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ORIGINAL ARTICLE

Discovery of a susceptibility factor for Fusarium head blight on chromosome 7A of wheat

Bhavit Chhabra[1] · Vijay Tiwari[1] · Bikram S. Gill[2] · Yanhong Dong[3] · Nidhi Rawat[1]

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Abstract

Key message **Discovery and mapping of a susceptibility factor located on the short arm of wheat chromosome 7A whose deletion makes plants resistant to Fusarium head blight.**

Abstract Fusarium head blight (FHB) disease of wheat caused by *Fusarium* spp. deteriorates both quantity and quality of the crop. Manipulation of susceptibility factors, the plant genes facilitating disease development, offers a novel and alternative strategy for enhancing FHB resistance in plants. In this study, a major effect susceptibility gene for FHB was identified on the short arm of chromosome 7A (7AS). Nullisomic–tetrasomic lines for homoeologous group-7 of wheat revealed dosage effect of the gene, with tetrasomic 7A being more susceptible than control Chinese Spring wheat, qualifying it as a genuine susceptibility factor. Five chromosome 7A inter-varietal substitution lines and a tetraploid *Triticum dicoccoides* 7A substitution line showed similar susceptibility as that of Chinese Spring, indicating toward the commonality of the susceptibility factor among these diverse genotypes. The susceptibility factor was named as *Sf-Fhb-7AS* and mapped on chromosome 7AS to a 48.5–50.5 Mb peri-centromeric region between del7AS-3 and del7AS-8. Our results showed that deletion of *Sf-Fhb-7AS* imparts 50–60% type 2 FHB resistance and its manipulation can be used to enhance resistance against FHB in wheat.

Introduction

Fusarium head blight (FHB) is one of the most destructive diseases of wheat worldwide affecting the yield and safety of grain. Severe outbreaks of FHB may cause up to 50% yield loss, in addition to the serious risk to grain safety because of the associated mycotoxins (Snijders 1990; Parry et al. 1995). In the USA alone, during 1991–1996, FHB outbreaks caused an estimated $3 billion crop loss (McMullen et al. 1997). During the 2015–16 crop year, economic loss due to FHB in the USA was estimated at $1.2 billion (Wilson et al. 2018). FHB is caused by a complex of *Fusarium* species (Parry et al. 1995). In North America, the predominant causal organism is *Fusarium graminearum* (McMullen et al. 1997; Goswami and Kistler 2004). In wheat, typical symptoms are pre-mature bleaching of infected spikelets resulting in aborted or shriveled seeds and hence, reduced yield. Associated mycotoxins, such as deoxynivalenol (DON), endanger safety of the grain (Snijders 1990; Chen et al. 2019). DON is phytotoxic and can cause wilting, chlorosis and necrosis (Cutler 1988). It inhibits protein synthesis in mammals by binding to 60S subunit of eukaryotic ribosomes (Rocha et al. 2005). US Food and Drug Administration has set an advisory limit of 1 ppm DON for wheat and barley products for human consumption, 10 ppm for cattle and poultry, 5 ppm for swine and all other animals (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-and-fda-advisory-levels-deoxynivalenol-don-finished-wheat-products-human accessed Oct 5, 2020). In wheat, DON acts as a virulence factor for *Fusarium*, helping the pathogen to colonize host tissue (Bai et al. 2002; Jansen et al. 2005).

Integrated management practices incorporating genetic resistance, chemical and agronomic control measures are used for controlling FHB (Wegulo et al. 2010; Salgado et al. 2014). Genetic resistance is the most economical, environment-friendly and effective component of the overall

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✉ Nidhi Rawat
nidhirwt@umd.edu

[1] Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742, USA

[2] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA

[3] Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

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strategy to control FHB (Bai and Shaner 2004). Mesterházy et al. (1999) described five types of genetic resistance to FHB, out of which, Type 1 (resistance to initial infection) and Type 2 (resistance to spread within the spike) are most widely studied in wheat. Type 2 resistance is less affected by non-genetic variables as compared to Type 1 resistance (Bai and Shaner 1994). Resistance against FHB is quantitatively controlled. Around 500 QTL (104 major effect) providing varying levels of resistance have been reported in the literature (Buerstmayr et al. 2019). *Fhb1*, the first quantitative trait locus (QTL) for resistance against FHB in wheat, was discovered in 1999 and has been extensively investigated (Waldron et al. 1999; Rawat et al. 2016; Su et al. 2019; Li et al. 2019). Another FHB resistance QTL *Fhb7*, derived from *Thinopyrum elongatum*, was characterized as a glutathione S-transferase gene (Wang et al. 2020). However, even after decades of efforts, achieving high levels of FHB resistance in wheat varieties using these QTL has been a challenge (Buerstmayr et al. 2019; Gorash et al. 2020). This necessitates exploring alternative strategies to engineer FHB resistance in wheat.

Plant genes that facilitate pathogen infection and colonization may be manipulated for enhancing resistance of plants against pathogens (van Schie and Takken 2014; Fabre et al. 2020). Eckardt (2002) first introduced the term 'susceptibility factors' for such genes while describing Arabidopsis powdery mildew susceptible mutant *pmr6*. A well-known example of a susceptibility gene used in crop breeding is the barley *mlo* gene. This loss of function mutation has provided broad spectrum resistance against powdery mildew for over 35 years in many plant species (Büschges et al. 1997; Engelhardt et al. 2018). Susceptibility factors can be divided into three categories based on their role during different stages of infection: (1) help pathogen in establishment; (2) involved in modulating/regulating plant defenses; (3) involved in providing nutrition to the pathogen (van Schie and Takken 2014). Manipulating susceptibility genes by knocking out or knocking down their expression provides a novel and alternative breeding strategy for protection against pathogens (Fabre et al. 2020). Genetically, susceptibility factors can be considered as dominant genes whose manipulation will lead to recessive resistance (Pavan et al. 2009). Benefits of utilizing susceptibility genes are that the resistance acquired is recessive, broad spectrum and more durable as compared to dominant resistant genes (Gorash et al. 2020).

Garvin et al. (2015) reported that deletion of ~ 19% of long arm of chromosome 3D in a susceptible cultivated variety Apogee increased FHB resistance of the resulting lines by ~ 59%. Likewise, Hales et al. (2020) reported a 31.7 Mb region on the short arm of chromosome 4D to contain prospective FHB susceptibility factors, whose deletion made plants resistant to the disease. Ma et al. (2006) screened a set of 30 ditelosomic (Dt) lines of Chinese Spring wheat for their response to *F. graminearum* infection and found that the genotypes varied in their response to FHB. Five out of thirty ditelosomic lines (Dt6BS, Dt4DL, Dt7BL, Dt3BS and Dt7AL) had lower proportion of scabby spikelets ($p < 0.01$) and four lines (Dt6AL, Dt6DS, Dt4DL and Dt7AL) had significantly less DON content ($p < 0.05$) as compared to Chinese Spring control. Dt7AL (missing 7AS) showed lowest amount of FHB severity as well as minimal DON content in their study (Ma et al. 2006). This may be because of the presence of either a potential susceptibility factor(s) or a negative regulator of FHB resistance on 7AS, as several small to large effect FHB resistance QTL have been reported on the long arm of chromosome 7A (Semagn et al. 2007; Qi et al. 2008; Zhang et al. 2010).

In the present work, we explored the putative susceptibility factor(s) present on chromosome 7AS of Chinese Spring indicated by Ma et al. (2006) with the following four objectives: (1) confirmation of the effect of deletion of chromosome 7AS on plants' FHB response, (2) study of the effect of enhancing dosage of 7A on FHB response, (3) study of the effect of 7A chromosome substitution from 6 diverse wheat varieties/species and (4) deletion bin-mapping of the susceptibility factor on 7AS. If chromosome 7A harbors a susceptibility factor, whose deletion enhances FHB resistance in multiple varieties, it will be a good target of manipulation for developing a durable broad-spectrum resistance in wheat varieties.

Materials and methods

Plant material

Experiments were conducted over three years (2018, 2019 and 2020) in Research Greenhouse Complex, University of Maryland, College Park. Plant materials used in the experiments are listed in Table 1. All of the plant materials used were in Chinese Spring genetic background; therefore, wild-type Chinese Spring was used as a positive control in all the experiments. Temperature conditions were 23–25 °C during daytime and 16–18 °C during nighttime. Photoperiod profile of 16 h (day): 8 h (night) was used. Five plants for each line were grown in 6″ pots. Nulli-tetrasomic lines and ditelosomic lines were tested only in year 2020, whereas deletion and substitution lines were analyzed in all three sets (2018, 2019 and 2020). Del7AS-6 and del7AS-3 could not be tested in year 2019 because of some technical mishaps. A subset of deletion lines critical for locating the susceptibility factor was tested again in an additional fourth set in 2020 along with control Chinese Spring.

