Identification of Genetic Loci of Black Point in Chinese Common Wheat by Genome-Wide Association Study and Linkage Mapping

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Abstract

Black point is a common disease in wheat all over the world. The disease could downgrade wheat quality and cause human health problems. In this study, 406 wheat cultivars were used to investigate black point resistance. In the field tests, 20, 65.5, and 14.5% of the tested cultivars were resistant, moderately resistant, and susceptible, respectively, suggesting that improving black point resistance is necessary in Chinese wheat breeding. A genome-wide association study (GWAS) identified 386 single-nucleotide polymorphisms (SNPs) significantly related to black point resistance in the tested wheat cultivars, and they were located on all chromosomes. Linkage mapping in a biparental population identified

three quantitative trait loci (QTL) for black point resistance—*QBP.hau-3A*, *QBP.hau-6D*, and *QBP.hau-7D*—with 6.76, 7.79, and 8.84% phenotypic variation explained, respectively. Based on both the GWAS and linkage analyses, *QBP.hau-6D* covered six significant SNPs from the GWAS, and the position of these SNPs indicated that this QTL is a new locus for black point resistance. This study provides valuable germplasm for breeding wheat cultivars with resistance to black point and information for further understanding of molecular and genetic basis of black point resistance.

Keywords: common wheat, black point, GWAS, linkage mapping

Black point is one of the important common diseases in wheat all over the world (Sissons et al. 2010). The obvious symptom of black point is dark-brown to black discoloration of the embryo end of wheat kernels, and it can cover most of the surface of the kernels (Conner and Davidson 1988; Fernandez and Conner 2011). The disease can lead to grain quality and value losses (Rees et al. 1984). Black point could influence many traits of wheat such as falling number, dough stability, germination, seed emergence, plant vigor, and plant growth, and even causes seedling blight (Cromey and Mulholland 1988; Dexter and Matsuo 1982; Fernandez et al. 2014; Hudec 2007). Previous reports showed that black point was associated with fumonisin contamination and might cause esophageal cancer and neural-tube defects in some human populations (Busman et al. 2012; Desjardins et al. 2007; Marasas 2001; Palacios et al. 2015).

Some studies showed that black point was mainly caused by abiotic stresses and high humidity, and extreme temperatures during grain filling easily lead to wheat black point (Fernandez et al. 2014; Maloy and Specht 1988). However, the cause of black point is still unclear and controversial. In most studies, fungi are proved as the main causal agent of black point; for example, *Alternaria alternata* (Busman et al. 2012; Perelló et al. 2008; Somma et al. 2019; Southwell et al. 1980), *Fusarium proliferatum* (Busman et al. 2012; Desjardins et al. 2007), and *Bipolaris sorokiniana* (Kumar

et al. 2002). However, a single fungal species may not be the only cause of black point. For example, the presence of *A. alternata* in both susceptible and resistant wheat cultivars was observed (Hudec 2007; Williamson 1997), indicating no direct relationship between fungal infection and black point. The formation of black point may be caused by enzymatic browning after suffering from stress such as polyphenol oxidase (Fuerst et al. 2014), peroxidases (POD) (Fernandez et al. 2014; March et al. 2007), and lipoxygenase (Porta and Rocha-Sosa 2002). These enzymes catalyze oxidation of phenolic compounds to brown or black pigments (melanins and quinines) (Fuerst et al. 2014; Tomás-Barberán and Espín 2001). Black point may be triggered by high humidity, extremes of temperature, and pre-germination during the late stages of grain filling and ripening. Some cultivars showing susceptibility to black point possess relatively high POD and phenylalanine ammonia-lyase, an enzyme involved in phenolic acid biosynthesis activities (March et al. 2007).

There are some strategies to control black point such as cultural, biological, and chemical control but the most effective, economical, and environmentally sustainable way is to plant resistant cultivars (El-Gremi et al. 2017; Lehmensiek et al. 2004; Li et al. 2014; Liu et al. 2016). The development of black point mainly depends on environmental conditions, and severity is mostly influenced by wheat genotype (Fernandez and Conner 2011; Lehmensiek et al. 2004). The incidence of black point ranges from 0.3 to 66.7% (El-Gremi et al. 2017; Ellis et al. 1996; Li et al. 2014; Walker et al. 2008), and the heritability of black point is approximately 50% (Li et al. 2014).

Studies on the genetic basis of black point resistance have commonly involved traditional linkage-mapping methods using biparental populations (Lehmensiek et al. 2004; March et al. 2007). Lehmensiek et al. (2004) used low-density simple-sequence repeat (SSR) markers and identified black point-resistant quantitative trait loci (QTL) on chromosomes 1D, 2B, 3D, 4A, 5A, and 7A in the population Sunco/Tasman, and on 2A, 2D, and 7A in the population Cascades/AUS1408, with phenotypic variation explained (PVE) values of 4 to 15% and 12 to 18%, respectively. Liu et al. (2016) mapped 9 QTL on chromosomes 2AL, 2BL, 3AL, 3BL, 5AS, 6A, 7AL (2), and 7BS in a recombinant inbred line (RIL) population, and found 25 loci on chromosomes 2A, 2B, 3A, 3B (2), 3D, 4B (2), 5A (3),

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5B (3), 6A, 6B, 6D, 7A (5), 7B, and 7D (2) (Liu et al. 2017), with PVE values of 7.9 to 18.0%, using the genome-wide association study (GWAS) approach.

