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BREEDING FOR ORGANIC AGRICULTURE: RESISTANCE TO FUSARIUM HEAD BLIGHT IN DURUM WHEAT FOR INCREASED FOOD SAFETY

GRADUATION THESIS; MS.c.

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ZÜCHTUNG FÜR DEN BIOLANDBAU: WIDERSTAND GEGEN ÄHRENFUSARIOSEN IN HARTWEIZEN FÜR ERHÖHTE LEBENSMITTELSICHERHEIT

DIPLOMARBEIT

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
B.C.	Before Christ
bw	body weight
cm	centimeter
FHB	Fusarium head blight
F_7	seventh filial generation
ha	hectare
kg	kilogram
L.	Linnaeus (Carl Linnaeus, author of the names of the species)
LSD	least significant difference
m	meter
mio	million
min	minute
mL	milliliter
m^2	square meter
Mha	million hectar
MMT	million metric tonne
ng	nanogram
QTL	quantitative trait locus/loci
Rht	reduced height (gene)
SR	stripe rust
W	Watt
°C	degree Celsius
µg	microgram
µl	microliter

ABSTRACT

One of the main concerns when breeding for organic agriculture is creating cultivars with good resistance to aggressive pathogens. Fusarium head blight (FHB) is a fungal disease that affects all cereals and causes severe yield and quality losses as also mycotoxin contamination of the grain. Durum wheat (*Triticum durum* Desf.*)* especially, suffers from above average FHB attack. Since breeding for resistance in durum wheat is impeded by the lack of suitable resistant cultivars in tetraploid wheat, a major focus lies on transferring FHB resistance from hexaploid wheat to durum wheat. In this study, the introgression of the hexaploid FHB resistance quantitative trait locus *Fhb1* into durum wheat was assessed. Two populations of 100 F_7 recombinant inbreed lines (RILs) were developed from crosses of the resistant and tall durum line DBC-480-1 with two susceptible and short durum wheat cultivars Durobonus and SZD1029K, respectively. DBC-480-1 was obtained by marker-assisted backcrossing of *Fhb1* from the donor line Sumai-3 into the durum wheat cultivar Semperdur. The two RIL populations were grown in the field and evaluated for FHB resistance after spray inoculation with *Fusarium culmorum* and for plant height. The effect of the segregating alleles at the *Fhb1* locus and the plant height controlling locus *Rht-B1* was analyzed for these two traits. The alleles at these loci were detected in plant material using the molecular markers *Umn10* and *Barc147* associated with *Fhb1,* and allele specific markers for *Rht-B1a* (wild-type allele) and *Rht-B1b* (semi-dwarf allele). Additionally, flowering date and severity of stripe rust were recorded in the field. In this study we showed that the effect of *Fhb1* was dependent on the genetic background: *Fhb1* resistance allele significantly reduced FHB severity in one population while showing no significant effect in the other population. In both populations plant height was tightly negatively linked with FHB severity, and was significantly affected by the *Rht-B1* locus. *Rht-B1b* semi-dwarfing allele was found to have a strong effect on FHB severity. Although flowering date was significantly correlated with FHB resistance only in one population; it seems to be affecting FHB severity due to the climatic conditions occurring at the flowering time.

Key words: Fusarium head blight, durum wheat, *Triticum durum*, *Fusarium culmorum*, *Fusarium graminearum*, mycotoxins, Deoxynivalenol, organic, *Fhb1*, *Rht-B1*, plant height

ZUSAMMENFASSUNG

Ein besonders großes Interesse wenn man für den biologischen Landbau züchtet, liegt in der Entwicklung von Sorten mit ausgeprägter Resistenz gegenüber aggressiven Krankheitserregern. Als Pilzerkrankung betreffen Ährenfusariosen alle Getreidearten und verursachen schwerwiegende Ertrags- und Qualitätsverluste, sowie eine Kontamination der Getreidekörner mit Mykotoxinen. Unter einem Befall mit Ährenfusariosen leidet vor allem der Durumweizen (*Triticum durum* Desf.) überdurchschnittlich stark. Die Resistenzzüchtung im Durumweizen ist aufgrund des Fehlens von passenden resistenten Sorten im tetraploiden Genpool erschwert, weswegen man die Übertragung der Ährenfusariosen – Resistenz von hexaploiden Weizensorten in den Durumweizen versucht. In dieser Studie wurde die Introgression des Resistenzlocus *Fhb1* aus hexaploiden Weizen in den Durumweizen untersucht. Zwei Populationen von je 100 F_7 Linien wurden entwickelt aus Kreuzungen des resistenten Durumweizen DBC-480-1 mit den zwei anfälligen Durumweizensorten, Durobonus und SZD10209K. Die experimentale Linie DBC-480-1 wurde durch markergestützte Rückkreuzung von *Fhb1* der Donorlinie Sumai-3 in die Durumsorte Semperdur erstellt. DBC-480-1 zeigt eine hohe Wuchshöhe, die beiden Durumsorten sind hingegen kurz. Die zwei Kreuzungspopulationen wurden im Feld auf Ährenfusarioseresistenz nach Sprühinokulation mit *Fusarium culmorum* untersucht, zusätzlich wurde die Pflanzenhöhe erfasst. Die Auswirkung der Allelzustände am *Fhb1* Locus und am Locus für Pflanzenhöhe *Rht-B1* wurde analysiert. Dafür wurden die 200 Linien mit molekularen Markern genotypisiert: *Umn10* und *Barc147 sind* mit *Fhb1* assoziiert, und den allelspezifischen Markern für *Rht-B1a* (wild-Typ Allel) und *Rht-B1b* (Kurzstroh-Allel). Zusätzlich wurde im Feld der Blühzeitpunkt und der Schweregrad des Gelbrostbefalls bonitiert. Die Studie zeigt, dass der Effekt von *Fhb1* von dem genetischen Hintergrund abhängig ist: das *Fhb1* – Resistenz-Allel zeigte in einer Population eine signifikante Reduzierung des Fusariumbefalls, in der anderen Population jedoch konnte kein eindeutiger Effekt detektiert werden. In beiden Populationen war die Pflanzenhöhe hoch negativ mit dem Auftreten von Ährenfusariosen korreliert und wurde signifikant durch den *Rht-B1* Locus beeinflusst. Das *Rht-B1b* Kurzstroh-Allel wirkte sich stark auf den Schweregrad der Ährenfusariosen aus. Der Blühzeitpunkt korrelierte nur in einer Population signifikant mit der Fusariumbefallsstärke und scheint von den vorherrschenden Witterungsverhältnissen während der Blüte verursacht.

Schlüsselwörter: Ährenfusariose, Durumweizen, *Triticum durum*, *Fusarium culmorum*, *Fusarium graminearum*, Mykotoxine, Desoxynivalenol, ökologisch, *Fhb1*, *Rht-B1*, Pflanzenhöhe

1 INTRODUCTION

1.1 FOOD SAFETY AND ENVIRONMENTAL HAZARDS IN MODERN AGRONOMY

The world population is rapidly growing; the number has more than doubled since 1960s, reaching roughly 7.5 billion in 2016 (Geohive, 2016). Subsequently, the demand for food supplies is increasing. To face this challenge, the actors of food chain are constantly searching for new practices and technologies to increase food yields without jeopardizing the consumer's food safety, in accordance with strict surveillance on food contamination of public health authority (Käferstein and Abdussalam, 1999; Antle, 1999). Both natural and human induced contaminants are of concern and can be detected in the lowest concentration in every step of food supply chain. Their upper limits for risk assessment are acceptable daily intake (ADI) and tolerable daily intake (TDI) expressed per body weight (mg/kg bw/day) (Nasreddine and Parent-Massin, 2002; Binder et al., 2007).

Contamination of food commodities with *Fusarium* mycotoxins is a major agricultural issue (Bryden et al., 2001).The fight against these natural toxins starts already with suppressing their cause, the fungal pathogen, beforehand. Numerous cereals and other crops are a common host of fungi on the field. Preventing their attack and suppressing their growth not only increases food safety but also omits economic losses (Leskowicz, 2006; Matz, 1991).

A well set measure in pre-harvest pathogen fighting is pesticide application resulting in a yearly pesticide consumption of about two million tones worldwide (De et al., 2014). Subsequent, negative externalities have arisen. Due to pest's adaptation and higher tolerance to agrochemicals, stronger concentrations, increased frequency and new chemicals need to be applied in order to fight the pests, causing damage to agricultural land, fauna and flora (Wilson and Tisdell, 2001). In this way, pesticide may work very unsustainable. Different pesticides have also been found in drinking water in the regions with high agricultural intensity (Alavanja, 2009). Many formerly used chemicals have been banned by the authorities due to their strong persistence in the environment and/or acute toxicity to whether human or other fauna (EU Comission, 2016). Overall, pesticides have been proven to work immunosuppressive, thus inhibit immune responses to pathogens and tumor (Repetto and Baliga, 1997).

In order to minimize risks of food contaminations mentioned so far, as well as diminish environmental hazards and sustain desired yields, sustainable low-input farming approach can be applied. Although organic farming has undergone a cogent development in the recent years, it remains handicapped because of deficiency of variables suitable for conservational farming systems. Since there are not plenty varieties produced that favor sustainable and organic agriculture, the organic sector is dependent on breeding programs that grow robust varieties (Lammerts van Bueren, 2008; Konvalina et al., 2011). Principle of breeding for organic agriculture is forming genotypes that are stable and reliable in performance under all environmental conditions. This means the varieties must not possess any detrimental flaw in a trait that is important for growth and productivity

(Stagnari et al., 2013; Wolfe, 2008). Organic wheat genotypes require an interaction of early crop vigor for nutrient uptake, weed competition and pathogen resistance. Achieving high plant vigor occurs through buffering capacity of internal self-regulatory mechanisms instead of external control measures such as chemical phytopharmaceuticals (Wolfe et al., 2008; Lammerts van Bueren, 2002). Hence, understanding the functional relationships between production systems and desired physiological responses ensures us a foundation for desired improvement of plant genetic material for organic agriculture. By providing that, we do not only accomplish satisfactory level of food and environmental contamination, but also contribute to soil structure and soil fertility improvement as well as higher overall production quality of grain in specific functional properties. Further we benefit cereal straw, which is extensive for organic animal husbandry and manure composting (Wolfe et al., 2008; Cavoski et al., 2015). The projected organic crop ideotype should not only be of interest to actors of organic farming systems but also conventional sector decreasing excessive inputs of chemical fertilizers, pesticide as well as energy and thus also decreasing food, feed and environment contamination (Lammerts van Bueren, 2002).

1.2 OBJECTIVES

We investigated resistance to Fusarium head blight (FHB) on durum wheat (*Triticum turgidum* subsp. *Durum*) at the Institute of Biotechnology in Plant Production in Tulln, part of the University of Natural Resources and Life Sciences. The reason for this research is the fact that tetraploid wheat, especially durum wheat, is highly susceptible to FHB, resulting in yield losses and lower food safety. The only effective damage control against FHB is development of resistant cultivars. In this project, DBC-480-1, derived from the back-cross of the bread wheat FHB resistant source Sumai-3 with durum wheat, has been used as resistant parental line in two crosses with durum wheat cultivars Durobonus and SZD1029K, respectively. The two populations derived from these crosses allow the evaluation of FHB resistance.

The aim of this project was to:

- Characterize variation for FHB resistance in durum plant material,
- Evaluate the effect of the major common wheat FHB resistance quantitative trait locus (QTL), *Fhb1,* in two durum wheat backgrounds,
- Evaluate the effects of morphological and developmental traits (date of flowering, plant height) on the disease resistance,
- Evaluate the effect of the plant height gene *Rht-B1* on disease resistance,
- Analyze the possible interference of stripe rust disease on FHB resistance.

2 LITERATURE REVIEW

2.1 DURUM WHEAT

2.1.1 Botanic description

Durum wheat, *Triticum durum* Desf., is also known as durum, hard wheat or macaroni wheat. Like all the wheats it belongs to the genus *Triticum* of grass family (*Poaceae* or *Gramineae*), *Triticeae* tribe and *Triticinae* subtribe (CFIA, 2006).

Durum wheat is a monocotyledonous, predominantly spring or semi-winter annual grass. Originally it is a mid-tall annual grass (1.2-1.5 m), however new semi-dwarf durums have been developed that adapt better to modern cultivation (Bozinni, 1988; Abinasa et al., 2011). Durum plants base is a relatively strong adventitious root system arising from the underground nodes of the culm and can branch up to one meter deep in optimal conditions. It grows into erect, usually hollow cylindrical stem from which the leaves grow distichously. Florets are typically hermaphroditic. Clustered together they form sessile to semi-sessile spikelet, lastly forming a spike. At the base of the spikelets, there are two well-developed, firm, narrow shouldered bracts called glumes, where the florets form. The axis carrying the spikelets is called rachis and rachilla is the axis of the spikelet that attaches the rachis on its fragments. Florets vary from two to six per pair of glumes. At the base of the flower there are two small bodies called lodicules that become tumid at the beginning of the blooming stage, hence open the glumes and then the florets (Bozinni, 1988). Each floret is enclosed by bract like structures called the lemma, with long awns, and the palea. The florets are perfect flowers and can produce a one-seeded fruit, caryopsis (CFIA, 2006; Bozinni, 1988).

Figure 1: *Triticum turgidum* plant (illustratedgarden.org, 2016).

Durum wheat is widely adapted to a range of environmental conditions; from temperate colder climates to warm tropical and semiarid conditions (Kadkol and Sissons, 2016; Bozzini, 1988). It can grow on heavy clay to sandy soils, though well drained loams are utmost suitable for its cultivation (Singh et al., 2010). The majority of world's durum wheat is grown in rainfed semiarid conditions of rather dry climate, warm days and cool nights and high drought-stress frequency during the vegetation stage (Kadkol and Sisson, 2016). High summer temperatures and low humidity favor grain quality. Rainy harvest season can cause problems as durum wheats are susceptible to sprouting (Matsuo, 1994). Spring durum types predominate. In warmer climates they are planted in late autumn or early winter and harvested in summer, oppose to more temperate climates, where they are planted in spring and harvested in late autumn. The winter varieties are planted in autumn and harvested in summer (Kadkol and Sissons, 2016). Winter durum wheats require whether vernalization or long days and short nights whereas spring durums neither. Under normal cultivation conditions each durum plant consists of one to three tillers, each one developing into one head. Depending on weather condition, blooming of a spike lasts three to six days. Normally, durum wheats are self-fertile, although depending on variety and environmental conditions, outcrossing can reach 5 %. Humidity is more favorable to selfing, whereas drier conditions favor outcrossing. Two to three weeks after fertilization, the embryo is physiologically functional, thus able to precede its development into a new plant. Thirty to fifty days after fertilization the seed starts to become ripe and accumulates major reserves within the last three weeks of ripening. When the water content drops under 15 % it reaches complete ripeness (Bozinni, 1988).

Durum kernels are larger, hard-vitreous and amber in color, later is due to the presence of carotenoids (Wrigley, 2016; Joppa and Williams, 1988; Ficco et al., 2014). Vitreous kernels have glassy and translucent appearance, whereas nonvitreous kernels starchy and opaque (Dowell, 2000). The largest morphological part of the grain is endosperm (~ 82% of grain weight), consisting of starch granules embedded in proteins. The bran, rich with proteins and minerals, and the embryo represent ~15 % and ~3 % of the grain weight, respectively (Holopainen-Mantila, 2016; Corke, 2016). Bran, embryo, semolina and flour are the main milling fractions (Joppa and Williams, 1988). Semolina, crushed coarse endosperm fraction remaining on top of a U.S: 100 sieve, is the largest part of durum wheat harvest and is used to produce pasta products (Corke, 2016; Joppa and Williams, 1988; Lafiandra et al., 2012). Important quality characteristics of durum kernel are yield of semolina, hard and vitreous endosperm, a high protein and yellow pigment content, and a good gluten protein composition. Overall, the higher the protein content, the higher the kernel vitreousness and the higher semolina milling yield (Lafiandra et al., 2012; Joppa and Williams, 1988). Physical defects negatively affect semolina and pasta products. Black colorations are very undesirable. Redness or decreased yellow pigment is caused by Fusarium head blight, a fungal infection resulting also in kernel shriveling and mycotoxin contamination. Before milling, grain moisture content needs to be determined to assure desired moisture content of semolina, which is 14-15 %. Higher moisture content also increases microbiological growth. Excessive ash content is a sign of high contamination of semolina by the bran (Sissons, 2016). The ultimate test of durum grain quality, especially when accepting new durum lines, is evaluation of final pasta

products. Good cooking quality, promoted by gluten strength, and bright yellow appearance are besides aroma and texture desired traits of durum pasta by the consumers (Kadkol and Sissons, 2016; Sissons, 2016).

2.1.2 Origin and spreading

Durum wheat has 14 pairs of chromosomes organized in two genomes (A and B) while common wheat harbors 21 pairs of chromosome, sharing the A- and B- genome with durum wheat, and an additional D-genome. The genomes, and the chromosomes of corresponding number (e.g., 1A vs 1B vs. 1D), are homoeologous – that is, they are similar but not identical (homologous). For instance, homoelogous genes for red grain color are located on chromosome 3A, 3B and 3D (Akhunov, 2016).

