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# Phenotypic and genotypic characterization of CIMMYT's 15th international Fusarium head blight screening nursery of wheat

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**Abstract** As an important cereal disease in humid and semi-humid areas, Fusarium head blight (FHB) has caused severe epidemics on wheat (*Triticum aestivum* L.) in different countries worldwide. By causing both yield loss and quality degradation, FHB presents a two-fold threat to farmers and consumers. Since the beginning of FHB research at the International Maize and Wheat Improvement Centre (CIMMYT) in the early 1980s, a large-scale FHB screening has been conducted to identify and incorporate new resistance genes into elite CIMMYT germplasm. Candidates of the 15th Fusarium head blight screening nursery (FHBSN) were derived from different CIMMYT wheat breeding programs and were tested for 3 years successively in El Batán, Mexico, before being included in the 15th FHBSN set. From 2010 to 2012, a set of 44 out of 2794 lines were gradually selected depending on their FHB indices, pedigree information, and phenological traits like plant height and days to heading. The performance of these lines varied across years under different disease pressure, but they all showed high level of resistance compared to the susceptible checks. In 2013, the nursery was again evaluated in El Batán, as well as in artificially inoculated field trials in Norway, Uruguay, the Netherlands, and Japan (2014), and in naturally infected experiments in Toluca, Mexico, and Canada. Although not all lines demonstrated strong resistance across environments, promising lines with good FHB resistance can still be identified in each location. The

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genotypes were haplotyped with PCR-based markers for ten loci on seven chromosomes associated with known FHB resistance, and the results suggested that 24 of the genotypes (55 %) carried the 4BS QTL as in Wuhan 1, which was the most frequent QTL in this nursery, and the 7A QTL as in *T. dicoccoides* was noticed in five (11 %) of the genotypes. The resistance QTLs on chromosomes 3B, 5A and 6B as in Sumai 3 and 3A as in *T. dicoccoides* were not detected in any of the genotypes denoting the uniqueness of these lines. Fifteen (34 %) of the genotypes may not carry any of the ten QTLs examined. The results provide valuable information that could be successfully utilized by breeders to select resistant parents for crosses since novel resistance sources were detected for better targeted crosses toward diversifying and/or pyramiding FHB resistance.

**Keywords** FHB screening · Resistance · *Fusarium* spp. · *Triticum aestivum* L

## Introduction

Fusarium head blight (FHB) is one of the most economically important diseases of small grains and continues to adversely impact crops. It is caused by numerous *Fusarium* species that infect florets at anthesis and produce similar symptoms. Yield and test weight reduction, contamination with the mycotoxin deoxynivalenol (DON), and additional costs on seed cleaning have caused high economic losses for farmers and the industry (McMullen et al. 2012). Economically important hosts of FHB include bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Desf.), barley (*Hordeum vulgare* L.), and oats (*Avena sativa* L.).

FHB epidemics are monocyclic since spike infection generally takes place during anthesis and early stages of kernel development (Leonard and Bushnell 2003; Audenaert et al. 2009). At flowering, airborne ascospores and rain splashed conidia land on open florets and get access to the host easily (Leonard and Bushnell 2003). Frequent rainfall and high relative humidity from spike emergence through anthesis favour inoculum production on cereal debris and ensure disease development (Khonga and Sutton 1988; Fernando et al. 1997). Under favourable weather conditions, high amount of primary inoculum and growing susceptible cultivars give rise to epidemics.

Mycotoxins such as zearalenone, HT-2 toxin, T-2 toxin, nivalenol, and DON and its acetylated forms (3-ADON and 15-ADON) are frequently formed in Fusarium-infected wheat and barley (Salas et al. 1999; Buerstmayr et al. 2012). DON is considered to be the most economically important toxin produced by *F. graminearum* (Culler et al. 2007) and has been shown to be a virulence factor in FHB (Bai et al. 2002; Jansen et al. 2005). Recently, severe epidemics have occurred repeatedly and research on this disease has become very important in the Americas, East Asia and Europe. Attributable to high yield losses that may reach 50–60 %, FHB has become a major threat to the global food supply and safety and is considered by the International Maize and Wheat Improvement Centre (CIMMYT) as a major limiting factor of worldwide wheat production (Dubin et al. 1997). In the EU, legally enforceable thresholds in grain and food products allow a maximum DON content in unprocessed cereals other than durum wheat, oats and maize of 1.25 ppm, in bread and biscuits of 0.5 ppm and in baby food for infants and young children of 0.2 ppm (European commission 2006).

Incorporating durable resistance to FHB in wheat is a challenging task for breeders since it is quantitatively inherited and is considerably affected by environment and pathogen populations (Miedaner et al. 2001; Buerstmayr et al. 2002). Wheat has different resistance mechanisms that act synergistically to combat fungal attacks. Mesterhazy et al. (1999) proposed five resistance components, i.e. resistance to fungal invasion (Type I) and spread (Type II), resistance to toxin accumulation (Type III), resistance to kernel infection (Type IV) and resistance to yield reduction (Type V). Additionally, the cuticular wax may decrease water availability and thus constrain fungal germination and penetration. The height, thickness and strength of a plant stem may indirectly affect its resistance to FHB, because the soil-borne spores can easily reach the heads of short or lodged plants. The results of a study conducted by Graham and Browne (2009) concluded that selection for anther extrusion (AE) among European wheat could improve FHB resistance, without negatively impacting on agronomic traits. Furthermore, it was shown that high AE led to low infection rate, contributing to Type I resistance (Skinnes et al. 2010). FHB severities were negatively correlated with both AE and plant height (PH) after spray and spawn

inoculation as reported by Lu et al. (2013) and Kubo et al. (2013). The two dwarfing genes *Rht-B1b* and *Rht-D1b*, especially the latter, have been reported to be associated with increased Type I FHB susceptibility (Draeger et al. 2007; Holzapfel et al. 2008; Srinivasachary et al. 2009). According to Lu et al. (2011), two major resistance QTL are required to counteract the negative effect of *Rht-D1b*. As for *Rht-B1b*, it conferred Type II resistance in several studies (Srinivasachary et al. 2009; Lu et al. 2013), despite its possible effect on reducing Type I resistance.

The quantitative nature of the inheritance of FHB resistance is a subject to several resistance mechanisms that are not necessarily genetically linked (Miedaner 1997), its regular association with detrimental agronomic traits and the large effect of environment makes breeding for FHB resistance a very difficult task in addition to the concern about reproducibility of testing for FHB resistance (Bai and Shaner 2004), and disease evaluation process *per se* is also a tedious process. In this regard, molecular markers can be very helpful to supplement phenotyping and classical breeding in selecting major resistance QTLs (Buerstmayr et al. 2009; Agostinelli et al. 2012) as well as to investigate novel resistance sources. Numerous QTL mapping studies have been performed since last decade, and resistance has been reported on all the 21 chromosomes (Liu et al. 2009).

As a communication platform and a promoter of international cooperation, CIMMYT has developed extensive FHB collaborations with research organizations in both developed and developing countries (Duveiller et al. 2008) and worked on the incorporation of FHB resistance into high yielding, semi-dwarf and rust resistant CIMMYT wheat (He et al. 2000). CIMMYT established a series of FHB Screening Nurseries (FHBSN, previously known as Scab Resistance Screening Nursery, SRSN), which were distributed worldwide and are available to anyone attentive to wheat FHB resistance improvement. The current name FHBSN was adopted in 2010, and the 13th and 14th FHBSN nurseries were distributed in 2011 and 2012, respectively (He et al. 2013a, b). The aim of this study was to identify and characterize the 15th FHBSN regarding field resistance, post-harvest indices of Fusarium damaged kernels (FDK) and DON, as well as phenological and morphological traits like PH, days to heading (DH), and AE.

