Dwarfing Genes \textit{Rht-B1b} and \textit{Rht-D1b} Are Associated with Both Type I FHB Susceptibility and Low Anther Extrusion in Two Bread Wheat Populations

Xinyao He\textsuperscript{1,2}, Pawan K. Singh\textsuperscript{1}*, Susanne Dreisigacker\textsuperscript{1}, Sukhwinder Singh\textsuperscript{1}, Morten Lillemo\textsuperscript{2}, Etienne Duveiller\textsuperscript{1}

\textsuperscript{1} International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6–641, 06600 Mexico DF, Mexico, \textsuperscript{2} Department of Plant Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

* pk.singh@cgiar.org

Abstract

It has been well documented that dwarfing genes \textit{Rht-B1b} and \textit{Rht-D1b} are associated with Type I susceptibility to Fusarium head blight (FHB) in wheat; but the underlying mechanism has not been well delineated. Anther extrusion (AE) has also been related to Type I resistance for initial FHB infection, where high AE renders FHB resistance. In this study, two doubled haploid populations were used to investigate the impact of the two dwarfing genes on FHB resistance and AE, and to elucidate the role of AE in \textit{Rht-} mediated FHB susceptibility. Both populations were derived by crossing the FHB susceptible cultivar ‘Ocoroni F86’ (\textit{Rht-B1a}/\textit{Rht-D1b}) with an FHB resistant variety (\textit{Rht-B1b}/\textit{Rht-D1a}), which was ‘TRAP#1/BOW/Taigu derivative’ in one population (the TO population) and ‘Ivan/Soru#2’ in the other (the IO population). Field experiments were carried out from 2010 to 2012 in El Batán, Mexico, where spray inoculation was adopted and FHB index, plant height (PH), and AE were evaluated, with the latter two traits showing always significantly negative correlations with FHB severity. The populations were genotyped with the DArTseq GBS platform, the two dwarfing genes and a few SSRs for QTL analysis, and the results indicated that \textit{Rht-B1b} and \textit{Rht-D1b} collectively accounted for 0–41% of FHB susceptibility and 13–23% of reduced AE. It was also observed that three out of the four AE QTL in the TO population and four out of the five AE QTL in the IO population were associated with FHB resistance. Collectively, our results demonstrated the effects of \textit{Rht-B1b} and \textit{Rht-D1b} on Type I FHB susceptibility and reducing AE, and proposed that their impacts on Type I FHB susceptibility may partly be explained by their effects on reducing AE. The implication of the relationship between the two dwarfing genes and AE for hybrid wheat breeding was also discussed.
Introduction

Fusarium head blight (FHB) is a notorious wheat disease prevailing in warm and humid environments, exerting global impact on food and feed safety due to the presence of mycotoxins produced by *Fusarium* species, the causal agents of FHB [1, 2]. Deoxynivalenol (DON) has been considered the most important FHB-related mycotoxin and legislation has been set up in many countries/organizations for controlling DON content in food and feed [3].

Host resistance to FHB is of quantitative inheritance and influenced significantly by environment [4], making breeding for this trait a difficult task. Multiple mechanisms of host resistance to FHB have been recognized, including Type I for resistance to initial infection, Type II for spread of pathogen in spike tissues, Type III for DON accumulation, Type IV for kernel infection, and Type V for yield reduction [5, 6]. In relation to food safety, Type III resistance is the most important; but so far no validated QTL specific for this resistance mechanism has been identified [7], and some researchers still regard it as a consequence of FHB infection and not an independent trait [1]. Of the first two resistance mechanisms, Type I resistance exhibited more frequent association with phenological, morphological, and flower biology traits, such as days to heading (DH), plant height (PH) and anther extrusion (AE) [8–11].

The negative association between PH and FHB susceptibility in wheat has long been observed, and it happened also in barley and oat [12–14]. Three possible mechanisms have been proposed for the association, i.e. disease escape, pleiotropy of reduced height (*Rht*) genes, and tight linkage [3]. In the last decade researches provided molecular evidence for this relationship and several QTL responsible for both FHB and PH were identified, including *Rht-B1*, *Rht-D1* and *Rht8* [9]. Dwarfing genes *Rht-B1b* and *Rht-D1b* (formally known as *Rht1* and *Rht2*, respectively) were derived from the Japanese cultivar ‘Norin 10’ and contributed greatly to the Green Revolution [15]. Strong evidences are available for the association between *Rht-D1b* and Type I FHB susceptibility in European varieties [16–20]. For example, *Rht-D1b* increased FHB severity by 52% in a ‘Mercia’ background and 38% in a ‘Maris Huntsman’ background [20]. Lu et al. [21] demonstrated in a mapping population that two major resistant QTL may be required to counteract the negative effect of *Rht-D1b*. In an association mapping study on European winter wheat materials, Miedaner et al. [22] also reported the significant association of *Rht-D1b* with increased FHB susceptibility, but to a lesser degree than reported previously; the authors concluded that the negative effects of *Rht-D1b* in bi-parental populations may have been overestimated. In the case of *Rht-B1b*, Srinivasachary et al. [23] found that it showed little or no negative impact on Type I FHB resistance under moderate FHB pressure, but exerted negative effects similar to *Rht-D1b* under severe infection. Miedaner and Voss [20] also reported the different performance of *Rht-B1b* under different genetic backgrounds. In the three mapping populations tested by Buerstmayr et al. [24], *Rht-B1b* was associated with increased FHB susceptibility, with phenotypic effects ranging from 3–18%. The negative effects of the two dwarfing genes on field FHB resistance have also been reported in Chinese and US wheat materials [25, 26]. In point inoculated experiments, *Rht-B1b* exhibited significant effects on Type II resistance, whereas *Rht-D1b* showed little or no effects on this type of FHB resistance [19, 23, 26–28]. Many researchers ascribed the association to the pleiotropic effects of dwarfing genes [17, 19, 24]; but Yan et al. [28] claimed that it was the micro-environmental condition around spikes that contributed to the relationship, since the negative effects on Type I resistance disappeared when *Rht-B1b* and *Rht-D1b* near-isogenic lines were physically raised to the same heights as their tall counterparts.

