# Evaluation of three winter triticale (x *Triticosecale* Wittmack) populations and a collection of triticale cultivars and breeding lines for resistance against Fusarium head blight

Master thesis

Submitted by

Simon Reinhold Dürr

H0840444

Supervision

Univ.Prof. Dipl.-Ing. Dr.nat.techn. Hermann Bürstmayr

Dipl.-Ing. Dr. Barbara Steiner

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University of Natural Resources and Life Sciences, Vienna

Department of Agrobiotechnology, Institute of Biotechnology in Plant Production, Tulln

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# List of Abbreviations

**AUDPC** – area under the disease progress curve for five visual scorings

**°C** – degree(s) Celsius

**BOKU** – University of Natural Resources and Life Sciences, Vienna, Austria

**cm** – centimeter

**$F_1$** – first filial generation

**$F_2$** – second filial generation

**$F_{4:5}$** – fifth filial generation, derived from one plant of the fourth filial generation

**FHB** – Fusarium head blight (scab)

**FLD** – flowering date (days after the 30$^{th}$ of April, 2014)

**g** – gram

**ha** – hectare (10.000 $m^2$)

**IFA-Tulln** – Department of Agrobiotechnology, University of Natural Resources and Life Sciences (BOKU); located in Tulln, Austria

**kg** – kilogram

**L** – liter

**m** – meter

**$m^2$** – square meter

**mL** – milliliter

**mm** – millimeter

**%FDK** – percentage of *Fusarium* damaged kernels

**%FHB** – percentage of visually infected spikelets per plot for the fifth scoring (26 days after full anthesis)

**PLH** – plant height (cm)

**POM** – severity of powdery mildew infections

**QTL** – quantitative trait loci

**RIL** – recombinant inbred line

**YER** – severity of yellow rust infections

## Abstract

Fusarium head blight (FHB, scab) is a devastating fungal disease of small grain cereals. Under sporadic epidemics, essential yield and quality losses have been observed globally. The contamination of grain with mycotoxins produced by the causal agents of the genus *Fusarium* poses an acute risk on human and animal consumption. So far, no completely resistant line has been found in any of the cereal species. Knowledge about FHB mechanisms and resistance in cereal species other than wheat and barley is rather scarce. In the thesis at hand, we present results of a field trial for FHB resistance in triticale conducted in 2014. Three recombinant inbred line (RIL) populations and a fourth population of currently registered cultivars and advanced breeding lines were phenotypically examined for a number of FHB related traits and other important parameters (i.e. plant height, flowering date, severity of powdery mildew and yellow rust infections). A wide variation with highly FHB resistant as well as susceptible lines was found in all four populations. Lines with high FHB resistance in combination with other desirable agronomic features could be detected in all three RIL populations. A separate analysis of advanced breeding lines and registered varieties in population 4 revealed that the group of breeding lines from former FHB resistance trials as such exhibited the best resistance among all populations tested. The registered cultivars in turn were more susceptible to FHB than any other population. The findings of this thesis suggest that with the current breeding program significant progress can be made in regard to FHB resistance in triticale. Future investigations about mycotoxin accumulation in kernels and yield potential as well as molecular based analyses for selected lines will help to increase our knowledge about FHB resistance mechanisms in triticale and hopefully result in the selection of high yielding FHB resistant triticale lines for cultivar registration.



Key words: Fusarium head blight, *Fusarium culmorum*, mycotoxins, triticale, resistance breeding

# Zusammenfassung

Die Pilzkrankheit Ährenfusariose gehört zu den bedeutendsten Erkrankungen an Getreide. Sporadisch auftretende Epidemien führten weltweit zu enormen Ertrags- und Qualitätseinbußen. Die Verursacher der Krankheit aus der Gattung *Fusarium* produzieren eine Reihe von gefährlichen Mykotoxinen, die beachtliche Risiken für die Ernährung von Mensch und Tier darstellen. Bisher wurde keine vollständig resistente Linie in einer der befallenen Getreidearten entdeckt. Abgesehen von Weizen und Gerste liegen für die übrigen Getreidearten nur begrenzt Informationen über Wirkungsmechanismen und Resistenz gegenüber dieser Krankheit vor. In der vorliegenden Masterarbeit werden die Ergebnisse eines Feldversuchs aus 2014 über die Resistenz von Triticale gegenüber Ährenfusariose präsentiert. Drei unterschiedliche Inzuchtpopulationen sowie eine vierte Population aus zugelassenen Sorten und fortgeschrittenen Züchtungslinien wurden phänotypisch auf mehrere Parameter untersucht, die mit Ährenfusariose zusammenhängen. Eine weite Streuung mit Ährenfusariose-resistenten wie auch anfälligen Genotypen konnte in allen vier Populationen beobachtet werden. Linien mit hohem Resistenzlevel, die gleichzeitig gute agronomische Eigenschaften (z.B. geringe Anfälligkeit gegenüber Mehltau, niedrige Wuchshöhe) aufwiesen, konnten in allen drei Inzuchtpopulationen gefunden werden. Die Ergebnisse der vorliegenden Arbeit lassen darauf schließen, dass durch das neu etablierte Züchtungsprogramm ein wesentlicher Fortschritt in Hinblick auf die Resistenz gegen Ährenfusariose in Triticale erzielt werden kann. Weitere Untersuchungen (Mykotoxinkontaminationen im Erntegut, Ertragspotential, molekulare Analysen) werden an ausgewählten Linien in weiteren Projektstadien durchgeführt werden. Diese Tests werden dabei helfen, die zugrundeliegenden Resistenzmechanismen in Triticale besser zu verstehen und hoffentlich die Selektion hochresistenter, ertragreicher Triticale-Linien für die Sortenzulassung ermöglichen.

Schlüsselwörter: Ährenfusariose, *Fusarium culmorum*, Mykotoxine, Triticale, Resistenzzüchtung

# 1.) Introduction

In the master thesis at hand, the most important outcome of a field trial for Fusarium head blight resistance in triticale (x *Triticosecale* Wittmack) will be presented. The screening of three recombinant inbred line (RIL) populations and a collection of triticale cultivars and breeding lines was carried out at the fields and laboratories of the Institute of Biotechnology in Plant Production of the University of Natural Resources and Life Sciences, Vienna, in 2014.

The content of the master thesis at hand will be structured as follows: First, the theoretical background of Fusarium head blight, resistance breeding, and triticale will be illustrated. Following this introduction, a section of materials and methods utilized for the practical work of this master thesis will be provided. In the next chapters, results of all screenings conducted will be demonstrated and discussed. In a final conclusion, the most relevant outcome will be summarized and a future outlook will be given.

## 1.1.) Fusarium head blight (FHB) in small grain cereals – threat to quality and yield

Fusarium head blight (FHB), also known as Fusarium ear blight (FEB) or scab, belongs to the most important and destructive fungal diseases on small grain cereals (Dean et al., 2012). So far, the disease occurred in most cereal-growing areas worldwide and sporadic epidemics have been reported from around the globe (Bai and Shaner, 1994; Buerstmayr et al., 2012; Gale, 2003; Martin and Johnston, 1982; McMullen et al., 2012; Obanor et al., 2013; Parry et al., 1995).

All small grain cereals, including wheat (*Triticum aestivum* L., *Triticum durum* Desf., and other wheats), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), triticale (x *Triticosecale* Wittmack), and oats (Avena sativa L.) might be attacked by *Fusarium* species causing FHB (Arseniuk et al., 1999; Atanasoff, 1920; Martin and Johnston, 1982) (for more detailed descriptions of the pathogens, see chapter 1.2.). The same pathogens of the genus *Fusarium* might also cause diseases on other plant organs than cereal heads (e.g. seedling blight, crown rot, foot rot) (Miedaner, 1997) and additionally infect corn (*Zea mays* subsp. *mays* L.), leading to head rots (Mesterházy et al., 2012; Sutton, 1982) and stalk rot (Gilbertson et al., 1985). Nevertheless, for

small grain cereals, the infection of the heads appears to be most critical (Ruckenbauer et al., 2001), as it may lead to severe yield losses and reductions of quality (Atanasoff, 1920; Bai and Shaner, 1994; Martin and Johnston, 1982; McMullen et al., 1997; Parry et al., 1995; Pugh et al., 1933; Snijders and Perkowski, 1990; Sutton, 1982).

Reductions in yield might be as high as 80 percent (McMullen et al., 2012; McMullen et al., 1997), having an enormous financial impact on agriculture and related sectors (McMullen et al., 1997; Windels, 2000). A high percentage of small, shriveled grains with low weight that might be lost during harvest or a complete failure in kernel development are responsible for these yield losses (Atanasoff, 1920; Buerstmayr et al., 2012) (for symptom development, see chapter 1.3.).