## Material and methods

### Plant material and field trials

Entries of the 15th FHBSN were selected from an initial set of 2794 advanced breeding lines with known pedigrees which were developed at CIMMYT. Field experiments were conducted using the FHB sick plot established at El Batán (Table 1, with an average annual precipitation of 625 mm), CIMMYT, Mexico. The genotypes were sown and evaluated for FHB resistance in the summer season (May–September) from 2010 to 2013. In 2010, the experiment was done in 1 m double rows without replication, whereas from 2011 to 2013 it was done in two replications. Selection was made from 2010 to 2012 based on FHB index (Stack and McMullen 1994), PH and DH, as well as pedigree information to maintain a high genetic diversity of the nursery. A final selection of the 15th FHBSN was made in 2012 and the nursery was verified again in 2013. Checks included three susceptible lines Gamenya, Ocoroni F 86 and Falcin/*Ae. squarrosa* (312)/3/THB/CEP7780//SHA4/Lira (referred to as Falcin# hereafter) and two resistant lines Sumai 3 and Heilo. The screening nursery was misted from flowering to early dough stage by a programmable misting system with DAN modular micro sprinklers (NaanDan Jain Irrigation Ltd.) arranged in 3 × 4 m spacing. The system operated automatically from 9 a.m. to 8 p.m., with 10 min of spraying per hour, to create a humid environment favourable for FHB development.

### Inoculation and phenotyping assays

Annually about 70–90 *Fusarium* strains are collected in late summer from naturally infected wheat spikes from different farms in Mexico to ensure the viability and virulence of pathogen. The isolates were firstly verified with *F. graminearum* sensu lato specific primer set FG16 N F/R (Nicholson et al. 1998) and then with the TOXP1/2 primer set for their chemotype classification (Li et al. 2005). For those DON-producing *F. graminearum* isolates, a rice medium assay was employed to determine DON productivity (He et al. 2013b). Briefly, the isolates were inoculated on 30 g autoclaved polished rice and incubated for 2 weeks, and then a subsample of 2 g was used to measure DON level. Subsequently, around ten strains

**Table 1** Geographical information of the experimental stations used in this study

| Name | State/province | Country | Latitude | Longitude | Altitude (m) |
|---|---|---|---|---|---|
| El Batán | Mexico | Mexico | 19.5°N | 98.8°W | 2240 |
| Toluca | Mexico | Mexico | 19.2°N | 99.5°W | 2585 |
| Ås | Akershus | Norway | 59.7°N | 10.8°E | 85 |
| INIA La Estanzuela | Colonia | Uruguay | 34.3°S | 57.7°W | 75 |
| Dronten | Flevoland | Netherlands | 52.5°N | 5.7°E | −5 |
| Minto | Manitoba | Canada | 49.4°N | 100.0°W | 487 |
| Kitami | Hokkaido | Japan | 43.8°N | 143.7°E | 193 |

with high DON production capacity were selected and evaluated in greenhouse for their aggressiveness on two resistant (Sumai 3 and Heilo) and three susceptible genotypes (SERI/CEP80120, BCN//DOY1/*Ae. squarrosa* (447), and Gamenya). Two *F. graminearum* strains with known aggressiveness that had been used for field inoculation in the previous year were used as control in greenhouse tests. The spikes were evaluated at 7, 14, and 21 days post inoculation (dpi) by counting symptomatic spikelets and rachis segments. Based on DON productivity and aggressiveness, four highest ranked isolates were selected and mixed with a control strain with known aggressiveness to generate the new inoculum for the year's field screening. Inoculum was produced in liquid mung bean [*Vigna radiata* (L.) Wilczek.] medium as mentioned in Buerstmayr et al. (2002). Inoculum concentration was adjusted to 50,000 conidia/ml (55,000 conidia/ml in 2013) for field application.

At anthesis, ten spikes of each line were labelled in the morning and spray inoculated in the afternoon, using a precision $CO_2$ backpack sprayer with flat fan nozzles at a constant pressure of 40 psi and a rate of about 60 ml/$m^2$. The inoculation was repeated 2 days later. FHB symptoms were scored on the ten tagged spikes at 25 dpi by counting the numbers of total and infected spikelets of each spike, and FHB index was calculated using the formula $FHB\ index\ (\%) = (Severity \times Incidence)/100$ (Stack and McMullen 1994), where 'Severity' stands for the averaged percentage of diseased spikelets, and 'Incidence' for the percentage of spikes which showed infection. Plots were sickle harvested at maturity, and spikes were threshed with a belt thresher set at low wind speed to retain scabby kernels. FDK was estimated only in 2013, from a random grain sample in a petri dish, with a scale of 0–9. For DON analysis, a sample of 20 g grain of each accession was pulverized, and a 2 g sub-sample was tested using the Ridascreen Fast DON ELISA kit (R-Biopharm GmbH, Darmstadt, Germany) following the manufacturer's instruction. DON data was available from 2011 to 2013. AE was recorded only in 2013 based on a linear scale from 0 (no extrusion) to 9 (full extrusion) according to Skinnes et al. (2010).

## Field evaluation in international locations

In addition to El Batán, six more locations were used for evaluation of the nursery, including Dronten in the Netherlands, Toluca in Mexico, Minto in Canada, Ås in Norway, INIA La Estanzuela in Uruguay (all in 2013), and Kitami in Japan (in 2014). See Table 1 for the detailed geographical information of these stations.

In the Netherlands, the nursery was sown in April 3 in 1 m triple row plots without replication. The inoculum consisted of a mixture of a *F. culmorum* and a *F. graminearum* strain (with a ratio of 7:3), and it was adjusted to approx. 25,000 conidia/ml for field application by a tractor mounted boom sprayer. The inoculation was repeated four times in the evening in June 25 and 28, and July 1 and 3, and disease notes were taken two times in July 19 and 30 with FHB severity estimated from 0 to 100 %.

Toluca is a humid location with an average annual rainfall of 800 mm concentrated in the growing season from May to September. FHB infection occurs naturally in this location and it had been used as CIMMYT's main FHB screening site until 2005. The nursery was sown in Toluca in May 8 in 0.75 m double row plots without replication, and no artificial inoculation was done. Disease scoring was taken on Zadoks GS 80–85, with visual estimation of incidence and severity on the plot basis for calculating FHB index.

In Canada, the nursery was sown in June 4 in 6.7 m single row plots spaced 40 cm apart without replication,

and no artificial inoculation was applied as there was severe natural infestation. Precipitation during the growing season was 339 mm. Visual FHB scoring was done in August 26, when most of the lines were at the stage of 25 days after anthesis. A composite estimation based on % of heads infested with FHB and % of spikelets infested was adopted with a range from 0 to 100 %.

In Norway, the nursery was planted on May 20, in hill plots of 0.40 × 0.45 m spacing with two replications. *F. graminearum* infected oat kernels were used as spawn inoculum and applied in the field at Zadoks GS 37–39. Mist irrigation was applied for 10 min at hourly intervals from 7 to 10 p.m. in the evening until the plants reached maturity. FHB evaluation was done at the beginning of maturity, when a bundle of 10–15 spikes was counted to determine disease severity. For more detailed information on FHB screening in this location, refer to Lu et al. (2013).

In Uruguay, the nursery was sown in July 17 in 1 m single row without replication. Maize kernels infected with a mixture of ten *F. graminearum* isolates of known aggressiveness and representativeness were applied twice in the field as spawn inoculum, with the first application at Zadoks GS 45 of the early maturity genotypes and the second at 3 weeks later. Each time, 40 g/m$^2$ of inoculum was applied. Misting system worked from 2 to 3 weeks prior to flowering to milk grain stage, with two sprayings of 15 min in the morning and two in the afternoon. The nozzles used were NaanDanJain microsprinklers DANSPRINKLERS 03 (NaanDan Jain Irrigation Ltd.). Disease scoring was made at Zadoks GS 80–85 (late milky to soft dough stages), with disease incidence and severity estimated to calculate FHB index. In Japan, the nursery was planted in April 23, 2014, in single row plots spaced 40 cm apart without replication. Spawn inoculation was carried out at Zadoks GS 45, with oats seeds infested by a single isolate of *F. graminearum* of known aggressiveness. Approximately 5 g of spawn was spread for each row and the field was watered with a sprinkler system for 8 min per hour after heading to maintain high humidity. FHB severity was scored visually on 15 spikes at 21 dpi, using a linear scale of 0 through 8, which were subsequently transformed to percentages of 0, 5, 15, 25, 50, 60, 75, 90, and 100 %, respectively.

