannin and Deo of NIR data, NIR calibration range values developed by Dykes et al. (2014) were used. Based on these calibration estimates, genotypes with phenols less than 10 mg gallic acid equivalents per g and tannins less than 14 mg catechin equivalents per g were considered phenols and tannins free.

**Data analysis:** Percentage scores of fungal colonies were transformed using log transformation for statistical analysis. Data were analyzed using Microsoft Excel and PROC MIXED, PROC REG and PROC CORR procedure of SAS 9.3 software (SAS 9.3, SAS Institute, 2010). Fungal isolation frequencies and relative percentage of isolated fungal genera were calculated according to Gonzalez et al. (1995) and Mahmoud et al. (2013).

## **Results and Discussion**

Traditional fungal taxonomy is based on morphological and pathogenic characterization. Molecular marker based approaches for fungal plant pathogen characterizations are more reliable and sound. For genus level grain mold fungal identification, sequence comparisons based on the nuclear encoded ribosomal DNA (rDNA) gene of the ITS is commonly used. This is because of its great variability among fungal organisms (White et al., 1990). The translation elongation factor (TEF) 1-a gene has high phylogenetic utility at the species level classification in *Fusarium*. This is because of the facts that this gene encodes an essential part



of the protein translation machinery and for this gene non-orthologous copies were not found from the *Fusarium* genus (O'Donnell et al., 1998). Using ITS and *ef* primers, 20 fungal colonies isolated based on morphology were differentiated into 10 different groups (Table 1.1). Yeast's colonies were counted but not identified. *Fusarium* fungal colonies were classified using *ef* primers into 2 species groups. 99.50% of these colonies were *Fusarium thapsinum* and 0.50% was *F. chlamydosporum*.

The frequencies of fungi genera differed from sample to sample. *Alternaria* was the most frequently isolated genus (60.44%), followed by *Cladosporium* (14.35%) and yeast (13.45); while *Cochliobolus* (0.02%), *Curvularia* (0.09%), *Penicillium* (0.09%) and *Aspergillus* spp. (0.55) were the least frequently isolated genera (Table 1.1; Figure 1.1). Non-pathogenic fungi *Rhizopus* (bread mold) and yeast together constitute 19.53 % of total isolated fungal genera. This suggested that these genera are common contaminants, which invade sorghum kernels at any time due to their relatively versatile environmental requirements. The high percentage of these genera could be due to their presence as commensal residents on the kernel surface, which did not wash off, even after surface sterilization of whole and bisected kernels with 10% bleach. *Cochliobolus* had the highest coefficient of variation (CV) followed by *Penicillium*, *Curvularia*, and *Phoma* (Table 1.1). The high CV value reflected the inconsistent distribution of these genera in the sorghum seed samples. 2.86 % of kernel samples plated on PDA plates were free from fungal growth, which will be called fungal free kernel (FFK). The sorghum genotypes tested in this study had 0.00 to 19.44% FFK's. Thus, the frequency of kernels free from fungal infection showed possible mold resistant sorghum genotypes. Tx3407 (19.44%) had a higher value than the standard grain mold resistant check, Sureño (15.56%). Other genotypes with high frequency of infection-free kernels were Tx3372 (12.22%), Tx3374 (7.78%), Tx3390 (5.56%), and Tx3389 (4.59%) (Table 1.2). All of these genotypes had a high percentage of FFK's even compared to the grain mold resistant check, Tx2911 (3.89%).

Most of the grain mold resistant genotypes tested in this study had low levels of infection from *Fusarium*, *Curvularia*, *Phoma*, *Aspergillus*, and *Penicillium* spp. These genotypes showed variable levels of infection from *Cladosporium* (5.26 to 24.47%) and

*Alternaria* (45.65 to 77.89%). Based on overall frequency scores comparisons for fungi and FFK of sorghum genotypes with checks, the top eight and bottom eight genotypes were selected (Table 1.2).

NIR range values calibration optimized by Dykes et al. (2014) revealed that total phenol and tannins are negligible in sorghum genotypes used for this study i.e. they are basically phenol and tannin free (Table 1.2, Figure 1.2). Based on the statistics for the quality traits (Table 1.3, Figure 1.2), the sorghum genotypes were characterized as medium sized kernels with high HI. Mean weight and diameter were 25.05 mg and 2.17 mm respectively. Protein content was intermediate to high with high starch content. Deo, Fat, fiber, ash and fat per cent were very low. Past studies confirmed that sorghum lines with high grain hardness, grain density, tannin, phenol and high proportion of corneous to floury endosperm contents are resistant to mold but none confer complete resistance (Harris and Burns, 1973; Glueck and Rooney, 1980; Jambunathan et al 1991; Mukuru, 1992; Waniska et al., 1992; Martizen et al., 1994; Menkir et al. 1996; Melake-Berhan et al., 1996; Audilakshmi et al., 1999; Reddy et al., 2000; Waniska et al., 2001; Dykes and Rooney, 2006). Grain traits like high protein, fiber and starch are important in determining nutritional value of sorghum but breeding these traits is very challenging and time consuming. Physical traits and NIR study analysis revealed that sorghum genotypes Tx3364, Tx3366, Tx3369, Tx3373, Tx3376, Tx3377, Tx3378 exhibit moderate to high protein, starch, fiber content with low level of phenol and tannin accumulation (Table 1.4).

Correlation studies between grain mold fungi revealed significant positive correlation between *Fusarium* and *Phoma* ( $r = 0.53$ ), *Aspergillus* and *Penicillium* ( $r = 0.35$ ), and *Rhizopus* and Yeast ( $r = 0.71$ ) (Table 1.5, Figure 1.4). Significant negative correlations were identified for *Fusarium* and *Alternaria* ( $r = -0.36$ ), *Cladosporium* and *Phoma* ( $r = -0.30$ ), *Cladosporium* and *Alternaria* ( $r = -0.62$ ), *Alternaria* and Yeast ( $r = -0.68$ ) (Table 1.5, Figure 1.4). This indicates that some fungal genera have interaction or association effects. There is a need more in-depth research into these associations to learn more about their behavior, degree of relationship between the interaction and about competition between fungi that occupy nominally the same eco-niche. However, under field conditions because of complex

interaction of host-pathogen-environment these data might be of limited value. It could be possible that isolated strains behavior under natural environmental conditions will be totally different in terms of growth rate and frequency from artificial media under laboratory conditions.

Strong consistent correlations do not exist between quality traits and grain mold pathogens (Table 1.6). Correlations between fungus and quality traits (Table 1.6) revealed that *Fusarium* has a significant negative correlation of 0.33 with fiber per cent. *Aspergillus* has significant negative correlation with HI ( $r = -0.36$ ). *Cladosporium* showed significant negative correlation with TPP ( $r = -0.50$ ). *Phoma* showed positive correlation with TPP ( $r = 0.31$ ). *Rhizopus* has significant negative correlation with moisture per cent ( $r = -0.31$ ). Significant correlations were identified among quality traits (Table 1.7). HI exhibited significant negative correlation with KW ( $r = -0.57$ ), KD ( $r = -0.68$ ) and Deo ( $r = -0.36$ ) but a significant positive correlation with fiber per cent ( $r = 0.51$ ). These results suggested that harder kernels were smaller in size with low kernel weight, high fiber and low Deo content. TPP was significantly negatively correlated with PDP ( $r = -0.31$ ) and starch per cent ( $r = -0.61$ ). PDP expressed a significant positive correlation with starch per cent ( $r = 0.35$ ) and fiber per cent ( $r = +0.39$ ) but a significant negative correlation with fat per cent ( $r = -0.30$ ). Based on these results protein will be higher in kernels with lower starch per cent and PDP. Previous studies showed that proteins in sorghum are located within starchy endosperm, which comprises around 70 % the sorghum total grain protein (Duodu, 2003). This suggests that sorghum kernels with lower starchy endosperm will have higher protein content. Poor protein digestibility in cooking is a nutritional constraint of sorghum as a food/feed. A protein with high digestibility would have better nutritional value than one with low digestibility. This is because, high digestibility protein provides more amino acids for absorption on proteolysis compared to low digestibility protein. Factors such as interaction of protein with non-protein components like starch and lipids, and endogenous factors, which arise out of changes in sorghum proteins, such as like protein and Disulphide cross linking, contribute to the poor digestibility of sorghum proteins (Duodu, 2003; Wong et al., 2009). Results indicate that high fiber and starch content increases PD but the positive correlation of PD with starch is contradictory with previous research, is not easily explainable and need

further verification. KW was positively correlated with KD ( $r = 0.86$ ) and Deo ( $r = 0.43$ ) but negatively correlation with fiber per cent ( $r = -0.53$ ) (Figure 1.4) and starch per cent ( $r = -0.50$ ) (Table 1.7). Negative correlations of KW with fiber and starch per cent need further investigation to better explain and understand this finding. Fiber per cent was negatively correlated with KW ( $r = -0.53$ ), KD ( $r = -0.44$ ) and Deo ( $r = -0.49$ ). This suggests that sorghum kernels with small size and weight will have high fiber content which would be further influenced by Deo content. These findings are also not easily explainable and require further investigation for possible explanation. Starch per cent is negatively correlated with KW ( $r = -0.53$ ), KD ( $r = -0.43$ ) TP ( $r = -0.61$ ), Deo ( $r = -0.37$ ) and positively correlated with PD ( $r = 0.35$ ) and fiber per cent ( $r = 0.50$ ). A reasonable explanation for the negative correlation of starch per cent is that large sorghum kernels have bigger triploid endosperm while smaller sorghum kernels have smaller endosperm. Deo was negatively correlated with HI ( $r = -0.36$ ) but positively correlated with KW ( $r = 0.43$ ) and KD ( $r = 0.35$ ). These findings also need to be further investigated for better understanding and possible explanation of the outcomes.

Principal component analysis (PCA) plot for fungal pathogens and quality traits explained 20.00 % variability between them through principal component (PC) 1. PC 2 explained 12.10 % variability (Figure 1.5). Quality traits KD, KW, Deo, Tannins, Phenol, TPP are present in PC 1 with fungal groups *Fusarium*, *Phoma*, *Aspergillus*, Yeast, *Rhizopus* and *Cladosporium*. This suggested that these quality traits might have some kind of positive association with fungal groups *Fusarium*, *Phoma*, *Aspergillus*, Yeast, *Rhizopus* and *Cladosporium*. Similarly, quality traits HI, PDP, fiber per cent, starch per cent, moisture per cent and fat per cent are present in PC 2 with fungal groups *Alternaria*, *Cochliobolus* and *Penicillium*. These results also suggested that quality traits and fungal groups present in PC 1 have weak correlation with quality traits and fungal groups present in PC 2.

Grain nutritional components such as high protein, protein digestibility (PD), fiber, and starch are important in determining nutritional value of sorghum but breeding for these traits is challenging and time consuming. Physical trait and NIR analysis revealed that sorghum genotypes Tx3364, Tx3366, Tx3369, Tx3372, Tx3373, Tx3376, Tx3377, and

Tx3378 express moderate to high protein, starch, and fiber content with good HI, PD, KD and KW. Replicated evaluations for grain mold resistance of sorghum genotypes from previous studies revealed that out of 44 genotypes, 33 genotypes had a mean score of 2 or 3, which were equivalent to the performance of the resistant checks (Rosenow et al., 2014). Of the top eight genotypes (Tables 1.4), five genotypes (Tx3364, Tx3366, Tx3372, Tx3373, and Tx3377) had a mean score of 3, two genotypes (Tx3369 and Tx3378) had a score of 4, and only one genotype (Tx3376) had a mean score of 2 (Rosenow et al., 2014). The top eight genotypes had lodging scores of 1. These genotypes exhibited lodging resistance than the grain mold resistant checks Sureño and Tx2911, which recorded a mean lodging score of 2 (Rosenow et al., 2014).

## **Conclusion**

Breeding for increased grain mold resistance requires identification of sources with high levels of grain mold resistance and higher grain yield. Sorghum lines with good grain quality are required to meet the varying demands for end-use products. Understanding the chemical and physical properties of sorghum kernels would facilitate screening of genotypes for resistance and for economically important quality traits before subsequent inclusion in a breeding program. Use of a multiple-trait selection index may enhance efficiency and accuracy in selecting sorghum genotypes with a combination of desirable attributes with better mold resistance. Based on fungal isolation frequencies, grain mold scores, lodging scores and quality trait analysis Tx3364, Tx3366, Tx3369, Tx3372, Tx3373, Tx3376, Tx3377, Tx3378 are genotypes potentially useful in developing germplasm with improved grain mold resistance. Identified lines from this study can be used in future breeding programs for developing mapping population to identify QTLs (Quantitative trait loci) and hybrids harboring grain mold resistance genes and for development of parental lines with improved resistance to grain mold.

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**Table 1.1. Summary statistics for fungal genera isolated from sorghum genotypes**

Fungus	Freq <sup>a</sup>	Range <sup>b</sup>	SD <sup>c</sup>	CV% <sup>d</sup>
<i>Fusarium</i>	4.67	1.05-13.68	2.84	60.89
<i>Cladosporium</i>	14.35	5.26-24.47	4.2	29.28
<i>Alternaria</i>	60.44	45.65-77.89	7.45	12.32
<i>Curvularia</i>	0.09	0.00-1.06	0.27	311.86
<i>Aspergillus</i>	0.55	0.00-2.54	0.65	116.44
<i>Penicillium</i>	0.09	0.00-1.59	0.27	312.91
<i>Phoma</i>	0.26	0.00-3.68	0.64	243.79
<i>Cochliobolus</i>	0.02	0.00-1.08	0.16	692.8
Yeast	13.45	4.00-18.00	3.34	24.81
<i>Rhizopus</i>	6.08	2.11-10.46	1.92	31.54
FFK	2.86	0.00-19.44	4.31	150.60

Freq<sup>a</sup> = Frequency (%); Range<sup>b</sup> = Average minimum value-Average maximum value; SD<sup>c</sup> = Standard deviation; CV<sup>d</sup> = Coefficient of variation; FFK = Fungus free kernel (FFK = [Total number of fungal free bisected kernels per sorghum genotype counted on PDA plates/Total number of bisected kernels plated on PDA media plates]\*100)

**Table 1.2. Fungal isolation frequencies for selected sorghum genotypes**

Genotypes	Fus	Cla	Alt	Cur	Asp	Pen	Pho	Coc	Yea	Rhi	FFK
Top eight											
Tx3407	7.35	13.73	62.68	0.00	0.98	0.00	0.00	0.00	10.00	5.26	19.44
Tx3372	6.32	13.16	58.95	0.00	1.05	0.53	0.00	0.00	13.00	7.00	12.22
Tx3374	2.69	16.13	64.85	0.00	0.54	0.00	0.54	0.00	11.00	4.26	7.78
Tx3390	5.26	15.79	54.48	0.00	0.00	0.00	0.53	0.00	16.00	7.94	5.56
Tx3389	6.12	14.80	55.14	0.00	0.00	0.00	0.00	0.00	17.00	6.94	4.59
Tx3379	3.68	13.60	58.78	0.00	0.00	0.00	0.00	0.00	16.00	7.94	4.44
Tx3402	1.55	17.53	64.12	0.00	1.55	0.00	0.00	0.00	11.00	4.26	4.44
Tx3404	2.69	17.20	64.85	0.00	0.00	0.00	0.00	0.00	11.00	4.26	4.40
Bottom eight											
Tx3386	4.69	17.71	55.21	0.00	0.00	0.00	0.00	0.00	17.00	5.40	0.00
Tx3388	3.16	17.37	63.16	1.05	0.00	0.00	0.00	0.00	10.00	5.26	0.00
Tx3394	2.66	13.30	68.78	0.00	0.00	0.00	0.00	0.00	10.00	5.26	0.00
Tx3395	2.13	24.47	45.74	0.00	0.00	0.00	0.00	0.00	18.00	9.66	0.00
Tx3398	3.80	19.57	60.82	0.00	0.54	0.00	0.00	0.00	11.00	4.26	0.00
Tx3377	3.09	16.49	65.42	0.00	0.00	0.00	0.00	0.00	11.56	3.43	0.56
Tx3378	4.62	16.41	58.44	0.00	0.00	0.00	0.00	0.00	15.00	5.54	0.00
Tx3367	2.65	19.58	68.78	0.53	1.06	0.53	0.00	0.00	4.00	2.80	0.00
Checks											
RTx430	3.16	5.26	77.89	0.00	0.53	0.00	1.05	0.00	10.11	1.11	0.00
RTx2737	10.16	6.42	74.33	0.00	0.53	0.00	1.07	0.00	4.00	3.49	0.00
Tx2911	6.95	18.18	53.92	0.00	0.00	0.00	0.00	0.00	14.00	6.95	3.89
Sureño	1.06	10.58	65.08	0.00	0.00	0.00	0.00	0.00	15.00	7.78	15.56

Fus = *Fusarium*; Cla = *Cladosporium*; Alt = *Alternaria*; Cur = *Curvularia*; Asp = *Aspergillus*; Pen = *Penicillium*; Pho = *Phoma*; Coc = *Cochliobolus*; Yea = Yeast; Rhi = *Rhizopus*; FFK= Fungus free kernel (FFK = [Total number of fungal free bisected kernels per sorghum genotype counted on PDA plates/Total number of bisected kernels plated on PDA media plates]\*100)

**Table 1.3. Summary statistics for quality traits**

Fungus	Freq <sup>a</sup>	Range <sup>b</sup>	SD <sup>c</sup>	CV% <sup>d</sup>
Phenol (mg GAE/g)	1.89	0.00-4.94	1.43	76.02
Tannins (mg CE/g)	3.18	0.00-11.72	3.67	115.50
Deo (abs/ml/g)	14.83	0.52-43.73	8.17	55.12
Moisture (%)	7.65	5.41-8.43	0.48	6.32
Fat (%)	4.61	3.76-5.34	0.32	6.93
Fiber (%)	1.95	1.46-2.22	0.18	9.21
Ash (%)	1.18	1.03-1.25	0.04	3.79
Starch (%)	65.25	62.81-67.02	0.97	1.48
HI	90.91	53.98-107.03	11.00	12.10
KW (mg)	25.05	19.59-31.59	3.00	11.99
KD(mm)	2.17	1.9-2.58	0.19	8.70
TPP	13.06	11.15-15.07	0.94	7.18
PDP	62.81	52.28-72.78	5.30	8.44

Phenol= < 10 (mg gallic acid equivalents per g) = 0 (phenol-free); Tannins = < 14 (mg catechin equivalents per g) = 0 (tannin-free); Deo = 3-Deoxyanthocyanidins; HI = Hardness index; KW = Kernel weight (mg); KD = Kernel diameter (mm); TPP = Total protein per centage (dry basis); PDP = Protein digestibility per cent; Freq<sup>a</sup> = Frequency (%); Range<sup>b</sup> = Average minimum value-Average maximum value; SD<sup>c</sup> = Standard deviation; CV<sup>d</sup>= Coefficient of variation

**Table 1.4. Means of quality traits for selected sorghum genotypes**

Genotype	HI	KW	KD	TPP	PDP	Deo	Moi	Fat	Fib	Ash	Sta
<i>Top eight</i>											
Tx3366	100.80	23.00	2.00	13.10	57.70	7.50	7.90	4.80	2.00	1.20	65.50
Tx3369	107.00	22.10	2.00	13.50	55.40	7.10	7.70	4.70	2.10	1.20	64.80
Tx3372	101.10	24.20	2.00	13.60	68.90	13.30	7.80	4.50	2.10	1.20	65.90
Tx3373	102.70	23.60	2.00	13.80	68.70	18.60	7.80	4.50	2.10	1.20	65.50
Tx3376	105.40	21.90	2.00	14.20	60.20	13.00	7.80	4.80	2.10	1.20	64.90
Tx3377	101.30	23.30	2.00	12.40	61.20	25.40	7.80	4.90	2.00	1.20	65.80
Tx3378	102.20	21.30	2.10	12.40	72.30	5.10	7.90	4.70	2.20	1.20	65.10
Tx3364	97.90	24.90	2.10	14.70	55.80	25.70	7.50	4.90	1.80	1.20	63.50
<i>Mean</i>	<i>102.30</i>	<i>23.00</i>	<i>2.00</i>	<i>13.50</i>	<i>62.50</i>	<i>14.50</i>	<i>7.80</i>	<i>4.70</i>	<i>2.10</i>	<i>1.20</i>	<i>65.10</i>
<i>Bottom eight</i>											
Tx3365	78.50	29.40	2.30	13.40	60.00	11.20	7.80	4.40	1.80	1.20	65.50
Tx3368	97.70	23.90	2.10	12.40	64.60	10.20	8.20	4.70	1.80	1.30	65.80
Tx3388	93.20	26.10	2.20	11.40	67.80	20.20	7.90	4.00	2.20	1.20	66.40
Tx3396	80.80	22.70	2.20	12.90	59.60	1.80	7.10	5.10	2.20	1.10	66.40
Tx3397	93.00	24.60	2.10	12.30	66.20	11.40	8.20	4.10	2.00	1.20	66.10
Tx3398	92.30	25.30	2.20	12.40	64.50	13.00	8.10	4.50	2.10	1.20	65.90
Tx3404	96.60	21.40	2.00	12.90	70.90	8.60	8.00	4.60	2.00	1.20	66.20
Tx3389	95.90	23.10	2.00	12.10	65.90	7.50	7.40	4.60	2.20	1.20	66.20
<i>Mean</i>	<i>91.00</i>	<i>24.60</i>	<i>2.10</i>	<i>12.50</i>	<i>64.90</i>	<i>10.50</i>	<i>7.80</i>	<i>4.50</i>	<i>2.00</i>	<i>1.20</i>	<i>66.10</i>
<i>Top vs. Bottom</i>											
<i>p value</i>	<i>0.00</i>	<i>0.14</i>	<i>0.02</i>	<i>0.02</i>	<i>0.38</i>	<i>0.26</i>	<i>0.68</i>	<i>0.12</i>	<i>0.87</i>	<i>1.00</i>	<i>0.01</i>
<i>Checks</i>											
RTx2737	93.70	26.40	2.20	14.20	63.70	0.50	7.90	5.30	1.90	1.10	66.10
RTx430	92.90	31.60	2.50	14.80	61.80	18.20	7.60	4.50	1.80	1.10	64.10
Sureño	98.90	25.70	2.20	13.20	68.50	24.80	5.40	3.80	1.90	1.00	64.20
Tx2911	64.60	30.60	2.50	12.20	64.00	10.80	7.60	4.60	1.60	1.20	64.70

Deo = 3-Deoxyanthocyanins (Abs per ml per g); Moi = Moisture %; Fat = Fat %; Fib = Fiber %; Ash = Ash %; Sta = Starch %; HI = Hardness index; KW = Kernel weight (mg), KD = Kernel diameter (mm); TPP = Total protein per cent (dry basis); PDP = Protein digestibility per cent



**Table 1.5. Correlation (*r*) matrix for isolated grain mold fungi**

Fungi	Fus	Cla	Alt	Cur	Asp	Pen	Pho	Coc	Yea	Rhi	FFK
Fus	1.00	-0.13	-0.36*	-0.05	0.01	-0.12	0.53**	-0.13	0.00	0.05	0.00
Cla		1.00	-0.62**	0.21	0.09	0.02	-0.30*	-0.16	0.12	0.22	-0.23
Alt			1.00	-0.13	-0.09	0.10	-0.14	0.12	-0.68**	-0.73	0.13
Cur				1.00	-0.01	-0.02	-0.10	-0.04	-0.04	0.13	-0.15
Asp					1.00	0.35*	0.06	-0.12	-0.16	0.05	-0.05
Pen						1.00	-0.04	-0.05	-0.19	-0.19	0.12
Pho							1.00	0.06	0.02	0.05	-0.03
Coc								1.00	0.06	-0.08	0.00
Yea									1.00	0.71**	-0.01
Rhi										1.00	0.07
FFK											1.00

\*\*Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

Fus = *Fusarium*; Cla = *Cladosporium*; Alt = *Alternaria*; Cur = *Curvularia*; Asp = *Aspergillus*; Pen = *Penicillium*; Pho = *Phoma*; Coc = *Cochliobolus*; Yea = Yeast; Rhi = *Rhizopus*; FFK= Fungus free kernel (FFK = [Total number of fungal free bisected kernels per sorghum genotype counted on PDA plates/Total number of bisected kernels plated on PDA media plates]\*100)

**Table 1.6. Correlation (*r*) matrix for grain mold pathogens and quality traits**

Traits	Moi	Fib	HI	TPP
<i>Fusarium</i>	0.14	-0.33*	0.13	0.21
<i>Cladosporium</i>	0.09	0.02	-0.23	-0.50**
<i>Alternaria</i>	0.04	0.15	0.16	0.21
<i>Curvularia</i>	-0.01	0.02	-0.07	-0.08
<i>Aspergillus</i>	0.25	-0.25	-0.36*	0.01
<i>Penicillium</i>	0.25	0.09	0.08	-0.16
<i>Phoma</i>	0.15	-0.04	0.24	0.31*
<i>Cochliobolus</i>	0.01	0.12	0.21	0.06
Yeast	-0.25	-0.03	-0.05	-0.05
<i>Rhizopus</i>	-0.31*	-0.04	-0.22	-0.02
FFK	-0.09	-0.03	0.21	0.16

\*Significant at  $p < 0.05$ ; \*\*Significant at  $p < 0.01$

Moi = Moisture %; Fib = Fibre %; Ash = Ash %; HI = Hardness index; TPP = Total protein per cent (dry basis); FFK = Fungus free kernel (FFK = [Total number of fungal free bisected kernels per sorghum genotype counted on PDA plates/Total number of bisected kernels plated on PDA media plates]\*100).

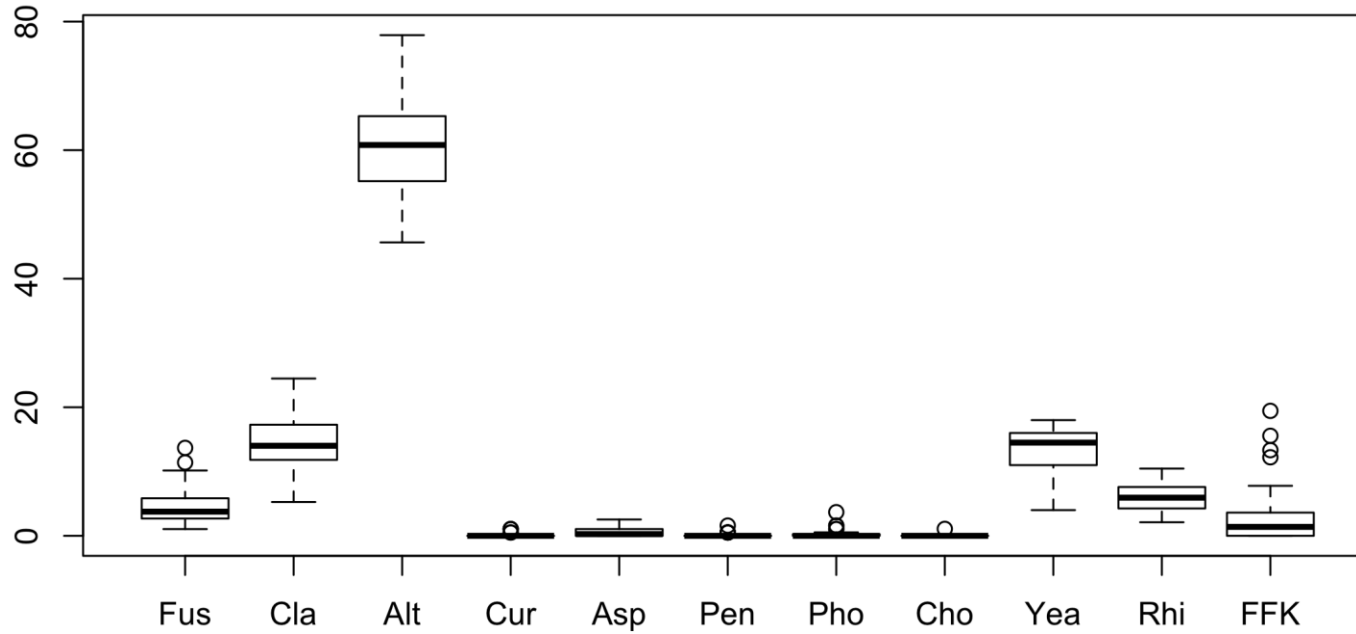
**Table 1.7. Correlation (*r*) matrix for quality traits**

Traits	HI	KW	KD	TPP	PD	Deo	Moi	Fat	Fib	Ash	Sta
HI	1.00	-0.57**	-0.68**	0.10	0.26	-0.36*	0.06	-0.10	0.51**	0.06	0.15
KW		1.00	0.86**	0.15	-0.21	0.43**	-0.02	0.04	-0.53**	-0.01	-0.50**
KD			1.00	0.10	-0.18	0.35*	-0.03	0.17	-0.44**	-0.01	-0.43**
TPP				1.00	-0.31*	-0.03	-0.07	0.22	-0.27	-0.20	-0.61**
PDP					1.00	-0.17	0.03	-0.30*	0.39**	0.03	0.35*
Deo						1.00	-0.16	-0.24	-0.49**	0.03	-0.37*
Moi							1.00	0.12	0.03	0.70**	0.21
Fat								1.00	0.01	0.09	0.00
Fib									1.00	-0.03	0.50**
Ash										1.00	0.09

\*Significant at  $p < 0.05$ ; \*\*Significant at  $p < 0.01$

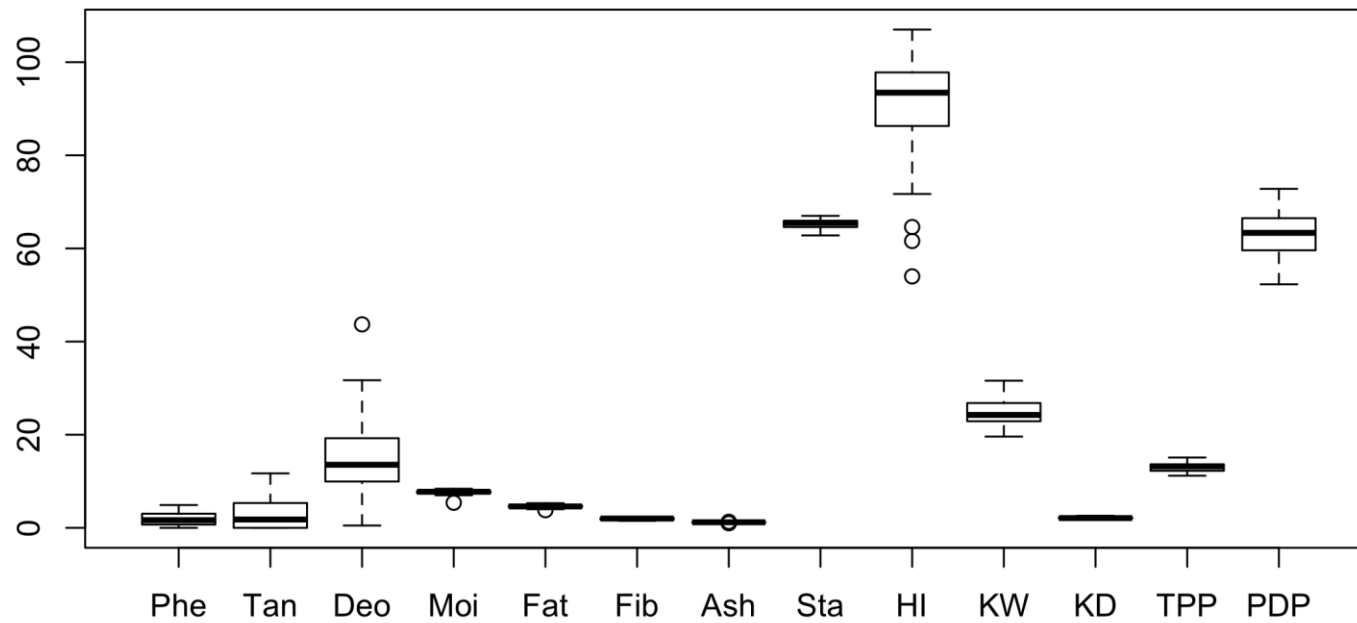
HI = Hardness index; KW = Kernel weight, KD = Kernel diameter; TPP = Total protein per cent (dry basis); PDP = Protein digestibility per cent; Deo = 3-Deoxyanthocyanin concentration (Abs per ml per g); Moi = Moisture %; Fat = Fat %; Fib = Fiber %; Ash = Ash %; Sta = Starch %

**Figure 1.1. Box plot for different fungal genera and FFK**



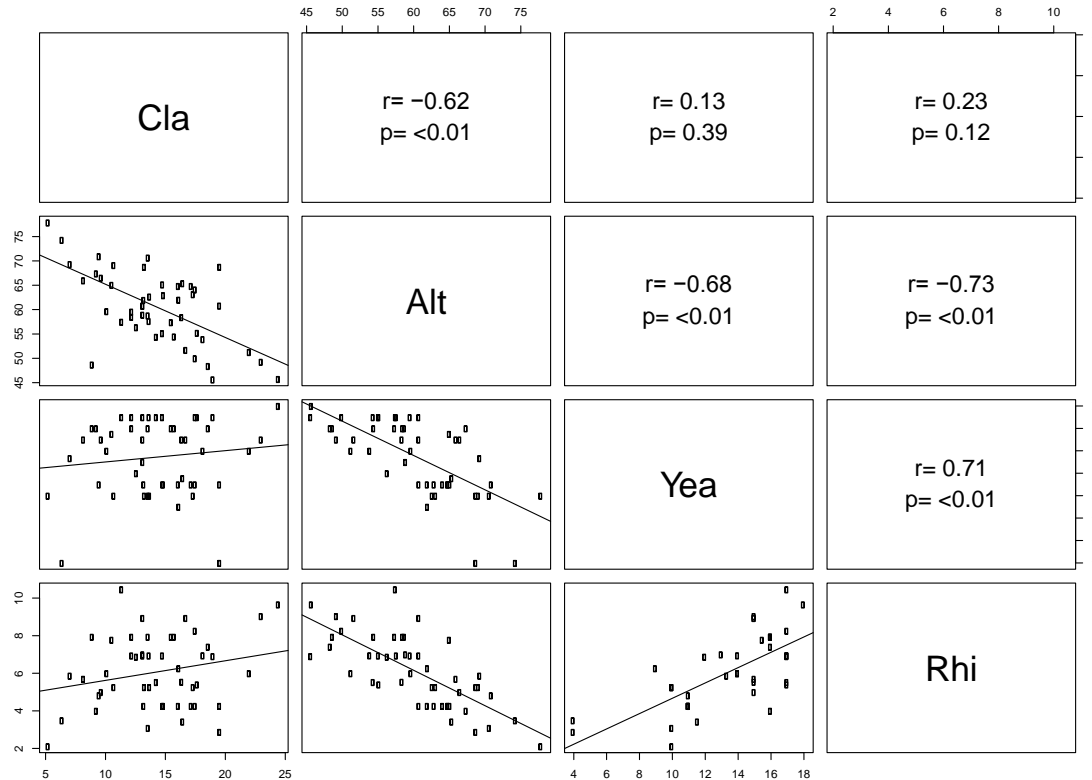
Alt = *Alternaria*; Fus = *Fusarium*; Cla = *Cladosporium*; Cur = *Curvularia*; Asp = *Aspergillus*; Pen = *Penicillium*; Pho = *Phoma*; Coc = *Cochliobolus*; Yea = Yeast; Rhi = *Rhizopus*; FFK = Fungus free kernel (FFK= [Total number of fungal free bisected kernels per sorghum genotype counted on PDA plates/Total number of bisected kernels plated on PDA media plates]\*100).

**Figure 1.2. Box plot for quality traits**



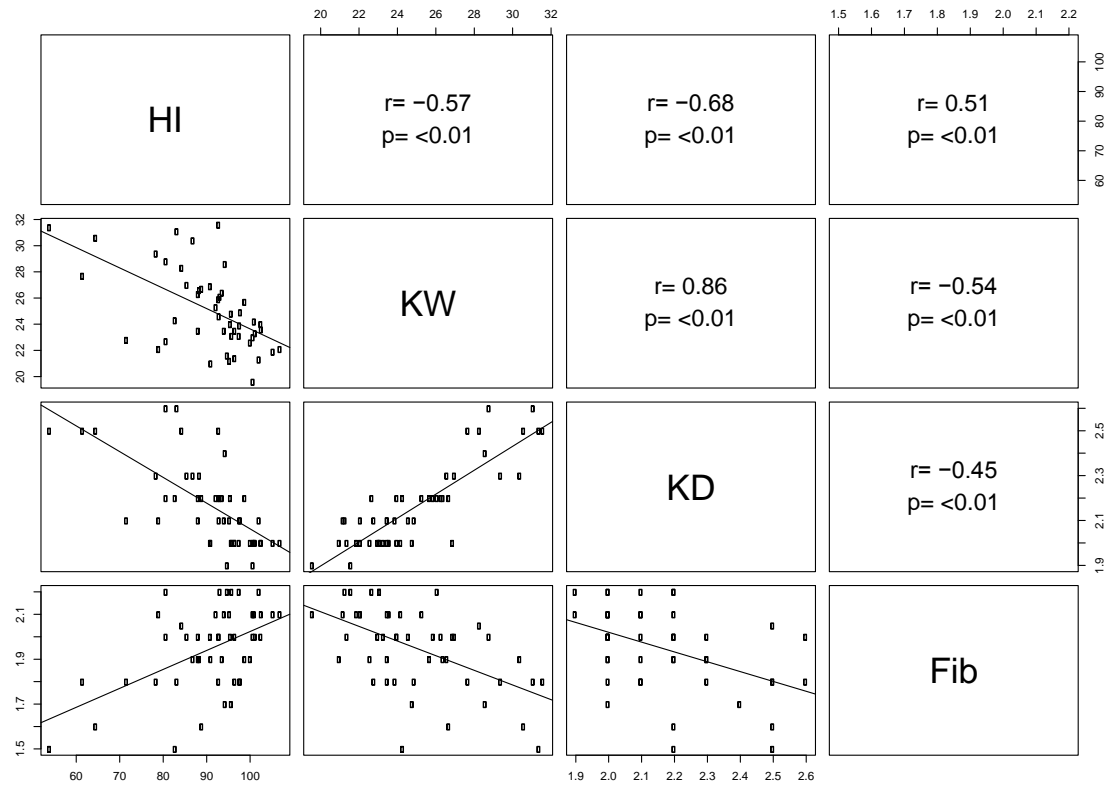
Phe = Total phenolic acids (mg gallic acid equivalents per g); Tan = Total tannins (mg catechin equivalents per g); 3-Deo = 3-Deoxyanthocyanin concentration (Abs per ml per g); Moi = Moisture %; Fat = Fat %; Fib = Fiber %; Ash = Ash %; Sta = Starch %; HI = Hardness index, KW = Kernel weight (mg), KD = Kernel diameter; TPP = Total protein per cent (dry basis); PDP = Protein digestibility per cent

**Figure 1.3. Pairs plot for *Cladosporium*, *Alternaria*, *Yeast* and *Rhizopus*. Pairwise relationships between variables *Cladosporium*, *Alternaria*, *Yeast* and *Rhizopus* are shown in above figure through scatterplot and correlation matrix. The lower corners of pairs plot shows scatter plots with regression lines between these traits. The upper corner or portion of this plot shows Pearson's correlation between *Cladosporium*, *Alternaria*, *Yeast* and *Rhizopus*.**



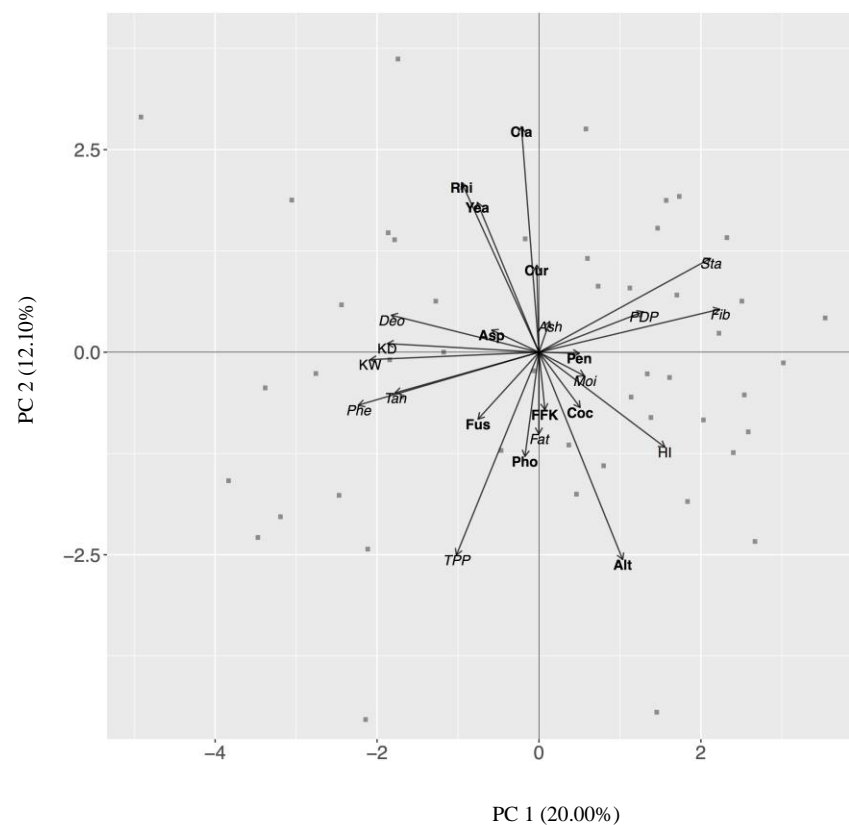
Alt = *Alternaria*; Cla = *Cladosporium*; Yea = *Yeast*; Rhi = *Rhizopus*

**Figure 1.4. Pairs plot for HI, KW, KD and fiber per cent. Pairwise relationships between variables HI, KW, KD and fiber per cent are shown in above figure through scatterplot and correlation matrix. The lower portion of plot shows scatter plots with regression lines between HI, KW, KD and fiber per cent. The upper portion of this plot shows Pearson's correlation between these traits.**



HI = Hardness index; KW = Kernel weight (mg); KD = Kernel diameter (mm); Fib = Fiber %;

**Figure 1.5. Principal component analysis (PCA) plot for quality traits and fungal pathogens. In Figure, fungi are in bold letters and quality traits are in italics.**



Alt = *Alternaria*; Fus = *Fusarium*; Cla = *Cladosporium*; Cur = *Curvularia*; Asp = *Aspergillus*; Pen = *Penicillium*; Pho = *Phoma*; Coc = *Cochliobolus*; Yea = Yeast; Rhi = *Rhizopus*; FFK= Fungus free kernel; Phe = Total phenolic acids (mg gallic acid equivalents/g); Tan = Total tannins (mg catechin equivalents/g); Deo = 3-Deoxyanthocyanin (Abs/ml/g); Moi = Moisture %; Fat = Fat %; Fib = Fiber %; Ash = Ash %; Sta = Starch %; HI = Hardness index; KW = kernel weight, KD = kernel diameter; TPP = Total protein per cent (dry basis); PDP = Protein digestibility per cent



## **Chapter 2 - Effect of time x NaOCl stringency on extraction of grain mold complex fungus from naturally weathered sorghum seeds surface**

### **Abbreviations**

DDW = Double distilled water; DNA = Deoxyribonucleic acid; FIP = Fungal isolation per cent; GM = Grain mold; GW = Grain weathering; GMC = Grain mold complex; ITS = Internal transcribed spacer; PDA = Potato dextrose agar; SAP = Sorghum association panel

## Abstract

The purpose of surface sterilization is to destroy or remove non-pathogenic fungi and bacteria on the grain surface without killing pathogenic fungi and other microflora present on pericarp and internal part of kernel tissue. Standardized surface sterilization protocol for fungal genera isolation from bisected kernels is already in use. The main objectives of this laboratory experiment were to optimize surface sterilization protocol for fungal genera extraction from the kernel surface (pericarp) and to study the effect of bleach percentage and time of treatment. Seven treatments using sterilized double distilled water (0 % bleach (v/v)) and different bleach (NaOCl) concentrations were used with a time interval of 2.5, 5, 7.5 and 10 min. Contamination of Potato dextrose agar (PDA) plates with non-pathogenic fungi like bread mold *Rhizopus*, bacteria and yeast were reported. These contaminations decreased with increased in time x NaOCl concentration stringency suggesting that the rate of contamination of PDA plates and time x NaOCl concentration stringency have an inverse relationship. Optimized surface sterilization in the range of 7.5 to 15 % bleach (v/v) for 7.5 to 10 min resulted least contamination and fungal genera isolation from the surface of the kernel. Isolated fungal colonies were classified via sequencing using genus specific primers, revealed that *Fusarium* and *Alternaria* are the two major fungal genera isolated from kernel surface and internal tissue.

## Introduction

Grain mold (GM) and grain weathering (GW) are globally important panicle diseases of sorghum. Together they are referred as “grain mold complex (GMC)” (Forbes et al. 1992; Das et al., 2011; Little et al., 2012). More than 40 genera of fungi are reported to be responsible for grain mold complex (Williams and Rao, 1981; Navi et al., 1999; Singh and Bandyopadhyay, 2000; Thakur et al., 2006; Das et al., 2011; Little et al. 2012). Important fungal genera include *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Epicoccum*, *Exserohilum*, *Fusarium*, *Nigrospora*, *Olpi-trichum*, *Penicillium*, *Phoma*, *Rhizopus*, and *Trichoderma* (Little et al., 2012). GM differs from GW in that it is a condition in which fungal infection and colonization of spikelet tissues occur before grain maturity. Mostly, GM fungi colonize the internal tissues of the floret and developing kernel i.e. on living tissue. GW, on the other hand, is a situation in which fungi colonize the developing kernel after physiological maturity, before harvest as defined by black layer deposition. GW fungi colonize the primarily non-living tissue i.e. mostly external surface of the kernel (Forbes et al. 1992; Bandyopadhyay et al., 2000; Little et al., 2012).

