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Anther Extrusion and Its Association with Fusarium Head Blight in CIMMYT Wheat Germplasm

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Abstract: Pronounced anther extrusion (AE) is associated with field resistance to Fusarium head blight (FHB), one of the most devastating diseases of wheat globally. In this study, two recombinant inbred line (RIL) populations were used to map quantitative trait loci (QTL) for AE and field FHB resistance and to investigate the association of both traits at the genetic level. Furthermore, two panels of International Maize and Wheat Improvement Center (CIMMYT) wheat breeding lines were evaluated to describe the phenotypic association between the two traits in detail. Highly significant negative correlation was identified between AE and FHB severity in the two populations and the two panels, with *r*-values ranging from 0.55 to 0.74. QTL analysis in the two RIL populations identified 12 QTL for AE and nine for FHB resistance, of which five QTL located on chromosomes 3BL, 4BS, 4DS, 5AL, and 5BL were associated with both AE and FHB, collectively explaining over 50% of phenotypic variation for FHB. The QTL on chromosomes 4BS, 4DS, 5AL, and 5BL were closely linked to *Rht-B1*, *Rht-D1*, *Vrn-A1*, and *Vrn-B1* genes, respectively. In conclusion, AE is closely related to field FHB resistance and could be used as a morphological marker in wheat breeding for field FHB resistance.

Keywords: wheat; Fusarium head blight; anther extrusion; plant height; days to heading

1. Introduction

Bread wheat (*Triticum aestivum* L.) is a staple food crop and one of the most widely grown around the world [1]. To meet future food demands, wheat yield and quality must be enhanced. Fusarium head blight (FHB) is one of the most devastating diseases of wheat globally, which leads to yield losses, quality degradation, and mycotoxin contamination, greatly threatening food and feed safety [2].

Host resistance to FHB is quantitatively inherited and influenced significantly by the environment, making breeding for this trait a difficult task. Multiple mechanisms of host resistance to FHB have been recognized, including Type I for initial infection, Type II for spread of pathogen in spike tissues, Type III for deoxynivalenol (DON) accumulation, Type IV for kernel infection, and Type V for yield reduction [3]. For the wheat plant, Type I resistance exhibits frequent association with phenological, morphological, and flower biology traits, such as plant height (PH), days to heading (DH), and anther extrusion (AE) [4–6].

Similar to FHB, AE is characterized as a quantitative trait controlled by many genes and is currently widely studied to promote the cross-pollination ability in hybrid wheat [7,8]. It is also suggested as one of the resistance mechanisms to FHB [4,5,9]. Selection of wheat lines with high AE could be a good strategy for breeders to adopt when breeding against FHB [10]. AE usually shows a negative correlation with FHB severity in the field, where partially extruded anthers are associated with increased FHB

infection and fully extruded anthers are associated with reduced FHB infection [5,9,11]. Anthers are a nutrition-rich organ and have high concentrations of growth stimulants such as betaine and choline, facilitating the initial infection by the FHB pathogen [12–14]. If the anthers are only partially extruded, or stuck between palea and lemma, they serve as a bridge to facilitate the infection of FHB into the floret cavity; however, if the anthers are fully extruded, it becomes more difficult for the FHB pathogen colonized on the anthers to infect other floret tissue [4].

In the last decade, quantitative trait loci (QTL) for AE were detected on numerous wheat chromosomes, including 1A, 1B, 1D, 2A, 2B, 2D, 3B, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, and 7A [1,4,8,9,15–17]. However, most of these QTL were of minor effects except for two on 4BS and 4DS linked to *Rht-B1* and *Rht-D1*, respectively, and one on 5AL [9,15,17]. In addition to genetic factors, environmental factors such as temperature and humidity also contribute to the degree of AE in wheat [18].

PH is another trait closely related to field FHB resistance; it usually shows a negative correlation with FHB severity in barley, oat, and wheat [9,19–21]. In the last two decades, researches provided molecular evidence for this relationship, and many QTL responsible for both FHB and PH were identified, including *Rht-B1*, *Rht-D1*, and *Rht8* [6]. Three possible mechanisms, i.e., disease escape, pleiotropy of reduced height (*Rht*) genes, and tight linkage, were proposed for the association [16]. DH is another trait that frequently shows association with field FHB resistance, and, depending on weather conditions, the correlation could be both positive and negative [22].

The aim of this study was to characterize the genetics of AE and FHB resistance, as well as their potential association with PH and DH, through phenotypic and genotypic analysis. Such an association was reported in several of our previous publications, in which QTL responsible for multiple of the abovementioned traits were mapped [9,23]. The current study presents results from two recombinant inbred line (RIL) populations, "NASMA" × "IAS20*5/H567.71" and "NASMA" × "RPB709.71/COC", and two panels of CIMMYT breeding lines, to further investigate the interrelationships among AE, FHB, PH, and DH.