## Statistical analysis

The phenotypic data was analysed by the SAS program ver. 9.2. Analysis of variance (ANOVA) was carried out with the PROC GLM module. The data in the ANOVA table were used for calculating the heritability estimates, using the formula $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$ for single year and $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{g*y}^2/y + \sigma_e^2/ry)$ for multiple years; in which $\sigma_g^2$ stands for genetic variance, $\sigma_{g*y}^2$ for genotype-by-year interaction, $\sigma_e^2$ for error variance, $y$ for the number of years, and $r$ for the number of replications (Lu et al. 2013). In order to facilitate the identification of stably resistant lines across environments, a composite index was calculated, i.e. the sixth root of the product of FHB index (or severity) in El Batán (2013), Norway (2013), Uruguay (2013), the Netherlands (2013), Canada (2013), and Japan (2014). FHB parameters, PH, and AE were normalized with the PROC STANDARD function prior to the principal component analysis (PCA) using the PAST software ver. 3.01 (Hammer et al. 2001).

## Haplotyping

To identify the genetic basis behind the FHB resistance in the 15th FHBSN entries, 17 PCR-based markers linked to ten validated FHB resistance QTLs on seven chromosomes were chosen for haplotyping, to assess the 15th FHBSN entries for the possible presence of QTLs as in Wuhan 1, CJ 9306, Frontana, Sumai 3, and *T. dicoccoides*. Leaf tissue was harvested from the second leaf for DNA extraction, following the CTAB method recommended by the European Community Reference Laboratories for the isolation of maize DNA as cited in Brunner et al. (2009). The lines were genotyped at the GenServe Laboratories, Saskatoon, SK, Canada. The markers were fluorescently labelled (Schuelke 2000) and the PCR system and cycling program followed the endorsed protocols of each marker. All PCR reactions were performed in an Applied Biosystems Veriti 96 well thermal cycler. PCR products were analysed using an ABI 3500xl Genetic Analyzer through capillary electrophoresis; allele calling was conducted using GENEMAPPER version 4.0 (Applied Biosystems, Foster City, CA,

USA). The strategy for confirming a QTL was according to the following strategy; a resistance QTL was assumed to be present only when both contiguous markers showed the resistance alleles which accordingly marked as '+ +'. Similarly, '− −' represented the absence of the resistance allele, whereas '+ −' indicated that only one of the two flanking markers showed resistance genotype. For the 3BS QTL as in Sumai 3, the 3AS QTL as in *T. dicoccoides*, and the 3AL QTL as in Frontana, only one closely linked marker was genotyped for declaration of presence or absence of the corresponding QTL. *Rht-B1* and *Rht-D1* were genotyped in the LGC Company (http://www.lgcgroup.com) with the KASP assay.

## Results

ANOVA results indicated significant 'year' effect for FHB, having the largest mean square (MS) value that was almost two times higher than that of the 'entry' effect; this was more significant for DON, where the year MS was 60 times higher than the entry MS (Table 2), ascribable to both the different disease levels in El Batán across years and the positive selection error. Nevertheless, the 'entry' effect was significant for all the three FHB parameters at $P < 0.001$ level, and so did the genotype-by-environment effects for FHB and DON. FHB exhibited the highest heritability estimate of 0.82, followed by FDK of 0.81 and DON of 0.69.

In 2010, FHB index ranged from 0.6 to 83.5 %, but over 75 % of the entries had a value lower than 25 % (Fig. 1a). The two resistant checks, Sumai 3 and Heilo, showed very low disease index, whereas the two moderately susceptible checks, Ocoroni and Falcin#, exhibited FHB index around 40 %, and the susceptible check Gamenya had the highest disease severity (>90 %) (Fig. 1b). Based on the field screening data, 1109 lines with an FHB index lower than 15 %, DH less than 80 days and PH lower than 110 cm, were selected for further screening in 2011.

In 2011, the disease level was lower than in the previous year, which was evidenced from the disease distribution patterns of both the candidates (Fig. 1a) and checks (Fig. 1b). Accordingly, DON distribution skewed markedly to the direction of low value, with 90 % of the lines having DON content less than 3 ppm (Fig. 1c). Selection was made to retain 311 entries showing FHB index <5 % and DON content <3 ppm. The disease level in 2012 was similar to that in 2011, and finally 44 resistant lines with FHB index <2.5 % and DON content <1.5 ppm were selected and compiled as the 15th FHBSN, wherein maximum two sister lines per cross were included (Table 3).

**Table 2** Analysis of variance of the 15th FHBSN evaluated in El Batán for FHB index, DON content, FDK and their heritability estimates

| Trait | Source | DF | MS | *F* value | *Pr* > F | Heritability |
|---|---|---|---|---|---|---|
| FHB | Entry | 45 | 627.46 | 24.56 | <0.001 | 0.82 |
| | Year | 2 | 11,747.55 | 459.86 | <0.001 | |
| | Rep (year) | 3 | 37.12 | 1.45 | 0.2302 | |
| | Entry × Year | 90 | 127.19 | 4.98 | <0.001 | |
| | Error | 135 | 25.55 | | | |
| DON | Entry | 45 | 6.81 | 6.22 | <0.001 | 0.69 |
| | Year | 1 | 415.34 | 379.02 | <0.001 | |
| | Rep (year) | 2 | 0.80 | 0.73 | 0.4850 | |
| | Entry × Year | 45 | 2.69 | 2.46 | <0.001 | |
| | Error | 90 | 1.10 | | | |
| FDK | Entry | 45 | 3.85 | 5.21 | <0.001 | 0.81 |
| | Rep | 1 | 0.27 | 0.37 | 0.5471 | |
| | Error | 45 | 0.74 | | | |

Only the 15th FHBSN entries and the two checks (Sumai 3 and Gamenya) were included in this analysis. FHB data were from 2011, 2012 and 2013, DON from 2012 and 2013, and FDK from 2013. FHB 2010 and DON 2011 were not included since the experiments were not replicated. All the data were measured in El Batán, Mexico