The importance of anthers in FHB infection has long been observed. Pugh et al. [29] observed that retained anthers were the first tissues to be colonized as a base for further infection. Strange and Smith [30] also found this phenomenon and reported that the presence of
anthers favoured greatly the FHB infection, whereas emasculation significantly reduced the disease severity. They ascribed this to the fungal growth stimulants in anthers, of which choline and betaine were the two major components [31]. Based on these findings, Strange et al. [32] suggested the selection of wheat lines with low anther retention (or high AE) to facilitate FHB resistance breeding. Three decades later, Skinnes et al. [33] and Graham and Browne [34] reported the association of FHB with AE in European wheat varieties, where those having high AE tended to have low FHB severity. The positive correlation between AE and FHB resistance has also been reported in Chinese, Japanese and CIMMYT germplasm [35–37]. QTL mapping studies revealed the underlying mechanisms for this relationship by identifying linked or coincided QTL for the two traits [8, 11, 38, 39]. Like PH, AE was also found to be associated with Type I FHB resistance [11].

Considering the associations of Type I FHB resistance with both PH and AE, it is tempting to investigate the association between the latter two traits, and clues do exist in literature. In the Shanghai-3/Catbird x Naxos population, Rht-B1 explained 10% of the phenotypic variation of AE [11], and in the Hermann x Skalmeje population, lines with Rht-B1b or Rht-D1b showed reduced AE and double dwarfs (Rht-B1b/Rht-D1b) had a high degree of anther retention (+99%) compared to tall lines with Rht-B1a/Rht-D1a [40]. It was also observed by hybrid wheat breeders that PH and AE are positively correlated [41]. The objectives of the current study were to map QTL for FHB and its related traits and to evaluate the impacts of dwarfing genes Rht-B1b and Rht-D1b on field FHB resistance and AE in two mapping populations.

Materials and Methods

Plant material

Two doubled haploid populations were used in this study. The first one was developed from a cross between ‘TRAP#1/BOW//Taigu derivative’ and ‘Ocoroni F86’ with 135 progenies (referred to as the TO population hereafter), while the second was from ‘Ivan/Soru#2’ and ‘Ocoroni F86’ with 92 progenies (the IO population). Both the two female parents were FHB resistant lines bred at CIMMYT, while ‘Ocoroni F86’ (pedigree JUPATECO-73/(SIB)EMU// (SIB)GRAJO) is a CIMMYT breeding line moderately susceptible to FHB [42]. Both of the two resistant parents carried Rht-B1b/Rht-D1a whereas the susceptible parent had the Rht-B1a/ Rht-D1b genotype, resulting in both dwarfing genes segregating in the two populations.

Field trials and phenotyping

The field FHB experiments were conducted at the El Batán experimental station (altitude of 2,240 meters above sea level, coordinate 19.5°N, 98.8°W, with an average annual precipitation of 625 mm) of CIMMYT, Mexico, during the summer season (May to September) when rainfall is concentrated. The two populations were evaluated from 2010 to 2012, sown in 1 m double rows with randomized complete block design with three replications. Each year, a mixture of 5 aggressive F. graminearum isolates were collected, characterized, and used for field inoculation, following the protocols described by He et al. [42]. Spray inoculation was targeted to each line’s anthesis stage with an inoculum of 50,000 spores/ml and was repeated two days later. From anthesis to early dough stages, the nursery was misted from 9 am to 8 pm with 10 minutes of spraying each hour, to create a humid environment favourable 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 had been tagged at anthesis. Numbers of infected spikes and symptomatic spikelets of each spike were counted for calculating FHB index with the formula: \( FHB\ index = \frac{\text{Severity}}{\text{Incidence}} \)
where Severity stands for the averaged percentage of diseased spikelets, and Incidence for the percentage of symptomatic spikes. Plots were sickle harvested and threshed with a belt thresher set at low wind speed to retain scabby kernels. Fusarium damaged kernels (FDK) was estimated only in 2012 for the two populations through visually evaluating a random sample in a petri dish, where both scabby and shrivelled kernels were regarded as FDK. DON content was quantified in 2010 and 2012 for the TO population and in 2011 and 2012 for the IO population, based on 2 g flour sampled from 20 g ground grain of each accession, using the Ridascreen Fast DON ELISA kit (RBiopharm GmbH, Darmstadt, Germany) following the manufacturer’s instructions. AE and PH were scored in 2011 and 2012 for the IO population and in 2012 for the TO population. In 2015, the TO population was planted in 40x15 cm hill plots with two replications for an additional evaluation of AE and PH. AE was rated with a linear scale from 0 (no extrusion) to 9 (full extrusion) according to Skinnes et al. [8], and PH was measured before harvest from ground to the average spike tips excluding awns in each plot. Days to heading (DH) was scored for the two populations in all the experiments.

**Statistical analyses**

The phenotypic data was analysed by the SAS program ver. 9.2. 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 $h^2 = \sigma_g^2 / \left( \sigma_g^2 + \frac{\sigma_y^2}{y} + \frac{\sigma_e^2}{r} \right)$ for single years and $h^2 = \sigma_g^2 / \left( \sigma_g^2 + \frac{\sigma_y^2}{y} + \frac{\sigma_e^2}{r} \right)$ for multiple years; in which $\sigma_g^2$ stands for genetic variance, $\sigma_g^2\gamma$ for genotype-by-year interaction, $\sigma_e^2$ for error variance, $y$ for the number of years, and $r$ for the number of replications [11].

**Genotyping**

The two populations were genotyped with the DArTseq genotyping-by-sequencing (GBS) platform at the Genetic Analysis Service for Agriculture (SAGA) in Guadalajara, Mexico. This genotyping method is a combination of complexity reduction methods developed for array-based DArT and sequencing of resulting representations on next-generation sequencing platforms, for detailed information please check Li et al. [44]. Additionally, two dwarfing genes Rht-B1 and Rht-D1 were also genotyped, using the KASPar technology (KBioscience) based SNP markers developed at CIMMYT [45]. A few SSR markers linked to known FHB resistance QTL [7] were also applied. Markers with missing data points greater than 20% and segregation ratio beyond the range 0.5–2.0 were discarded from further analysis.