2. Materials and Methods

2.1. Plant Material

Two RIL populations were used in this study for QTL mapping. The first one was developed from a cross between "NASMA" × "IAS20*5/H567.71" with 197 progenies (referred to as the NIH population hereafter), while the second was from "NASMA" × "RPB709.71/COC" with 185 progenies (the NRC population). Both male parents are CIMMYT breeding lines with high AE and FHB resistance, while the female parent "NASMA" is a Moroccan line of low AE and susceptible to FHB. Both resistant parents carried *Rht-B1b* and *Rht-D1a*, whereas the susceptible parent had the *Rht-B1a/Rht-D1b* genotype, resulting in both dwarfing genes segregating in the two populations. It should be noted that QTL mapping for FHB, Fusarium damaged kernels (FDK), and DON in the NIH population was published [23]; however, AE and its association with FHB in this population was not reported. Furthermore, two germplasm panels, the 16th Fusarium head blight screening nursery (FHBSN) and a Panel of Parents (POP), both consisting of 36 lines mostly of CIMMYT origin, were included to verify the relationship between AE and FHB resistance (Table S1).

2.2. Field Trials and Phenotyping

The FHB field experiments were conducted at the CIMMYT El Batán experimental station (altitude of 2240 m above sea level, coordinate 19.5° north (N), 98.8° west (W), with an average annual precipitation of 625 mm), in Mexico during the summer season (May to September) when rainfall is concentrated [9]. The two RIL populations were evaluated from 2013 to 2014, sown in 1-m double rows with randomized complete block design with two replications. Each year, a mixture of five aggressive *Fusarium graminearum* isolates were collected, characterized, and used for field inoculation, following the protocols described by He et al. [24]. Spray inoculation was targeted to each line's anthesis stage

with an inoculum of 50,000 spores/mL and was repeated two days after the first spray. From anthesis to early dough stages, the nursery was misted from 9:00 a.m. to 8:00 p.m. with 10 min of spraying each hour, to create a humid environment favorable for FHB development. A wheat/maize rotation and conservation agricultural practices were followed in the nursery to enhance natural inoculum.

FHB symptoms were evaluated at 25 days post inoculation (dpi) on the 10 spikes that were tagged at anthesis. Numbers of infected spikes and symptomatic spikelets of each spike were counted for calculating FHB index with the formula FHB index = severity × incidence. Severity was measured as the averaged percentage of diseased spikelets, and incidence as the percentage of symptomatic spikes. In 2013 and 2014, AE, DH, and PH were scored for the two RIL populations. AE was rated with a linear scale from zero (no extrusion) to nine (full extrusion) according to Skinnes et al. [4] with minor modification. Briefly, AE evaluation was performed at approximately seven days after anthesis on a whole-plot basis. Additionally, five randomly selected spikes were manually checked to see if the proportion of AE corresponded to the plot-based score, to prevent possible mistakes when anthers of high AE materials were blown away by wind or washed away by rain. PH was measured before harvest from ground to the average spike tips excluding awns in each plot. DH was scored in all the experiments. Additionally, AE and FHB severity were also evaluated for FHBSN and POP populations using the above protocols in the same screening nursery in 2014.

2.3. Statistical Analyses

The phenotypic data were analyzed using SAS ver. 9.2 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was carried out with the PROC GLM module, and Pearson correlation coefficients were calculated using the PROC CORR function. The results of ANOVA were used for calculating the heritability estimates, using the formula $h^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_e^2/r}$ for single years and $h^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_{g*y}^2/y+\sigma_e^2/ry}$ for multiple years, where σ_g^2 stands for genetic variance, σ_{g*y}^2 stands for genotype-by-year interaction, σ_e^2 stands for error variance, y stands for the number of years, and r stands for the number of replications [5]. The phenotypic data averaged from two replications of each experiment were used for QTL analyses.

2.4. Genotyping

Genomic DNA of the two mapping populations and their parents were extracted from young leaves using the CTAB method, and were genotyped with the Illumina iSelect 15K Beadchip provided by TraitGenetics GmbH, Germany. Additionally, alleles for the plant height and vernalization genes *Rht-B1*, *Rht-D1*, and *Vrn-A1* were also determined, using Kompetitive allele specific PCR (KASP) assays (LGC Genomics applications note, http://www.kbioscience.co.uk/reagents/KASP_Taqmancomparison.pdf) described in Dreisigacker et al. [25]. Additionally, some simple sequence repeat (SSR) markers were previously mapped in these two populations for studying Septoria tritici blotch resistance [26]. Markers with missing data points greater than 20% and segregation ratio beyond the range 0.5–2.0 were discarded from further analysis.

2.5. Linkage and QTL Mapping

Linkage groups (LGs) were constructed with the program JoinMap v.4.0 (Kyazma B.V., Wageningen, Netherlands), using LOD scores from 5–15 for grouping, and the maximum likelihood algorithm for calculating the order and position of markers within each LG. LGs were then assigned to chromosomes based on the Illumina 90K SNP map in Wang et al. [27]. QTL mapping was carried out with MapQTL v6.0 [28], in which interval mapping (IM) was firstly performed to detect potential QTL with LOD higher than 2.0 for each trait, followed by multiple QTL mapping (MQM) for each QTL, using the closest linked markers to each QTL detected in IM as cofactors. A significant QTL in MQM was defined in this study as one with an LOD score higher than 3.0; minor QTL with LOD values higher than 2.0 were also reported if they were significant in at least one environment. LGs and LOD curves were drawn by the software MapChart ver. 2.3 (Wageningen, Netherlands). Physical positions of markers in IWGSC RefSeq v1.0

were obtained from either T3/Wheat (https://triticeaetoolbox.org/wheat/viroblast/viroblast.php) or URGI (https://urgi.versailles.inra.fr/blast_iwgsc/blast.php) via BLASTN searches.