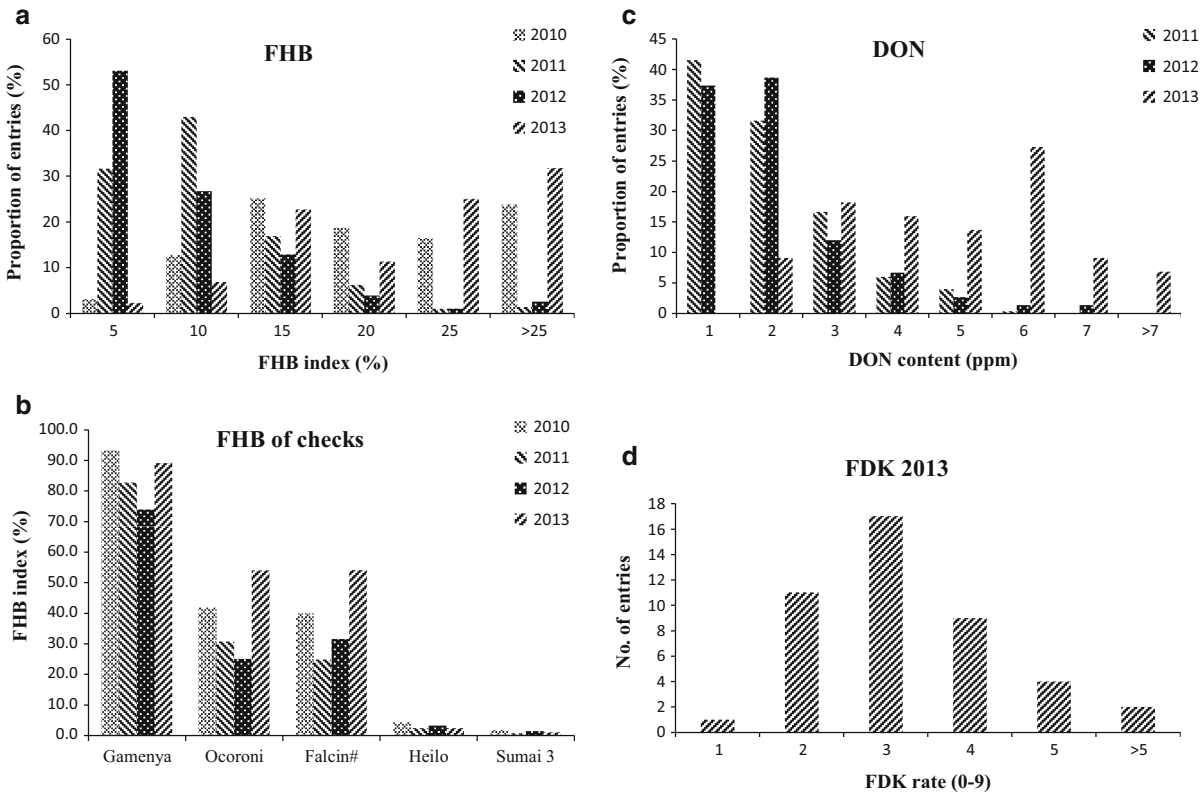


**Fig. 1** Frequency distribution of FHB index, DON content and FDK in different years in El Batán, Mexico. *Note* For the FHB chart (**a**), 2794 lines were evaluated in 2010, 1109 in 2011, 311 in 2012, and 44 in 2013; for the DON chart (**c**), 301 lines were measured in 2011, 75 in 2012, and 44 in 2013; for the FDK chart (**d**), only the 44 entries of the 15th FHBSN were evaluated

In 2013, the average FHB index of all genotypes was 23.1 %, much higher than those in previous years (Tables 3 and S1; Fig. 1), indicating a high disease pressure. DON content was also much higher than in 2011 and 2012 (Fig. 1c), with the highest value of 7.9 ppm, even higher than that of the susceptible check Gamenya (6.1 ppm). However, FDK values ranging from 1.0 to 5.5 (Fig. 1d) were not as high as would be predicted from the DON values.

In the Netherlands, the disease severity was very high with a grand mean FHB severity of 43.6 %, wherein only 12 lines exhibited values less than 30 %. Under the natural infection in Toluca, 2013, the entries showed very low disease with an average FHB index of merely 2 % for all genotypes, while 0 for Sumai 3 and 56 % for Gamenya (Table S1). Although no artificial inoculation was used in Canada, the severity was notably higher than Toluca under the natural infection, having a grand mean FHB severity of 29.6 %. In Norway, a grand mean FHB severity of 34.9 % was obtained, with ten lines showed more severe infection than Gamenya; but there were 18 lines being statistically non-significantly different from Sumai 3. Similar disease distribution happened in Uruguay, with the only marked difference being the lower grand mean value of 17.5 %. Whereas in Japan 2014, where spawn inoculation was applied, the grand mean FHB severity was 19.2 %, and only seven lines had FHB severity >30 % (Tables 3 and S1).

Across year/environment, Sumai 3 and Gamenya performed quite consistently, being the (or among the) most resistant and the most susceptible, respectively. On the other hand, no line other than Sumai 3 was consistently being highly resistant in all the experiments; but there were several lines being resistant in most experiments, which can easily be identified by the composite index, such as FRNCLN/HEILO//FRNCLN (CIMMYT germplasm bank identifier, GID, 6340966), WAXWING*2/TUKURU*2//HEILO (GID 6340862), ATTILA/PASTOR/3/ATTILA/

**Table 3** Phenotypic data of the 15th Fusarium head blight screening nursery (15th FHBSN)

| GID | Entry name | Mexico (El Batán) | | | | | | | | International locations | | | | | Composite index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FHB 2010 | FHB 2011 | DON 2011 | FHB 2012 | DON 2012 | FHB 2013 | DON 2013 | FDK 2013 | NO 2013 | UY 2013 | NE 2013 | CA 2013 | JP 2014 | |
| 6342075 | ATTILA/3*BCN*2//BAV92/3/HEILO | 7.6 | 5.2 | 2.8 | 1.4 | 1.2 | 10.9 | 5.0 | 2.5 | 31.6 | 36.0 | 55.0 | 30.0 | 10.0 | 24 |
| 6340362 | ATTILA/3*BCN*2//BAV92/3/HEILO/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 | 12.7 | 4.9 | 0.6 | 1.3 | 0.2 | 13.4 | 3.0 | 2.5 | 16.6 | 6.0 | 55.0 | 10.0 | 18.0 | 15 |
| 6000734 | ATTILA/BAV92//PASTOR/3/ATTILA*2/PBW65/4/ATTILA/PASTOR | 14.3 | 2.9 | 1.3 | 3.2 | 0.5 | 24.6 | 6.7 | 2.5 | 43.2 | 15.0 | 37.0 | 10.0 | 15.0 | 21 |
| 6000696 | ATTILA/PASTOR/3/ATTILA/BAV92//PASTOR/4/PBW343*2/TUKURU | 2.4 | 0.5 | 0.3 | 3.5 | 0.9 | 20.8 | 2.2 | 3.0 | 37.9 | 2.0 | 10.0 | 10.0 | 7.0 | 10 |
| 6343618 | BABAX/LR42//BABAX*2/3/PAVON 7S3, +LR47/4/HEILO | 3.8 | 6.4 | 1.4 | 0.6 | 1.0 | 35.9 | 4.5 | 1.0 | 46.4 | 64.0 | 60.0 | 30.0 | 9.0 | 35 |
| 6342108 | BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/HEILO | 12.5 | 3.9 | 1.8 | 3.0 | 0.9 | 20.8 | 4.4 | 2.0 | 22.9 | 2.0 | 37.0 | 30.0 | 13.0 | 15 |
| 6340565 | CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/GONDO/CBRD | 8.9 | 2.7 | 0.4 | 2.7 | 1.0 | 20.3 | 1.7 | 3.5 | 19.3 | 14.0 | 17.0 | 10.0 | 6.0 | 13 |
| 6340604 | CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92/4/HEILO/5/FRET2/KUKUNA//FRET2 | 8.9 | 4.1 | 2.9 | 1.7 | 2.4 | 23.9 | 5.3 | 4.0 | 28.4 | 0.3 | 65.0 | 50.0 | 24.0 | 15 |
| 6000632 | CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/ATTILA*2/PBW65/6/PBW343*2/TUKURU | 2.0 | 3.1 | 0.2 | 1.9 | 2.5 | 30.0 | 3.9 | 3.0 | 48.6 | 8.0 | 25.0 | 30.0 | 30.0 | 25 |
| 6342187 | FRET2/KUKUNA//FRET2/3/HEILO | 10.3 | 3.7 | 1.6 | 1.4 | 0.6 | 28.6 | 3.0 | 2.5 | 17.0 | 24.0 | 65.0 | 50.0 | 6.0 | 25 |
| 6340649 | FRET2/KUKUNA//FRET2/3/HEILO/4/BLOUK #1 | 2.8 | 3.6 | 2.3 | 1.9 | 0.7 | 17.4 | 1.9 | 2.0 | 30.5 | 42.0 | 50.0 | 30.0 | 14.0 | 28 |
| 6340966 | FRNCLN/HEILO//FRNCLN | 11.3 | 4.4 | 0.7 | 3.3 | 0.5 | 4.3 | 2.4 | 4.0 | 27.7 | 1.0 | 17.0 | 10.0 | 11.0 | 8 |
| 6001555 | GOUBARA-1/2*SOKOLL | 0.9 | 0.5 | 2.2 | 2.1 | 1.5 | 25.4 | 6.2 | 3.0 | 43.1 | 40.0 | 25.0 | 30.0 | 9.0 | 26 |
| 6001364 | KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/PASTOR/FLORKWA-1//BAV92 | 2.3 | 1.7 | 1.0 | 3.3 | 0.8 | 51.9 | 3.4 | 4.0 | 29.0 | 7.0 | 55.0 | 50.0 | 30.0 | 31 |
| 6000673 | KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/WHEAR/5/ATTILA/BAV92//PASTOR | 14.3 | 1.4 | 1.3 | 1.8 | 1.0 | 11.0 | 6.0 | 2.0 | 50.0 | 8.0 | 37.0 | 30.0 | 25.0 | 22 |
| 6176474 | KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU | 8.6 | 1.8 | 1.2 | 3.1 | 1.4 | 15.2 | 6.0 | 1.5 | 24.8 | 10.0 | 25.0 | 10.0 | 52.0 | 19 |
| 6342246 | KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO | 4.3 | 3.1 | 0.6 | 3.1 | 0.7 | 12.7 | 3.6 | 2.5 | 23.9 | 7.0 | 55.0 | 50.0 | 6.0 | 18 |
| 6340672 | KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 | 2.1 | 3.4 | 1.0 | 1.8 | 1.5 | 13.1 | 5.3 | 2.0 | 22.1 | 18.0 | 37.0 | 50.0 | 9.0 | 21 |