With the rapid development of sequencing techniques, high-density single-nucleotide polymorphism (SNP) markers have been widely used to detect new genes or QTL in common wheat through GWAS and linkage mapping for complex traits. Black point is becoming one of the important diseases in the Huanghuai Valley, the most important wheat region in China (with 60 to 70% of the total wheat production area). This study aims to screen wheat germplasm resistant to black point and identify novel resistance loci for marker-assisted selection as well as provide useful information for further understanding of the molecular and genetic basis of black point resistance in common wheat.

Materials and Methods

Plant material. Two wheat panels were used in this study. Panel I consisted of 163 common wheat cultivars from Henan, Hebei, Shaanxi, Shanxi, and Shandong Provinces, and they were selected based on their pedigree, released region, agronomic performance, importance in breeding, and cultivated area (Chen et al. 2019; Sun et al. 2017). Panel II consisted of 243 common wheat cultivars or advanced breeding lines released or developed after 2014, representing

Table 1. Representative wheat accessions with different levels of resistance to black point disease

Disease incidence (%)	Cultivars
0.1–4.9	Yanyou 361, Zhengzhou 8761, Linkang 5027, Xinong 6028, Yumai 8, Shannong 7859, Chinese spring, Xingyu 7, Zhongfengmai 2, Xuke 877, Xinyanmai 98, Xiaoyan 6, Xinong 979, Jinmai 49, Longmai 157, Neixiang 184, Aifeng 3, Zhongnong 28, Jinmai 47
5–14.9	Xinyin 13-2, Lumai 14, Beinongda 92, Jinmai 33, Yuanfeng 898, Wenmai 29, Xuke 158, Zhongmai 10, Lunxuan 167, Xiza 5, Shunmai 8, Yunong 805, Hemai 6, Hangmai 6, Jimai 26, Lumai 15, Huayan 328, Jiamai 329, Zhongle 8
15–29.9	Yanzhan 4110, Han 97-5297, Yumai 3, Pindong 34, Yumai 57, Xinmai 3306, Wenyu 019, Aomai 18, Luyuan 502, Tianle 6, Yumai 58, Xinmai 8, Yuanxie 62, Huabei 187, Caizhi 204, Zhengmai 9694, Zhoumai 23, Shunmai 299, Han 4589, Ningnong 718, Taixue 30
30	Yan 893, Xuan, Yimai 8

Fig. 1. Distribution of black point index in Chinese wheat cultivars in Panel I (A) and Panel II (B) in four environments.

current breeding situations in the Huanghuai Valley (Yang et al. 2019). In 2017, Panels I and II were planted at the Scientific Research and Education Center (SREC) of Henan Agricultural University, in Luohe, and in Zhengzhou, with two replications at each location. In 2018, panel I was planted in Zhengzhou and Yuanyang, with two replicates at each location, and panel II was planted in Xiping and Suiping.

An F_{10} recombinant inbred line (RIL) population with 187 lines derived from a cross between UC1110 and PI610750 (UP) was used to map QTL for black point resistance. The RIL population was

planted in the Zhengzhou SREC in 2017. All of the wheat accessions were performed using a completely randomized design. Each plot contained 12 2-m-long rows with 23 cm between neighboring rows and 10 cm between neighboring plants.

Phenotyping. After harvest and threshing, 100 grains of each accession were randomly selected from a well-mixed sample for phenotyping black point with three replications. Kernels with black point were calculated as the ratio of black point kernel number to the total kernel number. The mean value of black point in two replicates from

Fig. 2. Manhattan and Q-Q plots for black point identified by genome-wide association study (GWAS) in **A**, panel I and **B**, panel II; and **C**, distribution of significant single-nucleotide polymorphisms (SNPs) on different chromosomes.

Table 2. Multienvironmentally significant single-nucleotide polymorphisms (SNPs) identified by genome-wide association study in this study