It is estimated that roughly 10,000 years ago first domestication of ancient wild cereal species occurred on the Fertile Crescent territory of South-West Asia. Amongst them were einkorn (*Triticum monococcum* L.), diploid wheat with genomes "AA", and a tetraploid domesticated emmer (*Triticum dicoccon* (Schrank) Schübl.), possessing genomes "AABB". As known, all the wheat species originate from wild diploid grass ancestors, one being einkorn (*T. urartu*), the donor of "A" genome, and the other, an unknown close relative of *Aegilops speltoides* Tausch as donor of genome "B". Through their crossing and gene duplication durum wheat arose and further, by crossing with diploid *Ae. tauschii*, the donor of the "D" genome, led to the emergence of hexaploid (AABBDD) common wheat. An important trait as a result of domestication is non-brittle rachis that retains the seeds on the plant and enables the harvest (Miedaner, 2014; Bozzini et al, 2012; Dubcovsky and Dvorak, 2007). Emmer wheat spread further to Italy and Greece passing the coast of North Africa and the Mediterranean islands from the years 5000 to 3500 B.C. In the second millennium B.C. it established great importance for the nutrition amongst cereal crops in Mediterranean. Emmer remained under cultivation until 300 B. C. when it was replaced by durum wheat. From there it was adopted to other European and African countries as well as carried across the ocean (Miedaner, 2014; Bozzini et al., 2012; Kadkol and Sissons, 2016).

2.1.3 World production and use

Wheat is the principal cereal crop of the temperate regions of the world and a staple food of about half the population of the world. It ranged 3rd in the worldwide production of grains in 2013, with 716.19 million metric tons, after corn (1,018.11 MMT) and rice (740.9 MMT) (statista, 2016). Durum wheat represents 5 % of the total wheat production and is after bread wheat the second most important *Triticum* species for human consumption (Matsuo, 1994). The biggest share of durum wheat supply produce countries of the EU (10 mio tons of grain on 2.8 mio ha); Italy (50% of the total durum area in EU), Greece and Spain are the leading ones and make up for 80 % of total durum production in EU (Porceddu and Blanco, 2014; Grant et al., 2012). In the recent years Canada has increased its durum wheat production surpassing the production of North African countries Morocco, Algeria and Tunisia as well as production of Turkey and Syria. Other producers include Kazakhstan, Russia, United States of America, Mexico, India, Far East Asia and

Australia (Gillen, 2013; Grant et al., 2012; Matsuo, 1994; Matz, 1991; Kadkol and Sissons, 2016). Austrian durum production amounts 15,000 ha with an average yield of 67,000 tones (BMLFUW, 2016).

Organic agriculture with 43.7 million hectares (year 2014) represents 0.99 per cent of total world agricultural area. In Europe the share is more than double, 2.4 % of total European agricultural land or 11.6 million hectares, which is more than a quarter of total organic cultivated area worldwide. Organic crops yields equal on average 80 % of conventional yields (Ponti et al., 2012; Ponisio et al., 2014). In 2014, at least 3.3 mio ha of cereals were under organic management, which is 40 % of overall organic surface (0.5 % of the total world cereal area). Austria, for instance, greatly exceeds the global 0.5 per cent with 12.2 % organic cereal share (Lernoud and Willer, 2016). In Italy organic cereal share equals 5.9 % , amongst which durum wheat is the most diffused, grown on 0.074 million hectares (Lernoud and Willer, 2016; SINAB, 2014 in Camerini et al., 2016).

The major Mediterranean durum-producing countries, such as Italy, Spain and France, are large organic producers with 1 069 339 ha, 388 031 ha and 420 000 ha of organic production, respectively. Of the non-EU Mediterranean countries, Turkey is the largest organic producer in terms of area with 44 552 ha. In the Mediterranean countries, cereals are major organic crops in Egypt, France, Greece, Israel, Italy, Lebanon, Portugal and Spain (De Castro et al., 2002).

Durum wheat is almost entirely intended for human consumption (Ranieri et al., 2012). In Western Europe and North America durum wheat is used primarily for the production of semolina as a raw material for pasta products. Diets of millions in the Near East and North Africa are based on various durum products, with local durum breads accounting for half of their durum consumption. Single- and two-layer bread, burghul, cous-cous and frekeh are as well on their regular diet (Matz, 1991; Matsuo, 1994). Because durum wheat kernels are extremely hard, longer milling times and more milling energy are required. Durum flour therefore consists of coarse endosperm particles with greater amount of damaged starch. Role of protein and starch in wheat is significant for viscosoelastic properties of the dough, which determines performance in processing. Durum flour semolina produces bread of smaller loaf volume, has a prolonged shelf-life and an intensive yellow color, which is latter well reflected in pasta products and cous-cous. High cooking quality and stability to overcooking are further typical qualities of semolina (Matsuo, 1994; Matz, 1991; Sisson, 2008; D'Egidio, 2001; Peña, 2003).

2.2 FUSARIUM HEAD BLIGHT

Fusarium head blight (FHB), also Fusarium ear blight or scab, is a devastating disease of small grain cereals caused by up to 17 different species (Parry et al., 1995). *Fusarium graminearum* and *Fusarium culmorum* are two most common and aggressive FHB pathogens, still moderately present are *F. avenaceum* and *F. poae* (Miedaner, 2012). Although many pathogens causing FHB can as well induce other diseases on crops, like seedling blight or foot rot, FHB is far the most important concern (Wagacha and Muthomi, 2007). Yield losses under severe circumstances of hot and wet conditions can attain even

up to 30 %, including quality decrease that negatively affects the sale of cereal and endangers food security (Miedaner, 2012). Durum wheat in particular possesses severe susceptibility to FHB, much higher than common wheat (Prat et al., 2014; Ban and Watanabe, 2001).

2.2.1 Morphology

Fusarium is a genus of ascomycete fungi containing many common soil saprophytes, as well as endophytes and plant pathogens, and is frequently found in cereal grains. Many species of *Fusarium* produce a number of secondary metabolites, which evoke physiological and pharmacological responses in organisms (Vesonder and Golinski, 1989). *F. graminearum* and *F. culmorum* of the section *Discolor* are utmost important FHB pathogens, less aggressive but still moderately present are *F. avenaceum* and *F. poae*. *F. graminearum* is also the most ubiquitous of them all. *F. culmorum* only occupies certain regions of the world (Miedaner, 2012; Liddell, 2003).

The primary morphological features of *Fusarium* are the shape and size of macroconidia, microconidia and chlamydospores, and also type and presence of conidiogenous cells. Further criteria of division include hyphae feature in the culture, pigments, odor, growth rate and secondary metabolites (Leslie and Summerell, 2013). Cereal fusaria are principally distinguished on the basis of macroconidia morphology, considering they only form microconidia under certain cultural conditions. The macroconidia have a thick wall, are distinctly septate, fusiform to falcate with beaked or fusoid apical cell. Chlamydospores are usually present and may form either from hyphae or from the cells of the macroconidia (Wagacha and Muthomi, 2007). The effective survival strategies, including chlamidiospores, thickened hyphae, and resistant perithecia as well as good adaptation to grass hosts are substantial reasons why the FHB is difficult to combat (Liddell, 2003).

The red pigmentation of *F. graminearum* and related species that cause stem and head blight of cereals is due to the deposition of aurofusarin in the cell walls (Malz et al., 2005).

2.2.2 Lifecycle

Fusarium species are low specialized soil-borne fungi with a facile life-cycle with ability to colonize the host at its every life stage (Miedaner, 2012). They perform multiple sexual lifestyles: asexual, homothallic and heterothallic. While the sexual reproduction does not predominate in the field in most species, the asexual stage and hyphae are produced continuously under sufficient available food resources. Whether or not species that appear to have limited sexual development in the field still might reproduce sexually remains uninvestigated. *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch], a homothallic species, depends on sexual development for spore dissemination to host plants, which is essential for disease outbreak (Trail, 2013). *F. culmorum* on the other hand, is up to date known to reproduce only asexually with spores (conidia). Both however have similar life-cycle. Additionally, *F. culmorum* builds permanent spores (chlamydospores) in the soil (Miedaner, 2012).

The infestation occurs through mycelium spores resting on organic soil material or harvest residues of preceding crop. Additionally, the infection of seedling might as well occur through infected seed (Miedaner, 2012). With increased relative humidity the ascospores (sexual spores) mature in perithecial and are forcibly discharged in the air (Trail et al., 2002). Largely by means of wind and rain, macroconidia (asexual spores) as well as ascospores land on the host - durum wheat in our case (Bushnell et al., 2003). Penetration causing the most external damages arises through inflorescence. Because of the thick-walled cells that build florets, and the enclosing glumes, the fungus cannot penetrate directly through epidermis inside the floret. Several potential pathways on *Fusarium* active penetration have been proposed, suggesting stomata entry in glume, the lemma and palea, mouth of the apex or crevice between the palea and lemma (Bushnell, 2001). Either way, the penetration through cuticle is presumably assisted by diverse hydrolyzing enzymes (i. e. cutinases, lipases) secreted by the fungi. Additionally, it has also been demonstrated that fungal growth stimulants may be present in anthers (Walter et al, 2010). The infection follows as described by Urban and Hammond-Kosack (2013): *Fusarium* spore germinates at the tip of the floret followed by hyphae growth ectopically on stamen, filaments and inner side of palea and lemma. After invasion the ovary development terminates. Further hyphae growth proceeds from floret to floret within the same spikelet then to rachis, causing rachis browning. The infection now spreads to vascular system and moves upwards as well as downwards, leading to bleaching of the spikelets. The disease advances and grows into peduncle. Once the infection is present on the inflorescence, the fungi grow gradually both intra- and intercellularly throughout caryopsis. *F. graminearum* can grow between cells without entering them, and establish biotrophic parasite-host association (Bushnel, 2001). A crucial aggressiveness factor in FHB spread within the spike has been shown to be the mycotoxin deoxinivalenol (DON) produced by *F. graminearum* and *F. culmorum* (Snijders, 2004). When two different virulent strains of *F. graminearum*, one DON-producing and the other DON-nonproducing, were compared, the DON-producing *F. graminearum* caused significantly more disease symptoms and yield reduction. However, DON production is not essential for initial infection (Bai et al., 2001).

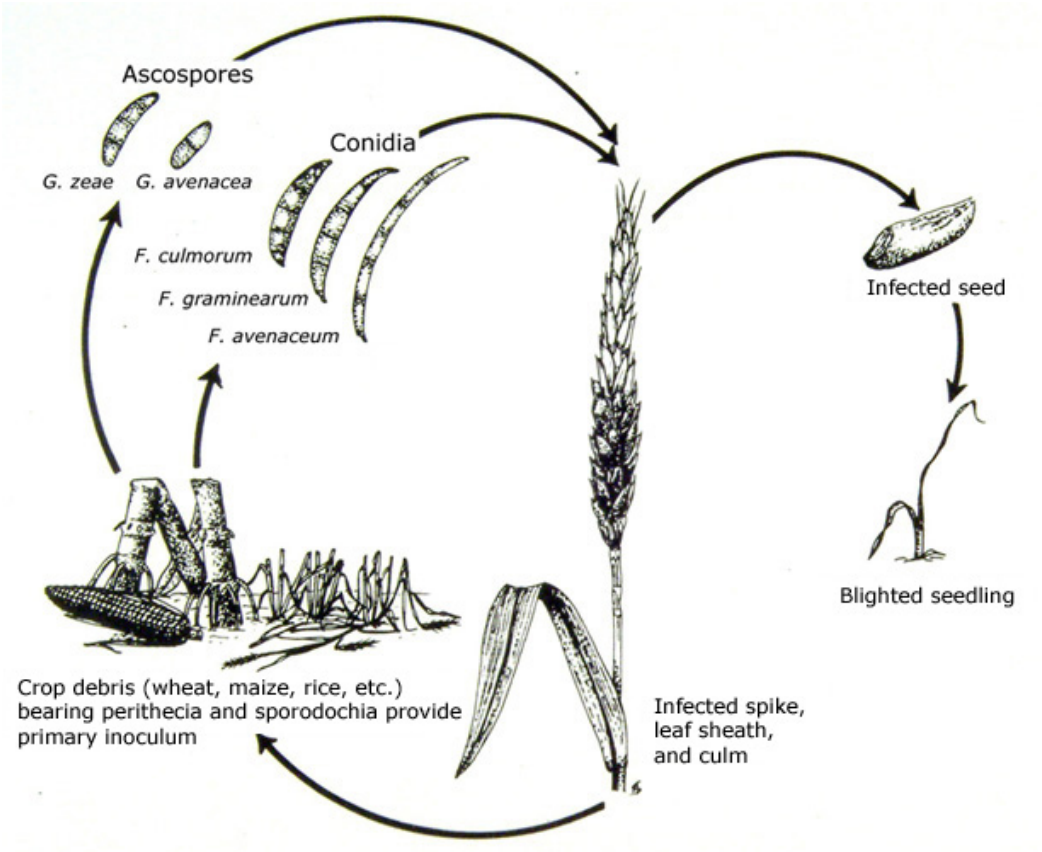

Figure 2: Life-cycle of FHB (American Phytopathological Society, 2016).

The main sources of spike infection are the harvest residues of preceding crop and sufficient precipitation (3-4 mm within one day or 2 mm within few days) during anthesis. The susceptibility to *Fusarium* infection is highest from the middle point of ear emergence to the end of flowering and causes the most damage. *Fusarium graminearum* requires temperature above 17 ºC and *F. culmorum* 10 – 14 ºC (Miedaner, 2012). After anthesis or later at the milk to soft dough stage of kernel development the cereals are less susceptible, although further spreading of fungus in the spikes and accumulation of mycotoxins is critical under sufficient humidity until the seeds ripen (Bushnell et al., 2003).

During the first few days, symptoms of FHB attack remain latent, soon after brown, dark purple to black necrotic lesion on the florets and glumes can be observed (Urban and Hammond-Kosack, 2013; Goswami and Kistler, 2004). Further sings may be discolored peduncle in brown or purple. After a period of time, florets become blighted, bleached and tan, appearing prematurely and unevenly ripe. Awns are frequently deformed, twisted and curved downwards (Richard, 2007; Goswami and Kistler, 2004). In most susceptible cultivars, the formation of the grains could be inhibited completely or partially, resulting in shrivel grains of blanched appearance (tombstone kernels) and high in mycotoxin content (Bushnel et al., 2003; Richard, 2007). With abundant precipitation the fungi can form mycelium of light pink to salmon-orange color (Miedaner, 2012; Goswami and Kistler, 2004). Furthermore, infected seeds bear lower germination rate and seedling vigor (Stenglein and Rogers, 2010).

2.2.3 Mycotoxins

Mycotoxins are secondary metabolites produced by ascomycete filamentous fungi on agricultural commodities only few hours after infection on the field (Shwab and Keller, 2008; Krska, 2009; Miedaner, 2012). Plant pathogenic species of *Fusarium* produce numerous phytotoxic mycotoxins during infection of host plants which disrupt host defense responses and suppress plant growth (Nishiuchi, 2013). *F. graminearum* and *F. culmorum* build the same mycotoxins, these are foremost the group of Trichothecene: Deoxynivalenol (DON) and Nivalenol (NIV); as well as Zearalenone (ZEN) (Miedaner, 2012). The primary toxic effect of trichothecenes is protein synthesis inhibition (Rocha et al., 2005). It is suggested that DON and NIV production ability of *F. graminearum* and *F. culmorum* reflects their aggressiveness level thus contribute to the virulence of the fungi (Mesterházy, 2002; Nishiuchi, 2013). A fungus strain of *F. graminearum* and *F. culmorum* can produce whether only DON or NIV toxins, but never both together (Miedaner et al., 2012). Nevertheless, *F. graminearum* can still produce both DON and ZEN which can lead to co-contamination of cereals with both metabolites (Nishiuchi, 2013). DON concentration in contaminated grain is also more frequent and at higher levels than NIV (Turner, 2010).

Among thousands of known mycotoxins only a scatter presents food safety challenges (Murphy et al., 2006). *Fusarium* mycotoxins trichothecenes, zearalenone (ZEN) and fumonisins occur most commonly in cereal grains, animal feeds and forages worldwide (D'Mello et al., 1999; Desjardins and Proctor, 2007). They can cause mycotoxicoses; diseases derived from dietary, respiratory, dermal and other exposures to mycotoxins (Bennet and Klich, 2003). Because of nature of this report, we will be focusing only on trichothecenes and zearalenones, the product of the leading FHB pathogens *F. graminearum* and *F. culmorum*. Trichocenes frequently occur in cereal grains and are most strongly associated with chronic and fatal animal toxicoses (Hazel and Patel, 2004; Desjardins and Proctor, 2007). Conditional upon dose, frequency and duration of exposure as well as type of immune function assay, they act both, immunostimulatory or immunosuppressive (Pestka, 2008). It has been shown that ingestion of feed contaminated *F. graminearum* leads to hemorrhagic syndrome, estrogenic syndrome, and feed refusal in farm animals (Desjardins, 2006). Acute and chronic mycotoxicoses have as well been reported worldwide (Turner, 2010; D'Mello et al., 1999). Because of fair heat stability of trichothecenes, they survive the production processes employed. Some decrease due to high water solubility is possible (Hazel and Patel, 2004). Lancova et al. (2008) showed baking at 210 ºC for 14 min had no significant effect on DON levels. ZEN, together with its metabolites possesses estrogenic activity in farm livestock which interferes with their reproductive performance (D'Mello, 1999). The highest concentration of DON and ZEN are located in the bran, therefore the most contaminated milling products are the ones containing whole or the outer portions of the grain and the least reduction flours (Hazel and Patel, 2004; EFSA, 2011).