3. Results

3.1. Phenotypic Analysis

In both years, AE in the NIH and NRC populations showed continuous and broad variation with a range of mean value across replications and years from 1.0 to 8.3 in the NIH and 0.5 to 8.3 in the NRC population, in agreement with the quantitative inheritance of the trait. Transgressive segregation of AE was observed in both populations, and the AE scores of NASMA (with a mean value of 2.0) were always much lower than those of the two male parents (5.5 for IAS20*5/H567.71 and 6.0 for RPB709.71/COC). In FBHSN and POP populations, AE also showed large variation, with a range from 2.0 to 9.0 in the former and 1.0 to 8.5 in the latter. In all four populations, variation in "genotype" for AE were significant ($p < 0.0001$), as well as the "genotype × year" effects in the two RIL populations. The heritability estimates of AE in these populations were from 0.74 to 0.86 (Table 1 and Figure 1).

Table 1. Analysis of variance for anther extrusion (AE) and its heritability estimates in the "NASMA" × "IAS20*5/H567.71" (NIH), "NASMA" × "RPB709.71/COC" (NRC), 16th Fusarium head blight screening nursery (FHBSN), and Panel of Parents (POP) populations.

Population	Source	DF	Mean Square	*F*-Value	*p*-Value	Heritability
NIH	Genotype	196	14.97	9.66	<0.0001	0.83
	Year	1	324.92	209.67	<0.0001	
	Genotype × year	196	2.52	1.63	<0.0001	
	Rep (year)	2	23.27	15.02	<0.0001	
	Error	392	1.55			
NRC	Genotype	184	14.07	6.82	<0.0001	0.74
	Year	1	231.62	112.28	<0.0001	
	Genotype × year	184	3.61	1.75	<0.0001	
	Rep (year)	2	26.43	12.81	<0.0001	
	Error	368	2.06			
FHBSN	Genotype	35	4.67	5.67	<0.0001	0.82
	Rep	1	1.68	2.04	0.1620	
	Error	35	0.82			
POP	Genotype	35	9.44	7.04	<0.0001	0.86
	Rep	1	0.06	0.04	0.8399	
	Error	35	1.34			

In terms of FHB index, the two male parents, IAS20*5/H567.71 (with a mean value of 24.9%) and RPB709.71/COC (32.3%), showed significantly lower infection than the female parent NASMA (69.9%). Continuous distribution patterns were found in both populations, indicating quantitative inheritance of FHB resistance. Transgressive segregation was observed for both high and low disease directions. As for FHBSN and POP, FHB index showed a range of mean values across replications from 1.5% to 73.1% in the former and 1.0% to 82.4% in the latter. The heritability estimates for the FHB index were from 0.74 to 0.93, and the "genotype" effects were significant in all four populations ($p < 0.0001$) (Table 2).

AE exhibited consistently significant negative correlation with FHB index in the four populations used in this study, with *r*-values ranging from −0.55 in the NRC population to −0.74 in the FHBSN population (Figure 1). PH and DH also showed continuous distribution patterns in the studied populations (Table S1), and they often exhibited significant correlations with FHB and AE. These two phenological traits showed constantly better correlations with FHB than with AE (Table S2).