Year, location, SNP	Chr ^a	Position (bp)	P value	R ² (%) ^b
2017_Luohe, 2017_Zhengzhou, 2018_Yuanyang				
<i>RAC875_rep_c71149_738</i>	2B	782,536,180	0.00052581	12.29
<i>Excalibur_c10055_221</i>	6A	28,017,180	6.87E-06	18.20
<i>GENE-4167_145</i>	6A	611,328,876	6.87E-06	18.20
<i>BS00099401_51</i>	6A	613,256,522	6.87E-06	18.20
2017_Luohe, 2018_Yuanyang				
<i>GENE-1337_298</i>	2A	107,383,862	9.11E-05	16.21
<i>Kukri_c17_1587</i>	2A	774,850,845	2.63E-05	17.80
<i>BS00097147_51</i>	2B	786,033,209	6.29E-05	16.68
<i>Tdurum_contig51605_1130</i>	3B	822,680,599	0.00085425	13.44
<i>Tdurum_contig51605_194</i>	3B	822,682,075	0.00085425	13.44
<i>BS00087695_51</i>	3B	822,826,547	0.00085425	13.44
<i>Tdurum_contig11114_335</i>	3B	822,828,808	0.00085425	13.44
<i>Tdurum_contig50364_1051</i>	6B	711,702,135	0.00058243	13.91
<i>GENE-4021_496</i>	6D	463,743,572	0.00016166	15.49
2018_Yuanyang, 2018_Zengzhou				
<i>Excalibur_rep_c77404_218</i>	1A	33,205,346	1.24E-05	18.78
<i>D_contig25392_201</i>	1D	31,425,108	1.24E-05	18.78
<i>RAC875_rep_c71149_798</i>	2B	782,536,120	1.24E-05	18.78
<i>Kukri_c3831_2136</i>	4A	634,841,828	1.24E-05	18.78
<i>Kukri_c22504_459</i>	4A	636,574,055	1.24E-05	18.78
<i>Kukri_c2757_1535</i>	4A	638,174,291	1.24E-05	18.78
<i>Excalibur_rep_c102533_116</i>	5B	677,154,676	1.24E-05	18.78
<i>w SNP_CAP11_c3570_1730776</i>	6A	61,409,322	1.24E-05	18.78
<i>CAP7_c9162_265</i>	6A	61,409,514	1.24E-05	18.78
<i>Excalibur_c35871_596</i>	6A	615,467,141	0.00028336	14.79
<i>Tdurum_contig25789_260</i>	6B	702,751,203	1.24E-05	18.78
<i>RAC875_c50572_106</i>	6B	720,474,930	3.24E-06	20.55
<i>RAC875_c57219_1058</i>	6B	720,507,929	1.24E-05	18.78
2017_Luohe, 2017_Zhengzhou				
<i>AX-109939573</i>	2A	733,030,778	8.36E-05	18.54

^a Chromosome.^b R² of model with SNP. **Fig. 3.** Comparison of black point between cultivars with superior and inferior alleles in four environments: **A**, *Tdurum_contig11114_335*; **B**, *BS00087695_51*; **C**, *BS00097147_51*; and **D**, Combination of *Tdurum_contig11114_335*, *BS00087695_51*, and *BS00097147_51*. Asterisks (**) indicate $P < 0.01$.

the same environment was used for further analysis. Best linear unbiased prediction (BLUP) values were calculated across environments using the 'lme4' package in R software, version 3.5.2. Function 'lmer' was used to fit a linear mixed-effects model. Afterward, function 'ranef' was used to get the random effect of BLUP. The final adjusted phenotype values were obtained by adding the random effect to averaged phenotype values. Based on a previous report (Kang et al. 1999), wheat with a black point percentage of 0.1 to 4.9% was regarded as resistant, 5 to 14.9% as moderately resistant,

15 to 29.9% as moderately susceptible, and greater than 30% as susceptible.

Genotyping and GWAS. Panels I and II were genotyped using the wheat 90K and 660K SNP arrays, respectively, as previously described (Chen et al. 2019; Sun et al. 2017; Yang et al. 2019). The quality pre-treatment of genotyping data was carried out for SNP call rate and minor allele frequency with the PLINK software using the thresholds of maf 0.02 and geno 0.1 (<http://zzz.bwh.harvard.edu/plink/tutorial.shtml>) (Purcell et al. 2007). Population structure was assessed using

Fig. 4. Haplotype analysis of significant single-nucleotide polymorphism (SNPs) on chromosome 2B, 5B, and 7A. **A**, Linkage disequilibrium heatmaps. **B**, Phenotypic comparison of cultivars with different haplotypes. **C**, Phenotypic comparison of pyramiding haplotypes. Asterisks (**) indicate $P < 0.01$.

STRUCTURE software 2.3.4 with unlinked markers ($r^2 = 0$) (Pritchard et al. 2000). The number of subpopulations (k) was implemented by a burn-in of 1,000 iterations followed by 1,000 Monte Carlo Markov Chain replicates in a putative range of 1 to 10. Linkage disequilibrium (LD) among markers was calculated for the A, B, and D genomes using the PLINK software. The window size for LD calculation was set

based on the number of SNPs located in each genome. Pairwise LD was measured using the squared allele frequency correlations, according to Weir (Williams 1996), and assessed by calculating r^2 for pairs of SNP loci. GWAS was implemented using the mixed linear model (PCA+K) using the GAPIT package in R software (Zhang et al. 2010). The threshold for P value was calculated using a modified

Fig. 5. Genome-wide composite interval mapping results of the UC1110 × PI610750 population. **A**, The quantitative trait loci (QTL) map and **B**, detailed information about three QTL. Chr = chromosome and cM = centimorgans.