Mycotoxin contamination of food and feed is a worldwide issue (Antonissen et al., 2014). To avoid potential health risk, the control organs of food safety regulation, such as the European Commission, US Food and Drug Administration, World Health Organization and FAO, have determined science based levels of acceptable mycotoxin content in food and

feed (Krska, 2009; Miedaner, 2012). Exposure assessments usually focus on estimating toxin exposures of population based on dietary intake and typical levels of contamination (Turner, 2010). Analytical methods for detection and determination of mycotoxins in cereals and cereal-based products can be obtained after extraction and clean-up steps. Quantitative determination of mycotoxins are usually performed by chromatographic methods, including gas-chromatography coupled with electron capture, flame ionization or mass spectrometry detectors, and high-performance liquid chromatography (HPLC) coupled with ultraviolet, diode array, fluorescence or MS detectors. Enzyme-linked immunosorbent assays (ELISA) for screening purposes is as well frequently used, and many novel methods are on the rise (Pascal, 2009).

Deoxynivalenol (DON)

Deoxinivalenol (often called vomitoxin for its side effects) is the most frequent *Fusarium* toxin (Lancova et al., 2008; Pestka, 2010). It prompts ribotoxic stress, thus disrupting macromolecule synthesis, cell signaling, differentiation, proliferation, and apoptosis. Like other trichothecenes mycotoxins , DON targets at macrophages, T cells, and B cells of the immune system causing whether immunostimulation or immunosuppression (Pestka, 2010; Pestka, 2008). Dietary exposure to DON both directly with foods of plant origin and indirectly through products of animal origin (e.g. kidney, liver, milk, eggs) causes acute temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever (Sobrova et al., 2010). Additionally, chronic exposure in experimental animals results in impeded weigh gain, anorexia, decreased nutritional efficiency and immune deregulation (Pestka, 2008). In order to avert such health risk, maximum levels of unprocessed wheat and tolerable daily intake (TDI) have been adopted by national and international control organs. In EU, unprocessed durum wheat must not exceed DON content of 1750 µg/kg. Maximum allowed levels for pasta, bakery and pastry, and baby food hold 750, 500 and 200 µg/kg, respectively (Commission regulation (EC) No 1881/2006, 2006). TDI was set to 1 µg/kg bw/day by Scientific Committee for Food (SCF) of European commission in 2002 and in 2013 confirmed by European food safety authority (EFSA) (European Commission, 2002; EFSA, 2013). Determination of DON content is predominantly carried out by gas chromatography (HPLC) and also mass spectrometric methods are applied (Wilson et al., 1998).

Nivalenol (NIV)

Nivalenol occurs in the same products as DON and has apart from one additional hydroxyl group at C-4 also the same structure. Although NIV is frequently detected in much lower concentrations than DON, it tends to be more toxic (Frisvad et al., 2007; Kosova et al., 2009). Its mode of action is quantitative breakdown of polyribosomes in H-HeLa cells, a process which is inhibited by anisomycin, cycloheximide, or trichodermin. NIV is a strong and highly selective inhibitor of polypeptide chain initiation in eukaryotes (Cundliffe et al., 1974). The Scientific committee on Food of European Commission set a temporary NIV TDI of 0.7 µg/kg bw/day (European Commission, 2000).

Zearalenone (ZEN)

A mycoestrogen zearalenone is a phenolic resorcyclic acid lactone mycotoxin and has a close structural relationship to numerous fungal antibiotic metabolites (EFSA, 2011). ZEN has potential of disrupting sex steroid hormone functions that are known to cause infertility in livestock. Alternatively, its derivatives are also used in livestock feeds for growth promotion purpose (Murphy et al., 2006). Although ZEN represents a risk for animals for its estrogenic activity, the phytotoxic impact appears insignificant (Nishiuchi, 2013; Desjardins and Proctor, 2007). More commonly than in cereal grain ZEN is found in maize (EFSA, 2011). In 2000, Scientific committee on food of European Commission determined temporary TDI for ZEA of 0.2 mg/kg of body weight (Commission regulation (EC) No 1881/2006, 2006). The TDI was slightly corrected by European Food Safety Authority in 2011 to 0.25 µg/kg bw (EFSA, 2011). Maximum level of TDI for ZEN in unprocessed cereals other than maize is 100 µg/kg, for cereals intended for direct human consumption 75 µg/kg, and for bread and pastry products 50 µg/kg bw. ZEN in baby food products should not exceed 20 µg/kg bw (Commission regulation (EC) No 1881/2006). For determination and analysis, gas chromatography-tandem mass spectrometry method is applied (Wilson et al., 1998).

2.3 FHB MANAGEMENT

In comparison with other cereals, durum wheat is far more susceptible to FHB. DON content of durum wheat in dry areas can be also few times higher than in common wheat, and the contamination of durum in humid areas comparing to dry areas can be two folded or more (Miedaner, 2012). Preventive control measures should therefore unconditionally be applied in order to minimize the yield loss and avoid mycotoxin contamination of the grains (Wegulo et al., 2015). Since no single strategy is effective enough a combined integration of agronomical actions will give best results (Gilbert and Haber, 2013). Crop rotation, tillage, application of effective chemical and biological control, and using less susceptible cultivars are major practices that mitigate FHB infection and formation of DON in durum wheat (Wegulo et al., 2015; Gilbert and Haber, 2013; Landschoot et al., 2013).

2.3.1 Crop rotation and soil tillage

Crop rotation is one of the most efficient agronomic control measures that can be adopted and plays a fundamental part in organic farming (Pirgozliev et al., 2003; Lammerts van Bueren, 2008). By rotating with non-host crops the density of inoculums is reduced (Mehta, 2014). For instance, Golkari et al. (2008) showed that pea and canola planted after wheat and oat can break the cycle of *F. graminearum* inoculum increase. When wheat is grown after corn it is more susceptible to FHB than wheat following other crops (e.g. soybean, canola, peas) (Dill-Macky and Jones, 2000; Mesterhazy, 2003; Miedaner, 2012). In such case, the increase of epidemic severity can be two- to three-fold of the one where wheat follows a non-host preceding crop (Mesterhazy, 2003). If for economic reasons wheat and maize must be planted sequential, then tillage cannot be avoided. The severity of FHB infection depends greatly on the amount of inoculated left-over stubble on the ground, hence the best way of diminishing the FHB attack is to remove or incorporate

the debris deep into the soil by plowing. Such measures are not appropriate for the soils of high erosion. Mulching on the other hand does not give sufficient results, considering that the debris is placed on the level of sowing (Miedaner, 2012). Furthermore, in a study by Champeil et al. (2004) direct drilling system resulted in the highest FHB severity compared to other systems containing tillage.

2.3.2 Fungicide treatment

Complementary to aforesaid control measures, phytopharmaceutical compounds can be employed. It is crucial that fungicide application against FHB do not only result in disease deficiency but also regulate mycotoxin content in caryopsis. How substantial effect a fungicide will have depends on several factors. Firstly, most suitable fungicide must be selected. Because of controversial reports, a uniform assessment on an explicit fungicide does not exist, although some reviews showed simmilar outcome (Mesterhazy, 2003). The most widely used fungicides are triazole (demethylation inhibitor) group and strobilurins (quinone inhibitor), where main part do not translocate from leaves to head (Wegulo et al., 2015; Mesterhazy, 2003; Butkute et al., 2008). Triazole group includes metconazole, propiconazole, prothioconazole, tebuconazole, and prothioconazole + tebuconazole (Wegulo et al., 2015). Blandino et al. (2006) did not observe FHB decline in seed dressing with tebuconazole. The single treatment at anthesis showed to be as efficient as double treatment at the end of shooting and mid anthesis. Combination of triazoles and strobilurin did however show higher DON content as untreated control, although still increasing yields. This phenomenon could be attributed to gene *Tri5*, responsible for the DON synthesis pathway, which can be significantly influenced (increased) by factors such as fungicide treatment. Menniti et al. (2003) established that prochloraz, tebuconazole, epoxiconazole or bromuconazole only provided good control at low to medium infection pressure and with main pathogens *F. graminearum* and *F. culmorum* whereas strobilurin kresoxin-methyl showed a low efficacy at controlling the disease. Likewise, Fernandez et al. (2014) observed the most consistent decrease level of disease when applying tebuconazole fungicide at anthesis, and double application was again not more efficient. The timing of fungicide application is primordial for efficient disease control. Although early application might protect the infection of the leaves, it will have no effect on the emerging heads. Hence, fungicide treatment should not be executed before all heads have emerged and in practice uneven flowering can be a deteriorating factor (Mesterhazy, 2003; Wegulo et al., 2015). Furthermore, good coverage whit fungicides will substantial improve the efficiency, therefore spraying from both sides is desirable (Mesterhazy, 2003). Variability in efficiency of fungicides is also attributed to differences in isolate aggressiveness, and above all climatic conditions (Mesterhazy, 2003; Ioos et al., 2015 Champeil et al. 2004; Butkute et al., 2008). Unfavorable climatic conditions not only negatively influence the incidence of FHB and DON content but also enable application of fungicide at optimal time beforehand (Wegulo et al., 2015). Finally, the efficacy of the fungicide in durum wheat is much lower than in bread wheat (Bagga, 2008). Conventional fungicide can be fairly effective in combating FHB, yet their persistence in the environment impairs the attractiveness (Schisler et al., 2002).

2.3.3 Biological control agents

A promising alternative to chemical measures are increasingly researched environmental-friendly biological control agents which comprise predominantly of bacterial strains of the genus *Pseudomonas* or the endospore-forming, Gram-positive genera *Bacillus* and *Paenibacillus*, as well as genera of filamentous and yeast fungi. The mode of action aims on disruption of *F. graminearum* lifecycle, starting already with decreasing of inoculum on plant debris. Other strategies intervene with *Fusarium* spikelet infection or systemic movement within the rachis of the spike, either seedling blight development in blighted seeds and even inoculum production by the pathogen. Mechanisms of action include antibiosis, competition, mycoparasitism, induced resistance, and metabolic inhibition of mycotoxin synthesis (Luz et al., 2003). Petti et al. (2008) showed that a head spray of *P. fluorescens* (*Pseudomonas*) strain MKB 158 can inhibit the development of Fusarium seedling blight disease on wheat and barley as well as diminishes DON contamination in the field by inducing disease resistance. A study of antagonistic bacteria and yeast strains by Schisler et al. (2002) demonstrated efficiency against *G. zeae* on durum wheat after spraying the heads with bioassay. All antagonists reduced FHB severity on cultivar Renville, and three of the four reduced severity on cultivar Ben, with *Bacillus subtilis* strain AS 43.3 decreasing FHB severity by as much as 90 %. Another successful antagonist lysobacter enzymogenes strain C3 was presented by Jochum (2006) showing drastic decline of FHB severity of infected spikes in comparison to non-treated control. In a survey by Hue et al. (2009), antibiotic effect of a strain of *Clonostachys rosea* sprayed onto wheat heads prior to inoculation with *G. zeae* displayed significantly reduced infected spikelets by 58-71 % and fusarium damaged kernels by 59-73 % compared to the untreated disease control. In addition, biofumigation as a control agent has also shown potential of FHB inhibitor. Fan et al. (2008) presented *Brassica oleracea* var. *caulorapa* as suitable material for this purpose. Further, chickpea's volatile compounds may negatively impact pathogenic *Fusarium* spp. (Cruz et al., 2012). Although FHB biocontrol agents from recent reports show high potential and would be invaluable in organic production systems, they are not commercially available yet (Pirgozliev et al., 2003; Wegulo et al., 2015).

2.3.4 Host resistance

Control measures against FHB revised so far cannot completely prevent the risk of mycotoxin contamination in durum wheat, especially in years of high FHB epidemics. More effective approach of combating this devastating disease is utilization of FHB-resistant durum cultivars, which is a very cost-efficient and sustainable strategy (Kosova et al., 2009; Oliver et al., 2008; Prat et al., 2014). The advantage of FHB-resistance is its horizontal and race non-specific nature which already facilitates the breeding (Mesterhazy, 1997). Although progress has been done up to date in this research field, the work has been restrained by limited effective sources of FHB-resistance in the gene pool of cultivated durum wheat (Oliver et al., 2008; Buerstmayr et al., 2012). Resistant sources have been identified in hexaploid genotypes, such as Chinese common wheat cultivar Sumai 3 and its derivatives, although attempts to introgress their resistance to tetraploid wheat are restrained by the differences in ploidy levels (see 2.1.2) (Oliver et al.,

2008; Zhang et al., 2004). It has been suggested, that the absence of D genome impedes resistance to FHB in tetraploid wheat (Ban and Watanabe, 2001). Breeders are therefore focusing on germplasm of wild tetraploid relatives and cultivated durum varieties (Prat et al., 2014). For instance, FHB-resistance has been so far determined in tetraploid wheat *T. dicoccum*, *T. turgidum carthilicum*, and *T. dicoccoides,* as well as in tetraploid wheatgrass (*Thinopyrum junceiforme*) (Oliver et al., 2008; Talas et al., 2011; Jauhar and Peterson, 1998). Introgression of FHB-resistance genes from these cultivars however does not give the best results since other agronomical unfavorable traits are inherited as well (i.e. linkage drag - presence of two different tightly linked loci with different effects, or pleiotrophy - two different phenotypic effects on one locus) (Kosova, 2009). However, amongst thousands of lines tested for FHB-resistance, moderate resistance has been shown in some Tunisian and Syrian landraces (Talas et al., 2011; Ghavami et al., 2011). Unfortunately, the germplasm of non-native cultivars is often not adapted to European environmental conditions (Kosova, 2009). Striving for stable durum FHB-resistant ideotype therefore still remains.

Types of resistance

In the course of time, plants have developed a variety of defense mechanism against biotic elicitors (Ribeiro do Vale et al., 2001). Host plant resistance can be of physiological (active) and/or morphological (passive). Passive resistance against FHB observed in wheat shows several avoidance mechanisms, by which the plant is able to escape from pathogen in time and space: plant height, time and type (extrusion/retention of anthers) of flowering (Mesterhazy, 1995). It has also been shown, that presence of awns increases disease severity, as well as lower natural infection where spikelet-density is inferior (Mesterhazy, 1995; Ban, 1997). Particularly in durum wheat, the effect of spike compaction together with anther retention at flowering acts much more in favor of FHB development compared to common wheat (Prat et al., 2014). Active resistance on the other hand is a response of plants by which the host plant suppresses the pathogen (Mesterhazy, 1995; Ribeiro do Vale et al., 2001). High hydrolase activity of infected *Fusarium*-resistant plants has been observed. Moreover, resistant wheat varieties indicated a great amount of fructose at penetration site of *Fusarium* spp. Resistant plants infected by *Fusarium* spp. actually neutralize the high proteolytic activity of the parasite by increasing their trypsin inhibitor content (Klechkovskaya et al., 1997).

Phenotypic expression of FHB resistance can be assigned to five different components of resistance: resistance to initial infection (type 1) and resistance to subsequent spread within the spike (type 2) (Schroeder and Christensen, 1963), resistance to kernel infection (type 3) and tolerance (type 4), resistance to mycotoxin accumulation (type 5) (Mesterhazy, 1995).

To systematically evaluate inheritance of FHB resistance in the collections of durum wheat, appropriate artificial inoculation method must be applied. Depending on type of resistance, a greenhouse experiment and/or a field experiment is carried out (Buerstmayr et al., 2014a). Single-floret inoculation method under controlled conditions in the greenhouse is a tool for assessing type 2 resistance. Assessing severity to FHB in the

field by spraying the spikelets with inoculum mimics the pathogen pressure of epidemic and gives results for type 1 and type 2 resistance (Prat et al., 2014; Rudd et al., 2001). Alternatively, grain spawn mimics the natural inoculation, raising the traits of morphological resistance, such as spike form and plant height (Rudd et al., 2001; Miedaner, 2012). FHB expression in wheat is genetically controlled and affected by environmental conditions, therefore vary in results in time and space. To obtain reliable value of genetic performances it is necessary to execute evaluations over several seasons and/or environments (Ban, 1997; Buerstmayr et al., 2014a). Type 1 resistance is more difficult to assess, and is measured as disease incidence usually together with type 2 resistance in the field trial (Buerstmayr et al., 2009). Type 3 resistance is determined by *Fusarium*-damaged kernels (FDK), type 4 by measuring the yield of grains that show no FHB symptoms and type 5 measuring DON concentration at given level of FHB (Rudd et al., 2001). Phenotypic selection is rather labourious and time-consuming, therefore application of QTL (quantitative trait loci) mapping approach is further alternative tool to study FHB resistance (type 1 resistance QTL, type 2 and resistance to DON accumulation) (Buerstmayr et al., 2014a; Buerstmayr et al., 2009; Prat et al., 2014).