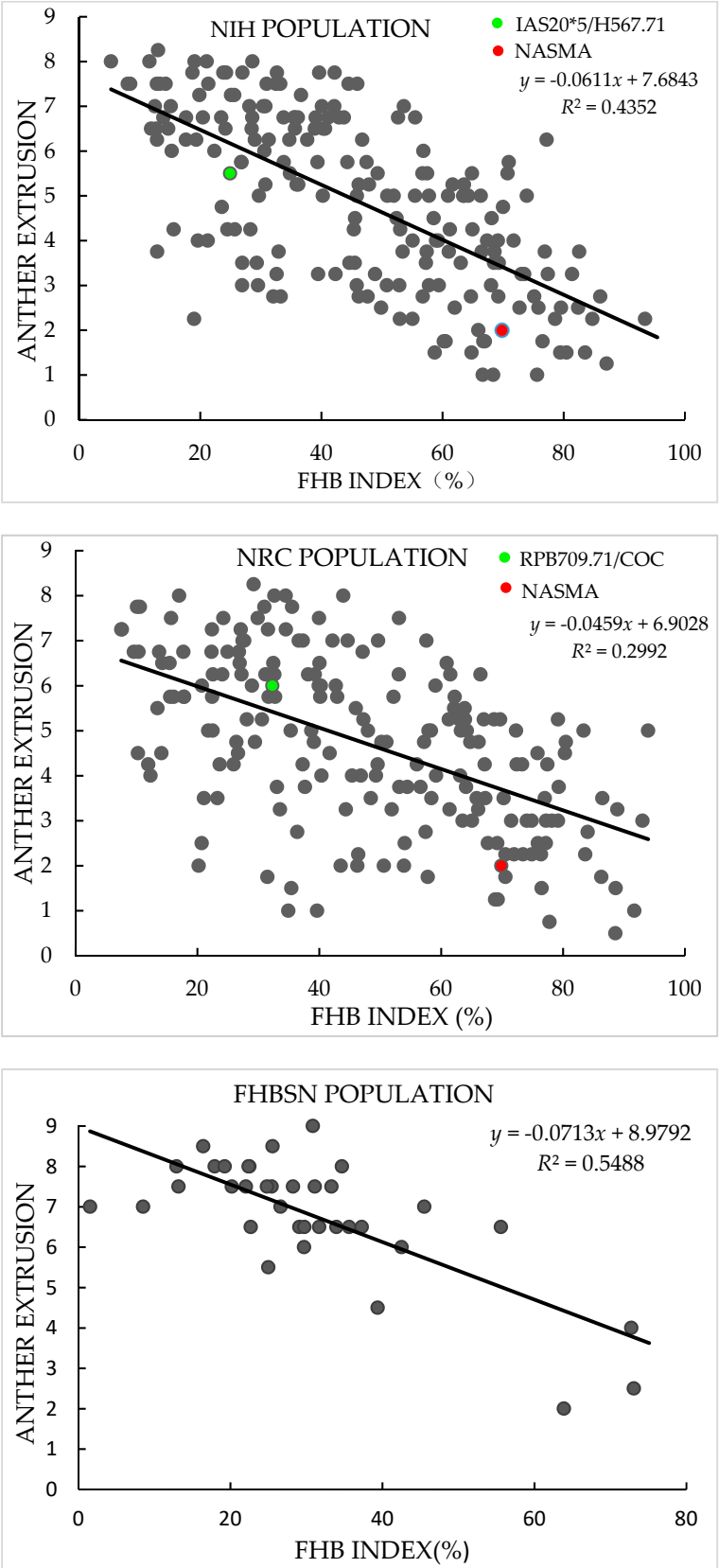

Figure 1. *Cont.*

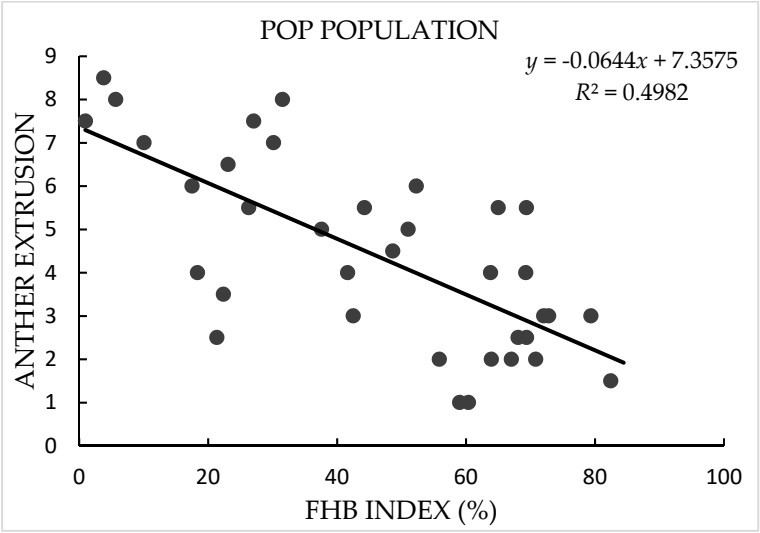

Figure 1. Scatter plots of Fusarium head blight (FHB) index against anther extrusion in "NASMA" × "IAS20*5/H567.71" (NIH), "NASMA" × "RPB709.71/COC" (NRC), 16th Fusarium head blight screening nursery (FHBSN), and "Panel of Parents" (POP) populations, using overall means. The red dot represents NASMA, and the green dot represents IAS20*5/H567.71 or RPB709.71/COC. The correlations are all significant at $p < 0.0001$.

Table 2. Analysis of variance for Fusarium head blight (FHB) and its heritability estimates in "NASMA" × "IAS20*5/H567.71" (NIH), "NASMA" × "RPB709.71/COC" (NRC), 16th Fusarium head blight screening nursery (FHBSN), and "Panel of Parents" (POP) populations.

Population	Source	DF	Mean Square	*F*-Value	*p*-Value	Heritability
NIH	Genotype	196	1734.59	18.2	<0.0001	0.92
	Year	1	297.47	3.12	0.0781	
	Genotype × year	195	136.17	1.43	0.0016	
	Rep (year)	2	1173.78	12.32	<0.0001	
	Error	390	95.308			
NRC	Genotype	184	1927.59	19.33	<0.0001	0.74
	Year	1	6655.90	66.73	<0.0001	
	Genotype × year	184	279.66	2.80	<0.0001	
	Rep (year)	2	699.51	7.01	0.001	
	Error	362	99.74			
FHBSN	Genotype	35	504.16	10.79	<0.0001	0.91
	Rep	1	27.90	0.60	0.4448	
	Error	35	46.72			
POP	Genotype	35	1135.29	13.52	<0.0001	0.93
	Rep	1	1454.45	17.32	0.0002	
	Error	35	83.99			

3.2. Genotyping and Linkage Analysis

In the NRC population, 1182 SNPs together with *Rht-B1*, *Rht-D1*, *Vrn-A1*, and four SSRs were used for linkage map construction. Thirty-one LGs were generated, covering 4430 cM with an average density of 2.2 cM between markers. All the 21 wheat chromosomes were represented in this map, and nine LGs were not assigned to a chromosome due to a lack of anchored markers. Regarding the NIH population, 1316 markers were used for linkage mapping, and its map had a very similar marker density to the NRC population, as described in He et al. [23].