**Table 3** continued

| GID | Entry name | Mexico (El Batán) | | | | | | | | International locations | | | | | Composite index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FHB 2010 | FHB 2011 | DON 2011 | FHB 2012 | DON 2012 | FHB 2013 | DON 2013 | FDK 2013 | NO 2013 | UY 2013 | NE 2013 | CA 2013 | JP 2014 | |
| 6340708 | KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 | 10.3 | 3.4 | 2.0 | 2.3 | 1.3 | 22.1 | 4.7 | 2.5 | 25.5 | 28.0 | 50.0 | 70.0 | 12.0 | 30 |
| 6340845 | MUNAL//SHA3/CBRD/3/PAURAQ | 7.3 | 4.3 | 1.3 | 1.8 | 0.4 | 10.9 | 2.7 | 1.5 | 35.9 | 21.0 | 60.0 | 10.0 | 12.0 | 20 |
| 6342263 | OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO | 8.1 | 3.9 | 0.9 | 2.7 | 1.1 | 13.9 | 6.0 | 2.0 | 38.9 | 0.3 | 55.0 | 10.0 | 51.0 | 12 |
| 6342266 | OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO | 8.2 | 3.8 | 0.2 | 2.5 | 0.5 | 8.5 | 2.3 | 2.0 | 35.2 | 5.0 | 50.0 | 50.0 | 9.0 | 18 |
| 6340765 | PBW343/WBLL1//PANDION/3/HEILO/4/PAURAQ | 7.4 | 2.9 | 1.5 | 2.6 | 0.7 | 21.8 | 3.8 | 3.0 | 29.8 | 8.0 | 17.0 | 30.0 | 13.0 | 18 |
| 6340803 | PFAU/WEAVER*2//BRAMBLING/3/HEILO/4/WAXWING*2/TUKURU | 3.6 | 6.7 | 1.4 | 2.4 | 0.9 | 39.3 | 4.5 | 2.0 | 26.6 | 8.0 | 65.0 | 30.0 | 11.0 | 24 |
| 6343651 | PFAU/WEAVER*2//TRANSFER#12,P88.272.2/3/HEILO | 17.9 | 2.7 | 0.9 | 1.4 | 0.7 | 16.0 | 2.6 | 2.5 | 19.1 | 18.0 | 50.0 | 30.0 | 9.0 | 20 |
| 5999927 | PROINTA SUPERIOR/4/RL6043/4*NAC//PASTOR/3/BAV92/5/KLEIN SAGITARIO | 0.9 | 0.8 | 1.1 | 3.8 | 1.6 | 36.6 | 5.3 | 4.5 | 49.8 | 12.0 | 60.0 | 10.0 | 15.0 | 24 |
| 6000034 | QG 4.37A/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92 | 3.7 | 1.7 | 1.2 | 2.1 | 1.2 | 24.3 | 5.4 | 4.0 | 35.9 | 16.0 | 25.0 | 50.0 | 8.0 | 23 |
| 6000970 | SOKOLL*2/ROLF07 | 13.6 | 0.6 | 0.4 | 2.3 | 1.9 | 28.0 | 7.8 | 3.0 | 53.1 | 12.0 | 37.0 | 50.0 | 24.0 | 30 |
| 6000906 | SOKOLL*2/TROST | 3.9 | 2.5 | 0.7 | 3.3 | 1.4 | 45.2 | 7.7 | 5.5 | 41.6 | 24.0 | 55.0 | 50.0 | 56.0 | 44 |
| 6001180 | SOKOLL//FRTL/2*PIFED | 8.0 | 2.9 | 1.1 | 3.1 | 3.1 | 39.8 | 5.9 | 3.0 | 55.4 | 30.0 | 25.0 | 30.0 | 40.0 | 35 |
| 6000931 | SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR | 7.1 | 2.1 | 1.0 | 1.7 | 1.1 | 27.4 | 5.4 | 4.0 | 56.2 | 48.0 | 55.0 | 30.0 | 24.0 | 38 |
| 6001093 | SOKOLL/ROLF07 | 9.0 | 1.7 | 1.9 | 3.0 | 2.2 | 58.7 | 7.9 | 5.0 | 42.3 | 24.0 | 25.0 | 30.0 | 52.0 | 36 |
| 6000939 | SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA/4/ARREHANE | 11.2 | 0.6 | 1.5 | 2.4 | 1.7 | 24.4 | 7.0 | 3.5 | 34.6 | 45.0 | 37.0 | 30.0 | 13.0 | 29 |
| 6342336 | TAM200/PASTOR//TOBA97/3/HEILO | 6.5 | 1.6 | 1.4 | 2.8 | 2.2 | 7.7 | 5.0 | 2.5 | 45.0 | 1.0 | 25.0 | 50.0 | 21.0 | 14 |
| 6342353 | TAM200/PASTOR//TOBA97/3/HEILO | 18.7 | 3.3 | 0.6 | 1.1 | 0.5 | 11.8 | 1.3 | 4.5 | 48.2 | 18.0 | 65.0 | 10.0 | 13.0 | 21 |
| 6343684 | THELIN/2*WBLL1//HEILO | 18.6 | 4.5 | 1.0 | 3.0 | 0.7 | 5.8 | 1.6 | 2.5 | 35.9 | 16.0 | 60.0 | 10.0 | 12.0 | 17 |
| 6342383 | TOBA97/PASTOR//HEILO | 10.0 | 3.7 | 2.0 | 1.8 | 1.1 | 24.7 | 4.4 | 4.0 | 26.0 | 30.0 | 55.0 | 50.0 | 6.0 | 26 |
| 5999807 | VORB/4/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM | 2.7 | 2.2 | 0.0 | 1.5 | 1.0 | 35.2 | 5.5 | 5.5 | 35.2 | 6.0 | 55.0 | 30.0 | 24.0 | 26 |
| 6340858 | WAXWING*2/TUKURU*2//HEILO | 3.9 | 2.5 | 1.3 | 2.0 | 2.5 | 39.4 | 4.4 | 3.0 | 42.1 | 36.0 | 50.0 | 30.0 | 12.0 | 32 |
| 6340862 | WAXWING*2/TUKURU*2//HEILO | 11.1 | 4.2 | 0.7 | 1.7 | 0.5 | 11.3 | 2.6 | 1.5 | 32.9 | 1.0 | 50.0 | 10.0 | 3.0 | 9 |
| 6343369 | WBLL1*2/BRAMBLING*2//GONDO/TNMU | 16.4 | 3.8 | 1.3 | 2.7 | 3.1 | 15.9 | 5.9 | 1.5 | 22.6 | 21.0 | 60.0 | 10.0 | 25.0 | 22 |
| 6342460 | WBLL1*2/KIRITATI//HEILO | 9.7 | 4.1 | 2.0 | 1.5 | 1.1 | 20.5 | 3.6 | 3.5 | 31.1 | 28.0 | 65.0 | 30.0 | 13.0 | 28 |