Quantitative trait loci for FHB resistance

FHB-resistance is known to be quantitative and polygenic, which means that is controlled by multiple genes on different loci and is therefore complex, but durable and stable. Its expression is greatly modulated by environmental conditions (Kosova et. al., 2009; Buerstmayr et al., 2014a; Ban, 1997; Mesterhazy, 1997). Quantitative resistance diminishes the rate of development of an epidemic, which depends on the aggressiveness of a pathogen (Frantzen, 2000). Application of molecular markers allows the identification of QTL of resistance (Keller et al., 2000). In hexaploid wheat over 100 QTL for FHB-resistance have been documented on all wheat chromosomes except chromosome 7D (Buerstmayr et al., 2009). The most precisely described QTL come from Sumai-3-derived populations; *Fhb1* on chromosome 3BS, *Qfhs.ifa-5A* on chromosome 5AS and *Fhb2* on chromosome 6BS (Buerstmayr et al., 2009). *Fhb1* (3BS), flanked by SSR markers *Xgwm533* and *Xgwm493,* is the most reported of them all and possesses excellent type 2 resistance, and has been shown to be involved in DON detoxification (Buerstmayr et al., 2009; Kosova et al., 2009; Lemmens et a., 2008). In tetraploid wheat on the other hand, only thirteen QTL with small to moderate FHB-resistance effect have been detected on 11 chromosomes (2A, 2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, 7A and 7B) (Prat et al., 2014). Although recent results suggest introgression of *Fhb1* QTL from Sumai-3 into durum wheat via recurrent back-crossing, *Qfhs.ifa-5A* introgression has not been able to peruse further, due to reduced spike fertility of the durum wheat back-cross lines (Prat et al., 2014). Breeding with moderately resistant durum cultivars such as “Ben”, “Creso”, “Enduro”, “Eupoda 3” etc. could result in an additive effect and lead to transgressive segregation as with high FHB-resistant cultivar Sumai-3, which parents are both only moderately resistant to FHB (Royo et al., 2009; Gilbert and Haber, 2013; Chen et al., 1997).

Furthermore, several QTL for FHB-resistance are associated with other agronomic traits, such as heading date, flowering time and plant height (Stenglein and Rogers, 2010).

Flowering time and plant height have a dramatic effect on FHB symptoms, particularly when the inoculation techniques mimic the natural disease infection (Buerstmayr et al., 2009). The correlations between FHB resistance and plant height are complex (Mao et al., 2010). Although difference in FHB incidence could be an effect of plant height *per se*, where microclimatic conditions play a great part in better type1 resistance of taller plants, this effect might also be due to morphological escape or result of linkage drag or pleiotropy (Yan et al., 2011; Mesterhazy 1995; Draeger et al., 2007; Buerstmayr, 2009). Several FHB resistance QTL overlap with plant height (Kosova, 2009). Srinivasachary et al. (2008) demonstrated association between enhanced initial FHB infection (type 1) and the semi-dwarfing allele *Rht-D1b* (also known as *Rht2*). Negative association of reduced height *Rht* genes (*Rht-B1*, *Rht-D1* and *Rht8*), controlling plant height, with FHB resistance was confirmed by many studies (Mao et al., 2010; Buerstmayr et al., 2012; Klahr et al., 2007). The cause of correlation of flowering time and heading date, respectively, with FHB incidence are often environment specific factors, most likely weather conditions around flowering and inoculation time (Buerstmayr et al., 2014b). Additionally, significant association of low FHB incidence and narrow flower opening was found (Gilsinger et al., 2005).

2.4 STRIPE RUST

Stripe rust pathogen (*Puccinia striiformis* Westend.) is a fungus of phylum *Basidiomycota*, family *Pucciniaceae* and genus *Puccinia*. Depending on which grass it colonizes, several subspecies have been characterized, whilst *P. striiformis* f. sp. *tritici* Erikss. (*P. striiformis*) causing stripe rust on wheat, including durum wheat (Chen et al., 2014). In regions of cool and moist weather conditions during growing season *P. striiformis* can cause severe epidemics to the cereals, such as in the USA in year 2000 and in China between 2001 and 2002 (Chen et al., 2014; Chen et al., 2002; Wan et al., 2004). *P. striiformis* is a macrocyclic, heteroecious parasite that requires both primary (wheat or grasses) and surrogate (*Berberis* or *Mahonia* spp.) host to conclude its lifecycle. Furthermore, the inoculum (urediniospores) can travel even thousands of kilometers from the primary infection site. The urediniospores colonize mostly leaves, but also leaf sheaths, glumes and awns, which reflects as long, narrow yellow to orange stripes subsequently causing chlorosis and necrosis. Rapid germination of spores occurs with free moisture on leaf surfaces and temperatures between 7 and 12 °C. At higher temperatures or during the later growing stages of the host, black telia are often produced (Chen et al., 2014).

Stripe rust (SR) is an important fungal disease in durum wheat. Resistance to SR in durum is provided by recessive genes. Some durum cultivars possess major genes for SR resistance, and additionally general resistance called “slow rusting”. Slow rusting is a superior ability for recovering from infection, found in some cultivars. Although resistant cultivars arose, often a virulent new SR strain appears and overcomes the resistance (Matz, 1991).

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

Our field trial was carried out in 2015 at IFA-Tulln (16°04,16′E, 48°19, 08′N, 177 m above sea level), Lower Austria, 30 km west of Vienna. The soil type is a meadow-czernosem and the climate is temperate but warm with mean temperature of 9.7 °C and 625 mm precipitation per annum (climate-data.org, 2016).

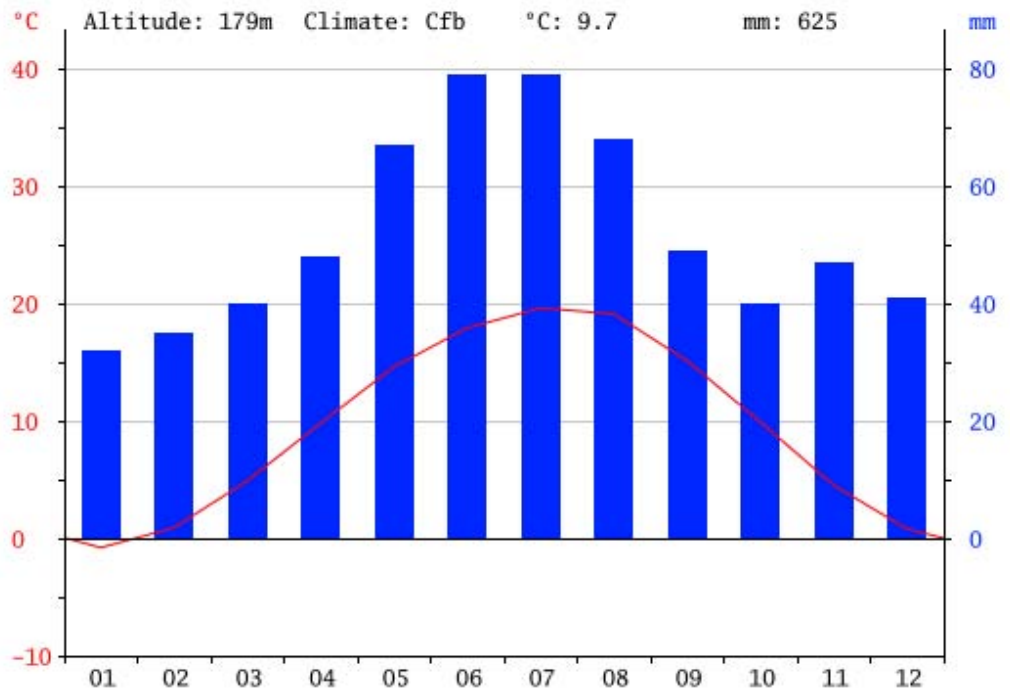

Figure 3: Average monthly temperatures (˚C) and precipitation (mm) in Tulln (climate-data.org, 2016).

3.2 PLANT MATERIALS AND FIELD TRIALS

Two mapping population, each encompassing 100 F_7 RILs were developed from crosses of resistant tetraploid parent DBC-480-1 and the susceptible European durum wheat parents, Durobonus and SZD1029K, respectively. DBC-480-1 was developed at the IFA-Tulln and possesses an introgression carrying *Fhb1* from the Asian resistant bread wheat cultivar Sumai-3 after back-crossing of the latter into the Austrian cultivar Semperdur. It is a tall experimental line which carries the wild-type allele *Rht-B1a*. Durobonus is an Austrian durum wheat commercial variety developed by Saatzucht Donau and SZD1029K is an advanced durum wheat breeding line from Saatzucht Donau breeding program. They both carry the semi-dwarfing allele *Rht-B1b*.

3.3 EXPERIMENTAL DESIGN

The populations were sown in three blocks (randomized block design) in early spring with one week time shift between each block. Double-rowed plots were of 1 m length and 17 cm spacing. Management was executed in accordance with good agronomical practice by Buerstmayr et al. (2002).

3.4 INOCULUM PRODUCTION, INOCULATION, AND RESISTANCE EVALUATION

As a pathogen causing FHB on our experimental plant material *F. culmorum* isolate 'Fc91015' at a conidial concentration of 2.5×10^4 mL^{-1} was produced as described in extension. Wheat and oat kernels in 3:1 ratio were soaked in a jar of water overnight, then autoclaved and inoculated with freshly agar grown culture of *F. culmorum*. The mixture was incubated for two weeks under diffused light at 25 °C followed by three weeks in the dark at 5 °C, resulting in production of macroconidia. At the end of incubation, macroconidia were rinsed off of the colonized grains with deionized water followed by a microscopic determination of conidia concentration using Bürker-Türk counting chamber. To achieve desired concentration of inoculum, the suspension was additionally diluted with deionized water. Spore suspension was stored at -80°C until application, when it was finally diluted with tap water to concentration of 2.5×10^4 conidia mL^{-1}.

Inoculation of each block started when its first plot reached 50 percent anthesis and was repeated in two-day intervals until two days after its last plot had flowered. Approximately 100 mL m^{-2} inoculum amount was sprayed on wheat heads with a motor-driven backpack sprayer in the late afternoon. Furthermore, an automatic mist irrigation system applied for 20 hours after inoculation providing sufficient humidity for the FHB development.

FHB severity was visually rated five times as percentage of infected spikelets per plot (0–100 %, see Figure 5), beginning with day 14 after inoculation and repeated every four days until 30 days after inoculation. This scoring indicates the number of infected spikes per plot (type 1 resistance) and the number of infected spikelets per spike (type 2 resistance) all in one (Miedaner, 2011). The values were further used to calculate the area under the disease progress curve (AUDPC) as an integrated measure for disease severity (Steiner, 2003).

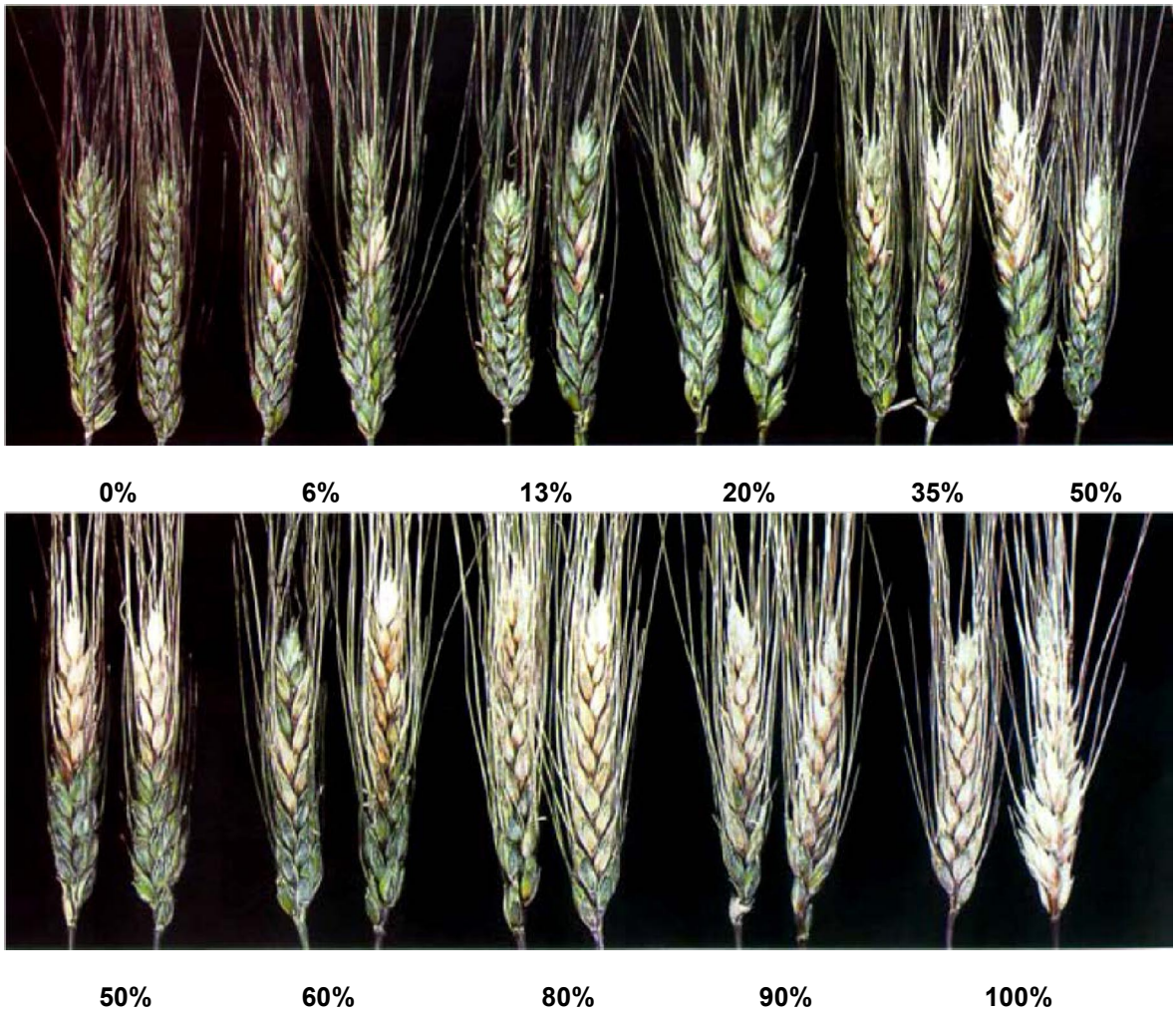

Figure 4: Scale of FHB severity (Stack and McMullen, 2011).

3.5 OTHER TRAITS

3.5.1 Flowering date

Flowering was recorded when 50 % of the plot reached anthesis on a timescale starting May 31st. On the same day that a plot was flowering, it was also inoculated with *F. culmorum* suspension.

3.5.2 Plant height

In each plot, plant height was measured in centimeter. Plant height was defined as a mean value of three measures of plant height, distancing from the soil surface to the top of each measured plant head, excluding awns.

3.5.3 Severity of stripe rust infections

On June 11, severity of stripe rust was recorded for each plot of the first and second block. Scoring was carried out with a scale for SR scoring (see Figure 6), where the overall severity of a plot was expressed as percent (1-100 %) of infected leaf surface.

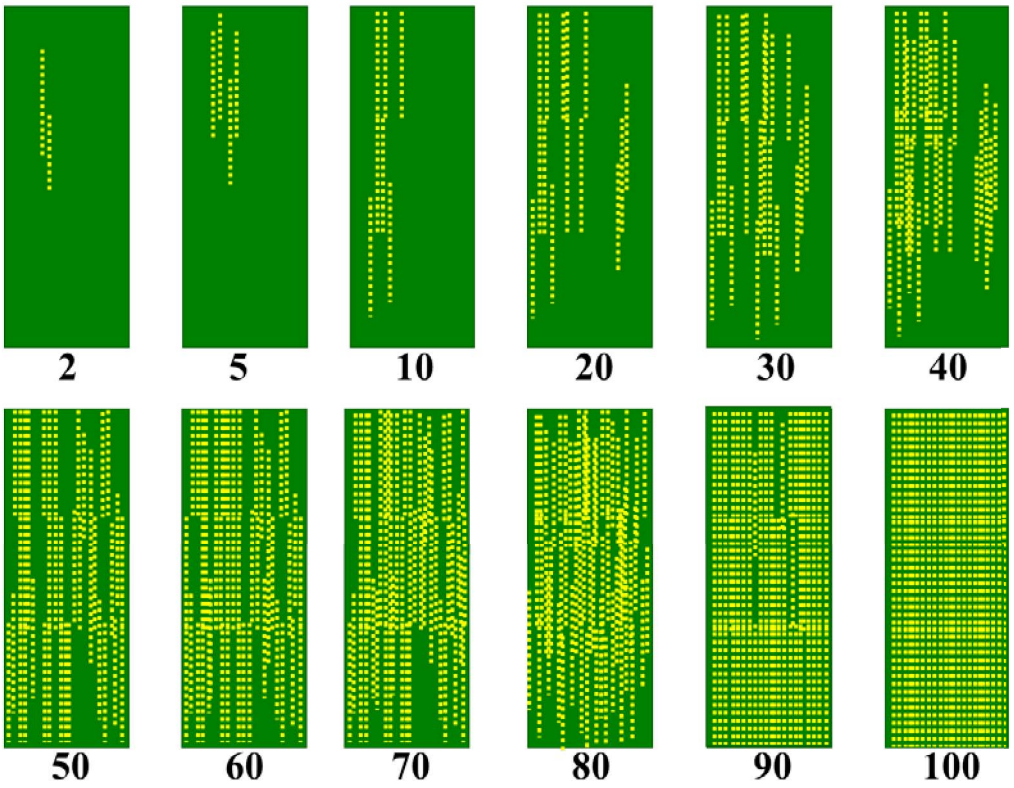

Figure 5: Scale of stripe rust severity in percentage (Washington State University, 2016).