FHB is affecting quality in manifold ways: First of all, as mentioned above, kernels will be small, leading to low test and thousand kernel weight (Jones and Mirocha, 1999; Snijders and Perkowski, 1990). Secondly, the milling, baking, and brewing quality (‘gushing’ of beer) of affected grain will be significantly reduced by a destruction of starch granules, storage proteins, and cell walls (Bechtel et al., 1985; Schildbach, 2013). Thirdly, germination rates and seedling vigor of even slightly infected kernels will be low (Argyris et al., 2003; Bai and Shaner, 1994). Fourthly, and most importantly, FHB infections lead to a contamination of kernels with so-called mycotoxins, toxic chemical compounds produced and released by the fungi (Bai and Shaner, 1994; Miedaner, 1997; Parry et al., 1995; Snijders and Perkowski, 1990; Sutton, 1982). *Fusarium* mycotoxins, having various detrimental effects on human and animal health (see chapter 1.4. for further details), have been detected in grain, food, and feed stuff worldwide (Placinta et al., 1999). Their occurrence is the main reason why researchers from all continents are currently working on a solution to combat FHB efficiently.

## 1.2.) Pathogens causing FHB

Up to 17 distinct *Fusarium* species can cause FHB in small grain cereals (Parry et al., 1995). The genus *Fusarium* includes several of the anamorphic stages of the genera *Gibberella* and *Nectria* (Ascomycota, Hypocreales, Nectriaceae). The most important FHB pathogens belong to the *Fusarium* sections *Discolor, Roseum, Gibbosum*, and *Sporotrichiella* (Liddell, 2003).

On a worldwide basis, *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch), *Fusarium culmorum* (Wm. G. Smith) Sacc. (no teleomorph known), and *Fusarium avenaceum* (Fr.) Sacc. (teleomorph: *Gibberella avenacea* R.J. Cook) are most often associated with FHB. The same is true for Europe, although the frequencies of occurrence of the three species vary widely within the European countries (Bottalico, 1998; Bottalico and Perrone, 2002; Gale, 2003). Due to differing temperature requirements, *Fusarium graminearum* will predominate in warmer climates (e.g. continental areas, such as central and south-eastern Europe) while *Fusarium culmorum* and *Fusarium avenaceum* are more often found in cooler regions (e.g. maritime European areas or northern Europe) (Bottalico and Perrone, 2002; Parry et al., 1995). Nevertheless, all three species (and others) can be found on the same field or even on the same plant (tissue) (Bottalico, 1998; Clear and Patrick, 1990; Gale, 2003). As for *Fusarium graminearum*, modern phylogenetics have revealed that the fungus is not a single species, but that it is rather a species complex with at least 13 biogeographically distinct species or lineages (O'Donnell et al., 2008).

*Fusarium culmorum* and *Fusarium graminearum* are not only the species most often found in infected cereal heads, but they are also the most aggressive FHB pathogens (Stack and McMullen, 1985; Wilcoxson et al., 1988; Wong et al., 1992). Hence, these two species have been used for research purposes most often and they have been investigated most thoroughly. Both *F. culmorum* and *F. graminearum* belong to the section *Discolor*, so they do not produce microconidia (Liddell, 2003). *F. graminearum* produces mycelium, macroconidia, chlamydospores, and in its teleomorphic stage (*Gibberella zeae*) also ascospores. *F. culmorum* on the other hand, does not produce ascospores (no teleomorph known) (Buerstmayr et al., 2012; Liddell, 2003). The two fungi can survive within soil and as saprophytes on crop debris and infect the roots, foot, and heads of cereals (Liddell, 2003).

## 1.3.) Life cycle of FHB pathogens

In general, FHB pathogens overwinter as mycelium in soil or saprophytically on crop debris (Bushnell et al., 2003; Goswami and Kistler, 2004; Liddell, 2003; Shaner, 2003). In addition to their cereal hosts (and corn), they have been found to colonize and survive on various gramineous or dicotyledonous weeds and soybean (Fernandez and Fernandes, 1990; Jenkinson and Parry, 1994a; Martin and

Johnston, 1982; Summerell and Leslie, 2011). Crop residue has been described as the main source of inoculum (Atanasoff, 1920; Bai and Shaner, 1994; Parry et al., 1995). All forms of fungal biomass (mycelium, chlamydospores, micro-, macroconidia, ascospores) can function as inoculum, but ascospores and macroconidia are the dominating forms (Bai and Shaner, 1994; Bushnell et al., 2003; Goswami and Kistler, 2004; Paulitz, 1996; Sutton, 1982). Warm, moist weather in spring leads to development of conidia and the production of ascospores from perithecia (Markell and Francl, 2003). Spore dispersal is dependent on wind (for ascospores) or rain splash (for conidia) (Bai and Shaner, 1994; Jenkinson and Parry, 1994b), but arthropods have also been suggested as vectors (Parry et al., 1995). Conidia and ascospores have been shown to be equally valuable as inoculum (Stack, 1989).

Fusarium species causing FHB are non-host-specific, i.e. they attack cereals, corn, and other grasses without preference for a certain host (Gale, 2003; van Eeuwijk et al., 1995). The symptoms caused by different FHB pathogens are more or less the same (Bai and Shaner, 1994). Infections on heads can occur from early flowering to soft dough stage, with a peak in susceptibility at anthesis (Andersen, 1948; Atanasoff, 1920; Pugh et al., 1933; Strange and Smith, 1971). Due to the relatively short period of high susceptibility, FHB is in general a monocyclic disease (Bai and Shaner, 1994; Trail, 2009).

Infection of a cereal head starts when ascospores or conidia land on a spikelet, germinate (usually within a few hours (Pritsch et al., 2000)), and invade extruded anthers (Bai and Shaner, 1994). Initially, the two chemical compounds choline and betaine, found in extruded anthers, were supposed to be fungal growth stimulants (Strange et al., 1974; Strange and Smith, 1971), but later it was shown that they did neither affect spore germination nor hyphal growth (Engle et al., 2004). After infection of the anthers, the pathogen colonizes the developing caryopsis, floral bracts, and rachis (Bai and Shaner, 1994). Direct penetration of the glume, lemma, palea, ovary, and rachilla is also possible (Kang and Buchenauer, 2000) (for more information on infection structures see Boenisch and Schäfer (2011), Wanjiru et al. (2002), and Brown et al. (2010)). The first symptoms on infected spikelets will appear as brown, water-soaked spots on the glumes (Bai and Shaner, 1994; Sutton, 1982). Fungal hyphae may extend internally as well as externally, leading to infections on further

spikelets (Bushnell et al., 2003). FHB pathogens use vascular bundles to spread from floret to floret within a spikelet and from spikelet to spikelet within the head (Kang and Buchenauer, 2000; Ribichich et al., 2000). From the initial point of infection, the disease may spread basally and apically (Bai and Shaner, 1996; Bushnell et al., 2003). Infected heads will show typical head blight symptoms, i.e. bleached, pale-straw color like mature heads, pinkish, orange, salmon, or red discoloration on glumes, base of spikelet or kernels, black perithecia on bleached spikes (for species with a teleomorph), and often premature wilting of upper parts of the head (Atanasoff, 1920; Bai and Shaner, 1994; Buerstmayr et al., 2012; Miedaner, 1997; Shaner, 2003). The wilting of parts above the actual infection results from a clogging of the vascular bundles by the fungus (Schroeder and Christensen (1963) in Bai and Shaner (1994)). Infected kernels are shriveled (due to water deficit and premature ripening), small, light in weight, might turn white, gray or brown, show the above mentioned discolorations, and have a floury discolored interior (Abramson et al., 1987; Buerstmayr et al., 2012; Ruckenbauer et al., 2001). Heavily infected kernels are referred to as *Fusarium* damaged kernels (FKD), visually scabby kernels (VSK) or 'tombstone' kernels (Bechtel et al., 1985; Clear and Patrick, 1990; Jones and Mirocha, 1999; Miedaner et al., 2004). If infections start at a very early stage, kernels might not develop at all (Bai and Shaner, 1994). The symptoms of FHB on triticale heads and kernels can be seen in figure 1.

**Figure 1: Symptoms of FHB on triticale. Symptoms on heads (left and middle), symptoms on triticale kernels (right, upper picture), healthy triticale kernels (right, lower picture).**

During infection, water-soluble mycotoxins might be transported within the head (Pritsch et al., 2001). There is quite some evidence that mycotoxins might play an important role in fungal spread, disease development, and aggressiveness of *Fusarium* species (as so-called virulence factors) (Desjardins and Hohn, 1997; Desjardins et al., 1996; Langevin et al., 2004; McCormick, 2003; Mesterházy, 2002) (for more information on mycotoxins, see chapter 1.4.). Different isolates of the same species were shown to differ in their toxicity (Miller et al., 1985) and virulence or aggressiveness (Miedaner et al., 1996; Wang and Miller, 1988a). It has also been reported that natural field populations from different sites vary in their aggressiveness (Miedaner and Schilling, 1996; Miedaner et al., 2001b).