**Table 3** continued

| GID | Entry name | Mexico (El Batán) | | | | | | | | International locations | | | | | Composite index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FHB 2010 | FHB 2011 | DON 2011 | FHB 2012 | DON 2012 | FHB 2013 | DON 2013 | FDK 2013 | NO 2013 | UY 2013 | NE 2013 | CA 2013 | JP 2014 | |
| 6343743 | WBLL1*2/KUKUNA//HEILO | 7.3 | 4.3 | 0.6 | 0.6 | 0.7 | 12.9 | 2.4 | 2.5 | 34.0 | 12.0 | 17.0 | 10.0 | 13.0 | 15 |
| 5999852 | YAR/AE.SQUARROSA (518)/3/PRL/SARA//TSI/VEE#5/4/ATTILA/5/BERKUT | 1.1 | 2.4 | 2.5 | 2.7 | 0.9 | 18.9 | 3.2 | 4.5 | 59.1 | 9.0 | 37.0 | 10.0 | 25.0 | 21 |
| **10004** | **SUMAI #3 (resistant check)** | **1.5** | **1.4** | **0.1** | **1.0** | **0.0** | **1.0** | **0.3** | **1.0** | 7.6 | 1.0 | 3.0 | 10.0 | 7.0 | 3 |
| **5536** | **GAMENYA (susceptible check)** | **68.0** | **78.7** | **3.9** | **72.0** | **7.5** | **60.4** | **6.1** | **8.5** | 44.6 | 24.0 | 70.0 | 70.0 | 65.0 | 52 |

Additional information to this table is available in Table S1. Phenotyping data include FHB index (%), DON content (ppm) and FDK (%) from El Batán, Mexico, FHB severity (%) from Norway (NO), the Netherlands (NE), Canada (CA), and Japan (JP), and FHB index (%) from Uruguay (UY). The composite index was the sixth root of the product of FHB2013 (in El Batán), NO2013, UY2013, NE2013, CA2013, and JP2014

The two checks Sumai 3 and Gamenya are bolded

BAV92//PASTOR/4/PBW343*2/TUKURU (GID 6000696), etc. (Table 3).

As expected, Sumai 3 and Gamenya were scattered away from the 15th FHBSN entries in the biplot, wherein the most promising lines were found in the second quadrant (Fig. 2), including the lines nominated above. Regarding the correlation among FHB traits, those evaluated in El Batán were highly interrelated and their vectors clustered together in the biplot, with only DON 2011 and DON 2013 being outliers. Of the five international locations, Norway, Japan, and Canada were more similar to El Batán than Uruguay and the Netherlands, although positive correlation was always evidenced between these locations and El Batán.

AE, a trait associated with Type I resistance, ranged between 5.5 and 8.0 for the entries in El Batán, whereas a low AE of 3.0 was observed for Gamenya (Table S1). Negative correlation was observed between AE and most FHB traits (Fig. 2). PH did not differ greatly among the entries in different locations and showed marginally negative correlation with FHB traits in the most cases.

The haplotyping results proposed that 24 (55 %) of the genotypes carried the 4BS QTL as in Wuhan 1, which was the most frequent QTL in this nursery. Another frequent one was the 7A QTL as in *T. dicoccoides*, which was noticed in five (11 %) of the genotypes. In contrast, the resistance QTLs on chromosomes 3B, 5A and 6B as in Sumai 3 and 3A as in *T. dicoccoides* were not detected in any of the genotypes (Table 4). It is noteworthy that 15 (34 %) of the genotypes appeared to carry none of the ten QTLs examined. Results of allelic variation at *Rht-B1* and *Rht-D1* are presented in Table S1. All but one of the entries had the *Rht-B1b* dwarfing allele, whereas none had the *Rht-D1b* dwarfing allele, i.e. most entries were of the *Rht-B1b/Rht-D1a* genotype.

## Discussion

To successfully identify novel FHB resistance resources, an effective screening protocol is crucial, in which genetic background and haplotyping results must be taken into consideration in addition to FHB parameters, to maintain good level of diversity. FHB resistance could be best estimated in the field by FHB index since it considers both severity and incidence

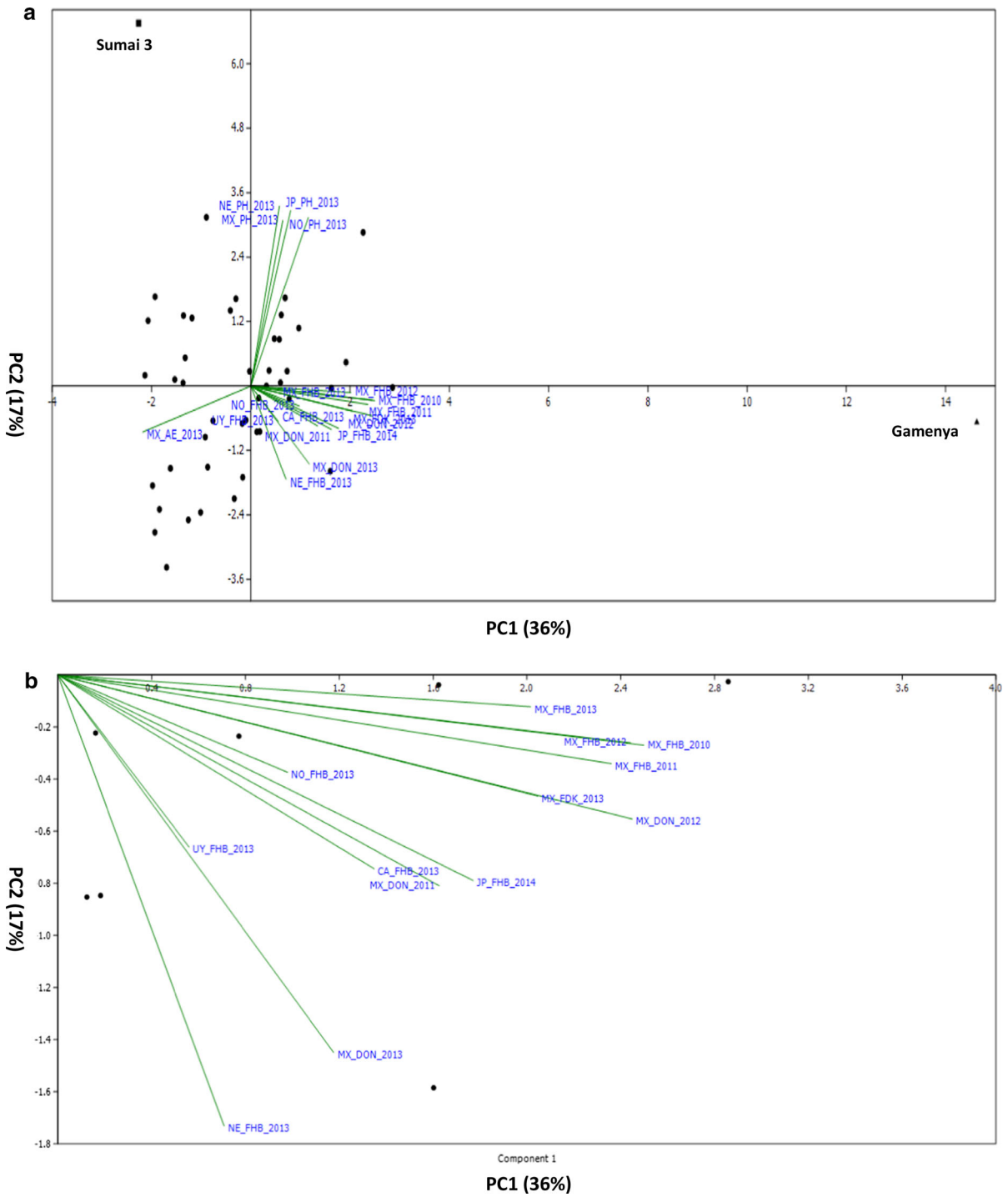


**Fig. 2** Biplot of the 15th FHBSN based on principal component analysis (PCA) on FHB parameters, PH and AE values. *Note* Both the original one (**a**) and its magnified part showing the FHB related vectors (**b**) are shown. Cosine of the angle between vectors indicates correlation between variables in the dimension of the first two principal components (PCs)