3.6 MOLECULAR MARKERS

DNA from parental lines and 200 RILs was extracted from young leaves using the CTAB method. The plant material was screened with molecular markers which are known to be tightly associated with the well-known major resistance QTL *Fhb1* located on chromosome 3B. The populations were also genotyped using markers associated with major plant height gene *RhtB1* located on chromosome 4B.

3.6.1 PCR markers (*Umn10* and *Barc147*)

Genotyping for *Fhb1* was performed with markers *Umn10* and *Barc147* via polyacrylamide gel electrophoresis. 10 µl reaction mix for M13-tailed PCR markers was conducted in the following order:

- 2 µl (30 ng μl^{-1}) of each DNA sample was pipetted into a single well on a 96-well plate (12 x 8) and put in the freezer.

PCR master mix was prepared for a 96-well plate. For one reaction:

- 1 µl dNTPmix (2nM), 1 µl buffer, 0.02 µl forward primer and 0.2 µl reverse primer,
- 0.18 µl of M-13 primer for CBS-gel was included; Cy5 and FAM for *Umn10* and *Barc147*, respectively,
- 5.5 µl deionized water and 0.1 µl Taq-polymerase were added.

Plates were well shaken and centrifuged, and placed in the PCR termocycler.

PCR program for 'M13 tailed' primers was: initial incubation at 94 °C for 2 min, 30 cycles with 94 °C for 1 min, 0.5 °C/sec to 51 °C, 51 °C for 30 sec, 0.5 °C/sec to 72 °C, 72 °C for 1 min; 72 °C for 5 min. PCR was performed on a 'Primus' 384 well thermocycler (MWG Biotech).

After DNA amplification, we loaded our samples with eight-channel Hamilton syringe on the 12 % polyacrylamide gel using a 96-well shark tooth comb with 0.4 mm spacer, and let them run for two hours. Electrophoresis was performed at constant power of 40 W and a temperature of 48 °C. In the end, the gel was scanned and the images were scored optically for the PCR markers.

3.6.2 Rht markers

The effect of *Rht-B1* was assessed by performing allelic discrimination which classifies unknown samples as hetero- and homozygous for the wild-type *Rht-B1a* or the mutant semi-dwarfing allele *Rht-B1b*. For this purpose we conducted a following assay:

- 2 µl (30 ng μl^{-1}) DNA and
- 3 µl of master-mix: 2.5 µl TaqMan Master Mix + 0.5 H_2O + 0.07 primer Mix.

Samples were shaken and centrifuged, and transferred to a 384-well reaction plate. As a control, cultivars with known alleles at the *Rht-B1* locus were used. Durobonus, SZD1029K and Bobwhite carried the allele *Rht-B1b*, while *Rht-B1a* allele was carried by DBC-480-1, Capo and Monsun. The competitive allele specific PCR (KASP) was performed on CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad) with only manually selected wells. Cycle conditions for the KASP analysis were following: 94 °C for 15 min, 94 °C for 20 sec and 65-57 °C for 1 min, both in 10 steps, 94 °C for 20 sec and 57 °C for 1 min, both in 26 steps, and 37 °C for 1 minute. Results of allelic discrimination were displayed on a scatterplot and allele calls were manually assigned.

3.7 STATISTICAL DATA ANALYSIS

All data described in chapter 3 were statistically analyzed with free statistical software RStudio using analysis of variance (one-way ANOVA). To explain the impact of other traits on main investigated trait of FHB resistance, correlation test was carried out. For the trait "FHB severity" repeated disease assessments (14, 18, 22 and 26 days after anthesis) were joint into so called area under the disease progress curve (AUDPC), which is

commonly used for assessing quantitative disease resistance in crop cultivars, and is calculated as following:

$$\mathrm{AUDPC} = \int \frac{\mathrm{dt}}{1 + \mathrm{Ae}^{-\mathrm{rt}}}$$

A real, continuous function y=f(t) (with y>0) is expressed as integral, limited by the t_0 and T, and $T>t_0$. A equals $(1-y_0)/y_0$, where y_0 is the value of y at $t_0=0$, and r is a rate parameter (Jeger and Viljanen-Rollinson, 2001).

Final equation in or case is

$$\mathrm{AUDPC} = 9\ \mathrm{x}\ \mathrm{B1}\ +\ 4\ \mathrm{x}\ \mathrm{B2}\ +\ 4\ \mathrm{x}\ \mathrm{B3}\ +\ 4\ \mathrm{x}\ \mathrm{B4},$$

where B1, B2, B3 and B4 stand for values of FHB severity (percentage of infected spikelets) on day 14, 18, 22 and 26 post anthesis, respectively.

Measurements are mostly exposed to variety of errors, causing variability between the measured value and the true value. Reliability however, depends also on the investigated population. With repeatability study we exclude the possibility of bias between measurements (Bartlett and Frost, 2008). Repeatability was tested for all traits with following equation:

$$\mathrm{Repeatibility} = 1 - \frac{\mathrm{MeanSq\_residuals}}{\mathrm{MeanSq\_genotype}}$$

MeanSq_residuals … mean square of residuals (ANOVA)
MeanSq_genotype … mean square of genotypes (ANOVA)

Favorable repeatability values are near value 1.

4 RESULTS

4.1 FHB SEVERITY

Overall results for the trait percentage of infected spikelets and AUDPC, respectively, are given in Table 1 and 2. The results show much lower FHB severity as well as AUDPC values of minimum, maximum and median in population 1 (Table 1). In both populations though, percentage of infected spikelets reach up to 100 per cent in some genotypes.

Table 1: Minimum (Min), maximum (Max), mean, median and values of parents DBC-480-1 and Durobonus for population 1 for traits FHB severity and AUDPC, respectively.

Trait	Min	Max	Mean	Median	DBC-480-1	Durobonus
FHB severity (%)	7.0	100.0	47.1	36.3	21.0	88.8
AUDPC	103.5	1380.0	535.9	438.5	249.8	1090.2

Table 2: Minimum (Min), maximum (Max), mean, median and values of Parents DBC-480-1 and SZD1029K for population 2 for traits FHB severity and AUDPC, respectively.

Trait	Min	Max	Mean	Median	DBC-480-1	SZD1029K
FHB severity (%)	14.0	100.0	72.4	85.0	21.0	98.00
AUDPC	129.0	1545.0	897.0	996.5	249.8	1427.0

Genetic variation among the population 1 was highly significant for FHB severity, including AUDPC (Table 3). Likewise, genetic variation in population 2 was highly significant again for both traits (Table 4).

Table 3: ANOVA for traits percentage of infected spikelets (FHB severity %) and AUDPC for population 1. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

FHB severity (%)	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	184589	1865	12,47	$<2 \times 10^{-16}$
Repetition	1	20849	20849	139.49	$<2 \times 10^{-16}$
Residuals	199	29743	149		
AUDPC	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	28300643	285865	13.53	$<2 \times 10^{-16}$
Repetition	1	20849	20849	139.49	$<2 \times 10^{-16}$
Residuals	199	4205526	21133		

Table 4: ANOVA for traits percentage of infected spikelets (FHB severity %) and AUDPC for population 2. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

FHB severity (%)	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	181917	1838	20.81	$< 2 \times 10^{-16}$
Repetition	1	5233	5233	59.26	6.33×10^{-13}
Residuals	199	17573	88		
AUDPC	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	43184001	436202	23.47	$<2 \times 10^{-16}$
Repetition	1	2442382	2442382	131.41	$<2 \times 10^{-16}$
Residuals	199	3698579	18586		

Population 1 and population 2 exhibited good repeatability values for FHB severity (percent of infected spikelets 26 days post anthesis) and AUDPC. Both repeatability values were slightly lower in population 1 than in population 2 (Table 5).

Table 5: Repeatability values for FHB severity (percent of infected spikelets 26 days post anthesis) and AUDPC in population 1 and 2, respectively.

	Repeatability	
Trait	Population1	Population2
FHB severity (%)	0.92	0.95
AUDPC	0.93	0.96

An insight into parental disease development from day 14 to day 26 after the anthesis is given in Figure 7. DBC-480-1 displayed the slowest but continuous development and the lowest overall results for all four measurements. Durobonus and SZD1029K both showed rapid continuous FHB manifestation.

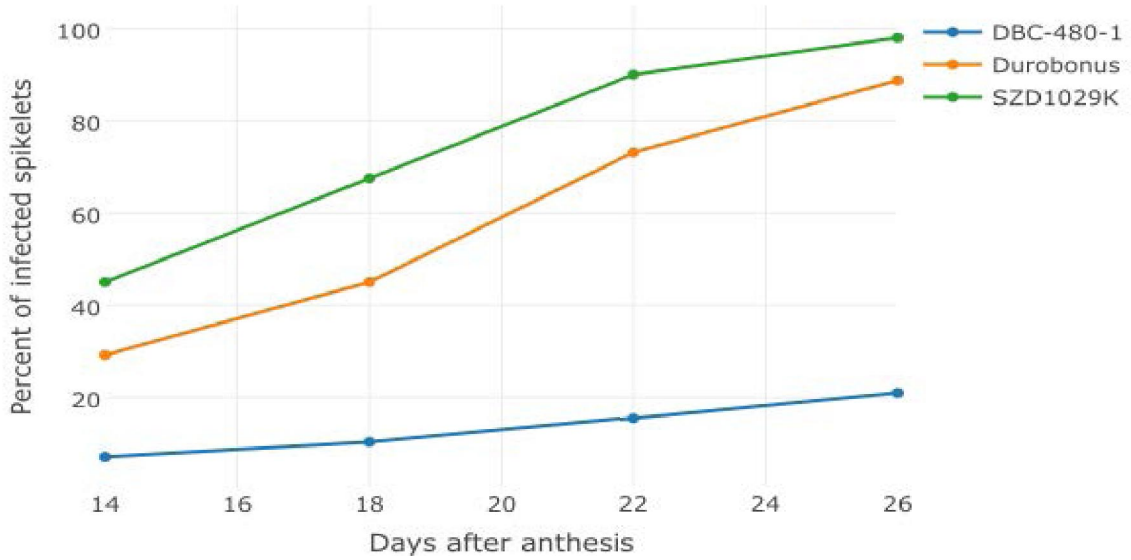

Figure 6: Disease development (percent of infected spikelets from day 14 to day 26 after anthesis) for the parental lines DBC-480-1, Durobonus and SZD1029K, respectively.

Histograms of frequency distribution for the trait of FHB severity are shown in Figure 8. Population 1 shows higher frequency peaks in the first half of the histogram, exhibiting lower FHB severity. Population 2 on the other hand, displayed its highest peaks in the very end of the severity axis, showing a great number of very susceptible genotypes. Parental lines differed significantly in both populations.

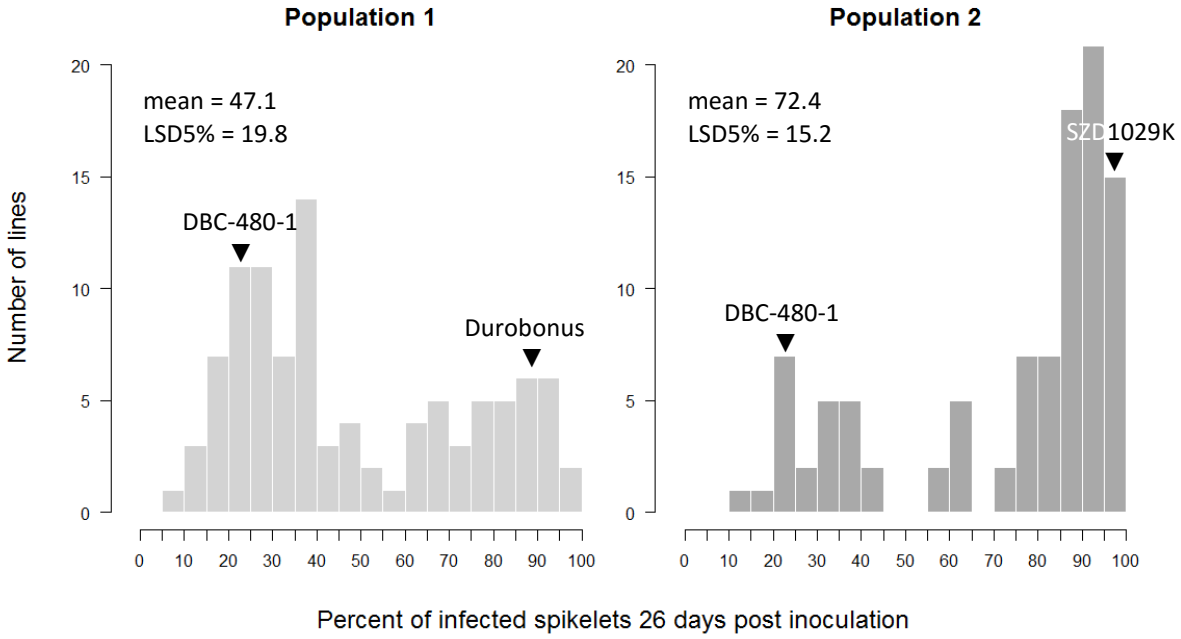

Figure 7: Frequency distribution of genotypes for FHB severity (percent of infected spikelets 26 days post anthesis), population mean and the least significant difference (LSD)

for comparison of lines means (a = 0.05) within population 1 and population 2, respectively. Parental lines are indicated by arrows.

A similar frequency distribution can be seen in Figure 9 where genotypes are distributed along AUDPC range. Again in the population 1, a high number of genotypes occupies first half of the histogram, which clearly expresses lower FHB severity oppose to those in population 2, where again a large number of genotypes is within the second half of the histogram. The same as observed in Figure 8, parental lines differed significantly in AUDPC in both populations.

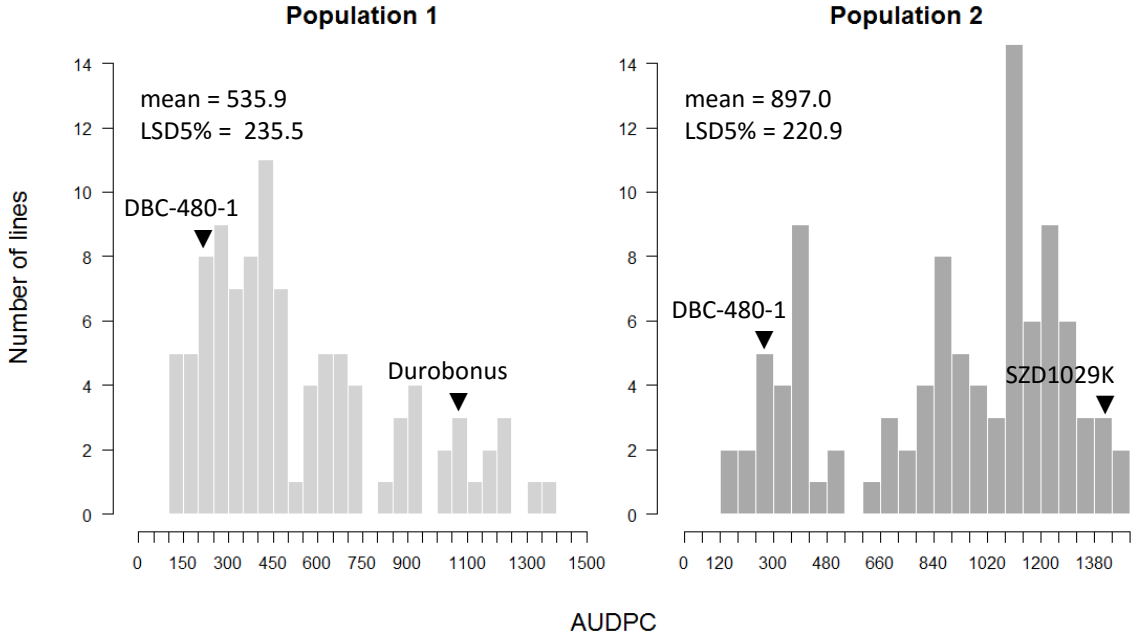

Figure 8: Frequency distribution of genotypes for AUDPC, population mean and the least significant difference (LSD) for comparison of lines means (a = 0.05) within population 1 and population 2, respectively. Parental lines are indicated by arrows.

4.2 OTHER TRAITS

The overall values according to the populations are displayed in Table 6 and 7.

Table 6: Minimum (Min), maximum (Max), mean, median and values of Parents DBC-480-1 and Durobonus for population 1 for traits plant height, flowering date and SR infection [%], respectively.

Trait	Min	Max	Mean	Median	DBC-480-1	Durobonus
Plant height [cm]	60.0	120.0	91.1	93.8	109.0	64.4
Flowering date [days after June 1st]	3.0	8.0	5.3	5.0	6.2	7.5
SR infection [%]	10.0	35.0	21.0	20.0	17.5	13.9

Table 7: Minimum (Min), maximum (Max), mean, median and values of Parents DBC-480-1 and SZD1029K for population 2 for traits plant height, flowering date and SR infection [%], respectively.

Trait	Min	Max	Mean	Median	DBC-480-1	SZD1029K
Plant height [cm]	50.0	120.0	72.4	85.0	109.0	58.8
Flowering date [days after June 1st]	4.5	10.0	7.0	7.0	6.2	8.0
SR infection [%]	2.0	22.5	6.8	6.5	17.5	3.0

In population 1 and 2, genetic variation was significant for plant height, flowering date and SR severity (Table 8 and 9).