In assessing GM and GW fungal invasion, kernels are surface sterilized and placed on half or full strength potato dextrose solution (PDA) or on agar medium. The purpose of surface sterilization protocol is to destroy or remove non-pathogenic fungi and bacteria from the surface without killing pathogenic fungi and other microflora present on pericarp and internal part of kernel tissue. In case of extracting GM fungi from the internal part of kernel, first the whole kernels are incubated in a 10% bleach solution (commercial bleach containing 5.25% sodium hypochlorite i.e. NaOCl) for 10 minutes. It was followed by repeated washing (3-4 times) with autoclaved double distilled water for 10 min. Surface sterilized kernels are then bisected and incubated again in 10 % bleach solution for 10 min. After NaOCl treatment bisected kernels are rinsed with sterile distilled water for additional 10 minutes to remove residual NaOCl. These kernels are transferred on filter paper for surface drying under lamina air flow, followed by plating endosperm-side down on half-strength Potato dextrose agar (PDA) plates in order to expose interior caryopsis tissues directly to the fungal isolation medium (Noll et al., 2010; Chintapalli et al., 2006; Tomar et al., unpublished). But for extracting GW fungi from kernel

surface, there are no standard protocols available. Melake-Berhan et al. (1996) treated harvested kernels with 2 % NaOCl for 3 min followed by rinsing with sterile water and blotted dry on sterile paper before plating on acidified potato dextrose agar. Singh and Navi (2001) treated molded kernels with 0.1 HgCl<sub>2</sub> for 2 min followed by repeated washing with distilled sterile water followed by plating kernels on oat meal agar. Thakur et al. (2006) used 1 % NaOCl for 3 min followed by several washes with sterile distilled water for treating molded sorghum kernels before plating on oat meal agar plates. In another study, 5 % NaOCl was used for treating sorghum kernels for 10-20 min followed by 3 to 10 washes with sterile water before plating on solidified agar (Hussaini et al., 2009; Yassin et al., 2010). Das et al. (2012) used 4% NaOCl for 5 minutes for surface sterilization of sorghum kernel samples. NaOCl treated kernels were washed twice with double distilled water followed by air-drying under laminar flow hood. Kange et al. (2015) surface sterilized sorghum kernel samples with 2.5 % NaOCl for 1 min followed by rinsing twice with sterile water. Kernels were further treated with 70% ethanol for 30 s followed by rinsing with sterile water. Prom et al. (2015) soaked kernels in 10 % NaOCl for 1 min followed by washing with distilled water thrice and were dried under laminar flow before plating on half strength PDA. Effect of bleach percentage and time of treatment on the extraction of GW fungi from sorghum kernels surface (pericarp) is never investigated.

The objectives of this study were to (i) investigate potential GM and GW fungal pathogens colonizing kernels surface as well as internal kernel tissue in selected four sorghum lines (ii) assess the relationship between bleach percentage and time of treatment on extraction GW fungi from sorghum kernels (iii) optimize bleach concentration and time of treatment for extracting GW fungus from naturally weathered sorghum kernel surface with minimum contamination from non-pathogenic fungi, and to (iv) confirm the same fungal genera are responsible for GW and GM in these lines.

## **Material and Methods**

**Seed source:** A total of 227 genotypes of sorghum association panel (SAP) were evaluated for grain mold infection following standard artificial inoculation in two replications at North farm, Kansas State University, Manhattan, Kansas in 2014. Four lines included as checks (Sureño and

Tx2911: resistant; RTx2536 and BTx378: susceptible) in this experiment were used for studying the effect of bleach percentage and time of treatment on extraction of GW fungi from sorghum kernels surface (pericarp) and for extracting fungal genera. Three open heads per genotypes per replication were harvested from these four lines at physiological maturity and bulked separately. Lab experiment was performed in a two way factorial arranged on a completely randomized design. Treatments were combination of bleach levels and different time durations.

**Isolation of fungi from sorghum pericarp:** Kernels from each sorghum line were given seven treatments for four different time intervals. Sun Flo bleach 5.25 %, (Kansas Correctional Industries, Lansing, Kansas) was used. In first treatment, autoclaved double distilled water (DDW) (0 % bleach (v/v)) was used for surface sterilization for 2.5, 5, 7.5 and 10 min time intervals. In second treatment 2.5 % bleach (v/v) was used for surface sterilization for 2.5, 5, 7.5 and 10 min time intervals. Similarly, 5 , 7.5 , 10 , 12.5 and 15 % bleach solution were in used in other six treatments for 2.5, 5, 7.5 and 10 min time intervals. After each bleach treatment, sorghum kernels were washed repeatedly with sterile water 3-4 times to remove residual NaOCl. Kernels were blotted dry on sterile paper under lamina airflow before plating on half-strength PDA. For better comparison, kernels were plated directly on PDA plates without any treatment as negative control. 10 kernels per line were plated per PDA plate and each experiment per treatment was done in three replications. Bisected kernels from these four-line were also plated half-strength PDA plates following steps described in introduction section. The PDA media was amended with streptomycin (20 µg per 1000 ml), tetracycline (10 µg per1000 ml), and penicillin (10 µg per 1000 ml) in order to prevent bacterial contamination. Plates were allowed to incubate for 3 to 4 days at 26°C. Fungal colonies growing from kernels were counted and classified into different groups based on morphology and were further classified up to genus level via sequencing using genus specific Internal Transcribed Spacer (ITS) primers.

**Identification of isolated fungi:** For genus level fungal identification, single-spored fungal isolates were plated in PDA plates for 72 hours at 26°C. The resultant mycelium was used to extract deoxyribonucleic acid (DNA) by Epicentric- an Illumina company (MasterPure™ DNA extraction Kit). Isolated DNA was suspended in TE buffer (1X Tris-EDTA; pH 6.0), and stored at 4°C. DNA was quantified using a ND-1000 Nanodropper spectrophotometer (NanoDrop

Technologies, Wilmington, Delaware, USA). Using ITS primers [ITS4 (5' TCCTCCGCTTATTGATATGC- 3' and ITS5 (5'GGAAGTAAAAGTCGTAACAAGG- 3')] morphological groups were identified to genus via sequencing (White et al., 1990). Primers (ITS 4 and ITS 5) were used in a ratio of 1.0 µl (0.25 pmoles per µl) each for amplifying fungal DNA (1-20 ng per µl) (White et al., 1990). Standard PCR reaction mixture (25 µl) consists of 12.5 µl PCR master mix (Promega), 2 µl each of forward and reverse primers, 2 µl DNA template and 6.5 µl Nuclease free water. For amplification profile PCR cycling condition were: initial denaturation of the template DNA at 94°C for 1 min, followed by 34 cycles at 94°C for 30 sec (denaturation), 50 °C for 45 sec, 72 °C for 1 min (annealing) and followed by cycle at 72 °C for 7 min (extension). Data were analyzed using Microsoft Excel and, PROC MIXED, of SAS v. 9.3 software (SAS 9.3, SAS Institute, 2010).

## **Results and Discussion**

A significant effect of time, NaOCl concentration and line (resistant or susceptible) on fungal frequency isolation was reported (Table 2.1). This suggests that time x NaOCl concentration stringency significantly effect fungal isolation from kernel surface which is further influenced by the line (resistant or susceptible). Classification of fungus via sequencing using ITS primers revealed that only two fungal genera (*Fusarium* and *Alternaria*) were isolated from kernel surface. The fungus *Aspergillus* was identified in addition to *Fusarium* and *Alternaria* from the bisected kernels (internal tissue) of the susceptible line RTx2536 (Table 2.2). The results clearly indicated that *Fusarium* and *Alternaria* are two major fungal genera responsible for GW and GM infection. Noll et al. (2010) reported similar results.

Comparison of fungal genera isolated from kernel surface using different time x NaOCl concentration stringency and bisected kernel revealed that *Alternaria* fungal frequency was major followed by *Fusarium*. With the increase in time x NaOCl concentration stringency, the frequency of *Alternaria* increases whereas, the frequency of *Fusarium* decreases in both grain mold resistant and susceptible lines used in this study (Figure 2.1-2.4). This suggests that *Fusarium* infects more superficially on kernel surface. Due to prolonged time x NaOCl concentration stringency treatment and repeated washes with autoclaved double distilled water,

*Fusarium* was washed away from kernel surface. On the other hand, *Alternaria* might be presented deeper in the kernel. The sorghum kernel is a “caryopsis” and in this kernel endosperm is fused directly with the pericarp. This suggested us that *Alternaria* would be major GM pathogen in these lines. From graphs (Figure 2.1 and 2.3), it is clear that high *Alternaria* frequency was isolated at 7.5 min treatment of sorghum kernels with different NaOCl concentration. In the case of *Fusarium*, frequencies of isolation were higher at 10 min treatment for different NaOCl concentration (Figure 2.2 and 2.4).

## **Conclusion**

From this experiment, it was observed that contamination of PDA plates with non-pathogenic fungi like bread mold *Rhizopus*, bacteria and yeast decreased with increase in time x NaOCl concentration stringency which suggested that rate of contamination of PDA plates and time x NaOCl concentration stringency have an inverse relationship. 100 % contamination with *Rhizopus* was observed, in the PDA plates, when kernels without any treatment (direct plating). 80 to 100% contamination with *Rhizopus* was observed in case of kernels treated with only sterilized double distilled water for different time interval treatments were plated on PDA plates. PDA plates treated for at least 7.5 bleach (v/v) for 7.5 to 10 min showed the least contamination (0 to 20%). No contamination in PDA plates was observed in the case kernels treated with 10 to 15% of kernels bleach (v/v) for 10 min and considered as the ideal treatment combination for extraction of pathogenic grain mold fungi from the kernel surface. Based on our findings the proposed protocol for extracting fungus from kernel surface will be:

First, whole kernels will be incubated in a minimum of 10% to 15% bleach solution (commercial bleach containing 5.25% sodium hypochlorite i.e. NaOCl) for 10 minutes. Treated kernels should be washed repeatedly for 10 min with sterilized double distilled to remove residual NaOCl. These kernels are transferred on filter paper for surface drying under lamina airflow for 25-30 minutes. After surface drying, kernels would be plated on media plates for fungal isolation using standard laboratory protocol.

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**Table 2.1. Effect of time, NaOCl concentration and line (resistant or susceptible) on fungal frequency isolation**

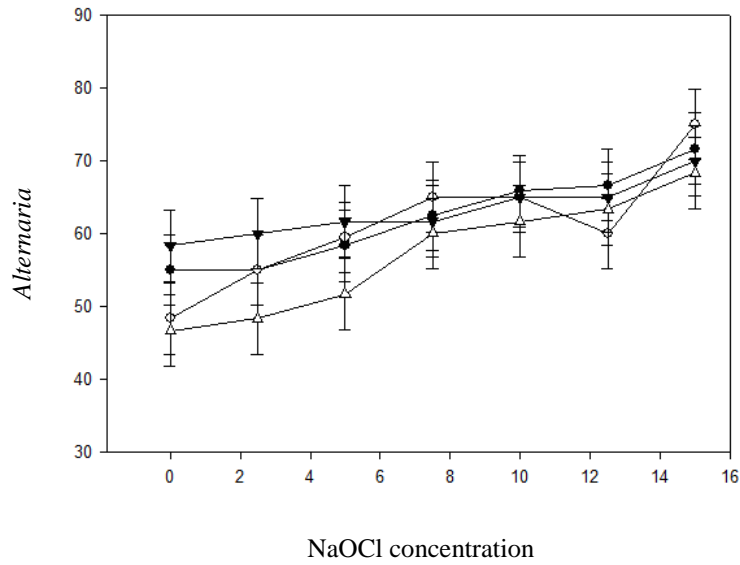
Factors	DF	<i>Fusarium</i>	<i>Alternaria</i>
Time	3	0.0002**	0.0003**
Conc	6	0.0286*	0.0298*
Time*Conc	18	0.0140*	0.0142*
Line	1	0.0001***	0.0001***
Line*Time	3	0.1546	0.1515
Line*Conc	6	0.0180*	0.0176*
Line*Time*Conc	18	0.2387	0.2387

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$   
 \*Conc= Concentration of NaOCl

**Table 2.2. Fungal frequency from bisected kernels**

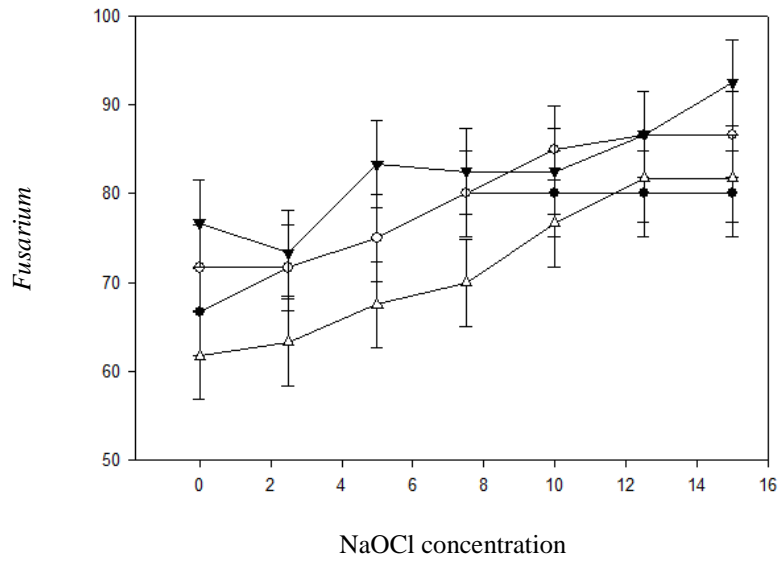
S.No	Line	<i>Fusarium</i>	<i>Alternaria</i>	<i>Aspergillus</i>
1	Sureno	3.33	96.66	0.00
2	Tx2911	15.00	85.00	0.00
3	RTx2536	10.00	85.00	5.00
4	BTx378	10.00	90.00	0.00

**Figure 2.1. Effect of time x NaOCl stringency on *Alternaria* frequency isolated from the kernel surface of resistant lines Sureño and Tx2911**



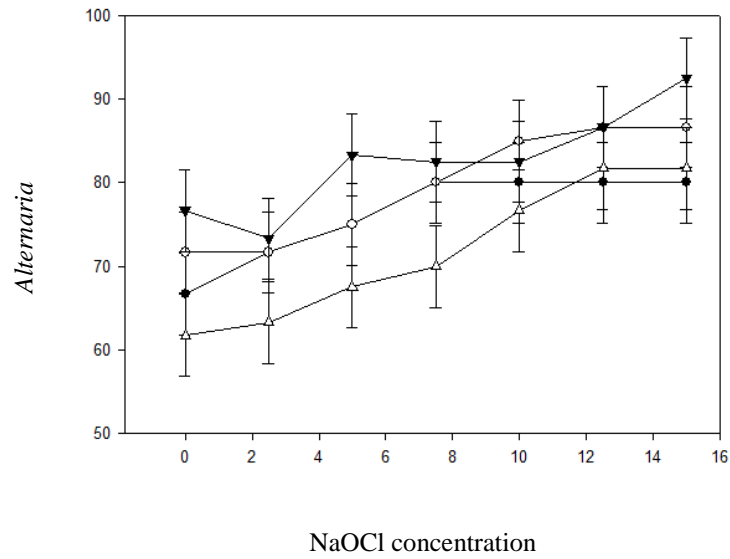
- *Alternaria* frequency at different NaOCl concentration for 2.5 min treatment
- *Alternaria* frequency at different NaOCl concentration for 5.0 min treatment
- ▼ *Alternaria* frequency at different NaOCl concentration for 7.5 min treatment
- △ *Alternaria* frequency at different NaOCl concentration for 2.5 min treatment

**Figure 2.2. Effect of time x NaOCl stringency on *Fusarium* frequency isolated from the kernel surface of grain mold resistant lines Sureño and Tx2911**



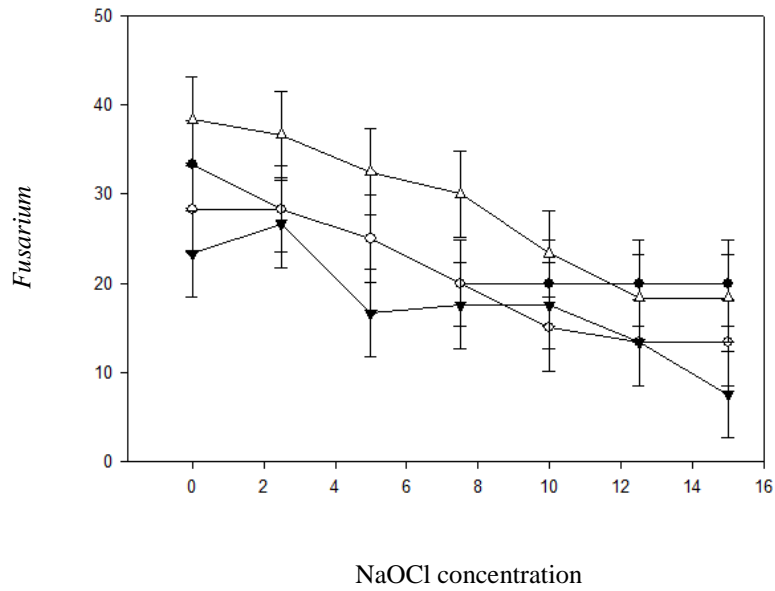
- *Fusarium* frequency at different NaOCl concentration for 2.5 min treatment
- *Fusarium* frequency at different NaOCl concentration for 5.0 min treatment
- ▼ *Fusarium* frequency at different NaOCl concentration for 7.5 min treatment
- △ *Fusarium* frequency at different NaOCl concentration for 2.5 min treatment

**Figure 2.3. Effect of time x NaOCl stringency on *Alternaria* frequency isolated from the kernel surface of grain mold susceptible lines RTx2536 and BTx378**



- *Alternaria* frequency at different NaOCl concentration for 2.5 min treatment
- *Alternaria* frequency at different NaOCl concentration for 5.0 min treatment
- ▼ *Alternaria* frequency at different NaOCl concentration for 7.5 min treatment
- △ *Alternaria* frequency at different NaOCl concentration for 2.5 min treatment

**Figure 2.4. Effect of time x NaOCl stringency on *Fusarium* frequency isolated from the kernel surface of grain mold susceptible lines RTx2536 and BTx378**



- *Fusarium* frequency at different NaOCl concentration for 2.5 min treatment
- *Fusarium* frequency at different NaOCl concentration for 5.0 min treatment
- ▼— *Fusarium* frequency at different NaOCl concentration for 7.5 min treatment
- △— *Fusarium* frequency at different NaOCl concentration for 2.5 min treatment

## Chapter 3 - Genome-wide association study for grain mold resistance and related quality traits in Sorghum

### Abbreviations

Ash = Ash per cent; Asp = *Aspergillus*; Alt = *Alternaria*; CV = Coefficient of variation; DF = Days to 50% flowering; DF = Days to flowering; DPM = Days to physiological maturity; Cur = *Curvularia*; DPM = Days to physiological maturity; DNA = Deoxyribonucleic acid; Deo = 3-Deoxyanthocyanidins; Fat = Fat per cent; Fib = Fiber per cent; FV = *Fusarium verticillioides*; FT = *Fusarium thapsinum*; FP = *Fusarium proliferatum*; FIESC = *Fusarium incarnatum-equiseti complex*; Freq = Frequency per cent; G = Genotype; GM = Grain mold; GW = Grain weathering; GMC = Grain mold complex; GMDC = Grain mold disease complex; GWAS = Genome wide association study; GL = Glume length; GW = Glume width; GI = Glume index; GC = Glume color; G = Genotype; HI = Hardness index; ITS = Internal transcribed spacer; IB = Panicle inoculated and bagged for seven days after inoculation; KW = Kernel weight; KD = Kernel diameter; KGP = Kernel germination per cent; KVP = Kernel viability per cent; LPBL = Lowest primary branch length; Lod = Lodging; MLM = Mixed linear model; MAT = Mean air temperature; MRH = Mean relative humidity; NIR = Near infrared reflectance; NB = Panicle non-inoculated but bagged for seven days after inoculation; NN = Panicle non-inoculated and non bagged; NAM = Nested association mapping; PGMR = Panicle grain mold rating; PDA = Potato dextrose agar; PH = Plant height; PL = Panicle length; PT = Panicle type; PC = Principal component; Phe = Total phenolic acids; QTL = Quantitative trait loci; RIL = Recombinant inbred line; SAP = Sorghum association panel; SNP = Single nucleotide polymorphism; SC = Seed color; SD = Standard deviation; SG = Staygreen; STP = Seasonal total precipitation; Sta = Starch per cent; SKCS = Single kernel characterization system; TGMR = Threshed grain mold rating; TSW = Thousand seed weight; TG= Total genotypes; TEF = Translation elongation factor; T = Treatment; TPP = Total protein per cent; Tan = Total tannins; Moi = Moisture per cent; VI = Vigor index; Y = Year



## Abstract

Grain mold (GM) is an important biotic constraint limiting yield and market value of sorghum grains. It results in kernel discoloration and deterioration. Such kernels have reduced viability and low food and feed quality. Breeding for grain mold resistance is challenging because of complex nature of host-pathogen-environment interactions. Worldwide there are few known sources of grain mold resistance. It might be possible that these sources will be overcome by new race/strains of grain mold pathogens. Characterization of germplasm for resistance to GM pathogens under different environments is very important for identifying new sources of resistance and subsequently developing hybrids and cultivars resistant to GM pathogens prevailing in that environment. With such intentions, a grain mold screening experiment was conducted at Manhattan, Kansas during 2014 and 2015 seasons where the entire set of sorghum association panel containing 229 accessions were screened for resistance to the major grain mold pathogen, *F. thapsinum*. We studied the effects of different agronomic and panicle architecture traits on grain mold incidence and severity. Effects of grain mold on kernel quality traits were also studied. Based on the first year field screening data, we selected 46 accessions representing a range of resistance response (having grain mold ratings 1-5 with 1 = < 1% panicle kernel molded; 5 = > 50% panicle kernel molded) for further study aimed at understanding the possible interaction between fungal pathogen to affect the physical and chemical kernel traits. Seed germination, vigor index, and tetrazolium viability test were performed to study effect of grain mold infection on kernel health and vigor. *Alternaria*, *Fusarium thapsinum*, *Fusarium verticillioides* and *Fusarium proliferatum* were the main fungal genera isolated from bisected kernels. Genome wide association studies (GWAS) were also used to map key QTLs and understand genetic basis of grain mold resistance. We reported two loci associated with grain mold resistance. Based on two year screening, SC623, SC67, SC621, SC947 and SC1494 were most resistant based on both PGMR and TGMR rating while SC370, SC833, SC1484, and SC1077 showed the most susceptible reaction and this was consistent for individual location analysis.

## Introduction

Sorghum is an important cereal crop, which grows well under harsh, and erratic weather conditions of arid and semi-arid regions. But the production, grain quality and market value of the grains are limited by several biotic and abiotic constraints. Drought and GM are important abiotic and biotic constraints limiting yield and market value of sorghum (Reddy et al., 2000; Waddington et al., 2010; Prom et. al., 2014; Cuevas et al., 2015). GM is an important panicle disease under warm and humid weather conditions, which results in kernel discoloration and deterioration. Such kernels have reduced seed viability, low food and feed quality and fetch less market value (Forbes et al., 1992; Thakur et al., 2006; Ambekar et al., 2011). Global annual economic losses of US\$ 130 million from grain mold infection have been reported from Asia and Africa (ICRISAT, 1992). Indian Institute of Millet Research (IIMR) conducted a survey, Hyderabad, India based on cropping area and production for 10 years time period (2001 to 2010) for 5 major sorghum-producing states in India (Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu and Gujarat). It was estimated on an average annual economic loss of 3000 to 5000 million Indian rupees (US\$ 50 to 83 million) due to grain mold (Das and Patil, 2013; Das et al., 2014).

*Fusarium thapsinum* and *Curvularia lunata* are two principal GM pathogens (Forbes et al. 1992; Das et al., 2011; Little et al. 2012). GM and grain weathering (GW) are two terms often used synonymously to describe molded and deteriorated sorghum kernels. In the case of GM, fungal infection and colonization of spikelet tissues and developing kernel occur before physiological maturity. But if fungi colonize the kernel after physiological maturity as denoted by black layer formation, it is termed as GW (Forbes et al., 1992; Das et al., 2011; Little et al., 2012). GM is caused by only pathogenic fungi, which colonize the internal tissues of the floret and developing grain. But GW on other hand is caused by pathogenic fungi (infect living tissue of developing kernel) as well as by saprophytic fungi (infect dead grain tissue). More than 40 fungal genera are associated with GM and GW, together called as grain mold disease complex (GMDC) or simply grain mold complex (GMC) (Forbes et al. 1992; Das et al., 2011; Little et al. 2012). Differentiation between pathogenic and non-pathogenic fungal colonization of kernels (early infections and post-maturity colonization) could be challenging under field condition. If

sorghum kernels harvested after physiological maturity is used to isolate fungi from kernel surface and from bisected kernels after surface sterilization using non or semi-selective media, deterioration of kernels and fungal genera responsible for GM can be identified (Singh and Navi, 2001; Noll et al., 2010).

Breeding for GM resistance is pretty challenging because of complex nature of host-pathogen-environment interactions (Rodriguez-Herrera et al., 2000; Das et al., 2011). Moreover, multiple mechanisms of resistance (major or minor genes with epistatic gene interactions) and undesirable agronomical traits linkage with mold resistance made breeding efforts for mold resistance pretty cumbersome (Audilakshmi et al., 1999; Audilakshmi et al., 2000; Audilakshmi et al., 2005; Audilakshmi et al., 2011). Due to complex nature of host-pathogen-environment interactions, control methods like avoidance, chemical control and bio-control agents have limited success under experimental conditions (Thakur et al., 2006; Das et al., 2011). Host plant resistance is needed to develop high yielding mold resistance lines.

Associated morphological traits including panicle traits have been associated mold resistance. Role of morphological traits like days to flowering (DF) (Thakur et al., 2009; Rao et al., 2013), days to physiological maturity (DPM) (Das et al., 2013), plant height (Klein et al., 2001; Kumar et al., 2011; Das et al., 2013), panicle compactness (Sharma et al., 2010; Das et al., 2013), glume color, glume coverage (Audilakshmi et al., 1999; Ambekar et al., 2011; Das et al., 2013) seed color, glume index (Audilakshmi et al., 1999) in mold resistance is well documented.

Grain quality, seed viability, kernel density and kernel weight are influenced by changes in physical and chemical kernel traits due to mold infection. Previous studies reported a negative correlation of kernel density and kernel weight with panicle grain mold rating (PGMR) (Klein et al., 2001; Das et al., 2012). Kernel quality and viability are important for determining the biological value of kernel as potential seed and for determining the feed value of kernel. Seed germination, vigor index and Tetrazolium violet indicator dye test are important tests for determining the kernel tissue damage and its value as a seed (Noll et al., 2010; Das et al., 2011; Das et al., 2012). Chemical and physical properties of sorghum kernels would facilitate screening sorghum genotypes for resistance and economic quality traits before subsequent inclusion in

breeding program. Sorghum genotypes with good grain quality are important for varying demands for end-use products. Important quality traits that are closely correlated with grain mold resistance are kernel hardness (Audilakshmi et al., 1999; Das et al., 2012; Thakur et al., 2009), kernel density and integrity (Waniska et al., 1992; Klein et al., 2001), kernel size (Rodriguez-Ballesteros et al., 2009), pericarp color (Reddy et al., 2000; Thakur et al., 2008), phenolic compounds (e.g. phenolic acids) (Waniska et al., 1992; Rodriguez-Ballesteros et al., 2008), tannins (Menkir et al., 1996; Melake-Berhan et al., 1996), and flavonoids (Jambunathan et al., 1991; Martizen et al. 1994).

There are few known sources of grain mold resistance worldwide. Sureño, RTx2911, IS8545 and IS14384 are important examples (Kumar et al., 2008; Noll et al., 2010; Sharma et al., 2010). It might be possible that resistance in sources may overcome by new race or strains of grain mold pathogens. Characterization of germplasm for resistance to GMC pathogens in different environments is very important for identifying new sources of resistance and developing cultivars with improved resistance to the disease. Identifying new genes or quantitative trait loci (QTLs) and precise characterization of sorghum germplasm for traits associated with GM resistance should be part of the effort to enhance GM resistance.

The diversified sorghum genotypes used in this study are part of the US sorghum association panel (SAP), which has been studied thoroughly for traits like drought tolerance (Mutava et al., 2011), plant architecture (Morris et al., 2013a), grain quality, polyphenol concentrations and flavonoid pigmentation (Sukumaran et al., 2012; Morris et al., 2013b; Rhodes et al., 2014) stalk rot resistance (Adeyanju et al., 2015) and, grain yield components (Boyles et al., 2016). But this panel is never screened for finding grain mold resistance sources. Characterization of SAP for GM resistance would help in finding genomic regions associated with GM resistance by performing a genome-wide association study (GWAS). GWAS is tool for finding genomic regions associated with phenotype of interest by performing genome wide scan for statistical association between genotypic and phenotypic variations (Myles et al., 2009). GWASs are advantageous over bi-parental QTL mapping approaches. This is because GWASs are performed using natural populations like landraces, cultivars, advanced breeding lines and diversity panels. Due to this, it is faster and cheaper approach because no research time is

required for developing mapping populations. Also, because it is performed using diverse plant material with diversified genetic background, it captures greater genetic diversity. In addition to this, GWAS accounts for greater mapping resolution by taking into consideration historical recombination events that took place in existing populations over time (Zhu et al., 2008; Morris et al., 2013; Adeyanju et al., 2015; Boyles et al., 2016). In crop species, GWAS has successfully been applied in cereal crops like rice (Huang et al., 2010; Zhao et al., 2011; Begum et al., 2015), wheat (Mora et al., 2015; Juliana et al., 2015), barley (Massman et al., 2011; Wang et al., 2012), maize (Poland et al., 2011; Kump et al., 2011; Riedelsheimer et al., 2012) and sorghum (Morris et al., 2013a; Morris et al., 2013b; Rhodes et al., 2014; Adeyanju et al., 2015; Boyles et al., 2016).

The objectives of this study were to: (i) characterize diverse sorghum genotypes for resistance to grain mold pathogen and determine the impact of the disease on quality traits (ii) map genomic regions associated with GM resistance and grain quality traits (iii) assess the relationship within and between GM fungi, and quality traits, and to test hypothesis; (a) sorghum lines with low grain mold rating will have lower level of grain mold infection and better seed quality, and (b) seed color, glume color, panicle architecture and other agronomic traits influence grain mold development on panicles.

## **Materials and Methods**

**Genetic materials:** Two sets of experiments were conducted. The first experiment involved field evaluation of 229 genotypes from the sorghum SAP for reaction to panicle inoculation by grain mold fungi, *Fusarium thapsinum*. The genotypes were planted at the Kansas State University Agronomy Research Farm, Manhattan, KS in 2014 and 2015 seasons. The panel also consisted of known resistant and susceptible entries that were used as checks (resistant - Sureño and RTx2911; moderately resistant or susceptible - Dorado, BTx3197, BTx378, and susceptible - RTx2536, RTx430 and RTx2737). The experiment was laid in randomized complete design with two replications. The second experiment is a laboratory study conducted on a subset of accessions. Based on the 2014 data, a total of 46 accessions representing a range of grain mold

reaction were selected and included in the laboratory study. The selections also included five checks: Sureño and RTx2911 (resistant); Dorado, BTx3197, BTx378 (moderately resistant to moderately susceptible) and RTx2536 (susceptible) that represent all of the 1-5 PGMR and TGMR scores.

**Inoculum preparation and inoculation:** Pure culture of *F. thapsinum* was obtained from the row crops pathology laboratory, Department of Plant Pathology, KSU, Manhattan, KS. *F. thapsinum* inoculum was produced using potato dextrose broth by incubating the suspension on rotary shaker for four days at room temperature. After four days of incubation, liquid conidial suspension cleaned off the mycelial mass using a cheesecloth and the clean suspension collected. Conidial counts in the cleaned suspension were estimated using a hemacytometer under the microscope. For field inoculation, the conidial suspension was diluted to  $1 \times 10^6$  conidia per ml by using 10 mmol (pH 7.2) Phosphate buffer saline solution. At 50% flowering, nine plants per line per replication were selected and tagged. The first three plants had their panicles inoculated at 50% flowering with *F. thapsinum* using small hand operated sprayer and bagged for seven days (IB); the next three plants were simply bagged for seven days without inoculation (NB) and the remaining three plants remained un-inoculated and not bagged (NN). All panicles were scored for PGMR at physiological maturity and for TGMR after harvesting on a scale 1 to 5 (1 = < 1% and 5 = > 50% threshed seeds molded) (Bandyopadhyay et al., 1988; Audilakshmi et al., 1999; Audilakshmi et al., 2011).

**Isolation of fungi from sorghum kernels:** 3 panicles per treatment per replication for the 46 genotypes selected for the lab experiment were first harvested; the grains separated from the chaff and kept in a paper bag. For fungal isolation, the grain samples were incubated in a 10% bleach solution (NaOCl) for 10 min followed by repeated washing with autoclaved double distilled water and dried. The kernels were then bisected longitudinally using a stainless knife and were treated again with 10% bleach for 10 min followed by repeated washing with autoclaved double distilled water as above. Bisected kernels were then transferred to filter paper under lamina-airflow for drying. Individual bisected kernels were divided into two pools, A and B. Half bisected kernels of pool A per genotype per treatment per replication, were plated endosperm-side down on half-strength potato dextrose solution (PDA) (10 half bisected kernels

per plate). This was to expose the interior caryopsis tissues directly to the fungal isolation medium. The media was amended with streptomycin (20 µg per L), tetracycline (10 µg per L), and penicillin (10 µg per L) to prevent bacterial contamination. Plates were allowed to incubate for 3 to 4 days at 26°C. Similarly, full kernels from NN treatment were surface sterilized and plated (10 kernels per plate). Grain mold fungal colonies growing from bisected kernels were counted and identified via sequencing using Internal Transcribed Spacer (ITS) and genus-specific primers (Translation elongation factor (TEF) 1- $\alpha$ ) using protocol used by Noll et al. (2010). Estimation of fungal isolation frequencies and relative percentage of isolated fungal genera were done following Gonzalez et al. (1995) and Mahmoud et al. (2013).

**Tetrazolium viability assay and seed germination test:** Another half bisected kernels of pool B per line per treatment were used for tetrazolium viability assay using a protocol suggested by Noll et al. (2010). Fifty seeds per line per replication per treatment were used for germination test using protocol from Noll et al. (2010). Germination percentage, shoot length and root length data were recorded. Using this data, vigor index (VI) was calculated according to Abdul-Baki et al. (1973) and Raju et al. (1999).

#### **Data collection:**

**Phenotypic and weather data:** The 9 plants per line per replication were tagged at 50% flowering were used to collect data on days to flowering (DF), days to physiological maturity (DPM), glume length (GL), glume width (GW), glume index (GI), lowest primary branch length (LPBL), plant height (PH), panicle length (PL), and 1000-seed weight (TSW). Days to flowering (DF) were calculated as number of days from planting to when 50% of the plants in a plot reached half-bloom stage. Days to physiological maturity (DPM) were recorded as the number of days from planting to until grains in the lower one-third section of the panicle formed black layer. Randomly selected glumes from the lowest primary branch of sorghum inflorescence were used for calculating GL and GW. GI was calculated by dividing GL by GW (Audilakshmi et al., 1999). Data were also collected on panicle type (shape and compactness) following a 2-12 scale as per the sorghum descriptors published by ICRISAT (ICRISAT, 1993). Scale 2 is for the most loose and erect type panicles and scale 10 for the most compact panicle while scale 12 was a

broom corn type panicle. Visual scoring of glumes and seeds were also done. Based on visual scoring of glume color (GC), genotypes were classified into five categories (purple, red, black, beige or pale yellow and pale orange). Genotypes were also classified into four categories based on seed color (white, yellow, red and brown). Seed color scores were further compared with results published by Rhodes et al. (2013). At physiological maturity, the entries were also scored for lodging (Lod) on a scale of 1 to 9 (1 = 0-10% lodging; 9 = 81-100% lodging) and staygreen (SG) on a scale of 1 to 5 (1 = leaves have natural green color; 2 = 1/3<sup>rd</sup> of yellow leaves; 3 = intermediate; 4 = 1/3<sup>rd</sup> of leaves green, and 5 = all leaves yellow or dead) (Reddy et al., 2007; Rosenow et al., 2014). At physiological maturity panicle grain mold ratings (PGMR) and after harvesting threshed grain mold rating (TGMR) was collected.

Weather data (Maximum and minimum temperature, rainfall, and humidity) 2014 and 2015 were collected using KSU mesonet (<http://mesonet.k-state.edu/weather/>).

**Grain quality data:** Bulk seed samples harvested from 3 panicles per genotype per replication for 46 genotypes selected based on 2014 field screening for lab study were analyzed for grain quality at the USDA Grain Marketing and Research Laboratory, Manhattan, KS using the single kernel characterization system (SKCS) procedure (Bean et al., 2006) to determine hardness index (HI) and physical grain traits including kernel weight (KW), kernel diameter (KD). Similarly seed samples harvested for 229 genotypes (3 panicles bulked in each treatment and replication in 2014 and 2015) were analyzed for chemical grain characteristics (phenolic acids, tannins, protein, moisture, fat, fiber, ash, starch, and 3-deoxyanthocyanin (Deo) content using near-infrared reflectance (NIR) spectroscopy at Texas A&M University, College Station (Dykes et al., 2014).

**Statistical analysis:** Data were analyzed using Microsoft Excel, SAS 9.3 software (SAS Institute, 2010a), JMP 10 software (SAS Institute, 2010b) and R Studio version 3.2.3 (R core team, 2015). Data were analyzed using mixed linear model (MLM) in PROC MIXED procedure of SAS software. For the entire association panel the genotypes were treated as random effect and so are the block, year, year × genotype interaction effects. But the 46 genotypes for the lab study were treated as fixed effects during the analysis of variance. PROC REG and PROC



CORR procedure of SAS software were used for correlation analysis. Using PROC GLM procedure of SAS software variance for different traits were estimated which were used in estimation of broad sense heritability ( $H^2$ ) according to Boyles et al. (2016) for both field data and NIR spectroscopy data sets. Using PROC FREQ procedure of SAS, Fisher F test for ordinal and qualitative data was performed.

**Genomic data and genomic analysis:** Genotypic data (404,627 SNP markers) available from Morris et al. (2013a) and Rhodes et al (2014) was used for performing GWAS for agronomic and NIR traits data using Genome Association and Prediction Integrated Tool (GAPIT) package in R studio software (Lipika et al., 2012). For GWAS, genotypic data available for 178 lines out of 227 accessions in 2014 and 229 accessions in 2015 were used. GWAS was performed using a general linear model (GLM) and mixed linear model (MLM) (Yu et al., 2006) considering kinship (K), and kinship and population structure (Q+K). Kinship was estimated automatically in GAPIT from genotypic data using the VanRaden method (VanRaden, 2008). Principal components (PCs) based approach (Zhao et al., 2007) in GAPIT was used for running models with Q+K. Initially GWAS was performed using GLM, MLM with K and MLM with Q+K using 404, 627 SNP markers. In order to remove noise due to missing values in SNPs, first we filter SNPs at 10% missing values (around 191K SNPs) in R and ran GWAS. But, we did not notice much reduction in noise. So, we further filtered SNPs at 5% missing value and reduced SNP number to around 94K. For identifying significant associations, Bonferroni correction at 0.05 level with  $P$ -value  $< 10^{-7}$  was used.

For complex quantitative traits, simple GWAS methods like GLM will yield spurious signals with elevated associations (false positive) (Morris et al., 2013b). Compared to GLM, MLM method reduces false positives by considering population structure and kinship (Wang et al., 2014). In this study GLM was used as a control method to compare association peaks between GLM and MLM. We did not observe much difference in results between MLM with K and MLM with Q+K based on Bonferroni correction at 0.05 levels with  $p$ -value  $< 10^{-7}$ . We are reporting results based on MLM with Q+K for taking into consideration both population structure and kinship. For GWAS, *Tannin 1* (*tan-1*) gene was used as positive control (alleles *tan-1-a* and *tan-1-b*), which is associated with S4\_61667908 SNP marker (Morris et al., 2013b; Wu

et al., 2012). For finding major and minor alleles responsible for mold resistance, TASSEL 5 software package was used for performing GWAS for grain mold ratings using MLM model with Q+K (Bradbury et al., 2007). Irrespective of the models used for GWAS, mean data per treatment per year was used for running GWAS. GWAS analysis was followed by identification of candidate genes. It was performed using *Sorghum bicolor* v1.4 reference genome available from Gramene website ([http://ensembl.gramene.org/Sorghum\\_bicolor](http://ensembl.gramene.org/Sorghum_bicolor)). Phytozome was used to find the genes in the nearby regions, their positions and about gene function if they are fairly old. Gene names and functions were further verified using Morokoshi (<http://sorghum.riken.jp/morokoshi/Home.html>) and Gramene (<http://www.gramene.org>). Genes located within 100 kb of a SNP that were associated with different traits using *Sorghum bicolor* v1.4 reference genome studied were reported.

## **Results and discussion**

**Environmental conditions:** The seasonal total precipitation (STP) at Manhattan during 2014 and 2015 seasons was 27.45 and 24.43 cm, respectively (Table 3.1). Average mean relative humidity (MRH) was 61.5% in 2014 and 58% in 2015. Similarly, variability in mean daily air temperature (MAT) was 13.16 °C to 28.94 °C in 2014 and 14.38 °C to 30.22 °C in 2015. During both years, maximum temperature was reported in August around flowering time. For GM development, humidity and moisture at DF is important. In 2015, MRH and STP during inoculation period were relatively lower than 2014. Supplemental irrigation was provided twice at two weeks interval during the 2015 season.

**Response of genotypes to grain mold screening for pathogen *F. thapsinum*:** Response to GM infection by pathogen *F. thapsinum* was evaluated for 229 genotypes. The combined analysis for the field data revealed significant environment (year) effect for all agronomic and panicle traits (Table 3.2). Similarly, the genotype and environment by accession (Y x G) interaction effects were significant for most of the traits including PGMR, LPBL, TGMR, TSW, GL, GW, and GI. The genotype effects for PH and PL however were not significant. Tukey means separation test revealed that genotypes SC623, SC67, SC621, SC947 and SC1494 were most resistant based on

both PGMR and TGMR rating while SC370, SC833, SC1484, and SC1077 showed the most susceptible reaction and this was consistent for individual location analysis. For traits like SG, Lod, DF, and DPM field responses were recorded per genotype per replication per year. For Lod (1.23 in 2014 and 1.22 in 2015) and DF (67.86 in 2014 and 68.68 in 2015) mean values were almost similar across both years. But, for SG (2.30 in 2014 and 2.98 in 2015) and DPM (108 in 2014 and 126 in 2015) there are differences in mean values across years. The response of the check genotypes was as expected. The resistant genotype Sureño maintaining the most resistance reaction to *F. thapsinum* (PGMR rating = 1.00 in both 2014 and 2015 for IB) while RTx2911 showing some degree of susceptibility (PGMR rating = 2.00 in both 2014 and 2015 for IB) (Table 3.4). The susceptible check, RTx430 had a highly susceptible reaction (IB = 4.00 (2014) and 3.50 in 2015) followed by the other susceptible checks RTx2536 (IB = 5.00 in 2015) and RTx2737 (IB = 3.80 in 2015). Among medium resistant to medium susceptible checks, Dorado showed low susceptibility to *F. thapsinum* in both years (IB = 1.50 in 2014 and 2015). The other two checks i.e. BTx3197 (PGMR rating = 3.50 for IB in 2014, and 2015 IB = 3.00) and BTx378 (PGMR rating = 2.70 for IB in 2014, and 2015 IB = 3.00) showed moderate susceptibility to *F. thapsinum* in both 2014 and 2015 seasons.

Broad sense heritability for the traits was variable ranging from low to high depending on the traits. Robinson et al. (1949) classified heritability into three categories i.e. low (<0.30), moderate (0.30-0.60) and high (>0.60). In the present study heritability for SG ( $H^2$ ) was low (0.26) as compared with previous studies. Xu et al. (2000) reported high heritability of 0.88 for SG trait in RIL population of sorghum developed from crossing B35 and Tx7000. Kebede et al. (2001) reported medium to high heritability (0.58-0.83) for SG in RIL population developed by crossing SC56 and TX7000 under different environments. Heritability for traits like PH and LBPL were high (>0.60). Brown et al. (2006) reported high heritability of 0.94 and 0.93 for PH and branch length. But DF, DPM and TSW had moderate  $H^2$  range (0.30-0.60) while previous research on the same panel of accessions reported high heritability (DF = 0.90, DPM = 0.93 and TSW = 0.83) for these traits (Boyles et al. 2015). Heritability for the grain mold ratings falls in the moderate range (PGMR = 0.37 and TGMR = 0.46). Rami et al. (1998) reported medium (0.55) and low heritability (0.27) for grain mold ratings on two different RIL populations.

Lodging score had moderate  $H^2$  of 0.45. Bittinger et al. (1981) reported  $H^2$  of 0.58 on random mating sorghum population PP9.