The weather during flowering is the third crucial factor (besides the two factors 'amount of inoculum' and 'aggressiveness of present FHB pathogens') influencing the severity of FHB infections and mycotoxin accumulation (Atanasoff, 1920; Doohan et al., 2003; Gautam and Dill-Macky, 2008; Hart et al., 1984; Xu et al., 2008; Xu, 2003) (concerning the factor 'host resistance', see chapter 1.5.4.). Increasing temperature and wetness favor a fast disease development and, hence, mycotoxin accumulation. The optimum temperature for spike infections was found to be 25°C, while at 15°C, disease progress was very slow or did not occur at all. For a high level of disease severity and incidence, high humidity is necessary during a period of at least 24 hours (Andersen, 1948; Parry et al., 1995; Pugh et al., 1933). Under optimum conditions, the first symptoms might be visible after three to five days after infection (Miedaner, 1997; Parry et al., 1995).

## 1.4.) Production of mycotoxins

The accumulation of mycotoxins in grain infected with FHB has often been reported (Arseniuk et al., 1999; Cosic et al., 2008; Morgavi and Riley, 2007a; Snijders and Perkowski, 1990; Yoshida and Nakajima, 2010). While a single *Fusarium* species can produce various characteristic mycotoxins, a certain mycotoxin can also be produced by several *Fusarium* species (de Nijs et al., 1996; Desjardins, 2006; Desjardins et al., 1993). A description of the most important *Fusarium* species pathogenic to small grain cereals and their associated mycotoxins can be seen in table 1 (Abramson et al., 1993; Bottalico, 1998; Bottalico and Perrone, 2002; Buerstmayr et al., 2012; Glenn, 2007; Kokkonen et al., 2010).

**Table 1: Important FHB pathogens and their associated mycotoxins (Bottalico, 1998; Buerstmayr et al., 2012; Glenn, 2007).**

| Species | Associated mycotoxins |
|---|---|
| *F. acuminatum* | T2, HT2, DAS, MAS, MON, NEO |
| *F. avenaceum* | MON, BEA, ENN |
| *F. crookwellense* | NIV, FUS, ZEN, ZOH |
| *F. culmorum* | DON, ZEN, NIV, FUS, ZOH, AcDON |
| *F. equiseti* | DAS, ZEN, ZOH, NIV, DAcNIV, MAS, FUS |
| *F. graminearum* | DON, ZEN; NIV, FUS, AcDON, DAcDON, DAcNIV |
| *F. oxysporum* | MON, BEA, ENN |
| *F. poae* | DAS, MAS, NIV, FUS, T2, HT2, NEO |
| *F. sporotrichioides* | T2, HT2, NEO, MAS, DAS |
| *F. subglutinans* | BEA, MON, FUP |
| *F. tricinctum* | MON |

AcDON = mono-acetyldeoxynivalenol (3-AcDON, 15-AcDON); BEA = beauvericin; DAcDON = di-acetyldeoxynivalenol; DAcNIV = di-acetylnivalenol; DAS = diacetoxyscirpenol; DON = deoxynivalenol; ENN = enniatins; FUP = fusaproliferin; FUS = fusarenone; HT2 = HT-2 toxin; MAS = monoacetoxyscirpenol; MON = moniliformin; NEO = neosolaniol; NIV = nivalenol; T2 = T-2 toxin; ZEN = zearalenone; ZOH = zearalenols.

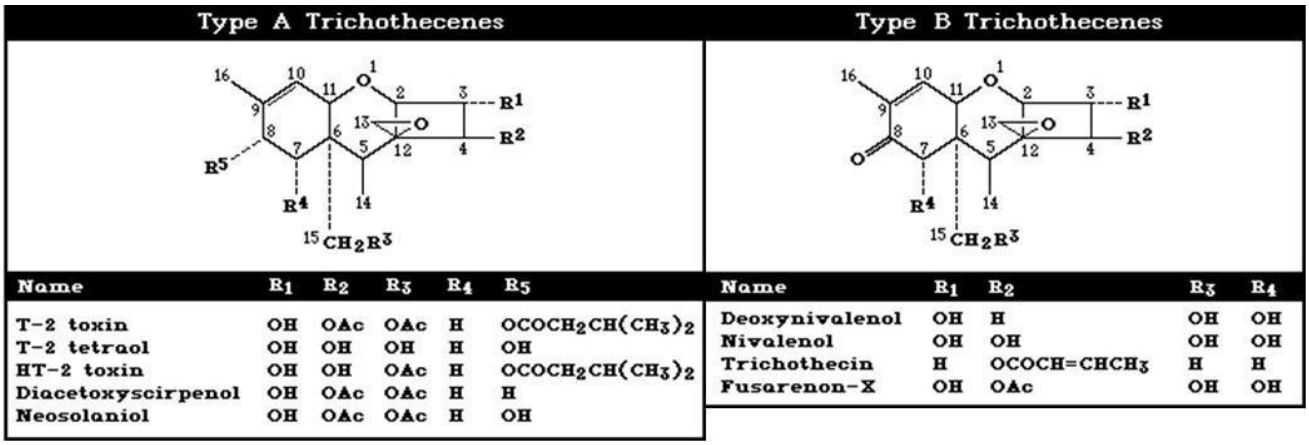
As already mentioned above, hazardous levels of mycotoxins have been found in grain, food, and feed stuff worldwide. The mycotoxins of highest concern and distribution are the two trichothecenes deoxynivalenol (DON) and nivalenol (NIV), and zearalenone (ZEN) (Binder et al., 2007; Desjardins, 2006; Placinta et al., 1999; WHO, 1990). The two most important FHB pathogens *Fusarium culmorum* and *Fusarium graminearum* are able to produce all three of these toxic metabolites (see table 1). For the analysis of mycotoxins in cereals, food, and feed, a number of methods have been established: Mycotoxin antibody-based techniques like ELISA (enzyme-linked immunosorbent assay) allow an easy and fast detection. For the distinction of different mycotoxins and parallel exact quantification, more sophisticated methods are used. These include HPLC (high performance liquid chromatography), GC-MS (gas chromatography in combination with mass spectrometry), TLC (thin layer chromatography; qualitative or semi-quantitative analysis), and LC-MS/MS (liquid chromatography with tandem mass spectrometry)

(Desjardins, 2006; Krska et al., 2001; Krska et al., 2007; Mirocha et al., 1998; Mirocha et al., 2003; Simsek et al., 2012). In addition, molecular tools like multiplex PCR (polymerase chain reaction) assays have been developed to distinguish different *Fusarium* mycotoxin chemotypes for prediction of grain contamination (Pasquali et al., 2010).

Due to their high significance and occurrence, the chemistry, toxicity, and mode of action of the two trichothecenes deoxynivalenol and nivalenol, and zearalenone will be described in more detail.

**Trichothecenes:**

Trichothecene mycotoxins are named after the fungus *Trichothecium roseum*, the producer of trichothecin, the first toxin of this class described in 1949 (Desjardins, 2006; Glenn, 2007). *Fusarium* trichothecenes (e.g. DON, NIV, T2, HT2, DAS, see table 1 and figure 2) are tricyclic sesquiterpenes with a double bond between carbons 9 and 10 and a 12,13-epoxide ring. Various patterns of oxygenation and esterification occur on carbon atoms 3, 4, 7, 8, and 15. They are characterized as simple or nonmacrocyclic trichothecenes (no linkage between carbons 4 and 15). *Fusarium* trichothecenes can be classified as Type A (no keto group at carbon 8) or Type B (keto group at carbon 8) trichothecenes (Desjardins, 2006). The chemical structure of Type A and B trichothecenes can be seen in figure 2.




| Name | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| T-2 toxin | OH | OAc | OAc | H | $OCOCH_2CH(CH_3)_2$ |
| T-2 tetraol | OH | OH | OH | H | OH |
| HT-2 toxin | OH | OH | OAc | H | $OCOCH_2CH(CH_3)_2$ |
| Diacetoxyscirpenol | OH | OAc | OAc | H | H |
| Neosolaniol | OH | OAc | OAc | H | OH |

| Name | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| Deoxynivalenol | OH | H | OH | OH |
| Nivalenol | OH | OH | OH | OH |
| Trichothecin | H | $OCOCH=CHCH_3$ | H | H |
| Fusarenon-X | OH | OAc | OH | OH |