**Linkage and QTL analysis**

Linkage groups (LGs) were constructed using the JoinMap v.4 software [46], where groupings were based on LOD values from 5 to 10, and ordering within each LG was done with the Maximum Likelihood algorithm. LGs were assigned to chromosomes according to the consensus GBS map by Li et al. [44]. QTL mapping was carried out with MapQTL v6.0 [47], in which interval mapping (IM) was first performed to detect potential QTL 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. QTL were taken as significant and were reported if they were over the LOD threshold of 3 in at least one environment or over the threshold of 2 in multiple environments. LGs and LOD curves were drawn by the software MapChart ver. 2.3 [48].
Results

FHB development of the two populations was satisfactory, ranging from slight infection to around 50% of FHB index in all the three years (Fig 1). The resistant parents ‘TRAP#1/BOW/ Taigu derivative’ and ‘Ivan/Soru#2’ showed always significantly higher resistance than the susceptible parent ‘Ocoroni F86’, in terms of all three FHB parameters. In both populations, ‘year’ effect contributed the most variation of FHB and DON, followed by ‘genotype’ and ‘genotype x year’ effects which were also significant except for DON in the TO population (Table 1). Usually high heritability estimates were obtained for the FHB parameters, but DON in the TO population had a value of merely 0.22 (Table 1). Significantly positive correlations were found among all the FHB traits, although in several cases the r values were low (Table 2).

AE and PH showed wide segregation in both populations (Fig 1). High heritability estimates of 0.79 and 0.87 were obtained for AE in the TO and IO populations, respectively, and in the case of PH the values were even higher (Table 1). The two traits showed significantly negative correlations with FHB in the TO population (r = -0.73 for PH vs. FHB, and r = -0.65 for AE vs. FHB, p<0.0001), and the corresponding correlations were also significant in the IO population but with lower r values (r = -0.63 for PH vs. FHB, and r = -0.48 for AE vs. FHB, p<0.0001).

In the TO population, 1,858 GBSs together with seven SSRs and the two dwarfing genes were used for LG construction. Thirty three LGs were generated, covering 4,053cM with an average density of 2.2 cM/marker. All but 1D chromosome were represented in this map and three LGs were not assigned to a chromosome due to a lack of anchored markers. Regarding the IO population, 1,986 GBSs, Rht-B1, Rht-D1 and four SSRs were used for linkage mapping and 35 LGs were obtained. Total length of the LGs was 4,430cM with a very similar density as
Table 1. Analysis of variance for Fusarium head blight and associated traits and their heritability estimates in the ‘TRAP#1/BOW//Taigu derivative’ x ‘Ocoroni F86’ (TO) and ‘Ivan/Soru#2’ x ‘Ocoroni F86’ (IO) populations.

| Traits                  | Source     | DF  | Mean square | F value | P value | Heritability |
|-------------------------|------------|-----|-------------|---------|---------|--------------|
|                         |            |     |             |         |         |              |
| TO FHB                  | Genotype   | 138 | 743.80      | 6.27    | <0.0001 | 0.84         |
|                         | Year       | 2   | 15025.92    | 126.62  | <0.0001 |              |
|                         | Genotype x Year | 275 | 118.67     | 3.07    | <0.0001 |              |
|                         | Rep (Year) | 6   | 280.06      | 7.24    | <0.0001 |              |
|                         | Error      | 811 | 38.70       |         |         |              |
| DON                     | Genotype   | 138 | 41.83       | 1.26    | 0.0879  | 0.22         |
|                         | Year       | 1   | 1583.00     | 47.71   | <0.0001 |              |
|                         | Genotype x Year | 138 | 33.18      | 1.35    | 0.0121  |              |
|                         | Rep (Year) | 3   | 210.54      | 8.59    | <0.0001 |              |
|                         | Error      | 413 | 24.51       |         |         |              |
| FDK                     | Genotype   | 129 | 1182.55     | 9.16    | <0.0001 | 0.89         |
|                         | Rep (Year) | 1   | 35.53       | 0.28    | 0.6008  |              |
|                         | Error      | 126 | 129.13      |         |         |              |
| Anther extrusion        | Genotype   | 138 | 16.19       | 4.67    | <0.0001 | 0.79         |
|                         | Year       | 1   | 29.38       | 8.47    | <0.0001 |              |
|                         | Genotype x Year | 138 | 3.47      | 3.47    | <0.0001 |              |
|                         | Rep (Year) | 3   | 0.24        | 0.24    | 0.8673  |              |
|                         | Error      | 414 | 1.00        |         |         |              |
| Plant height            | Genotype   | 138 | 2276.49     | 37.67   | <0.0001 | 0.97         |
|                         | Year       | 1   | 675.22      | 11.17   | <0.0001 |              |
|                         | Genotype x Year | 138 | 60.44      | 4.26    | <0.0001 |              |
|                         | Rep (Year) | 3   | 161.13      | 11.37   | <0.0001 |              |
|                         | Error      | 414 | 14.18       |         |         |              |
| IO FHB                  | Genotype   | 93  | 602.42      | 3.90    | <0.0001 | 0.74         |
|                         | Year       | 2   | 6630.69     | 42.90   | <0.0001 |              |
|                         | Genotype x Year | 186 | 154.57     | 3.73    | <0.0001 |              |
|                         | Rep (Year) | 6   | 112.33      | 2.71    | 0.0133  |              |
|                         | Error      | 546 | 41.42       |         |         |              |
| DON                     | Genotype   | 93  | 8.06        | 2.48    | <0.0001 | 0.62         |
|                         | Year       | 1   | 65.38       | 20.11   | <0.0001 |              |
|                         | Genotype x Year | 93  | 3.25      | 2.48    | <0.0001 |              |
|                         | Rep (Year) | 3   | 25.59       | 19.52   | <0.0001 |              |
|                         | Error      | 269 | 1.31        |         |         |              |
| FDK                     | Genotype   | 93  | 2460.63     | 8.25    | <0.0001 | 0.88         |
|                         | Rep (Year) | 2   | 1410.71     | 4.73    | 0.0100  |              |
|                         | Error      | 177 | 298.13      |         |         |              |
| Anther extrusion        | Genotype   | 93  | 25.11       | 6.04    | <0.0001 | 0.87         |
|                         | Year       | 1   | 25.11       | 6.24    | <0.0001 |              |
|                         | Genotype x Year | 93  | 4.16      | 4.30    | <0.0001 |              |
|                         | Rep (Year) | 4   | 1.75        | 1.81    | 0.1266  |              |
|                         | Error      | 372 | 0.97        |         |         |              |
| Plant height            | Genotype   | 93  | 1685.82     | 35.60   | <0.0001 | 0.98         |
|                         | Year       | 1   | 7634.09     | 161.23  | <0.0001 |              |
|                         | Genotype x Year | 93  | 47.35     | 2.81    | <0.0001 |              |
|                         | Rep (Year) | 4   | 185.38      | 11.01   | <0.0001 |              |
|                         | Error      | 372 | 16.84       |         |         |              |
that of the TO population. In this case, only chromosome 6D was not represented and six LGs were not assigned to chromosomes.