**Trait correlations:** Results of correlation analysis between grain mold ratings and panicle traits are presented in Table 3.5. The analysis revealed that grain mold ratings have significant negative correlation with TSW (2014: -0.13 with TGMR and -0.19 with PGMR, and in 2015: -0.15 with TGMR and -0.19 with PGMR), PH (2014: -0.27 with TGMR and -0.10 with PGMR, and in 2015: -0.15 with TGMR and PGMR), LPBL (2014: -0.22 with TGME and -0.14 with PGMR, and in 2015: -0.10 with TGMR and -0.07 with PGMR), and Lod (2014: -0.14 with TGMR and -0.07 with PGMR, and in 2015: -0.07 with TGMR and PGMR) in both years but it had positive correlation with PT in 2014 (0.25 with TGMR and 0.13 with PGMR) and 2015 (0.15 with TGMR and 0.14 with PGMR) (Table 3.5). Sharma et al. (2010) and Das et al. (2013) too reported positive correlation between grain mold ratings and PT. Klein et al. (2001) and Thakur et al. (2009) reported negative correlation of grain mold ratings with PH. Das et al. (2012) reported negative correlation between grain mold score and seed weight. In 2014, grain mold scores have significant negative correlation DF, and DPM. But this correlation with grain mold ratings was absent in 2015. Results of 2015 are in accordance with results from Navi et al. (2006) in pearl millet and Thakur et al. (2009) in sorghum. Both of these studies did not report any correlation between grain mold score and DF. PH is often positively associated with DF and DPM; thus the negative correlation between grain mold rating and these characters may have to do with the timing of flowering which has an impact on grain mold infection than the traits per se. The absence of significant correlation between grain mold infection and these traits in 2015 a relatively less humid season shows that mold infection is more related to environmental conditions, primarily humidity. PL has significant negative correlation with grain mold ratings in 2015. In 2014, it showed negative correlation only with TGMR. TGMR showed significant negative correlation with GL (-0.16), GW (-0.09), and GI (-0.07) in 2014. But in 2015, such associations were absent. This supports the common knowledge that panicle structure has significant bearing on grain mold infection. Genotypes with open panicles and more glume coverage such as the bicolor and guinea races tend to have low grain mold infection. Such genotypes normally have larger GW, and GL, so that the negative correlation between grain mold infection and these traits was expected. Audilakshmi et al. (1999) also did not report any

correlation between grain mold scores and GI. Additionally, TSW showed significant positive correlations with PL (0.15), GL (0.11), GW (0.27), DF (0.19) and DPM (0.16), and significant negative correlations with PGMR (-0.13), TGMR (-0.19), GI (-0.20) and awns (-0.15) in 2014. In 2015, TSW showed positive correlation with GL (0.08) and GW (0.07), and negative correlations with TGMR (-0.19) and PGMR (-0.15).

Data in Table 3.6 shows that PGMR and TGMR are dependent on PT (2014:  $p < 0.001$ ; 2015:  $p < 0.001$ ), GC (2014:  $p < 0.01$ ; 2015:  $p < 0.05$ ), and seed color (2014:  $p < 0.001$ ; 2015:  $p < 0.001$ ) in both test seasons. However, Lod and SG were not dependent with TGMR in both years. PGMR and Lod were dependent in 2014 ( $p < 0.01$ ), and PGMR and SG were dependent only in 2015 ( $p < 0.01$ ).

The data in Table 3.7 shows that kernels with light seed color (white and yellow) from 2014 experiment have higher PGMR ratings (PGMR for IB = 2.50 for white and yellow) than kernels with darker seed color (Brown; PGMR for IB = 2.10, and for red; PGMR for IB = 2.00). But in 2015, there is not much variation in PGMR ratings in 2015 based on seed color. In 2015, Red, white and yellow seeds have mean PGMR rating for IB was 2.5 and for brown seed, it was 2.40. This also agrees with previous studies where significant correlation was reported between grain mold incidence and seed color (Audilakshmi et al., 1999) and glume color (Das et al., 2012). Similarly glume color appears to associate with grain mold disease with genotypes with red or darker glumes tend to have lower grain mold infection. In general both grain color and glume color appear to have some degree of relationship with grain mold incidence (Tables 3.7 and 3.8) but they seem to be confounded by environmental factors.

Another panicle characteristics, the panicle type also seems to have strong bearing on grain mold infection (Table 3.9). Genotypes with compact panicles tend show high grain mold incidence as compared to those with open panicles. Compact oval (PGMR mean rating for IB: 3.67 (2014) and 4.07 (2015)) and compact elliptical (PGMR mean rating for IB: 2.76 (2014) and 2.81 (2015)) panicles recorded highest PGMR rating for IB treatment panicles. This agrees with previous studies as well as common sense observations that because of the unique micro-environment (high humidity) that favors the growth of grain mold pathogens, genotypes with

compact panicles tend to suffer from grain mold than open panicles types. In addition, because compact panicles limit light penetration, moisture and dew that accumulate in the panicles overnight take longer time to evaporate in the mornings thus facilitate the establishment of the parasite by giving enough time for inoculum to grow and penetrate the host cells.

**Change in chemical composition of kernels in response to grain mold screening for pathogen *F. thapsinum*:** The combined analysis for the NIR data revealed significant environment (year) effect for all quality traits (Table 3.10). Similarly, the genotype and environment by accession (Y x G) interaction effects was significant for quality traits. Summary statistics and broad sense heritability ( $H^2$ ) are reported for quality traits in Table 3.11. Based on Tukey means separation test, it is clear that there is variation in quality traits between treatments. But, this variability across treatments is not huge. Grain mold infection usually results in reduction in kernel quality traits like protein and starch content of infected kernels.

The low protein per cent and starch per cent in inoculated panicle kernels compared to non-inoculated panicle kernels could be due to higher GM infection in kernels of inoculated panicles which deteriorates chemical composition of inoculated panicle kernels. From Table 3.11, it is clear that  $H^2$  for all traits are high. This would be justified by statement that in collecting quality traits data using NIR spectroscopy, environmental variation and human error were least. Rami et al. (1998) reported heritability of 0.68 and 0.70 for TPP on two different sorghum RIL populations used for mapping QTL for grain quality. Similarly, Melchinger et al. (1998) reported high heritability for TPP (0.71) and MP (0.73) for maize kernels from F<sub>3</sub> maize population. Campbell et al. (2001) reported high heritability for TPP (0.90) kernel traits from wheat RIL's population developed by crossing NY18 and Clark's cream.

Table 3.12 classified mean of quality traits per genotype per treatment per year based on seed color. Based on this it is clear that lowest mean TPP content (9.82 in 2014 and 11.24 in 2015 for IB) was in yellow kernels followed by white (9.32 in 2014 and 10.87 in 2015 for IB). Mean Moi (9.54 in 2014 and 10.87 in 2015) was reported higher in white kernels followed by yellow kernels. In yellow kernels, Moi was (9.69 in 2014 and 8.39 in 2015). This suggested that kernels with light color and higher moisture are prone to GM infection. Similarly across all

Kernels type based on seed color, it was evident that mean Deo concentration was higher in IB treated kernels compared to other two treatments. Highest Deo content was reported in IB red kernels (26.72 in 2014 and 26.22 in 2015). This result is consistent with previous findings, which suggested that Deo production increases under stress conditions like GM fungal pathogen invasion (Lo et al., 1999; Waniska and Rooney, 2000; Seitz, 2004).

**Correlation between grain mold rating and grain quality traits:** The grain mold ratings were shown to have negative correlation (Table 3.13) with TPP (2014: -0.21 with TGMR; -0.11 with PGMR, and in 2015: -0.17 with TGMR and -0.13 with PGMR), Fat (2014: -0.23 with TGMR; -0.13 with PGMR, and in 2015: -0.05 with TGMR and -0.06 with PGMR), Fib (2014: -0.19 with TGMR and -0.14 with PGMR, and in 2015: -0.17 with TGMR and -0.18 with PGMR), Phe (2014: -0.25 with TGMR and -0.26 with PGMR, and in 2015: -0.13 with TGMR and -0.15 with PGMR) and Tan (2014: -0.26 with TGMR and -0.25 with PGMR, and 2015). These results suggested that grain mold infection deteriorate TPP, Fat, Fib, Phe and Tan content in kernels. In both years, grain mold ratings showed positive correlation with Moi (2014: 0.17 with TGMR and 0.11 with PGMR, and in 2015: 0.15 with TGMR and PGMR), Ash (2014: 0.21 with TGMR and 0.15 with PGMR, and in 2015: 0.06 with TGMR and 0.10 with PGMR) and Deo (2014: 0.19 with TGMR and 0.16 with PGMR, and 2015: 0.21 with TGME and 0.16 with PGMR). Positive correlation between grain mold ratings and Moi suggested that seeds with higher moisture per cent are prone to grain mold infection. Similarly, positive association of Ash with grain mold ratings suggested that with increase in grain mold infection there would be more kernel deterioration, which ultimately results in more Ash production. Deo is a phytoalexins produced in response to fungal or mold invasion or under other stress conditions in sorghum (Lo et al., 1999; Waniska and Rooney, 2000; Seitz, 2004). Consistent positive correlation between grain mold rating and Deo would also suggest that with increase in gain mold infection there is increase in Deo production. TSW showed negative correlation with Phe (-0.15 in 2014 and -0.09 in 2015) and Tan (-0.25 in 2014 and -0.06 in 2015) in both years, this suggested that TSW would be higher when Phe and Tan content of kernel will be lower. On the other side, TSW showed positive correlation with TPP (0.11), Fat (0.14) and Ash (0.07) in 2014, with TPP (0.14) and Fib (0.07) in 2015. TPP showed negative correlation with Sta (-0.59 in 2014 and -0.56 in 2015) and Moi (-0.11 in 2014 and -0.28 in 2015) in both years, and with Ash in 2015 (-0.23). These results suggested that Sta,

Moi and Ash affect kernel protein concentration. TPP showed positive correlation with Fat (0.61 in 2014 and 0.66 in 2015), Fib (0.17 in 2014 and 0.15 in 2015), and Deo (0.08 in 2014 and +0.06 in 2015) in both years. With Phe (0.11) and Tan (0.11), TPP has positive correlation only in 2014. These findings would suggest that these quality traits have mutual synergistic effect on TPP kernel concentration. Sukumaran et al., (2012) also reported negative correlation between TPP and Sta using SAP genotypes kernels. Deo have consistent negative correlation with Fib (-0.17 in 2014 and -0.16), Sta (-0.13 in 2014 and -0.26 in 2015), Phe (-0.08 in 2014 and 2015), and Tan (-0.09 in 2014 and -0.10 in 2015). These results clearly suggest that Deo has synergistic relation with TPP but with Fib, Sta, Phe, and Tan it has antagonistic interactions. Fat has negative correlation with Sta (-0.43 in 2014 and -0.42 in 2015), Ash (-0.18 in 2014 and -0.38 in 2015), Phe (-0.15 in 2014 and -0.12 in 2015) and Tan (-0.15 in 2014 and -0.13 in 2015) in 2014 and 2015. But, it has consistent positive correlation with TPP (0.61 in 2014 and 0.66 in 2015) and Fib (0.41 in 2014 and 0.29) in both years. Sukumaran et al., (2012) also reported negative correlation between Fat and Sta.

### **Genome-wide association studies**

**GWAS for grain mold resistance:** GWASs for grain mold resistance and agronomic field traits reported statistically significant SNPs ( $p$ -value  $< 10^{-7}$ ) for grain mold ratings, PH, LPBL, GL, GI, SC, Lod, DFF and DPM (Table 3.14). But for traits such as PL, TSW, Awn, SG, PT, GC and GW no SNP was reported based on Bonferroni correction at 0.05 levels with  $p$ -value  $< 10^{-7}$ . Single SNP per trait was reported for DFF (Figure 3.1) and DPM (Figure 3.2) in 2014. For DFF, SNP (S9\_57512180) was present on chromosome 9 which have negative allelic effect (-15.27) on DFF i.e. this SNP promote late flowering. For DPM, SNP was reported on chromosome 5 (S5\_56712991), which showed positive allelic effect (6.79) on DPM, which suggested that this SNP has role in late maturation. For Lod, 18 SNPs in 2014 (Figure 3.3) and 29 SNPs in 2015 (Figure 3.4) were reported. 12 SNPs in 2014 and 16 SNPs in 2015 showed negative allelic effect for Lod, which suggested that these SNP reduce lodging effect. Three SNPs S5\_20322083, S5\_61278370 and S6\_48743331 were present above threshold cut off of in both 2014 and 2015 GWAS results for Lod. Allelic effect for S5\_20322083 was positive (1.73 in 2014 and 1.42 in 2015). For S5\_61278370 (-1.87 in 2014 and -1.80 in 2015) and S6\_48743331 (-1.44 in 2014 and



-1.09 in 2015) allelic effect were negative in both years for Lod. For LPBL, 10 SNPs in 2014 (Figure 3.5) and 38 SNPs in 2015 were reported. We reported SNPs for LBPL on chromosome 1, 2, 3, 4, 5, 7, 8, and 10. Similarly, Morris et al. (2013) reported SNPs on chromosome 1, 2, 3, 4, 5, 9, and 10 but for different loci. For PH, we reported 27 SNPs, which were shared in 2014 (Figure 3.6) and 2015 (Figure 3.7), and were present on chromosome 6 and 9, which harbor *dw2* and *dw1*, dwarfing loci. Morris et al. (2013a) reported association peaks for PH on chromosome 6 and 9 using diverse sorghum germplasm of 971 accessions from worldwide collection. Zou et al. (2012) reported QTLs for PH using sorghum RIL population on chromosome 6 and 7. In case of GL, 4 SNPs were in 2014 (Figure 3.8) and 13 in 2015 (Figure 3.9) were reported. All four SNPs (S1\_43415793, S1\_43415807, S1\_43415822, S3\_67033014) reported in 2014 were present in 2015 too. For GI, four SNPs (S3\_67033014, S5\_8042669, S6\_60367299, S8\_30544247) was reported in 2014, and were present on chromosome 3, 5, 6, and 8. For SC, one statistically significant SNP (S8\_43259635) in 2014 was reported on chromosome 8 and is having positive allele effect of 2.97 on SC (Figure 3.11).

For grain mold ratings, in 2014 no SNP was reported above threshold cut off ( $p$ -value  $< 10^{-7}$ ) (Figure 3.12). In 2015, two SNPs for PGMR (S1\_43223535 at Chromosome 1 and S9\_58695115 at chromosome 9) were reported (Figure 3.13). In case of S1\_43223535 at Chromosome 1, C is the major allele, which has allelic effect of -1.93 on PGMR rating and T is the minor allele with 0.00 allelic effects on PGMR rating. C allele for this SNP is present in all 177 genotypes and T allele in 1. For SNP S9\_58695115 at chromosome 9, G was major allele (present in 169 out of 178 genotypes) with allelic effect of -0.58 on PGMR rating and A was minor allele with allelic effect of -0.14 (present in 5 out of 178 genotypes), and was heterozygous. Minor allele (A) for this SNP is present in genotypes: SC309, SC213, SC833, SC971 and SC1047. Based on two-year mean of PGMR for these genotypes, SC309 (PGMR = 2) and SC1047 (2.2) are moderately resistant genotypes; SC971 (PGMR = 3) is moderately susceptible; SC213 (PGMR = 3.8) and SC833 (PGMR = 4.4) are susceptible genotypes. These genotypes can be further used to validate this allele by generating segregating population. RTx430 and SC9701 is one of the genotype used for developing nested association mapping population (NAM) by crossing it with RTx430. RILs from this specific cross can be used for further validation of this loci in multiple environments to further validation.

Most of the SNPs for grain mold ratings fall below threshold level cut off ( $p$ -value  $< 10^{-7}$ ). Three SNPs between GL and GI were common across years (S3\_67033014, S6\_60367299, S8\_30544247). No other trait shared loci across years. Candidate genes for field screening traits for first ten statistically significant SNPs per trait based on  $p$ -value are reported in Table 3.16.

**GWAS for quality traits:** GWASs for quality traits reported SNPs above threshold cut off ( $p$ -value  $< 10^{-7}$ ) for Tan, Phe, TPP, Starch, Moi, and Deo (Table 3.15). We did not report SNPs, which were for traits - Sta, Ash, Fat, Fib. This is because of complex nature of these traits, which resulted in marker traits association falling below threshold cut off ( $p$ -value  $< 10^{-7}$ ). Boyle's et al. (2016) similarly reported low peaks below significant threshold ( $P = 10^{-5}$ ) for yield related traits using MLM model on genotypes from SAP. For Deo, 14 SNP's (IB =1; NB = 10; NN= 3) in 2014 (Figure 3.14) and 24 SNPs (NB = 14; NN = 10) in 2015 (Figure 3.15) were reported which were above threshold cut off. Out of 14 SNPs reported from 2014, 10 SNPs have negative allelic effect on Deo concentration. Similarly, in 2015 out 24 SNPs reported, 9 SNPs have negative allelic effect on Deo. SNPs reported based on GWAS results for Deo are distributed across 1-10 sorghum chromosome set. Rhodes et al. (2014), similarly reported SNPs for proanthocyanidin distributed across whole chromosome set of 1-10. For TPP, one SNP from 2014 data was reported (S4\_3943073 on chromosome 4,  $p$ -value  $< 10^{-8}$ ) from IB treatment having positive allelic effect (0.79) on TPP concentration (Figure 3.16). One SNP (S4\_3943073) was shared between traits - Deo and TPP ( $p$ -value  $< 10^{-8}$ ). For Moi, 5 SNPs in 2014 (IB =2; NB = 2; NN =1) and 2015 (IB = 3; NB = 1; NN = 1) were reported. 3 SNPs in 2014 (Figure 3.17) and all 5 SNPs in 2015 have negative allelic effect on Moi (Figure 3.18). Only one SNP for Phe was reported across all three treatments above threshold cut off ( $p$ -value  $< 10^{-7}$ ) from 2015 samples having positive allelic effect for Phe (IB = 4.19. NB = 4.20 and NN = 4.26) and was present on chromosome 4 (Figure 3.19). Morris et al. (2013b) and Rhodes et al. (2014) similarly reported SNPs above cut off threshold for polyphenols on chromosome 4. Same SNP for Tan (S4\_60837496,  $p$ -value  $< 10^{-7}$ ) were reported too from 2015 data set on Chromosome 4 having positive allelic effect for Tan per cent (IB = 9.34, NB = 10.47 and NN = 9.95) in kernels (Figure 3.20). This SNP is with in 100 kb range of SNP associated with *tan-1* gene. This suggested that these SNP's show pleiotropic effect. Both in 2014 and 2015, our positive control SNP

S4\_61667908 on chromosome 4 for *tan-1* was below cut off threshold  $p$ -value  $< 10^{-7}$ . This is due to small population size, which reduced the power of this SAP to demonstrate statically significant associations for this SNP. This suggested that peak's for other traits will be below threshold cut off of  $p$ -value  $< 10^{-7}$  too. Candidate genes for quality traits for first ten statistically significant SNP per trait based on  $p$ -value are reported in supplementary Table 3.16.

### ***In vitro* screening of selected sorghum lines:**

**Fungal identification:** 3 panicles harvested per treatment and replication for 46 genotypes kernels were used for extraction GMC fungus. 15 fungal colonies were isolated based on morphology. They were further differentiated into five fungal genera via sequencing using ITS primers: *Alternaria*, *Curvularia*, *Aspergillus*, *Penicillium*, and *Fusarium*. Using TEF- $\alpha$  primers, *Fusarium* samples were further differentiated into four species i.e. *F. thapsinum* (FT), *F. verticillioides* (FV), *F. proliferatum* (FP) and *F. incarnatum-equiseti* complex (FIESC). Using nucleotide BLAST search at National Center for Biotechnology Information (NCBI) website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) percentage identity and accession numbers for non-*Fusarium* specimens were differentiated (Table 3.17). For *Fusarium* samples, percentage identity and accession numbers were identified using Fusarium-ID database (<http://isolate.fusariumdb.org/index.php>) (Table 3.17). Among fungal isolates, a highest fungal genus isolated was *Alternaria* (51.40%) followed by *Fusarium* (47.60 %). Other fungal genera (*Curvularia*, *Aspergillus*, and *Penicillium*) were present in traces (Table 3.18). Out of 47.59 % of *Fusarium*, 25.04 were FV followed by FT (19.11%) and FP (2.93%). Results from Table 3.19 showed that *Fusarium* (57.87) was the primary genus isolated from IB treated kernels followed by *Alternaria* (42.08). Among *Fusarium* isolates, FT was the primary isolate extracted from IB treated seeds followed by FV. But in case of NB and NN treated panicle kernels, FV followed FT were the major isolates. These results suggested that there is antagonistic association between FT and FV. In case of NB and NN, *Alternaria* followed by *Fusarium* were the main fungal genera isolated.

In Table 3.20, summary statistics (mean, range, standard deviation and coefficient of variation as per cent) were reported for kernel viability, kernel germination per cent, vigor index,

and quality traits for 46 sorghum genotypes across treatments. For grain mold ratings (IB: 2.47 for PGMR and 2.90 TGMR) and Deo mean (20.69) was higher in IB than other two treatments. Additionally, for HI, it was found that HI value was lower in kernels collected from IB (63.35) treated panicle than NB (67.44) and NN (66.19) indicating that there is deterioration of kernel per endosperm hardness due to further fungal infection. Results from kernel viability per cent (KVP) and germination per cent (KGP) test clearly reported that GM infection deteriorate seed viability causing poor seed germination. KVP and KGP were minimum in kernels from IB (KVP = 71.94; KGP = 57.53) followed by NB treated (KVP = 91.33; KGP = 73.98) and NN (KVP = 93.44; KGP = 81.60) panicles. Based on mean comparison, it is clear that VI was the lowest in IB (1132.68) followed by NB (1527.92) treated and NN (1755.65) panicles.

**Correlation among fungal genera:** The study revealed significant negative correlation of FV with FT (-0.48) and *Alternaria* (-0.29). FT reported significant negative correlation with FP (-0.18), FIESC (-0.12), *Alternaria* (-0.44) and *Penicillium* (-0.14). These results suggested that FV has antagonistic interaction with FT and *Alternaria*. Similarly, FT has antagonistic interaction with FP, FIESC, *Alternaria* and *Penicillium*. FP reported significant positive correlations with FIESC (0.14), *Aspergillus* (0.19) and *Penicillium* (0.25). Similarly, *Penicillium* has positive correlation with FIESC (0.25) and *Aspergillus* (0.15). These positive associations between suggested that these fungal genera have some kind of positive interaction or association effect. (Table 3.21)

**Correlation between fungal genera and traits related to grain mold resistance and quality traits:** Grain mold ratings have significant negative correlations with KVP (-0.39), KGP (-0.49) and VI (-0.51) suggesting that with increase in grain mold infection there is reduction in kernel viability and germination potential. Noll et al. (2010) reported negative correlation between FT and seed viability.

Grain mold ratings have negative correlations with HI (-0.15 with PGMR; -0.19 with TGMR). Audilakshmi et al. (1999) reported similar results between grain mold ratings and HI (Table 3.22). PGMR and TGMR have negative correlation with FV (-0.18) but positive correlation was reported with FT (0.25). TGMR reported negative correlation with *Alternaria* (-

0.22) suggesting that in presence of FT other fungal genera do not freely grow in same niche. TGMR reported positive correlation with KW and KD suggesting that bigger kernels are more susceptible to grain mold infection. Rodriguez-Ballesteros et al. (2009) reported positive correlation between grain mold rating and kernel size. Results of grain mold ratings with other quality traits followed similar associations, which we reported for quality traits for 229 genotypes in Table 3.13. KGP has significant positive correlation with KVP and VI. Noll et al. (2010) reported similar significant positive correlation between kernel germination and viability. KGP and KVP have significant positive relationship with Fib (0.20 with PGMR; 0.24 with TGMR) and significant negative correlation with Deo (-0.17 with PGMR and TGMR). KVP significantly showed positive correlation with Sta and HI. Similarly, KGP had significant positive correlation with TPP (0.13) and significant negative correlation with Moi (-0.13). With FV and *Alternaria*, KGP had significant positive correlation but with FT showed significant negative correlation. VI reported significant positive correlation with TPP (0.22), Fat (0.16) and HI (+0.14). With Moi (-0.17), KW (-0.27) and KD (-0.24), significant negative correlations were recorded. These findings suggested that quality and physical traits could influence seed viability and germination. .

FT has significant negative correlation with Fib (-0.18) and Sta (-0.16) (Table 3.23). FV had positive correlation with Ash content. These findings suggested that with increase in FT infection there is reduction in fiber and starch per cent in kernel and will result in increase in ash which is basically seed debris resulted from kernel deterioration or rotting. FV showed significant positive correlation with Sta (0.14) and Fib (0.17), and with Moi, it has significant negative correlation (-0.15). *Alternaria* has significant positive correlation with TPP (0.15) and Fat (0.15). Ash content had significant positive correlation with *Curvularia*. With *Aspergillus*, significant negative correlation (-0.16) was observed. Positive associations between quality traits and fungal genera need more detailed study to understand possible behavior and mechanism which could give better explanation for such associations.

HI revealed significant positive correlation with Phe (0.15) and significant negative correlation with Deo (-0.26), KW (-0.53) and KD (-0.54) (Table 3.24). This result suggested that Phe and Deo influence kernel hardness. It also suggested that harder kernels were smaller in

size with low KW, KD and Deo. In chapter 1 using sorghum genotypes from Texas, we reported that HI had significant negative correlation with KW, KD and Deo. KW and KD reported significant positive correlations with Fib (0.42 with KD; 0.48 with KD) and Deo (0.18 with KW; 0.19 with KD). In same study we reported positive correlation of Deo with KW and KD, but with Fib we reported significant negative correlation. KW and KD reported significant negative correlation with Phe (-0.18 with KW and -0.14 with KD) and Tan (-0.16 with KW and -0.13 with KD). This finding suggested that larger kernels are rich in Fib, Deo but are low in Phe and Tan. Between KD and KW, there was significant positive correlation (+0.93).

## **Conclusion**

This study characterized sorghum association panel for grain mold pathogen *F. thapsinum*. We studied the effect of different agronomic and panicle architecture traits on grain mold incidence and severity. Effects of grain mold on grain quality traits were also studied. Selected 46 genotypes were screened for grain mold ratings 1-5 to understand interaction between grain mold fungal pathogens, physical and chemical kernel traits. Seed germination test, vigor index, and tetrazolium viability test were performed to study the effect of grain mold infection on kernel viability and vigor. Based on field and *in vitro* studies, accessions performed better than checks were reported. Tukey means separation test revealed that accessions SC623, SC67, SC621, SC947 and SC1494 were most resistant based on both PGMR and TGMR rating while SC370, SC833, SC1484, and SC1077 showed the most susceptible reaction and this was consistent for individual location analysis. GWAS was used to understand the genetic basis of grain mold resistance. We reported loci associated with grain mold resistance. SC309, SC213, SC833, SC971 and SC1047 are genotypes having identified allele for grain mold resistance. We also reported loci and candidate genes associated with traits like LPBL, GL, Lod, DFF, DPM, Deo, Moi, Phe and Tan. Based on 2014 and 2015 field screening the following genotypes with TSW > 30 g; TPP >10 %; Sta > 60% and grain mold ratings <2 were selected for further breeding work:

- White seed - Dorado, SC301, SC467, SC982, P898012, SC520, SC575
- Yellow seed - SC373, SC372, SC391
- Red seed - SanChiSan, SC25, SC1429, SC63, SC525, SC1047, SC1471
- Brown seed - SC332, SC135, SC563, SC60, SC328, SC155, SC1322

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**Table 3.1. Mean maximum and minimum air temperature, mean relative humidity, and total precipitation for 2014 and 2015 sorghum – growing season in Manhattan, Kansas.**

Period	MAT <sub>Max</sub> (°C)	MAT <sub>Min</sub> (°C)	MRH (%)	STP (cm)
During inoculation period (10 Aug to 6 Sep, 2014)@ 50% flowering	32.44	17.00	62.4	12.36
During PGMR scoring period at physiological maturity (15 Oct- 5 Nov, 2014)	22.66	3.61	58.1	0.22
Overall seasonal (19 June to 5 Nov, 2014)	28.94	13.16	61.5	27.45
During inoculation period (10 Aug to 9 Sep, 2015)@ 50% flowering	33.72	17.00	55.5	1.24
During PGMR scoring period at physiological maturity (17 Oct- 7 Nov, 2015)	20.77	5.50	60.4	5.43
Overall seasonal (20 June to 7 Nov, 2015)	30.22	14.38	58	24.43

MAT = Mean air temperature; MRH = Mean relative humidity; STP = Seasonal total precipitation; PGMR = Panicle grain mold rating



**Table 3.2. Type 3 test of fixed effects for 2014-2015 field traits.**

Traits	Y	T	Y*T
PGMR	***	***	***
TGMR	***	***	***
TSW	***	***	***
PH	***	***	NS
PL	***	***	***
LPBL	***	***	***
GL	***	***	***
GW	***	***	***
GI	***	***	***
SG	***	***	***
Lod	***	***	***
DF	***	***	***
DPM	***	***	***

\*\*\* Significant at  $p < 0.001$

Y= Year; T=Treatment; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; TSW = 1000 Seed weight; PH = Plant height; PL = Panicle length; LPBL = Lowest primary branch length; GL = Glume length; GW = Glume width; GI = Glume index; SG = Staygreen; Lod = Lodging; DF = Days to flowering; DPM = Days to physiological maturity

**Table 3.3. Overall summary statistics for 2014-2015 field traits.**

Traits	H <sup>2</sup>	Year	Mean <sup>a</sup>			Range <sup>b</sup>			SD <sup>c</sup>			CV% <sup>d</sup>		
			IB	NB	NN	IB	NB	NN	IB	NB	NN	IB	NB	NN
PGMR	0.37	2014	2.31a	1.68b	1.36c	1.00-5.00	1.00-4.00	1.00-3.00	1.18	0.84	0.61	51.29	51.10	45.62
		2015	2.47a	1.40b	1.11c	1.00-5.00	1.00-3.67	1.00-2.33	1.04	0.44	0.21	42.23	31.36	18.94
TGMR	0.46	2014	2.72a	1.74b	1.42c	1.00-5.00	1.00-5.00	1.00-2.67	1.08	0.67	0.50	39.97	38.21	35.28
		2015	2.69a	1.63b	1.21c	1.00-5.00	1.00-5.00	1.00-3.00	0.99	0.52	0.23	36.79	32.01	19.20
TSW	0.51	2014	25.71a	27.48b	28.37c	15.87-40.80	18.00-42.00	19.07-43.07	3.92	3.82	3.84	15.23	13.88	13.52
		2015	25.18c	26.33b	27.33a	16.67-39.10	17.50-40.17	18.23-41.03	3.43	3.53	3.46	13.60	13.10	12.88
PH	0.84	2014	131.95a	131.63a	130.9a	60.00-320.00	61.00-320.00	60.00-317.50	38.04	37.98	37.92	28.73	28.81	28.55
		2015	137.95a	138.39a	139.64a	64.33-330.00	68.00-330.17	68.33-327.67	38.40	38.42	38.53	27.68	27.59	27.43
PL	0.45	2014	24.31a	24.57a	24.33a	12.00-40.00	12.00-40.00	11.50-41.00	4.93	9.80	4.89	20.13	39.68	19.98
		2015	25.34a	25.32a	26.15a	13.00-41.67	12.67-40.8	11.00-41.00	4.59	6.77	4.63	18.16	26.75	17.75
LPBL	0.66	2014	6.62b	6.81a	6.88a	1.80-18.43	2.07-22.67	2.53-19.77	2.33	2.45	2.38	34.71	35.51	34.42
		2015	7.95a	8.21a	8.23a	3.33-21.67	3.17-21.7	3.67-25.33	2.19	2.86	2.17	27.16	34.78	26.12
GL	0.63	2014	0.44a	0.42a	0.41a	0.30-0.88	0.32-0.88	0.30-0.88	0.07	0.07	0.07	16.13	16.04	15.94
		2015	0.46ab	0.47a	0.46a	0.30-1.10	0.30-0.90	0.25-1.00	0.08	0.07	0.08	17.19	15.47	17.04
GW	0.43	2014	0.32a	0.31b	0.32a	0.20-0.48	0.20-0.48	0.20-0.48	0.05	0.05	0.05	15.71	15.97	15.38
		2015	0.34a	0.33b	0.34a	0.20-0.50	0.20-0.50	0.20-0.55	0.06	0.05	0.05	19.56	16.76	17.24
GI	0.42	2014	1.36a	1.38a	1.36a	0.91-2.65	0.87-2.79	0.87-2.74	0.23	0.24	0.22	16.74	17.31	16.64
		2015	1.44a	1.46a	1.41b	0.88-2.50	0.75-3.00	0.83-2.50	0.26	0.27	0.26	17.97	18.51	18.30
SG*	0.26	2014		2.3			1.00-5.00			0.85			36.69	
		2015		2.98			1.00-5.00			0.57			19.22	
Lod*	0.45	2014		1.23			1.00-6.00			0.77			62.12	
		2015		1.22			1.00-9.00			0.98			79.15	
DF*	0.42	2014		67.86			53.00-102.00			6.59			9.70	
		2015		68.68			44.00-97.00			8.08			11.76	
DPM*	0.36	2014		107.85			77.00-127.00			4.70			4.35	
		2015		125.98			118.00-144.00			6.89			5.47	

\*For these traits, field responses were recorded per genotype per replication per year; IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; TSW = 1000 Seed weight; PH = Plant height; PL = Panicle length; LPBL = Lowest primary branch length; GL = Glume length; GW = Glume width; GI = Glume index; SG = Staygreen; Lod = Lodging; DF = Days to flowering; DPM = Days to physiological maturity

**Table 3.4. Grain mold ratings for checks in 2014 and 2015.**

Checks	2014						2015					
	PGMR			TGMR			PGMR			TGMR		
	IB	NB	NN	IB	NB	NN	IB	NB	NN	IB	NB	NN
Sureno	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.25	1.00	1.00
RTx2911	2.00	1.00	1.00	2.15	1.15	1.00	2.00	1.25	1.00	1.85	1.15	1.00
Dorado	1.50	1.00	1.00	2.50	1.50	1.00	2.50	1.50	1.00	2.00	1.25	1.00
BTx3197	3.50	3.00	2.00	3.50	3.00	2.00	3.00	2.00	1.20	2.80	2.00	1.20
BTx378	2.70	2.00	1.00	3.00	1.15	1.00	3.00	2.00	1.15	3.20	1.60	1.20
RTx2536	4.00	2.00	1.00	4.00	2.70	2.00	3.50	2.15	1.20	2.30	1.20	1.00
RTx430	NA	NA	NA	NA	NA	NA	5.00	2.70	1.25	4.30	2.30	1.70
RTx2737	NA	NA	NA	NA	NA	NA	3.80	2.20	1.20	4.00	2.00	1.70

NA = Not available; IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating

**Table 3.5. Correlation (Spearman's) coefficients for 2014 and 2015 field traits. Upper right side of diagonal represents correlation coefficients for 2014 field traits and lower left side of the diagonal represent correlation coefficients for 2015 field data.**

Traits	PGMR	TGMR	TSW	PH	PL	LPBL	GL	GW	GI	SG	Lod	Awn	PT	DF	DPM
PGMR	1.00	0.47 ***	-0.13 ***	-0.10 **	-0.01	-0.14 ***	-0.05	0.04	-0.13	0.00	-0.07 **	-0.01	0.13 **	-0.11 **	-0.11 **
TGMR	0.76 ***	1.00	-0.19 ***	-0.27 ***	-0.18 ***	-0.22 ***	-0.16 ***	-0.09 **	-0.07 *	-0.03	-0.14 ***	-0.02	0.25 ***	-0.08 **	-0.08 **
TSW	-0.19 ***	-0.15 ***	1.00	0.03	0.15 ***	0.05	0.11 ***	0.27 ***	-0.20 ***	-0.01	-0.04	-0.15 ***	0.03	0.19 ***	0.16 ***
PH	-0.15 ***	-0.15 ***	0.02	1.00	0.14 ***	0.19 ***	0.18 ***	0.04	0.16 ***	0.09 **	0.26 ***	0.04	-0.20 ***	-0.03	0.02
PL	-0.07 *	-0.09 ***	0.05	0.13 ***	1.00	0.51 ***	0.08 **	0.06 *	-0.03	-0.16 ***	-0.08 **	0.00	-0.40 ***	0.18 ***	0.16 ***
LPBL	-0.07 **	-0.10 ***	0.07 *	0.16 ***	0.38 ***	1.00	0.16 ***	0.06 *	0.09 **	-0.12 ***	0.02	0.02	-0.30 ***	0.17 ***	0.24 ***
GL	-0.03	0.00	0.08 **	0.11 ***	0.11 ***	0.14 ***	1.00	0.51 ***	0.28 ***	-0.08 **	0.14 ***	0.16 ***	-0.10 **	0.05	0.08 **
GW	0.00	0.05	0.07 *	0.04	0.15 ***	0.06 *	0.41 ***	1.00	-0.50 ***	-0.01	-0.06 *	0.10 **	-0.09 **	0.03	0.09 **
GI	-0.02	-0.04	-0.03	0.09 ***	-0.03	0.08 **	0.43 ***	-0.50 ***	1.00	-0.06 *	0.18 ***	0.02	-0.01	0.00	-0.04
SG	0.01	0.05	-0.05	-0.16 ***	0.01	0.02	0.07 **	0.03	0.03	1.00	0.18 ***	-0.08 **	-0.06 *	-0.24 ***	-0.23 ***
Lod	-0.07 *	-0.07 *	-0.04	0.15 ***	-0.06 *	0.03	0.08 **	0.00	0.10 **	0.05	1.00	0.18 ***	-0.17 ***	-0.13 ***	-0.06 *
Awn	-0.04	-0.03	-0.05	0.02	0.02	0.04	0.16 ***	0.12 **	0.02	0.06 *	0.09 **	1.00	-0.14 **	0.02	0.03
PT	0.14 ***	0.15 ***	0.04	-0.22 ***	-0.39 ***	-0.26 ***	-0.07 ***	-0.14 ***	0.05	-0.01	-0.04	-0.14 ***	1.00	0.03	-0.08 **
DF	0.01	-0.04	0.01	-0.04	0.06 *	-0.01	0.00	0.02	-0.01	-0.17 ***	-0.05	-0.03	0.11 ***	1.00	0.68 ***
DPM	0.01	-0.06	-0.03	-0.05	0.02	0.00	-0.03	-0.03	0.01	-0.13 ***	-0.07 *	-0.03	0.09 **	0.89 ***	1.00

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; TSW = 1000 Seed weight; PH = Plant height; PL = Panicle length; LPBL = Lowest primary branch length; GL = Glume length; GW = Glume width; GI = Glume index; SG = Staygreen; Lod = Lodging; PT = Panicle type; DF = Days to flowering; DPM = Days to physiological maturity

**Table 3.6. Fisher F test for ordinal and qualitative data set.**

Traits	2014	2015
	Chi square value	Chi square value
PGMR vs Panicle type	195.47***	360.63***
PGMR vs Glume color	39.24**	93.16*
PGMR vs Seed color	90.51***	163.36**
PGMR vs Lodging	37.83**	NS
PGMR vs Staygreen	NS	120.66**
TGMR vs Panicle type	174.55**	258.73**
TGMR vs Glume color	88.57**	98.23*
TGMR vs Seed color	203.48***	276.42***
TGMR vs Lodging	NS	NS
TGMR vs Staygreen	NS	NS

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating

**Table 3.7. Mean for grain mold ratings (PGMR and TGMR) per treatment in 2014 and 2015, based on seed color (SC).**

Seed color	TG	Year	T	PGMR mean	PGMR range	TGMR mean	TGMR range
Brown	79	2014	IB	2.10	1.00-5.00	2.60	1.00-5.00
			NB	1.60	1.00-4.00	1.60	1.00-3.70
			NN	1.30	1.00-3.00	1.30	1.00-2.70
	79	2015	IB	2.40	1.00-5.00	2.80	1.00-5.00
			NB	1.40	1.00-3.70	1.70	1.00-5.00
			NN	1.10	1.00-2.30	1.20	1.00-3.00
Red	34	2014	IB	2.00	1.00-5.00	2.60	1.00-5.00
			NB	1.40	1.00-4.00	1.50	1.00-3.30
			NN	1.30	1.00-3.00	1.30	1.00-2.30
	34	2015	IB	2.50	1.00-5.00	2.80	1.20-4.80
			NB	1.40	1.00-2.30	1.70	1.00-3.30
			NN	1.10	1.00-1.70	1.20	1.00-2.80
White	96	2014	IB	2.50	1.00-5.00	2.80	1.00-5.00
			NB	1.80	1.00-5.00	1.90	1.00-4.00
			NN	1.50	1.00-3.00	1.50	1.00-2.70
	98	2015	IB	2.50	1.00-5.00	2.50	1.00-5.00
			NB	1.40	1.00-2.70	1.60	1.00-3.20
			NN	1.10	1.00-2.00	1.20	1.00-2.30
Yellow	18	2014	IB	3.10	1.00-5.00	2.60	1.00-5.00
			NB	1.90	1.00-4.00	1.80	1.00-3.30
			NN	1.60	1.00-3.00	1.30	1.00-2.70
	18	2015	IB	2.50	1.00-5.00	3.00	1.00-5.00
			NB	1.70	1.00-3.30	1.40	1.00-3.30
			NN	1.10	1.00-2.30	1.30	1.00-2.20

TG = Total genotypes; T= Treatments; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged

**Table 3.8. Mean for grain mold ratings (PGMR and TGMR) per treatment in 2014 and 2015, based on glume color.**

Glume color	TG	Year	T	PGMR mean	PGMR range	TGMR mean	TGMR range
Black	13	2014	IB	2.60	1.00-5.00	2.30	1.00-4.70
			NB	1.60	1.00-4.00	1.70	1.00-3.00
			NN	1.40	1.00-3.00	1.50	1.00-2.30
	13	2015	IB	2.60	1.00-5.00	2.60	1.00-4.70
			NB	1.50	1.00-2.30	1.60	1.00-2.70
			NN	1.10	1.00-1.30	1.20	1.00-1.70
Beige	81	2014	IB	2.50	1.00-5.00	2.80	1.00-5.00
			NB	1.80	1.00-4.00	1.90	1.00-3.30
			NN	1.40	1.00-3.00	1.50	1.00-2.70
	81	2015	IB	2.50	1.00-4.70	2.80	1.20-5.00
			NB	1.40	1.00-2.70	1.70	1.00-5.00
			NN	1.20	1.00-2.00	1.10	1.00-2.80
Purple	40	2014	IB	2.60	1.00-5.00	2.30	1.00-5.00
			NB	1.60	1.00-5.00	1.70	1.00-3.70
			NN	1.30	1.00-3.00	1.40	1.00-2.70
	40	2015	IB	2.60	1.00-5.00	2.60	1.00-5.00
			NB	1.50	1.00-2.70	1.60	1.00-3.20
			NN	1.10	1.00-2.30	1.30	1.00-3.00
Red	73	2014	IB	2.20	1.00-5.00	2.70	1.00-5.00
			NB	1.50	1.00-3.30	1.70	1.00-4.00
			NN	1.30	1.00-3.00	1.40	1.00-2.70
	75	2015	IB	2.30	1.00-5.00	2.70	1.00-5.00
			NB	1.40	1.00-3.30	1.60	1.00-3.30
			NN	1.10	1.00-2.30	1.20	1.00-2.20
Pale orange	10	2014	IB	2.50	1.00-3.70	2.30	1.00-4.00
			NB	1.50	1.00-2.30	1.80	1.00-2.00
			NN	1.40	1.00-2.00	1.40	1.00-2.00
	10	2015	IB	2.30	1.20-4.70	2.40	1.00-4.70
			NB	1.40	1.00-2.70	1.40	1.00-2.30
			NN	1.10	1.00-1.70	1.10	1.00-1.30



TG = Total genotypes; T= Treatments; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged

**Table 3.9. Mean for grain mold ratings per treatment in 2014 and 2015, based on panicle compactness and shape (Panicle type).**

<b>Panicle type</b>	<b>TG</b>	<b>Year</b>	<b>T</b>	<b>PGMR Mean</b>	<b>PGMR Range</b>	<b>TGMR Mean</b>	<b>TGMR Range</b>
2	2	2014	IB	1.25	1.00-2.00	1.58	1.00-2.30
			NB	1.00	1.00-1.00	1.15	1.00-1.30
			NN	1.00	1.00-1.00	1.08	1.00-1.30
	2	2015	IB	2.50	1.00-3.70	2.68	1.00-3.80
			NB	1.63	1.00-2.80	1.68	1.00-2.30
			NN	1.33	1.00-2.00	1.23	1.00-1.70
3	4	2014	IB	1.50	1.00-5.00	1.84	1.00-2.70
			NB	1.38	1.00-4.00	1.36	1.00-2.30
			NN	1.00	1.00-1.00	1.04	1.00-1.30
	4	2015	IB	1.50	1.00-3.70	1.65	1.00-3.70
			NB	1.16	1.00-2.30	1.28	1.00-2.30
			NN	1.00	1.00-1.00	1.05	1.00-1.20
4	7	2014	IB	1.57	1.00-4.00	2.54	1.00-4.70
			NB	1.36	1.00-3.00	1.84	1.00-2.70
			NN	1.07	1.00-2.00	1.51	1.00-2.70
	7	2015	IB	2.43	1.00-4.30	2.63	1.00-5.00
			NB	1.45	1.00-2.50	1.53	1.00-2.70
			NN	1.15	1.00-1.70	1.17	1.00-1.70
5	12	2014	IB	1.33	1.00-3.00	2.21	1.00-3.30
			NB	1.13	1.00-2.00	1.58	1.00-2.30
			NN	1.04	1.00-2.00	1.43	1.00-1.70
	12	2015	IB	1.50	1.00-2.30	1.80	1.00-2.70
			NB	1.16	1.00-1.70	1.35	1.00-2.20
			NN	1.01	1.00-1.30	1.12	1.00-1.30
6	8	2014	IB	2.00	1.00-5.00	2.16	1.00-5.00
			NB	1.31	1.00-3.00	1.36	1.00-2.30
			NN	1.13	1.00-3.00	1.25	1.00-2.30
	8	2015	IB	1.97	1.00-3.80	2.32	1.00-3.80
			NB	1.37	1.00-2.70	1.43	1.00-2.20
			NN	1.08	1.00-1.70	1.19	1.00-1.80
7	8	2014	IB	2.19	1.00-4.00	2.52	1.00-5.00