Table 1 Description of plant materials used in the study

Accession[a]	Description	Abbreviation[b]
TA 3008	Chinese Spring	CS
TA 3110	Chinese Spring Ditelosomic 7AS	CS Dt7AS
TA 3111	Chinese Spring Ditelosomic 7AL	CS Dt7AL
TA 3121	Chinese Spring Ditelosomic 7BS	CS Dt7BS
TA 3122	Chinese Spring Ditelosomic 7BL	CS Dt7BL
TA 3130	Chinese Spring Ditelosomic 7DS	CS Dt7DS
TA 3071	Chinese Spring Ditelosomic 7DL	CS Dt7DL
TA 3282	Chinese Spring Nulli 7A-Tetra 7B	CS N7A-T7B
TA 3283	Chinese Spring Nulli 7B-Tetra 7A	CS N7B-T7A
TA 3281	Chinese Spring Nulli 7A-Tetra 7D	CS N7A-T7D
TA 3285	Chinese Spring Nulli 7D-Tetra 7A	CS N7D-T7A
TA 3221	Chinese Spring-Timstein Disomic Substitution 7A T(7A CS)	CS-T DS7A
TA 3447	Chinese Spring-Dicoccoides Disomic Substitution 7A TDIC(7A CS)	CS-TDIC DS7A
TA 3242	Chinese Spring-Cheyenne Disomic Substitution 7A CNN(7A CS)	CS-CNN DS7A
TA 3782	Chinese Spring-Thatcher Disomic Substitution 7A TH(7A CS)	CS-TH DS7A
TA 3200	Chinese Spring-Hope Disomic Substitution 7A H(7A CS)	CS-H DS7A
TA 3451	Chinese Spring-Axminster Disomic Substitution 7A AM(7A CS)	CS-AM DS7A
TA 4536	Chinese Spring Deletion 7AS-1	CS del7AS-1
TA 4546	Chinese Spring Deletion 7AS-2	CS del7AS-2
TA 4546	Chinese Spring Deletion 7AS-3	CS del7AS-3
TA 4546	Chinese Spring Deletion 7AS-4	CS del7AS-4
TA 4546	Chinese Spring Deletion 7AS-5	CS del7AS-5
TA 4546	Chinese Spring Deletion 7AS-6	CS del7AS-6
TA 4546	Chinese Spring Deletion 7AS-8	CS del7AS-8
TA 4546	Chinese Spring Deletion 7AS-9	CS del7AS-9
TA 4546	Chinese Spring Deletion 7AS-10	CS del7AS-10
TA 4518	Chinese Spring Deletion 7AS-11	CS del7AS-11
TA 4546	Chinese Spring Deletion 7AS-12	CS del7AS-12

[a]Accession numbers of the Wheat Genetics Resource Center (WGRC), Kansas State University

[b]Abbreviations according to Raupp (1995)

Marker development, PCR conditions and Gel electrophoresis

For monitoring the identity and size of deletions in the set of deletion lines used, genome-specific markers were developed every 10 Mb of 7AS arm, selecting gene sequences at 10 Mb interval on the Chinese Spring wheat reference genome sequence version 1.0 (IWGSC 2018). To cover the entire ~ 360 Mb-long chromosome 7AS (IWGSC 2018), a total of 36 markers were designed starting from the telomeric end of the chromosome (Table 2). Genome-specific primer design software GSP (Wang et al. 2016) was used with default settings for designing primers specific to chromosome 7A. A touchdown polymerase chain reaction profile from 64 °C to 58 °C (95 °C for 5 min, 7 cycles of 95 °C for 45 s, 64–58 °C for 45 s with a decrease of 1 °C per cycle, 72 °C for 1 min, followed by 27 cycles of 95 °C for 45 s, 58 °C for 45 s, 72 °C for 1 min and a final extension of 72 °C for 7 min) was used. PCR product obtained was run on ethidium bromide stained 1.5–2% agarose gel for 1–1.5 h. and visualized under UV light.

Fungal inoculum preparation and inoculation

F. graminearum isolate GZ3639, which is known for its high virulence (Desjardins et al. 1997; Rawat et al. 2016), was used for inoculation in all the experiments. For macroconidia production, 3–4 plugs of Potato Dextrose Agar mycelial culture of the fungus were inoculated in Mung bean broth (MBB). For preparing MBB, 20 g of mung bean seeds, purchased from a local grocery store, were steeped in 500 ml of boiling distilled water for 20 min. The resulting liquid was filtered using a cheesecloth, autoclaved and cooled. After inoculation with fungal mycelial plugs, the broth was kept shaking at a speed of 200 rpm at 28 °C for 7–10 days. Macroconidia were counted on a hemocytometer and inoculum was prepared by diluting the culture to a concentration of 1×10^5 spores/ml using sterile water.

Table 2 Names and sequences of genome-specific primers used to genotype 7AS deletion lines

Primer name	Forward primer sequence 5′–3′	Reverse primer sequence 5′–3′
FHB-SF7AS-1-F	AACATTGGTGGTGAAATTCG	AATGATTCAAATTTATGGTGGC
FHB-SF7AS-2-F	AACAGGACCAACCGGTACTTCTC	TATGTAGTACGTACCTCGAGCGG
FHB-SF7AS-3-F	TTAAGCCACCACAAAACTCC	CGTCCCTCTGCCTGAGACTATC
FHB-SF7AS-4-F	TGTTTTATTGTCTGTTGCCTACG	AGTCTTGCTTAATTGAAGAGCG
FHB-SF7AS-5-F	ACCTCTCCGTGGTGTTGC	CTTCCACAAATTGCAACTCATC
FHB-SF7AS-6-F	CCTGAAAAGTATTGGAGGAGGC	AAGTGACAACAGTCCTCATGTGC
FHB-SF7AS-7-F	GTTTACTTGTGCTGAAGGAGGG	TCCACCATGTATCCAGAAATCG
FHB-SF7AS-8-F	ATGTTAAGCTCTGAAAGTGCTCG	ACTTCCTGCCCGAACGAG
FHB-SF7AS-9-F	CTGTCTACACTGCCATTTACACC	AAAATACTGCAAAGAGCAGCC
FHB-SF7AS-10-F	CAACAAAGATCTATGAGCCACTC	GGCATATGTAAGCAAACAACG
FHB-SF7AS-11-F	ACACAATCACACAACCACACAC	ATCATTAGTACATACCAGCAGGC
FHB-SF7AS-12-F	TTGTCTTTGTGGAATGTGATG	GACTGCGAGAAACCAATAGC
FHB-SF7AS-13-F	TGGTTTGGATGGAACTTGG	GATGTATAGACTGGCCAAGTAGC
FHB-SF7AS-14-F	AGCTCAAGGACAAGAAGTGC	CACCATGTGATGAGTGATCC
FHB-SF7AS-15-F	CTACTCCAAGTTGCCTGGTG	TCTCGTCTTCGCTGTCGTC
FHB-SF7AS-16-F	CTTTTACGGTCCATCACTTACC	TAAGGAGGGAGTATCGTCCTG
FHB-SF7AS-17-F	ACTGCAGGAAATATCCACTAACC	TGGGGGACCAAAATATAATGC
FHB-SF7AS-18-F	GTTCGTGTCTCCCTCTTCCC	CTCCCTGTGGATGATTCG
FHB-SF7AS-19-F	AGAGGTTGTAGGCTTTCCG	CTGGACAGAGAAGATGGTTAGG
FHB-SF7AS-20-F	AGGGTGGTGATCAAGTCTTGTG	GCGTGCAAACTCTCTCTGG
FHB-SF7AS-21-F	ACTCGAGAAGCAGGAGCG	CATGAAGATGTCCACAGCGG
FHB-SF7AS-22-F	CGTCCATAATTCAGAGGACATCG	GTCTGTTCTTTTCAGTCGGCTC
FHB-SF7AS-23-F	GCACCTACAGTACCTGACAGC	GTCAAAGCTTCAGGACGGTG
FHB-SF7AS-24-F	TCCTGCAGGGTAGGAGTACTTG	GTGATCCCATTCTCATCTAGCAG
FHB-SF7AS-25-F	TCTCGGTACCGTTCAGGTAG	GCAGCTCAGCTCAACACAG
FHB-SF7AS-26-F	GCAGCTCAGCTCAACACAG	AGGCCAAATAGGTATATGAGGCA
FHB-SF7AS-27-F	TGACAGACTTCTCTAGGATCACC	AGAGGATGTTTCCTCCACACAC
FHB-SF7AS-28-F	TCCGTTCCAAGTTGGTAGTGC	TGACCGAGAATATCTCTTGTGCT
FHB-SF7AS-29-F	TCCAGCAGTTAATCATGCAGC	CAGGATGGCGATGATGACC
FHB-SF7AS-30-F	CTCCGTTTATAAAAGTGAAGCTG	ATTACTGTACTGCAGCGAAGG
FHB-SF7AS-32-F	TAAAGTCTTGAAAAGCAATCGTG	TTCAGTTCAGGATCGTCATCAAG
FHB-SF7AS-34-F	AGGTTTGGCCGGTCTGGT	CGAGGGAGTACTAATGATTGGC
FHB-SF7AS-36-F	AATGGAGAGCCTTCAGCGTG	TGTTTGGTAGATAATGAACGGTG

Inoculation was performed at pre-anthesis stage on spikes, which was about 2 days prior to anthers emerging out of the spikes. Tenth and eleventh spikelets counted from the base of the spikes were marked with a black sharpie pen, and 10 µl macroconidial inoculum was injected between the lemma and palea of the florets (one floret/spikelet), avoiding injury to any other part of the florets. Spikes were covered with moisture saturated zip lock bags for 72 h to provide high humidity for optimal fungal growth. Each genotype had 5 plants (1 plant/pot). All plants in Chinese Spring genetic background produced 10–15 tillers in our growing conditions, out of which initial 5–6 healthy spikes were used per plant for inoculations. Therefore, a total of 25–30 spikes were inoculated for each genotype in each experiment.

FHB severity, AUDPC and FDKs

FHB severity in the inoculated plants was recorded at 14, 21 and 28 days after inoculation (dai) and was found to be maximum at 28 dai in Chinese Spring control. Therefore, final comparisons of FHB severity for all the experiments were made at 28 dai in all the experiments. FHB severity was recorded by counting the number of bleached spikelets, including the inoculated 10th spikelet, downward from the point of inoculation. To calculate the percentage of symptomatic spikelets (PSS), the number of bleached spikelets below the point of inoculation was divided by 10 and converted to a percentage. To compare the FHB severity progression among different genotypes, area under the disease progress curve (AUDPC) values were calculated from the

average of PSS at 14, 21 and 28 dai for each genotype. Formulae given by Simko and Piepho (2011) were used for calculating AUDPC values:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left[\left(Y_i + Y_{i+1}\right)/2\right]\left(T_i - T_{i+1}\right)$$

where Y_i is the average of PSS (in percentage) at the ith observation, T_i is time (days) at the ith observation, and n is the total number of observations.