Table 8: ANOVA for plant height, flowering date and SR severity (SR %), respectively, for population 1. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

Plant height	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	103128	1041.7	28.25	$<2 \times 10^{-16}$
Repetition	1	496	496.1	13.46	0.000313
Residuals	199	7337	36.9		
Fl. date	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	358.3	3.62	4,66	$<2 \times 10^{-16}$
Repetition	1	186.2	186.25	240.010	$<2 \times 10^{-16}$
Residuals	199	154.4	0.78		
SR %	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	6993	70.6	2,001	0.000325
Repetition	1	2865	2865.2	81.179	1.58×10^{-14}
Residuals	199	3494	35.3		

Table 9: ANOVA for plant height, flowering date and SR severity (SR %), respectively, for population 2. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

Plant height	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	119024	1202.3	47.80	$<2 \times 10^{-16}$
Repetition	1	2211	2211.1	87.91	$<2 \times 10^{-16}$
Residuals	199	5006	25.20		
Fl. date	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	456.9	4.62	4,49	$<2 \times 10^{-16}$
Repetition	1	235.4	235.45	229.05	$<2 \times 10^{-16}$
Residuals	199	204.6	1.03		
SR %	Df	Sum Sq	Mean Sq	F-value	*p*-value
Genotype	99	3206	32.39	1556,000	0.014494
Repetition	1	293	292.82	14.064	0.000298
Residuals	199	2061	20.82		

Repeatability of plant height, flowering date and SR severity measurements are given in table 10.

Table 10: Repeatability values for plant height [cm], flowering date [June], and SR severity [%] in population 1 and 2, respectively.

	Repeatability	
Trait	Population1	Population2
Plant height [cm]	0.96	0.98
Flowering date	0.78	0.78
SR severity [%]	0.50	0.36

Figure 10 - 12 display the histograms of frequency distribution for each trait. As we can see, population 1 flowered roughly earlier as the population 2 (Figure 10). Greater number of higher plants could be found in population 1, and in population 2 semi-dwarf plants prevailed (Figure 11). Severity of SR was higher in the population 1 (Figure 12).

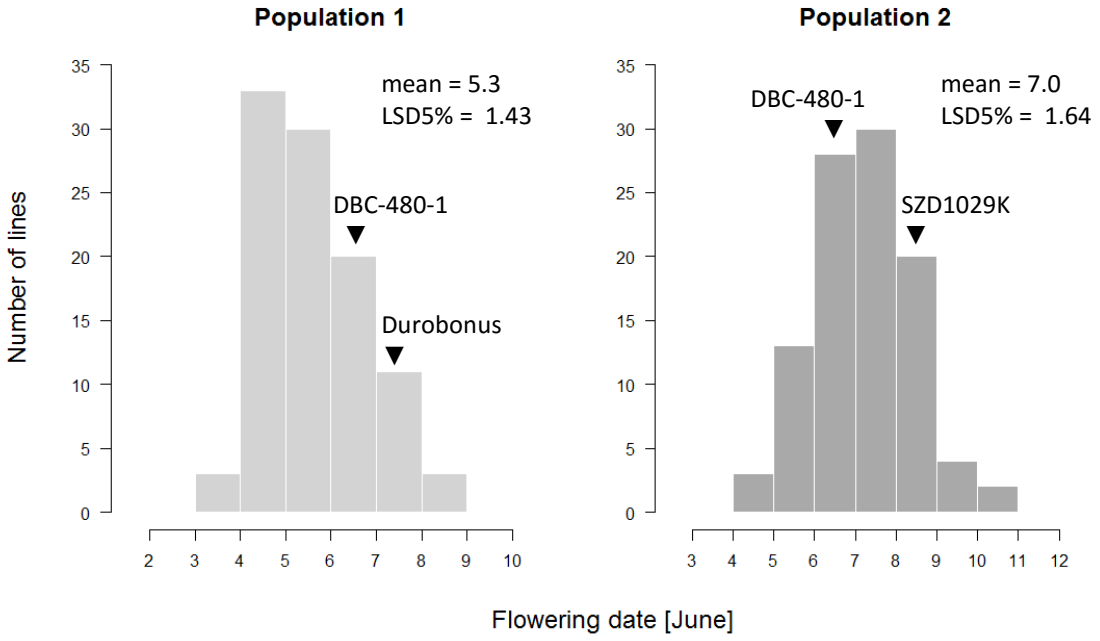

Figure 9: Frequency distribution of the trait flowering date [June], population mean and the least significant difference (LSD) for comparison of lines means (a = 0.05) within population 1 and population 2, respectively. Parental lines are indicated by arrows.

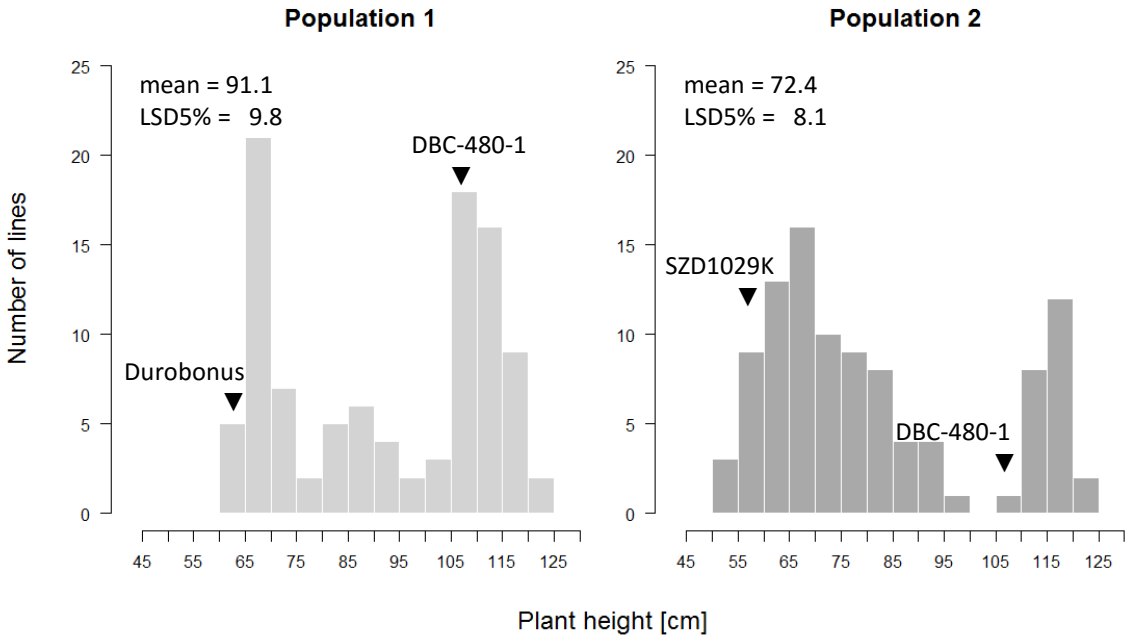

Figure 10: Frequency distribution of the trait plant height [cm], population mean and the least significant difference (LSD) for comparison of lines means (a = 0.05) within population 1 and population 2, respectively. Parental lines are indicated by arrows.

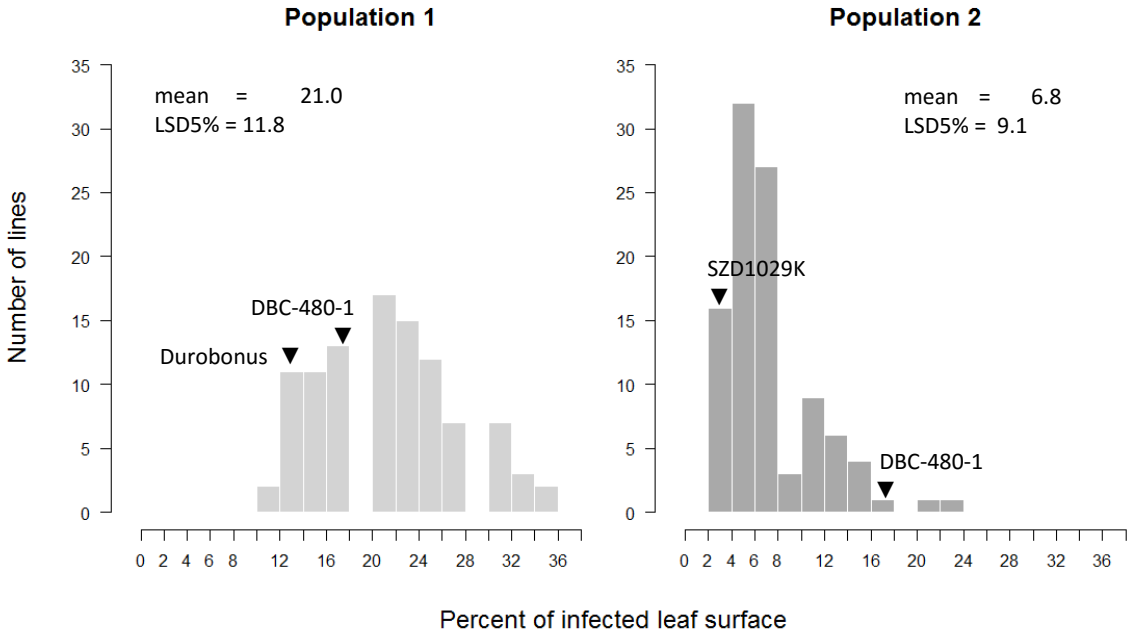

Figure 11: Frequency distribution of the trait severity of stripe rust infection [percent of infected leaf surface], population mean and the least significant difference (LSD) for comparison of lines means (a = 0.05) within population 1 and population 2, respectively. Parental lines are indicated by arrows.

4.3 TRAIT CORRELATION

Correlations were established between traits of FHB severity (percent of infected spikelets 26 days post anthesis) and AUDPC versus other traits. Additionally, correlation between FHB severity and AUDPC were obtained and exhibited significant high positive value in both populations (r=0.96, p<0.001). In the population 1, FHB severity and AUDPC were significantly correlated with plant height and SR, no significant correlation was found with flowering date (Table 11). In population 2, the FHB severity and AUDPC were correlated with all three other traits (Table 12). The highest and very significant (p<0.001) negative correlation factor was found for plant height, higher in population 2 (r=-0.86 and r=-0.84) than population 1 (r=-0.69 and r=-0.64). The correlation can be also seen in scatterplots (Figure 14 and 15) where higher plants tend to be less susceptible to FHB. Flowering date in population 2 was positively correlated with FHB severity and AUDPC, respectively. The correlation with SR was negative.

Table 11: Pearson's correlation coefficient and *p*-values for the given traits in population 1.

population 1	Plant height	Flowering date	SR %	AUDPC
FHB severity (%)	-0.69***	ns	-0.4***	0.96***
AUDPC	-0.64***	ns	-0.39***	
significant correlation, p-value<0.001 (***)/<0.01(**)/<0.05 (*); not significant correlation (ns), p-value>0.05				

Table 12: Pearson's correlation coefficient and *p*-values for the given traits in population 2.

population 2	Plant height	Flowering date	SR %	AUDPC
FHB severity (%)	-0.86***	0.64*	-0.52***	0.96***
AUDPC	-0.84***	0.66**	-0.53***	
significant correlation, p-value<0.001 (***)/<0.01(**)/<0.05 (*); not significant correlation (ns), p-value>0.05				

Correlations are displayed in figures 13 -17.

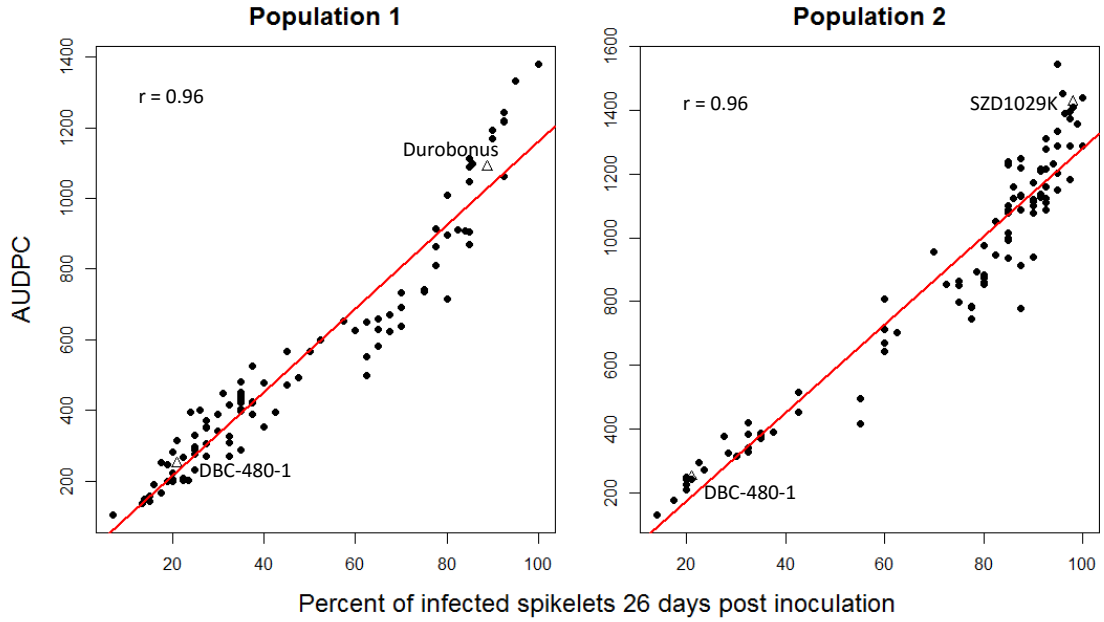

Figure 12: Relationship between variables FHB severity [%] and AUDPC, and correlation coefficient (r) for population 1 and population 2, respectively. Parental lines are depicted with a triangle and indicated with their name.

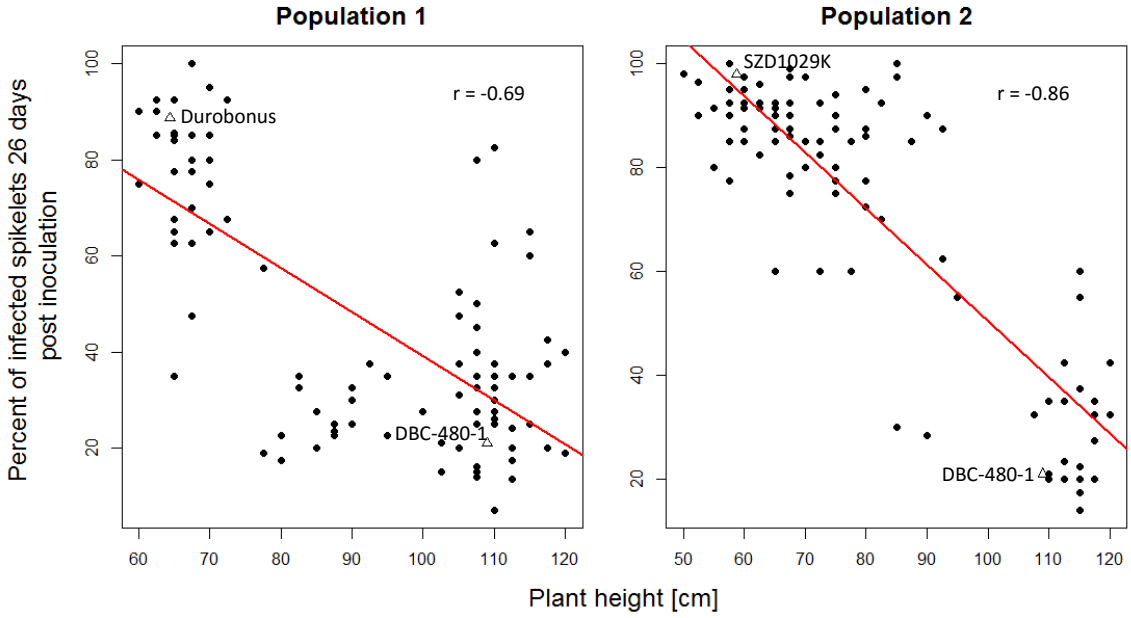

Figure 13: Relationship between variables plant height [cm] and FHB severity [%], and correlation coefficient (r) for population 1 and population 2, respectively. Parental lines are depicted with a triangle and indicated with their name.

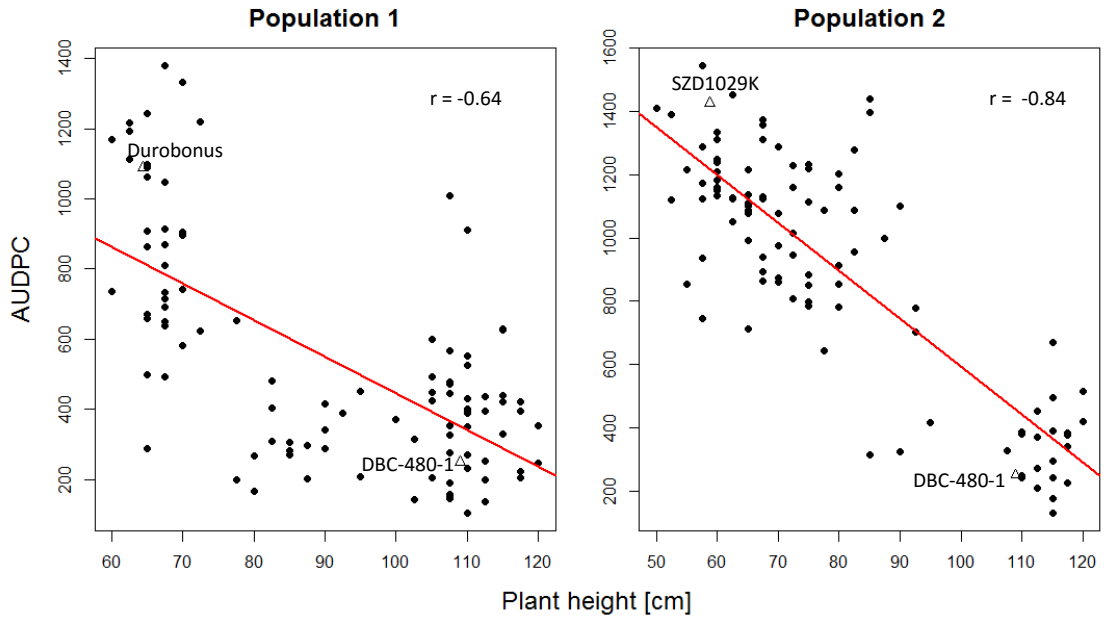

Figure 14: Relationship between variables plant height [cm] and AUDPC, and correlation coefficient (r) for population 1 and population 2, respectively. Parental lines are depicted with a triangle and indicated with their name.