			NB	1.25	1.00-3.00	1.73	1.00-3.00
			NN	1.06	1.00-2.00	1.44	1.00-2.30
			IB	1.69	1.00-3.70	2.28	1.00-3.80
	8	2015	NB	1.12	1.00-1.70	1.36	1.00-1.80
			NN	1.03	1.00-1.30	1.15	1.00-1.50
			IB	2.32	1.00-5.00	2.78	1.00-5.00
	109	2014	NB	1.62	1.00-5.00	1.69	1.00-4.00
			NN	1.33	1.00-3.00	1.37	1.00-2.70
8			IB	2.52	1.00-5.00	2.79	1.00-5.00
	111	2015	NB	1.40	1.00-3.30	1.67	1.00-5.00
			NN	1.11	1.00-2.30	1.21	1.00-2.20
			IB	2.76	1.00-5.00	2.95	1.00-5.00
	61	2014	NB	2.00	1.00-4.00	1.96	1.00-3.70
			NN	1.56	1.00-3.00	1.53	1.00-2.70
9			IB	2.81	1.20-4.70	2.87	1.00-5.00
	61	2015	NB	1.51	1.00-2.70	1.70	1.00-4.20
			NN	1.14	1.00-2.00	1.25	1.00-3.00
			IB	3.67	1.00-5.00	4.12	1.00-4.70
	3	2014	NB	2.83	1.00-4.00	2.17	1.00-3.70
			NN	2.00	1.00-3.00	1.90	1.00-2.70
10			IB	4.07	1.00-5.00	4.00	1.00-4.80
	3	2015	NB	2.17	1.00-3.70	2.23	1.00-3.70
			NN	1.48	1.00-2.30	1.70	1.00-2.80
			IB	1.30	1.00-3.00	1.95	1.00-3.30
	5	2014	NB	1.00	1.00-1.00	1.53	1.00-2.70
			NN	1.00	1.00-1.00	1.22	1.00-2.30
11			IB	1.91	1.00-3.00	2.57	1.00-4.20
	5	2015	NB	1.27	1.00-2.00	1.61	1.00-2.70
			NN	1.10	1.00-1.70	1.19	1.00-1.70
			IB	1.25	1.00-2.00	1.43	1.00-2.70
	2	2014	NB	1.00	1.00-1.00	1.25	1.00-2.00
			NN	1.00	1.00-1.00	1.00	1.00-1.00
12			IB	1.35	1.00-1.70	1.65	1.00-1.80
	2	2015	NB	1.00	1.00-1.00	1.28	1.00-1.30
			NN	1.00	1.00-1.00	1.08	1.00-1.30

TG = Total genotypes; T = Treatment; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; Panicle type-

2 = Very loose erect primary branches; 3 = Very loose drooping primary branches; 4 = Loose erect primary branches; 5 = Loose drooping primary branches; 6 = Semi-loose erect primary branches; 7 = Semi-loose drooping primary branches; 8 = Semi-erect compact elliptical; 9 = Compact elliptic; 10 = Compact oval; 11 = Half broom corn; 12 = Broom corn

**Table 3.10. Type 3 test of fixed effects for 2014-2015 NIR spectroscopy traits.**

Traits	Y	T	Y*A
TPP	***	***	***
Moi	***	***	***
Fat	***	***	***
Fib	***	***	***
Ash	***	***	***
Sta	***	***	***
Phe	***	***	***
Tan	***	***	***
Deo	***	***	***

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

Y= Year; T=Treatment; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib = Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g

**Table 3.11. Overall summary statistics for 2014-2015 NIR spectroscopy based quality traits.**

Traits	H <sup>2</sup>	Year	Mean			Range			SD			CV%		
			IB	NB	NN	IB	NB	NN	IB	NB	NN	IB	NB	NN
TPP	0.77	2014	9.78b	9.94b	10.49a	6.36-19.60	6.02-19.22	7.02-19.80	1.89	1.93	2.01	19.27	19.38	19.16
		2015	11.33c	11.60b	12.37a	7.72-19.00	7.98-20.28	8.93-20.60	1.96	1.99	1.94	17.27	17.16	15.64
Moi	0.85	2014	9.19a	9.03b	8.92b	1.54-12.34	0.00-11.73	0.38-11.73	1.23	1.26	1.21	13.41	13.90	13.60
		2015	7.97a	7.73b	7.60c	1.74-10.45	3.14-9.70	1.23-9.58	1.13	0.97	1.09	14.13	12.55	14.33
Fat	0.75	2014	2.70b	2.69b	2.83a	0.94-4.74	0.97-5.06	0.92-4.87	0.72	0.71	0.67	26.56	26.27	23.66
		2015	3.33b	3.33b	3.42a	1.35-5.26	1.37-5.22	1.59-5.15	0.73	0.73	0.62	21.80	21.90	18.15
Fib	0.83	2014	1.03a	1.02b	0.98c	0.87-2.11	1.14-2.31	1.17-2.28	0.20	0.18	0.19	12.54	11.11	11.02
		2015	1.62b	1.72a	1.73a	1.12-2.15	1.27-2.24	1.32-2.25	0.19	0.17	0.18	11.51	10.03	10.26
Ash	1.00	2014	1.21a	1.19b	1.19b	0.88-1.40	0.66-1.35	0.76-1.35	0.08	0.08	0.08	6.41	6.46	6.61
		2015	1.13a	1.11b	1.10c	0.80-1.29	0.84-1.30	0.70-1.28	0.07	0.07	0.08	6.44	6.47	7.47
Sta	0.72	2014	1.34a	1.33a	1.31b	61.56-71.89	62.17-71.76	62.26-71.66	1.58	1.51	1.52	2.36	2.22	2.24
		2015	65.77c	66.56b	66.82a	61.07-69.13	60.98-69.96	62.01-70.23	1.36	1.36	1.36	2.07	2.05	2.04
Phe	0.80	2014	6.58a	6.38ab	6.09b	0.00-37.80	0.00-36.42	0.00-40.97	4.79	4.85	5.13	79.64	77.13	79.14
		2015	4.84b	5.38a	5.72a	0.00-35.26	0.00-33.85	0.00-40.81	4.67	4.62	4.98	95.97	85.67	86.85
Tan	0.77	2014	11.30a	10.99a	9.79b	0.00-84.25	0.00-84.66	0.00-93.01	11.17	11.42	12.15	116.88	107.89	111.38
		2015	7.21b	8.17ab	8.80a	0.00-84.77	0.00-78.15	0.00-95.80	10.24	10.70	11.57	141.54	130.52	130.95
Deo	0.64	2014	18.81a	14.92b	14.36b	0.00-150.38	0.00-169.23	0.00-166.29	20.72	18.47	20.18	109.91	127.06	134.54
		2015	18.92a	10.68b	11.43b	0.00-107.56	0.00-88	0.00-103.39	18.89	13.53	14.58	99.85	126.91	127.77

IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib = Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g

**Table 3.12. Mean for quality traits per treatment based on seed color.**

Seed color	TA	Year	T	TPP	Moi	Fat	Fib	Ash	Sta	Phe	Tan	Deo
Brown	79	2014	IB	10.10	8.84	2.87	1.60	1.19	66.76	8.41	15.53	20.28
			NB	10.31	8.73	2.87	1.68	1.18	67.44	8.87	16.56	14.14
			NN	10.91	8.66	2.97	1.70	1.18	67.52	9.24	17.31	13.66
	79	2015	IB	11.78	7.68	3.43	1.64	1.11	65.45	7.03	11.93	18.79
			NB	11.97	7.57	3.45	1.73	1.10	66.23	7.39	12.86	8.71
			NN	12.80	7.35	3.53	1.75	1.08	66.46	8.04	14.18	10.42
Red	34	2014	IB	10.17	8.85	2.68	1.55	1.19	66.95	7.05	11.28	26.72
			NB	10.25	8.80	2.71	1.64	1.18	67.69	6.88	12.35	20.51
			NN	10.94	8.69	2.85	1.66	1.17	67.73	7.31	12.84	22.36
	34	2015	IB	11.55	7.87	3.44	1.57	1.12	65.55	5.77	9.16	26.22
			NB	12.17	7.65	3.40	1.69	1.11	66.32	6.22	10.67	15.77
			NN	12.96	7.69	3.45	1.71	1.10	66.84	6.23	10.32	13.99
White	96	2014	IB	9.32	9.54	2.52	1.57	1.25	67.53	4.15	5.11	15.27
			NB	9.48	9.34	2.51	1.64	1.23	68.12	4.36	6.20	12.46
			NN	9.95	9.20	2.68	1.67	1.22	68.17	4.40	6.32	12.98
	98	2015	IB	10.87	8.24	3.17	1.62	1.15	66.12	3.21	3.83	16.52
			NB	11.08	7.91	3.18	1.72	1.14	66.92	3.72	4.29	10.22
			NN	11.83	7.79	3.29	1.73	1.13	67.09	3.94	4.82	11.40
Yellow	18	2014	IB	9.82	9.69	3.01	1.64	1.22	66.95	4.52	7.05	16.18
			NB	9.82	9.24	2.96	1.75	1.19	67.86	5.34	9.22	14.29
			NN	10.60	9.23	3.03	1.76	1.18	67.78	5.21	8.29	17.29
	18	2015	IB	11.24	8.39	3.58	1.65	1.11	65.54	3.48	4.58	22.86
			NB	11.79	7.93	3.52	1.76	1.09	66.44	4.45	5.91	12.50
			NN	12.58	7.84	3.60	1.78	1.08	66.90	5.08	7.29	10.16

TG = Total genotypes; T=Treatment; IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib = Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g

**Table 3.13. Correlation (Spearman's) coefficients for 2014 and 2015 quality traits. Upper right side of diagonal represents correlation coefficients for 2014 traits data and lower left side of the diagonal represent correlation coefficients for 2015 data.**

Traits	TGMR	PGMR	TSW	TPP	Moi	Fat	Fib	Ash	Sta	Phe	Tan	Deo
TGMR	1	0.50	-0.13	-0.21	0.17	-0.23	-0.19	0.21	0.04	-0.25	-0.26	0.19
		***	***	***	***	***	***	***		***	***	***
PGMR	0.76	1	-0.17	-0.11	0.11	-0.13	-0.14	0.15	-0.05	-0.26	-0.25	0.16
	***		***	***	***	***	***	***		***	***	***
TSW	-0.18	-0.12	1	0.11	0.05	0.14	0.23	0.07	0.03	-0.15	-0.11	0.04
	***	***		***		***	***	**		***	***	
TPP	-0.17	-0.13	0.14	1	-0.11	0.61	0.17	-0.03	-0.59	0.11	0.11	0.08
	***	***	***		***	***	***		***	***	***	*
Moi	0.15	0.15	-0.03	-0.28	1	-0.02	-0.05	0.69	-0.06	-0.49	-0.43	-0.14
	***	***		***			*	***	*	***	***	***
Fat	-0.05	-0.06	0.02	0.66	-0.23	1	0.41	-0.18	-0.43	-0.11	-0.15	-0.05
	*	*		***	***		***	***	***	***	***	
Fib	-0.17	-0.18	0.07	0.15	-0.27	0.29	1	-0.29	-0.12	0.06	0.09	-0.17
	***	***	*	***	***	***		***	***	*	**	***
Ash	0.06	0.108	0.031	-0.23	0.65	-0.38	-0.45	1	-0.17	-0.44	-0.47	0.01
	*	***		***	***	***	***		***	***	***	
Sta	-0.25	-0.22	0.03	-0.56	0.01	-0.42	0.03	-0.03	1	-0.10	-0.11	-0.13
	***	***		***		***				**	***	**
Phe	-0.13	-0.15	-0.09	-0.02	-0.48	-0.12	0.03	-0.29	0.06	1	0.93	-0.08
	***	***	**		***	***		***	*		***	***
Tan	-0.12	-0.14	-0.06	-0.04	-0.47	-0.13	0.03	-0.33	0.07	0.93	1	-0.09
	***	***	*		***	***		***	**	***		***
Deo	0.21	0.16	0.00	0.06	0.02	0.04	-0.16	0.09	-0.26	-0.08	-0.10	1
	***	***		*			***	**	***	**	***	

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$



PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib = Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g

**Table 3.14. Chromosome locations, and other summary statistics for SNPs associated with different field traits in 2014 and 2015.**

Trait	Year	Treatment	SNP	P.value	MAF	Allelic Effect
DF	2014	LINE	S9_57512180	2.31E-08	0.01	-15.27
DPM	2014	LINE	S5_56712991	3.02E-07	0.02	6.79
GL	2014	IB	S3_67033014	1.57E-08	0.01	0.20
GL	2014	IB	S6_60367299	1.57E-08	0.01	0.20
GL	2014	IB	S5_24012095	1.64E-08	0.01	-0.17
GL	2014	NB	S3_67033014	1.43E-08	0.01	0.20
GL	2014	NB	S6_60367299	1.43E-08	0.01	0.20
GL	2014	NB	S5_24012095	1.51E-08	0.01	-0.17
GL	2014	NB	S4_65592008	5.95E-08	0.02	0.14
GL	2014	NN	S3_67033014	8.39E-09	0.01	0.21
GL	2014	NN	S6_60367299	8.39E-09	0.01	0.21
GL	2014	NN	S5_24012095	1.13E-08	0.01	-0.17
GL	2014	NN	S4_65592008	9.64E-08	0.02	0.14
GL	2015	IB	S3_67033014	6.03E-10	0.01	0.25
GL	2015	IB	S6_60367299	6.03E-10	0.01	0.25
GL	2015	IB	S8_30544247	6.88E-09	0.01	0.21
GL	2015	IB	S1_43415822	8.83E-09	0.01	-0.20
GL	2015	IB	S1_43415793	8.83E-09	0.01	0.20
GL	2015	IB	S1_43415807	8.83E-09	0.01	0.20
GL	2015	IB	S5_24012095	1.29E-08	0.01	-0.19
GL	2015	IB	S10_3268340	3.91E-08	0.01	0.17
GL	2015	IB	S2_45873489	3.96E-08	0.01	0.17
GL	2015	IB	S3_504045	5.29E-08	0.02	-0.15
GL	2015	NB	S1_43415793	6.61E-08	0.01	0.18
GL	2015	NB	S1_43415807	6.61E-08	0.01	0.18
GL	2015	NB	S1_43415822	6.61E-08	0.01	-0.18
GL	2015	NN	S3_67033014	2.44E-11	0.01	0.25
GL	2015	NN	S6_60367299	2.44E-11	0.01	0.25
GL	2015	NN	S1_43415793	2.75E-11	0.01	0.23
GL	2015	NN	S1_43415807	2.75E-11	0.01	0.23
GL	2015	NN	S1_43415822	2.75E-11	0.01	-0.23
GL	2015	NN	S10_3268340	4.22E-10	0.01	0.18
GL	2015	NN	S8_30544247	1.01E-09	0.01	0.20
GL	2015	NN	S5_24012095	1.02E-09	0.01	-0.19

GL	2015	NN	S2_6857323	1.19E-08	0.01	0.17
GL	2015	NN	S6_56032063	4.91E-08	0.02	-0.14
GL	2015	NN	S4_65592008	5.53E-08	0.02	0.14
GI	2014	IB	S3_67033014	2.24E-08	0.01	0.65
GI	2014	IB	S6_60367299	2.24E-08	0.01	0.65
GI	2014	NB	S3_67033014	4.49E-09	0.01	0.70
GI	2014	NB	S6_60367299	4.49E-09	0.01	0.70
GI	2014	NB	S8_30544247	8.06E-08	0.01	0.57
GI	2014	NB	S5_8042669	9.51E-08	0.02	0.37
GI	2014	NN	S3_67033014	3.85E-09	0.01	0.67
GI	2014	NN	S6_60367299	3.85E-09	0.01	0.67
GI	2014	NN	S8_30544247	6.59E-08	0.01	0.55
Lod	2014	LINE	S6_8196103	1.14E-11	0.02	1.89
Lod	2014	LINE	S5_61278370	5.78E-11	0.02	-1.80
Lod	2014	LINE	S2_74030881	3.73E-10	0.03	-1.41
Lod	2014	LINE	S4_64368929	1.93E-09	0.02	1.50
Lod	2014	LINE	S6_50165577	8.16E-09	0.02	-1.42
Lod	2014	LINE	S5_20322083	1.07E-08	0.02	1.43
Lod	2014	LINE	S6_48712306	2.21E-08	0.03	-1.26
Lod	2014	LINE	S6_48666386	2.34E-08	0.03	1.22
Lod	2014	LINE	S7_56624989	4.48E-08	0.02	-1.29
Lod	2014	LINE	S9_47197540	5.83E-08	0.04	-0.96
Lod	2014	LINE	S9_47197545	5.83E-08	0.04	-0.96
Lod	2014	LINE	S7_59483342	7.11E-08	0.02	-1.15
Lod	2014	LINE	S7_59483390	7.11E-08	0.02	-1.15
Lod	2014	LINE	S2_75033606	7.25E-08	0.01	-1.45
Lod	2014	LINE	S8_35623721	7.52E-08	0.01	1.75
Lod	2014	LINE	S6_48743331	7.56E-08	0.03	-1.10
Lod	2014	LINE	S6_48743332	7.56E-08	0.03	-1.10
Lod	2014	LINE	S3_8023907	8.98E-08	0.03	1.17
Lod	2015	LINE	S6_56429588	7.86E-11	0.01	2.14
Lod	2015	LINE	S8_1674514	8.42E-11	0.01	-2.12
Lod	2015	LINE	S10_16292917	2.57E-09	0.03	-1.38
Lod	2015	LINE	S4_24592944	6.14E-09	0.02	2.00
Lod	2015	LINE	S2_4762527	7.87E-09	0.02	-1.95
Lod	2015	LINE	S5_12075266	1.16E-08	0.02	1.57
Lod	2015	LINE	S6_8196103	1.22E-08	0.02	1.92
Lod	2015	LINE	S6_48743331	1.25E-08	0.03	-1.45
Lod	2015	LINE	S6_48743332	1.25E-08	0.03	-1.45
Lod	2015	LINE	S8_1688906	1.71E-08	0.02	-1.76

Lod	2015	LINE	S5_20322083	1.71E-08	0.02	1.74
Lod	2015	LINE	S4_3690465	1.73E-08	0.02	1.72
Lod	2015	LINE	S5_61278370	1.79E-08	0.02	-1.88
Lod	2015	LINE	S9_57837663	2.35E-08	0.01	2.19
Lod	2015	LINE	S7_6848600	2.55E-08	0.01	-2.01
Lod	2015	LINE	S10_14069029	2.58E-08	0.03	-1.34
Lod	2015	LINE	S10_34137357	3.03E-08	0.03	1.46
Lod	2015	LINE	S10_24061642	3.15E-08	0.01	1.99
Lod	2015	LINE	S4_3509809	3.65E-08	0.03	1.58
Lod	2015	LINE	S1_8195946	4.34E-08	0.02	-1.76
Lod	2015	LINE	S6_49961669	4.54E-08	0.01	-2.00
Lod	2015	LINE	S9_58202041	4.63E-08	0.02	-1.74
Lod	2015	LINE	S1_8131902	4.84E-08	0.02	-1.76
Lod	2015	LINE	S5_22241703	4.97E-08	0.02	1.81
Lod	2015	LINE	S6_48743333	5.25E-08	0.03	1.34
Lod	2015	LINE	S5_12075268	5.52E-08	0.03	-1.44
Lod	2015	LINE	S5_22335164	5.60E-08	0.03	-1.43
Lod	2015	LINE	S5_26558101	7.29E-08	0.03	1.44
Lod	2015	LINE	S1_7807789	9.62E-08	0.01	-1.78
LPBL	2014	IB	S1_38937804	2.66E-08	0.01	-6.00
LPBL	2014	IB	S1_47665610	2.87E-08	0.02	5.27
LPBL	2014	NB	S1_47665610	2.14E-08	0.02	5.73
LPBL	2014	NB	S7_41747887	2.23E-08	0.02	-5.31
LPBL	2014	NB	S10_45364785	5.29E-08	0.02	5.28
LPBL	2014	NB	S10_45804776	5.42E-08	0.01	-6.98
LPBL	2014	NB	S10_52007000	5.44E-08	0.05	2.86
LPBL	2014	NB	S2_76233142	7.04E-08	0.12	1.74
LPBL	2014	NB	S2_76233146	7.04E-08	0.12	1.74
LPBL	2014	NN	S1_38937804	1.90E-09	0.01	-5.15
LPBL	2014	NN	S4_67556910	5.63E-08	0.01	4.57
LPBL	2014	NN	S5_54297848	7.42E-08	0.01	4.18
LPBL	2015	IB	S7_41747887	2.20E-08	0.02	-4.22
LPBL	2015	IB	S1_47665610	2.20E-08	0.02	4.50
LPBL	2015	NB	S5_9215374	1.63E-14	0.01	9.89
LPBL	2015	NB	S2_1789087	1.84E-12	0.03	-6.90
LPBL	2015	NB	S2_1725555	3.21E-11	0.03	6.63
LPBL	2015	NB	S7_61136114	3.35E-11	0.01	-8.14
LPBL	2015	NB	S2_1725585	6.68E-11	0.03	6.15
LPBL	2015	NB	S2_1694283	7.59E-11	0.03	-6.43
LPBL	2015	NB	S3_65729139	3.94E-10	0.01	-8.91

LPBL	2015	NB	S4_62195064	9.94E-10	0.02	6.12
LPBL	2015	NB	S2_1794660	1.38E-09	0.03	5.03
LPBL	2015	NB	S10_37396402	1.44E-09	0.01	8.50
LPBL	2015	NB	S7_4918379	2.89E-09	0.02	6.49
LPBL	2015	NB	S5_22482695	6.54E-09	0.02	6.76
LPBL	2015	NB	S3_63905352	9.95E-09	0.01	-8.49
LPBL	2015	NB	S3_45981557	1.34E-08	0.02	5.53
LPBL	2015	NB	S3_45981558	1.34E-08	0.02	5.53
LPBL	2015	NB	S2_1693887	1.58E-08	0.02	-6.09
LPBL	2015	NB	S2_26486857	2.03E-08	0.02	-5.18
LPBL	2015	NB	S3_46900770	3.98E-08	0.02	5.12
LPBL	2015	NB	S8_46201871	4.02E-08	0.03	-5.42
LPBL	2015	NB	S2_5536622	4.31E-08	0.01	9.19
LPBL	2015	NB	S2_6773478	4.31E-08	0.01	9.19
LPBL	2015	NB	S4_60490544	4.31E-08	0.01	9.19
LPBL	2015	NB	S2_5536591	4.31E-08	0.01	-9.19
LPBL	2015	NB	S2_5536605	4.31E-08	0.01	-9.19
LPBL	2015	NB	S2_5536614	4.31E-08	0.01	-9.19
LPBL	2015	NB	S2_5946019	4.31E-08	0.01	-9.19
LPBL	2015	NB	S2_5999721	4.31E-08	0.01	-9.19
LPBL	2015	NB	S2_59754900	5.52E-08	0.03	-4.55
LPBL	2015	NB	S7_61275464	6.79E-08	0.05	-3.00
LPBL	2015	NB	S2_23102136	8.50E-08	0.01	-7.19
LPBL	2015	NN	S10_45804776	2.29E-10	0.01	-6.83
LPBL	2015	NN	S7_41747887	3.95E-10	0.02	-4.87
LPBL	2015	NN	S1_20159690	5.20E-10	0.01	-6.35
LPBL	2015	NN	S5_5373617	6.74E-10	0.01	-6.10
LPBL	2015	NN	S1_47665610	1.17E-09	0.02	5.02
LPBL	2015	NN	S10_45364785	3.24E-09	0.02	4.63
PGMR	2015	NB	S1_43223535	1.95E-08	0.01	1.10
PGMR	2015	NN	S9_58695115	9.48E-08	0.04	-0.28
PH	2014	IB	S6_41241264	1.34E-09	0.01	79.57
PH	2014	IB	S6_41241273	1.34E-09	0.01	79.57
PH	2014	IB	S6_44753204	3.29E-09	0.02	-68.57
PH	2014	IB	S6_44753242	3.29E-09	0.02	-68.57
PH	2014	IB	S6_44753240	3.29E-09	0.02	68.57
PH	2014	IB	S6_41240065	3.80E-09	0.02	-73.23
PH	2014	IB	S6_41240066	3.80E-09	0.02	-73.23
PH	2014	IB	S6_41240067	3.80E-09	0.02	-73.23
PH	2014	IB	S6_41240063	3.80E-09	0.02	73.23

PH	2014	IB	S6_41238791	3.93E-09	0.02	-69.97
PH	2014	IB	S6_41238816	3.93E-09	0.02	-69.97
PH	2014	IB	S6_38989060	5.85E-09	0.03	62.85
PH	2014	IB	S6_41238522	7.29E-09	0.02	-81.07
PH	2014	IB	S6_38650911	8.23E-09	0.03	-58.99
PH	2014	IB	S9_55804298	1.99E-08	0.02	55.00
PH	2014	IB	S6_41428532	2.10E-08	0.03	57.18
PH	2014	IB	S6_38823693	2.17E-08	0.03	-62.19
PH	2014	IB	S6_41241303	3.08E-08	0.03	52.63
PH	2014	IB	S6_41238845	3.31E-08	0.03	67.33
PH	2014	IB	S6_41317554	3.91E-08	0.04	51.25
PH	2014	IB	S6_37881800	4.67E-08	0.02	62.44
PH	2014	IB	S6_32259652	5.11E-08	0.03	49.80
PH	2014	IB	S6_41317209	5.41E-08	0.03	-57.73
PH	2014	IB	S6_41241916	6.00E-08	0.02	-61.10
PH	2014	IB	S6_41241925	6.00E-08	0.02	61.10
PH	2014	IB	S6_41317276	6.98E-08	0.02	-58.81
PH	2014	IB	S6_38753869	7.94E-08	0.02	-61.01
PH	2014	NB	S6_41241264	1.09E-09	0.01	79.84
PH	2014	NB	S6_41241273	1.09E-09	0.01	79.84
PH	2014	NB	S6_41238791	2.83E-09	0.02	-70.49
PH	2014	NB	S6_41238816	2.83E-09	0.02	-68.71
PH	2014	NB	S6_41240065	2.88E-09	0.02	-70.49
PH	2014	NB	S6_41240066	2.88E-09	0.02	-73.67
PH	2014	NB	S6_41240067	2.88E-09	0.02	-73.67
PH	2014	NB	S6_41240063	2.88E-09	0.02	73.67
PH	2014	NB	S6_44753204	3.26E-09	0.02	-68.31
PH	2014	NB	S6_44753242	3.26E-09	0.02	-68.31
PH	2014	NB	S6_44753240	3.26E-09	0.02	68.31
PH	2014	NB	S6_41238522	6.22E-09	0.02	-81.21
PH	2014	NB	S6_38989060	8.42E-09	0.03	61.85
PH	2014	NB	S6_38650911	9.82E-09	0.03	-58.35
PH	2014	NB	S6_38823693	2.09E-08	0.03	-62.01
PH	2014	NB	S9_55804298	2.34E-08	0.02	72.90
PH	2014	NB	S6_41241303	2.46E-08	0.03	52.89
PH	2014	NB	S6_41428532	2.97E-08	0.03	56.21
PH	2014	NB	S6_41238845	3.27E-08	0.03	67.03
PH	2014	NB	S6_41317554	4.23E-08	0.04	50.86
PH	2014	NB	S6_41241925	4.66E-08	0.02	61.47
PH	2014	NB	S6_41241916	4.66E-08	0.02	-61.47

PH	2014	NB	S6_41317209	4.94E-08	0.03	-57.72
PH	2014	NB	S6_32259652	4.98E-08	0.03	49.65
PH	2014	NB	S6_37881800	5.71E-08	0.02	61.78
PH	2014	NB	S6_41317276	6.25E-08	0.02	-58.85
PH	2014	NN	S6_41241264	2.22E-09	0.01	78.00
PH	2014	NN	S6_41241273	2.22E-09	0.01	78.00
PH	2014	NN	S6_44753204	5.14E-09	0.02	-67.09
PH	2014	NN	S6_44753242	5.14E-09	0.02	-67.09
PH	2014	NN	S6_44753240	5.14E-09	0.02	67.09
PH	2014	NN	S6_41240063	5.48E-09	0.02	72.06
PH	2014	NN	S6_41240065	5.48E-09	0.02	-72.06
PH	2014	NN	S6_41240066	5.48E-09	0.02	-72.06
PH	2014	NN	S6_41240067	5.48E-09	0.02	-72.06
PH	2014	NN	S6_41238791	6.04E-09	0.02	-68.71
PH	2014	NN	S6_41238816	6.04E-09	0.02	-68.71
PH	2014	NN	S6_38989060	1.44E-08	0.03	60.52
PH	2014	NN	S6_41238522	1.46E-08	0.02	-78.77
PH	2014	NN	S6_38650911	1.74E-08	0.03	-56.96
PH	2014	NN	S9_55804298	3.36E-08	0.02	71.87
PH	2014	NN	S6_38823693	4.03E-08	0.03	-60.39
PH	2014	NN	S6_41241303	4.24E-08	0.03	51.79
PH	2014	NN	S6_41428532	4.34E-08	0.03	55.19
PH	2014	NN	S6_41238845	5.30E-08	0.03	65.59
PH	2014	NN	S6_41317554	6.17E-08	0.04	49.93
PH	2014	NN	S6_41317209	8.65E-08	0.03	-56.40
PH	2014	NN	S6_41241916	8.70E-08	0.02	-59.95
PH	2014	NN	S6_41241925	8.70E-08	0.02	59.95
PH	2014	NN	S6_32259652	8.86E-08	0.03	48.45
PH	2014	NN	S6_37881800	9.33E-08	0.02	60.49
PH	2015	IB	S6_41241264	1.33E-09	0.01	79.13
PH	2015	IB	S6_41241273	1.33E-09	0.01	79.13
PH	2015	IB	S6_41238791	2.65E-09	0.02	-70.42
PH	2015	IB	S6_41238816	2.65E-09	0.02	-70.42
PH	2015	IB	S6_41240063	4.84E-09	0.02	72.27
PH	2015	IB	S6_41240065	4.84E-09	0.02	-72.27
PH	2015	IB	S6_41240066	4.84E-09	0.02	-72.27
PH	2015	IB	S6_41240067	4.84E-09	0.02	-72.27
PH	2015	IB	S6_41238522	4.96E-09	0.02	-81.60
PH	2015	IB	S6_44753204	7.74E-09	0.02	-66.38
PH	2015	IB	S6_44753242	7.74E-09	0.02	-66.38

PH	2015	IB	S6_44753240	7.74E-09	0.02	66.38
PH	2015	IB	S6_38989060	7.82E-09	0.03	61.93
PH	2015	IB	S6_38650911	1.03E-08	0.03	-58.26
PH	2015	IB	S9_55804298	1.32E-08	0.02	74.02
PH	2015	IB	S6_41428532	2.41E-08	0.03	56.60
PH	2015	IB	S6_38823693	3.04E-08	0.03	-61.13
PH	2015	IB	S6_41241303	3.11E-08	0.03	52.31
PH	2015	IB	S6_41317209	3.42E-08	0.03	-58.33
PH	2015	IB	S6_41238845	4.95E-08	0.03	66.02
PH	2015	IB	S6_37881800	4.98E-08	0.02	61.94
PH	2015	IB	S6_41241916	5.16E-08	0.02	-61.07
PH	2015	IB	S6_41241925	5.16E-08	0.02	61.07
PH	2015	IB	S6_41317554	5.38E-08	0.04	50.41
PH	2015	IB	S6_32259652	5.92E-08	0.03	49.26
PH	2015	IB	S6_41317276	5.95E-08	0.02	-58.81
PH	2015	IB	S6_38176360	6.71E-08	0.02	-60.94
PH	2015	IB	S6_38753869	7.54E-08	0.02	-60.77
PH	2015	NB	S6_41241264	1.33E-09	0.01	79.13
PH	2015	NB	S6_41241273	1.33E-09	0.01	79.13
PH	2015	NB	S6_41238791	2.65E-09	0.02	-70.42
PH	2015	NB	S6_41238816	2.65E-09	0.02	-70.42
PH	2015	NB	S6_41240063	4.84E-09	0.02	72.27
PH	2015	NB	S6_41240065	4.84E-09	0.02	-72.27
PH	2015	NB	S6_41240066	4.84E-09	0.02	-72.27
PH	2015	NB	S6_41240067	4.84E-09	0.02	-72.27
PH	2015	NB	S6_41238522	4.96E-09	0.02	-81.60
PH	2015	NB	S6_44753204	7.74E-09	0.02	-66.38
PH	2015	NB	S6_44753242	7.74E-09	0.02	-66.38
PH	2015	NB	S6_44753240	7.74E-09	0.02	66.38
PH	2015	NB	S6_38989060	7.82E-09	0.03	61.93
PH	2015	NB	S6_38650911	1.03E-08	0.03	-58.26
PH	2015	NB	S9_55804298	1.32E-08	0.02	74.02
PH	2015	NB	S6_41428532	2.41E-08	0.03	56.60
PH	2015	NB	S6_38823693	3.04E-08	0.03	-61.13
PH	2015	NB	S6_41241303	3.11E-08	0.03	52.31
PH	2015	NB	S6_41317209	3.42E-08	0.03	-58.33
PH	2015	NB	S6_41238845	4.95E-08	0.03	66.02
PH	2015	NB	S6_37881800	4.98E-08	0.02	61.94
PH	2015	NB	S6_41241916	5.16E-08	0.02	-61.07
PH	2015	NB	S6_41241925	5.16E-08	0.02	61.07



PH	2015	NB	S6_41317554	5.38E-08	0.04	50.41
PH	2015	NB	S6_32259652	5.92E-08	0.03	49.26
PH	2015	NB	S6_41317276	5.95E-08	0.02	-58.81
PH	2015	NB	S6_38176360	6.71E-08	0.02	-60.94
PH	2015	NB	S6_38753869	7.54E-08	0.02	-60.77
PH	2015	NN	S6_41241264	1.64E-09	0.01	78.97
PH	2015	NN	S6_41241273	1.64E-09	0.01	78.97
PH	2015	NN	S6_41238791	6.23E-09	0.02	-68.86
PH	2015	NN	S6_41238816	6.23E-09	0.02	-68.86
PH	2015	NN	S6_41238522	6.26E-09	0.02	-81.37
PH	2015	NN	S6_41240065	6.62E-09	0.02	-71.85
PH	2015	NN	S6_41240066	6.62E-09	0.02	-71.85
PH	2015	NN	S6_41240067	6.62E-09	0.02	-71.85
PH	2015	NN	S6_41240063	6.62E-09	0.02	71.85
PH	2015	NN	S6_44753204	8.34E-09	0.02	-66.54
PH	2015	NN	S6_44753242	8.34E-09	0.02	-66.54
PH	2015	NN	S6_44753240	8.34E-09	0.02	66.54
PH	2015	NN	S6_38989060	9.82E-09	0.03	61.78
PH	2015	NN	S6_38650911	1.13E-08	0.03	-58.39
PH	2015	NN	S9_55804298	1.34E-08	0.02	74.27
PH	2015	NN	S6_38823693	3.09E-08	0.03	-61.40
PH	2015	NN	S6_41428532	3.37E-08	0.03	56.26
PH	2015	NN	S6_41241303	4.46E-08	0.03	51.87
PH	2015	NN	S6_41238845	5.99E-08	0.03	65.92
PH	2015	NN	S6_41241925	6.12E-08	0.02	60.96
PH	2015	NN	S6_41241916	6.12E-08	0.02	-60.96
PH	2015	NN	S6_37881800	6.23E-08	0.02	61.72
PH	2015	NN	S6_32259652	6.63E-08	0.03	49.29
PH	2015	NN	S6_41317276	6.76E-08	0.02	-58.81
PH	2015	NN	S6_41317209	7.06E-08	0.03	-57.11
PH	2015	NN	S6_41317554	7.80E-08	0.04	50.00
PH	2015	NN	S6_38753869	8.84E-08	0.02	-60.68
SC	2014	IB	S8_43259635	8.62E-09	0.01	2.98

PGMR = Panicle grain mold rating; PH = Plant height; LPBL = Lowest primary branch length; GL = Glume length; GI = Glume index; Lod = Lodging; DF = Days to flowering; DPM = Days to physiological maturity; IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; Line = Data collected per genotype

**Table 3.15. Chromosome locations, and other summary statistics for SNPs associated with different NIR traits in 2014 and 2015.**

Trait	Year	Treatment	SNP	P.value	MAF	Allelic Effect
Deo	2014	IB	S1_27515950	9.31E-08	0.08	-16.19
Deo	2014	NB	S2_16670356	4.33E-09	0.02	28.03
Deo	2014	NB	S2_18596604	4.80E-08	0.03	-22.84
Deo	2014	NB	S3_53317716	9.94E-10	0.01	42.12
Deo	2014	NN	S3_53317716	1.16E-08	0.01	40.07
Deo	2014	NB	S6_45816090	5.63E-09	0.03	-29.88
Deo	2014	NB	S6_45861653	9.09E-09	0.03	23.16
Deo	2014	NB	S6_45861733	3.51E-09	0.03	-29.46
Deo	2014	NN	S6_45861733	9.73E-08	0.03	-26.99
Deo	2014	NB	S6_48246041	2.92E-08	0.03	-27.82
Deo	2014	NB	S6_48246053	2.92E-08	0.03	-27.82
Deo	2014	NB	S8_41129598	4.39E-08	0.01	-35.35
Deo	2014	NB	S9_57512180	5.79E-09	0.01	-40.52
Deo	2014	NN	S9_57512180	9.77E-08	0.01	-37.64
Deo	2015	NB	S1_22280313	1.33E-08	0.01	39.25
Deo	2015	NN	S1_22280313	2.91E-08	0.01	39.81
Deo	2015	NB	S1_27533638	1.33E-08	0.01	39.25
Deo	2015	NN	S1_27533638	2.91E-08	0.01	39.81
Deo	2015	NB	S10_15568565	1.33E-08	0.01	39.25
Deo	2015	NN	S10_15568565	2.91E-08	0.01	39.81
Deo	2015	NB	S2_5829058	4.34E-09	0.01	-36.47
Deo	2015	NB	S2_62561916	1.33E-08	0.01	39.25
Deo	2015	NN	S2_62561916	2.91E-08	0.01	39.81
Deo	2015	NB	S2_71074299	1.93E-08	0.02	25.79
Deo	2015	NB	S2_71074304	1.93E-08	0.02	-25.79
Deo	2015	NB	S3_68832001	1.33E-08	0.01	39.25
Deo	2015	NN	S3_68832001	2.91E-08	0.01	39.81
Deo	2015	NB	S3_72721409	1.33E-08	0.01	39.25
Deo	2015	NN	S3_72721409	2.91E-08	0.01	39.81
Deo	2015	NB	S4_3259546	2.02E-10	0.01	-36.66
Deo	2015	NN	S4_3259546	9.65E-08	0.01	-31.46
Deo	2015	NB	S4_3905697	1.33E-08	0.01	-39.25
Deo	2015	NN	S4_3905697	2.91E-08	0.01	-39.81
Deo	2015	NN	S4_3943073	9.53E-11	0.01	-38.54
Deo	2015	NB	S5_10151682	1.33E-08	0.01	39.25

Deo	2015	NN	S5_10151682	2.91E-08	0.01	39.81
Deo	2015	NN	S6_31673491	6.12E-09	0.01	-31.63
Deo	2015	NB	S7_1266866	1.33E-08	0.01	39.25
Deo	2015	NN	S7_1266866	2.91E-08	0.01	39.81
Deo	2015	NB	S8_55130953	1.33E-08	0.01	-39.25
Deo	2015	NN	S8_55130953	2.91E-08	0.01	-39.81
Deo	2015	NN	S9_54841588	1.77E-08	0.03	23.95
Moi	2014	NN	S4_59573016	8.33E-08	0.03	1.74
Moi	2014	NB	S5_41382017	4.50E-08	0.01	-2.35
Moi	2014	IB	S6_44564702	5.36E-08	0.02	2.22
Moi	2014	NB	S6_44564702	1.87E-08	0.02	2.31
Moi	2014	IB	S7_18825659	4.11E-08	0.01	-2.62
Moi	2015	IB	S4_4603470	6.85E-08	0.03	-1.39
Moi	2015	IB	S4_4603471	6.85E-08	0.03	-1.39
Moi	2015	NB	S4_62195064	1.77E-08	0.03	-1.38
Moi	2015	IB	S7_5195801	1.40E-08	0.03	-2.01
Moi	2015	NN	S7_5195801	2.69E-08	0.03	-1.91
Phe	2015	IB	S4_60837496	1.62E-07	0.06	4.20
Phe	2015	NB	S4_60837496	9.35E-08	0.06	4.20
Phe	2015	NN	S4_60837496	5.71E-07	0.06	4.27
Tan	2015	IB	S4_60837496	1.25E-07	0.06	9.34
Tan	2015	NB	S4_60837496	1.92E-08	0.06	10.47
Tan	2015	NN	S4_60837496	4.42E-07	0.06	9.96
TPP	2014	IB	S4_3943073	5.16E-08	0.01	0.79

IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib= Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g

**Table 3.16. Chromosome locations, and other summary statistics for SNPs associated with different field and NIR traits in 2014 and 2015 based on *Sorghum bicolor* v1.4.**

Trait	SNP	Gene ID	Start position, bp	End position, bp	Putative gene description
DF	S9_57512180	Sb09g028590	57,473,451	57,475,646	Ribosomal protein L18ae/LX family protein
DPM	S5_56712991	Sb05g023760	56,554,719	56,559,321	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein
GL	S1_43415793	Nothing close			
GL	S1_43415807	Nothing close			
GL	S1_43415822	Nothing close			
GL	S10_3268340	Sb10g003830	3,344,774	3,345,310	Zinc finger, C3HC4 type domain containing protein, expressed
GL	S2_45873489	Sb02g018880	45,889,744	45,894,156	Mnd1 family protein
GL	S2_6857323	Sb02g005750	6,810,247	6,812,784	Oxoglutarate/iron-dependent oxygenase
GL	S3_504045	Sb03g000680	484,190	485,887	AGC kinase 1.7
GL	S3_67033014	Sb03g039360	67,054,051	67,057,207	Heat shock protein 70
GL	S4_65592008	Sb04g035150	64,964,113	64,977,355	tRNA synthetase class I (I, L, M and V) family protein
GL	S5_24012095	Sb05g011561	23,969,838	23,969,963	Protein coding
GL	S6_56032063	Sb06g026980	55,969,525	55,971,076	RING/U-box superfamily protein
GL	S6_60367299	Sb06g032100	60,346,841	60,349,161	Ubiquitin C-terminal hydrolase 3
GL	S8_30544247	Sb08g011955	30,598,417	30,598,596	Protein coding
GI	S5_8042669	Sb05g005730	8,032,584	8,036,576	Protein coding
Lod	S6_8196103	Sb06g003790	81,192,816	8,195,181	Expressed protein
Lod	S5_61278370	Sb05g027130	61,258,641	61,261,974	Protein coding
Lod	S6_56429588	Sb06g027570	56427045	56,429,501	Tetratricopeptide repeat (TPR)-like superfamily protein
Lod	S8_1674514	Sb08g001655	1,674,373	1,674,596	
Lod	S2_74030881	Sb02g039840	73,990,863	73,991,912	Protein coding

Lod	S4_64368929	Sb04g033830	63,730,131	63,731,297	Mitochondrial transcription termination factor family protein
Lod	S4_24592944	Sb04g013785	24,574,794	24,596,184	Drought-responsive family protein
Lod	S2_4762527	Sb02g004190	4,746,355	4,747,963	Expressed protein
Lod	S6_50165577	Sobic.006G139400	50,161,760	50,167,120	Expressed protein
Lod	S5_20322083	Sb05g010050	20,342,767	20,344,139	Protein of unknown function (DUF1685)
LPBL	S5_9215374	Sb05g006165	9,277,312	9,282,383	Protein coding
LPBL	S2_1789087	Sb02g001803	1,766,959	1,768,694	Fucosyltransferase 8
LPBL	S2_1725555	Sb02g001760	1,697,054	1,699,234	F-box and associated interaction domains-containing protein
LPBL	S7_61136114	Sb07g025985	6,139,148	61,141,411	Protein coding
LPBL	S2_1694283	Sb02g001740	1,681,914	1,684,234	Putative uncharacterized protein
LPBL	S3_65729139	Sb03g037770	65,727,037	65,731,255	EAP30/Vps36 family protein
LPBL	S7_41747887	Nothing close			
LPBL	S1_20159690	Sb01g019080	20,124,386	20,126,981	Putative uncharacterized protein
LPBL	S5_5373617	Sb05g004250	5,350,261	5,353,154	Transmembrane amino acid transporter protein
LPBL	S4_62195064	Sb04g032210	62,208,798	62,212,363	Protein of unknown function (DUF630 and DUF632)
PGMR	S1_43223535	Sb01g026071	43,266,495	43,266,767	Protein coding
PGMR	S9_58695115	Sb09g030043	58,695,700	58,698,024	Protein coding
Deo	S4_3943073	Sb04g004120	3,927,433	3,929,790	Protein kinase family protein with leucine-rich repeat domain
Deo	S4_3259546	Sb04g003390	3,259,055	3,263,298	Eukaryotic translation initiation factor 4A1
Deo	S3_53317716	Sb03g026510	53,311,999	53,313,105	One-helix protein 2
Deo	S6_45861733	Sb06g016770	45,860,300	45,863,988	Cellulose synthase-like B4
Deo	S2_16670356	Sb02g010720	16,668,971	16,670,475	Expansin precursor
Deo	S2_5829058	Sb02g005030	5,809,278	5,810,592	Putative uncharacterized protein
Deo	S6_45816090	Sb06g016770	45,860,300	45,863,988	Cellulose synthase-like B4
Deo	S9_57512180	Sb09g028660	57,513,946	57,517,030	WRKY DNA-binding protein 71
Deo	S6_31673491	Sb06g011401	31,553,721	31,554,122	Drought-induced protein 1
Deo	S6_45861653	Sb06g017470	45,900,000	45,925,000	Peptide transporter PTR2
Moi	S7_5195801	Sb07g004100	5,154,354	5,158,043	Homeodomain-like superfamily protein
Moi	S7_18825659	Sb07g010440	18740731	18759606	Putative uncharacterized protein
Moi	S5_41382017	Sb05g016823	41,368,713	41,368,992	Putative uncharacterized protein