Seeds were manually threshed individually from each inoculated spike after maturity and bulked by genotypes. Fusarium damaged kernels (FDKs) were measured for each genetic line by counting visually scabby kernels among all the threshed seeds of that line in the particular experiment and converting into percentage values.

DON content

DON content of seeds was measured at USWBSI DON-testing laboratory at the University of Minnesota by GC/MS following Mirocha et al. (1998). Each sample was analyzed in three technical replications. Briefly, seeds from inoculated spikes of each genotype were pooled from different plants, and then ground to a fine powder. Each pooled genotype powder was divided in three technical replications of 1 g each. Each 1 g sample was extracted with 10 mL of acetonitrile/water (84/16, v/v) in 50 mL centrifuge tubes. Each sample was placed on a shaker for 24 h, and then, 4 ml of the extract was passed through a column packed with C18 and aluminum oxide (1/3, w/w). Two milliliter of the filtrate was evaporated to dryness under nitrogen at room temperature, and 100 µl of trimethylsilyl (TMS) reagent (TMSI/TMCS, 100/1,v/v) was added to the vial, rotating the vial so that the reagent makes contact with residue on the sides of the vial. The vial was placed on a shaker for 10 min, and then, 1 mL of isooctane containing 500 ng/mL mirex was added and shaken gently. HPLC water (1 ml) was added to quench the reaction and the vial was vortexed so that the milky isooctane layer became transparent. The upper layer was transferred into a GC vial for GC/MS analysis (Shimadzu GCMS-QP2020, Shimadzu Corporation, Kyoto, Japan), and readings were recorded.

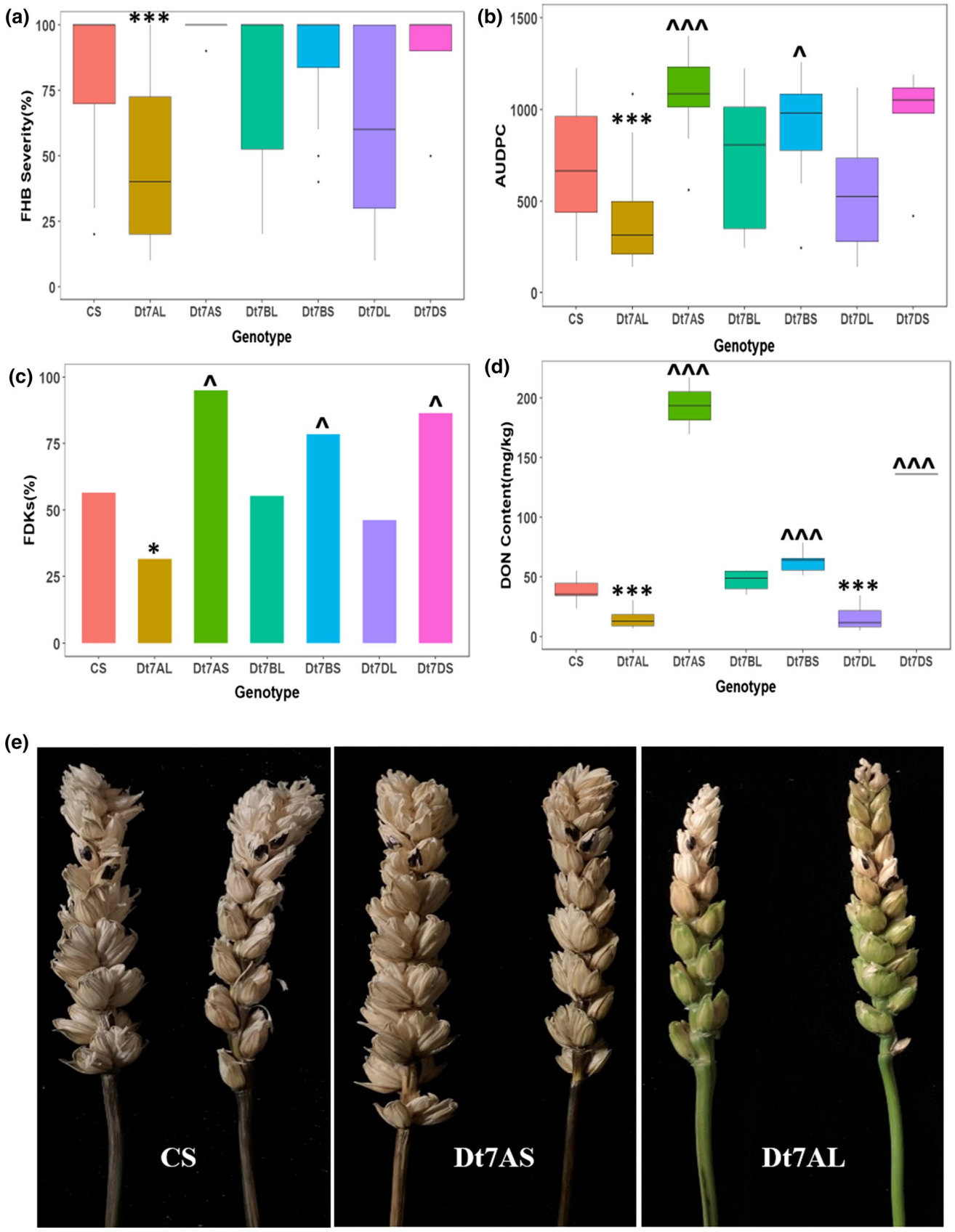
Statistical analysis

Data were analyzed in R (vR x64 3.6.3), R studio and Excel for all sets of experiments. All the experiments were conducted in a completely randomized design. R packages *lme4*, *car*, *ggplot2* were used for data analysis, ANOVA and making graphs, respectively, for all the recorded parameters. Parameters analyzed were: FHB severity, AUDPC, FDKs and DON content. Each spike tested was considered as an individual replicate. Each data set was first tested for normality and homogeneity of error variances before doing analysis. Normality was checked by plotting QQ-plots and performing Shapiro–Wilk tests. Homogeneity of error variances was checked by plotting residual vs fitted plots and performing Levene test. Experiments on nulli-tetrasomic and ditelosomic lines were performed only once in 2020, hence there is no variable year. For FHB severity, PSS was taken as dependent variable, whereas genotype was considered as independent variable with fixed effect. Kruskal–Wallis rank sum test was performed for 28 dai as this data set did not meet ANOVA assumptions of normality and homogeneity of error variances.

For FHB severity data of deletion lines and substitution lines, PSS was taken as dependent variable, whereas genotype and year were considered as independent variables. Both genotype and year were considered as fixed effect. A two-way analysis of variance (ANOVA) with interaction was performed for 28 dai over three years. For deletion lines, Type-1 two-way ANOVA was performed as we lacked two genotypes (del7AS-3 and del7AS-6) in 2019 set due to technical reasons; however, for substitution lines, type-3 two-way ANOVA was performed. Data points where significant Genotype * Year interaction was found, a simple one-way ANOVA was performed separately for each year. Type-3 one-way ANOVA was performed for both deletion and substitution lines. Data from which residuals were not normally distributed or did not appear independent of fitted values were log10 transformed, in order to meet the assumptions of data analysis.

In order to make pair-wise comparisons between control Chinese Spring and other genotypes tested in each season for all the experiments, a post hoc test (Dunnett test if analysis conducted by ANOVA or Dunn test if analysis conducted by Kruskal–Wallis rank sum test) was performed. For analysis of AUDPC values and DON content, same set of procedures was followed as that for FHB severity data of deletion lines for all the experiments. Data for DON content and AUDPC values which did not meet assumptions of ANOVA were square root transformed, in order to meet the assumptions of data analysis.

For analysis of FDK values, a single sample t test with unknown standard deviation was used as only one replicate/genotype was available for all experiments. First, standard deviation was calculated by considering whole data set. **A critical t value was obtained from the t-table on the basis of degrees of freedom and at $\alpha = 0.05$. t value was calculated between control and other genotypes separately for each experiment.


(a)
FHB Severity(%)
100
75
50
25
0
***
CS
Dt7AL
Dt7AS
Dt7BL
Dt7BS
Dt7DL
Dt7DS
Genotype
(b)
AUDPC
1500
1000
500
0
***
^^^
^
Genotype
(c)
FDKs(%)
*
^
^
^
Genotype
(d)
DON Content(mg/kg)
200
150
100
50
0
***
^^^
^^^
***
^^^
Genotype
(e)
CS
Dt7AS
Dt7AL

◂**Fig. 1** FHB response of the Chinese Spring (CS) control and ditelosomic lines of group-7 chromosomes. *X* axis denotes the genotypes, and *Y* axis denotes the parameters tested. **a** FHB Severity (%); **b** AUDPC values; **c** FDKs(%); **d** DON content(mg/kg); and **e** infected spikes of control and ditelosomic lines of 7A (photographs taken at 28 days after inoculation). *Depicts values lower than control Chinese Spring at $p<0.05$, and ***at $p<0.001$. ^Depicts higher significance values over control Chinese Spring at $p<0.05$, and ^^^at $p<0.001$. Horizontal line over the individual box plots depicts the median values

Results

Short arm of chromosome 7A carries a potential FHB susceptibility factor

Chinese Spring group 7 ditelosomic stocks lacking different arms of the group 7 chromosomes (Dt7AS-lacking 7AL, Dt7AL-lacking 7AS, Dt7BS-lacking 7BL, Dt7BL-lacking 7BS, Dt7DS-lacking 7DL and Dt7DL-lacking 7DS) were evaluated for their FHB response to confirm the presence of a potential FHB susceptibility factor as indicated in the survey by Ma et al. (2006). The overall FHB severity of ditelosomic lines varied from 10 to 100%. Significant genotypic differences at $p=1.99e^{-09}$ with Chi-square value of 51.859 were observed at 28 dai (Supplementary Table S1a). Chinese Spring parent had an average FHB severity of 80%. With an average FHB severity of 49%, Dt7AL had significantly less FHB severity at $p<0.001$, whereas all other ditelosomics were found to be statistically similar to control (Fig. 1a, e; Supplementary Fig. S1b). Dt7DL had an average FHB severity of 62%, which was numerically lower, but statistically similar to Chinese Spring control.