**Figure 2: Chemical structure of Type A and B trichothecenes (WHO, 1990).**

Trichothecenes are potent inhibitors of eukaryotic protein synthesis (inhibition of peptidyltransferase) (Desjardins, 2006; WHO, 1990). They are phytotoxic to a number of plants (Cossette and Miller, 1995; Glenn, 2007) and have been described as virulence factors for several pathosystems, as already mentioned earlier. In

addition, they have been associated with toxicoses in humans and animals (Goswami and Kistler, 2004; Placinta et al., 1999; WHO, 1990). Outbreaks of alimentary toxic aleukia and a similar disease called akakabi-byo have been associated with food contaminated with trichothecenes (Desjardins and Hohn, 1997; Desjardins et al., 1993). Trichothecene-contaminated feed stuff poses a high risk on animal health (Hoerr et al., 1982; House, 2007; Morgavi and Riley, 2007b; Pestka, 2007). All animal species investigated were found to be susceptible to deoxynivalenol, the trichothecene most often found in contaminated feed stuff. Pigs and other monogastric animals like cats or dogs were shown to be most sensitive, followed by poultry and ruminants. Feeding with deoxynivalenol (also called 'vomitoxin') leads to feed refusal, vomiting, decreased weight gain, anorexia, anemia, hemorrhage, decreased nutritional efficiency and altered immune function (Desjardins, 2006; Desjardins et al., 1993; Pestka, 2007). Nivalenol is found less frequently than deoxynivalenol (often in co-occurrence), but on the other hand, regarded as being even more toxic to animals (Bottalico and Perrone, 2002; Yoshida and Nakajima, 2010). In general, deoxynivalenol is a very stable compound. The content in contaminated grain might be reduced during milling processes (as DON is mostly found in the outer layers of the kernel). Baking and heating can also lead to a reduction or degradation of deoxynivalenol. The compound is soluble in water and lower contents can be achieved by cooking in larger amounts of water (Kushiro, 2008).

**Zearalenone:**

Zearalenone (ZEN, sometimes abbreviated as ZON or ZEA) is a structurally and functionally unique mycotoxin (Glenn, 2007). The chemical structure of this β-resorcyclic acid lactone can be seen in figure 3.

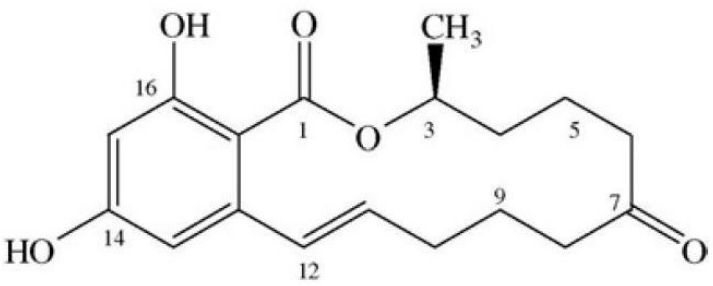



**Figure 3: Chemical structure of zearalenone (Krska et al., 2007).**

Zearalenone is not acutely toxic and has not been associated with fatal mycotoxicoses in humans or animals (Desjardins, 2006). Instead, the toxicity of zearalenone is based on its estrogenic property (Bottalico, 1998). The mycotoxin activates estrogen receptors, leading to functional and morphological changes in reproductive organs (Fink-Gremmels and Malekinejad, 2007). Pigs are again the most sensitive animal species (Bottalico, 1998; Fink-Gremmels and Malekinejad, 2007; Morgavi and Riley, 2007b). Among the problems caused are vulvovaginitus, tenesmus, vaginal and rectal prolapses, reduced libido, plasma testosterone, anestrus, reduced litter size, fetal resorption, and implantation failure (as reviewed in Morgavi and Riley (2007b)). Ruminants seem to be more resistant, but infertility, reduced milk production, and hyperestrogenism have been reported for cows (Placinta et al., 1999). Poultry are considered to be resistant (Morgavi and Riley, 2007b).

In general, the most effective strategy against mycotoxin accumulation is the preventive control of FHB infections (Desjardins et al., 1993; Jouany, 2007) (see also chapter 1.5.). Maximum levels for mycotoxins in grain and maize used for food production have been implemented in the European Union (Anonymous, 2007). For products intended for animal feeding, the European Commission recommended guidance values for certain mycotoxins (Anonymous, 2006). The methods for decontamination of heavily infected grain are limited (Desjardins et al., 1993; Jouany, 2007). Such samples can be used for nonfood/nonfeed purposes such as bioethanol production (Desjardins et al., 1993; McMullen et al., 1997), but the mycotoxins will be concentrated up to three times of their initial concentration in the ethanol production byproducts used for feeding (Wu and Munkvold, 2008). Alternatively, as concerns feeding, the contaminated grain might be diluted with sound grain, or the kernels might be detoxified chemically or biologically (House, 2007).

## 1.5.) Control of FHB

Control of FHB of small grain cereals is a very complex problem and methods are limited. Despite the decades of research for controlling FHB and mycotoxin accumulation, severe epidemics do still occur sporadically (McMullen et al., 2012; McMullen et al., 1997; Miedaner, 2007; Parry et al., 1995; Shaner, 2003). Weather during anthesis plays an important role in disease development, as mentioned earlier. Unfortunately, weather cannot be controlled. Therefore, especially under

unfavorable weather conditions or in regions with higher frequency of FHB epidemics, an integrated strategy has to be adopted to reduce the risk of severe FHB infections (Blandino et al., 2012; Martin and Johnston, 1982; McMullen et al., 2008). Such an integrated strategy should include optimum cultural practices, the use of fungicides and biocontrol agents (if available), and the cultivation of resistant varieties (Blandino et al., 2012; McMullen et al., 2012; Pirgozliev et al., 2003).

### 1.5.1.) Cultural practices

The adoption of reduced tillage or no-tillage systems as means of soil conservation, prevention of excessive erosion, and cost reduction has often been associated with higher incidence and severity of FHB infections. With these management techniques, crop residues are left at the soil surface, thereby increasing the inoculum load for subsequent crops. Hence, a change in tillage pattern might lead to a reduction of FHB infections in small grain cereals (Bai and Shaner, 1994; Champeil et al., 2004; Dill-Macky, 2008; Dill-Macky and Jones, 2000; McMullen et al., 2012; McMullen et al., 1997; Teich and Hamilton, 1985).

Another important agronomy-based method to reduce the risk of FHB occurrence is the choice of crop to plant. Dense rotations of natural FHB hosts (e.g. corn, wheat, barley, and other small grain cereals) were shown to be one of the main causes for heavy head blight infections. The integration of noncereal species into the rotation system are therefore highly recommended (Bai and Shaner, 1994; Dill-Macky and Jones, 2000; Koehler et al., 1924; McMullen et al., 2012; McMullen et al., 2008; McMullen et al., 1997; Parry et al., 1995; Sutton, 1982; Teich and Hamilton, 1985).

Controversial reports have been published about the effect of nitrogen application on FHB development. While Martin et al. (1991) described a higher incidence of FHB infections caused by supplementary nitrogen, no such correlation was found by Fauzi and Paulitz (1994), Pageau et al. (2007), and Yoshida et al. (2008). The factors soil phosphorus, potassium, and pH did not correlate significantly with levels of FHB or mycotoxins (Teich and Hamilton, 1985).

Lodging was reported to increase FHB infections and mycotoxin accumulation. The choice of cultivar and fertilization have to be adapted accordingly (Nakajima et al., 2008b). Plant growth regulators do also play an important role in prevention of lodging, especially in crops like rye or triticale (Rodemann and Mielke, 2007;

Schildbach, 2013). On the other hand, they could promote FHB infections as they lead to decreased plant height (Fauzi and Paulitz, 1994; Martin et al., 1991) (for more information on the parameter plant height, see chapter 1.5.4.).

The occurrence of foliar diseases (e.g. rusts, powdery mildew) usually leads to an increased susceptibility of small grain cereals to FHB (Mesterházy (1977) and Mesterházy and Rowaished (1977), both cited in Dill-Macky (2003); Mesterházy (2003a)). Therefore, appropriate control strategies have to be applied.

### 1.5.2.) Chemical control with fungicides

During the last decade, a number of new fungicides have been tested and released for the control of FHB. Results have been quite variable. The highest level of reduction in FHB severity and/or mycotoxin content have been achieved using either triazole or strobilurin fungicides (Bagga, 2008; Balaž et al., 2008; Butkutė et al., 2008; Champeil et al., 2004; Dubournet et al., 2008; Haeuser-Hahn et al., 2008; Lechoczki-Krsjak et al., 2008; Mankevičienė et al., 2008; McMullen et al., 2012; Mesterházy, 2003b; Mesterházy et al., 2011; Pirgozliev et al., 2003). Nevertheless, a reduction in disease incidence and severity does not necessarily lead to a reduction in mycotoxin content (Martin and Johnston, 1982).