Moi	S6_44564702	Sb06g016180	44,598,216	44,599,035	Protein of unknown function (DUF1279)
Moi	S4_4603470	Sb04g004825	4,621,071	4,621,433	Putative uncharacterized protein
Moi	S4_4603471	Sb04g004860	4,671,723	4,672,466	Putative uncharacterized protein
Moi	S4_59573016	Sb04g029490	59,578,941	59,583,318	RNA polymerase II, Rpb4, core protein
Phe	S4_60837496	Sb04g030880	60,875,943	60,878,836	Topoisomerase II
Sta	S4_3943073	Sb04g004200	3,991,737	4,002,024	Putative DNA polymerase delta catalytic subunit
Sta	S8_10465255	Sb08g006580	10,400,515	10,401,486	Putative uncharacterized protein
Sta	S4_66843496	Sb04g037210	66,880,218	66,891,041	Putative uncharacterized protein
Sta	S8_6642715	Sb08g005170	6,628,219	6,630,350	Putative uncharacterized protein
Tan	S4_60837496	Sb04g030830	60,829,092	60,831,886	Homeodomain-like superfamily protein
TPP	S4_3943073	Sb04g004170	3,973,735	3,974,866	Myosin heavy chain, putative, expressed

PGMR = Panicle grain mold rating; PH = Plant height; LPBL = Lowest primary branch length; GL = Glume length; GI = Glume index; Lod = Lodging; DF = Days to flowering; DPM = Days to physiological maturity; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib = Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g

**Table 3.17. Fungal genera isolated from 46 sorghum genotypes.**

Fungal isolate	Identity %	Accession No.	Database
<i>Alternaria</i>	100	KP267520	NCBI
<i>Curvularia</i>	100	HF934916	NCBI
<i>Aspergillus</i>	100	KR106454	NCBI
<i>Penicillium</i>	100	FJ884117	NCBI
<i>F. verticillioides</i>	100	FD_01388	Fusarium-ID
<i>F. thapsinum</i>	100	FD_01177	Fusarium-ID
<i>F. proliferatum</i>	100	FD_01380	Fusarium-ID
FIESC	100	FD_01643	Fusarium-ID

FIESC = *F. incarnatum-equiseti* complex

**Table 3.18. Overall summary statistics for fungal genera isolated from 46 sorghum genotypes.**

Fungus	Freq	Range	SD	CV%
<i>Fusarium</i> *	47.59	0.00-100	22.25	213.89
<i>FV</i>	25.04	0.00-90.00	21.35	117.25
<i>FT</i>	19.11	0.00-100	24.00	79.64
<i>FP</i>	2.93	0.00-45.00	8.19	35.73
<i>FIESC</i>	0.52	0.00-25	2.09	24.78
<i>Alternaria</i>	51.39	0.00-95	22.26	230.89
<i>Curvularia</i>	0.54	0.00-5.00	1.55	34.62
<i>Aspergillus</i>	0.28	0.00-25	2.48	11.20
<i>Penicillium</i>	0.35	0.00-25	2.23	15.76

Freq = Frequency (%); Range = Average minimum value-Average maximum value; SD = Standard deviation; CV = Coefficient of variation; *Fusarium*\* = All *F.* species combined; *FV* = *F. verticillioides*; *FT* = *F. thapsinum*; *FP* = *F. proliferatum*; *FIESC* = *F. incarnatum-equiseti* complex



**Table 3.19. Summary statistics for fungal genera isolated from 46 sorghum genotypes across treatments.**

Fungus	Freq			Range			SD			CV%		
	IB	NB	NN	IB	NB	NN	IB	NB	NN	IB	NB	NN
<i>Fusarium*</i>	57.87a	42.75b	42.33b	15.00-100	10.00-95.00	5.00-90.00	21.82	20.38	21.94	265.20	209.80	192.91
<i>FV</i>	15.78b	27.11a	32.22a	0.00-80.00	0.00-50.00	0.00-25.00	19.80	19.27	21.71	79.68	140.69	148.41
<i>FT</i>	41.28a	9.33b	6.72b	0.00-100.00	0.00-25.00	0.00-10.00	27.07	10.92	11.81	152.47	85.48	56.93
<i>FP</i>	0.56b	5.72a	2.50b	0.00-15.00	0.00-5.00	0.00-25.00	2.18	11.08	7.83	25.51	51.64	31.91
<i>FIESC</i>	0.28a	0.39a	0.89a	0.00-5.00	5.00-85.00	25.00-95.00	1.15	1.35	3.14	24.12	28.88	28.29
<i>Alternaria</i>	42.08b	55.22a	56.72a	0.00-85.00	5.00-90.00	10.00-95.00	21.56	20.57	21.89	195.21	268.40	259.09
<i>Curvularia</i>	0.51a	0.66a	0.44a	0.00-5.00	0.00-5.00	0.00-5.00	1.52	1.70	1.43	33.35	38.76	31.06
<i>Aspergillus</i>	0.00a	0.82a	0.00a	0.00-0.00	0.00-25.00	0.00-0.00	0.00	4.23	0.00	0.00	19.47	0.00
<i>Penicillium</i>	0.00a	0.55a	0.50a	0.00-0.00	0.00-15.00	0.00-25.00	0.00	2.29	3.09	0.00	23.98	16.16

Per centage means in rows with different letters are significantly different according to the Tukey means separation test ( $p < 0.05$ ). IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; Freq = Frequency (%); Range = Average minimum value-Average maximum value; SD = Standard deviation; CV = Coefficient of variation; *Fusarium\** = All *F.* species combined; *FV* = *F. verticillioides*; *FT* = *F. thapsinum*; *FP* = *F. proliferatum*; *FIESC* = *F. incarnatum-equiseti* complex; IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged

**Table 3.20. Overall summary statistics for grain mold ratings, days to flowering, and days to physiological maturity, seed viability, germination per cent, vigor index, and quality traits for 46 sorghum genotypes across treatments.**

Traits	Mean			Range			SD			CV%		
	IB	NB	NN	IB	NB	NN	IB	NB	NN	IB	NB	NN
PGMR	2.47a	1.82b	1.39c	1.00-5.00	1.00-5.00	1.00-5.00	1.29	1.00	0.63	193.54	183.85	221.36
TGMR	2.90a	1.87b	1.50c	1.00-5.00	1.00-5.00	1.00-5.00	1.37	0.84	0.60	213.65	226.42	252.27
DF*		66.42			53.00-79.00			5.70			1162.86	
DPM*		107.21			77.00-117.00			4.71			2273.83	
TSW	26.16c	27.77b	28.78a	19.06-36.93	18.00-36.93	19.07-38.00	4.01	4.06	4.08	652.60	684.69	704.36
KVP	71.94c	91.33b	93.44a	75.00-90.00	75.00-100.00	75.00-100.00	10.38	5.34	4.66	693.27	1709.65	2005.49
KGP	57.53c	73.98b	81.60a	60.00-86.00	48.00-88.00	60.00-94.00	20.41	10.00	7.12	281.96	740.11	1146.86
VI	1132.68c	1527.92b	1755.65a	816.00-2330.00	665.00-2442.72	816.00-2747.00	497.91	335.55	306.01	227.48	455.35	573.72
TPP	9.41b	9.58b	10.10a	7.02-14.92	6.02-15.21	7.02-18.05	1.54	1.64	1.97	612.72	584.78	512.73
Moi	9.25a	9.17a	9.13a	6.70-11.10	5.49-11.73	6.70-11.73	1.28	1.08	0.99	720.77	846.54	921.66
Fat	2.69a	2.69a	2.77a	1.33-4.17	0.99-4.02	1.33-4.12	0.63	0.58	0.60	425.53	463.80	464.53
Fib	1.57b	1.65a	1.68a	1.32-2.11	1.14-2.13	1.32-2.19	0.21	0.19	0.19	758.67	887.65	896.66
Ash	1.22a	1.21a	1.20a	1.03-1.40	0.97-1.37	1.03-1.37	0.08	0.08	0.07	1474.56	1595.79	1704.40
Sta	67.37b	67.97a	68.06a	64.83-71.10	64.61-71.76	64.83-71.30	1.67	1.41	1.39	4025.99	4827.52	4903.15
Phe	5.30a	5.87a	5.91a	0.00-17.54	0.00-17.91	0.00-17.07	4.41	4.31	4.55	120.13	136.15	129.88
Tan	8.26b	10.15a	9.96ab	0.00-44.88	0.00-39.93	0.00-43.48	10.79	10.74	11.68	76.52	94.58	85.33
Deo	20.69a	14.25b	13.02b	0.00-150.38	0.00-54.27	0.00-71.31	22.22	14.05	13.79	93.08	101.40	94.43
HI	63.35b	67.44a	66.19ab	0.00-102.14	4.20-97.01	0.00-102.59	17.00	14.42	15.17	372.76	467.62	436.38
KW (mg)	24.17b	24.92ab	25.12a	15.28-37.63	15.56-37.97	15.28-39.49	5.54	5.87	6.22	436.57	424.58	403.73
KD(mm)	2.44a	2.47a	2.48a	1.98-3.08	1.94-3.10	0.29-3.11	0.28	0.28	0.29	882.35	885.96	843.03

\*\*For these traits, field responses were recorded per accession per replication per year; Means in rows with different letters are significantly different according to the Tukey means separation test ( $p < 0.05$ ). IB = Panicles inoculated and bagged for seven days at 50% flowering; NB = Panicles non-inoculated but bagged for seven days at 50% flowering; NN = panicles non-inoculated and non-bagged; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; DF = Days to fifty per cent flowering; DPM = Days to physiological maturity; TSW = 1000 Seed weight; KVP = Seed viability; KGP = Seed germination per centage; VI = Vigor index; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib= Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g; HI = Hardness index; KW = Kernel weight (mg), KD = Kernel diameter (mm)

**Table 3.21. Correlation (Pearson's correlation) among isolated fungal genera.**

Fungi	FV	FT	FP	FIESC	Alt	Cur	Asp	Pen
FV	1	-0.48 ***	-0.08	-0.1	-0.29 ***	-0.05	-0.09	-0.04
FT		1	-0.18 **	-0.12 *	-0.44 ***	-0.04	-0.07	-0.14 *
FP			1	0.14 *	-0.07	-0.05	0.19 **	0.25 ***
FIESC				1	0.05	-0.06	-0.04	0.25 ***
Alt					1	0.11	-0.05	-0.07
Cur						1	-0.04	-0.06
Asp							1	0.15 *
Pen								1

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

FV = *Fusarium verticillioides*; FT= *Fusarium thapsinum*; FP = *F. proliferatum*; FIESC = *F. incarnatum-equiseti* complex; Cla = *Cladosporium*; Alt = *Alternaria*; Cur = *Curvularia*; Asp = *Aspergillus*; Pen = *Penicillium*

**Table 3.22. Correlation (Spearman's correlation) between grain mold ratings and seed viability, germination per cent, vigor index, and important fungal genera for 46 sorghum genotypes.**

Traits	KVP	KGP	VI	TPP	Moi	Fat	Fib	Sta	Deo	HI	KW	KD	FV	FT	Alt
PGMR	-0.39 ***	-0.49 ***	-0.51 ***	-0.24 ***	0.17 **	-0.22 **	-0.07	0.10	0.20 ***	-0.15 *	0.10	0.10	-0.18 **	0.25 ***	-0.15
TGMR	-0.45 ***	-0.57 ***	-0.57 ***	-0.29 ***	0.13 *	-0.31 ***	-0.14	0.24 ***	0.31 ***	-0.19 **	0.19 **	0.16 **	-0.16 **	0.28 ***	-0.22 **
KVP	1.00	0.54 ***	0.48 ***	0.09	-0.10	0.00	0.20 **	0.19 ***	-0.17 ***	0.13 **	0.02	0.02	0.39	-0.54	0.21
KGP		1.00	0.84 ***	0.13 *	-0.13 *	0.09	0.24 ***	0.02	-0.17 **	0.06	-0.05	-0.02	0.33 ***	-0.42 ***	0.15 *
VI			1.00	0.22 **	-0.17 **	0.16 **	0.10	-0.06	-0.16 **	0.14 *	-0.27 ***	-0.24 ***	0.26	-0.38	0.15 *

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; KVP = Kernel viability per cent; KGP = Kernel germination per cent; FV = *Fusarium verticillioides*; FT = *Fusarium thapsinum*; FP = *F. proliferatum*; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib = Fiber per cent; Sta = Starch per cent; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g; HI = Hardness index; KW = Kernel weight (mg), KD = Kernel diameter (mm)

**Table 3.23. Correlation (Pearson's correlation) for between quality traits and fungal genera.**

Traits	FV	FT	FP	Alt	Cur	Asp
TPP	0.00	-0.09	-0.05	0.15 *	0.03	-0.01
Moi	-0.15 *	0.14	-0.10	0.00	0.13	-0.07
Fat	-0.11	-0.03	-0.03	0.15 ***	-0.09	0.09
Fib	0.17 **	-0.18 **	0.06	0.08	-0.03	0.06
Ash	-0.10	0.13 *	-0.16 **	0.04	0.14 *	-0.16 *
Sta	0.14 *	-0.16 ***	0.09	-0.01	0.01	0.01

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

FV = *Fusarium verticillioides*; FT= *Fusarium thapsinum*; FP = *F. proliferatum*; FIESC = *F. incarnatum-equiseti complex*; Cla = *Cladosporium*; Alt = *Alternaria*; Cur = *Curvularia*; Asp = *Aspergillus*; TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib= Fiber per cent; Ash = Ash per cent; Sta = Starch per cent

**Table 3.24. Correlation (Pearson's correlation) between physical kernel traits and quality traits.**

Traits	HI	KW	KD
TPP	-0.10	0.03	0.02
Moi	-0.02	0.12	0.11
Fat	0.04	-0.02	0.00
Fib	-0.09	0.42 ***	0.48 ***
Ash	-0.11	0.13 *	0.07
Sta	0.11	-0.01	-0.02
Phe	0.15 *	-0.18 **	-0.14 *
Tan	0.12	-0.16 *	-0.13 *
Deo	-0.26 ***	0.18 **	0.19 **
HI	1.00	-0.53 ***	-0.54 ***
KW		1.00	0.93 ***
KD			1.00

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; \* Significant at  $p < 0.05$

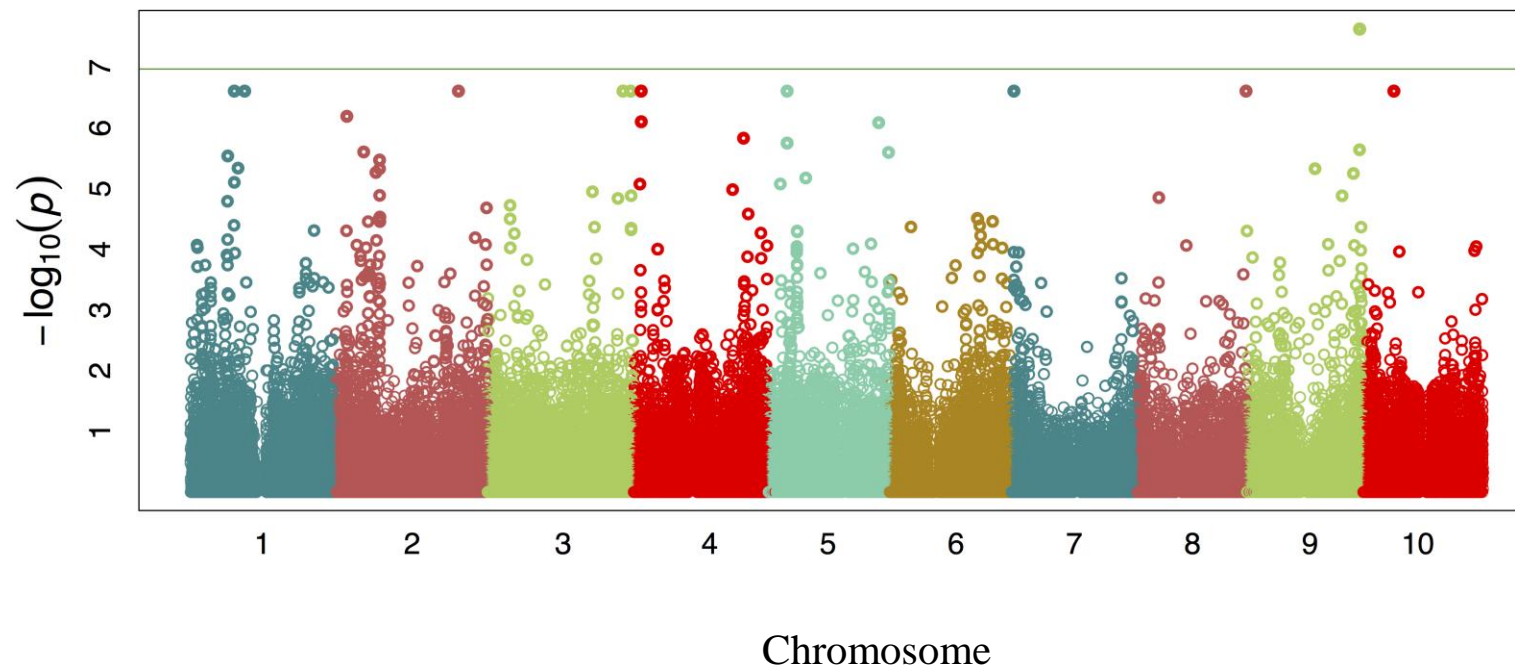
TPP = Total protein per cent; Moi = Moisture per cent; Fat = Fat per cent; Fib= Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g; HI = Hardness index; KW = Kernel weight (mg), KD = Kernel diameter (mm)

**Table 3.25. Overall summary statistics for grain mold ratings, days to fifty per cent flowering, and days to physiological maturity, seed viability, germination per cent, vigor index, and quality traits for 46 sorghum genotypes.**

Traits	Mean	Range	SD	CV%
PGMR	1.91	1.00-5.00	1.10	173.11
TGMR	2.11	1.00-5.00	1.15	183.08
DFP	66.31	53.00-79.00	5.71	1161.36
DPM	107.13	77.00-117.00	4.68	2289.02
TSW	27.56	16.40-38.00	4.17	660.43
KVP	85.57	45.00-100-.00	12.09	707.66
KGP	71.04	6.00-94.00	16.99	418.01
VI	1472.00	27.00-2747.00	465.56	316.18
TPP	9.70	6.02-18.05	1.74	556.28
Moi	9.18	1.54-11.73	1.12	818.10
Fat	2.72	0.99-4.17	0.60	451.28
Fib	1.63	1.08-2.19	0.20	819.55
Ash	1.21	0.88-1.40	0.08	1581.27
Sta	67.80	62.73-71.76	1.52	4456.52
Phe	5.69	0.00-17.91	4.42	128.88
Tan	9.46	0.00-44.88	11.07	85.46
Deo	15.98	0.00-150.38	17.41	91.83
HI	65.66	0.00-102.60	15.60	420.87
KW(mg)	24.74	15.00-39.50	5.80	421.16
KD(mm)	2.46	1.90-3.10	0.28	865.72

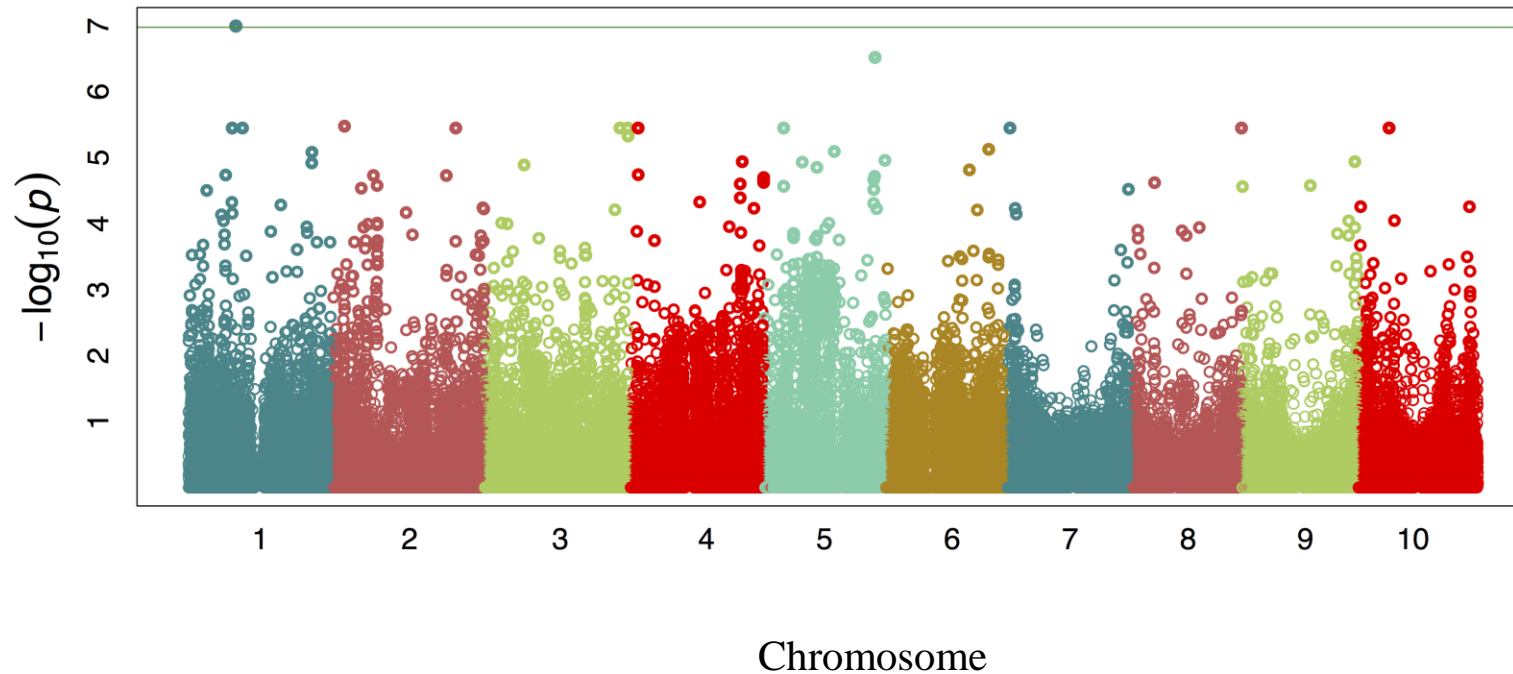
Freq = Frequency (%); Range = Average minimum value-Average maximum value; SD = Standard deviation; CV = Coefficient of variation; PGMR = Panicle grain mold rating; TGMR = Threshed grain mold rating; TSW = 1000 Seed weight; KVP = Seed viability; KGP = Seed germination per centage; VI = Vigor index; TPP = Total protein per cent; Moi =Moisture per cent; Fat = Fat per cent; Fib = Fiber per cent; Ash = Ash per cent; Sta = Starch per cent; Phe = Total Phenolic acids in mg GAE per g; Tan = Total tannins in mg CE per g; Deo = 3-Deoxyanthocyanins concentration in Abs per ml per g; HI = Single kernel characterization system (SKCS) hardness index; KW = Kernel weight (mg), KD = Kernel diameter (mm)

Figure 3.1. Manhattan plot of GWAS (2014) for days to flowering based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ .

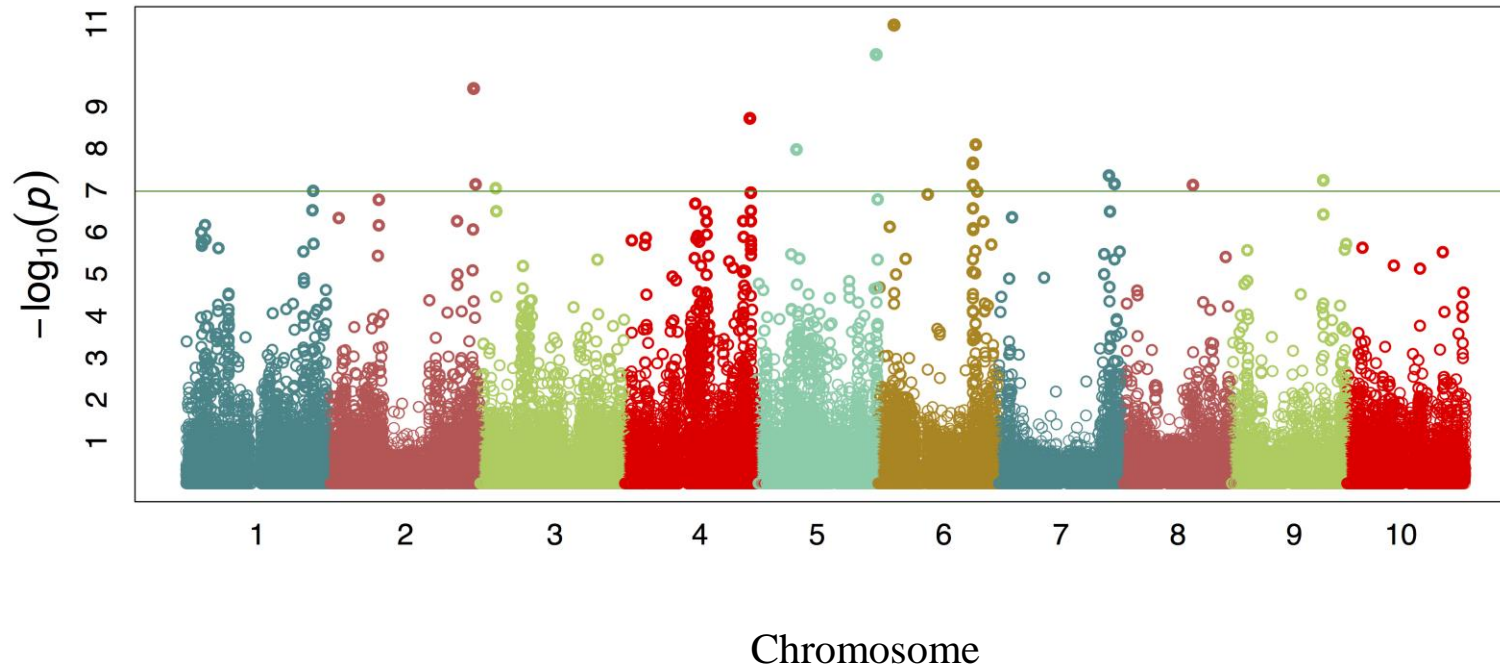




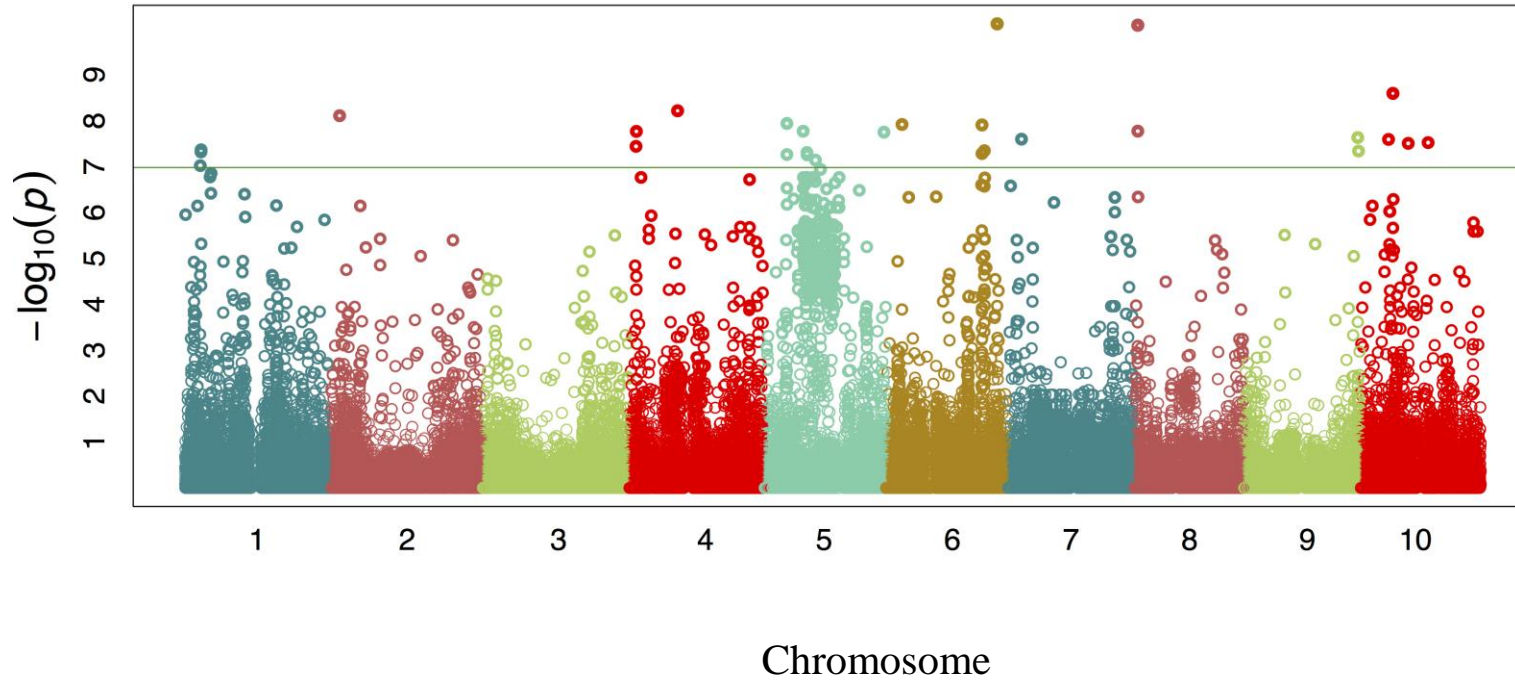
**Figure 3.2. Manhattan plot of GWAS (2014) for days to physiological maturity based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ .**



**Figure 3.3. Manhattan plot of GWAS (2014) for lodging based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ .**

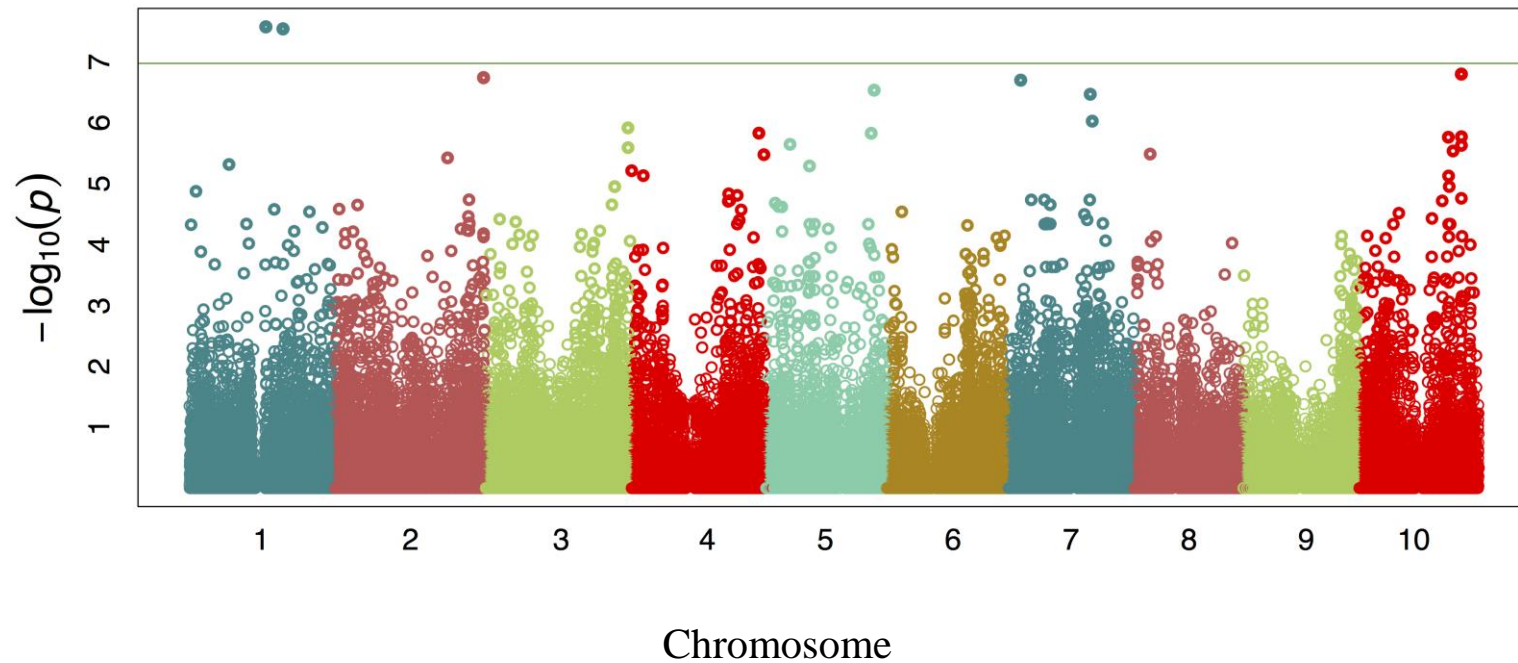


**Figure 3.4. Manhattan plot of GWAS (2015) for lodging (Lod) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ .**

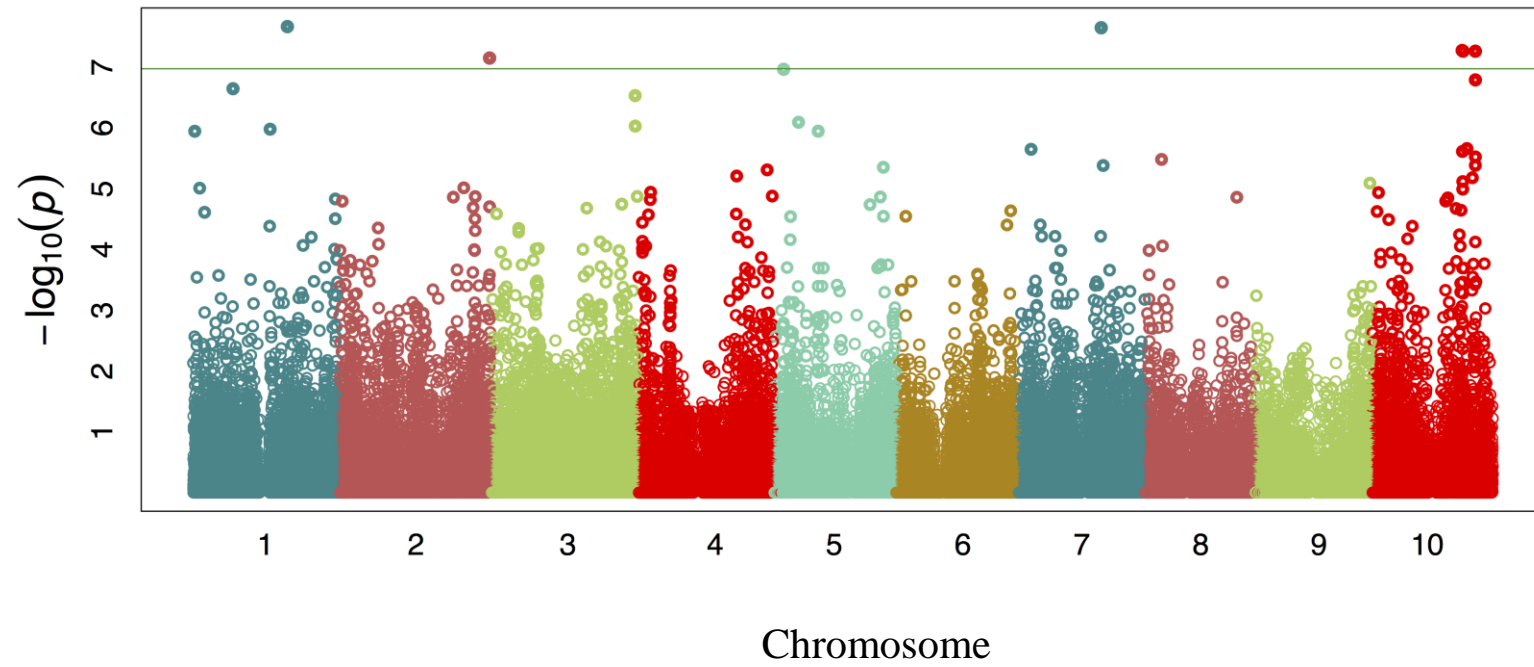


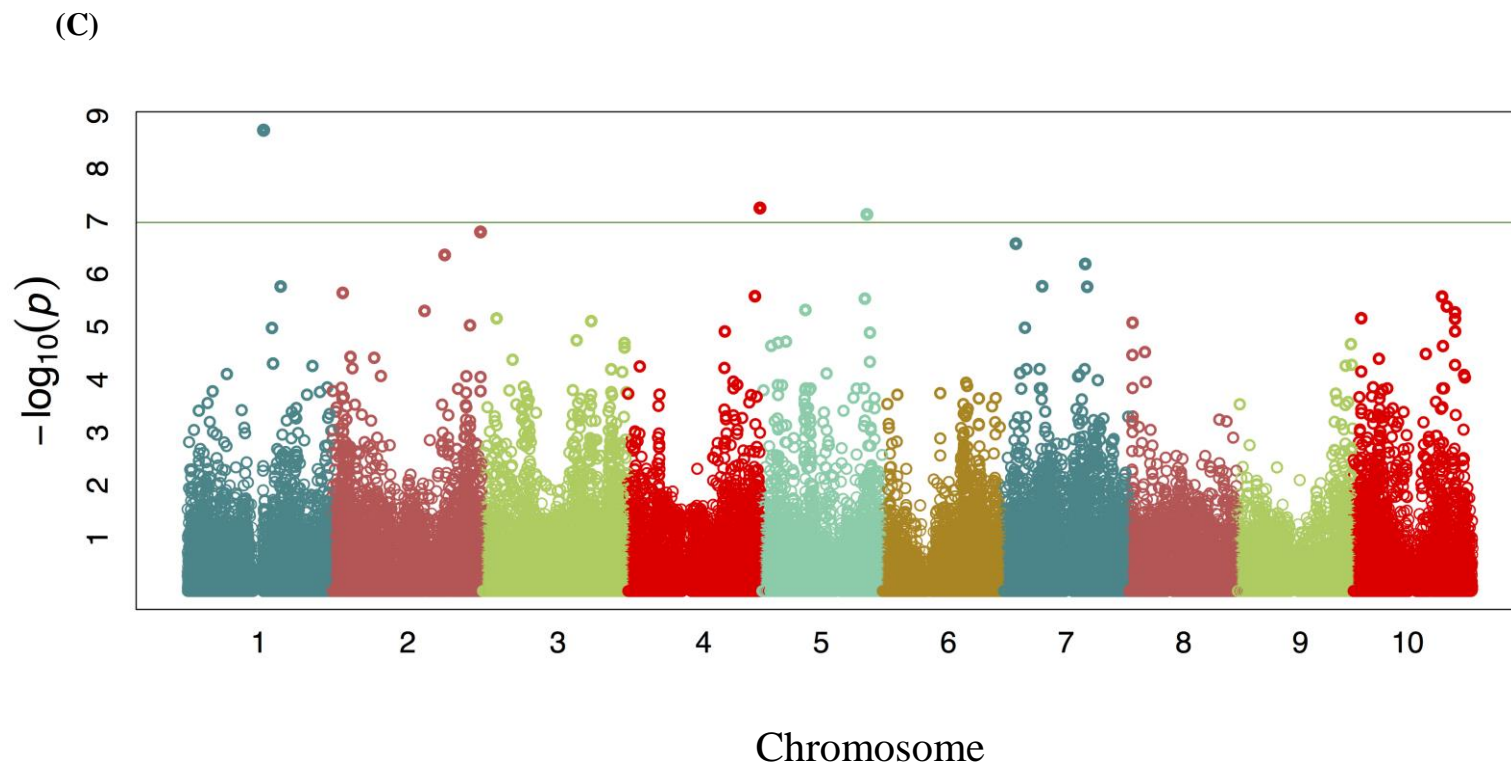
**Figure 3.5. Manhattan plots of GWAS (2014) for lowest primary branch length (LPBL) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

(A)

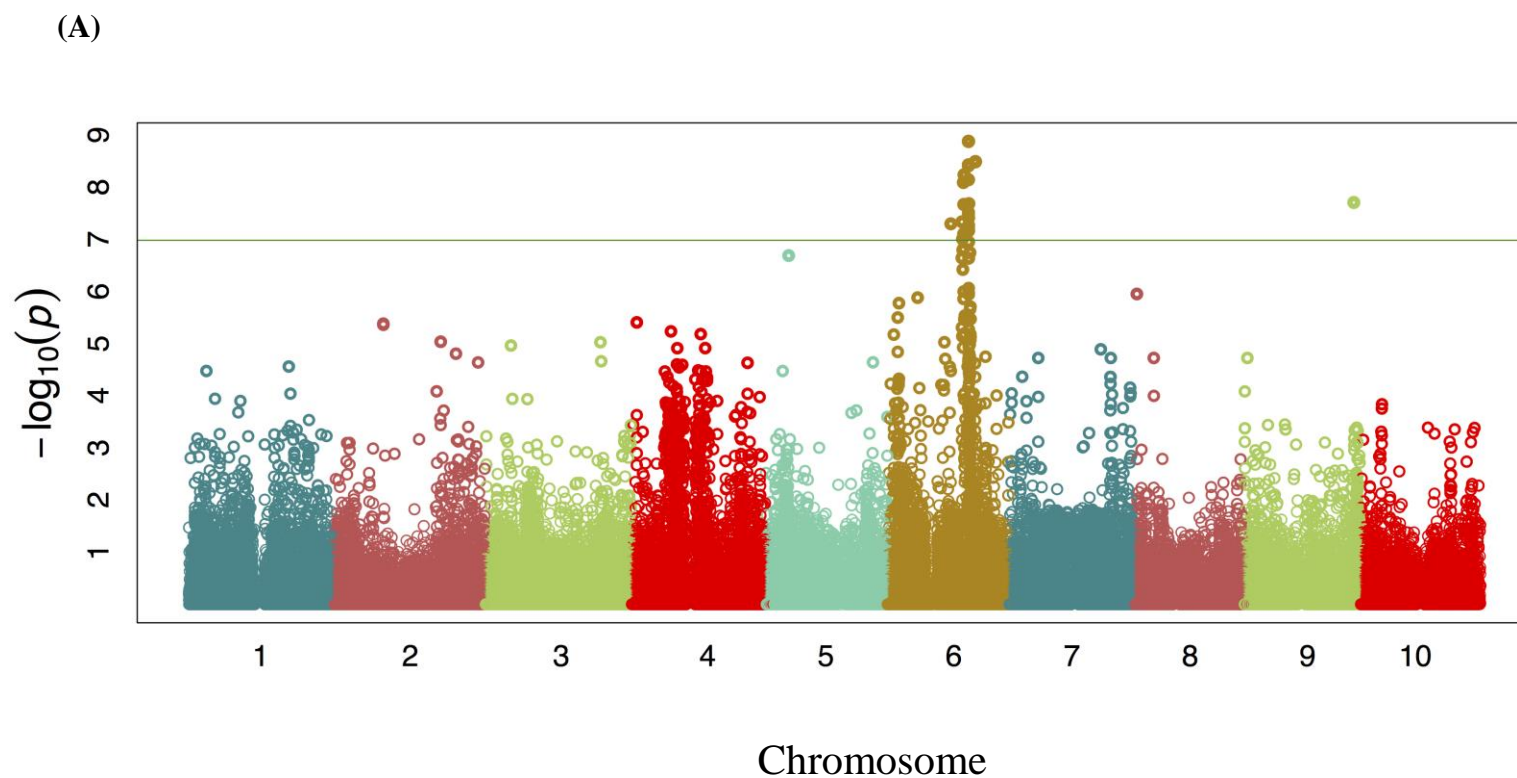


(B)