Three QTL with major effects were identified in both populations, i.e. Rht-B1 on 4BS, Rht-D1 on 4DS and a QTL on 5AL (Table 3, Fig 2). The latter was most likely at Vrn-A1, due to its strong effects on heading time, explaining 48.2% of the DH variation in the TO population and 14.9% in the IO population. The expression of the three QTL was more stable in the TO population, being associated with FHB parameters in most environments, accounting mostly 10–20% of phenotypic variation. Comparably, the magnitude of their phenotypic effects was similar in the IO population, but their expression was not detected in certain environments, e.g. the

### Table 2. Pearson correlation coefficients among FHB traits in the ‘TRAP#1/BOW//Taigu derivative’ x ‘Ocoroni F86’ (TO, below the diagonal) and ‘Ivan/Soru#2’ x ‘Ocoroni F86’ (IO, above the diagonal) populations.

|           | FHB10 | DON10(11) | FHB11 | FHB12 | DON12 | FDK12 |
|-----------|-------|-----------|-------|-------|-------|-------|
| FHB10     | 1     | 0.35**    | 0.48**| 0.37**| 0.52**| 0.36* |
| DON10(11)*| 0.62**| 1         | 0.67**| 0.54**| 0.42**| 0.42**|
| FHB11     | 0.72**| 0.49**    | 1     | 0.61**| 0.35* | 0.56**|
| FHB12     | 0.61**| 0.36**    | 0.65**| 1     | 0.55**| 0.74**|
| DON12     | 0.44**| 0.35**    | 0.43**| 0.61**| 1     | 0.36* |
| FDK12     | 0.56**| 0.29*     | 0.65**| 0.69**| 0.56**| 1     |

* P<0.01  
** P<0.0001  
*a* DON10 in the case of the TO population and DON11 in the case of the IO population.

### Table 3. QTL for FHB traits after spray inoculations in the ‘TRAP#1/BOW//Taigu derivative’ x ‘Ocoroni F86’ (TO) and ‘Ivan/Soru#2’ x ‘Ocoroni F86’ (IO) populations and their association with other traits.

| Linkage group | Position | Left marker | Right marker | FHB index | FDK | DON content | R source | Traits associated |
|---------------|----------|-------------|--------------|-----------|-----|-------------|----------|------------------|
|               |          |             |              | 2010      | 2011| 2012 Mean   |          |                  |
| TO 2A         | 78.4–90.4| 1219210     | 1004513      | 2.6       | 3.4 | 3.3         | 2.7      | 4.7              | T     | AE               |
| 4B            | 13.4–30.5| 1092528     | Rht-B1       | 11.2      | 13.7| 15.2        | 16.6     | 14               | 12.9  | O, PH, AE        |
| 4D            | 0–16.5   | 1059032     | Rht-D1       | 7.5       | 15.7| 9.6         | 20.4     | 5.8              | T     | PH, AE           |
| 5A            | 62.0–72.4| 1129347     | 2260918      | 29.6      | 17.5| 7.0         | 21.2     | 5.4              | 17.3  | T, DH, PH        |
|               |          |             |              | 2010 Mean | 2012 Mean |          |          |                  |
| Accumulated percentage of variation explained | 43.4 | 42.1 | 41.2 | 51.2 | 42.5 | 22.0 | 18.7 |
| IO 1B         | 17.7–22.6| 100007924   | 1024654      | 5.3       | 8.2 | 6.5         | I        |                  |
| 2A            | 124.1–131.3| 1027267   | 1694741      | 6.5       | 7.8 | 7.6         | 7.3      | I, AE            |
| 3B, 2         | 53.5–82.1| 997675      | 2268570      | 2.5       | 6.4 | 5.7         | I        | AE               |
| 3B, 3         | 78.6–85.0| 1001892     | 2278701      | 9.2       | 7.4 | I           |          |                  |
| 4B            | 33.2–65.5| Rht-B1      | 1238830      | 20.4      | 25.4| 7.9         | 6.6      | O, PH, AE        |
| 4D            | 0.0–26.4 | Rht-D1      | 993587       | 3.7       | 17.1| 5.9         | 15.2     | 4.8              | 5.7   | I, PH, AE        |
| 5A            | 238.8–275.1| 1067537   | 3064895      | 12.2      | 21.1| 17.1        | 13.7     | 7.6              | I     | DH               |
| 5B            | 156.7–168.6| 1066241   | 1216740      | 3.7       | 3.7 | 2.3         | 5.4      | I                |       |
| Accumulated percentage of variation explained | 21.2 | 45.9 | 46.3 | 42.3 | 46 | 36.7 | 17.7 | 35.9 |

The percentage of explained phenotypic variation in the multiple regression models is shown, QTL are listed if they were over the LOD threshold of 3 (in bold) in at least one environment or over the threshold of 2 in multiple environments.

*a* In the case of the IO population, DON content was measured in 2011  
*b* ‘TRAP#1/BOW//Taigu derivative’, ‘Ivan/Soru#2’, ‘Ocoroni F86’  
*c* AE anther extrusion, PH plant height, DH days to heading.