A one-way ANOVA for AUDPC revealed significant genotypic effect at $p<0.001$ (Supplementary Table S1b). Dt7AL showed significantly lower AUDPC values at $p<0.001$, whereas Dt7AS and Dt7BS had significantly higher AUDPC values at $p<0.001$ and $p<0.05$, respectively, compared to control (Fig. 1b). Dt7AL also showed significantly lower FDKs, whereas Dt7AS, Dt7BS and Dt7DS had significantly higher FDKs than control at $\alpha=0.05$ and Dt7BL and Dt7DL had FDKs similar to Chinese Spring (Fig. 1c).

For DON content, one-way ANOVA showed significant genotype effect at $p<0.001$(Supplementary Table S1c). Dt7AL and Dt7DL showed significantly lower DON content at $p<0.001$, whereas Dt7AS, Dt7BS and Dt7DS had significantly higher DON content at $p<0.001$ in comparison to control (Fig. 1d).

These results are in agreement with Ma et al. (2006) and indicate toward the presence of a major effect susceptibility factor(s) on the short arm of chromosome 7A. DON results of Dt7DL also indicate possibility of a homoeoallele of the 7AS factor or another weak susceptibility factor on 7DS influencing DON content (Fig. 1d).

7AS FHB susceptibility factor has a dosage effect

Several minor to major effect QTL have been reported on 7A long arm of wheat (Semagn et al. 2007; Qi et al. 2008; Zhang et al. 2010). So, the resistance in dt7AL may be due to the presence of either a negative regulator of resistance on 7AL, or a genuine susceptibility factor. It would be appropriate to call the underlying factor a susceptibility factor, if increasing the copies of 7A makes the plants more susceptible as compared to parental Chinese Spring (dosage effect). For this purpose, nullisomic–tetrasomic lines for chromosome 7A were evaluated for their response to *F. graminearum* infection.

FHB severity of nulli-tetrasomic stocks for chromosome 7A ranged from 10 to 100%. Kruskal–Wallis test explained statistical significance at $p=2.3643e^{-07}$ with Chi-square value of 36.426 (Supplementary Table S2a). Dunn test results showed all four of the tested nulli-tetra lines to be significantly different from control. N7A-T7B and N7A-T7D both showed significantly lower disease than Chinese Spring control, whereas N7B-T7A and N7D-T7A had significantly higher disease severity at $p<0.01$ (Fig. 2a, e), indicating that the underlying factor is a genuine susceptibility factor.

A one-way ANOVA for AUDPC showed significant genotype effect at $p<0.001$ (Supplementary Table S2b). Compared to control, N7A-T7B ($p<0.01$) and N7A-T7D ($p<0.05$) were found to have significantly lower AUDPC values, whereas N7B-T7A ($p<0.01$) and N7D-T7A ($p<0.001$) had significantly higher values (Fig. 2b). All nulli-tetrasomic lines had statistically similar FDKs as that of control Chinese Spring at $\alpha=0.05$; however, N7A-T7B and N7A-T7D had numerically lower FDKs among all the nullisomic–tetrasomic genotypes tested (Fig. 2c).

For DON content, a one-way ANOVA showed significant genotype effect at $p<0.001$ (Supplementary Table S2c). N7A-T7B had significantly lower DON at $p<0.05$ as compared to Chinese Spring control. N7B-T7A and N7D-T7A showed significantly higher DON content at $p<0.001$ and $p<0.05$, respectively, than Chinese Spring control. DON content of N7A-T7D was numerically lower, but statistically similar to that of Chinese Spring (Fig. 2d).

In the above experiments, both N7A-T7B and N7A-T7D are missing chromosome 7A and both showed significantly higher level of resistance than control Chinese Spring. This indicates the presence of a potential susceptibility factor(s) on chromosome 7A affecting FHB severity and DON. Furthermore, since N7B-T7A and N7D-T7A plants have four copies of chromosome 7A and thus four doses of putative FHB susceptibility factor and these plants, as expected, showed higher level of susceptibility to FHB and higher DON content. The results showed that the action of susceptibility gene was affected by chromosome 7A dosage; the deletion of chromosome 7A made the plants

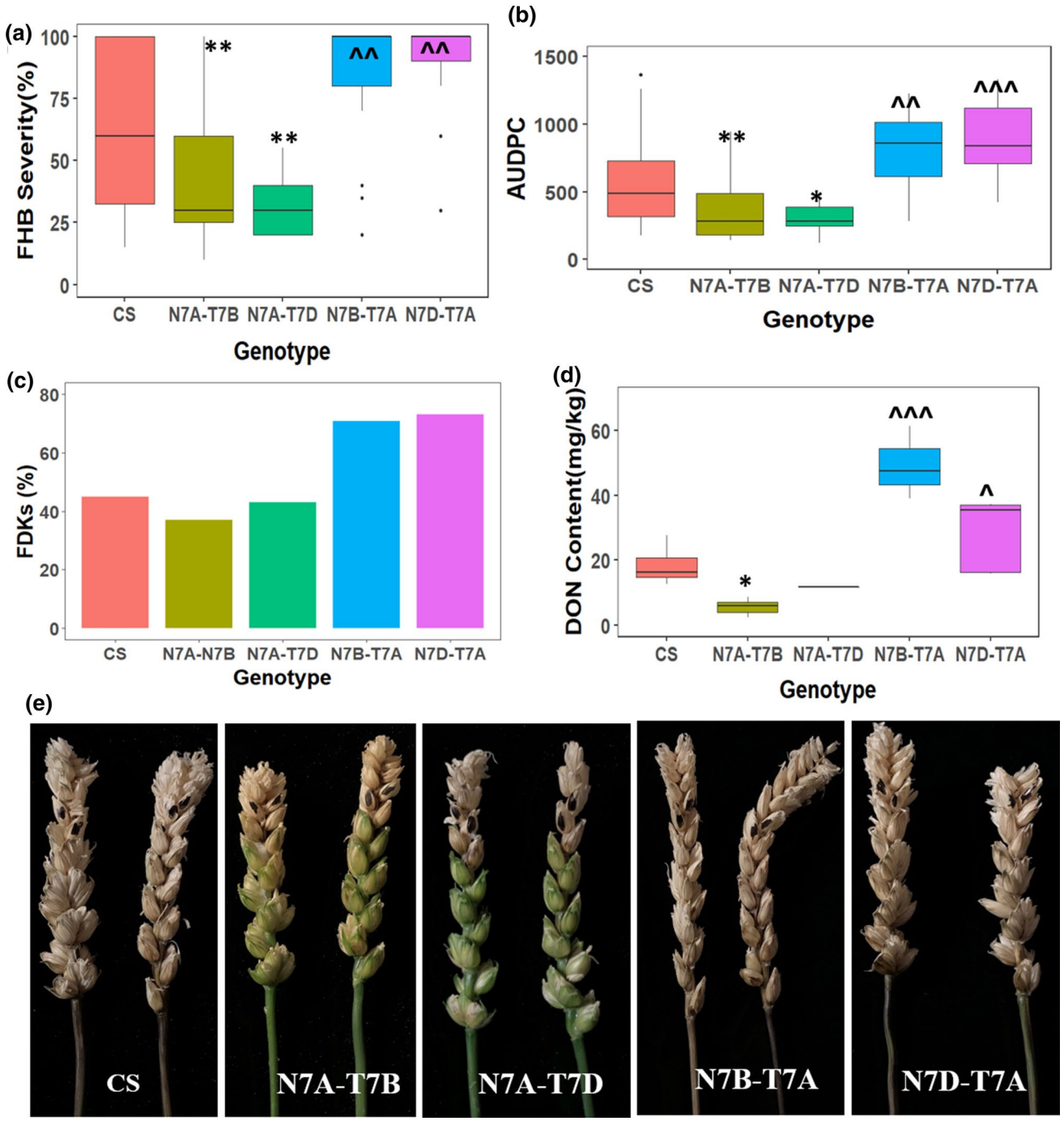


Fig. 2 FHB response of Chinese Spring (CS) control and chromosome 7A nullisomic–tetrasomic lines (*X* axis). *Y* axis denotes the parameters tested. **a** FHB Severity (%); **b** AUDPC values; **c** FDKs (%);**d** DON content (mg/kg); and **e** infected spikes of tested lines (photographs taken at 28 days after inoculation). *Depicts lower significance values than control Chinese Spring at $p < 0.1$, and **at $p < 0.01$. ^Depicts higher significance values over control Chinese Spring at $p < 0.05$, ^^at $p < 0.01$ and ^^^at $p < 0.001$. Horizontal line over the individual box plots depicts the median values

resistant, whereas extra copies of 7A made the plants more susceptible to FHB. Therefore, it is justified to call it an FHB susceptibility factor whose deletion increases resistance. The susceptibility factor was named *Sf-Fhb-7AS*.