The biggest challenges concerning the use of fungicides are still the timing of application and the application technology (McMullen et al., 2012; Nakajima et al., 2008a; Pirgozliev et al., 2008). Anthesis has been described as the growth stage best suited for an effective application, with a decline in effectivity afterwards (Reis et al. (1996) in Mesterházy (2003b), Pirgozliev et al. (2008)). Unfortunately, severity of FHB infections and mycotoxin accumulation will be highest under wet conditions during anthesis, as described above. The theoretically optimum time for an application might therefore collide with weather conditions making an application impossible in reality (McMullen et al., 2012). Fungicide applications at a late stage of cereal development might also result in fungicide residues in the harvested kernels (Bai et al., 2003; da Luz et al., 2003).

The standard fungicide application technology generally used to combat foliar diseases can often not be used for fungicide applications against FHB. Advanced application technologies have been introduced lately, but there are still a number of problems concerning equal distribution of the fungicide on cereal heads (McMullen et

al., 2012; Mesterházy et al., 2011). A disease forecasting system to support farmers in their decision whether to use fungicides or not has been established in the USA (McMullen et al., 2012). To our knowledge, no such system does yet exist in Europe.

### 1.5.3.) Biological control

Biological control (or biocontrol, i.e. the use of living organisms including viruses or their natural products to control the pathogens) might be another approach to diminish negative effects of FHB. This strategy could be of interest especially for organic farming systems where the application of chemical fungicides is not allowed (da Luz et al., 2003; McMullen et al., 2012). A number of bacterial and fungal organisms, and bioactive compounds have been investigated for their potential to control FHB pathogens (Gromadzka et al., 2008; Hu et al., 2014; Khan et al., 2001; Lemmens et al., 2008; Petti et al., 2008; Soleimani et al., 2007; Xue et al., 2008), but so far, no biocontrol agent is in practical use against FHB (McMullen et al., 2012).

### 1.5.4.) Breeding for resistance against FHB

It has been early recognized in FHB research that genotypes differ in their susceptibility (Pugh et al., 1933). The cultivation of resistant varieties is seen as the best option to control FHB and/or reduce its negative impact. Therefore, breeding for resistance against FHB is a very important aspect in controlling the disease (Bai et al., 2003; Buerstmayr et al., 2012; Miedaner, 1997; Ruckenbauer et al., 2001).

Resistance to FHB is of quantitative nature with oligogenic to polygenic inheritance (Buerstmayr et al., 2012; Mesterházy, 2003a; Ruckenbauer et al., 2001; Snijders, 1990b). So far, no specific resistances have been found against distinct *Fusarium* species, therefore, the resistance can be described as horizontal type across probably all FHB pathogens (Mesterházy, 2003a; Mesterházy et al., 2005; Miedaner, 1997; Miedaner and Schilling, 1996; Snijders, 1990a; Stack and McMullen, 1985; Tóth et al., 2008; van Eeuwijk et al., 1995). Genomic regions involved in resistance against FHB (so-called quantitative trait loci, QTL) have been identified on several chromosomes, at least in wheat (Anderson et al., 2007; Buerstmayr et al., 2012; Buerstmayr et al., 2009; Buerstmayr et al., 1999; Grausgruber et al., 1999; Liu et al., 2009; Löffler et al., 2009). A genome-wide distribution of resistance QTL might also be possible in other cereal species. Among the most important resistance QTL detected so far are the two QTL *Qfhs.ndsu-3BS* (syn. *Fhb1*) at the short arm of

chromosome 3B and *Qfhs.ifa-5A* at the short arm of chromosome 5A, which are both used for the study at hand (see also chapter 2.1.). *Fhb1* is primarily associated with type II resistance, while *Qfhs.ifa-5A* rather seems to influence type I resistance (Buerstmayr et al., 2009) (for resistance types see below).

Quite a number of individual active and passive resistance mechanisms have been described. The following types of active resistance have been observed:

I. Resistance against initial infection (Schroeder and Christensen (1963) in Buerstmayr et al. (2012), Mesterházy (2003a), Bai and Shaner (1994), Mesterházy et al. (1999), and Stack (2003))
II. Resistance against spread of the pathogen within the head (Schroeder and Christensen (1963) in Buerstmayr et al. (2012), Mesterházy (2003a), Bai and Shaner (1994), Mesterházy et al. (1999), and Stack (2003))
III. Resistance to kernel infections (Mesterházy, 1995)
IV. Ability to degrade or detoxify deoxynivalenol (Boutigny et al., 2008; Lemmens et al., 2005; Miller and Arnison, 1986)
V. Limited accumulation of trichothecenes in infected kernels (Boutigny et al., 2008; Miller et al., 1985; Snijders and Krechting, 1992)
VI. Tolerance of high trichothecene levels in kernels (Wang and Miller, 1988a)

Tolerance in its classical sense (i.e. resistance to yield losses despite high levels of severity of the disease) has also been reported for FHB (Mesterházy, 1995; Mesterházy et al., 1999).

Several morphological traits might act as passive resistance mechanisms. One such trait is plant height. Under natural conditions, higher plants usually are less susceptible to FHB infections (while artificially spray-inoculated plants often do not differ in their susceptibility according to plant height). This passive resistance mechanism can easily be explained, as for the inoculum resulting from crop debris there is a much larger distance to the heads in higher plants than in lower ones (Buerstmayr et al., 2009; Couture, 1982; Hilton et al., 1999; Ransom, 2008). In addition, plant height has been associated with type I resistance (resistance against initial infection) (Lu et al., 2013).

The degree of anther extrusion and the width of flower opening have also been described as passive resistance mechanisms. The extent of anther extrusion seems

to be negatively correlated with the incidence and severity of FHB (Kubo et al., 2013; Skinnes et al., 2010; Skinnes et al., 2008). Anther extrusion was also found to be associated with type I resistance (Lu et al., 2013). Besides, narrower flower openings were shown to be responsible for lower levels of FHB (Gilsinger et al., 2005; Kubo et al., 2010; Schuster and Ellner, 2008).

Numerous reports have been published about breeding strategies for FHB resistance in small grain cereals (Agostinelli et al., 2012; Anderson et al., 2007; Bai and Shaner, 1994; Buerstmayr et al., 2012; Buerstmayr et al., 2009; Buerstmayr et al., 2002; Buerstmayr et al., 2003; Liu et al., 2009; Löffler et al., 2009; McMullen et al., 2012; Mesterházy, 1997, 2003a; Mesterházy et al., 2007; Miedaner, 1997; Miedaner et al., 2008; Ruckenbauer et al., 2001; van Sanford et al., 2001; Wilde et al., 2007). Modern FHB resistance breeding programs do either rely on phenotypic or genotypic screening and selection or on a combination of the two principles. One of the biggest challenges remaining is the compatibility and combination of resistance QTL with genes for good agronomic performance (von der Ohe et al., 2010).

For phenotypic screenings in the laboratory, glasshouse or in the field, controlled conditions are necessary that mimic natural infections as good as possible (Hart et al., 1984; Snijders, 1990a; Snijders and Perkowski, 1990). Therefore, field screenings for FHB resistance are usually based on artificial inoculation (spray inoculation or spreading of infected grain) and mist irrigation for humidity control (Buerstmayr et al., 2012; Dill-Macky, 2003; McMullen et al., 2012; Wang and Miller, 1988b). Ascospores and conidia are most often used as inoculum and they are both equally valuable (Stack, 1989; Wang and Miller, 1988b). The preference of either one of the two spore forms (if the species of interest has a teleomorph) is mostly depending on equipment, cultivation, and preparation methods (Dill-Macky, 2003). The list of traits that can be assessed in phenotypic (field) screenings is large. Among the traits most often investigated are FHB incidence (percentage of infected heads per plot), FHB severity (percentage of infected spikelets per head), FHB index (incidence × severity), percentage of *Fusarium* damaged kernels (FDK), mycotoxin content, and various morphological or physiological plant traits (e.g. plant height, flowering date, etc.) (Buerstmayr et al., 2012; Dill-Macky, 2003; Groth et al., 1999; McMullen et al., 2012; Wilcoxson et al., 1992). For optimum genotypic differentiation, it has been recommended to perform several disease ratings during FHB development

(Miedaner, 1997; Parry et al., 1995). Multiple disease ratings can then be combined to calculate the area under the disease progress curve (AUDPC), representing disease progress over time (Shaner and Finney, 1977). Due to a high level of genotype × environment interactions for FHB (as usual for quantitative traits), genotypes have to be tested in several environments (i.e. years, locations) to obtain reliable data about genotypic differentiation (Buerstmayr et al., 2008; Miedaner, 1997).