**PLOS ONE** | **DOI**: 10.1371/journal.pone.0162499 | September 8, 2016
5AL QTL was not identified in 2012 and the Rht-B1 QTL was significant mainly in 2012. The two dwarfing genes showed similar negative effects on FHB resistance in the two populations, although the phenotypic variations explained by Rht-B1 were often a bit higher than those by Rht-D1 (Table 3). A QTL on 2AL was also shared by the two populations based on common markers, but it was only a minor QTL accounting for phenotypic variations less than 10% (Table 3). Additional minor QTL were found in the IO population, located on 1BL, 3BL (LG 3B_2), 3BS (LG 3B_3) and 5BL (Table 3). It could be observed that for DON in the TO population, QTL on 2AL and 5AL in 2010 were significant, but the ones at Rht-B1 and Rht-D1 were identified in 2012 (Table 3), explaining the non-significant ‘genotype’ effect and low heritability estimate for this trait (Table 1).
Several QTL for AE were localized and three were shared by the two populations, viz. Rht-B1, Rht-D1 and a QTL on 2AL (Table 4, Fig 2), all associated with FHB resistance (Table 3). Additional QTL were found on 2BL in the TO population and on 2DS and 3BL in the IO population (Table 4). The two dwarfing genes collectively explained around 20% of AE reduction in both populations and Rht-B1b was always more strongly associated with reduced AE than Rht-D1b (Table 4). As for PH, Rht-B1 and Rht-D1 collectively accounted for about 60% variation in the two populations, while additional QTL were found on 5AL (Vrn-A1) and 7B in the TO population and on 5BS in the IO population (Table 5).

Table 4. QTL for anther extrusion in the ‘TRAP#1/BOW//Taigu derivative’ x ‘Ocoroni F86’ (TO) and ‘Ivan/Soru#2’ x ‘Ocoroni F86’ (IO) populations.

| Linkage group | Position | Left marker | Right marker | Anther extrusion | Source of elongation |
|---------------|----------|-------------|--------------|------------------|---------------------|
| TO 2A         | 100.6–102.1 | 984869 | 3064488 | 9.8 | 7.8 | 10.5 | T |
| 2B            | 244.0–247.0 | 1127943 | 1125516 | 5.2 | 4.3 | 5.6 | T |
| 4B            | 28.5–31.3 | 3064743 | Rht-B1 | 10.1 | 8.8 | 11.1 | O |
| 4D            | 0–16.5 | Rht-D1 | 1059032 | 8.4 | 6.6 | 8.7 | T |

Accumulated percentage of variation explained 33.5, 27.5, 35.9.

IO 2A            | 117.0–128.1 | 1128135 | 1019498 | 11.7 | 6.9 | 10.6 | I |
| 2D            | 4.5–5.6 | 2261713 | 984698 | 3.8 | 5.4 | 5.4 | O |
| 3B_2          | 53.5–82.1 | 997675 | 2268570 | 6.9 | 10 | 9.7 | I |
| 4B            | 33.2–65.5 | Rht-B1 | 123830 | 7.8 | 16.2 | 13.2 | O |
| 4D | 0.0–26.4 | Rht-D1 | 993587 | 5.2 | 6.9 | 6.9 | I |

Accumulated percentage of variation explained 35.5, 45.4, 45.8.

The percentage of explained phenotypic variation in the multiple regression models is shown, QTL are listed if they were over the LOD threshold of 3 (in bold) in at least one environment or over the threshold of 2 in multiple environments.

a In the case of the IO population, AE was evaluated in 2011 and 2012
b T ‘TRAP#1/BOW//Taigu derivative’, I ‘Ivan/Soru#2’, O ‘Ocoroni F86’.

doi:10.1371/journal.pone.0162499.t004

Table 5. QTL for plant height in the ‘TRAP#1/BOW//Taigu derivative’ x ‘Ocoroni F86’ (TO) and ‘Ivan/Soru#2’ x ‘Ocoroni F86’ (IO) populations.

| Linkage group | Position | Left marker | Right marker | Plant height | Source of tallness |
|---------------|----------|-------------|--------------|--------------|-------------------|
| TO 4B         | 28.5–31.3 | 3064743 | Rht-B1 | 20.5 | 25.6 | 22.7 | O |
| 4D | 0–16.5 | Rht-D1 | 1059032 | 35.4 | 35.9 | 35.1 | T |
| 5A            | 70.9–71.7 | 1135154 | 2262549 | 14.3 | 5.2 | 9.6 | T |
| 7B            | 87.3–97.8 | 1018730 | 977335 | 3.5 | 3.2 | O |

Accumulated percentage of variation explained 70.2, 70.2, 70.6.

IO 4B | 16.8–33.2 | 998452 | Rht-B1 | 30.4 | 30.8 | 31.1 | O |
| 4D | 0–26.4 | Rht-D1 | 993587 | 28.7 | 25.2 | 27.6 | I |
| 5B | 0–1.68 | 2282143 | 1255587 | 5.0 | 3.0 | 4.0 | I |

Accumulated percentage of variation explained 64.1, 59, 62.7.

The percentage of explained phenotypic variation in the multiple regression models is shown, QTL are listed if they were over the LOD threshold of 3 (in bold) in at least one environment.

a In the case of the IO population, PH was measured in 2011 and 2012
b T ‘TRAP#1/BOW//Taigu derivative’, I ‘Ivan/Soru#2’, O ‘Ocoroni F86’.

doi:10.1371/journal.pone.0162499.t005
Discussion

FHB index after field spray inoculation was generally considered as for a combination of Type I and Type II resistance; but in our study it appeared that mainly the former took place considering the significantly high correlation of FHB with PH and AE (Fig 1) that did not happen in point inoculated experiments for Type II resistance [11]. Therefore, we considered that the results obtained in this study were based mainly on Type I resistance.

Dwarfing genes Rht-B1 and Rht-D1 and the vernalisation gene Vrn-A1 were segregating in both populations used in this study, resulting in that most of the phenotypic variation for FHB parameters was explained by these three loci, whereas other QTL only explained a small fraction of the variation (Table 3). The latter category comprised QTL with phenotypic effects below 10%, which were likely known QTL based on their locations [7].