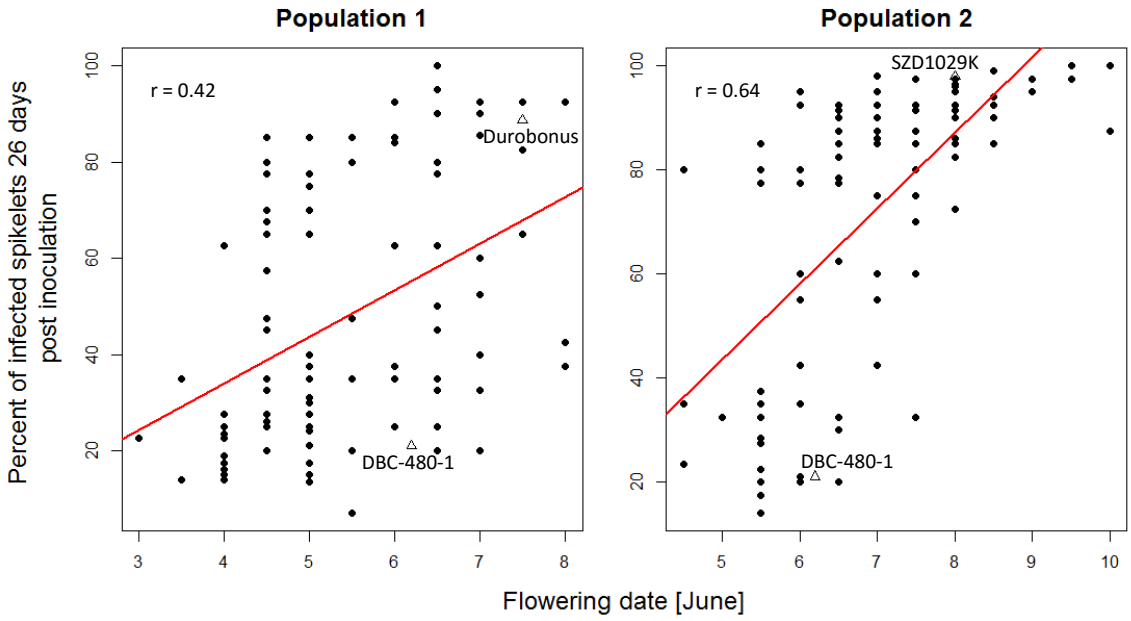

Figure 15: Relationship between variables flowering date [June] and FHB severity [%], and correlation coefficient (r) for population 1 and population 2, respectively. Parental lines are depicted with a triangle and indicated with their name.

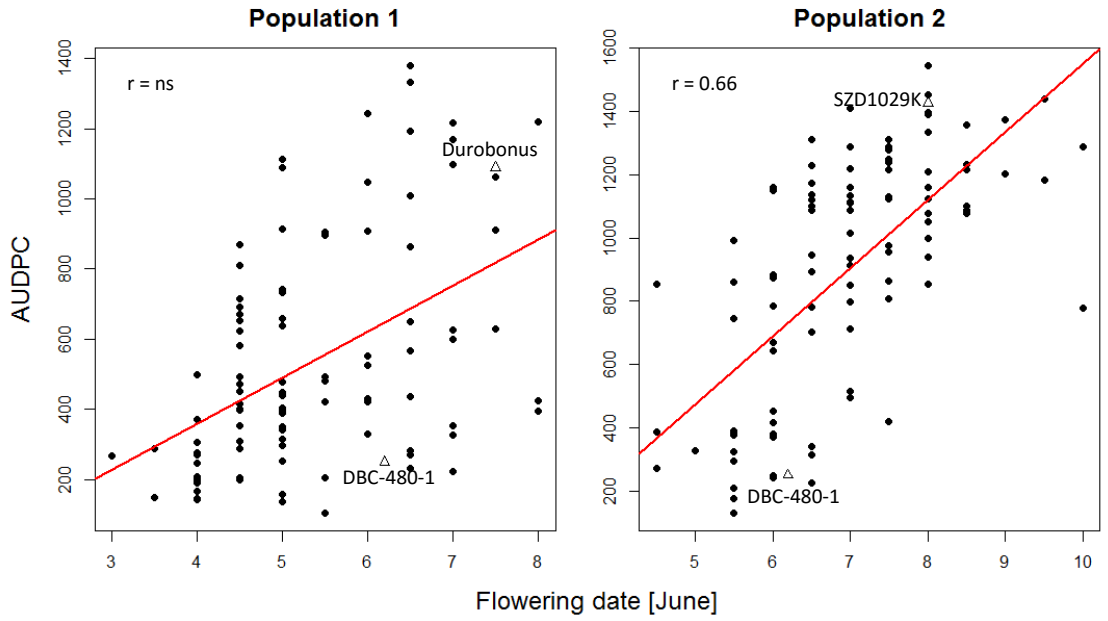

Figure 16: Relationship between variables flowering date [June] and AUDPC, and correlation coefficient (r) for population 1 and population 2, respectively. Parental lines are depicted with a triangle and indicated with their name.

4.4 MARKERS

An ANOVA was carried out to determine statistically significant difference between the means of two classes of plants possessing the *Fhb1* (*Fhb1+*) allele for FHB resistance of DBC-480-1 or absence of it (*Fhb1-*), which was obtained with markers *Umn10* and *Barc147*, as well as between means of the classes of plants with semi-dwarfing allele *Rht-B1b* of susceptible Durobonus and SZD1029K, and wild type tall allele of DBC-480-1 *Rht-B1a*.

Table 13: ANOVA for markers *Umn10*, *Barc147* and *Rht-B1*, respectively, affecting FHB response in population 1. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

	Df	Sum Sq	Mean Sq	F-value	*p*-value
Umn10	1	9495	9495	15.78	0.000135
Residuals	100	60190	602		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Barc147	2	8870	4435	7.219	0.00118
Residuals	99	60815	614		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Rht-B1	1	39071	39071	139.4	$<2 \times 10^{-16}$
Residuals	98	3494	35.3		

Table 14: ANOVA for markers *Umn10*, *Barc147* and *Rht-B1*, respectively, affecting AUDPC in population 1. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

	Df	Sum Sq	Mean Sq	F-value	*p*-value
Umn10	1	1761692	1761692	20.1	1.96×10^{-5}
Residuals	100	8763185	87632		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Barc147	2	1576639	788320	8.722	0.000325
Residuals	99	8948238	90386		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Rht-B1	1	4691579	4691579	88.16	2.56×10^{-15}
Residuals	98	5215512	53220		

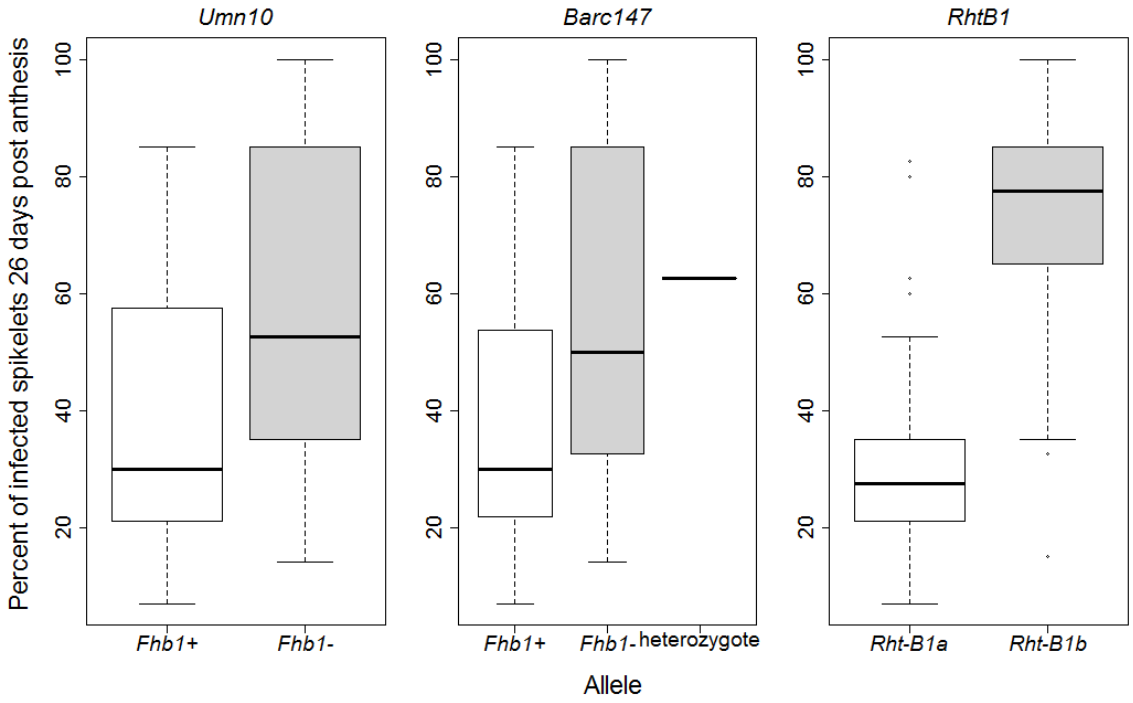

Figure 17: Boxplot distribution of the FHB severity [% of infected spikelets 26 days post anthesis] in population 1, according to allele status at the markers *Umn10*, *Barc147* and *Rht-B1*, respectively.

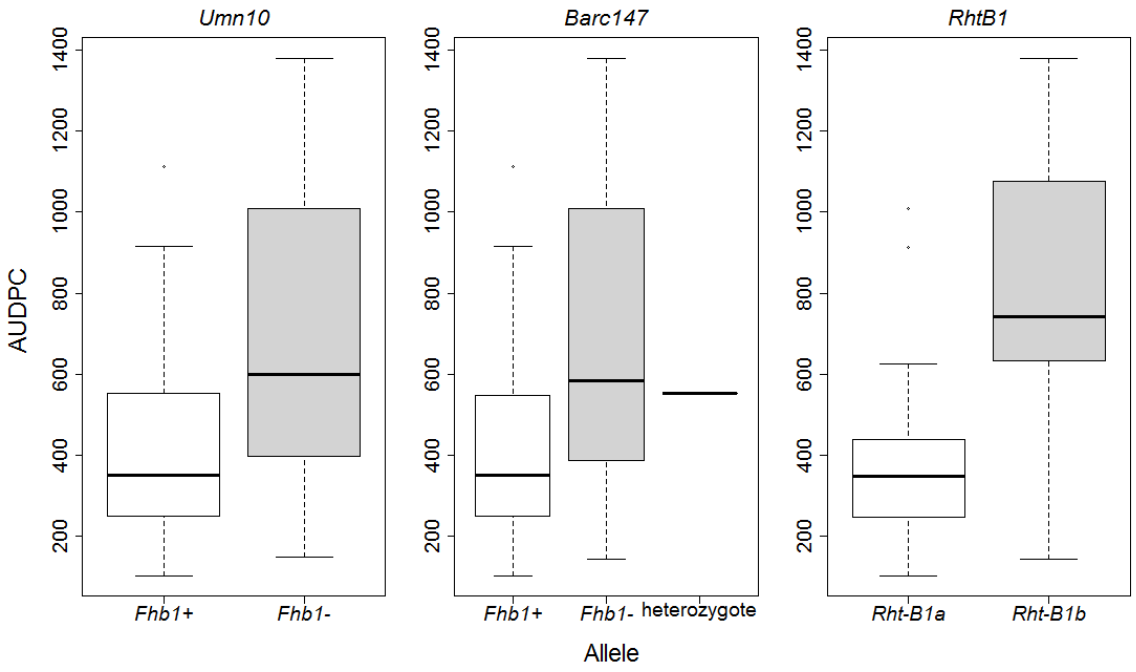

Figure 18: Boxplot distribution of the AUDPC in population 1, according to allele status at the markers *Umn10*, *Barc147* and *Rht-B1*, respectively.

For all three markers (*Umn10*, *Barc147* and *RhtB1*, respectively) the difference between means of classes for AUDPC is significant in population 1 (p<0.001). The comparison can be observed in Figure 19. When comparing the classes of each marker in population 2, there was no significant difference between AUDPC means within each marker for *Umn10* and *Barc147*, however *RhtB1* exhibited significant differences (p<0.001) between allele classes (Fig. 21). The same pattern as for AUDPC was observed when comparing means of classes for FHB severity (percentage of infected spikelets 26 days post anthesis); population 1 showed significant differences between means of classes (Fig. 18), and again only significant differences between the allele classes of *RhtB1* in population 2 when comparing the means of FHB severity were established (Fig. 20).

Table 15: ANOVA for markers *Umn10*, *Barc147* and *Rht-B1*, respectively, affecting FHB response in population 2. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

	Df	Sum Sq	Mean Sq	F-value	*p*-value
Umn10	1	74	74.5	0.105	0.747
Residuals	100	71128	711.3		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Barc147	2	176	87.8	0.117	0.89
Residuals	90	67546	750.5		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Rht-B1	1	16481	16481	31.38	1.94×10^{-7}
Residuals	98	5215512	53220		

Table 16: ANOVA for markers *Umn10*, *Barc147* and *Rht-B1*, respectively, affecting AUDPC in population 2. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

	Df	Sum Sq	Mean Sq	F-value	*p*-value
Umn10	1	95486	95486	0.65	0.422
Residuals	100	14692662	146927		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Barc147	2	152297	76148	0.498	0.609
Residuals	90	13761324	152904		
	Df	Sum Sq	Mean Sq	F-value	*p*-value
Rht-B1	1	2759265	2759265	23.7	4.31×10^{-6}
Residuals	98	11407403	116402		

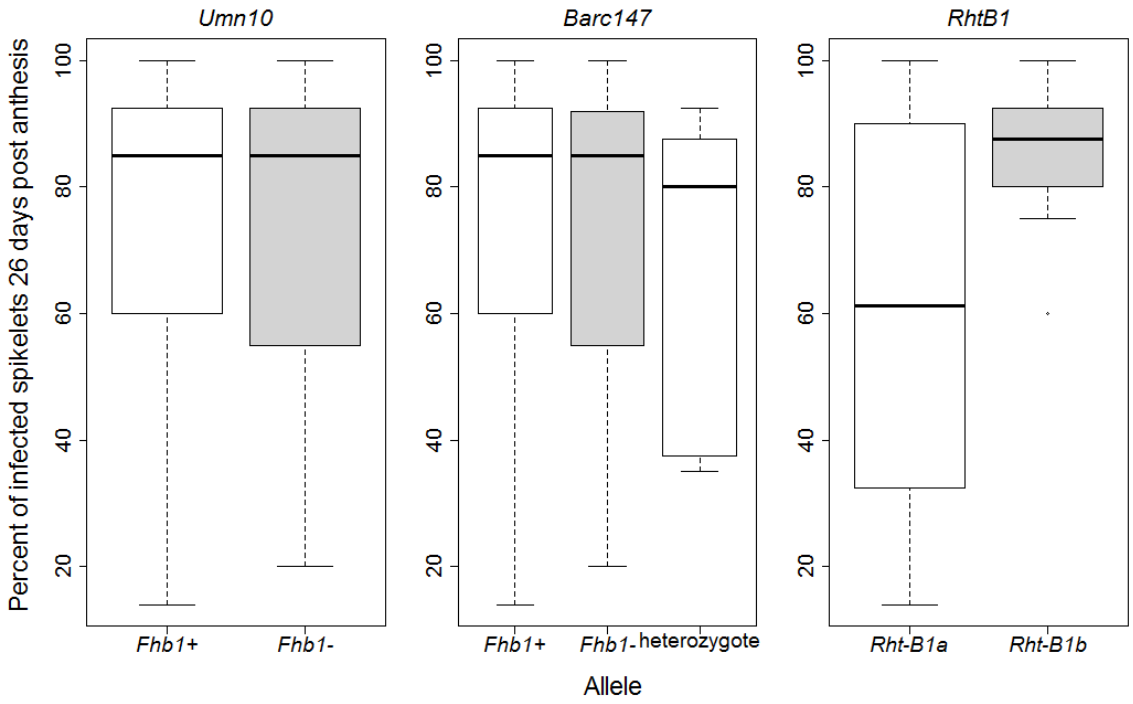

Figure 19: Boxplot distribution of the FHB severity [percent of infected spikelets 26 days post anthesis] in population 2, according to allele status at the markers *Umn10*, *Barc147* and *Rht-B1*, respectively.