3.3. QTL Mapping for AE

Seven QTL for AE were detected on chromosomes 1BS, 3BL, 4BS, 4DS, 5BL, 6BS, and 7AS in the NIH population, with accumulated percentage of phenotypic variation explained from 32.2% to 39.7% in different years, while six QTL were mapped on 3BL, 4AL, 4DS, 5AL, 6AS, and 7BS in the NRC population, collectively explaining 23.6% to 43.0% of phenotypic variation. Five QTL on 3BL, 4DS, 5BL, 6AS, and 7Ac were stably identified in both years (Table 3). The QTL on 4DS showed the biggest effects in both populations, explaining 4.6% to 16.1% of the phenotypic variation in the NIH population, and 11.8% to 19.7% in the NRC population. The QTL on 3BL explained 4.5% to 8.8% of phenotypic variation in the NIH population, and 3.1% to 6.9% in the NRC population. Alleles for high AE at both QTL were contributed by the male parents IAS20*5/H567.71 and RPB709.71/COC. Additionally, minor QTL were detected on 1BS, 5BL, 6BS, and 7Ac in the NIH population, which explained 3.8% to 6.8% of phenotypic variation, and on 4AL, 5AL, 6AS, and 7BS in the NRC population, explaining 3.9% to 6.1% of phenotypic variation (Table 3).

3.4. QTL Mapping for FHB Resistance

Nine QTL for FHB resistance were mapped on chromosomes 2AL, 2DS, 3BL, 4AL, 4BS, 4DS, 5AL, 5BL, and 7AS in the two mapping populations. Seven out of the nine QTL were stably identified in both years, and, among them, the QTL on 4DS and 5AL were shared by both populations and the one on 3BL was mapped to similar regions. These three QTL with their resistant alleles contributed by IAS20*5/H567.71 or RPB709.71/COC collectively explained 42.5% of phenotypic variation in NIH and 32.5% in NRC. The QTL on 5AL showed the biggest effects on FHB resistance in both populations, followed by 4DS and 3BL (Table 4, Figure 2).

Table 3. QTL for anther extrusion in "NASMA" × "IAS20*5/H567.71" (NIH) and "NASMA" × "RPB709.71/COC" (NRC) populations and their association with other traits.

Population	Chr.	Position	Left Marker	Right Marker	2013		2014		Source [b]	Traits Associated [c]
					LOD	PVE (%) [a]	LOD	PVE (%)		
NIH	1BS	46.9–54.1	wmc406	barc8	2.2	3			I	
	3BL	82.1–84.1	Ku_c37029_761	BS00049639_51	3.3	4.5	5.5	8.8	I	FHB
	4BS	44.1–46.3	Rht-B1	BS00021984_51	4.0	6.1			N	FHB, PH
	4DS	0–10.7	Rht-D1	barc105	10.6	16.1	3.0	4.6	I	FHB, PH
	5BL	98.4–111.8	Ex_c100531_251	Ku_c39809_164	4.4	6.2	4.3	6.8	N	FHB, DH
	6BS	65.5–66.0	WEC_3267186	Ku_c9507_142			3.4	5.2	I	
	7Ac	118.7–121.7	BS00006674_51	BW_c66165_77	2.8	3.8	4	6.8	N	
NRC	3BL	66.0–67.9	RFL_622_1601	BW_c45623_215	5.0	6.9	2.1	3.1	R	FHB
	4AL	78.4–79.4	Ex_c8658_335	Ex_9657856	3.4	4.7			N	
	4DS	0.0–21.3	Ku_c68594_530	barc105	12.6	19.7	6.0	11.8	R	FHB, PH
	5AL	149.4–150.2	BS00097986	Ex_c5998_10513766	4.1	5.6			R	FHB, PH, DH
	6AS	18.7–19.3	IACX2250	BS00037006_51	4.4	6.1	2.6	4.8	N	
	7BS	46.2–53.3	Ku_c101472_574	GENE-4333_211			2.1	3.9	R	DH

[a] Percentage variation explained. [b] The parent that contributes the allele for high anther extrusion. *I*, IAS20*5/H567.71; *N*, NASMA; *R*, RPB709.71/COC. [c] FHB, Fusarium head blight; PH, plant height; DH, days to heading. QTL mapping results for DH and PH are available in Table S3.

Table 4. QTL for Fusarium head blight (FHB) in "NASMA" × "IAS20*5/H567.71" (NIH) and "NASMA" × "RPB709.71/COC" (NRC) populations and their association with other traits.

Population	Chr.	Position (cM)	Left Marker	Right Marker	2013		2014		R Source [b]	Traits Associated [c]
					LOD	PVE (%) [a]	LOD	PVE (%)		
NIH	3BL	109.6–115.5	BW_c24364_73	wKc31407_41142340	4.0	4.8	3.0	4.4	I	AE
	4BS	44.1–46.3	Rht-B1	BS00021984_51	4.2	5.5			N	AE, PH
	4DS	0–10.7	Rht-D1	barc105	10.1	12.7	6.0	9.2	I	AE, PH
	5AL	147.6–147.9	WEC_5013188	Vrn-A1	15.8	21.2	13.2	21.7	I	AE, DH, PH
	5BL	92.1–92.4	BS00022673_51	R_c539_1789	4.1	4.8	2.3	3.4	N	AE, DH
NRC	2AL	91.3–96.9	BW_c4743_63	Ra_c38018_278			3.1	4.2	N	
	2DS	37.3–55.6	BS00022276_51	Ex_c39215_100	4.6	5.8	2.3	3.8	N	DH, PH
	3BL	64.9–65.6	Ra_c55214_932	Ra_c50787_146	3.1	3.8	2.6	3.6	R	AE
	4AL	129.3–136.8	IAAV1383	Ra_32274079	3.2	3.9	2.2	3	N	
	4DS	0.0–21.3	Ku_c68594_530	barc105	7.7	10.2	9.4	14.4	R	AE, PH
	5AL	149.4–164.7	BS00097986	Ra_c112818_307	13.4	18.7	6.4	9.2	R	AE, DH, PH
	7AS	54.9–61.6	Ex_c109881_701	BS00023225_51	6.1	9.7	2.9	4.7	R	DH, PH