Table 3. Comparison of significant single-nucleotide polymorphism (SNPs) in this and previous studies

Panel I				Panel II		
Chr ^a	Significant SNP numbers	Physical interval (bp)	R^2 (%) ^b	Significant SNP numbers	Physical interval (bp)	R^2 (%) ^b
1A	3	9,551,329–593,287,088	13.56–18.79	0	...	...
1B	5	10,367,413–680,997,890	10.66–13.84	18	600,095,813–673,248,437	10.8–20.33
1D	3	31,425,108–431,430,680	10.99–22.77	6	483,459,166–483,675,345	18.59–22.49
2A	8	107,383,862–774,850,845	10.87–27.33	4	731,247,133–733,091,499	10.26–19.61
2B	5	175,636,668–786,033,209	10.84–24.14	19	41,846,583–202,914,992	9.38–24.93
2D	6	117,112,325–618,153,084	14.49–23.07	3	29,842,702–636,069,885	10.71–12.58
3A	3	1,308,979–737,968,539	12.43–14.24	30	467,340,411–745,017,586	10.37–14.91
3B	18	18,822,229–822,828,808	10.41–20.63	4	769,228,573–827,300,622	10.03–21.83
3D	7	426,925,219–613,735,410	13.61–20.03	2	362,439,776–363,082,378	10.85–11.68
4A	10	11,811,173–712,863,183	13.27–22.77	44	13,659,934–640,211,103	12.05–21.90
4B	7	139,766,572–660,589,226	10.34–15.71	12	385,557,109–637,384,048	9.28–20.95
4D	2	329,008,125–403,809,743	12.28–14.00	1	450,325,005	12.34
5A	12	8,246,363–698,636,704	13.80–21.16	0	...	...
5B	7	223,985,697–697,377,736	11.60–18.79	55	42,393,883–687,764,237	9.29–18.63
5D	3	534,094,327–560,228,200	11.49–14.68	20	84,009,653–238,559,540	11.94–21.65
6A	15	5,872,955–615,467,141	10.55–25.63	8	9,376,588–610,930,261	10.17–18.78
6B	10	471,169,018–720,507,929	10.45–21.30	3	676,002,375–694,167,647	10.84–19.45
6D	4	2,101,148–463,743,572	11.76–22.78	4	3,838,603–456,500,399	10.03–13.03
7A	2	25,750,875–238,588,195	20.31–12.28	12	21,315,084–25,592,284	9.28–11.06
7B	2	392,031,609–624,784,151	12.36–17.45	1	733,155,385	12.08
7D	2	27,6935,787–394,068,656	16.11–18.53	3	22,427,719–636,561,521	9.88–18.75

(Continued on next page)

^a Chromosome.

^b R^2 of model with SNP.

^c QTL = quantitative trait loci.

Bonferroni correction (Genetic Type 1 Error Calculator, version 0.2). Haplotype analysis was performed using software Haploview 4.2.

QTL analysis and haplotype analysis. The genetic linkage map of the UP population previously constructed by Lowe et al. (2011) was composed of 1,494 polymorphic probes (SSRs, diversity arrays technologies, and expressed sequence tags), and 558 unique loci were used to detect QTL related to black point resistance through the genome-wide composite interval mapping (GCIM) method (Wang et al. 2016; Wen et al. 2018) using the software QTL.gCIMapping.GUI 1. Compared with the CIM and empirical Bayes methods, the GCIM method had a higher power in QTL detection, greater accuracy in QTL effect estimation, and stronger robustness under various backgrounds. The threshold of the logarithm of odds scores was set at 2.5.

Results

Resistance investigation of black point in Chinese common wheat. For the BLUP value across the different environments, the assessment of black point disease in Chinese common wheat showed a mean black point of 8.18 and 10.31% in panel I and panel II, respectively. The phenotyping results showed a wide range of black point incidences of 0 to 61% in panel I and 0 to 68% in panel II across the four environments (Fig. 1). Only four wheat accessions (Linkang 5027, Xinong 6028, Yanyou 361, and Zhengzhou 8761) had mean black point incidences of less than 1.5% in the two panels, indicating their big potential for use in wheat black point resistance breeding. Further analysis showed that there were 81, 266, 57, and 2 accessions having mean black point incidences of 0.1 to 4.99, 5 to 14.9, 15 to 29.9, and >30%, respectively. The data suggested that 14.5% of the tested Chinese wheat cultivars belong to the moderately susceptible or susceptible categories, and they should receive more attention for widely planting in black-point-endemic regions. Representative cultivars with different levels of black point resistance were shown in Table 1.