The susceptibility factor is conserved on 7A chromosome of multiple wheat cultivars

In order to test whether this susceptibility factor is conserved

across multiple wheat genotypes or not, six disomic substitution lines: five derived from wheat cultivars and one from wild tetraploid emmer wheat (chromosome 7A of *T. dicoccoides* substituted chromosome 7A of Chinese Spring) were tested for their phenotypic response to FHB in all three years. The mean FHB severity values ranged from 52% (CS-H DS7A)-79% (CS-AM DS7A) in 2018; 53% (CS-CNN DS7A)-69% (CS-TH DS7A) in 2019; and 58% (CS-CNN DS7A)-89% (CS-TDIC DS7A) in 2020. At 28 dai, a two-way ANOVA of FHB severity showed significant Genotype*Year interaction ($p < 0.05$) in the data set and no significant genotype effect (Supplementary Table S3a). Therefore, one-way ANOVA was not conducted separately for each year. All substitution lines had statistically similar FHB severity values to those of Chinese Spring control (Fig. 3a, e). Mean values of FHB severity observed in 2020 were highest among the three years for all the lines.

A two-way ANOVA of AUDPC with interaction for all three years of data showed significant genotype effect, year effect and Genotype*Year interaction at $p < 0.05$ (Supplementary Table S3b). As G*E effect was found, one-way ANOVA was calculated separately for each year. Data set from years: 2018 ($p < 0.001$) and 2020 ($p < 0.001$) showed significant genotype effect (Supplementary Tables S3c, S3d and S3e). For 2018, no substitution line was found to be statistically different from control. However, for 2020, two lines (CS-TDIC DS7A at $p < 0.001$ and CS-AM DS7A at $p < 0.01$) had significantly higher AUDPC values than control (Fig. 3b).

In the three years of testing, all the six analyzed substitution lines had similar or higher FDK values than those of control Chinese Spring. Four lines (CS-T DS7A, CS-CNN DS7A, CS-TH DS7A, CS-AM DS7A) in 2018, one line (CS-TDIC DS7A) in 2019 and five lines (CS-T DS7A, CS-TDIC DS7A, CS-TH DS7A, CS-H DS7A, CS-AM DS7A) in 2020 had higher FDKs over control at significance level of $\alpha = 0.05$ (Fig. 3c).

DON content measurement was done only for year 2020, and the DON values ranged from 9–79 mg/kg in the seven genotypes (Fig. 3d). A one-way ANOVA showed significant genotype effect ($p < 0.001$) (Supplementary Table S3f). All the substitution lines, except CS-CNN DS7A, showed significantly higher DON content than control $p < 0.001$. CS-CNN DS7A showed DON content similar to that of Chinese Spring (Fig. 3d).

These results indicate that the susceptibility factor(s) located in Chinese Spring is conserved across the 7A chromosomes of all the studied genotypes.

Sf-Fhb-7AS is located in the peri-centromeric region of chromosome 7AS

Molecular characterization of deletion lines

For deletion bin mapping of the susceptibility factor on 7AS, the deletion stock developed by Endo and Gill (1996) in Chinese Spring background was used. These deletion lines were originally sorted by Endo and Gill (1996) on the basis of the fraction length (FL) of retained chromosome using cytogenetic staining techniques as: del7AS-1 (FL = 0.89), del7AS-9 (FL = 0.89), del7AS-12 (FL = 0.83), del7AS-2 (FL = 0.73), del7AS-5 (FL = 0.59), del7AS-8 (FL = 0.45), del7AS-10 (FL = 0.45), del7AS-11 (FL = 0.33), del7AS-3 (FL = 0.29), del7AS-4 (FL = 0.26), del7AS-6 (FL = 0.21). To locate the exact Mb position of the physical boundaries of these deletion lines, A–genome-specific PCR-based molecular markers were developed 1 per 10 Mb along the short arm of chromosome 7A using the Chinese Spring Reference sequence assembly (IWGSC 2018). Genome specificity of the developed markers was confirmed using Chinese Spring and Chinese Spring N7A-T7B. Out of 36 markers developed, 33 were found to be specific for chromosome 7A. These confirmed genome-specific markers were then tested on all eleven deletion lines, and the results are shown in Table 3. Markers FHB-SF7AS-1, FHB-SF7AS-2 and FHB-SF7AS-3 were found to amplify only control Chinese Spring revealing terminal ~ 30 Mb to be deleted in all the deletion lines. Serially, FHB-SF7AS-4 was the first marker to amplify on del7AS-12 (FL = 0.83), and absent in all other deletion lines, showing del7AS-12 to have retained the maximum segment of chromosome 7AS among the deletion lines. The sizes of deletions for all the lines were deduced in a similar way (Table 3). The order of deletion lines deduced was found to be similar with the cytogenetic map of Endo and Gill (1996).

Deletion line del7AS-10 was found to have a small interstitial deletion in addition to its major deletion. Further application of more 7AS-specific PCR markers on del7AS-10 characterized the size of interstitial deletion in it to be ~ 1 Mb in size between 148.4 and 149.2 Mb on reference sequence of 7AS (Supplementary Table S4 and S5).

Deletion bin mapping of *Sf-Fhb-7AS*

To map *Sf-Fhb-7AS* to a specific chromosome interval, the eleven overlapping deletion lines of chromosome 7A short arm were tested for their FHB response in 2018, 2019 and 2020. Control Chinese Spring was found to be susceptible in all three years. A two-way ANOVA of FHB severity at 28 dai found significant genotype effect ($p < 0.001$) and Genotype * Year effect ($p < 0.001$) (Supplementary Table S6a). One-way ANOVA revealed significant genotype effect for all three years ($p < 0.01$ for 2018 and 2019; $p < 0.001$ for

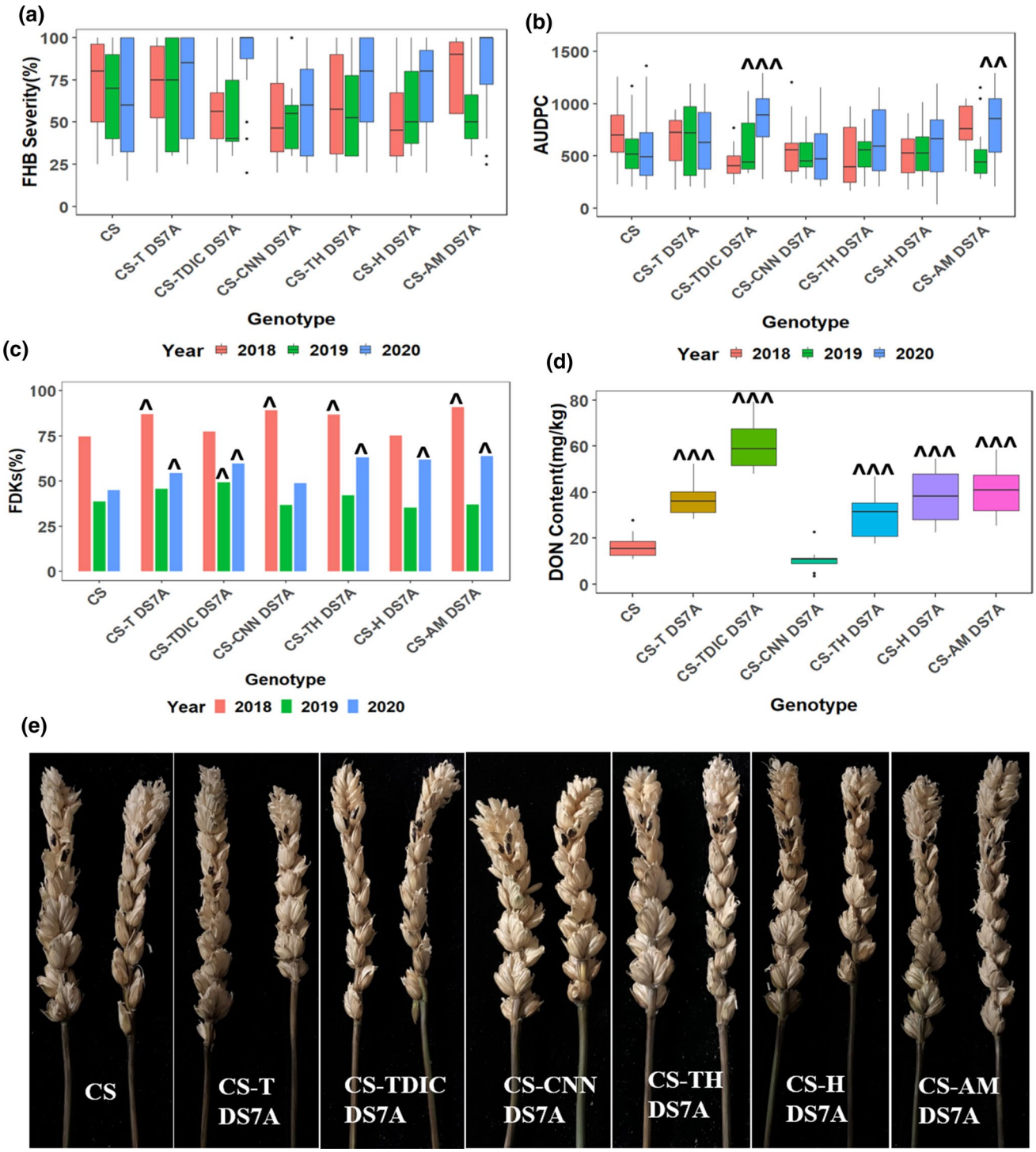


Fig. 3 FHB response of the Chinese Spring (CS) control and substitution lines. *X* axis denotes the genotypes, and *Y* axis denotes the parameters tested. **a** FHB Severity (%); **b** AUDPC values; **c** FDKs (%); **d** DON content (mg/kg); and **e** infected spikes of tested lines (photographs taken at 28 days after inoculation). ^Depicts higher significance values over control Chinese Spring at $p < 0.05$, ^^at $p < 0.01$ and ^^^at $p < 0.001$. Horizontal line over the individual box plots depicts the median values

2020; Supplementary Tables S6b, S6c and S6d). Mean FHB severity at 28 dai ranged from 22% (del7AS-4) to 73% (Chinese Spring) in 2018; 31% (del7AS-4) to 69% (del7AS-1) in 2019; and 26% (del7AS-6) to 88% (del7AS-11) in 2020 (Fig. 4a). It is important to note that del7AS-6 and del7AS-4 have peri-centromeric deletions and had significantly lower