As a first step of any genotypic selection for a quantitative trait like FHB resistance, the genomic regions governing resistance (i.e. resistance QTL) have to be identified via so-called QTL mapping that will also include phenotypic screenings. Genetic markers that are linked to these QTL can then be used to select for specific resistance QTL (marker assisted selection, MAS) (Anderson et al., 2007; Anderson et al., 2001; Buerstmayr et al., 2009; Buerstmayr et al., 2002; Buerstmayr et al., 2003; Liu et al., 2009; Löffler et al., 2009). In general, there are two types of QTL used in breeding programs – QTL from exotic resistance sources or QTL from locally adapted sources (Buerstmayr et al., 2012; Holzapfel et al., 2008; McMullen et al., 2012). The stacking or pyramiding of resistance QTL is highly recommended in terms of resistance stability (Miedaner et al., 2001a; Miedaner et al., 2006b).

Some research groups are working on transgenic lines of FHB resistant wheat and barley (Buerstmayr et al., 2012; Dahleen et al., 2001; Muehlbauer and Bushnell, 2003), but so far, no such line has been registered to our knowledge. Rye, triticale, and oats are not (yet) subject to transgenic approaches.

## 1.6.) Triticale – its origin, production, and use

Triticale (x *Triticosecale* Wittmack) is a man-made amphiploid cereal species derived from intergeneric hybridization with following chromosome doubling from a seed parent of the genus *Triticum* and a pollen parent of the genus *Secale*. Octoploid triticales (2n = 56 = AABBDDRR) originate from a hybridization of hexaploid wheat (*Triticum aestivum* L., 2n = 42 = AABBDD) and diploid rye (*Secale cereale* L., 2n = 14 = RR), hexaploid triticales (2n = 42 = AABBRR) are derived from tetraploid wheat (*Triticum durum* Desf., 2n = 28 = AABB) and rye. Triticales arisen from these original crosses are called ‘primary’ triticales, while triticales resulting from crosses of primary

triticales with each other or with other species (e.g. their parental species) are called ‘secondary’ triticales (Ammar et al., 2004).

The intention behind the creation of triticale was to combine the high quality of wheat with the high degree of resistance and adaptability found in rye (Rodemann and Mielke, 2007).

The first stable triticale line was bred by the German plant breeder Rimpau in 1888. Since then, a lot of effort has been put into improving stability as well as yield and quality traits of triticale. Modern triticale cultivars are usually secondary hexaploid triticales. In the last decades, the area planted to triticale has increased worldwide (Ammar et al., 2004; Mergoum et al., 2004). In 2013, triticale was grown on 44.996 hectares in Austria (i.e. on approximately 3,3 percent of the total acreage) (AGES, 2014). On a worldwide basis, circa 14 million tons of triticale are produced annually with a main focus on Europe (11 million tons) (data for 2007-2009) (Schildbach, 2013).

In high-yielding environments, triticale was shown to achieve little less or similar yields as wheat. In stressed environments on the other hand, triticale could even surpass wheat in terms of yield (Ammar et al., 2004; Mergoum et al., 2004; Rodemann and Mielke, 2007).

Triticale is not used as a food cereal (mainly because of the lack of the wheat D-genome), although it could be used for mixed breads same as rye, for flat breads or for oriental noodles (Peña, 2004). Instead, triticale is widely utilized for feeding due to its high protein and lysine content (Myer and Lozano del Rio, 2004; Rodemann and Mielke, 2007; Schildbach, 2013). Other options for triticale include the production of ethanol or energy (Rodemann and Mielke, 2007; Schildbach, 2013).

Although triticale is in general more resistant to adverse environmental conditions and biotic stressors than wheat, it is becoming more and more susceptible to diverse cereal pathogens and pests (as a result of the expanding acreage and/or adaptation of the aggressors) (Mergoum et al., 2004; Rodemann and Mielke, 2007). There is quite some evidence that triticale’s level of resistance to FHB and mycotoxin accumulation is similar to that of wheat and other small grain cereals (Arseniuk et al., 1999; Góral et al., 2013; Martin et al., 1991; Miedaner et al., 2001a; Miedaner et al., 2006a; Perkowski and Kaczmarek, 2002; Veitch et al., 2008). A number of authors

showed that cultivars and breeding lines of triticale were on average more resistant to FHB and mycotoxin contamination than those of wheat (Arseniuk et al., 1999; Góral and Ochodzki, 2006; Góral et al., 2013; Miedaner et al., 2001a; Miedaner et al., 2006a). On the other hand, triticale was also found to be more susceptible than wheat (Martin et al., 1991; Veitch et al., 2008). Triticale and rye often reacted pretty similar in terms of FHB and mycotoxin accumulation (Arseniuk et al., 1999; Miedaner et al., 2001a). So far, no completely resistant triticale line has been found. In general, a wide distribution of FHB reactions occurred also in triticale, indicating a large genetic variation for FHB resistance similar to that of other small grain cereals (Góral and Ochodzki, 2006; Góral et al., 2013; Miedaner et al., 2004; Miedaner et al., 2001a; Oettler et al., 2004; Oettler and Wahle, 2001). Hence, the resistance to FHB rather seems to be dependent on the genotype or line and not on the cereal species. Same as in wheat and other cereals, resistance to FHB in triticale seems to be quantitatively inherited with predominating additive effects (Miedaner et al., 2006a; Oettler et al., 2004; Oettler and Wahle, 2001). To minimize the risks FHB poses on triticale production and use (especially concerning mycotoxins in feed stuff), all possible control measurements should be taken into account. Due to the wide genetic variation for FHB resistance, breeding of resistant lines seems to be feasible.

## 1.7.) Aims and research questions

The work presented in the master thesis at hand is part of a two-year project about FHB resistance in triticale carried out at the Institute of Biotechnology in Plant Production of the University of Natural Resources and Life Sciences, in Tulln.

With a combination of phenotypic and genotypic approaches, the project aims at improving FHB resistance and limiting mycotoxin accumulation in triticale. For this, three recombinant inbred line (RIL) populations have been created carrying resistance QTL introgressed from wheat. Three distinct goals shall be achieved within the project:

1.) Basic information on the effectiveness of FHB resistance QTL in triticale in comparison to wheat and other cereals shall be gained. The study aims to be the first report about location and effect of FHB resistance genes in the genetic background of triticale.

2.) Molecular markers linked to effective FHB resistance QTL in triticale shall be discovered. These markers could then be used for the selection of improved FHB resistance in following generations and projects.
3.) Triticale lines that combine good agronomic performance with improved FHB resistance shall be selected for variety registration or as crossing partners for further breeding cycles.

The results of the first year's phenotypic screening will be presented in the master thesis at hand. The following research questions should be answered in the course of this master thesis:

1.) Is there variation for FHB resistance in the three RIL populations and the collection of cultivars and breeding lines after artificial inoculation with *Fusarium culmorum*?
2.) How do parameters related to FHB resistance (percentage of infected spikelets in the field, percentage of *Fusarium* damaged kernels) correlate with each other and with other developmental and morphological plant traits (e.g. plant height, flowering date) in triticale?
3.) Do other plant diseases such as stripe rust and powdery mildew interfere with FHB resistance?
4.) Can transgressive segregants with higher FHB resistance than the resistant parental line *G8.06* be detected?

# 2.) Materials and Methods

In the following section, all the material and methods used for the practical part of the thesis at hand will be explained in detail.

## 2.1.) Plant material

Three RIL populations derived from crosses of a highly resistant triticale line (*G8.06*) with three different triticale genotypes (cultivars *Tulus*, and *Elpaso* as well as the $F_1$ generation of the cross *Agostino* × *Grenado*, respectively) were tested in a field experiment for FHB resistance (see table 2). A fourth population consisting of both triticale genotypes currently registered as varieties in Austria and triticale lines from former FHB resistance breeding programs at IFA-Tulln was included in the experiments for comparison. For a complete list of all genotypes and their origin, see the appendix. In total, 441 genotypes were tested in the field experiment described in the thesis at hand. The distribution of these 441 genotypes within the four populations can be seen in table 3.

**Table 2: Parents of the three RIL populations used for FHB resistance testing.**

| Population | Crossing parents |
|---|---|
| Population 1 | *G8.06* × *Tulus* |
| Population 2 | *G8.06* × ($F_1$ of *Agostino* x *Grenado*) |
| Population 3 | *G8.06* × *Elpaso* |

**Table 3: Number of genotypes tested for FHB resistance within each of the four populations.**

| Population | Number of genotypes |
|---|---|
| Population 1 | 142 $F_{4:5}$ lines + parents *G8.06* and *Tulus* |
| Population 2 | 135 $F_{4:5}$ lines + (grand)parents *G8.06*, *Agostino*, and *Grenado* |
| Population 3 | 109 $F_{4:5}$ lines + parents *G8.06* and *Elpaso* |
| Population 4 | 35 varieties currently registered in Austria + 19 advanced breeding lines from IFA-Tulln (see appendix for complete list and information on breeding institution) |

The resistant parental triticale line *G8.06*, carrying the two resistance QTL *Qfhs.ndsu-3BS* (syn. *Fhb1*) at the short arm of chromosome 3B and *Qfhs.ifa-5A* at the short arm of chromosome 5A, was selected from a back-cross population (2nd back-cross generation, $BC_2$) of the highly resistant wheat line *CM-82036* (CIMMYT, Mexico) with the German triticale cultivar *Santop* (*CM-82036*/3 × *Santop*). Both resistance QTL mentioned above have a major effect on FHB resistance and both originated from the resistant wheat parent *CM-82036* (Buerstmayr et al., 2002; Buerstmayr et al., 2003). Crosses and selection for line *G8.06* were carried out at IFA-Tulln under supervision of Hermann Bürstmayr.