The association between the two dwarfing genes and FHB susceptibility has been reported in many studies, and three possible mechanisms including disease escape, pleiotropy and tight linkage have been proposed; but a conclusion has not been drawn as to which mechanism was actually taking place. Intuitively, this could be ascribed to PH per se or escape since tall plants were farther from soil surface where the inoculum was present (in the case of natural infection or spawn inoculation where FHB infected grain kernels were scattered in the field as inoculum) and ventilation was reduced that lead to high humidity favourable to FHB development [28, 49]. This mechanism must have contributed to the association in this study since the correlation remained significant in the sub-populations with homozygous Rht-B1 and Rht-D1 alleles (data not shown); despite the utilization of spray inoculation in this study, huge quantity of Fusarium inoculum was present on soil surface due to the adoption of wheat/maize rotation and conservation agricultural practices, supporting the escape mechanism. With the accumulation of molecular evidences in the last decade, more researchers took pleiotropy as the main mechanism for this association. Being DELLA protein producers, Rht-B1b and Rht-D1b have shown association with reduced resistance to biotrophic diseases including Type I FHB resistance (although FHB is regarded as a necrotrophic disease, it behaves more like a biotrophic disease at the early stages when Type I resistance takes place) but increased resistance to necrotrophic diseases like Type II FHB resistance [27]. Another evidence for the genetic effects of dwarfing genes instead of disease escape was that in sub-populations with homozygous Rht alleles, the correlation between PH and FHB disappeared or was significantly reduced [17, 21, 50], which was obviously not the case of the current study. However, this does not necessarily mean that the pleiotropic effects had no impacts on FHB in our study; it could function through controlling AE, which will be further explained below. Due to the limitation of map resolution, currently it is very difficult to separate pleiotropy and tight linkage; nevertheless clues supporting the latter have been reported. In the Soissons x Orvantis mapping population, Srinivasachary et al. [23] found that the peaks of FHB QTL were constantly located in a short distance away from the Rht-D1 locus. Similarly in our previous research, a QTL for FHB in close linkage with Rht-D1 appeared when PH was used as covariate [39]. So it appears that all the three mechanisms exist, but they are not necessarily simultaneously present in a single wheat line and their different combinations are expected.

The importance of AE in FHB resistance has long been recognized [29, 32], but genetic studies on AE were performed only in the last few years [8, 11, 38, 39]. In all these studies, the accumulated phenotypic variation explained by identified QTL for AE rarely exceeded 50% (usually around 30%), in accordance with the current study (Table 4), demonstrating a typical quantitative inheritance of AE. In the aforementioned four studies, totally 18 AE QTL have been identified, but only the QTL on 7AL found by Skinnes et al. [8] and Lu et al. [11] may be the same, and the one found on 4AL in He et al. [39] could be the same as reported by
Buerstmayr and Buerstmayr [38]. In the current study, six more AE QTL were found (Table 4), and not unexpectedly only the one on 2DS might be the same as found in our previous study [39], whereas others were all from new chromosome regions. Similar to previous results, four out of the six AE QTL were associated with FHB resistance (Tables 3 and 4), supporting the phenotypic association of the two traits.

The two dwarfing genes showed consistent effects on reducing AE in both populations across environments, collectively contributing about 20% of AE variation. The association may have its physiological basis. In Arabidopsis, the elongation of anther filament is stimulated by GA and repressed by DELLA proteins which are orthologous to wheat Rht-I gene products [51]. Thus it is reasonable to speculate that the GA insensitive mutants Rht-B1b and Rht-D1b in wheat have similar function in repressing anther elongation through over expression of DELLA proteins, resulting in the phenotype of anther retention or low AE. This finding partly explained the pleiotropic effects of Rht-B1b and Rht-D1b on Type I FHB susceptibility, i.e. the two dwarfing genes lead to low AE, which in turn caused increased Type I FHB susceptibility. The results have also implications for hybrid wheat breeding, in which the selection of male parent, the pollen provider, is very important. A good male parent is expected to have high AE and high pollen production, and these two traits were reported to be significantly positively associated with \( r = 0.82 \) by Joppa et al. [52] and was later validated by Johnson and Patterson [53] and Atashi-Rang and Lucken [54]. Thus the utilisation of wild type Rht alleles Rht-B1a and Rht-D1a will improve both traits. Still more, the tall stature of such lines is favourable for efficient pollination, since it was suggested that the male parent be taller than the female parent in hybrid seed production [55].

Supporting Information

S1 Table. Linkage map information for the ‘TRAP#1/BOW//Taigu derivative’ x ‘Ocoroni F86’ population.

(TXT)

S2 Table. Linkage map information for the ‘Ivan/Soru#2’ x ‘Ocoroni F86’ population.

(TXT)

Acknowledgments

Financial support by the CGIAR Research Program on Wheat and ‘Seeds of Discovery’-Sustainable Modernization of Traditional Agriculture project (MasAgro) is gratefully acknowledged. The first author was financially supported by a CGIAR scholarship, provided by the Research Council of Norway, through NFR project 208340/H30—Breeding for Fusarium resistance in wheat. The authors are grateful to Prof. Åsmund Bjørnstad, Norwegian University of Life Sciences, for his kind instruction on AE phenotyping, and to Francisco Lopez, Javier Segura and Nerida Lozano for technical assistance.

Author Contributions

**Conceptualization:** PS ED XH.

**Formal analysis:** XH.

**Methodology:** XH PS ED SD SS.

**Resources:** ED PS ML SD SS.

**Writing – review & editing:** XH ML PS.
References

1. Bai GH, Shaner G. Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol. 2004; 42:135–61. doi: 10.1146/annurev.phyto.42.040803.140340 PMID: IS0002242799000007.

2. Duveiller E, Mezzalama M, Legreve A. Good management practices for minimizing the risk of Fusarium head blight and mycotoxin contamination in nontraditional warmer wheat-growing areas. In: Leslie J, Logriec A, editors. Mycotoxin Reduction in Grain Chains: John Wiley & Sons, Inc.; 2014. p. 220–31.

3. Buerstmayr H, Anderson JA. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed. 2009; 128(1):1–26. PMID: IND4156637.

4. Gilbert J, Haber S. Overview of some recent research developments in Fusarium head blight of wheat. Can J Plant Pathol. 2013; 35(2):149–74.

5. Mesterhazy A. Types and components of resistance to Fusarium head blight of wheat. Plant Breeding. 1995; 114(5):377–86. PMID: IS0001995TY45300001.

6. Schroeder HW, Christensen JJ. Factors affecting resistance of wheat to scab caused by Gibberella zeae. Phytopathology. 1963; 53(7):831–8. PMID: IS0001A196318222C00036.

7. Liu SY, Hall MD, Griffey CA, McKendry AL. Meta-analysis of QTL associated with Fusarium head blight resistance in wheat. Can J Plant Sci. 2009; 49:1955–68. doi:10.2135/cropsci2009.03.0115 PMID: IS0002716073000002.

8. Skinnes H, Semagn K, Tarkegne Y, Marey AG, Bjørnstad Å. The inheritance of anther extrusion in hexaploid wheat and its relationship to Fusarium head blight resistance and deoxynivalenol content. Plant Breeding. 2010; 129(2):149–55.