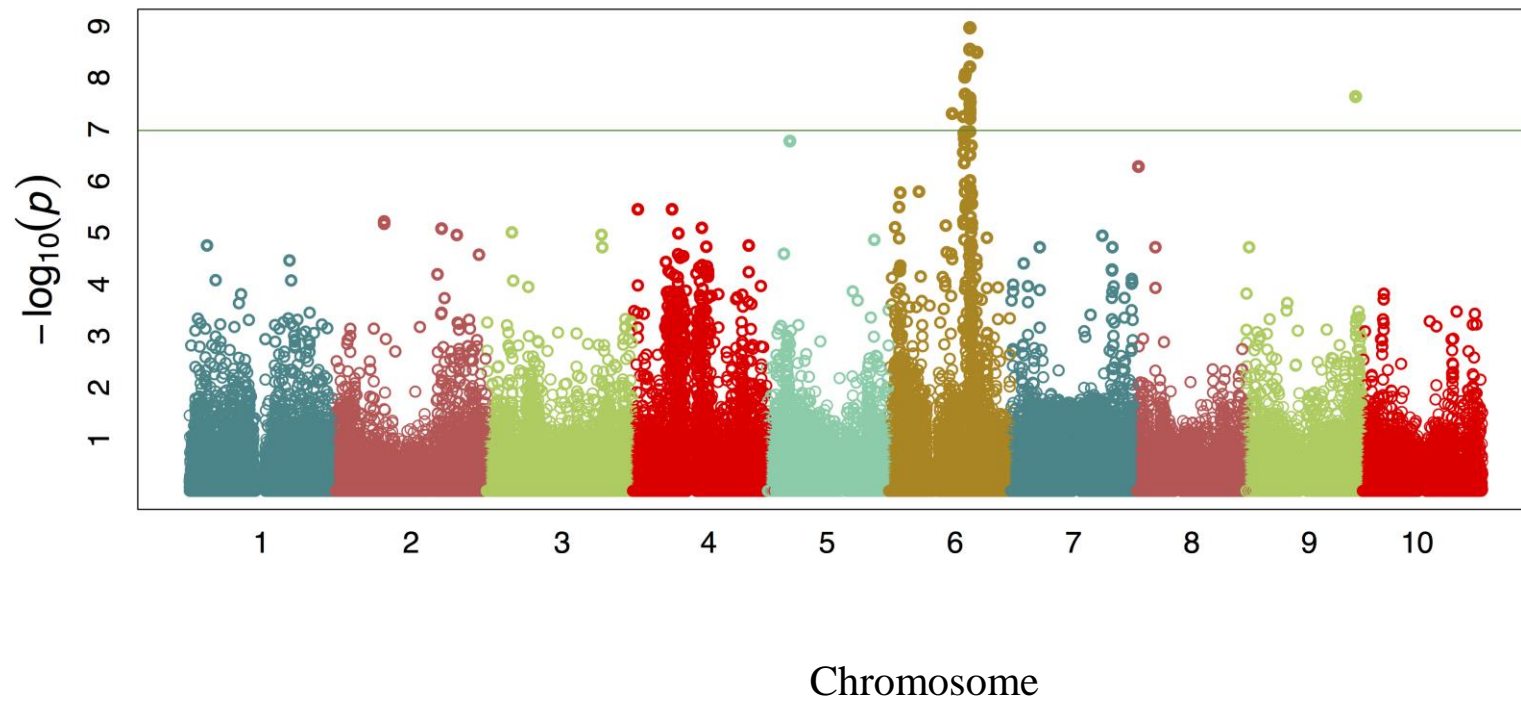




**Figure 3.6. Manhattan plots of GWAS (2014) for plant height (PH) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each SNP on chromosome 1-10. Each circle in plot represents a single nucleotide polymorphism (SNP). Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

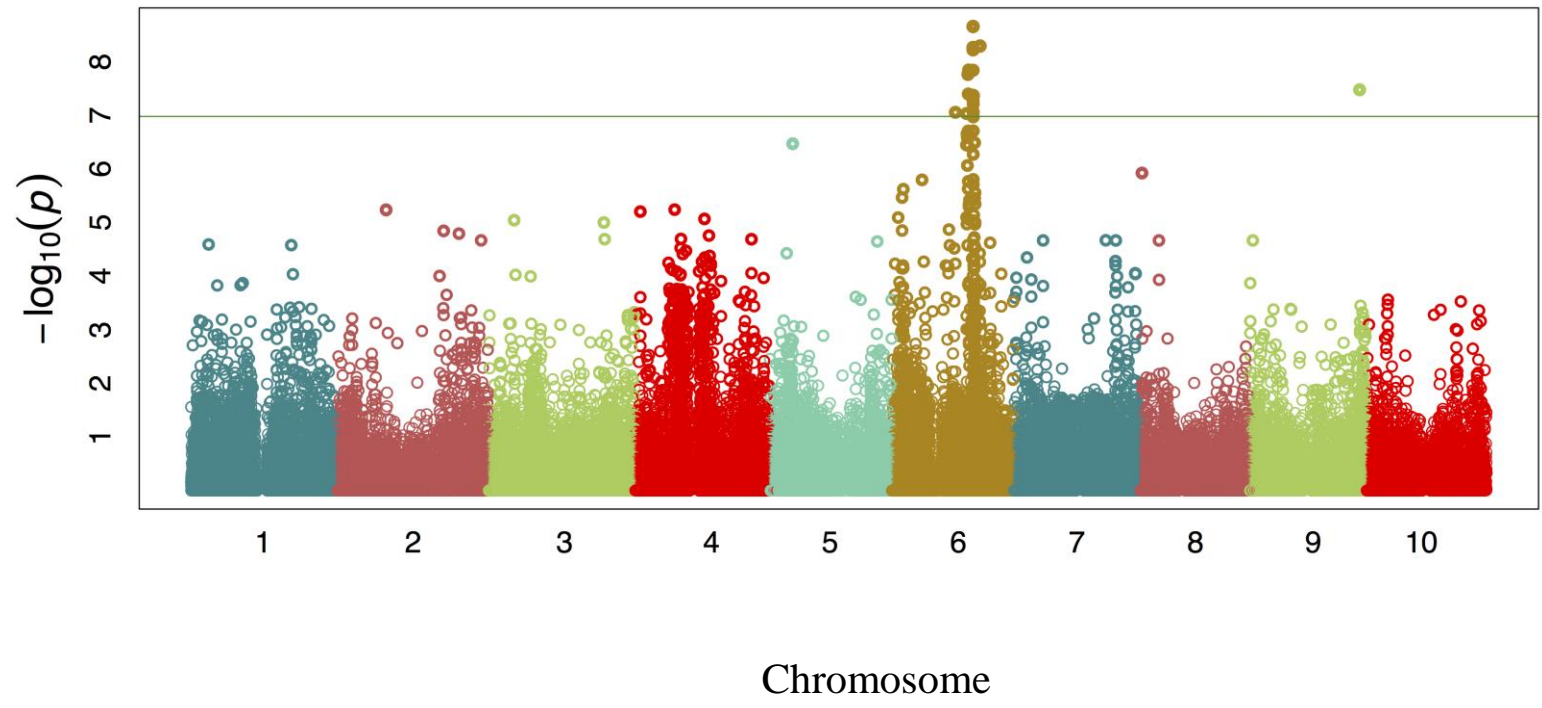


(B)



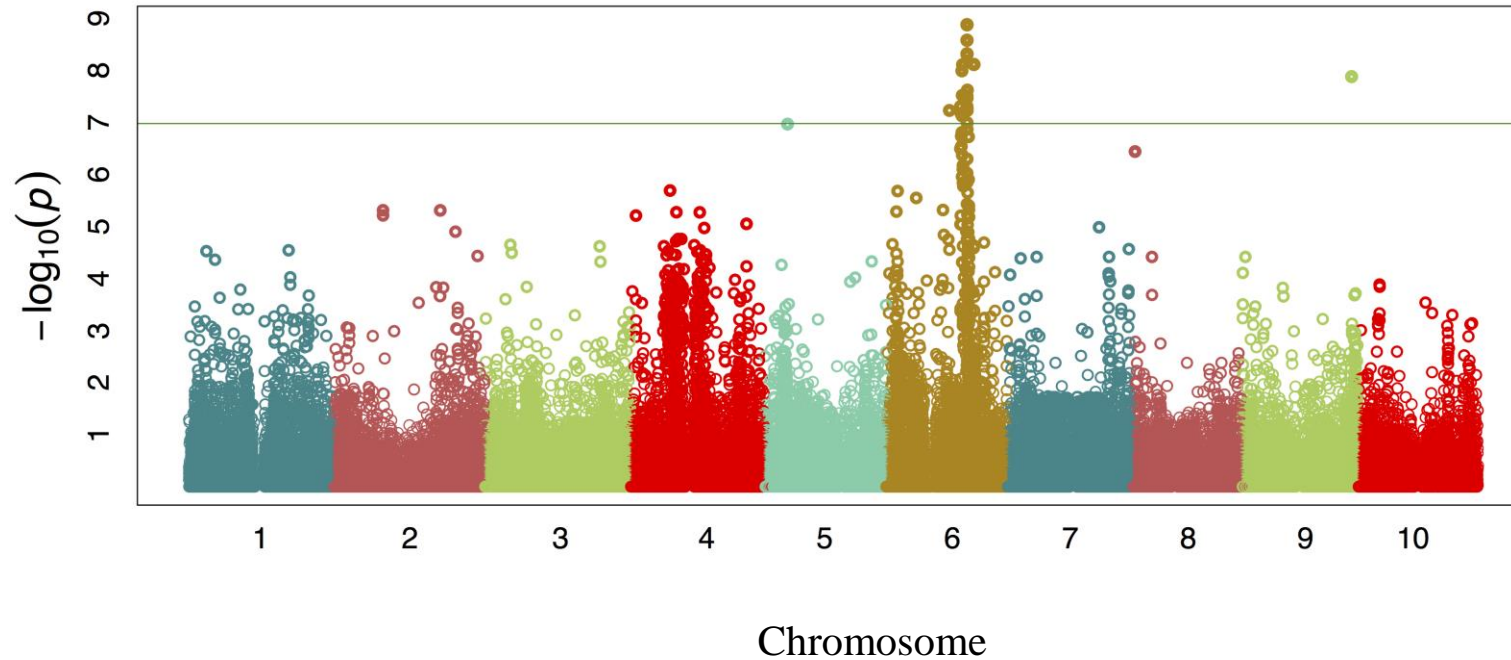


(C)

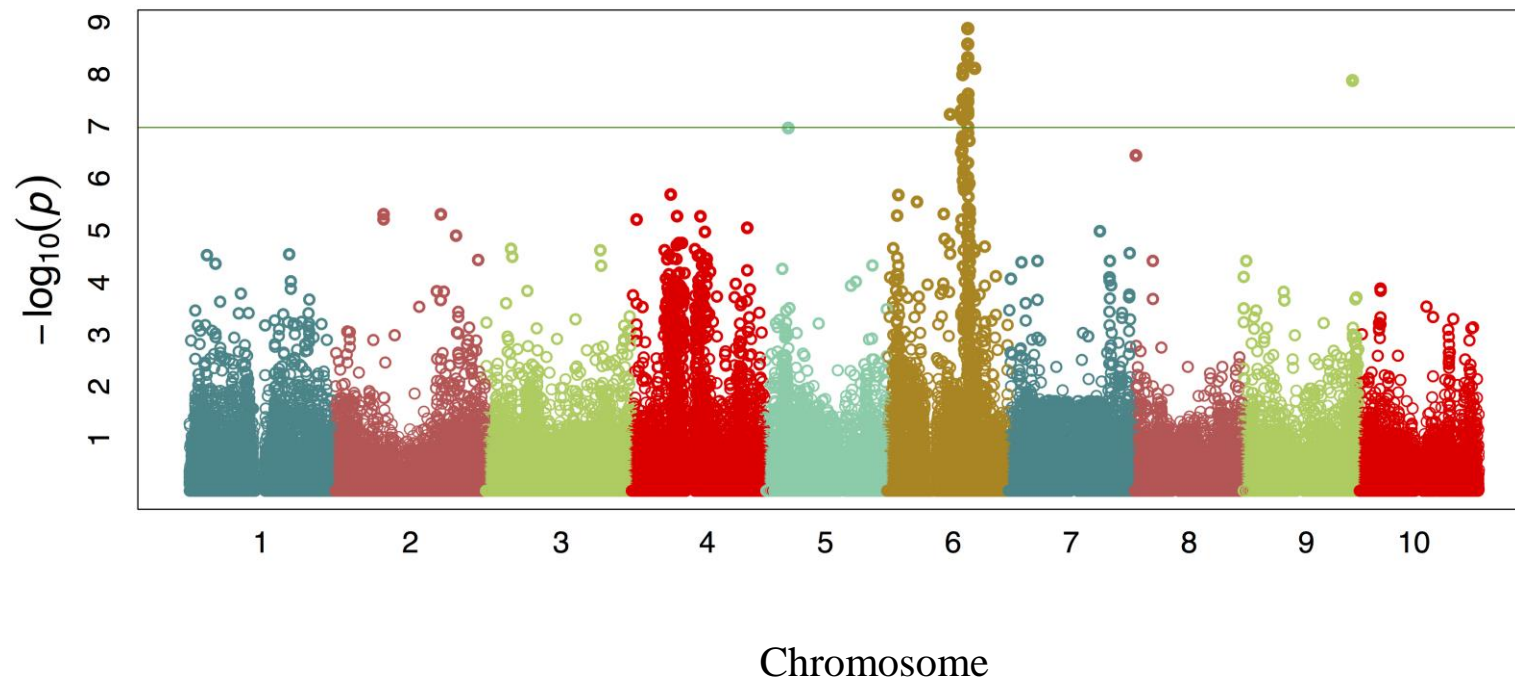


**Figure 3.7. Manhattan plots of GWAS (2015) for plant height (PH) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

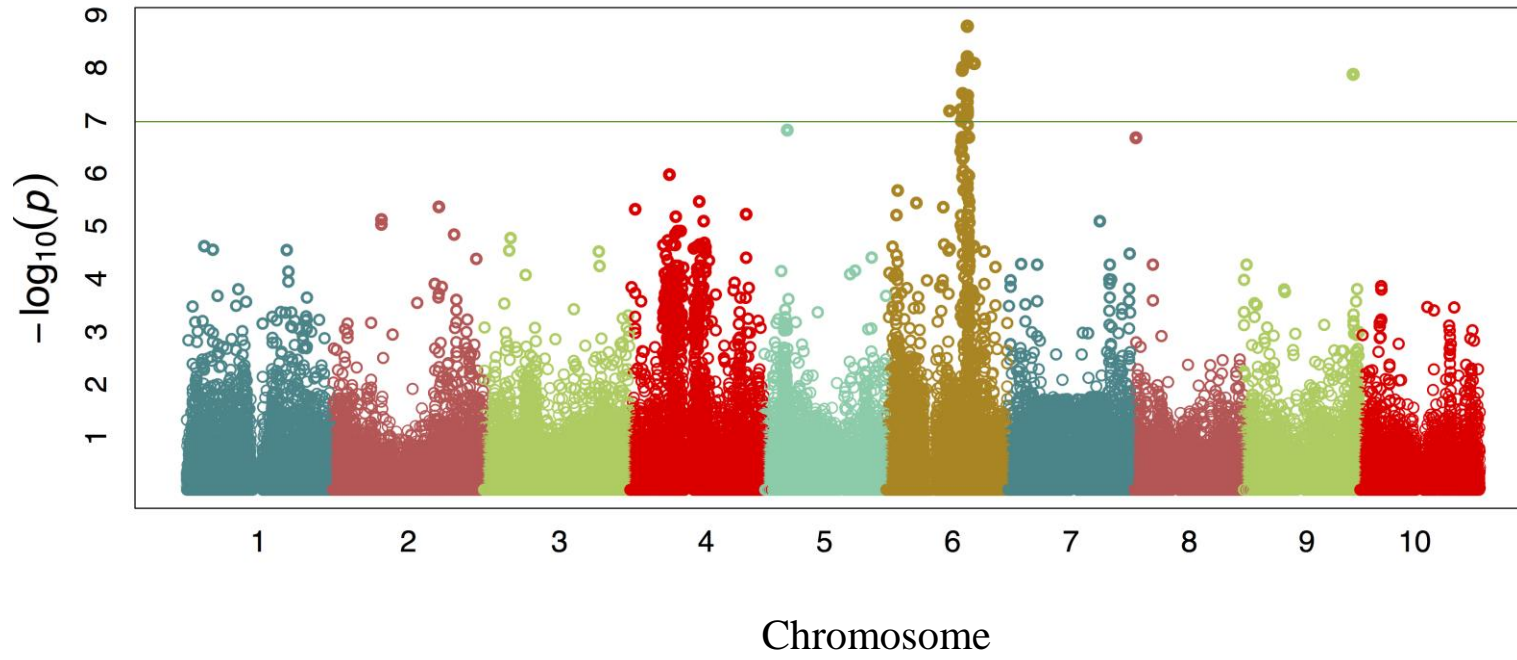
(A)



(B)

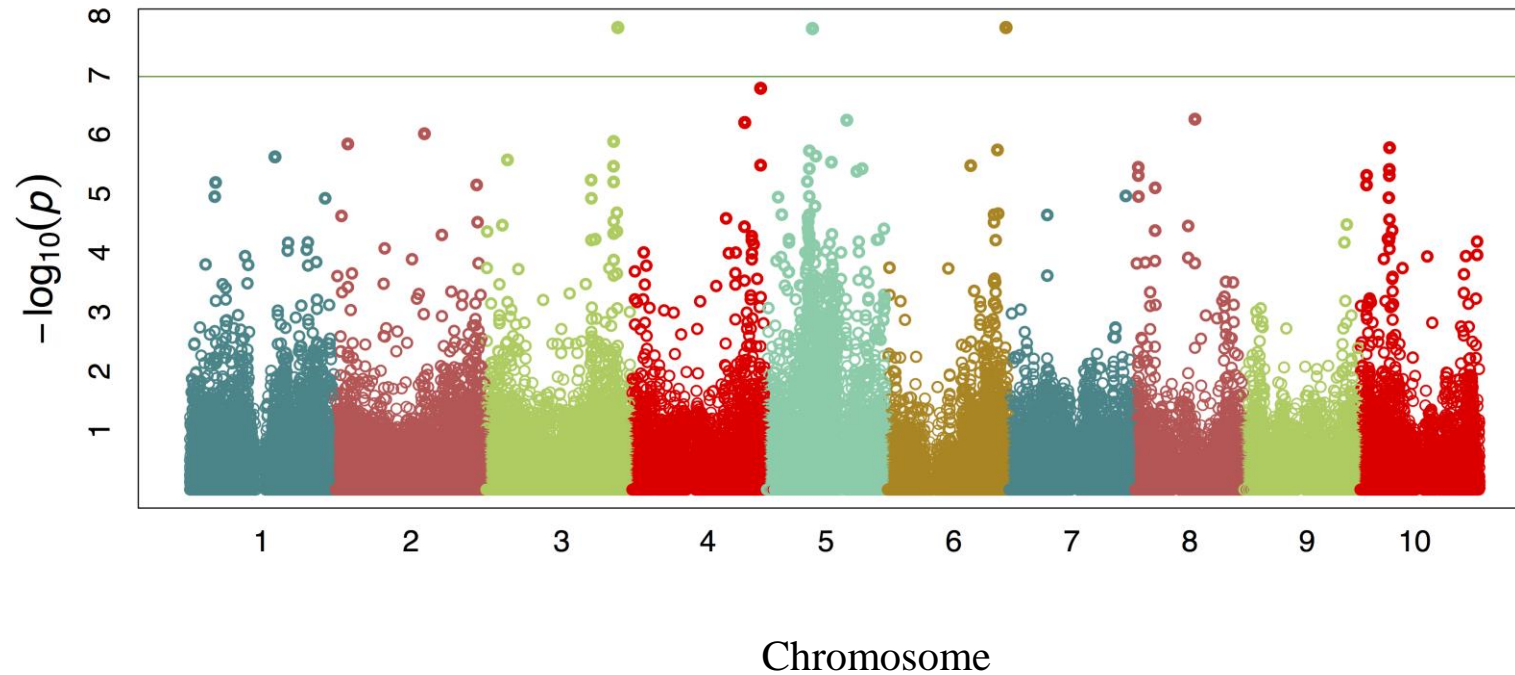


(c)

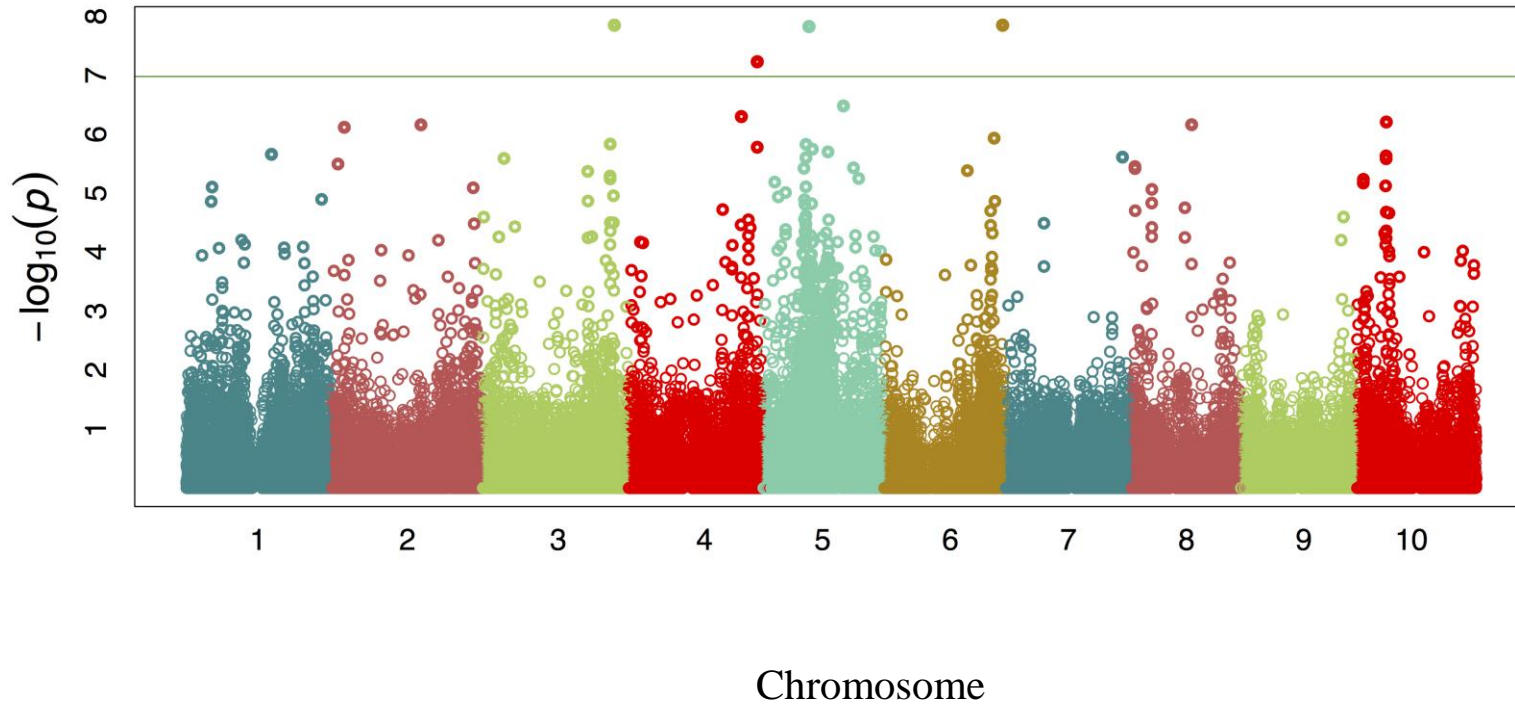


**Figure 3.8. Manhattan plots of GWAS (2014) for glume length (GL) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a single nucleotide polymorphism (SNP). Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

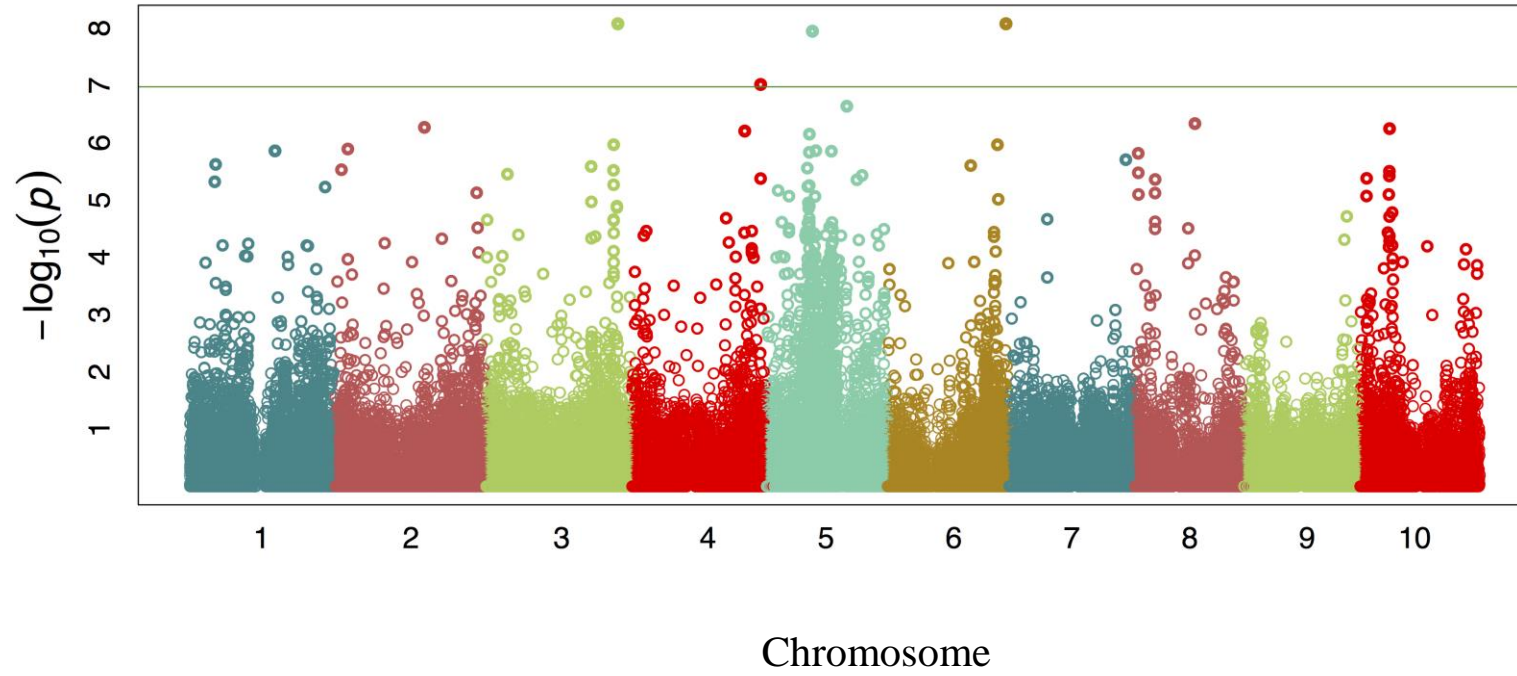
(A)



**(B)**

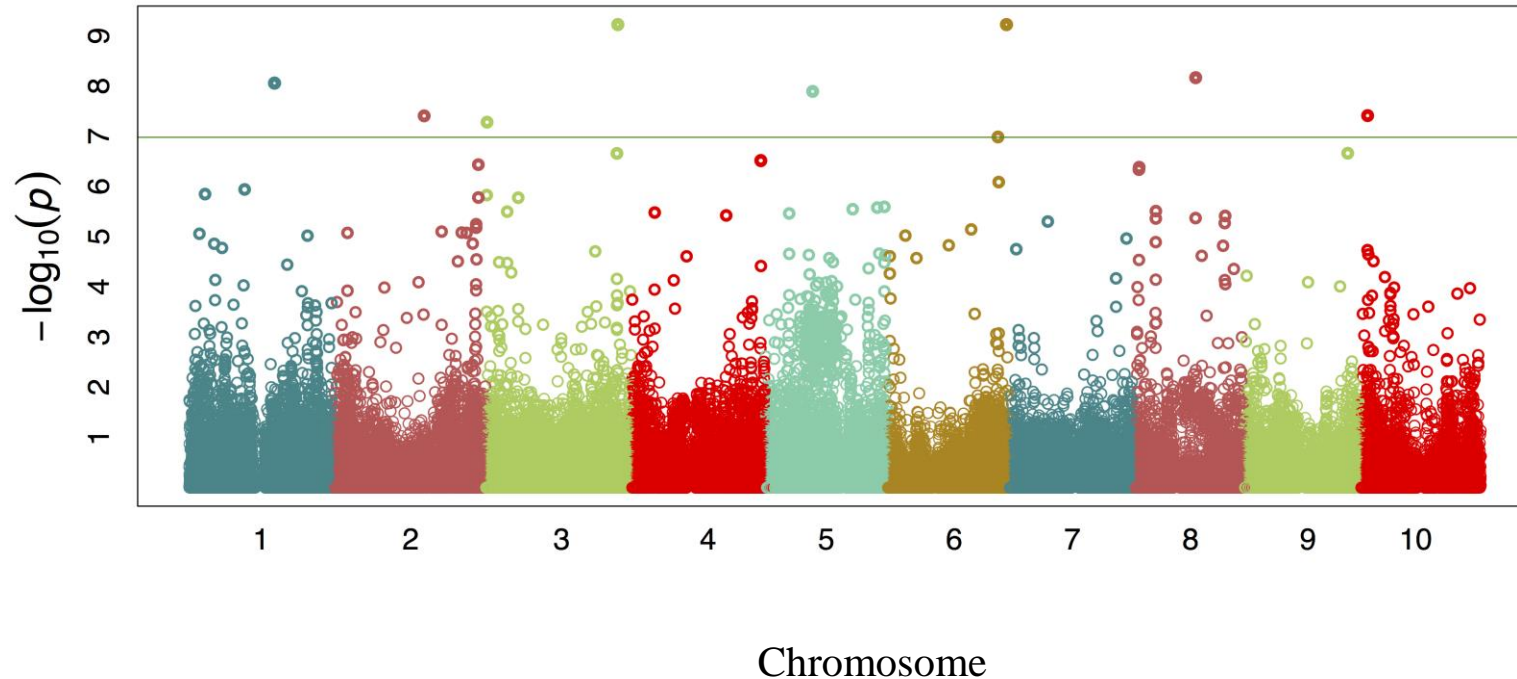


(C)



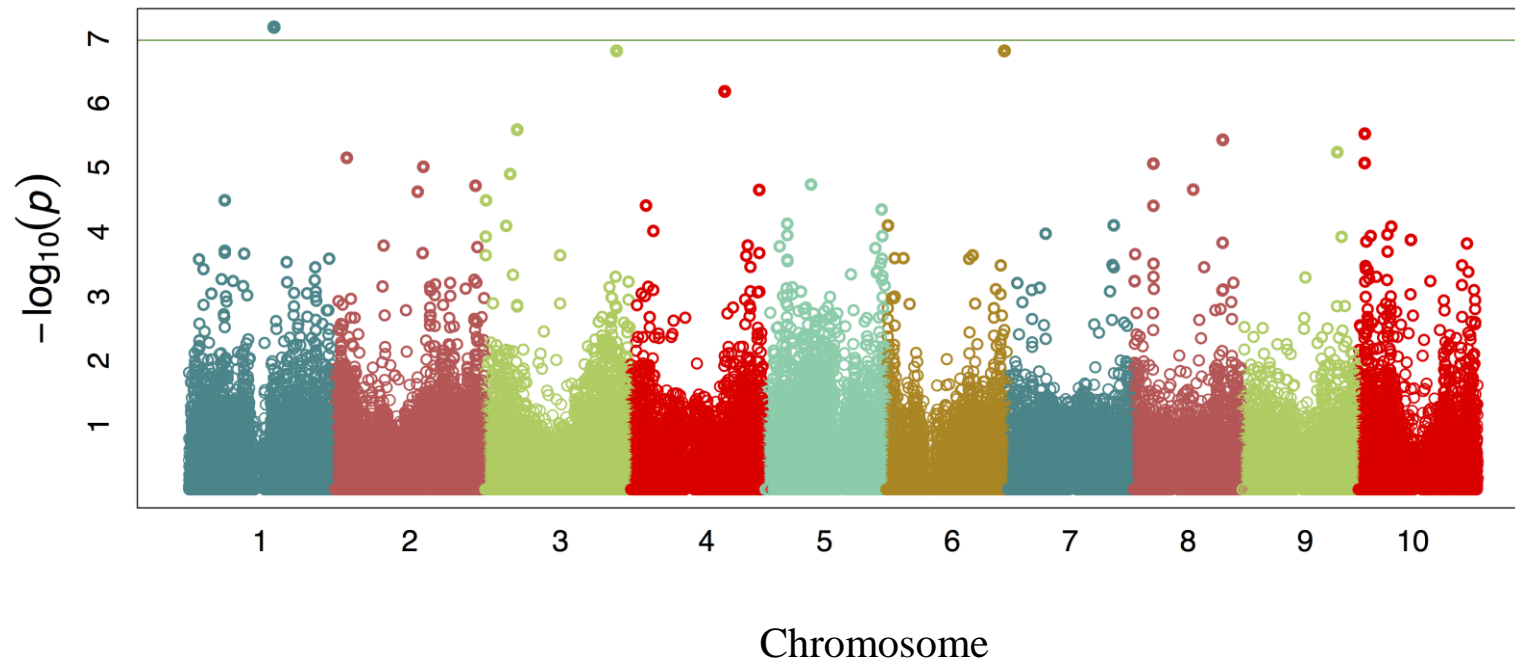
**Figure 3.9. Manhattan plots of GWAS (2015) for glume length (GL) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a single nucleotide polymorphism (SNP). Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

(A)

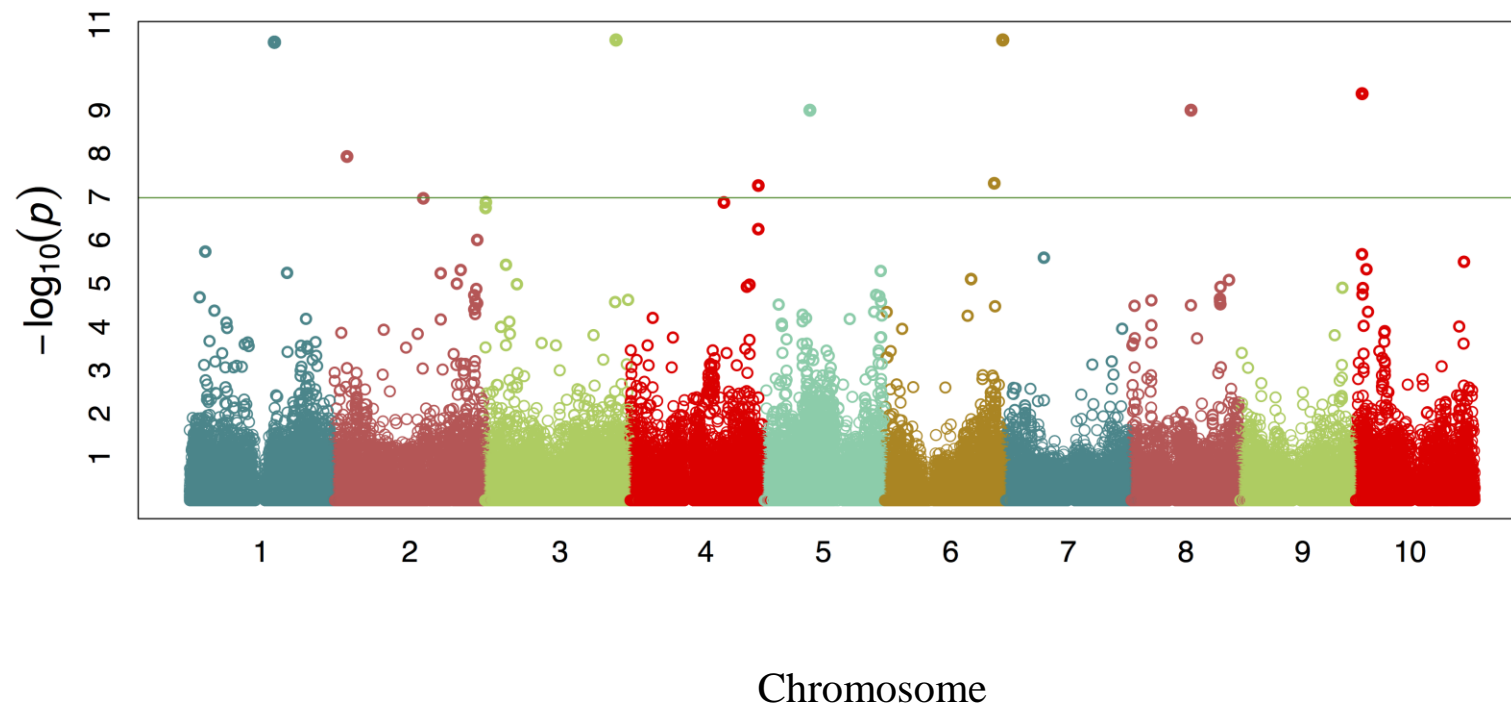




(B)

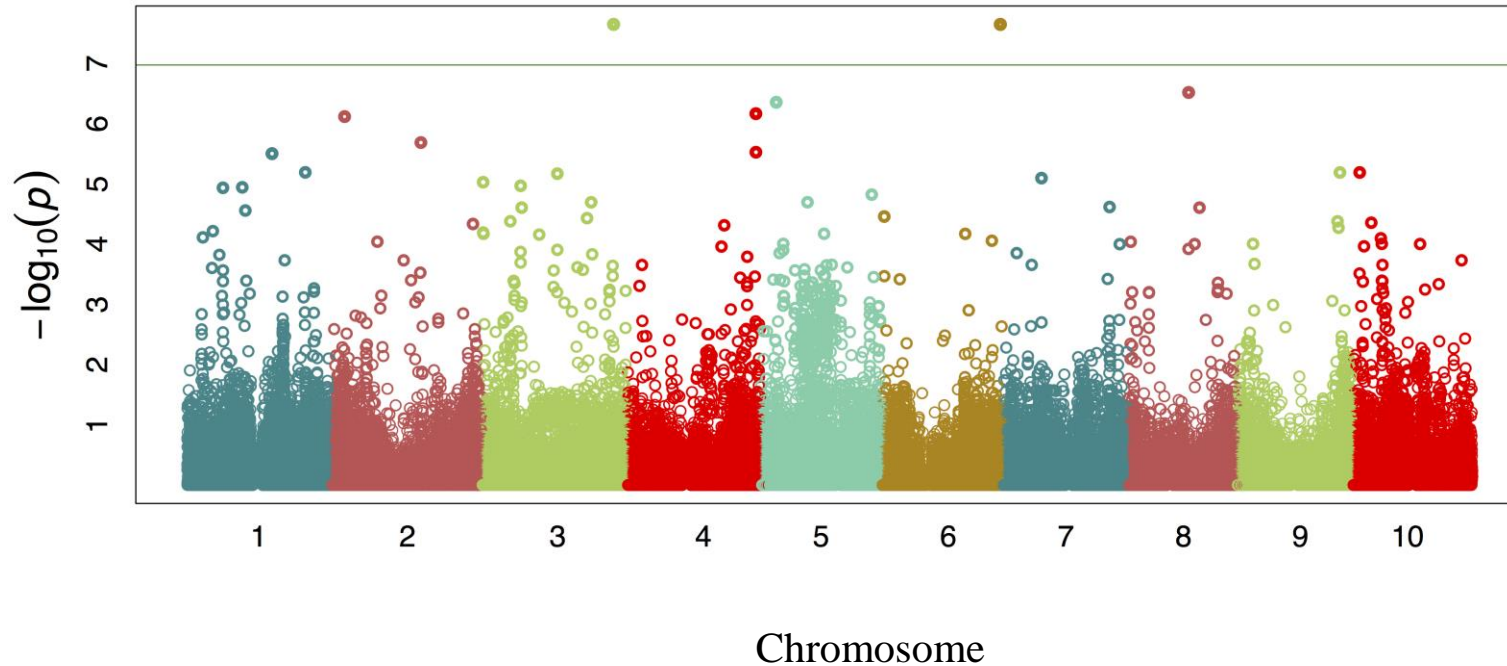


(C)

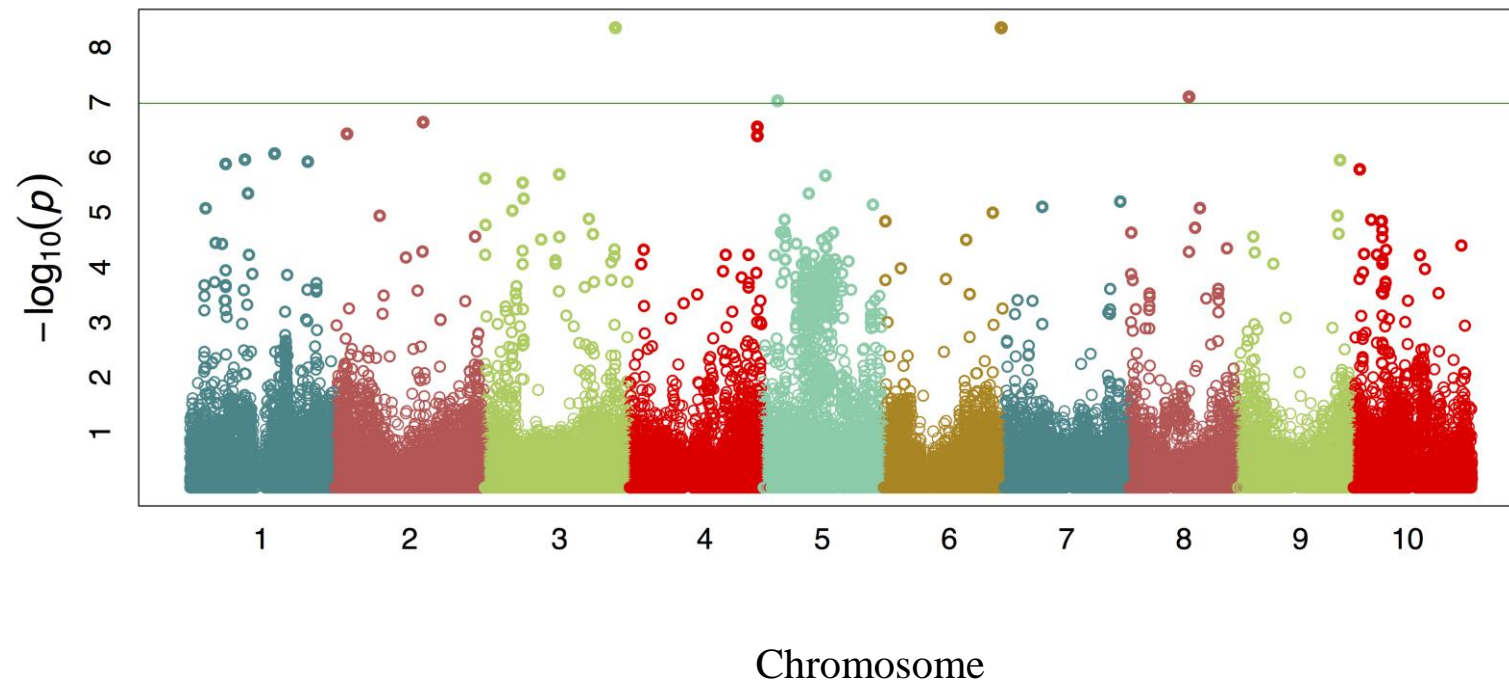


**Figure 3.10. Manhattan plots of GWAS (2014) for glume index (GI) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

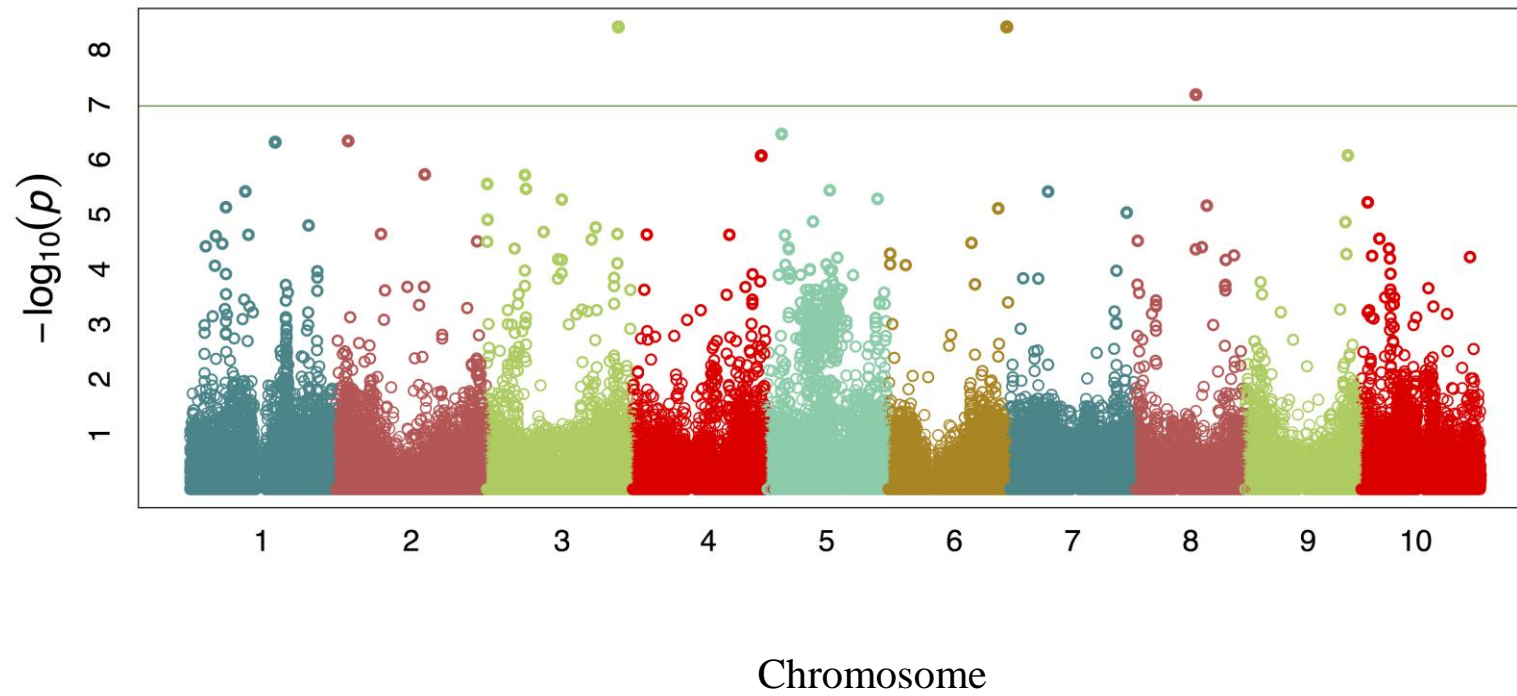
(A)