GWAS. After filtering, 41,561 SNPs for the wheat 90K SNP array and 395,783 SNPs for the 660K SNP array were retained for GWAS analysis. In total, 136 and 250 SNPs were significant in panels I and II, respectively, across the four environments (Fig. 2), and they were mainly

on chromosomes 3A, 4A, and 5B, with PVE of 9.29 to 24.93% (Supplementary Table S1). Of all significant SNPs, 27 were significant in multiple environments, and they were mainly on 2A, 2B, 3B, 4A, 6A, and 6B (Table 2). A *t*-test analysis indicated that three SNPs (*BS00097147_51*, *BS00087695_51*, and *Tdurum_contig11114_335*) were significant in four environments ($P < 0.01$) and their mean PVE values were 10.84, 13.45, and 20.63%, respectively. Cultivars with alleles TT at *BS00097147_51*, GG at *BS00087695_51*, and AA at *Tdurum_contig11114_335* showed significantly higher black point resistance and were regarded as superior alleles (Fig. 3). Pyramiding analysis of these three loci showed that cultivars with three superior and inferior alleles showed the averaged black point of 8.13 and 42.33%, respectively.

Haplotype analysis (Fig. 4) indicated that the representative significant SNPs on 2B were clustered into the block *BP_hap_2B1*, and the two haplotypes at this locus showed significant difference on black point (9.99% for *BP_hap_2B1a* and 16.72% for *BP_hap_2B1b*). The significant SNPs on 5B formed the block *BP_hap_5B1*, and cultivars with *BP_hap_5B1a* showed a significantly lower black point incidence (10.16%) than cultivars with *BP_hap_5B1b* (12.97%). The significant SNPs on 7A formed the block *BP_hap_7A1*, and cultivars with *BP_hap_7A1a* showed a significantly lower black point (9.89%) than cultivars with *BP_hap_7A1b* (14.31%). Blast of the significant SNPs in the genome database of Chinese Spring showed that these SNPs ranged from 42,179,859 to 42,187,832 bp on 2B, 685,349,950 to 685,392,702 bp on 5B, and 24,296,895 to 24,375,739 bp on 7A. Pyramiding analysis indicated that cultivars with *BP_hap_2B1a*, *BP_hap_5B1a*, and *BP_hap_7A1a* showed a mean black point incidence of 9.67%. There were no cultivars with the combination of *BP_hap_2B1b*, *BP_hap_5B1b*, and *BP_hap_7A1b*. Cultivars with *BP_hap_2B1a* and *BP_hap_5B1a* showed significantly lower black point (10%) than cultivars with *BP_hap_2B1b* and *BP_hap_5B1b* (18%), and those with *BP_hap_2B1a* and *BP_hap_7A1a* showed significantly lower black point (9.7%) than those with *BP_hap_2B1b* and *BP_hap_7A1b* (17.2%) (Fig. 4C).

Biparental QTL mapping for black point. Three QTL for black point resistance were detected in the UP population (Fig. 5), and

Table 3. (Continued from previous page)

QTL interval ^c	QTL, gene, markers	Intervals in Liu et al. (2017)
...	...	...
...	...	...
...	...	...
...	<i>IWB39123</i> (Liu et al. 2016)	709,831,643–709,831,743; 712,188,721–712,187,200
...	<i>abg356</i> , <i>wmc154</i> , <i>wmc149</i> (Lehmensiek et al. 2004); <i>IWB516</i> (Liu et al. 2016)	714,389,068–714,388,998
...	<i>gwm484</i> (Lehmensiek et al. 2004)	...
60,200,972±	<i>IWA94</i> (Liu et al. 2016)	9,605,904–9,605,974
...	<i>IWB633</i> (Liu et al. 2016)	58,767,930–58,768,000; 695,967,481–695,967,411
...	<i>wmc375</i> (Lehmensiek et al. 2004)	4,066,092–4,066,162
...	<i>germin</i> (Lehmensiek et al. 2004)	6,961,084–6,961,154; 504,944,902–504,944,832
...	...	...
...	...	...
...	<i>IWB13628</i> (Liu et al. 2016)	32,887,598–32,887,698; 535,780,381–535,780,311; 592,276,555–592,276,708
...	...	302,177,272–302,177,428; 531,539,253–531,539,323; 556,183,885–556,183,955
...	...	...
...	<i>IWB11102</i> (Liu et al. 2016)	94,211,460–94,211,390
...	...	676,210,414–676,210,344
5,806,474–47,062,712	...	217,194,463–217,194,533
...	<i>gwm60</i> (Lehmensiek et al. 2004); <i>IWB53563</i> , <i>IWA6562</i> (Liu et al. 2016)	88,862,791–88,862,721; 136,398,412–136,398,482; 609,508,901–609,508,971; 670,876,731–670,876,661; 708,211,110–708,211,040
...	<i>IWA7589</i> (Lehmensiek et al. 2004)	729,224,017–729,224,087
41,402,847–53,640,852	...	129,917,622–129,917,692; 615,826,844–615,826,914

they were named *QBP.hau-3A* (approximately *wms2* to *rPt-9057*), *QBP.hau-6D* (approximately *wPt-6661* to *wPt-1695*), and *QBP.hau-7D* (approximately *wPt-1100* to *wms295*), with PVE of 6.76, 7.79, and 8.84%, respectively. *QBP.hau-3A* and *QBP.hau-7D* were derived from UC1110 and *QBP.hau-6D* from PI610750. Further analysis of their physical positions in the Chinese Spring genome database showed that *QBP.hau-3A* was located at position 60,200,972 bp, *QBP.hau-6D* in a range between 5,806,474 and 470,627,654 bp, and *QBP.hau-7D* between 41,403,730 and 53,641,214 bp.