Table 3 Marker data on Chinese Spring (positive control), Nulli 7A (negative control) and deletion lines

Primer/genotype	CS	N-7A	7AS-12	7AS-1	7AS-9	7AS-2	7AS-5	7AS-11	7AS-10	7AS-8	7AS-3	7AS-4	7AS-6
FHB-SF7AS-1	+	−	−	−	−	−	−	−	−	−	−	−	−
FHB-SF7AS-2	+	−	−	−	−	−	−	−	−	−	−	−	−
FHB-SF7AS-3	+	−	−	−	−	−	−	−	−	−	−	−	−
FHB-SF7AS-4	+	−	+	−	−	−	−	−	−	−	−	−	−
FHB-SF7AS-5	+	−	+	+	+	−	−	−	−	−	−	−	−
FHB-SF7AS-6	+	−	+	+	+	−	−	−	−	−	−	−	−
FHB-SF7AS-7	+	−	+	+	+	−	−	−	−	−	−	−	−
FHB-SF7AS-8	+	−	+	+	+	−	−	−	−	−	−	−	−
FHB-SF7AS-9	+	−	+	+	+	−	−	−	−	−	−	−	−
FHB-SF7AS-10	+	−	+	+	+	+	−	−	−	−	−	−	−
FHB-SF7AS-11	+	−	+	+	+	+	−	−	−	−	−	−	−
FHB-SF7AS-12	+	−	+	+	+	+	−	−	−	−	−	−	−
FHB-SF7AS-13	+	−	+	+	+	+	−	−	−	−	−	−	−
FHB-SF7AS-14	+	−	+	+	+	+	+	−	−	−	−	−	−
FHB-SF7AS-15	+	−	+	+	+	+	+	+	−	−	−	−	−
FHB-SF7AS-16	+	−	+	+	+	+	+	+	−	−	−	−	−
FHB-SF7AS-17	+	−	+	+	+	+	+	+	−	−	−	−	−
FHB-SF7AS-18	+	−	+	+	+	+	+	+	−	−	−	−	−
FHB-SF7AS-19	+	−	+	+	+	+	+	+	−	−	−	−	−
FHB-SF7AS-20	+	−	+	+	+	+	+	+	+	−	−	−	−
FHB-SF7AS-21	+	−	+	+	+	+	+	+	+	−	−	−	−
FHB-SF7AS-22	+	−	+	+	+	+	+	+	−	−	−	−	−
FHB-SF7AS-23	+	−	+	+	+	+	+	+	+	+	−	−	−
FHB-SF7AS-24	+	−	+	+	+	+	+	+	+	+	−	−	−
FHB-SF7AS-25	+	−	+	+	+	+	+	+	+	+	−	−	−
FHB-SF7AS-27	+	−	+	+	+	+	+	+	+	+	+	+	−
FHB-SF7AS-28	+	−	+	+	+	+	+	+	+	+	+	+	−
FHB-SF7AS-29	+	−	+	+	+	+	+	+	+	+	+	+	−
FHB-SF7AS-30	+	−	+	+	+	+	+	+	+	+	+	+	−
FHB-SF7AS-32	+	−	+	+	+	+	+	+	+	+	+	+	+
FHB-SF7AS-34	+	−	+	+	+	+	+	+	+	+	+	+	+
FHB-SF7AS-36	+	−	+	+	+	+	+	+	+	+	+	+	+

Symbol '+' depicts presence and symbol '−' depicts absence of amplification with the specific primers. Markers are listed in the order of their physical location from the telomere to the centromere, and deletion lines are listed based on smallest to largest deletion of terminal 7AS segments

FHB severity in all three years. Telomeric, sub-telomeric and proximal deletion lines: del7AS-12, del7AS-9, del7AS-1, del7AS-2, del7AS-5, del7AS-11 and del7AS-8 were found to have either statistically similar or higher FHB severity as compared to Chinese Spring over all three years (Fig. 4a, e, and Supplementary Fig. S1b). Del7AS-10 showed FHB severity statistically similar to Chinese Spring in 2018 and 2019, and lower than control at $p < 0.01$ in 2020. Del7AS-3 showed significantly lower FHB severity than Chinese Spring ($p < 0.001$) in 2020, whereas in 2018, it had FHB severity similar to Chinese Spring. Because of the unclear patterns of del7AS-10 and del7AS-3 in both the years, an additional fourth set of exclusively the critical deletion lines (del7AS-10, del7AS-8, del7AS-3, del7AS-4 and del7AS-6) along with control Chinese Spring was tested again in 2020 for all the FHB parameters.

A two-way ANOVA of AUDPC for all three years showed significant genotype effect ($p < 0.001$) and Genotype * Year interaction ($p < 0.001$) (Supplementary Table S6e). A separate one-way ANOVA for each year revealed significant genotype effect at $p < 0.001$ (Supplementary Tables S6f, S6g, S6h). In 2018, del7AS-4 and del7AS-6 had significantly lower AUDPC values than Chinese Spring at $p < 0.001$. In 2019, only del7AS-4 showed significantly less AUDPC values at $p < 0.05$. In 2020, three lines del7AS-3, del7AS-4 and del7AS-6 had significantly

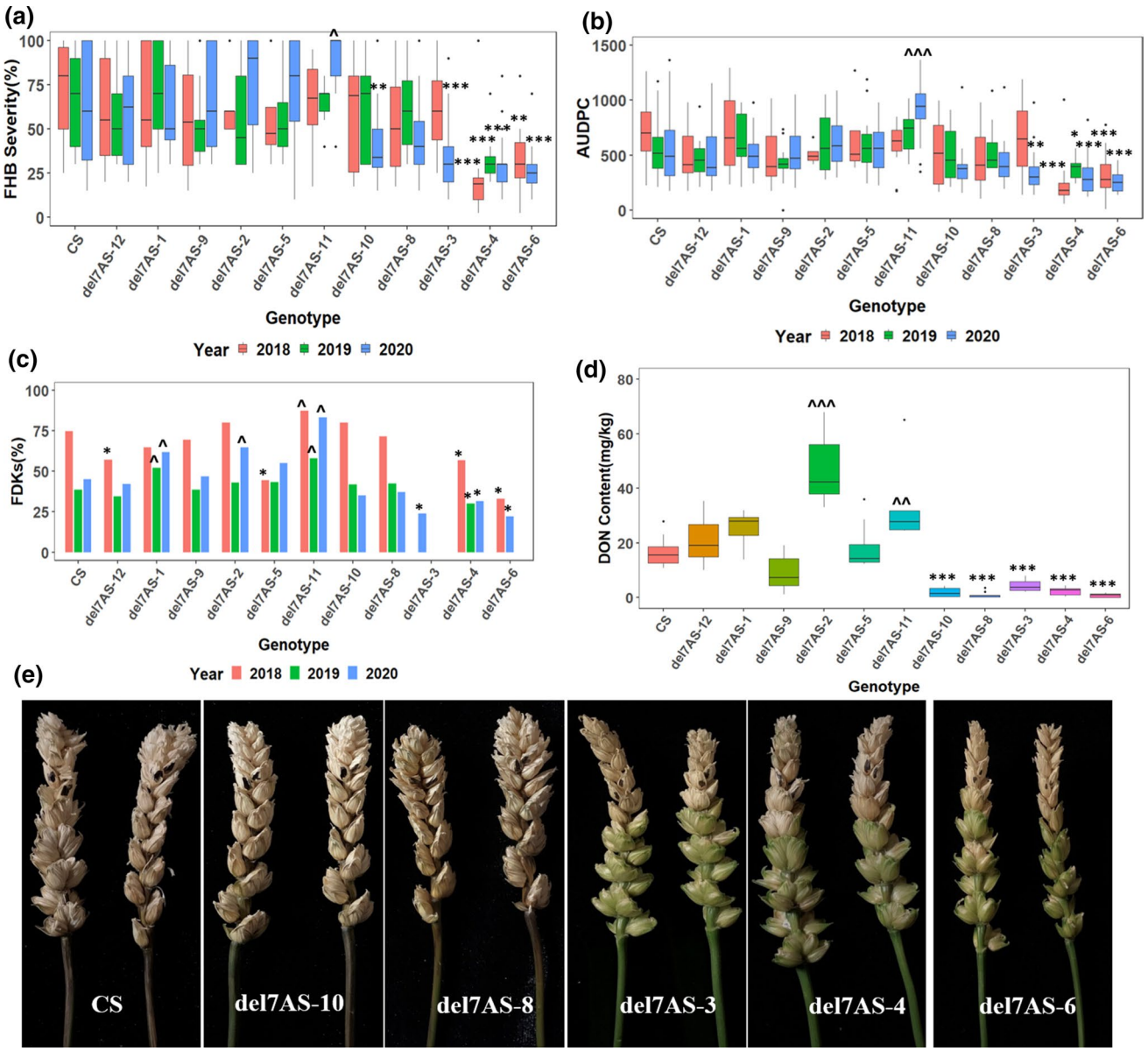


Fig. 4 FHB response of Chinese Spring (CS) control and tested deletion lines. *X* axis denotes the genotypes, and *Y* axis denotes the parameters tested. **a** FHB Severity (%); **b**: AUDPC values; **c** FDKs (%); **d** DON content (mg/kg); and **e** infected spikes of critical deletion lines (photographs taken at 28 days after inoculation). Lower significance values over control Chinese Spring are depicted with *at $p<0.05$, **at $p<0.01$ and ***at $p<0.001$. ^Depicts higher significance values over control Chinese Spring at $p<0.05$, ^^at $p<0.01$ and ^^^at $p<0.001$. Horizontal line over the individual box plots depicts the median values

lower AUPDC values, whereas del7AS-11 had higher AUPDC values over control Chinese Spring (Fig. 4b).