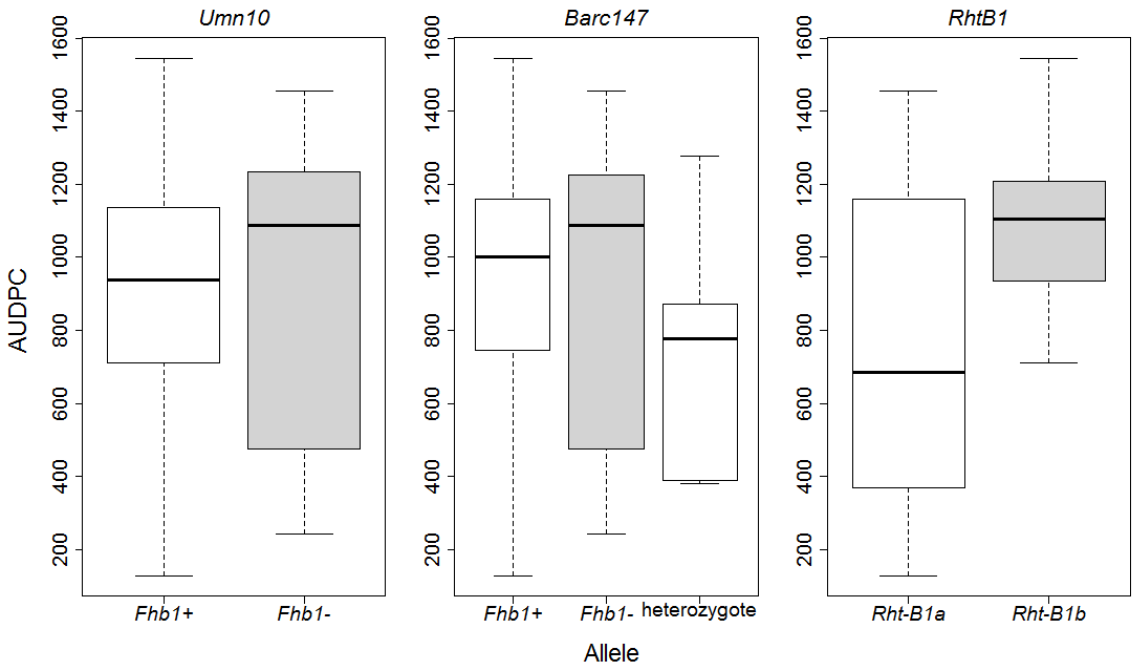

Figure 20: Boxplot distribution of the AUDPC in population 2, according to allele status at the markers *Umn10*, *Barc147* and *Rht-B1*, respectively.

Additionally, a display of *Rht-B1* marker for population 1 and 2 according to plant height is given in figure 22. The significant difference (p<0.001) between the means of classes with wild tall allele *Rht-B1a* and dwarf-type allele *Rht-B1b* within population 1 and 2 is indisputable, although the height within an allele in population 2 varies considerably more as in population 1.

Table 17: ANOVA for marker *Rht-B1* according to plant height for population 1 and 2, respectively. Results are shown as degrees of freedom (Df), sum squares (Sum sq), mean squares (Mean Sq), F- value and *p*-value, respectively.

Population 1	Df	Sum Sq	Mean Sq	F-value	*p*-value
Rht-B1	1	25046	25046	163.9	$<2 \times 10^{-16}$
Residuals	98	29743	149		
Population 2	Df	Sum Sq	Mean Sq	F-value	*p*-value
Rht-B1	1	21062	21062	90.03	1.56×10^{-15}
Residuals	199	22927	234		

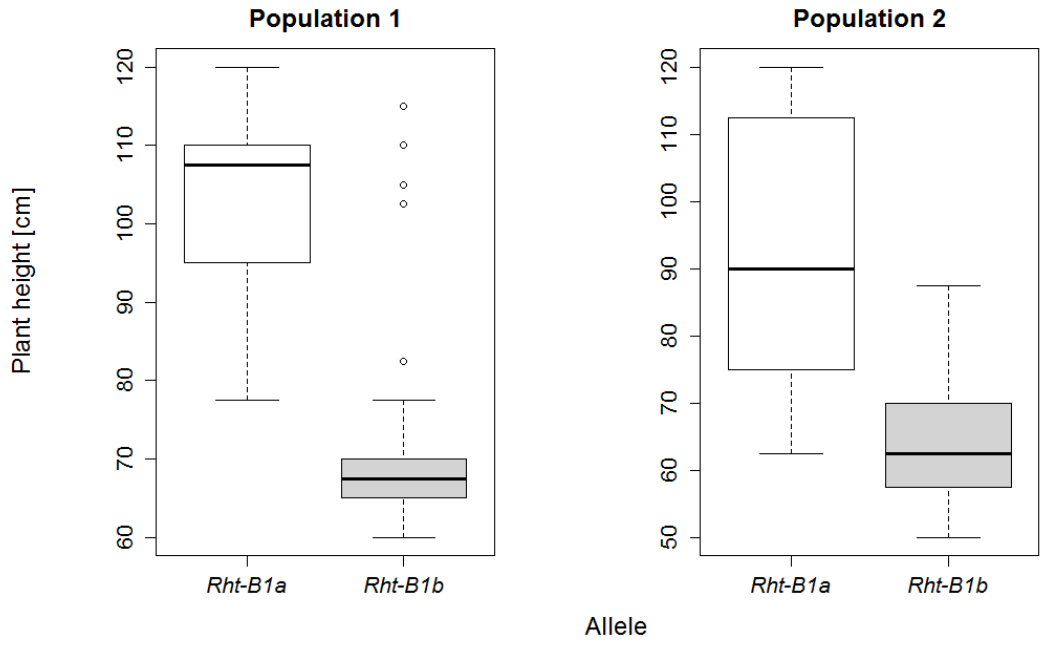

Figure 21: Boxplot distribution of the plant height [cm] according to allele status at the marker *Rht-B1* in population 1 and 2, respectively.

5 DISCUSSION

5.1 ASSESING RESISTANCE TO FHB

Assessing a quantitative trait such as FHB severity needs to be approached with high focus and indulgence. In our case, the execution was aggravated by fluctuating weather; from abundant daily precipitation and strong wind in the beginning of the severity estimation to high summer temperatures in the second half of our field work. Overall, the weather facilitated inoculation of durum plants with *Fusarium* pathogen and favored the development of FHB complementary to mist irrigation. Very last assessments needed to be carried out with extreme precaution though, due to senescence of the caryopsis which resembled the symptoms of FHB. Oliver et al., (2008) stress the important influence of environmental factors on FHB disease development, thus possible considerable variation of FHB response within a genotype in different field environments. Nevertheless, the obtained data was consistent (repeatability values between 0.92 and 0.96) and the levels of resistance in accordance with forgoing results (data not shown). High correlation values were obtained between FHB severity and AUDPC for both populations (0.96).

A total of 200 F_7 RIL durum genotypes together with parental lines were evaluated for FHB response (resistance type 1 and 2) after an artificial spray inoculation with *F. culmorum*. Percentage of infected spikelets was used as a measure of FHB severity and was further calculated into AUDPC to obtain a result of total disease development within a genotype. Population 1 and 2 shared the same first parental line DBC-480-1, while the susceptible parental line differed; Durobonus in population 1 and SZD1029K in population 2. ANOVA showed significant (p<0.001) genetic variation among the population 1 and 2 for FHB severity (AUDPC included). Population 1 consistently exhibited low to moderate levels of resistance and displayed lower overall severity to FHB (47.1 %) as population 2 (72.4 %). A vast majority of population 2 (70 %) suffered FHB severity from 70 to 100 %. In contrast, more than half of population 1 (56 %) exhibited FHB severity lower than 40 %. Likewise to FHB severity, population 1 displayed lower AUDPC (535.9) than population 2 (897.0). In compliance with RIL populations, Durobonus exhibited lower FHB severity and AUDPC (88.8 % or 1090.2) than SZD (98.0 % and 1427.0). Common parental line DBC-480-1 expressed much lower FHB severity (21 % or 249.8). In each population there was a significant difference between parental lines. Different susceptibility means between investigated populations show the importance of selecting an optimal adapted wheat line with good native resistance when introgressing FHB resistance (Brown-Guedira et al., 2008). This suggests the complexity of FHB resistance governed by several QTL for FHB resistance rather than only one major QTL (Buerstmayr et al., 2012; Mao et al., 2010; Buerstmayr et al., 2009; Draeger et al., 2007; Miedaner et al., 2011).

5.2 PLANT HEIGHT AND FHB RESISTANCE

Plant height has been consistently associated with FHB response in wheat plants (Yan et al., 2011; Mao et al., 2010; Srinivasachary et al., 2008; Klahr et al., 2007). Although plant height can be translated as passive resistance due to higher distance between *Fusarium* inoculum resting on the ground and ears where the fungus penetrates, as well as due to

unfavorable microclimatic condition for the FHB development in the canopy of shorter wheat plants (higher humidity than in tall genotypes), it is known for the plant height genes *Rht* to have an impact on FHB manifestation (Mesterhazy, 1995; Yan et al., 2011). For the purpose of reducing the cofounding effect of plant height, an artificial spray inoculation directly on the top of durum heads was carried out.

Plants in population 1 were on average higher (91.1 cm) than plants of population 2 (72.4 cm) and exhibited lower FHB severity (see 5.1). A strong negative correlation was established between plant height and FHB severity. In population 1, Pearson's correlation coefficient was slightly lower (r=-0.69) as in population 2 (r=-0.86). The same was observed with plant height and AUDPC, where Pearson's correlation coefficient was r=-0.64 in population 1 and r=-0.84 in population 2. In all cases *p*-value did not exceed 0.001. Buerstmayr et al. (2012) also reported high significant negative correlation between FHB severity and plant height in durum wheat. Significant negative correlation between FHB severity and plant height in common wheat has been observed by several authors in the past (Klahr et al., 2007; Buerstmayr et al., 2014b; Lu et al., 2013; Voos et al., 2008), though none of them were as high as in this investigation of durum wheat.

5.3 MARKERS AND FHB

Expression of resistance genes can be inhibited by confounding environmental effects such as temperature and humidity at flowering, thus visual symptoms of FHB might be misleading (Zhang et al., 2004). To support our results of visual FHB severity assessment and the correlation of plant height versus FHB severity (see 5.2), QTL detections with three different markers was carried out.

Fhb1 is a major gene controlling FHB resistance in common wheat (Cuthbert et al., 2006). It confers type 2 resistance by limiting the spread of the pathogen within the spike after initial infection (Schweiger et al., 2013). Incorporation of *Fhb1* into adapted plant material is a desired scenario for assuring FHB resistance in common wheat (Brown-Guedira et al., 2008). So far, the same attempt in durum wheat has been believed to be impeded by the absence of D genome (Oliver et al., 2008). RILs of durum wheat showing different levels of FHB resistance were used to identify *Fhb1*. PCR markers *Umn10* and *Barc147* were used as diagnostic markers for *Fhb1* detection. The genotypes carrying the allele of DBC-480-1 (*Fhb1*) exhibited lower FHB severity than genotypes with no *Fhb1* in population 1. In population 2, there was no significant difference between means of FHB resistance of classes with and without *Fhb1*.

Reduced height allele *Rht-B1b*, major factor of "green revolution" contributing to semi-dwarfism in wheat, has also showed to have an impact on FHB severity (Wilhelm et al., 2013). The response of cultivars to FHB and plant height was associated to the allelic composition for the *Rht-B1* locus. In population 1 as well as in population 2, semi-dwarf lines (carrying the *Rht-B1b* allele) exhibited significantly higher FHB severity whereas tall lines (carrying allele *Rht-B1a*) were better in FHB resistance. Presence of *Rht-B1* had also significant effect on AUDPC in both populations. However, the FHB severity varied more within each allele in population 2 compared to population 1. Although the relationship

between plant height and the FHB severity has been confirmed, the dwarfing genes affect FHB severity differently, and their performance of associating is complicated (Mao et al., 2010). Varying effect of *Rht-B1b* on FHB severity between durum populations was observed also by Buerstmayr et al. (2012). Srinivasachary et al. (2009) demonstrated however that *Rht-B1b* significantly increased type 2 resistance. They imply a through consideration when choosing semi-dwarfing genes for FHB resistance. The correlation between plant height and FHB severity in this study on one hand was stronger in population 2, yet when observing the effect of *Rht-B1* on FHB severity it seems to be more straightforward in population 1 due to smaller variation within allele. FHB severity appears to be due to plant height gene impact but also plant height *per se*.

The mean of the class carrying wild type tall allele *Rht-B1a* differed significantly in height from the mean of the class carrying semi-dwarf allele *Rht-B1b* in population 1 and 2, showing that *Rht-B1* affects plant height. Reduced stem elongation in lines containing *Rht-B1b* allele is generated by a limited response to the phytohormone gibberellin (Pearce et al., 2011).

As described in following paragraph (5.4), the varying weather condition during the time of inoculation might have also contributed to difference in FHB response.

5.4 OTHER TRAITS

Flowering date has been shown to play a significant part in FHB severity in durum and common wheat (Buerstmayr et al., 2012; Buerstmayr et al., 2014b). It is suggested that wheat lines that flower briefly and whose heads open narrowly decrease their risk of infection by reducing the area and time span in which *Fusarium* spores can penetrate the floret and herby infect the plant (Gilsinger et al., 2005). Our results showed no correlation with flowering date in population 1 whereas in population 2 flowering date was positively correlated with FHB severity and AUDPC, respectively. Medians for population 1 and population 2 were June 5 and 7, respectively. Regarding the fact that in population 1 FHB severity was not significantly correlated to flowering date there still might be some connection, considering that the plants of population 1 flowered earlier than plants of population 2, yet FHB severity in population 1 was lower than in population 2. Diaz de Ackermann and Kohli (1997) pointed out, that the susceptibility to FHB of a cultivar differs more according to weather condition at flowering time as on cultivar behavior. Parry et al. (1995) stressed the importance of humidity and temperature during the pathogenesis on the development of FHB. Although there was no rain occurring from June 3 to June 11 when the plants were flowering, a considerable variation in air temperature and air humidity might support this thesis. The relative air humidity from June 3 to June 6 did not exceed 64 % but right afterward started to increase and varied between 66.6 and 69.1 % from June 7 to June 11. Also the daily air temperatures from June 6 to June 8, when the population 2 was mostly flowering were increasing again (23.4, 22.9, 22.2 °C, respectively), thus favoring the FHB infection in population 2. Since the manifestation of the *Fusarium* pathogen depends partially also on the environmental conditions at the time of inoculation (e.g. flowering) (Ablova and Slusarenko, 1997), different time of flowering could have contributed to discrepancy in FHB severity of population 1 and 2.

Mist-irrigation can seriously contribute to development of foliar diseases such as stripe rust. Severe infection from these foliar diseases might also contribute to the early senescence of the same plants (Oliver et al., 2008). Stripe rust occurs in temperate regions with cool and moist weather conditions and is considered an economically important pathogen (Chen et al., 2014). We screened genotypes of both populations for SR symptoms by the same pattern as FHB. Population 1 was more prone to SR as population 2 (21 and 6.8 %). The same trend was observed with second parental line Durobonus and SZD1029K (13.9 and 3 %), however mutual FHB resistant parent DBC-480-1 exceeded both (17.5 %). The correlation between SR and FHB severity (and AUDPC) was negative with *p*-value below 0.001. Negative correlation suggests antagonistic effect of pathogens of FHB and SR. On the other hand, a positive correlation SR versus FHB severity in common spring wheat Frontana was recognized by Steiner (2003). Additionally, colocalization of major gene for SR resistance on chromosome 3B and a minor QTL for FHB severity was detected.

6 CONCLUSIONS

FHB of cereals causes multiple undesirable effects; yield and quality reduction, economic losses and mycotoxin toxicity (Chelkowski, 1989). Breeding for resistance to FHB not only improves plant vigor and productivity and decreases mycotoxin content but also favors organic agriculture eager for new low-input varieties. Introgression of FHB resistance from hexaploid wheat into tetraploid durum wheat has been hindered by the difference in ploidy levels and had only limited success in the past (Buerstmayr et al., 2009). With this experiment we evaluated introgression of hexaploid FHB resistance QTL into two different durum wheat RIL populations.

The major FHB resistance QTL *Fhb1* increases FHB resistance in durum wheat, as shown in population 1. Due to complexity of resistance gene expression and their interaction with environment, the effect of *Fhb1* on decreased FHB severity in population 2 could not be observed. Strong correlation between plant height and FHB severity has been established; shorter plants were more diseased than taller plants. Although plant height could have affected FHB severity due to microclimatic conditions, a significant effect of *Rht-B1* on FHB severity was established. *Rht-B1b* dwarfing allele highly influenced increased FHB severity in both populations. Flowering date was positively correlated with FHB severity only in population 2, however its impact seems to be more due to the environmental conditions during flowering period.

Investigated populations encompassed genotypes that varied significantly in resistance of FHB as well as in all other studied traits, which provides us with rich genetic pool for further durum wheat selection and breeding. In the future, highly FHB resistant *Fhb1* containing durum lines with good agronomical traits should be tested under organic management. Wolfe et al. (2008) stressed that breeding for organic agriculture must encompass entire organic system approach including crop rotation and practices that increase plant resilience. In this way we will select FHB resistant durum lines that are most suitable for organic production; an organic durum ideotype.

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