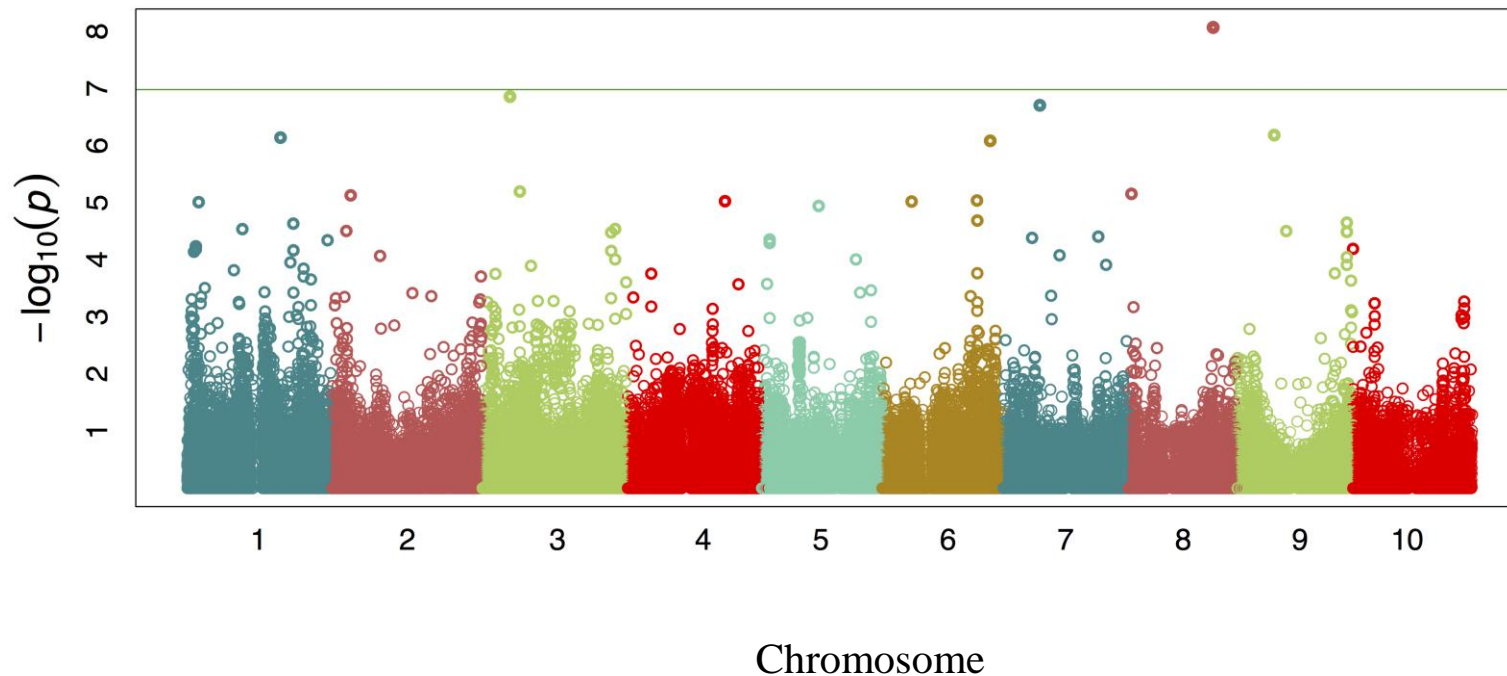
(B)



(c)

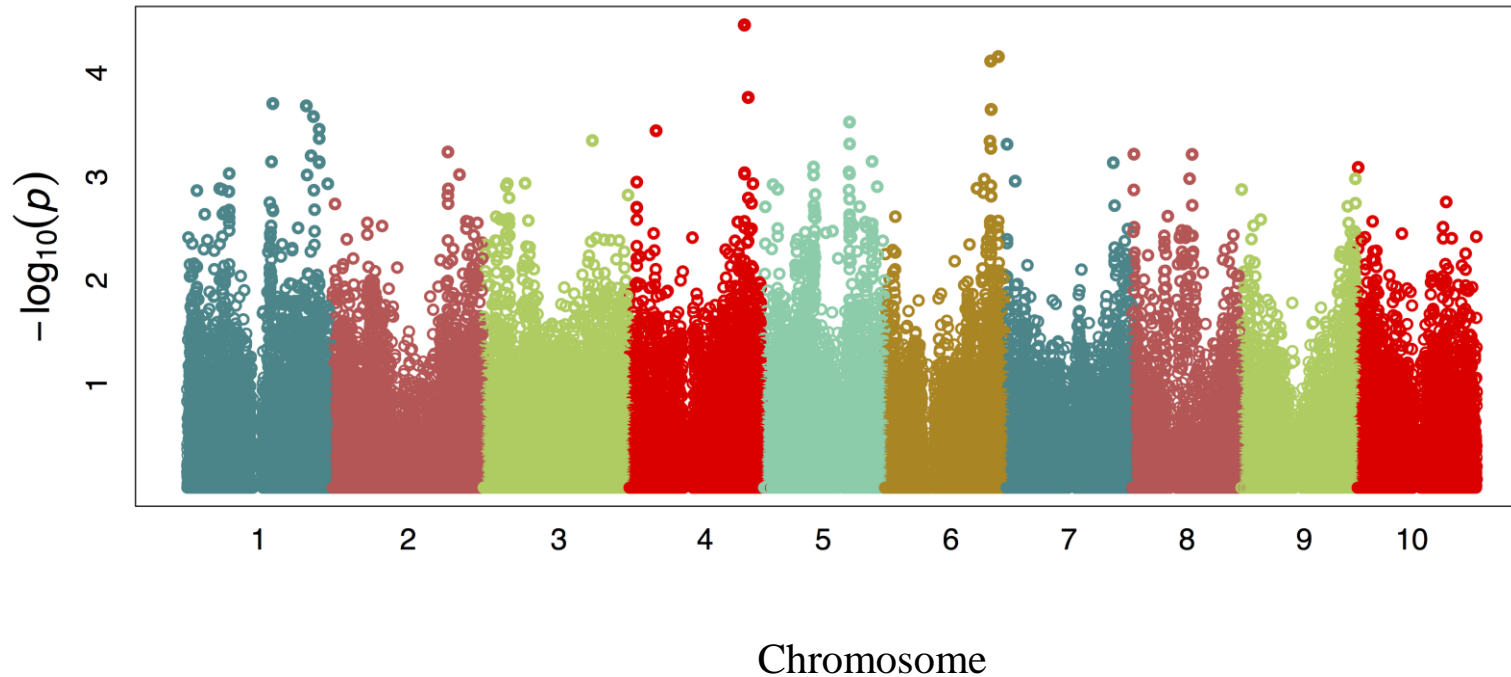


**Figure 3.11. Manhattan plots of GWAS (2014) for seed color (SC) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

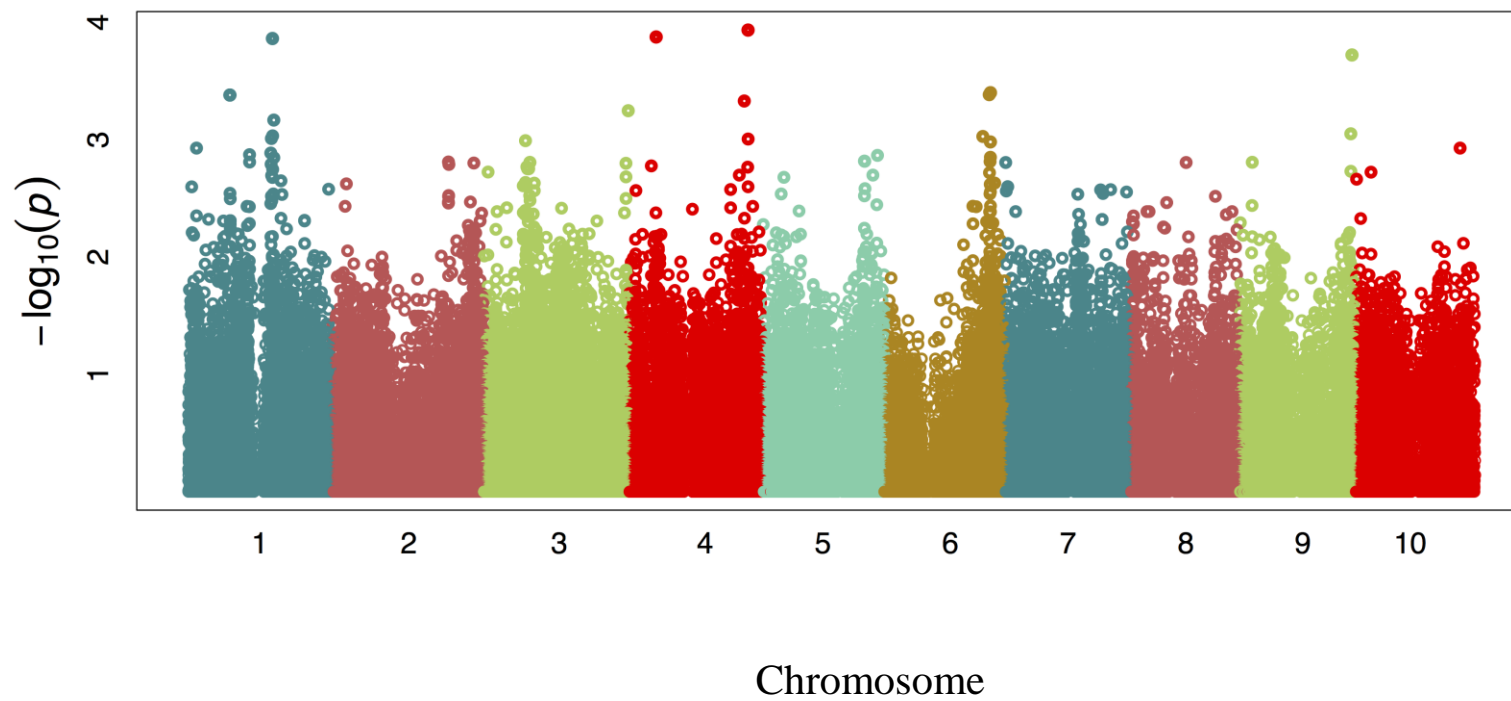


**Figure 3.12. Manhattan plots of GWAS (2014) for panicle grain mold rating (PGMR) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

(A)

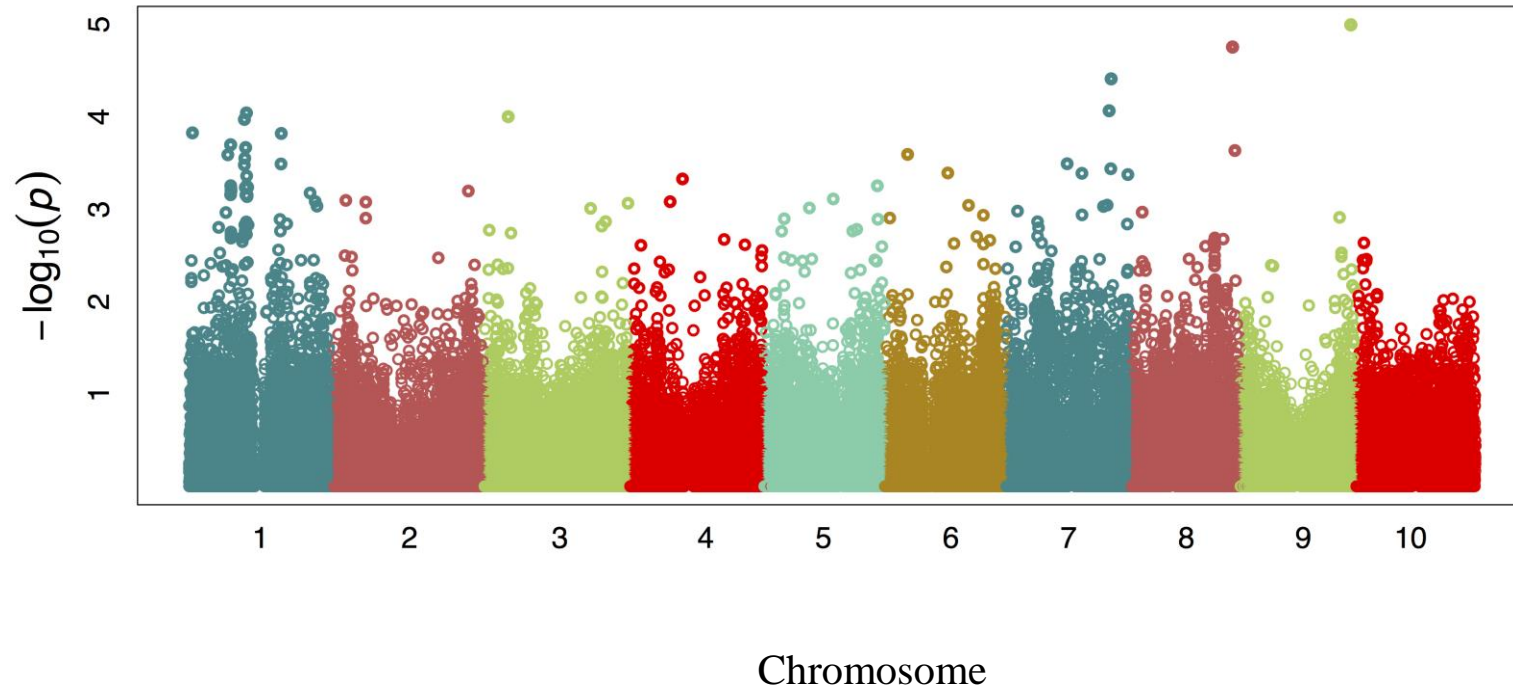


**(B)**



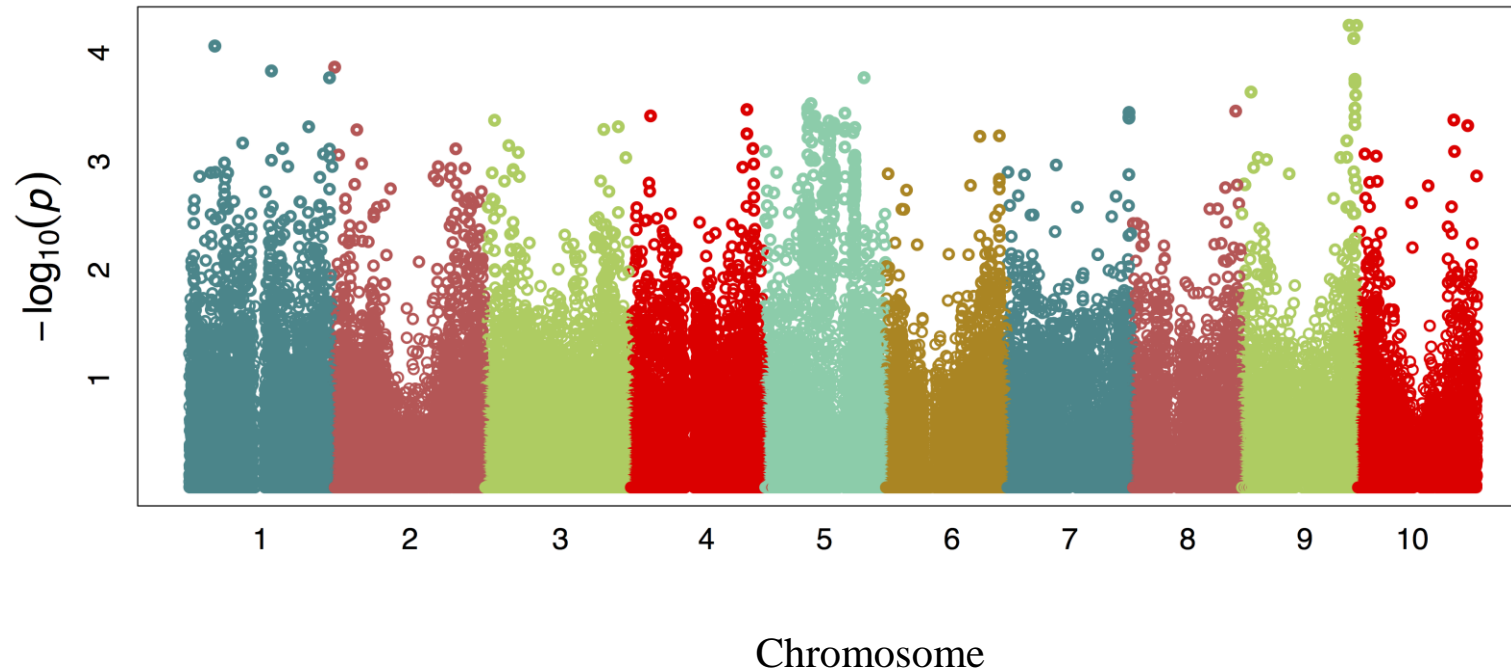


(C)

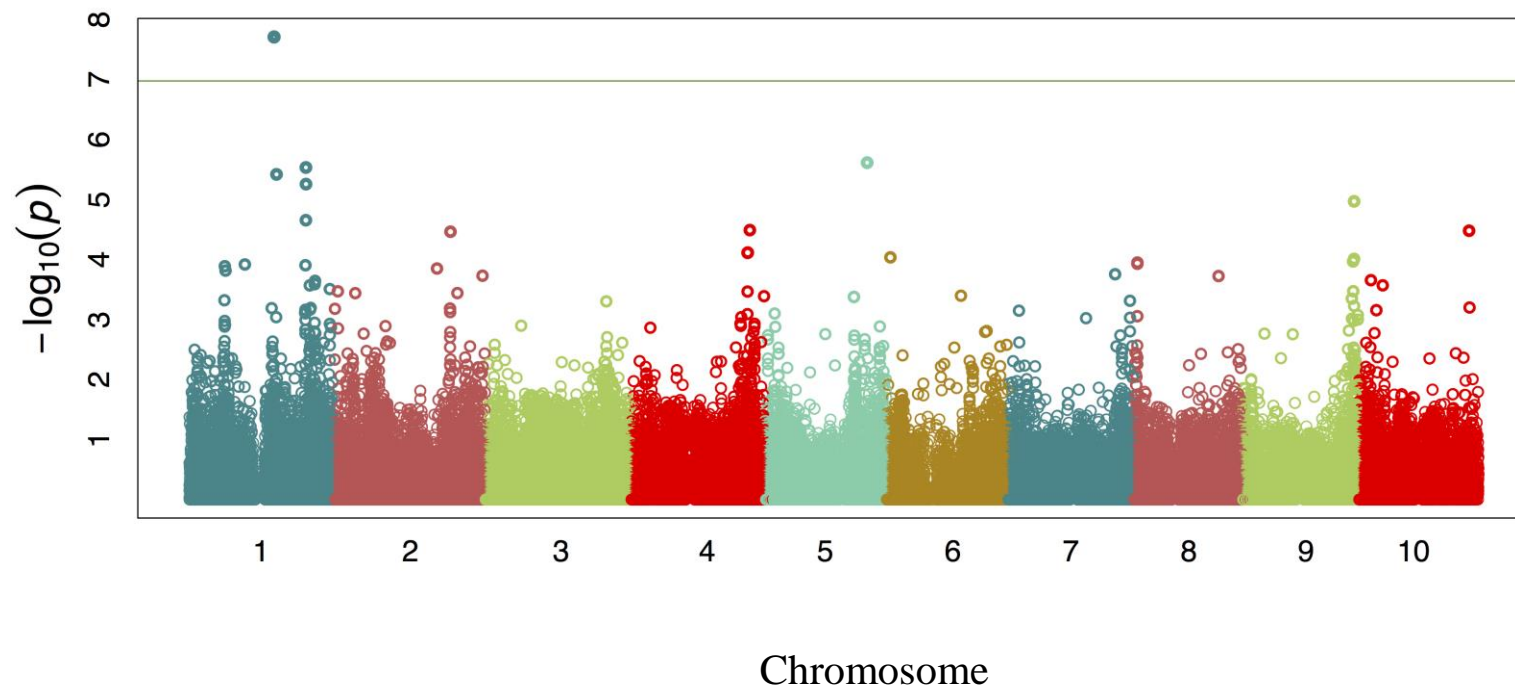


**Figure 3.13. Manhattan plots of GWAS (2015) for panicle grain mold rating (PGMR) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

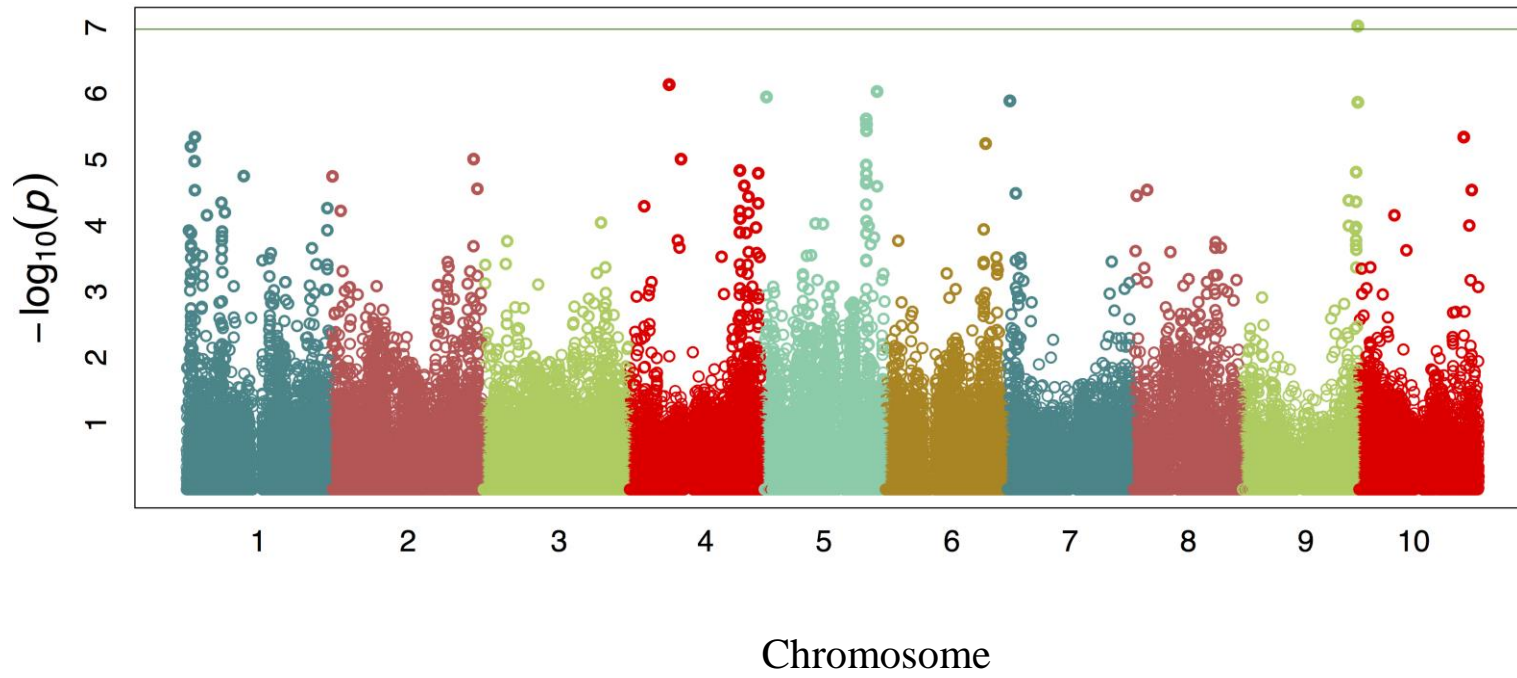
(A)



**(B)**

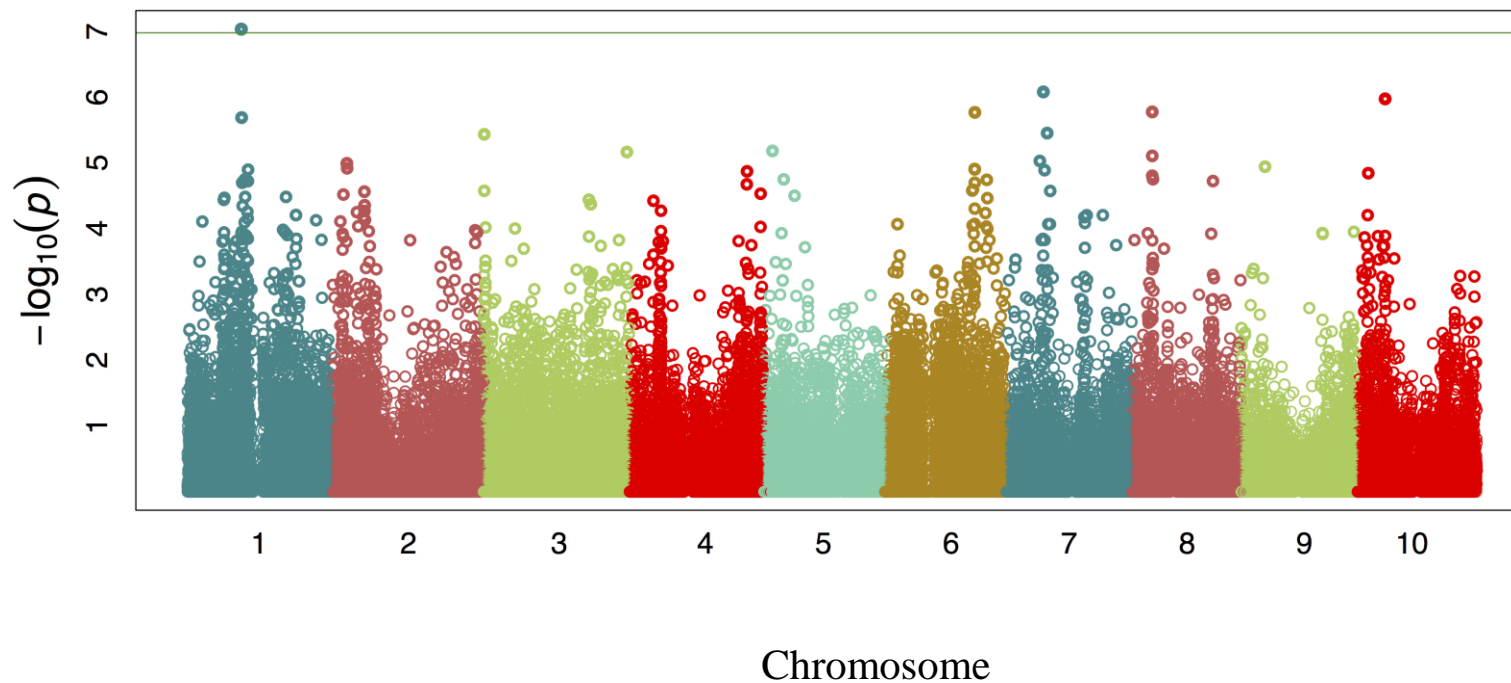


(c)

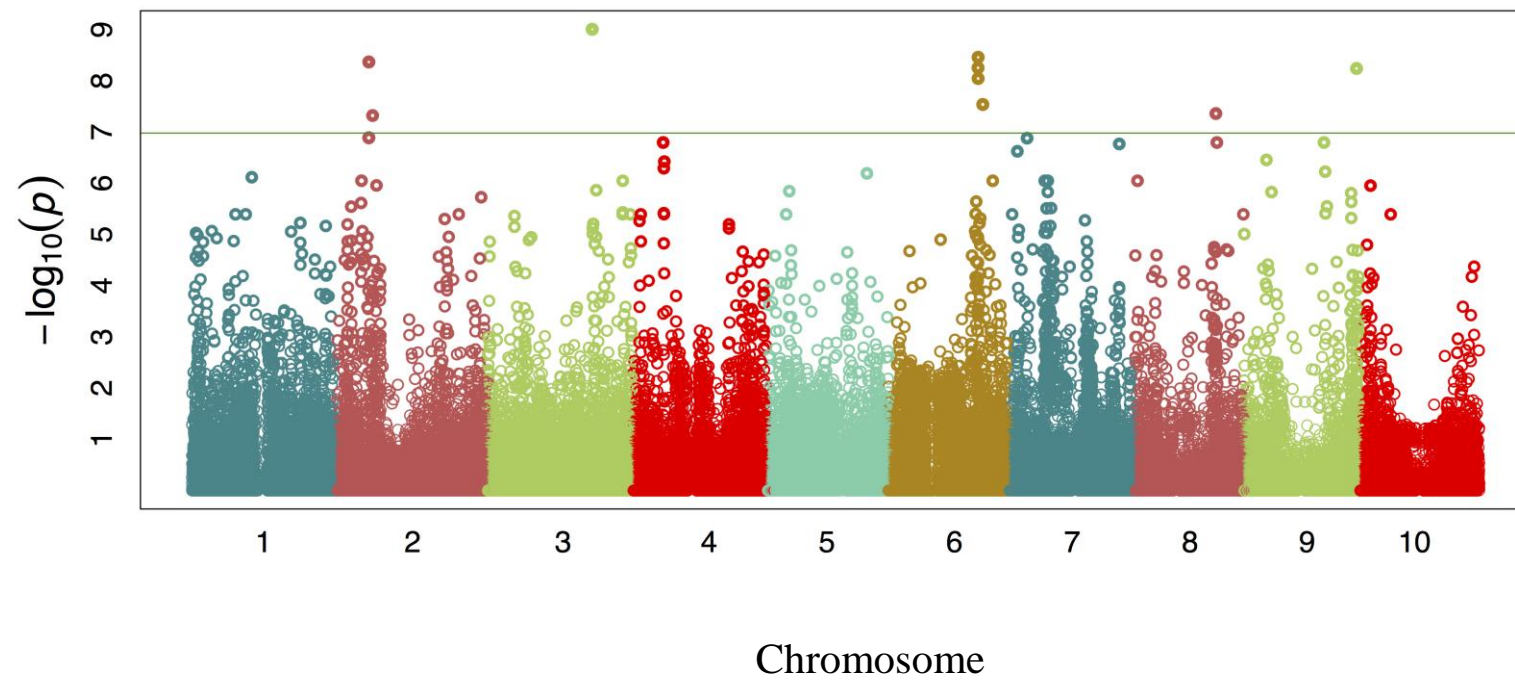


**Figure 3.14. Manhattan plots of GWAS (2014) for 3-deoxyanthocyanidins (Deo) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a single nucleotide polymorphism (SNP). Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

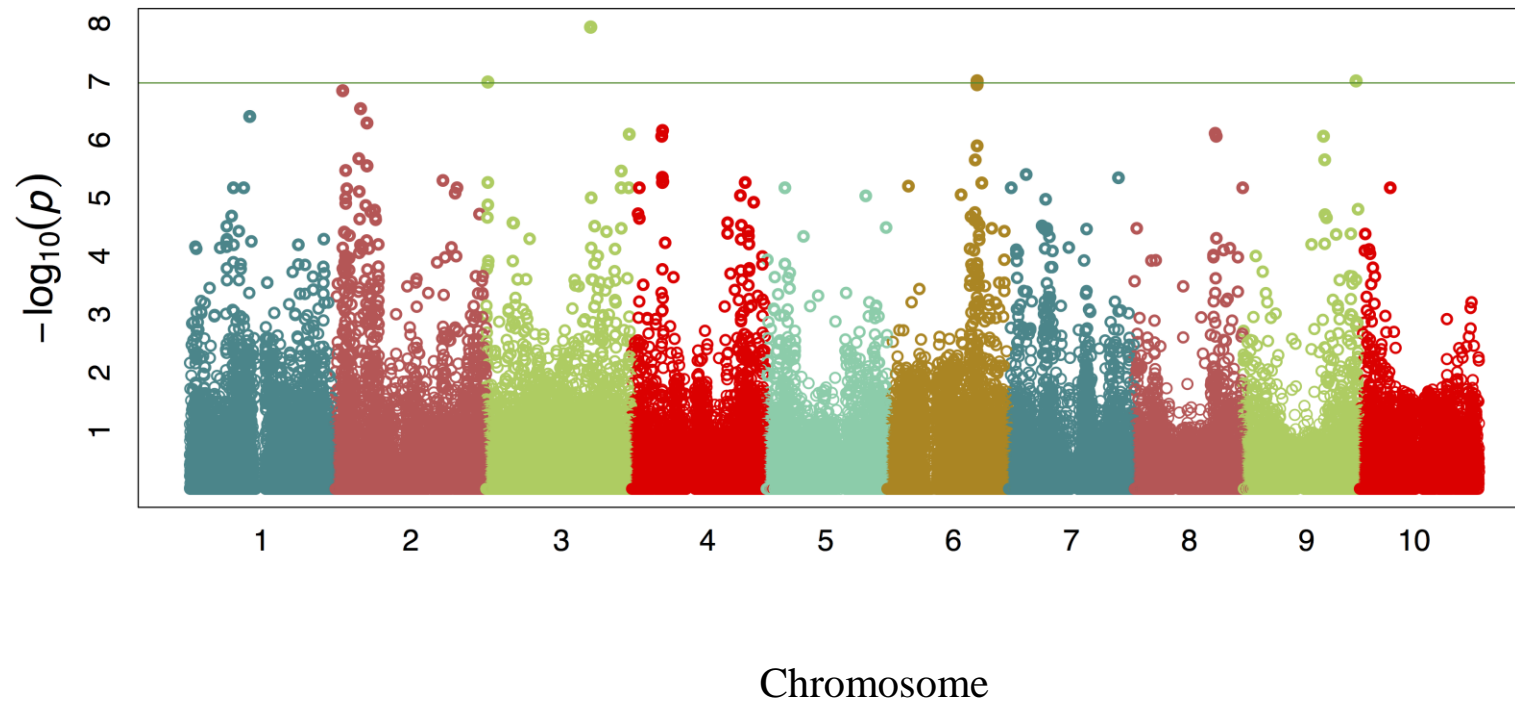
(A)



**(B)**

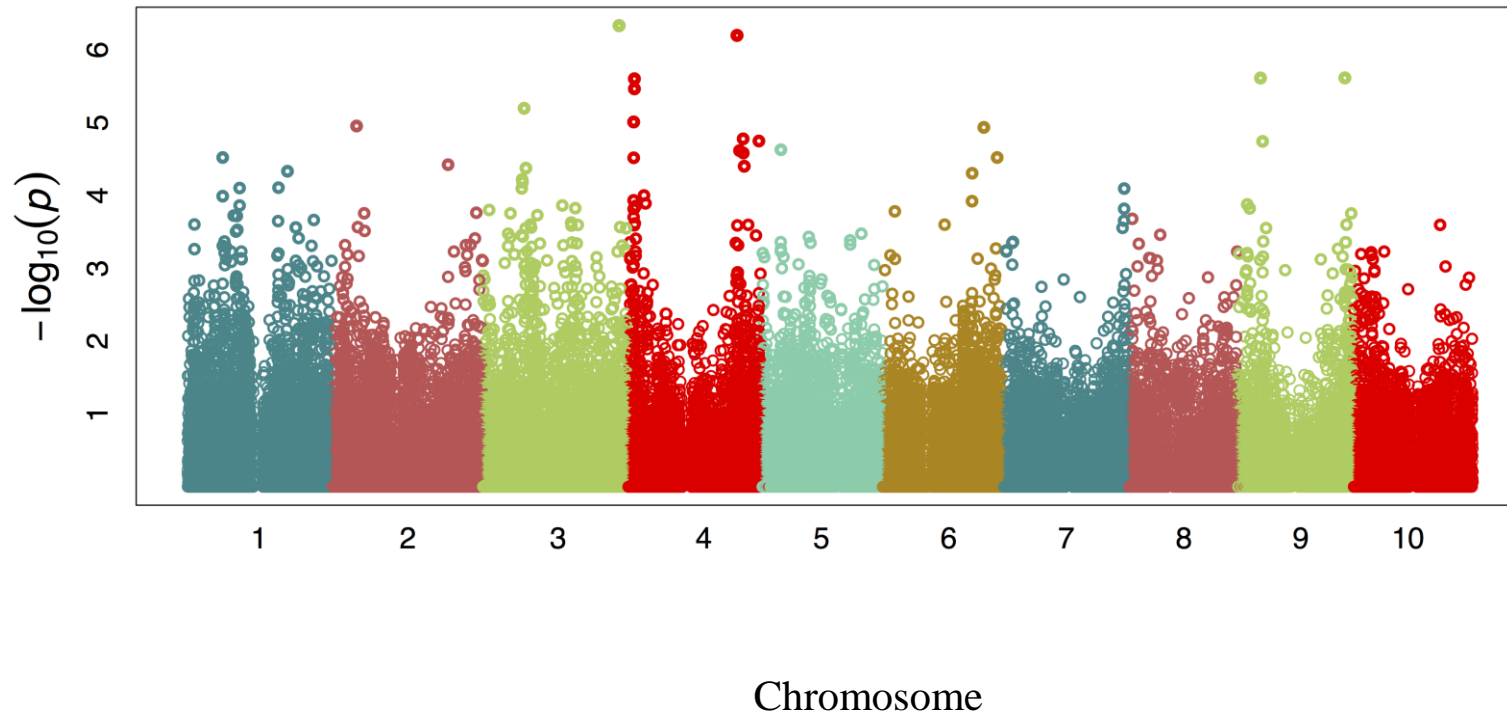


(c)



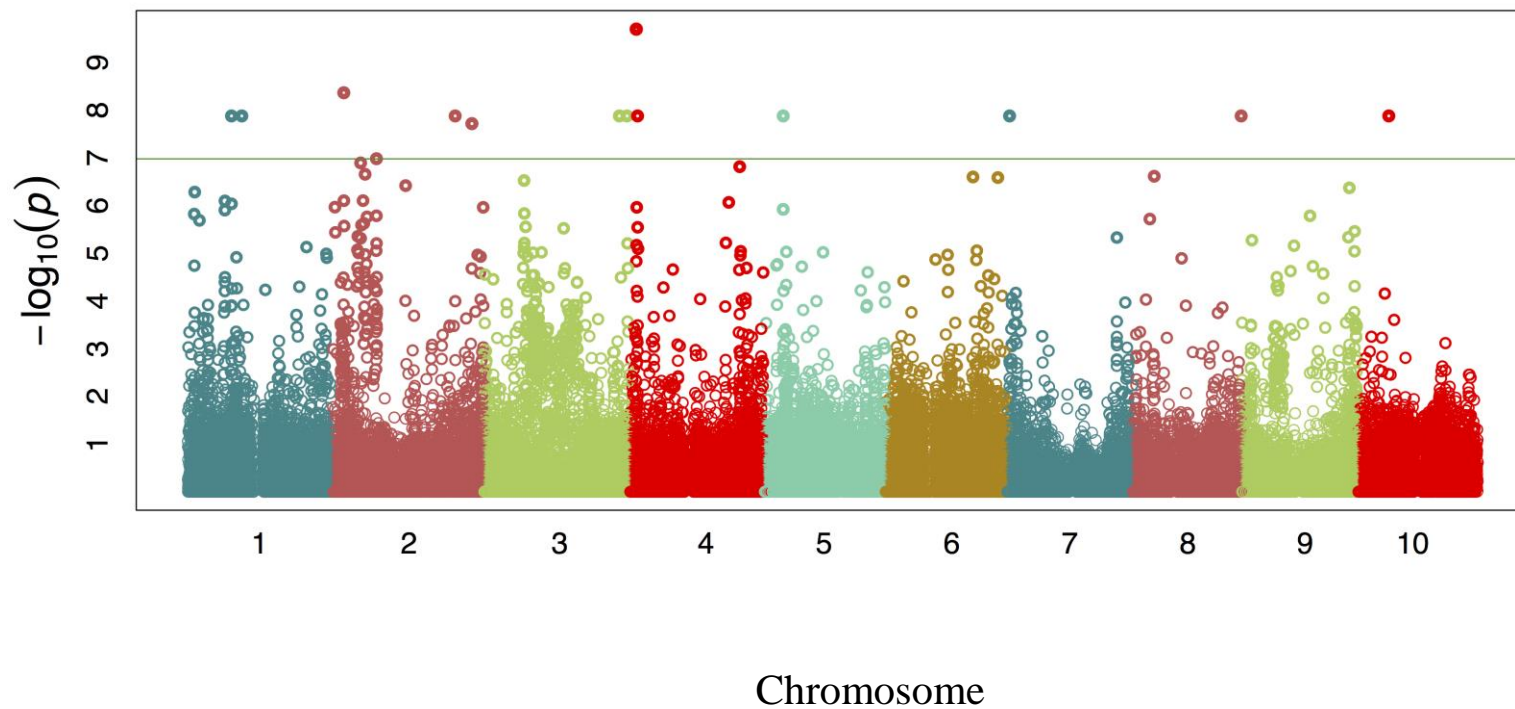
**Figure 3.15. Manhattan plots of GWAS (2015) for 3-deoxyanthocyanidins (Deo) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

(A)

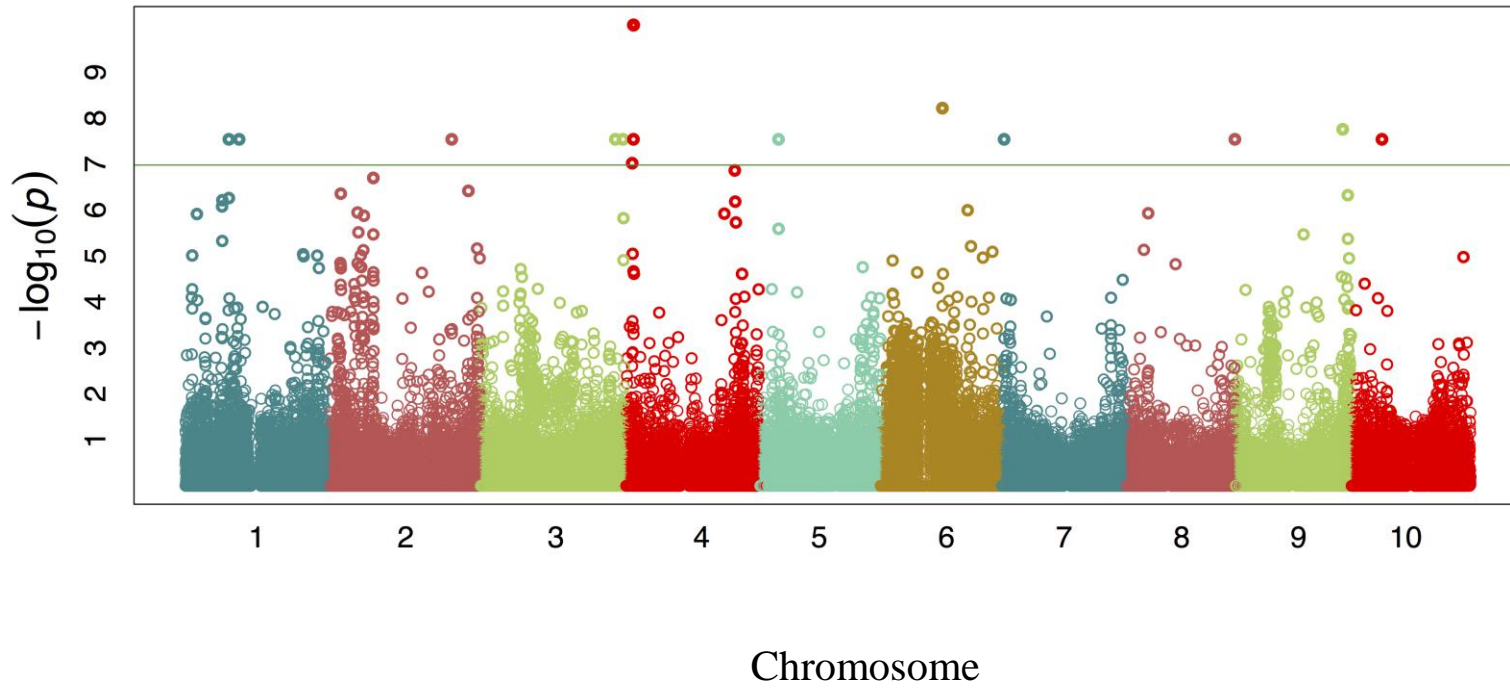




(B)

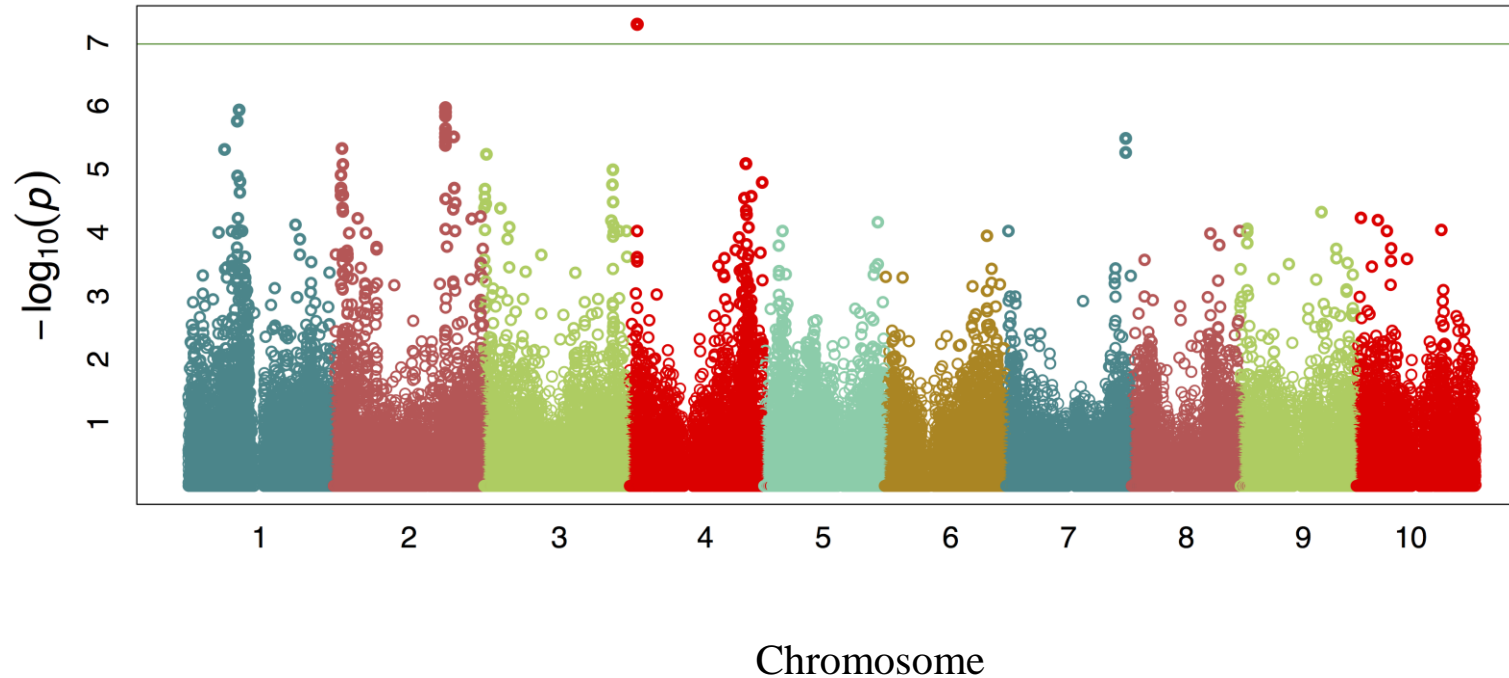


(c)