In 2018, four genotypes (del7AS-12, del7AS-5, del7AS-4 and del7AS-6) were found to have lower FDKs, whereas del7AS-11 had significantly more FDKs in comparison to Chinese Spring at $\alpha=0.05$. In 2019, del7AS-4 showed less FDKs and two genotypes (del7AS-1, del7AS-11) had significantly higher FDKs. In 2020, del7AS-3, del7AS-4 and del7AS-6 had significantly lower FDKs than Chinese Spring, whereas del7AS-1, del7AS-2 and del7AS-11 had significantly higher FDKs than that (Fig. 4c).

DON content measurement was done for year 2020 only. One-way ANOVA showed significant genotype effect at $p<0.001$ (Supplementary Table S6i). Two lines showed significantly higher DON than Chinese Spring (del7AS-11 at $p<0.01$ and del7AS-2 at $p<0.001$). Five deletion lines, namely del7AS-10, del7AS-8, del7AS-3, del7AS-4 and del7AS-6, were found to have significantly lower DON content at $p<0.001$ (Fig. 4d). DON content measurement

of these critical deletion lines was repeated in the additional fourth set in 2020 to reconfirm the patterns.

These three-year experiments localized the susceptibility factor *Sf-Fhb-7AS* to the peri-centromeric region of chromosome 7AS. Since the peri-centromeric deletion lines del7AS-10, del7AS-8, del7AS-3, del7AS-6 and del7AS-4 were critical in mapping the susceptibility factor and also we lost one year of data (in 2019) for a few of them due to a technical mishap, we tested a set of just these critical lines again in 2020 for their FHB response to robustly locate the susceptibility factor *Sf-Fhb-7AS*.

Confirmation of the peri-centromeric location of the major susceptibility factor using the critical deletion lines

At 28 dai, significant genotypic differences at $p = 4.165e{-}06$ with Chi-square value of 32.778 were observed (Supplementary Table S7a). Among the critical set of deletion lines tested, the three peri-centromeric deletion lines del7AS-6, del7AS-4 and del7AS-3 with mean FHB severity values of 22%, 33% and 27%, respectively, were found to have significantly lower disease spread as compared to Chinese Spring control. Del7AS-8 and del7AS-10 were statistically similar to control (Fig. 5a).

For AUDPC, a one-way ANOVA showed significant genotype effect at $p < 0.001$ (Supplementary Table S7b). Del7AS-10, del7AS-3 and del7AS-4, and del7AS-6 showed significantly lower AUDPC values than Chinese Spring control (Fig. 5b). Del7AS-8 was found to be statistically similar to control. Del7AS-3, del7AS-4 and del7AS-6 showed significantly lower FDKs than control at $\alpha = 0.05$, whereas del7AS-8 and del7AS-10 were similar in their FDK percentage to control Chinese Spring (Fig. 5c).

For DON content, Kruskal–Wallis test showed significant genotype effect at $p < 0.001$ with Chi-square value of 21.862 (Supplementary Table S7c). The three peri-centromeric

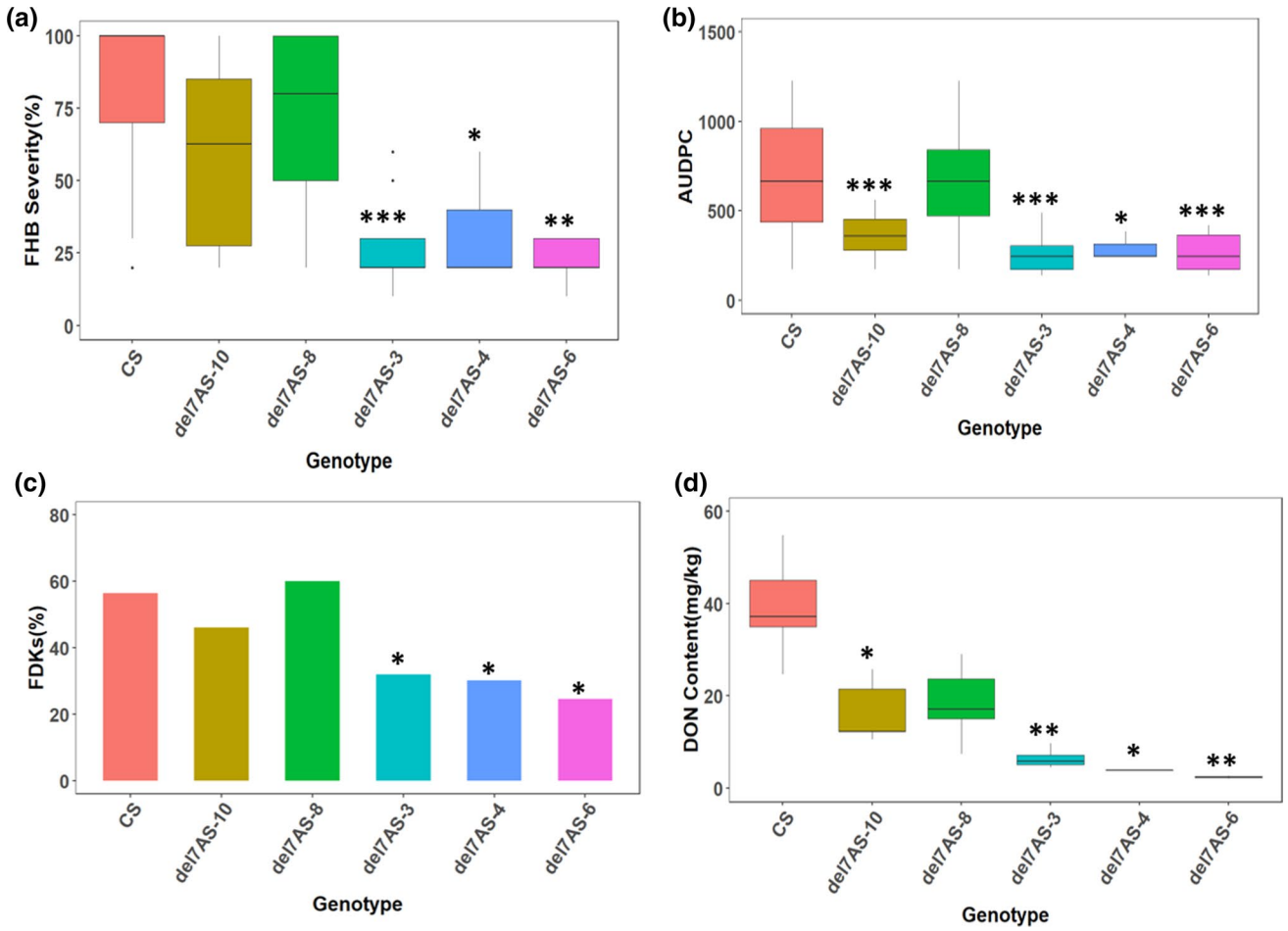


Fig. 5 FHB response of Chinese Spring (CS) control and critical deletion lines. *X* axis denotes the genotypes, and *Y* axis denotes the parameters tested. **a** FHB Severity (%); **b** AUDPC values; **c** FDKs (%); **d** DON content(mg/kg). *Depicts lower significance values over control Chinese Spring at $p < 0.05$, **at $p < 0.01$ and ***at $p < 0.001$. Horizontal line over the individual box plots depicts the median values

deletion lines del7AS-6, del7AS-4 and del7AS-3 also showed significantly lower DON content than control (Fig. 5d). Additionally, del7AS-10 also had lower DON content than Chinese Spring control at $p < 0.05$. Del7AS-8 had numerically lower, but statistically similar DON content to that of the control Chinese Spring (Fig. 5d).

Mapping the susceptibility factor *Sf-Fhb-7AS* to a ~ 50 Mb physical region on chromosome 7AS

Del7AS-6, del7AS-4 and del7AS-3 were found to show high level of type-2 (against the spread of the fungal pathogen) and type-4 (toward kernel infection) resistance against FHB, whereas all the other deletion lines were similar to control Chinese Spring in all the years of testing. Del7AS-10 had significantly lower FHB severity and DON content in year 2020. Integrated molecular and phenotypic analysis revealed the location of *Sf-Fhb-7AS* in the ~ 50 Mb region between 214 and 262.5 Mb on the short arm of chromosome 7A between del7AS-3 and del7AS-8 (Fig. 6). Some intriguing results were obtained for DON content. Del7AS-6, del7AS-4 and del7AS-3 were found to be resistant for FHB severity, AUDPC and FDKs as well as having low DON content. However, del7AS-10 showed FHB severity lower than Chinese Spring in 2020 complete set of deletion lines, whereas similar to Chinese Spring in 2020 critical set. However, del7AS-10 had significantly lower DON content than control Chinese Spring in both 2020 deletion lines complete set and 2020 critical set. Critical deletion line del7AS-8 sharing the deletion with del7AS-10, in addition to an extra 20-30 Mb deletion, showed significantly lower DON content than Chinese Spring during testing in the complete set of 2020. When tested again in the subset of critical lines, it was found to be numerically lower, but statistically similar to Chinese Spring. These results indicate toward the possibility of an additional DON-regulating factor to be present in the common deleted region of the chromosome in del7AS-8 and del7AS-10. Using marker analysis, we also observed an additional ~ 1 Mb interstitial deletion in del7AS-10 at ~ 211 Mb on chromosome 7A short arm (Supplementary Table S4), which is also contained in the major deletion of del7AS-8. The potential DON regulating factor may be either present either in the 1 Mb interstitial deletion or the major ~ 50 Mb deletion between del7AS-11 and del7AS-10. More experiments are needed to verify the presence of this potential factor and locate it on chromosome 7AS.