9. Mao S-L, Wei Y-M, Cao W, Lan X-J, Yu M, Chen Z-M, et al. Confirmation of the relationship between plant height and Fusarium head blight resistance in wheat (Triticum aestivum L.) by QTL meta-analysis. Euphytica. 2010; 174(3):343–56. doi: 10.1007/s10681-010-0128-9

10. Emrich K, Wilde F, Miedaner T, Piepho HP. REML approach for adjusting the Fusarium head blight rating to a phenological date in inoculated selection experiments of wheat. Theor Appl Genet. 2008; 117(1):65–73. doi: 10.1007/s00122-008-0753-z PMID: IS0002565260900008.

11. Lu Q, Lillemo M, Skinnes H, He X, Shi J, Ji F, et al. Another extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line ‘Shanghai-3/Catbird’. Theor Appl Genet. 2013; 126(2):317–34. Epub 10/12. doi: 10.1007/s00122-012-1981-9 PMID: 23052019.

12. Couture L. Réceptivité de cultivars de céréales de printemps à la contamination des graines sur inflorescence par les Fusarium spp. Can J Plant Sci. 1982; 62:29–34.

13. Zhu H, Gilchrist L, Hayes P, Kleinhoofs A, Kudrna D, Liu Z, et al. Does function follow form? Principal QTLs for Fusarium head blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a doubled-haploid population of barley. Theor Appl Genet. 1999; 99(7–8):1221–32. PMID: IS0000840149000016.

14. He X, Skinnes H, Oliver RE, Jackson EW, Bjørnstad A. Linkage mapping and identification of QTL affecting deoxynivalenol (DON) content (Fusarium resistance) in oats (Avena sativa L.). Theor Appl Genet. 2013; 126:2655–70. doi:10.1007/s00122-013-2163-0 PMID: 23959525.

15. Hedden P. The genes of the Green Revolution. Trends Genet. 2003; 19(1):5–9. PMID: 12493241.

16. Hilton AJ, Jenkinson P, Hollins TW, Parry DW. Relationship between cultivar height and severity of Fusarium ear blight in wheat. Plant Pathology. 1999; 48(2):202–8.

17. Draeger R, Gosman N, Steed A, Chandler E, Thomsett M, Srinivasachary, et al. Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor Appl Genet. 2007; 115(5):617–25. PMID: IND43944076.

18. Holzapfel J, Voss HH, Miedaner T, Korzun V, Haberle J, Schweizer G, et al. Inheritance of resistance to Fusarium head blight in three European wheat populations. Theor Appl Genet. 2008; 117(7):1119–28. doi:10.1007/s00122-008-0850-z PMID: 18670751.

19. Srinivasachary, Gosman N, Steed A, Simmonds J, Leverington-Waite M, Wang Y, et al. Susceptibility to Fusarium head blight is associated with the Rht-D1b semi-dwarfing allele in wheat. Theor Appl Genet. 2008; 116(8):1145–53. PMID: IND44052285. doi:10.1007/s00122-008-0742-7

20. Miedaner T, Voss HH. Effect of dwarfing rht genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Sci. 2008; 48(6):2115–22. doi:10.2135/cropsci2008.02.0107 PMID: IS0002615990000007.

21. Lu Q, Szabo-Hever A, Bjørnstad A, Lillemo M, Semagn K, Mesterhazy A, et al. Two major resistance quantitative trait loci are required to counteract the increased susceptibility to Fusarium head blight of the Rht-D1b dwarfing gene in wheat. Crop Sci. 2011; 51(6):2430–8. doi:10.2135/cropsci2010.12.0671
Skinnes H, Tarkegne Y, Dieseth JA, Bjornstad A. Associations between anther extrusion and Fusarium head blight resistance in European soft winter wheat. Mol Breed. 2011; 28(4):647–55. doi: 10.1007/s11032-010-9516-2. PMID: IS0:000297173800020.

23. Srinivasachary, Gosman N, Steed A, Hollins TW, Bayles R, Jennings P, et al. Semi-dwarfing Dwarfing Genes Associated with Type I FHB Susceptibility and Low Anther Extrusion in Wheat. Theor Appl Genet. 2009; 118(4):695–702. PMID: IND44163392. doi: 10.1007/s00122-008-0930-0

24. Buerstmayr M, Huber K, Heckmann J, Steiner B, Nelson JC, Buerstmayr H. Mapping of QTL for Fusarium head blight resistance and morphological and developmental traits in three backcross populations derived from Triticum dicoccum x Triticum durum. Theor Appl Genet. 2012; 125(8):1751–65. Epub 2012/08/29. doi: 10.1007/s00122-012-1951-2. PMID: 22926291; PubMed Central PMCID: PMC3493669.

25. Lv C, Song Y, Yao Q, Zhou R, Xu R, et al. Integration of QTL detection and marker assisted selection for improving resistance to Fusarium head blight and important agronomic traits in wheat. The Crop Journal. 2014; 2(1):70–8. http://dx.doi.org/10.1016/j.cj.2013.10.004

26. Liu S, Grifflce CA, Hall MD, McKendry AL, Chen J, Brooks WS, et al. Molecular characterization of field resistance to Fusarium head blight in two US soft red winter wheat cultivars. Theor Appl Genet. 2013; 126:2485–98. doi: 10.1007/s00122-013-2149-y. PMID: 23832049

27. Saville RJ, Gosman N, Burt CJ, Makepeace J, Steed A, Corbitt M, et al. The ‘Green Revolution’ dwarfing genes play a role in disease resistance in Triticum aestivum and Hordeum vulgare. J Exp Bot. 2012; 63(3):1271–83. doi: 10.1093/jxb/erz350. PMID: 22904435; PubMed Central PMCID: PMC3276090.

28. Yan W, Li HB, Cai SB, Ma HA, Rebetzke GJ, Liu CJ. Effects of plant height on type I and type II resistance to Fusarium head blight in wheat. Plant Pathology. 2011; 60(3):506–12. doi: 10.1111/j.1365-3059.2011.02426.x

29. Pugh GW, Johann H, Dickson J. Factors affecting infection of wheat heads by Gibberella saubietii. J Agric Res. 1933; 46(9):771–97.