A combination of GWAS and QTL results showed that six significant SNPs (*AX-109392024*, *GENE-3854_620*, *AX-94669779*, *AX-94973555*, *Jagger_c5046_63*, and *GENE-4021_496*) revealed by GWAS were within the interval of *QBP.hau-6D*, suggesting that *QBP.hau-6D* is an important novel QTL for resistance to black point because it was never reported before.

Discussion

Black point is a serious threat for wheat quality and production (Kahl et al. 2015). There was no absolute resistance in wheat cultivars and most cultivars would be easily affected by the disease under high moisture and other abiotic stresses (Mak et al. 2006; Sissons et al. 2010). Therefore, there is an urgent need to select resistant cultivars and find key genes for breeding new cultivars to control black point. Previously, Liu et al. (2017) used 166 accessions to evaluate for black point resistance and found that 86.1% (143) were susceptible (black point score >10%). Li et al. (2014) and El-Gremi et al. (2017) reported that the incidence of black point in wheat ranged from 0.3 to 66.7%. In the present study, all wheat accessions tested showed black point incidence of 0 to 68% in multiple environments. There were some cultivars showing high resistance across four environments, such as Yumai8, Yanyou 361, Xiaoyan6, Zhengzhou 8761, and Xinong 6028, and they could be widely used in wheat black point resistance breeding. However, some cultivars (i.e., Yan 893, Xuan, and Yimai 8) showed high susceptibility to black point in all environment and should be avoided for planting in black-point-endemic areas. The present study illustrated the distribution of black point reactions in current popular wheat cultivars of the Huanghuai Valley, and could provide valuable information for utilization of wheat cultivars in this region and also provide useful resistant germplasm for wheat breeding programs concerning black point.

In the past few decades, most of the previous studies on wheat black point mainly focused on pathogen identification, biological characteristic, disease cycle, and management (Busman et al. 2012; Kahl et al. 2015; Somma et al. 2019; Williamson 1997), though some studies identified genetic loci related to black point resistance (Lehmensiek et al. 2004; Liu et al. 2016, 2017) (Table 3). Lehmensiek et al. (2004) detected six QTL related to black point resistance with PVE of 4 to 15% in the population of Sunco/Tasman, and three of them on 2A, 2D, and 7A explained 12 to 18% of phenotypic variance. Liu et al. (2016) found nine QTL for black point resistance in an RIL population of Linmai2/Zhong 892 on chromosomes 2AL, 2BL, 3BL, 5AS, 6A, and 7AL. In the present study, we identified some important QTL for resistance to modulate wheat black point, and their physical positions overlapped with or covered previous QTL. However, three QTL (*QBP.hau-3A*, *QBP.hau-6D*, and *QBP.hau-7D*) that we identified in the RIL population have never been reported in previous studies, suggesting that they are possibly novel genetic loci for black point. Liu et al. (2017) found that the resistant SNPs were mainly located on 2A, 2B, 3A, 3B (2), 3D, 4B (2), 5A (3), 5B (3), 6A, 6B, 6D, 7A (5), 7B, and 7D (2) by GWAS, with PVE of 7.9 to 18.0%. Compared with this study, we found that some overlapped intervals were on 2A, 2B, 3A, 3B, 4A, 5A, 5B, 6A, 6B, 6D, and 7D, indicating that these intervals possibly have important genetic loci related to black point.

In the present study, significant SNPs related to black point were mainly distributed on 2B, 5B, and 7A. Haplotype analysis indicated that the cultivars with superior haplotype *BP_hap_2B1a*, *BP_hap_5B1a*, or *BP_hap_7A1a* showed significantly higher resistance to black point than cultivars with inferior haplotype *BP_hap_2B1b*, *BP_hap_5B1b*, or *BP_hap_7A1b*. Pyramiding analysis indicated that

192 cultivars such as Xinyanmai 98, Shenzhou 209, Kaimai 27, and Jinmai 109 with the superior haplotypic combination *BP_hap_2B1a*, *BP_hap_5B1a*, and *BP_hap_7A1a* showed a mean black point incidence of 9.67%, and they could be considered as germplasms resistant to black point for use in wheat breeding programs.