**Table 4** Haplotyping data of the 15th Fusarium head blight screening nursery (15th FHBSN)

| GID | Entry name | WU 2D | CJ 2D | FR 3A | SU 3B | DI 3A | WU 4B | SU 5A | FR 5A | SU 6B | DI 7A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6342075 | ATTILA/3*BCN*2//BAV92/3/HEILO | − − | − − | − | − | − | + − | − − | + − | − − | + − |
| 6340362 | ATTILA/3*BCN*2//BAV92/3/HEILO/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 | − − | − − | − | − | − | + + | − − | − − | − − | + + |
| 6000734 | ATTILA/BAV92//PASTOR/3/ATTILA*2/PBW65/4/ATTILA/PASTOR | − − | − − | − | − | − | + + | − − | + − | NA | − − |
| 6000696 | ATTILA/PASTOR/3/ATTILA/BAV92//PASTOR/4/PBW343*2/TUKURU | − − | − − | − | − | − | + − | − − | + − | − − | + − |
| 6343618 | BABAX/LR42//BABAX*2/3/PAVON 7S3, +LR47/4/HEILO | − − | − − | − | − | − | + + | − − | + − | − − | − − |
| 6342108 | BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/HEILO | − − | − − | − | − | − | + + | − − | + + | − − | + + |
| 6340565 | CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/GONDO/CBRD | − − | − − | − | − | − | + − | − − | − − | − − | + − |
| 6340604 | CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92/4/HEILO/5/FRET2/KUKUNA//FRET2 | − − | − − | − | − | − | − − | − − | − − | − − | + − |
| 6000632 | CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/ATTILA*2/PBW65/6/PBW343*2/TUKURU | − − | − − | − | − | − | − − | − − | + − | − − | + − |
| 6342187 | FRET2/KUKUNA//FRET2/3/HEILO | − − | − − | − | − | − | + + | − − | + + | − − | + − |
| 6340649 | FRET2/KUKUNA//FRET2/3/HEILO/4/BLOUK #1 | − − | − − | − | − | − | + + | − − | + − | − − | + − |
| 6340966 | FRNCLN/HEILO//FRNCLN | − − | − − | − | − | − | + + | − − | + − | − − | + − |
| 6001555 | GOUBARA-1/2*SOKOLL | − − | − − | − | − | − | + − | − − | − − | − − | − − |
| 6001364 | KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/PASTOR/FLORKWA−1//BAV92 | − − | + − | + | − | − | − − | − − | + − | − − | + − |
| 6000673 | KABY/BAV92/3/CROC_1/AE.SQUARROSA (224)//OPATA/4/WHEAR/5/ATTILA/BAV92//PASTOR | − − | − − | − | − | − | − − | − − | + − | − − | + − |
| 6176474 | KACHU #1/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/5/KACHU | − − | − − | − | − | − | + + | − − | + − | − − | − − |
| 6342246 | KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO | NA | − − | NA | − | − | + + | NA | + − | NA | + − |
| 6340672 | KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 | − − | − − | − | − | − | + + | − − | − − | − − | − − |
| 6340708 | KAUZ*2/MNV//KAUZ/3/MILAN/4/BAV92/5/HEILO/6/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 6340845 | MUNAL//SHA3/CBRD/3/PAURAQ | + + | − − | − | − | − | + − | − − | + − | − − | − − |
| 6342263 | OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO | − − | − − | − | − | − | + + | − − | + − | − − | + − |
| 6342266 | OASIS/SKAUZ//4*BCN*2/3/PASTOR/4/HEILO | − − | − − | − | − | − | + + | − − | + − | − − | + − |
| 6340765 | PBW343/WBLL1//PANDION/3/HEILO/4/PAURAQ | − − | − − | − | − | − | + − | − − | − − | − − | + + |
| 6340803 | PFAU/WEAVER*2//BRAMBLING/3/HEILO/4/WAXWING*2/TUKURU | − − | − − | − | − | − | + + | − − | − − | − − | − − |
| 6343651 | PFAU/WEAVER*2//TRANSFER#12,P88.272.2/3/HEILO | − − | − − | − | − | − | + + | − − | + − | − − | + − |
| 5999927 | PROINTA SUPERIOR/4/RL6043/4*NAC//PASTOR/3/BAV92/5/KLEIN SAGITARIO | − − | − − | − | − | − | + − | − − | − − | − − | − − |
| 6000034 | QG 4.37A/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92 | + − | − − | − | − | − | + + | − − | − − | − − | + + |

**Table 4** continued

| GID | Entry name | WU 2D | CJ 2D | FR 3A | SU 3B | DI 3A | WU 4B | SU 5A | FR 5A | SU 6B | DI 7A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6000970 | SOKOLL*2/ROLF07 | + − | − − | − | − | − | + + | − − | − − | − − | + + |
| 6000906 | SOKOLL*2/TROST | − − | − − | − | − | − | + − | − − | − − | + − | + − |
| 6001180 | SOKOLL//FRTL/2*PIFED | − − | − − | − | − | − | + − | − − | + − | − − | + − |
| 6000931 | SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR | − −− | − − | − | − | − | + − | − − | − −− | − − | + − |
| 6001093 | SOKOLL/ROLF07 | −− − | −− | − | − | − | + + | −− − | − − | − − | + − |
| 6000939 | SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA/4/ARREHANE | − − | − − | − | − | − | + + | − − | − − | − − | + − |
| 6342336 | TAM200/PASTOR//TOBA97/3/HEILO | + + | − − | − | − | − | − − | −− − | + − | − − | + − |
| 6342353 | TAM200/PASTOR//TOBA97/3/HEILO | − − | − − | − | − | − | + + | − − | + − | − − | − − |
| 6343684 | THELIN/2*WBLL1//HEILO | + − | − −− | − | − | − | + + | − − | + − | − − | − − |
| 6342383 | TOBA97/PASTOR//HEILO | −− −− | − − | − | − | − | + − | − − | + − | − − | + −− |
| 5999807 | VORB/4/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM | + − | − − | − | − | − | + + | − −− | − − | + − | + −− |
| 6340858 | WAXWING*2/TUKURU*2//HEILO | − − | − − | − | − | − | + − | − − | + − | − − | + − |
| 6340862 | WAXWING*2/TUKURU*2//HEILO | − − | − − | − | − | − | + − | − − | − − | − − | + − |
| 6343369 | WBLL1*2/BRAMBLING*2//GONDO/TNMU | − − | − − | − | − | − | + − | − − | − − | − − | − − |
| 6342460 | WBLL1*2/KIRITATI//HEILO | − − | − − | − | − | − | + + | − − | + − | − − | + − |
| 6343743 | WBLL1*2/KUKUNA//HEILO | − − | − − | − | − | − | + + | − − | + − | NA | − − |
| 5999852 | YAR/AE.SQUARROSA (518)/3/PRL/SARA//TSI/VEE#5/4/ATTILA/5/BERKUT | − − | − − | − | − | − | + + | − − | − − | + − | + − |
| **10004** | **SUMAI #3 (resistant check)** | − − | + + | − | + | − | − − | + + | − − | + + | + + |
| **5536** | **GAMENYA (susceptible check)** | + − | − − | − | − | − | + − | − − | − − | − − | − − |

Information on the sizes of PCR products is available in Table S1. Data from 17 markers linked to ten validated QTLs are presented, where WU stands for Wuhan 1, CJ for CJ 9306, FR for Frontana, SU for Sumai 3, and DI for *T. diccocoides*. '+ +' denotes the presence of the QTL supported by both flanking markers; '+ −', supported by only one marker; '− −' putative absence of a QTL; 'NA', not analyzed. For SU_3B, DI_3A, and FR_3A, only one flanking marker was applied to predict the presence/absence of QTL

The two checks Sumai 3 and Gamenya are bolded

(Wilcoxson et al. 1992). The selection for the 15th FHBSN was mainly based on FHB index and DON concentration, with an attention on the maintenance of genetic diversity based on pedigree information, in accordance with the selection for our previous FHBSNs (He et al. 2013a, b). Screening on DH and PH was done in 2010 primarily to discard very late and tall lines.