[a] Percentage variation explained. [b] Source of resistance. *I*, IAS20*5/H567.71; *N*, NASMA; *R*, RPB709.71/COC. [c] AE, anther extrusion; PH, plant height; DH, days to heading. QTL mapping results for DH and PH are available in Table S3.

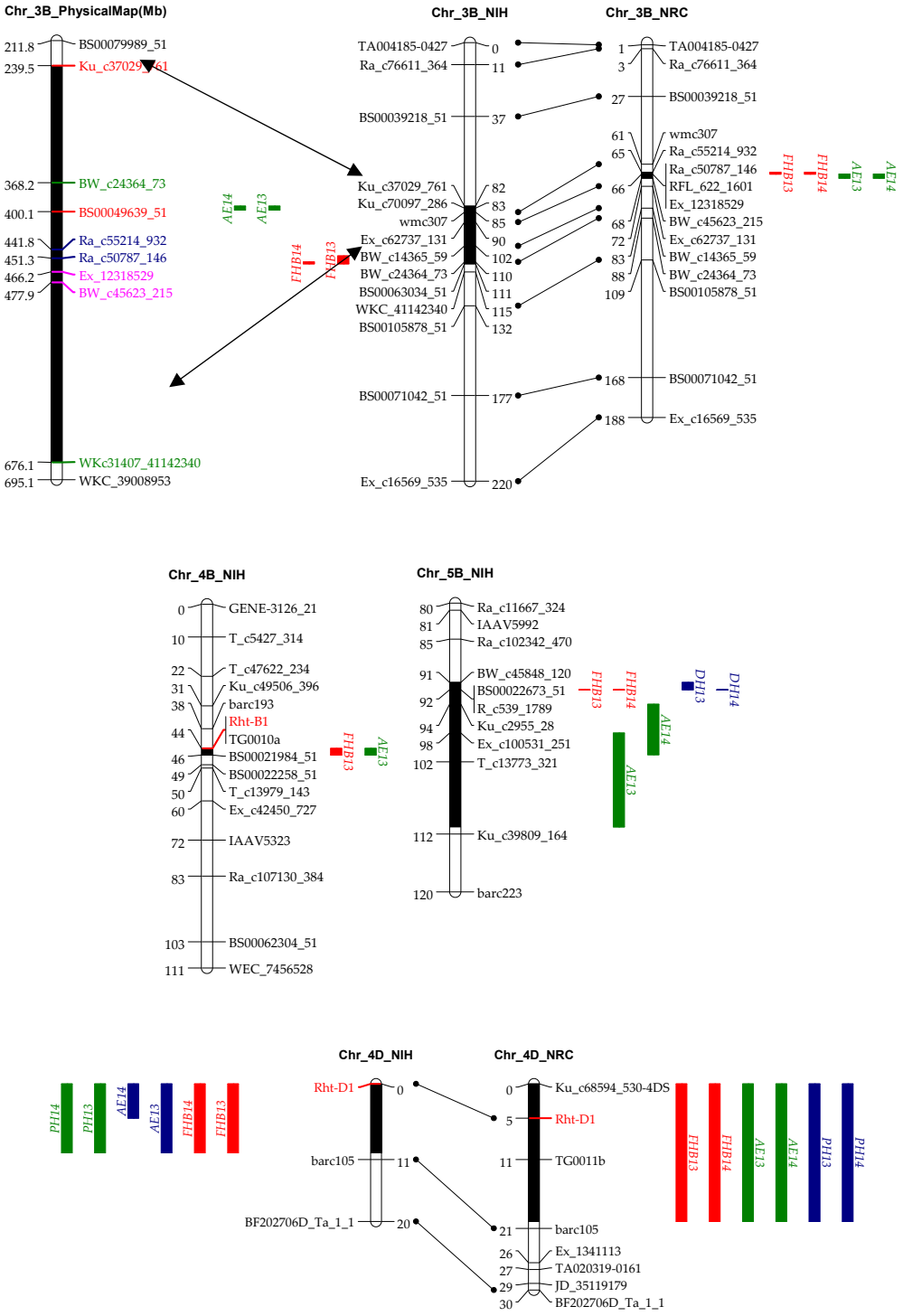

Figure 2. *Cont.*

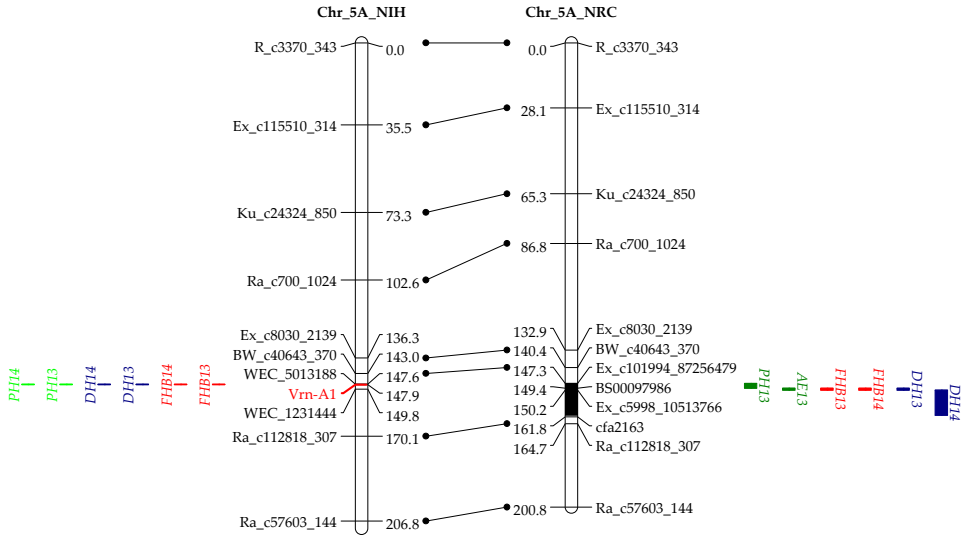

Figure 2. QTL for FHB resistance, anther extrusion (AE), plant height (PH), and days to heading (DH) on chromosomes 3B, 4B, 4D, 5A, and 5B in the "NASMA" × "IAS20*5/H567.71" (NIH) and "NASMA" × "RPB709.71/COC" (NRC) populations using the multiple QTL mapping (MQM) method. Genetic distances are shown in centimorgans to the inner or left side of the linkage groups, whereas QTL ranges are denoted to the outer or right side of the linkage groups. Only framework markers are presented except for the QTL regions.

In the NRC population, six QTL were detected for FHB resistance, i.e., 2DS, 3BL, 4AL, 4DS, 5AL, and 7AS. The QTL on 5AL that was associated with DH and AE and linked to *Vrn-A1* explained 9.2% to 18.7% of phenotypic variation. The QTL on 4DS, which was associated with AE and PH and linked to *Rht-D1*, also had major effects on FHB resistance, explaining 10.2%–14.4% of phenotypic variation. QTL on 2DS, 3BL, 4AL, and 7AS were consistently identified in both years and, thus, can be regarded as stable QTL, explaining 3.0% to 9.7% of phenotypic variation (Table 4). In the NIH population, four stable QTL on 3BL, 4DS, 5AL, and 5BL were detected, explaining 4.4% to 21.7% of phenotypic variation as previously reported in He et al. [23].