30. Strange RN, Smith H. A fungal growth stimulant in anthers which predisposes wheat to attack by Fusarium graminearum. Physiol Plant Pathol. 1971; 1(2):141–50. doi: 10.1016/0048-4059(71)90023-3

31. Strange RN, Major JR, Smith H. The isolation and identification of choline and betaine as the two major components in anthers and wheat germ that stimulate Fusarium graminearum in vitro. Physiol Plant Pathol. 1974; 4(2):277–90. doi: 10.1016/0048-4059(74)90015-0

32. Strange R, Deramo A, Smith H. Virulence enhancement of Fusarium graminearum by choline and betaine of Botrytis cinerea by other constituents of wheat germ. Transactions of the British Mycological Society. 1978; 70(2):1–7.

33. Skinnes H, Tarkegne Y, Dieseth JA, Bjornstad A. Associations between anther extrusion and Fusarium head blight in European wheat. Cereal Res Commun. 2008; 36:223–31. doi: 10.1556/Crc.36.2008.Suppl.B: .19. PMID: IS0:000259563800054.

34. Graham S, Browne RA. Anther extrusion and Fusarium head blight resistance in European wheat. J Phytopathol. 2009; 157(9):580–2. doi: 10.1111/j.1439-0434.2008.01524.x. PMID: IS0:000268707300009.

35. Osman M, He X, Singh RP, Duveiller E, Lillemo M, Pereyra SA, et al. Phenotypic and genotypic characterization of CIMMYT’s 15th international Fusarium head blight screening nursery of wheat. Euphytica. 2015; 205(2):521–37. doi: 10.1007/s10681-015-1425-0

36. He X, Singh PK, Schlang N, Duveiller E, Dreisigacker S, Payne T, et al. Characterization of Chinese wheat germplasm for resistance to Fusarium head blight at CIMMYT, Mexico. Euphytica. 2014; 195:383–95. doi: 10.1007/s10681-013-1002-3

37. Kubo K, Fujita M, Kawada N, Nakajima T, Nakamura K, Maejima H, et al. Minor differences in anther extrusion affect resistance to Fusarium head blight in wheat. J Phytopathol. 2013; 161(5):308–14.

38. Buerstmayr M, Buerstmayr H. Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo x Arina. Theor Appl Genet. 2015; 128(8):1519–30. doi: 10.1007/s00122-015-2527-8. PMID: 25982129; PubMed Central PMCID: PMC4477076.

39. He X, Lillemo M, Shi J, Wu J, Bjømstad A, Belova T, et al. QTL Characterization of Fusarium Head Blight Resistance in CIMMYT Bread Wheat Line Soru#1. Plos One. 2016; 11(6):e0158052. doi: 10.1371/journal.pone.0158052. PMID: 27351632

40. Buerstmayr H, Blöch D, Hasitschka S, Buerstmayr M, Maloku I, Schweiger W, et al. Association between anther-retention and Fusarium head blight susceptibility in wheat. Jahrestagung der Pflanzenzüchter und Saatgutkaufleute Österreichs. Raumberg-Gumpenstein2013. p. 69.
41. Langer SM, Longin CFH, Würschum T, Pillen K. Phenotypic evaluation of floral and flowering traits with relevance for hybrid breeding in wheat (Triticum aestivum L.). Plant Breed. 2014; 133(4):433–41. doi: 10.1111/pbr.12192

42. He X, Singh PK, Duveiller E, Schlang N, Dreisigacker S, Singh RP. Identification and characterization of international Fusarium head blight screening nurseries of wheat at CIMMYT, Mexico. Eur J Plant Pathol. 2013; 136(1):123–34. doi: 10.1007/s10658-012-0146-7

43. Stack RW, McMullen MP. A visual scale to estimate severity of Fusarium head blight in wheat. North Dakota State University Extension Service 1994. p. 1095.

44. Li H, Vikram P, Singh RP, Kilian A, Carling J, Song J, et al. A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. BMC Genomics. 2015; 16(1). doi: 10.1186/s12864-015-1424-5

45. Dreisigacker S, Zuniga LP, Navarro JC. Development of gene specific SNP assays open a new perspective on marker assisted selection in wheat. In: Dreisigacker S, Singh S, editors. Proceedings of the 21st International Triticeae Mapping Initiative Workshop. Mexico City, Mexico. 2011. p. 105.

46. Van Ooijen J. JoinMap® IV, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV: Wageningen, Netherlands. 2006.

47. Van Ooijen J. MapQTL® VI, Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma BV: Wageningen, Netherlands. 2009.

48. Voorrips RE. MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered. 2002; 93(1):77–9. Epub 2002/05/16. PMID: 12011185.

49. Klahr A, Zimmermann G, Wenzel G, Mohler V. Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in an European winter wheat cross. Euphytica. 2007; 154(1–2):17–28. doi: 10.1007/s10681-006-9264-7 PMID: ISI:000244191900003.

50. Voss HH, Holzapfel J, Hartl L, Korzun V, Rabenstein F, Ebmeyer E, et al. Effect of the Rht-D1 dwarfing locus on Fusarium head blight rating in three segregating populations of winter wheat. Plant Breed. 2008; 127(4):333–9. doi: 10.1111/j.1439-0523.2008.01518.x PMID: 180257151000002.

51. Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, et al. Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development. 2004; 131(5):1055–64. doi: 10.1242/dev.00992 PMID: 1500244191900003.

52. Joppa LR, McNeal FH, Berg MA. Pollen production and pollen shedding of hard red spring (Triticum aestivum em Thell.) and durum (T. durum Desf.) wheats. Crop Sci. 1968; 8(4):487–90. doi: 10.2135/cropsci1968.0011183X00800040028x

53. Johnson J, Patterson F. Pollen production of fertility restored lines of soft red winter wheats. Crop Sci. 1973; 13(1):92–5.

54. Atashi-Rang G, Lucken KA. Variability, combining ability, and interrelationships of anther length, anther extrusion, glume tenacity, and shattering in spring wheat. Crop Sci. 1978; 18(2):267–72. doi: 10.2135/cropsci1978.0011183X00800040028x

55. de Vries AP. Some aspects of cross-pollination in wheat (Triticum aestivum L.) 1. Pollen concentration in the field as influenced by variety, diurnal pattern, weather conditions and level as compared to the height of the pollen donor. Euphytica. 1972; 21(2):185–203.