The crosses for the three RIL populations (table 2) were carried out by Herbert Bistrich at the plant breeding station of Saatzucht-Donau in Reichersberg, Austria. The $F_2$ generations of these populations were then returned back to IFA-Tulln. At least 120 $F_2$ plants of each population were advanced to the $F_4$ generation by single seed descent at IFA-Tulln. Following that, single heads from $F_4$-head-rows were planted in the field in $F_5$-head-rows to gain enough seed for testing of $F_{4:5}$-lines. These $F_{4:5}$-lines were used for the experiments described within the thesis at hand.

The 19 advanced breeding lines in population 4 are all sister lines of *G8.06* with the wheat line *CM-82036* as resistance donor and *Santop* as recurrent parental line ($BC_2$; *CM-82036*/3 × *Santop*). They all possess the two resistance QTL *Fhb1* and *Qfhs.ifa-5A.*

## 2.2.) Field test site

All experiments described in this master thesis were conducted at the experimental station and fields of the Institute of Biotechnology in Plant Production, Department of Agrobiotechnology (IFA), University of Natural Resources and Life Sciences Vienna (BOKU). The field test site was located in Tulln, a city at the Danube river 30 km west of Vienna (48° 20′ N, 16° 3′ E). The experimental station of the IFA-Tulln lies 180 m above sea level within the Pannonian climate region. The long-term average temperature for Tulln is approximately 9,5°C, the annual precipitation amounts to circa 600 mm (data from the closest weather station in Langenlebarn, 48° 19’ N, 16° 7’ E; ZAMG (2000)). The mean monthly temperature and precipitation for the growth period 2013-2014 can be seen in figure 4 (data from BOKU Department of Crop Sciences (2014)). The weather data were evaluated from October 1st, 2013,

until harvesting at July 16th, 2014. The growth period 2013-2014 can be characterized as being rather dry with a total amount of precipitation of only 311,4 mm.

The soil-type of the field test site is a meadow-czernosem. The preceding crop was soybean (*Glycine max* (L.) Merr.).

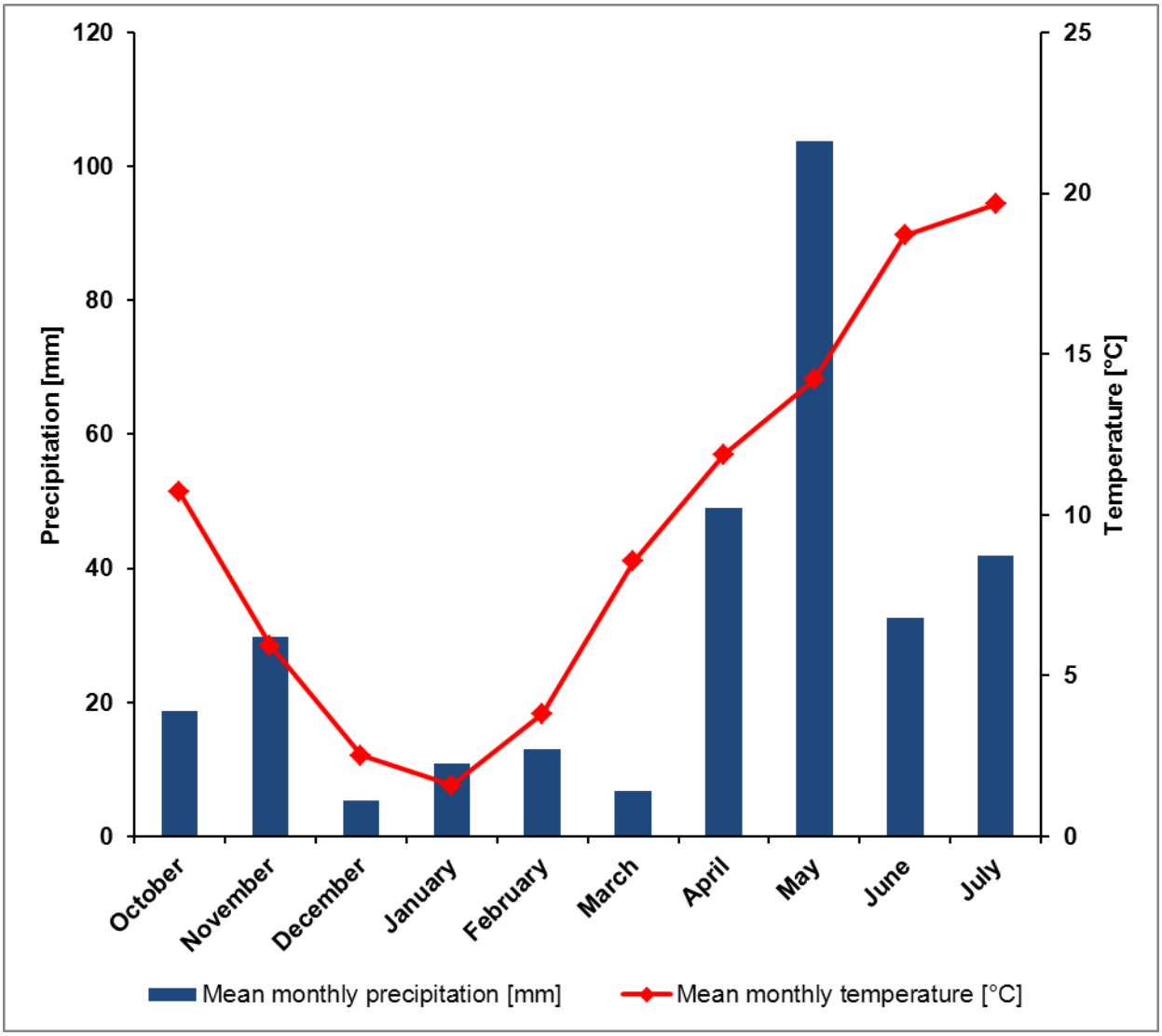



**Figure 4: Mean monthly precipitation [mm] and mean monthly temperature [°C] for Tulln from October 1st, 2013, until July 16th, 2014.**

## 2.3.) Experimental design and agronomic procedures

All four populations were planted separately as randomized complete blocks with two replications (2 times 450 plots, as several genotypes were planted more than once per replication). Each plot consisted of two rows of one genotype. The rows had a

length of 65 cm and were sown at a distance of 17 cm. In between plots, the row spacing was 33 cm. Hence, the plot size for each genotype was 0,325 $m^2$. Approximately 4,5 g of triticale seed were sown per double-row (corresponding to circa 140 kg per ha). Seeds were treated with Celeste Extra FS50 (at a rate of 200 ml/100 kg) before sowing. The sowing time for the first replication was October 29$^{th}$, 2013, for the second replication, it was November 18$^{th}$, 2013. The two replications were sown several weeks apart on purpose, so as to extend the period of anthesis, thereby minimizing negative effects of adverse environmental conditions during flowering.

Fertilizer was applied on April 3$^{rd}$, 2014 (300 kg/ha of a mixture of nitrogen, phosphorus, potassium, and sulfur at a rate of NPK 16:6:18 + 5S), and on May 9$^{th}$, 2014 (220 kg/ha of calcium ammonium nitrate (CAN) with 27% of nitrogen).

On April 8$^{th}$, 2014, the herbicide Andiamo Maxx (120 g/L Ioxynil, 120 g/L Bromoxynil, and 360 g/L Mecoprop-P) was applied at a rate of 1,5 L/ha. No other plant protection measures were carried out.

From the first day of inoculation on May 20$^{th}$, 2014, until June 5$^{th}$, 2014, two days after the last day of inoculation, a mist irrigation system was used over all plots to increase air humidity and ensure optimum conditions for FHB infection. The irrigation system was running in accordance with inoculation dates (for more information on inoculation, see chapter 2.5.). Hence, it started every second day at 3 pm and stopped at 12 pm on the following day. A single mist irrigation treatment lasted for 10 seconds. The interval of irrigation cycles was automatically regulated by a sensor for leaf-wetness. The minimum time period in between irrigation treatments was 20 minutes. On dry, hot days, mist irrigation treatments started every 20 minutes during the day and approximately once per hour during the night.