In agreement with the significant phenotypic correlation, QTL for AE and FHB often coincided or were tightly linked to each other. In this regard, QTL on chromosomes 3BL, 4BS, 4DS, 5AL, and 5BL in the NIH population, and those on 3BL, 4DS, and 5AL in the NRC population were responsible for both AE and FHB (Tables 3 and 4, Figure 2).

4. Discussion

4.1. AE as a Morphological Marker for FHB

A wide range of variation for AE among wheat genotypes was reported in recent studies. This trait was extensively studied as a factor promoting cross-pollination in hybrid breeding [7,8,15,29], and it showed a close relationship with FHB resistance in wheat [4,5,9,11,30]. It is generally acknowledged that cleistogamy and high AE have better Type I resistance against FHB [11]. This is in agreement with the results from the present study, where AE was consistently correlated with field FHB resistance.

The importance of AE in FHB resistance has long been recognized [10,31], but genetic studies on AE were performed only in the last decade [4,9,32]. In the aforementioned studies, numerous QTL for AE were identified, and many of them were reported to be responsible for both AE and FHB [4,5,9,33]. In this regard, the well-known ones are those on 4BS and 4DS, linking to *Rht-B1* and *Rht-D1* [9], respectively, as well as the closely linked ones on 5AS and 5Ac in the *Fhb5* region [5,34]. Considering the close relationship of AE with Type I FHB resistance, the former was proposed as a morphological marker for FHB resistance by many researchers [4,10]. In fact, this strategy was

practiced in the late 1970s by a Chinese breeder, who succeeded in selecting lines with good FHB resistance [35]. In recent years, breeders in Norway and Germany also started using this strategy, which proved to be promising [36,37].