Previous studies showed that QTL resistant to black point were mainly mapped to 2A, 2D, 7A 2AL, 2BL, 3BL, 5AS, 6A, and 7AL in different RIL populations (Lehmensiek et al. 2004; Liu et al. 2016). In the present study, *QBP.hau-3A*, *QBP.hau-6D*, and *QBP.hau-7D* were possibly novel genetic loci to control black point resistance because they were not previously reported in biparental populations, though a number of significant SNPs were within the intervals of these three QTL in some GWAS results using germplasm panels (Lehmensiek et al. 2004; Liu et al. 2016, 2017). We also found six significant SNPs within the *QBP.hau-6D* region in this study. Therefore, *QBP.hau-6D* should be used as a superior QTL for marker-assisted selection to improve wheat black point resistance. In addition, pyramiding several QTL conferring black point resistance is possibly more effective to improve black point resistance in wheat breeding programs.

Literature Cited

- Busman, M., Desjardins, A. E., and Proctor, R. H. 2012. Analysis of fumonisin contamination and the presence of *Fusarium* in wheat with kernel black point disease in the United States. *Food Addit. Contam.* 29:1092-1100.
- Chen, J., Zhang, F., Zhao, C., Lv, G., Sun, C., Pan, Y., Guo, X., and Chen, F. 2019. Genome-wide association study of six quality traits reveals the association of the *TaRPP13L1* gene with flour colour in Chinese bread wheat. *Plant Biotechnol. J.* 17:2106-2122.
- Conner, R. L., and Davidson, J. G. N. 1988. Resistance in wheat to black point caused by *Alternaria alternata* and *Cochliobolus sativus*. *Can. J. Plant Pathol.* 68:351-359.
- Cromey, M. G., and Mulholland, R. I. 1988. Black point of wheat: Fungal associations, cultivar susceptibility, and effects on grain weight and germination. *N. Z. J. Agric. Res.* 31:51-56.
- Desjardins, A. E., Busman, M., Proctor, R. H., and Stessman, R. 2007. Wheat kernel black point and fumonisin contamination by *Fusarium proliferatum*. *Food Addit. Contam.* 24:1131-1137.
- Dexter, J. E., and Matsuo, R. R. 1982. Effect of smudge and black point, mildewed kernels, and ergot on durum wheat quality. *Cereal Chem.* 59:63-69.
- El-Gremi, S. M., Draz, I. S., and Youssef, W. A. 2017. Biological control of pathogens associated with kernel black point disease of wheat. *Crop Prot.* 91: 13-19.
- Ellis, S. A., Gooding, M. J., and Thompson, A. J. 1996. Factors influencing the relative susceptibility of wheat cultivars (*Triticum aestivum* L.) to black point. *Crop Prot.* 15:69-76.
- Fernandez, M. R., and Conner, R. L. 2011. Black point and smudge in wheat. *Prairie Soils Crops* 4:158-164.
- Fernandez, M. R., Wang, H., and Singh, A. K. 2014. Impact of seed discoloration on emergence and early plant growth of durum wheat at different soil gravimetric water contents. *Can. J. Plant Pathol.* 36:509-518.
- Fuerst, E. P., Okubara, P. A., Anderson, J. V., and Morris, C. F. 2014. Polyphenol oxidase as a biochemical seed defense mechanism. *Front. Plant Sci.* 5:689.
- Hudec, K. 2007. Pathogenicity of fungi associated with wheat and barley seedling emergence and fungicide efficacy of seed treatment. *Biologia* 62:287-291.
- Kahl, S. M., Ulrich, A., Kirichenko, A. A., and Müller, M. E. H. 2015. Phenotypic and phylogenetic segregation of *Alternaria infectoria* from small-spored *Alternaria* species isolated from wheat in Germany and Russia. *J. Appl. Microbiol.* 119:1637-1650.
- Kang, Y., Zhang, Y., Li, H., and Guo, Y. 1999. Research status of wheat black point disease in China. *Acta Tritical Crops* 19:58-60.
- Kumar, J., Schäfer, P., Hükelhoven, R., Langen, G., Baltruschat, H., Stein, E., Nagarajan, S., and Kogel, K. H. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: Cytological and molecular approaches towards better control. *Mol. Plant Pathol.* 3:185-195.
- Lehmensiek, A., Campbell, A. W., Sutherland, M. W., Williamson, P. M., and Daggard, G. E. 2004. QTLs for black-point resistance in wheat and the identification of potential markers for use in breeding programs. *Plant Breed.* 123:410-416.
- Li, Q., Qin, Z., Jiang, Y., Shen, C., Duan, Z., and Niu, J. S. 2014. Screening wheat genotypes for resistance to black point and the effects of diseased kernels on seed germination. *J. Plant Dis. Prot.* 121:79-88.
- Liu, J., He, Z., Rasheed, A., Wen, W., Yan, J., Zhang, P., Wan, Y., Zhang, Y., Xie, C., and Xia, X. 2017. Genome-wide association mapping of black point reaction in common wheat (*Triticum aestivum* L.). *BMC Plant Biol.* 17:220.
- Liu, J., He, Z., Wu, L., Bai, B., Wen, W., Xie, C., and Xia, X. 2016. Genome-wide linkage mapping of QTL for black point reaction in bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 129:2179-2190.