Discussion

For decades, resistance genes have been known as the major players in imparting resistance to crop plants (Andersen et al. 2018; Kourelis and van der Hoorn 2018). However, little is known about host susceptibility genes facilitating pathogen infection or colonization of crop plants. Such genes can be good targets for manipulation to make it difficult for the pathogen to survive on the host, and hence making the plants resistant (Engelhardt et al. 2018; Fabre et al. 2020; Gorash et al. 2020). Following up on a survey of 30 ditelosomic lines of Chinese Spring for their FHB response by Ma et al. (2006), we selected Dt7AL (lacking short arm of chromosome 7A) for systematic investigation for the presence of a potential susceptibility factor(s) because it was reported to have lowest FHB severity as well as DON content among all the lines. Using a complete set of 6 ditelosomic lines (Dt7AS, Dt7AL, Dt7BS, Dt7BL, Dt7DS and Dt7DL), we

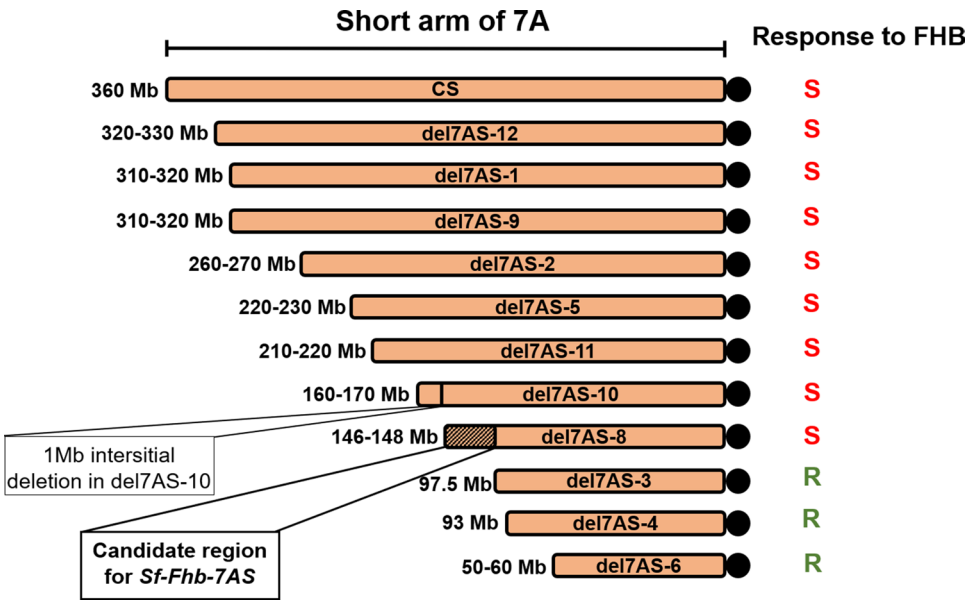


Fig. 6 Deletion-bin mapping of the candidate region for susceptibility gene *Sf-Fhb-7AS*. Deletion lines are depicted in decreasing order of the length of 7A short arm present in them. Response to FHB is shown as S (susceptible) or R (resistant) on right of each of the line. Diagonal pattern filled section of del7AS-8 shows the candidate region of *Sf-Fhb-7AS*. Location of 1 Mb interstitial deletion in del7AS-10 has also been shown in the figure

confirmed that loss of short arm in ditelosomic line Dt7AL made it resistant to FHB and DON accumulation. This verified the presence of a potential susceptibility factor(s) on 7AS. We also observed that Dt7DL had significantly lower DON content and numerically lower FHB severity than Chinese Spring (Fig. 1) indicating toward the presence of a homoeoallele(s) of the same or an additional minor susceptibility factor regulating DON content on 7DS. However, previously Ma et al. (2006) found Dt7DL to have either higher or similar FHB severity and DON content to that of Chinese Spring control. Instead in their study, Dt7BL was found to have lower FHB severity and DON content than Chinese Spring, whereas in the present study, Dt7BL was found to have higher/similar FHB severity and DON parameters. It should be noted that in the present study, the ditelosomic lines were analyzed only in one year. Nevertheless, the results of dt7AL FHB severity and DON content were same in both the studies. To analyze the contribution of different homoeologous copies and the effect of increasing or reducing their dosage, we studied nullisomic–tetrasomic lines of various group 7 chromosomes for their FHB response.

The presence of the major effect susceptibility factor was confirmed on chromosome 7A because N7A-T7B and N7A-T7D were found to have significantly lower FHB severity. Furthermore, increasing the dosage of 7A copy in N7B-T7A, and N7D-T7A, made the plants even more susceptible than Chinese Spring control. This experiment was conducted only in one year, and it indicated that the identified factor on chromosome 7A enhances susceptibility of the plants when present in multiple copies, and hence can be considered a genuine susceptibility factor. It is important to note that DON content of N7A-T7D plants was statistically similar to that of Chinese Spring control, indicating the possibility of a DON-regulating factor on 7D, which may/may not be homoeologous to *Sf-Fhb-7AS*. This pattern for DON content of 7D was similar to that observed for Dt7DL.

Using a set of six substitution lines from different varieties/species of wheat, we found that the *Sf-Fhb-7AS* factor(s) is conserved across not only multiple varieties, but also in a tetraploid species. All tested substitution lines were found to be statistically similar or showing higher disease susceptibility in comparison to Chinese Spring for all the four tested parameters. This indicates that the susceptibility factor is conserved among multiple varieties/species of wheat. Hence, developing a biparental genetic mapping population for mapping this trait would not be helpful. However, given its peri-centromeric location on the wheat chromosome 7AS, the conserved nature of the susceptibility factor as observed in tested varieties/species is not unexpected. It is known that genetic recombination events in wheat are limited to the telomeric and sub-telomeric regions. For example, in chromosome 3B of wheat, 82% of the crossover events are restricted to the distal ends of the chromosome, representing only 19% of the whole chromosome length (Saintenac et al. 2009; Darrier et al. 2017). However, more experiments are needed to confirm that the same conserved factor is leading to the susceptibility in all the substitution lines tested.

To physically localize the major susceptibility factor on 7AS, precise physical boundaries of the 11 overlapping deletion lines of 7AS were determined using genome-specific molecular markers based on the Wheat Reference Genome Sequence v 1.0 (IWGSC 2018). The order of deletions determined in all the lines was in agreement with that reported by Endo and Gill (1996). FHB response of the deletion lines was evaluated four times in the greenhouse for various FHB response parameters. For FHB severity, AUDPC and FDKs, peri-centromeric lines 7AS-6, 7AS-4 and 7AS-3 were found to be resistant, whereas all other lines were found to be susceptible. These three deletion lines also had low DON content. This indicates that other deletion lines (del7AS-12, del7AS-1, del7AS-9, del7AS-2, del7AS-5, del7AS-11, del7AS-10 and del7AS-8) harbor the major susceptibility gene(s), whereas loss of the chromosome segment carrying it makes the peri-centromeric lines (del7AS-3, del7AS-4 and del7AS-6) resistant. This allowed us to localize the major susceptibility factor(s) to a peri-centromeric region of 48.5–50.5 Mb on chromosome 7AS. It is interesting to note that del7AS-10 had lower DON content than Chinese Spring (Figs. 4b, 5b), although it retained the major effect susceptibility factor on 7AS. Application of more molecular markers on this deletion line showed it to contain an additional interstitial deletion of ~ 1 Mb (Supplementary Table S4). Possibly, this 1 Mb interstitial deletion in del7AS-10 or the 50 Mb major deletion of del7AS-10, both of which are common with del7AS-8 may harbor such a factor regulating DON content. However, more experiments are needed for validation of these propositions. In a similar study exploring susceptibility factor(s) on wheat chromosome 4DS, Hales et al. (2020) also reported presence of two different underlying genes, one providing resistance against FHB severity and the other against DON content.

In the present work, a new susceptibility factor(s) *Sf-Fhb-7AS* was physically mapped to a 48.5–50.5 Mbp on chromosome 7A short arm. Deletion of the region resulted in a 50–60% increase in resistance as compared to control Chinese Spring. It was also found that 7A from any of the six varieties studied in the 7A substitution genotypes set does not rescue the susceptible phenotype of Chinese Spring. This may have future implications on the utilization of this factor, as once the underlying gene is identified, it can be deleted/made non-functional in potentially several cultivars to make them resistant to FHB. It is worth mentioning here that most of the deletion lines did not show any major difference in their morphology or phenology from control Chinese Spring over all the three seasons. Deletion line 7AS-11 was late flowering and had compact and light green spikes. It was

susceptible and contained the *Sf-Fhb-7AS* region. However, the reason for the abnormal morphology of del7AS-11 may be the additional deletion reported in it on the short arm of chromosome 2B (Endo and Gill 1996). This indicated that the manipulation of *Sf-FHB-7AS* should theoretically not lead to any deleterious effect on the morphology or phenology of the plants. Fine mapping and identification of *Sf-FHB-7AS* gene will allow better understanding of its role in the plant and in the interaction with *F. graminearum* and further its utilization in developing FHB resistance in wheat varieties. The 48.5–50.5 Mb-long *Sf-FHB-7AS* region is still a big interval for selecting candidate gene(s) and needs to be reduced further. To do that, we plan to use a Gamma-irradiated Radiation-Hybrid panel of Chinese Spring (Tiwari et al. 2016) to detect smaller deletions in the mapped region and delineate the gene further. Manipulation of such susceptibility factors may provide alternative strategies for designing broad-spectrum durable FHB resistance in wheat varieties (Fabre et al. 2020; Gorash et al. 2020).

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Author Contribution statement BC performed the experiments, recorded data, conducted statistical analyses and co-wrote the manuscript with NR. VT, BSG and NR developed the idea, BSG provided the genetic stocks for conducting the study, YD performed DON content measurement, and NR designed the experiments and arranged funding resources for conducting the experiments. All co-authors provided their inputs in the manuscript.

Declarations

Conflict of interest Authors have no conflicts of interest.

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