4.2. Rht-B1 *and* Rht-D1 *with Major Phenotypic Effects on Both AE and FHB*

It is well known that dwarfing genes (*Rht-B1b* and *Rht-D1b*) reduce PH and AE and increase FHB index [9,38]. In the current study, *Rht-D1* was associated with QTL for AE and FHB in both RIL populations, exhibiting major phenotypic effects, whereas *Rht-B1* was associated with the two traits only in NIH with minor effects, and it was non-significant in NRC for neither AE nor FHB. This was different from our previous results, where *Rht-B1b* showed stronger effects on decreasing AE and FHB resistance than *Rht-D1b* in two mapping populations [9], implying a genetic background dependency of the genes; however, it is also possible that the minor effects of *Rht-B1b* were caused by environmental conditions that were not conducive for the expression of this gene. These two dwarfing genes encode forms of DELLA proteins that are insensitive to gibberellins (GAs) [39,40], which regulate plant height and several floral development processes, e.g., flower induction, pollen development, pollen tube growth, stamen development, filament extension, anther development and exertion [15,41,42]. Bioactive forms of GAs are most likely involved in establishing anther extrusion patterning in barley spikes [43]. Based on these facts, it is reasonable to speculate that *Rht-B1* and *Rht-D1* are the underlying genes for the AE QTL at the two loci, which influence FHB via two ways, i.e., (1) conditioning AE that in turn impacts FHB, and (2) controlling PH that is associated with FHB escape. *Rht-B1b* and *Rht-D1b* are widely used in breeding programs globally; however, considering their negative effects on FHB resistance and AE, *Rht24* that has no negative effects on FHB could be a good candidate to replace the former two genes [44].

4.3. QTL Associated with AE, DH, and FHB

In addition to the two dwarfing genes that showed association with multiple traits, we also detected two QTL on 5AL and 5BL, which were associated with AE, DH, and FHB (Tables 2 and 3, Figure 2). The QTL on 5AL encompasses *Vrn-A1*, spanning a region between 585.1 and 587.4 Mb in the Chinese spring (CS) reference genome. Recently, Muqaddasi et al. [8] reported a QTL on 5AL for AE, and its physical position was about 592.0 Mb, very close to ours and, thus, likely to be the same QTL. The QTL on 5BL responsible for AE, DH, and FHB spans a physical region between 583.6 and 613 Mb in the CS genome, closely linking to the *Vrn-B1*, with a distance of only 9.5 Mb. It could be possible that *Vrn-A1* and *Vrn-B1* are the underlying genes for these two QTL, and they affected FHB by regulating AE and DH.

Flowering of higher plants is a complex biological process and is regulated by both environmental and developmental factors. Many genes were reported to regulate flowering and are, thus, associated with DH, e.g., *VRN* and *Ppd* genes. The *VRN1* genes, including three homoeologous genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* that encode MADS-box proteins, are the major loci for vernalization requirement and are related to plant height and spikelet development in wheat [45]. *VRN1* is one of the central genes with strong epistatic interactions in the vernalization pathway to regulate heading time in wheat. Together with GAs, *VRN1* upregulate the expression of the *SOC1* gene that integrates GA signals, which is required for normal spike development and plant height [46,47]. Considering the biological functions of *VRN1* genes, it is reasonable that QTL at or closely linked to *VRN1* genes, like *Vrn-A1* and *Vrn-B1* in the current study, are associated with AE. Specific to the present study, the mechanism could be that *Vrn-A1a* and *Vrn-B1a* induce GA biosynthesis that promotes early heading and pronounced AE. However, in this study, early heading was associated with high FHB, whereas high AE was associated with low FHB; thus, the overall effects of *Vrn-A1a* and *Vrn-B1a* on FHB were more complicated than those of *Rht-B1a* and *Rht-D1a*, because the latter two led to tall stature and high AE that both were associated with FHB resistance. In this study, the overall effects of *Vrn-A1a* and *Vrn-B1a* were the increased FHB infection, implying that they had more impact on DH than on AE.

4.4. QTL on 3BL Was Also Important for AE and FHB Resistance

The QTL on 3BL for AE were always linked to those for FHB in the two mapping populations (Tables 3 and 4, Figure 2). In the NIH population, physical locations of 3B for AE (251.2–419.6 Mb) and those for FHB (386.1–709.0 Mb) overlapped, and, in the NRC population, the QTL for AE (488.9–501.1 Mb) were closely linked to those for FHB (463.3–473.2 Mb) (Figure 2). Distances among the QTL were short; thus, they may have the same underlying gene, or they have closely linked but different underlying genes. In our previous study, a major QTL for DON and FDK was located in the same region as that for FHB in the NIH population [22], making this chromosome region very important for FHB-related traits, warranting further investigation.

5. Conclusions

We demonstrated the close interrelationships among FHB, AE, PH and DH in the CIMMYT germplasm used in the current study and pointed out that *Rht-B1*, *Rht-D1*, *Vrn-A1* and *Vrn-B1* genes may have pleiotropic effects on those traits. Understanding of the complicated interrelationships is helpful in breeding for FHB resistance in wheat.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/47/s1, Table S1: Phenotypic data of the four populations investigated in the current study, along with their pedigree information and basic statistics. Table S2. Phenotypic correlations among Fusarium head blight (FHB), Anther extrusion (AE), Plant height (PH) and Days to heading (DH) in 'NASMA' × 'IAS20*5/H567.71' (NIH), 'NASMA' × 'RPB709.71/COC' (NRC), 16th FHBSN (FHBSN), and 'Panel of Parents' (POP) populations. Table S3. QTL for Days to heading (DH) and Plant height (PH) in 'NASMA' × 'IAS20*5/H567.71' (NIH) and 'NASMA' × 'RPB709.71/COC' (NRC) populations.

Author Contributions: P.K.S. and X.H. conceptualized and designed the experiments; X.H. and P.K.S. performed field trials; S.D. conducted all genotyping activities; K.X. and X.H. analyzed the data, K.X. wrote the first draft of the manuscript, and K.X., X.H., S.D., Z.H., and P.K.S. contributed to and approved the final draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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