- Lowe, I., Jankuloski, L., Chao, S., Chen, X., See, D., and Dubcovsky, J. 2011. Mapping and validation of QTL which confer partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. *Theor. Appl. Genet.* 123:143-157.
- Mak, Y., Willows, R. D., Roberts, T. H., Wrigley, C. W., Sharp, P. J., and Copeland, L. 2006. Black point is associated with reduced levels of stress, disease- and defence-related proteins in wheat grain. *Mol. Plant Pathol.* 7: 177-189.
- Maloy, O. C., and Specht, K. L. 1988. Black point of irrigated wheat in Central Washington. *Plant Dis.* 72:1031-1033.
- Marasas, W. F. 2001. Discovery and occurrence of the fumonisins: A historical perspective. *Environ. Health Perspect.* 109:239-243.
- March, T. J., Able, J. A., Schultz, C. J., and Able, A. J. 2007. A novel late embryogenesis abundant protein and peroxidase associated with black point in barley grains. *Proteomics* 7:3800-3808.
- Palacios, S. A., Susca, A., Haidukowski, M., Stea, G., Cendoya, E., Ramírez, M. L., Chulze, S. N., Farnochi, M. C., Moretti, A., and Torres, A. M. 2015. Genetic variability and fumonisin production by *Fusarium proliferatum* isolated from durum wheat grains in Argentina. *Int. J. Food Microbiol.* 201: 35-41.
- Perelló, A., Moreno, M., and Sisterna, M. 2008. *Alternaria infectoria* species-group associated with black point of wheat in Argentina. *Plant Pathol.* 57:379.
- Porta, H., and Rocha-Sosa, M. 2002. Plant lipoxygenases, physiological and molecular features. *Plant Physiol.* 130:15-21.
- Pritchard, J. K., Stephens, M. J., and Donnelly, P. J. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., Bakker, W. I. P., Daly, M. J., and Sham, P. C. 2007. Plink: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81:559-575.
- Rees, R. G., Martin, D. J., and Law, D. P. 1984. Black point in bread wheat. Effects on quality and germination and fungal associations. *Aust. J. Exp. Agric.* 24: 601-605.
- Sissons, M., Sissons, S., and Egan, N. 2010. The black point status of selected tetraploid species and Australian durum wheat and breeding lines. *Crop Sci.* 50:1279-1286.
- Somma, S., Amatulli, M. T., Masiello, M., Moretti, A., and Logrieco, A. F. 2019. *Alternaria* species associated to wheat black point identified through a multilocus sequence approach. *Int. J. Food Microbiol.* 293:34-43.
- Southwell, R. J., Brown, J. F., and Wong, P. T. W. 1980. Effect of inoculum density, stage of plant growth and dew period on the incidence of black point caused by *Alternaria alternata* in durum wheat. *Ann. Appl. Biol.* 96:29-35.
- Sun, C., Zhang, F., Yan, X., Zhang, X., Dong, Z., Cui, D., and Chen, F. 2017. Genome-wide association study for 13 agronomic traits reveals distribution of superior alleles in bread wheat from the Yellow and Huai Valley of China. *Plant Biotechnol. J.* 15:953-969.
- Tomás-Barberán, F. A., and Espín, J. C. 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric.* 81:853-876.
- Walker, K. R., Able, J. A., Mather, D. E., and Able, A. J. 2008. Black point formation in barley: Environmental influences and quantitative trait loci. *Aust. J. Agric. Res.* 59:1021-1029.
- Wang, S., Wen, Y., Ren, W., Ni, Y., Zhang, J., Feng, J., and Zhang, Y. 2016. Mapping small-effect and linked quantitative trait loci for complex traits in backcross or DH populations via a multi-locus GWAS methodology. *Sci. Rep.* 6:29951.
- Wen, Y., Zhang, Y., Zhang, J., Feng, J., Jim, M. D., and Zhang, Y. 2018. An efficient multi-locus mixed model framework for the detection of small and linked QTLs in F₂. *Brief. Bioinf.* 20:1-12.
- Williams, J. T. 1996. Genetic data analysis II: Methods for discrete population genetic data by Bruce S. Weir. *Hum. Biol.* 4:583-586.
- Williamson, P. M. 1997. Black point of wheat: In vitro production of symptoms, enzymes involved, and association with *Alternaria alternata*. *Aust. J. Agric. Res.* 48:13-19.
- Yang, X., Pan, Y., Singh, P. K., He, X., Ren, Y., Zhao, L., Zhang, N., Cheng, S., and Chen, F. 2019. Investigation and genome-wide association study for *Fusarium* crown rot resistance in Chinese common wheat. *BMC Plant Biol.* 19:153.
- Zhang, Z., Ersoz, E., Lai, C.-Q., Todhunter, R. J., Tiwari, H. K., Gore, M. A., Bradbury, P. J., Yu, J., Arnett, D. K., Ordoñez, J. M., and Buckler, E. S. 2010. Mixed linear model approach adapted for genome-wide association studies. *Nat. Genet.* 42:355-360.