Although the 15th FHBSN accessions generally exhibited low levels of infection in both El Batán and Toluca (Mexican environments), many turned out to be susceptible in other five locations, due to a significant genotype-by-environment interaction, which could be caused by one or all of the following reasons; different inoculation protocols, field management, weather condition, *Fusarium* isolates etc. Many studies have shown that FHB resistance in wheat is horizontal, not species- nor strain-specific (Van Eeuwijk et al. 1995; Mesterhazy et al. 1999, 2005). Therefore the differences in FHB levels resulting from variation in *Fusarium* isolates or species used throughout this study may not explain the resistance variation across locations, although significant differences in aggressiveness have been reported in *Fusarium* isolates/species of different geographic origins (Malihipour et al. 2012).

The environmental effects are obviously seen in 2013 at El Batan where the high precipitation during the epidemic season (227.1 mm, July–August) led to more FHB epidemics than those in the 2011 and 2012 seasons (August–September), with 39.5 and 126.7 mm of precipitation scored, respectively (El Batán weather station, CIMMYT). Though it is anticipated that the misting system could provide sufficient micro-environmental moisture and thus rainfall would not significantly contribute to increased disease development; results from El Batán 2013 rejected this hypothesis or at least raised questions about it. The reason could be due to the rain-splash facilitated pathogen spread; even though spray inoculation was adopted in this experiment, huge quantity of *Fusarium* pathogen was expected to be present in the soil after the long-term use of the field as FHB screening nursery, leading to what is called background infection. Similar situation was also found in Ontario, Canada (Tamburic-Ilincic et al. 2013) and in Nebraska, USA (Nopsa et al. 2012). Thus planting in the year was advanced half month compared to previous years, after realizing that early planting in El Batán usually leads to higher FHB disease pressure (He et al. 2014). This highlights the importance of multi-locational and/or multiple years FHB evaluation, whereby the potential resistant germplasm are exposed to diverse epidemic environments, facilitating the identification of genotypes with durable resistance as well as the selection of locally adapted lines useful to national breeding programs. As mentioned above, several lines were consistently resistant across environments and thus could be used as resistance sources; although their resistance is not as high as that of Sumai 3, especially under high disease pressure.

Generally, mycotoxin content is the most important FHB trait regarding food safety; but it is also laborious and costly to evaluate compared to FHB and FDK, especially in mass screening programs where thousands of accessions are tested annually. Accordingly it is obligatory to do a couple of field evaluations to reduce the total number of accessions to be tested for DON content by excluding lines which have high FHB index and/or FDK. This indirect screening strategy is based on the controversial association among different FHB parameters, most notably between FHB index and DON content. However the conclusions from different studies are debateable and the correlation between FHB index and DON ranges from no significant association to strong positive correlation (Paul et al. 2005, 2006). In the present study, DON content appeared to be the least stable FHB parameter across years in El Batán compared to the more stable FHB index as shown in the biplot, and no high correlation of FHB/DON and FDK/DON were found. The main reason for this could be ascribed to a lack of major QTL conditioning both field FHB and DON/FDK resistance, e.g. *Fhb1*, as proposed by Lu et al. (2013). Additionally, the temporal separation of evaluations for different FHB traits, invisible infections and wide diversity present in the studied lines are all possible reasons that could have caused the lack of expected correlations. This implies that low FHB and FDK do not necessarily lead to low DON, which complied well with our phenotypic data. Therefore, varieties with low FHB and FDK should be further tested for DON to determine their resistance components.

AE has been reported to be negatively correlated with FHB/DON and to be part of the Type I resistance after spawn and spray inoculation (Lu et al. 2013). In the present study, all the 15th FHBSN entries exhibited high AE rates, which could have conferred good Type I resistance that protected the materials very well in the low epidemic years of 2011 and 2012; but the protection was not sufficient in 2013 in El Batán, Norway, the Netherlands and Canada, implying weaker Type II resistance.

Unlike the 13th and 14th FHBSN, where the 2DL QTL as in Wuhan 1 was the predominant one (He et al. 2013a, b), the 15th FHBSN suggested a high frequency of 55 % of the 4BS QTL as in Wuhan 1. Although both were found in Wuhan 1, the 2DL QTL conferred Type II resistance, whereas the 4BS one contributed Type I resistance (Somers et al. 2003). The latter has been fine mapped by Xue et al. (2010) and designated as *Fhb4*. The QTL on 7A chromosome as in *T. dicoccoides* was the second frequent QTL, contributing to Type II resistance (Kumar et al. 2007); but it was found only in five lines. Considering also the very low frequencies or absence of other QTLs, the haplotyping results proposed a clear non-Sumai 3 resistance background of the 15th FHBSN, which lacked major Type II resistance QTLs such as 3BS (*Fhb1*) and 6BS (*Fhb2*) as in Sumai 3 (Cuthbert et al. 2006, 2007). CIMMYT has devoted great efforts on the identification and utilization of non-Sumai 3 resistance since the

last decade (He et al. 2013a), which was very successful as shown by the haplotyping results. *Fhb1* and *Sr2* are linked in repulsion (Flemmig 2012), thus the deployment of *Sr2* in high proportions of CIMMYT germplasm due to Ug99 (stem rust) threat has possibly further resulted in eliminating *Fhb1* gene from CIMMYT germplasm. Based on 2013 results, wherein very high disease was observed, it is imperative to introduce resistance genes/QTLs of both Sumai 3 and non-Sumai 3 origins, particularly the two Type II resistance genes *Fhb1* and *Fhb2*, into the CIMMYT germplasm to increase the resistance level.

It is well known from previous studies that the dwarfing genes *Rht-B1b* and *Rht-D1b* are associated with FHB susceptibility due to either genetic linkage, pleiotropic effect, or disease escape (Hilton and Hollins 1999; Schmolke et al. 2005; Holzapfel et al. 2008; Yan et al. 2011). However, the negative effect of *Rht-D1b* is more significant than that of *Rht-B1b*, and it has thus been advised to utilize the latter to achieve a desirable plant height at a relatively low cost of increasing FHB susceptibility (Miedaner and Voss 2008; Srinivasachary et al. 2009). According to our results, it was *Rht-B1b* instead of *Rht-D1b* that prevalent in this nursery, which is the ideal in terms of FHB resistance.

Taken together the phenotypic and genotypic data, this study demonstrated that the 15th FHBSN entries have generally good Type I and Type IV resistance, but lower Type III resistance. Although there was no direct evidence, their Type II resistance level may not be high, considering the high disease levels in El Batán, Norway, the Netherlands, and Canada in 2013, as well as the absence of major Type II resistance QTLs. Strategies are being adopted involving pyramiding of Sumai 3 and non Sumai 3 resistance in CIMMYT germplasm and breaking the repulsive linkage of *Sr2* and *Fhb1*. Resistant genotypes identified in this study could be successfully utilized by breeders as donors of novel FHB resistance in an attempt to diversifying and/or pyramiding FHB resistance.

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