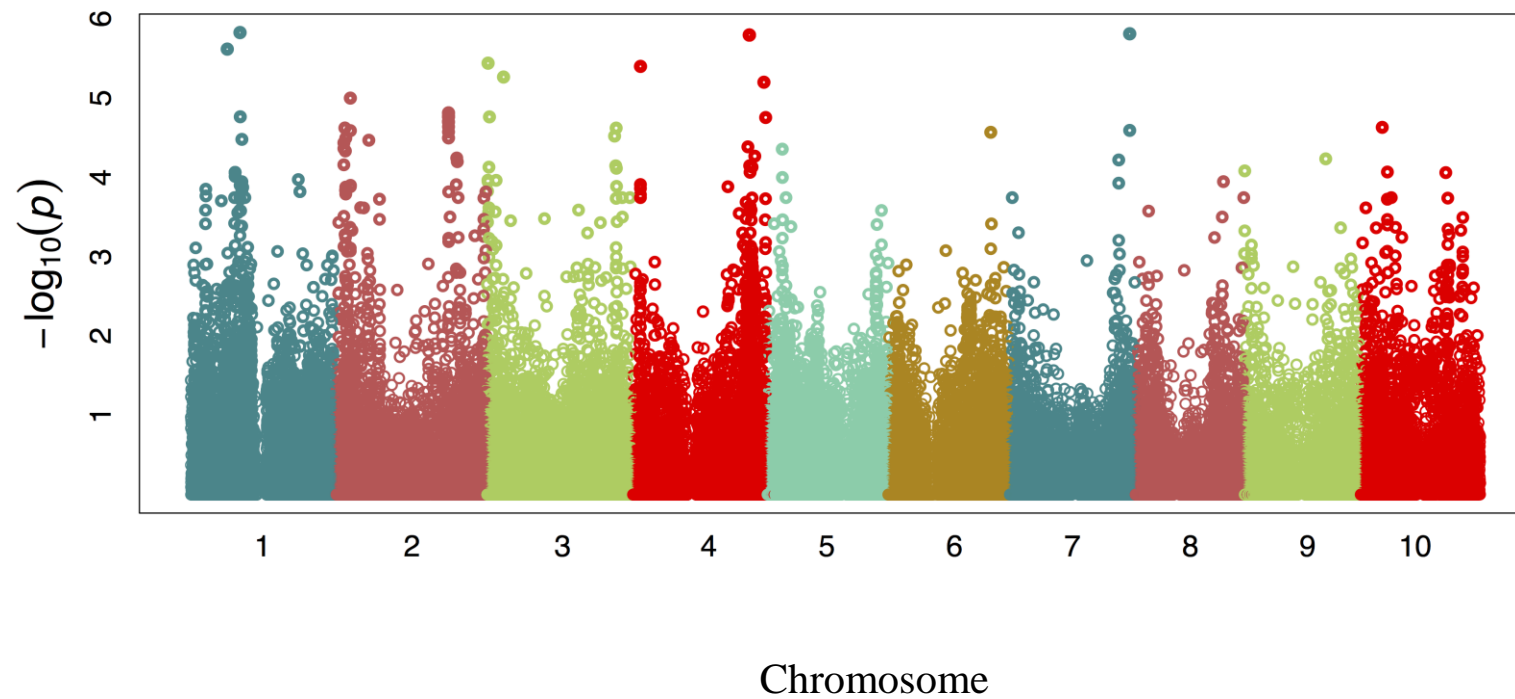


**Figure 3.16. Manhattan plots of GWAS (2014) for total protein per cent (TPP) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

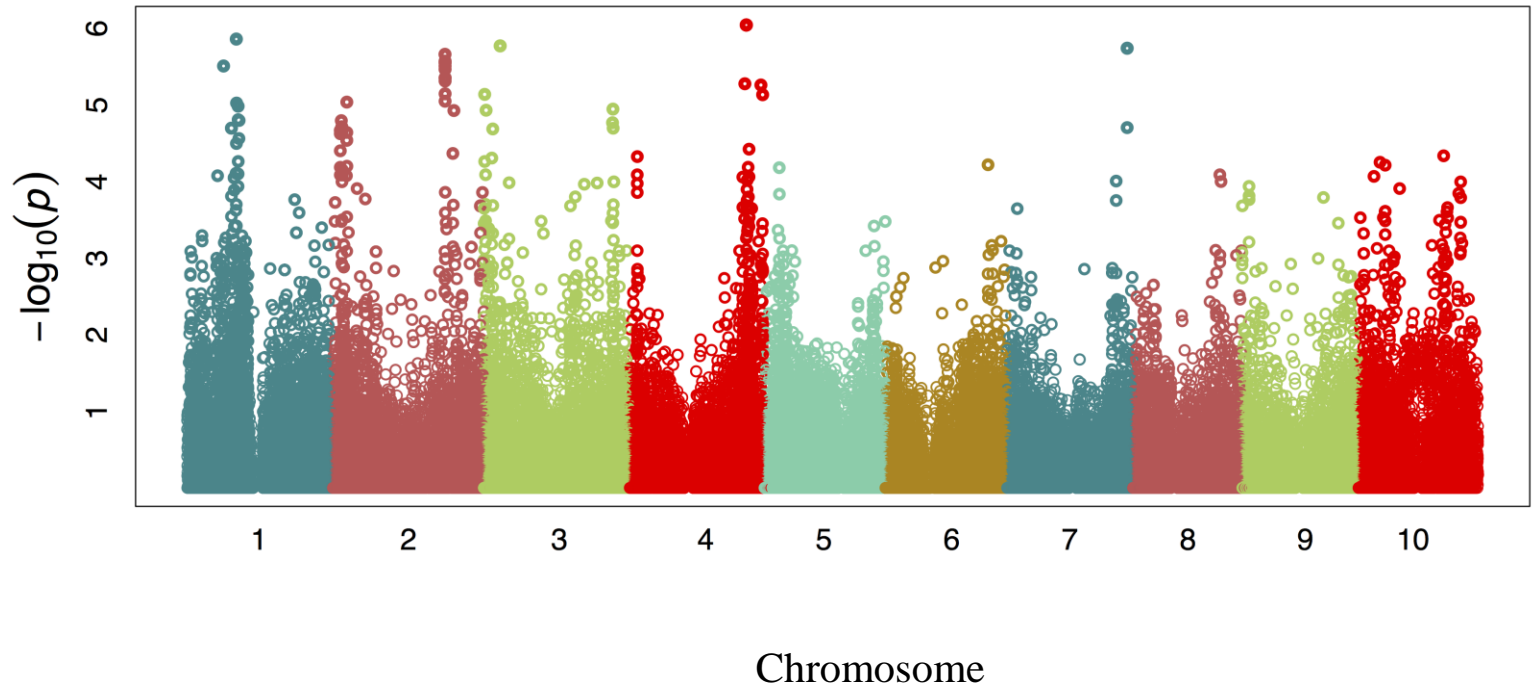
(A)



**(B)**

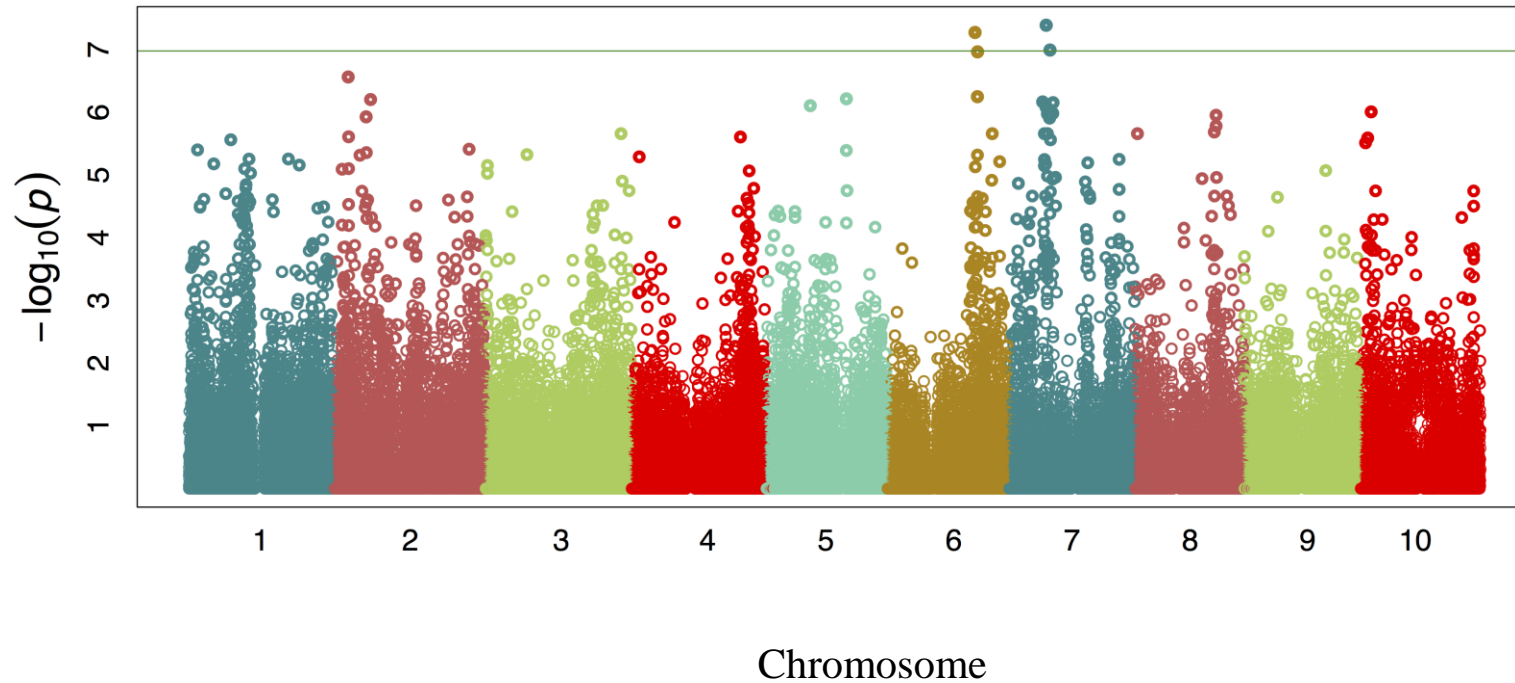


(C)

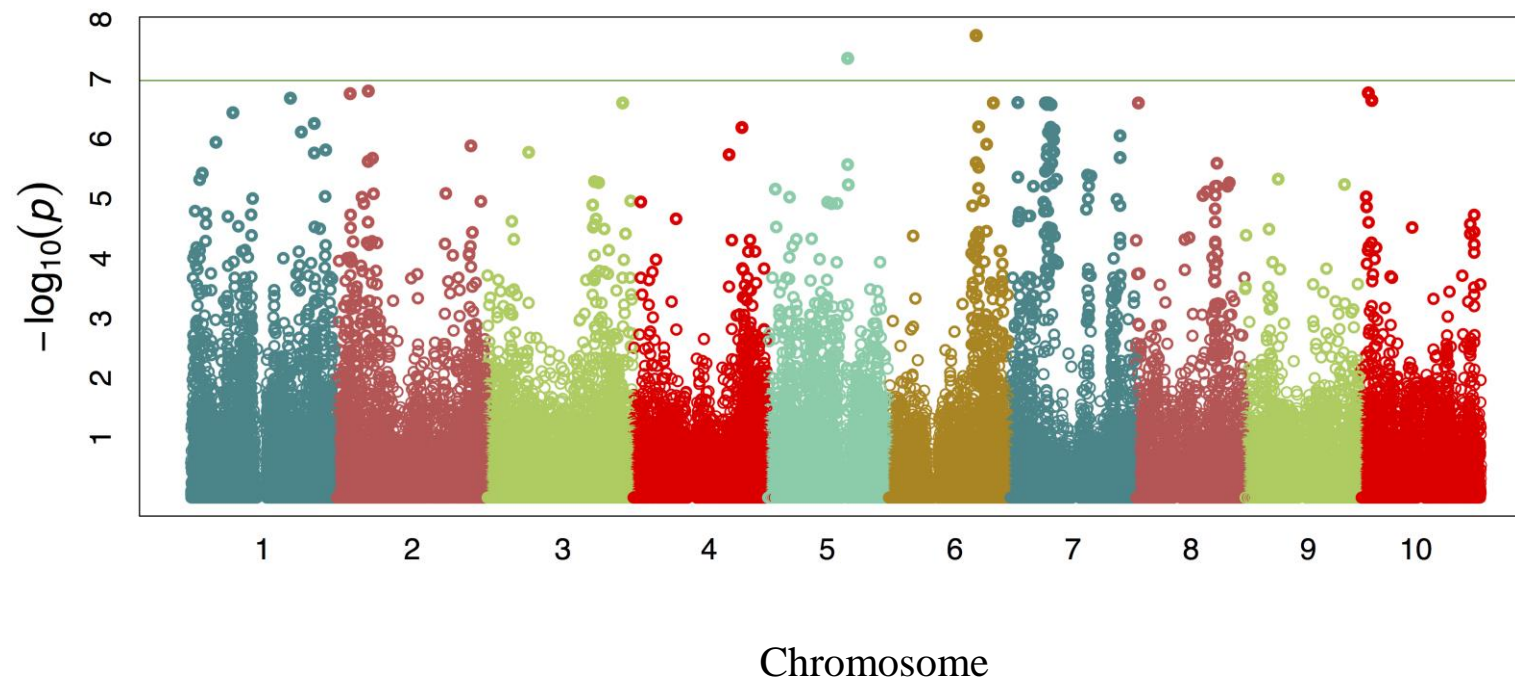


**Figure 3.17. Manhattan plots of GWAS (2014) for moisture per cent (Moi) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10}p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

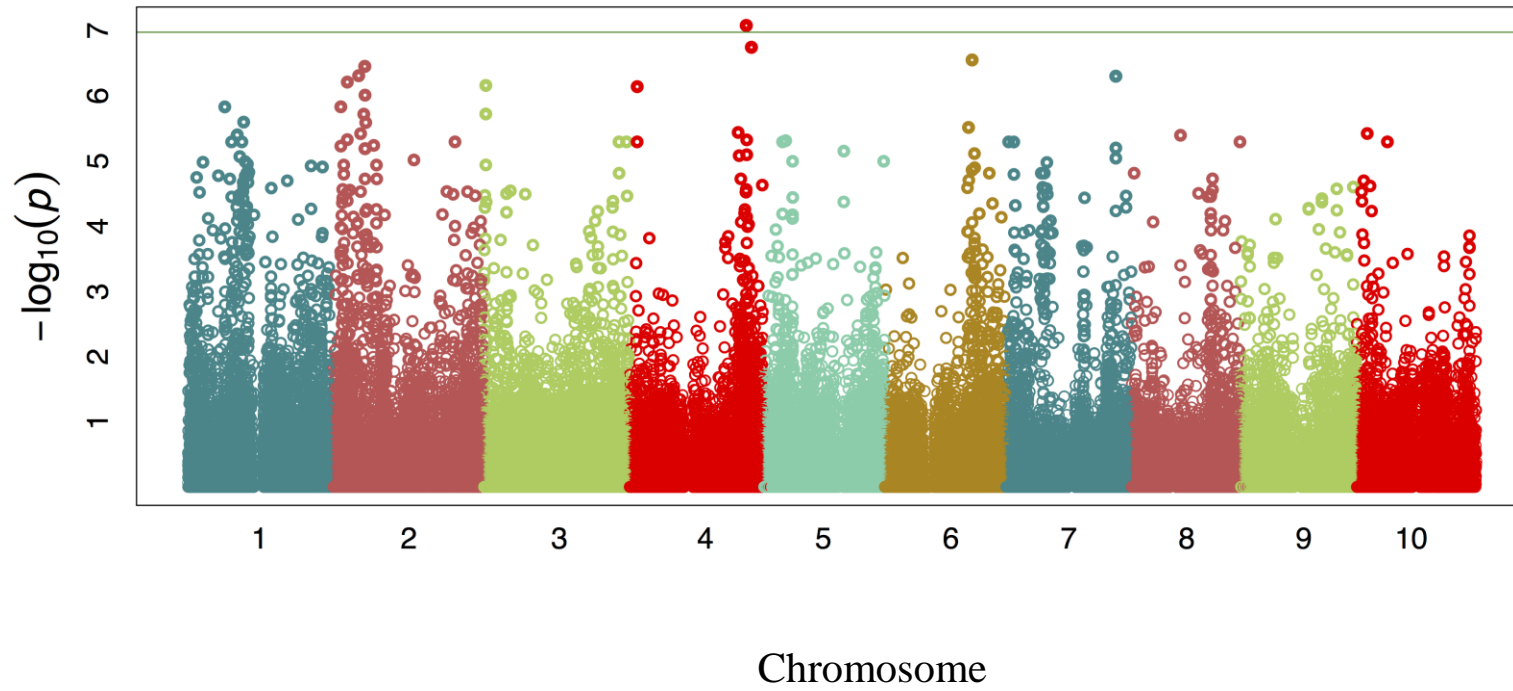
(A)



**(B)**



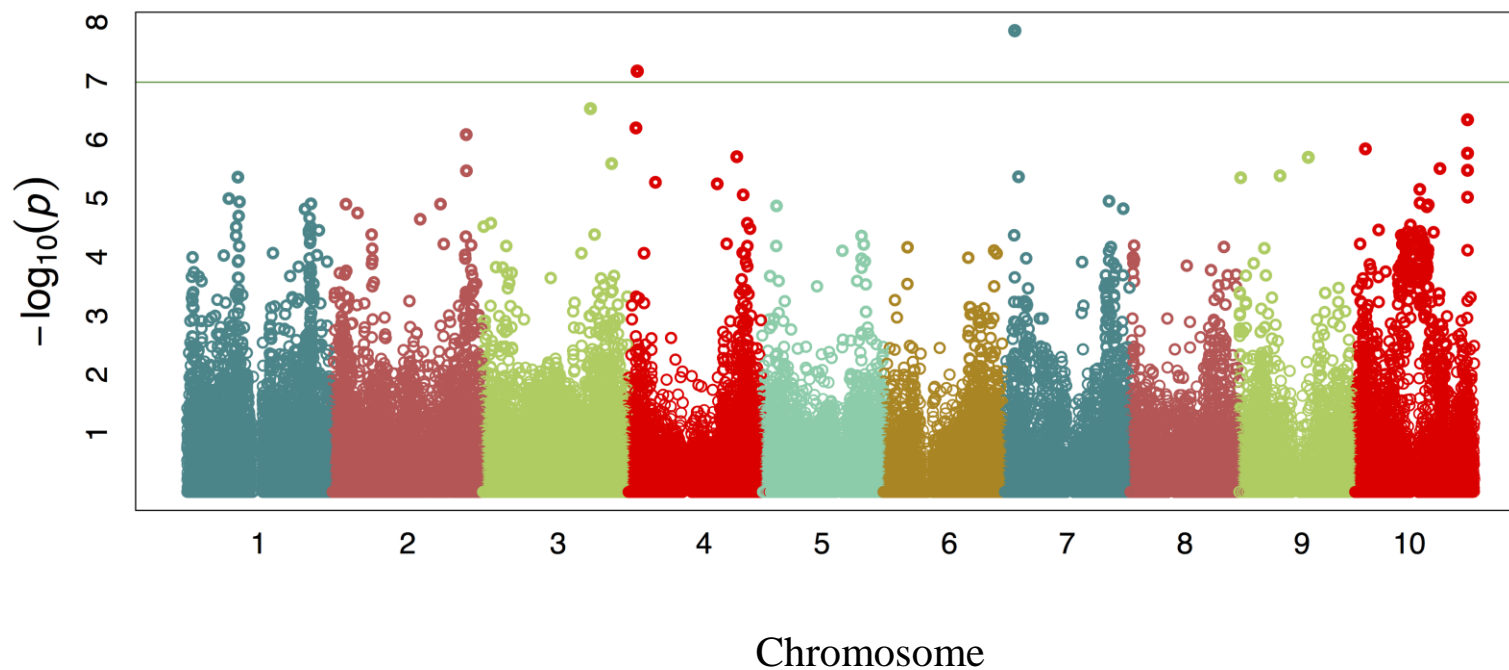
(c)



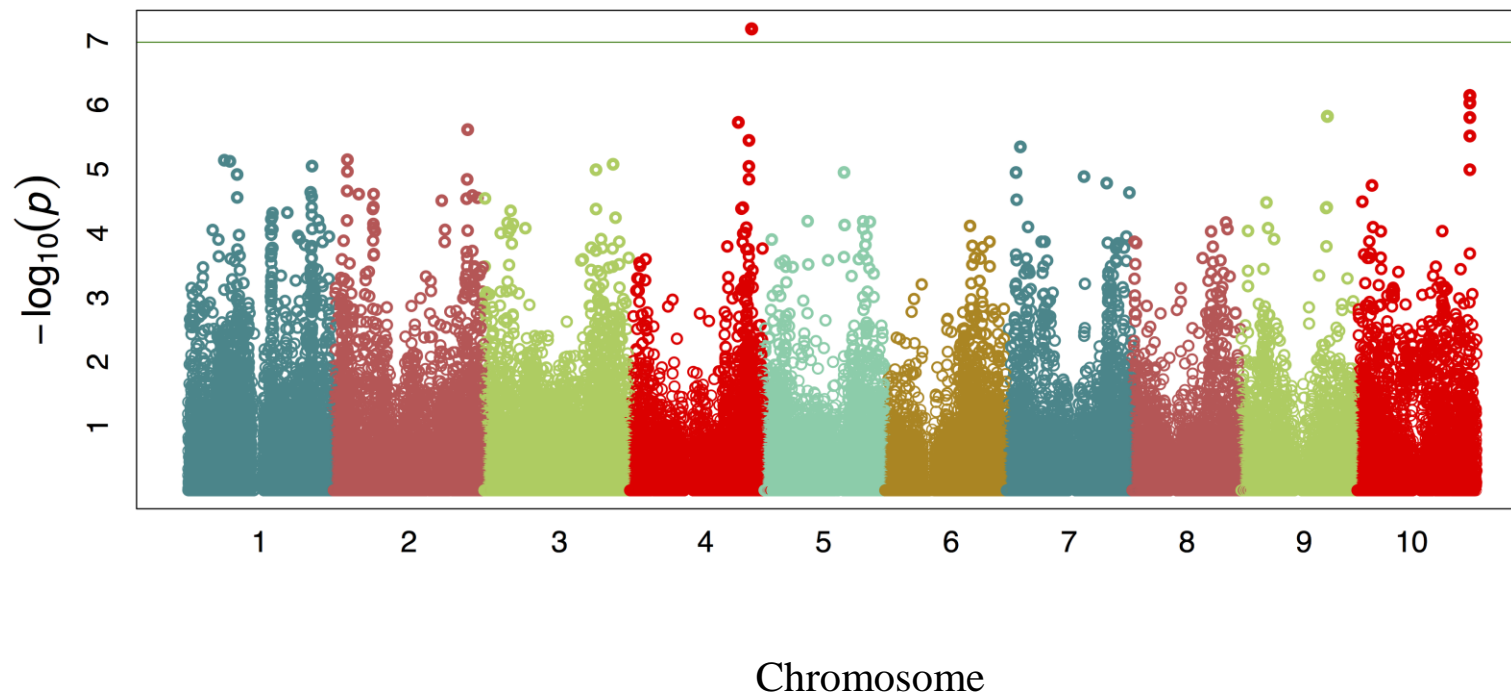


**Figure 3.18. Manhattan plots of GWAS (2015) for moisture per cent (Moi) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

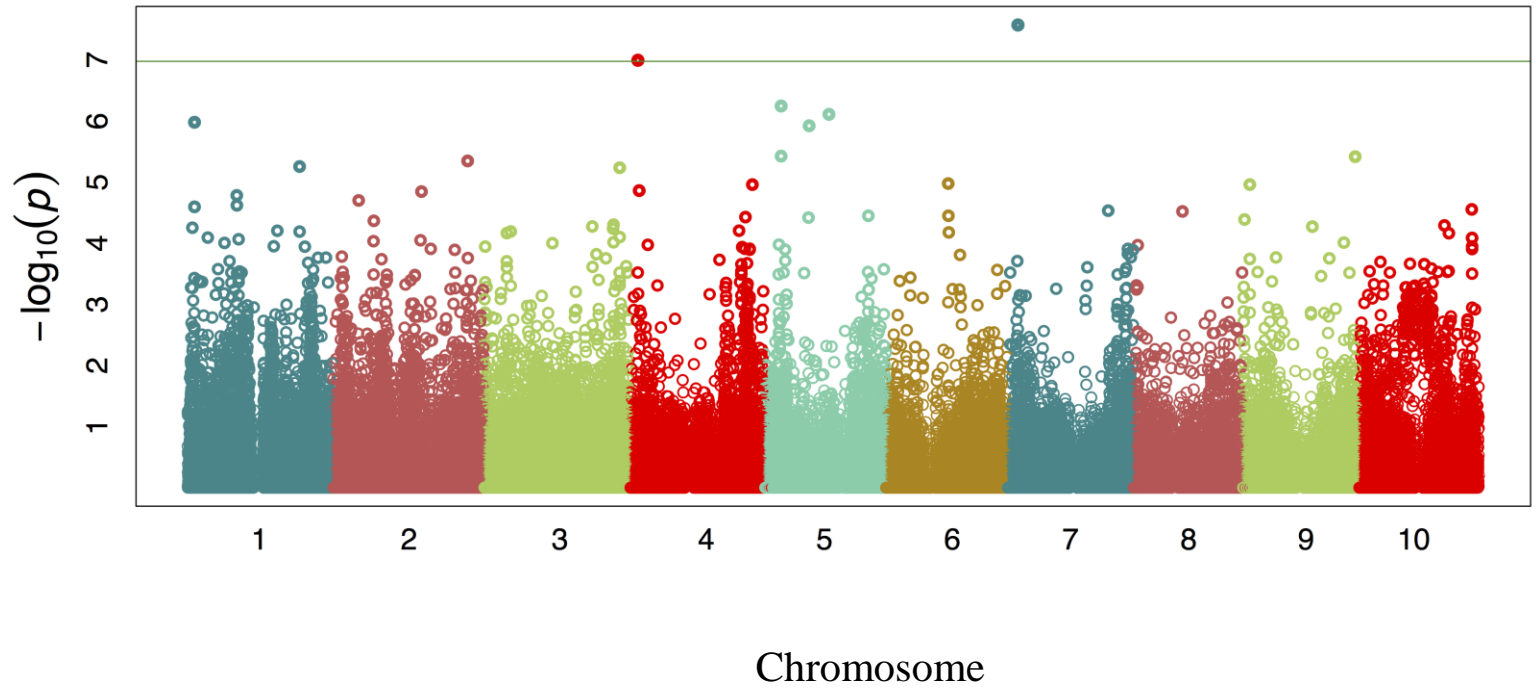
(A)



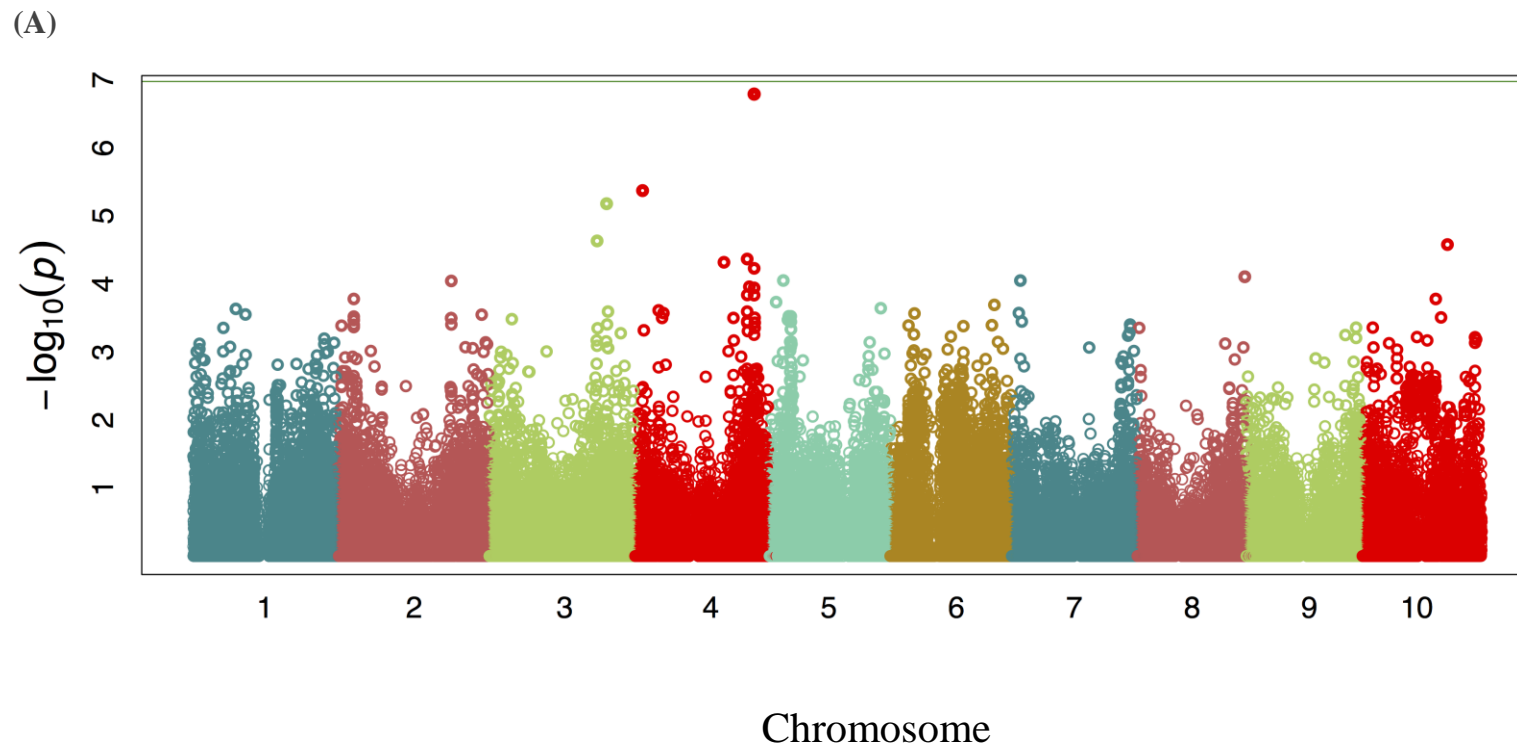
**(B)**



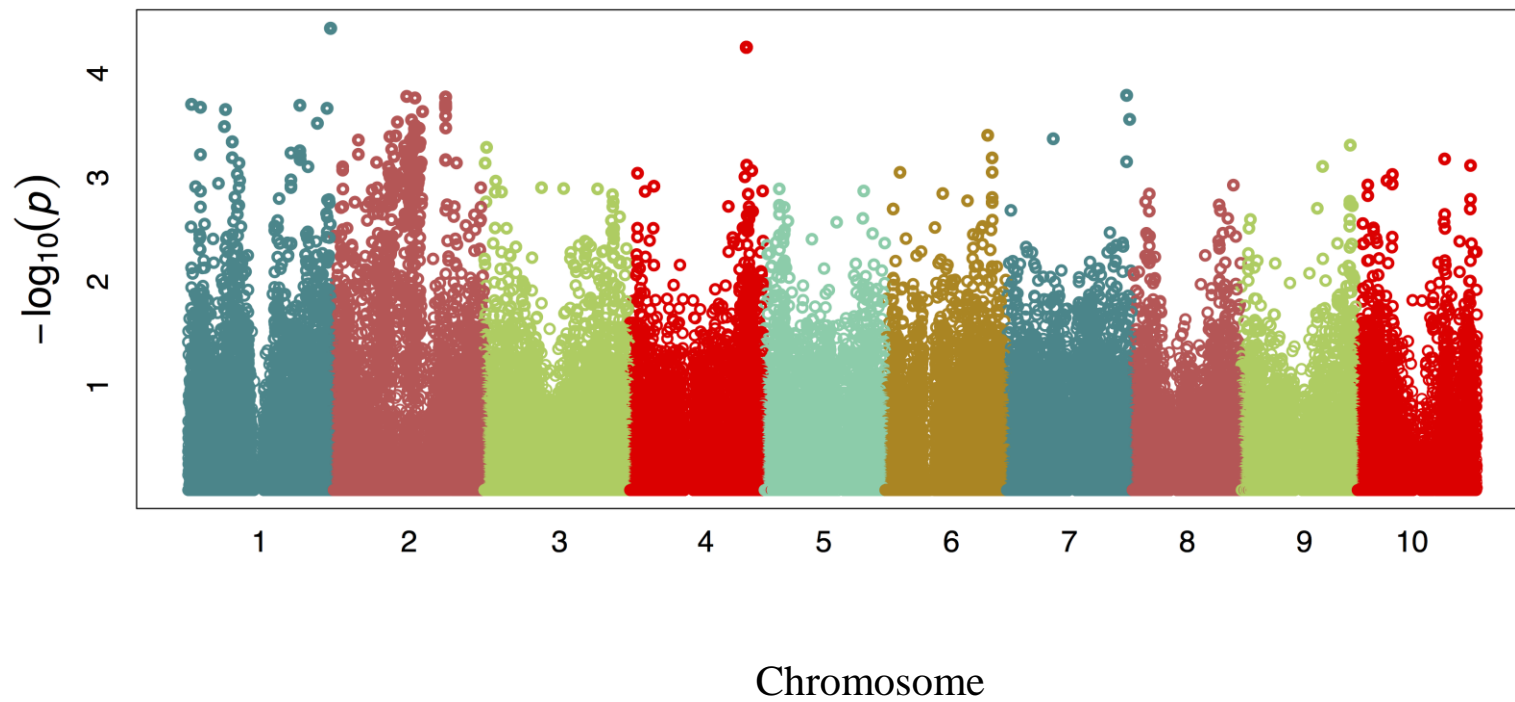
(c)



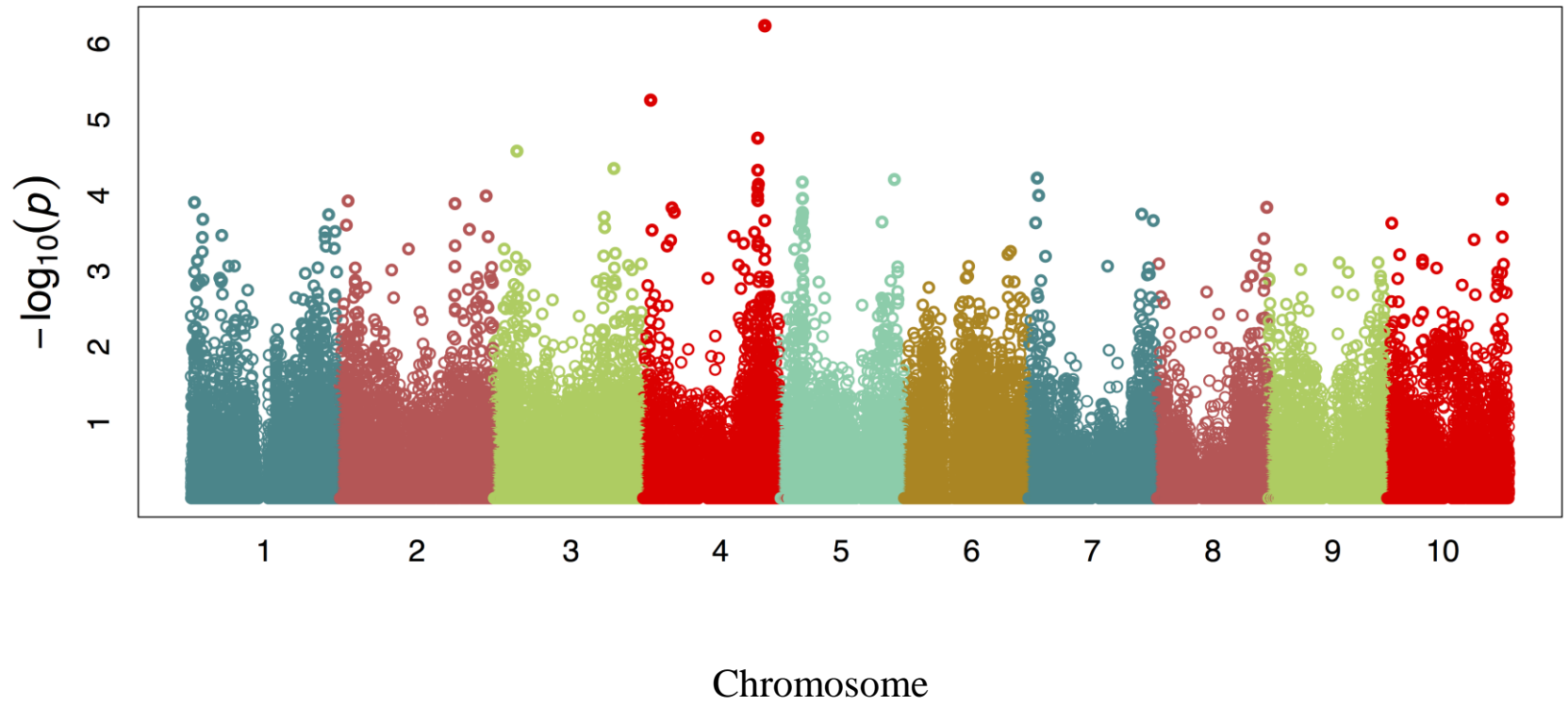
**Figure 3.19. Manhattan plots of GWAS (2015) for total phenolic acids (Phe) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**



**(B)**

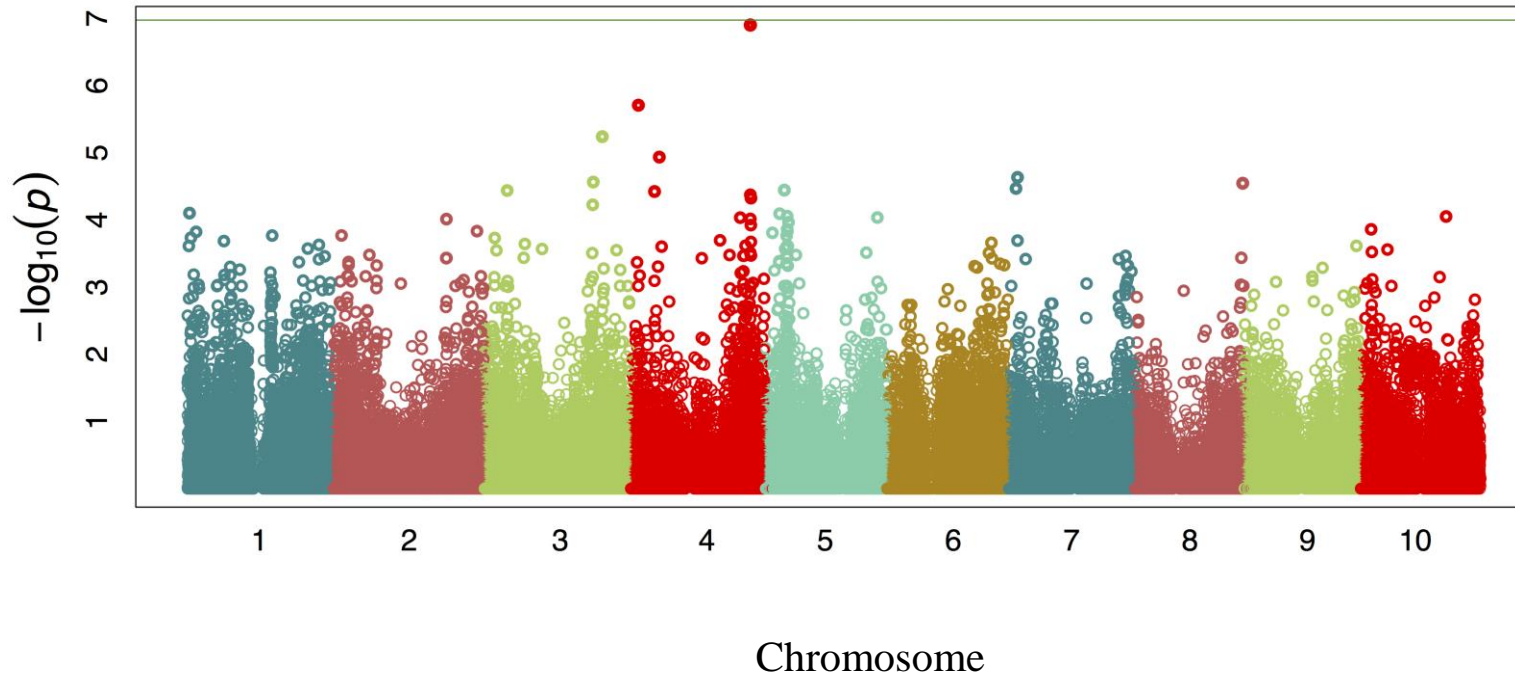


(c)

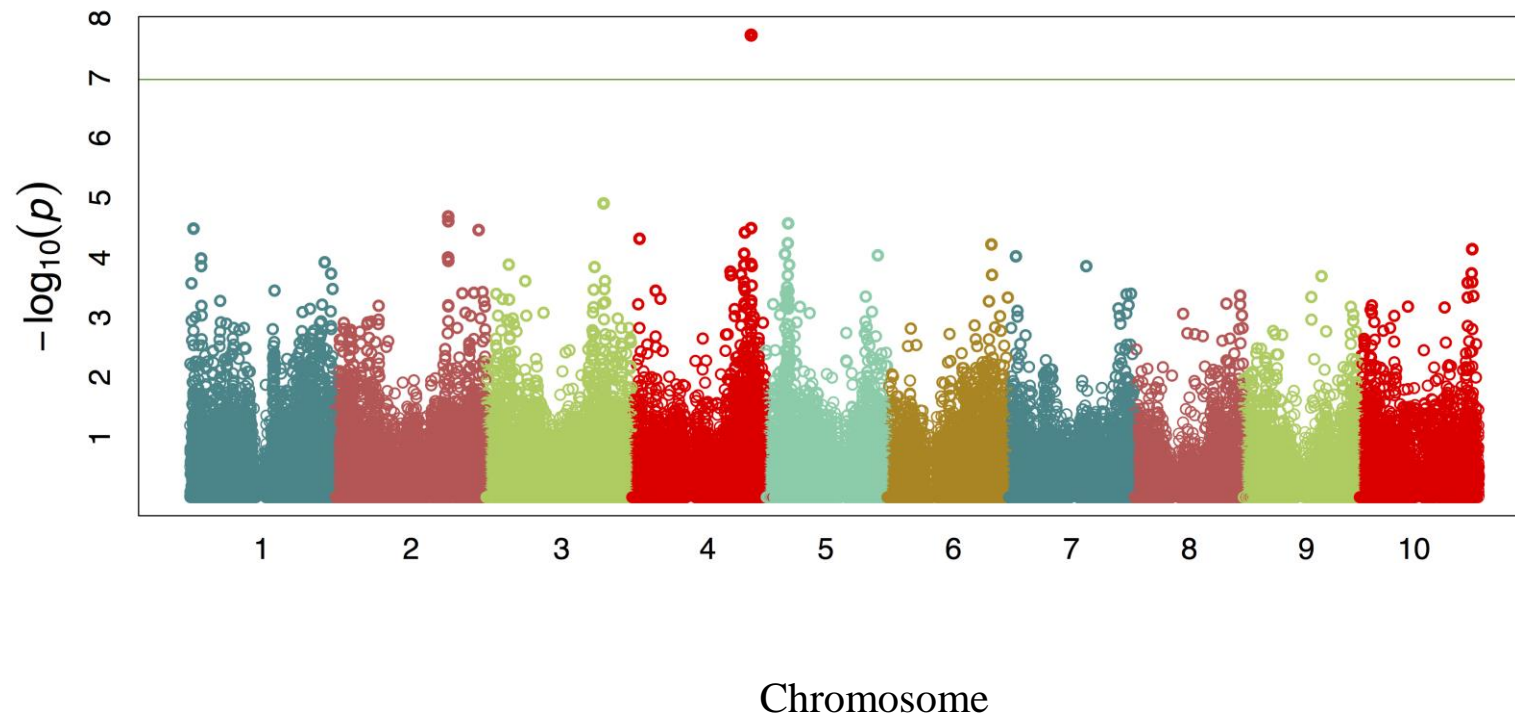


**Figure 3.20. Manhattan plots of GWAS (2015) for total tannins (Tan) based on mixed linear model (MLM) with kinship (K), and kinship and population structure (Q+K). The vertical axis (y axis) represents  $-\log_{10} p$ -values and the horizontal axis (x axis) represent genomic position of each single nucleotide polymorphism (SNP) on chromosome 1-10. Each circle in plot represents a SNP. Green horizontal line in plot represents Bonferroni-adjusted significant threshold at 0.05 levels with  $p$ -value  $< 10^{-7}$ . (A) Manhattan plot for panicles inoculated and bagged for seven days after inoculation at 50% flowering (IB), (B) Manhattan plot for panicles non-inoculated but bagged for seven days at 50% flowering (NB), and (C) Manhattan plot for panicles non-inoculated and non bagged (NN).**

(A)



**(B)**





(C)