## 2.4.) Production of inoculum

For all experiments described in the thesis at hand, the *Fusarium culmorum* isolate IFA 104 was used. The inoculum utilized for artificial inoculation of the field plots was prepared according to the standard operating procedure SOP 3-04 of the Institute of Biotechnology in Plant Production, Department IFA-Tulln (see the appendix). Briefly, a piece of agar medium containing freshly grown cultures of *Fusarium culmorum* IFA 104 was added to a mix of water-swollen wheat and oat kernels in a baby food jar,

allowed to grow for circa 2 weeks in diffuse daylight at room temperature, then put in a refrigerator at 4-8°C, and finally, spore suspensions were created from these jars by washing the kernels and diluting the washing suspension in osmose water (see also figure 5). The macroconidia of *Fusarium culmorum* IFA 104 in the washing suspension were counted with the help of a Bürker-Türk-chamber under a standard laboratory microscope according to the standard operating procedure SOP 3-06 of the Institute of Biotechnology in Plant Production, Department IFA-Tulln (see the appendix). The spore suspensions were set to a concentration so that a single tube containing a small amount of suspension (less than 10 mL) could be diluted in 10 L of tap water right before inoculation to give a final concentration of 50.000 conidia/mL. The spore suspensions were then kept at -80°C until inoculation. The spore viability was checked several times during the onset of this master thesis with spore germination tests on nutrient agar medium.


**Figure 5: Preparation of inoculum of *Fusarium culmorum* isolate IFA 104. Inoculation of swollen wheat and oat kernels with a fresh culture of the isolate (left); spore suspension obtained by washing infected kernels with osmose water (right).**

## 2.5.) Inoculation

All plots were artificially inoculated with the above described inoculum of *Fusarium culmorum* isolate IFA 104 at a rate of 50.000 conidia/mL (spore suspension diluted in 10 L of tap water). For a single inoculation treatment, 50 mL of inoculum were applied per plot with a motorized backpack sprayer with three nozzles for parallel inoculation of three plots (see figure 6). Inoculation was started as soon as the first plots of a replication reached the stage of flowering. Inoculation treatments were then carried out on a two-day interval. Usually, the last inoculation was performed two days after the last plots of a replication were at the stage of full anthesis (50% flowering), so that each plot was inoculated at least twice. For all plots, the date of full anthesis was evaluated. Always, the whole replication was inoculated (and not single plots). For replication 1, six inoculation treatments were applied from May 20$^{th}$, 2014, until May 30$^{th}$, 2014 (here, the last inoculation was performed four days after the last plots were flowering to guarantee a rather equal number of treatments for both replications). For replication 2, showing a wider range of flowering dates and starting two days later, seven inoculation treatments were applied from May 22$^{nd}$, 2014, until June 3$^{rd}$, 2014. Inoculations were always carried out in the evenings. The interval for the mist irrigation system was corresponding to the interval of inoculation, as mentioned above.


**Figure 6: Artificial spray inoculation of triticale heads with *Fusarium culmorum* isolate IFA 104 at anthesis.**

## 2.6.) Disease assessment

The severity of FHB infection was estimated by visual scorings. For this, the percentage of infected spikelets was evaluated on a whole plot basis, as can be seen in table 4. This pattern of severity evaluation allowed to estimate the combined effect of resistance types I and II, but not the effect of those resistances individually. Scorings were carried out (for each plot individually) 10, 14, 18, 22, and 26 days after full anthesis before ripening made further estimates impossible due to natural yellowing or bleaching of the heads.

**Table 4: Scoring guide for FHB severity on a whole plot basis used at IFA-Tulln.**

| Visually estimated average per plot | % diseased spikelets per plot (disease severity) |
|---|---|
| No symptoms visible | 0 |
| Traces of FHB visible | 0,1 |
| 0,1 spikelets per head infected | 0,5 |
| 0,2 spikelets per head infected | 1 |
| 0,4 spikelets per head infected | 2 |
| 0,6 spikelets per head infected | 3 |
| 1 spikelet per head infected | 5 |
| 2 spikelets per head infected | 10 |
| 3 spikelets per head infected | 15 |
| 4 spikelets per head infected | 20 |
| 5 spikelets per head infected | 25 |
| 6 spikelets per head infected | 30 |
| 8 spikelets per head infected | 40 |
| 10 spikelets per head infected | 50 |
| 12 spikelets per head infected | 60 |
| 14 spikelets per head infected | 70 |
| 16 spikelets per head infected | 80 |
| 18 spikelets per head infected | 90 |
| All spikelets per head infected | 100 |

All plots were harvested at ripening with a plot combine harvester (Nursery Master Elite, Wintersteiger, Ried im Innkreis, Austria) set to low wind speed so as not to lose small, light, and shriveled kernels. Harvesting was done on July 15$^{th}$ and 16$^{th}$, 2014. Afterwards, all harvested samples were cleaned with the help of a laboratory thresher (LD 350, Wintersteiger, Ried im Innkreis, Austria) to get rid of excess spikelets, hulls, and other impurities. The cleaned samples were then evaluated for the percentage of *Fusarium* damaged kernels (%FDK) by visual estimation. For this, reference samples were created (using a known number of healthy and diseased kernels) to facilitate visual scorings (see also figure 7). Smaller, shriveled, bleached, and/or orange, pinkish, and reddish kernels were characterized as being *Fusarium* damaged kernels.


**Figure 7: Examples for reference samples for visual scoring of the percentage of Fusarium damaged kernels.**

## 2.7.) Ratings for relevant traits other than FHB

Severity of powdery mildew infections:

Powdery mildew on cereals is caused by the pathogen *Blumeria graminis*, an obligate biotrophic fungus of the phylum Ascomycota with eight known formae specialis (f. sp.) linked to specific host species (Braun et al., 2000; Oku et al., 1985). Infections with powdery mildew lead to characteristic visual symptoms on leaves and

stems of cereals, namely creamy white pustules of mycelium and conidia, containing black fruiting bodies (cleistothecia) at the later stages of development (Schubiger, 2014a; Walker et al., 2011) (see also figure 8). Powdery mildew on triticale has only been recently found to gain more importance and it has been postulated that the causal agent is *Blumeria graminis* f. sp. *tritici* that expanded its host range from wheat to triticale (Troch et al., 2012; Walker et al., 2011). Visual scorings for the severity of powdery mildew infections have been integrated in the master thesis at hand as powdery mildew infections can cause reductions in both quality and yield (Conner et al., 2003; Everts et al., 2001). A simplified model based on a scaling of 1 to 9, where '1' represents 'no symptoms observed' and '9' represents 'very strong infection', was utilized for the disease assessment. The disease scorings were carried out on May 30$^{th}$, 2014, for replication one and on June 1$^{st}$, 2014, for replication two.

**Figure 8: Symptoms of powdery mildew on triticale leaves.**

Severity of yellow (stripe) rust infections:

Same as for powdery mildew, the causal agent of yellow rust is an obligate biotrophic fungus. However, the pathogen called *Puccinia striiformis* belongs to the phylum Basidiomycota. It is a macrocyclic, heteroecious rust fungus. Again, several host-specific formae specialis have been found, among them such that attack triticale (Hovmøller and Justesen, 2007; Randhawa et al., 2012; Schubiger, 2014b). Typical symptoms of yellow rust infections on cereal leaves are yellow longitudinal stripes on both sites of the leaves. These stripes are made of mycelium and yellow to orange fruiting bodies that contain uredospores (Schubiger, 2014b) (see also figure 9). As severe epidemics of yellow rust can cause significant reductions of quality and yield

(Hovmøller and Justesen, 2007; Randhawa et al., 2012; Tian et al., 2004), a visual scoring of the severity of stripe rust infections was included in this master thesis. Similar to the scoring scheme for powdery mildew, a simplified model based on a scaling of 1 to 9, where '1' represents 'no symptoms observed' and '9' represents 'very strong infection', was used to visually estimate the severity of yellow rust infections on triticale. The scorings were carried out on June 1st, 2014, for both replications.

**Figure 9: Symptoms of yellow (stripe) rust on triticale leaves.**

Plant height:

The average plant height of each genotype (plot) was measured to check whether this morphological trait is correlated with infections of FHB, powdery mildew or yellow rust. The plant height was measured from ground to the top of the plants (excluding awns) with a measuring stick. The measurements were carried out on June 11th, 2014, for both replications.

Homogeneity:

The parameter homogeneity (based on plant height, spike morphology, and spike color) was evaluated as quality control of all other scorings. Inhomogeneous genotypes were not included in the statistical analysis as scorings for such types did show a high variance even within a single plot (lines of the $F_{4:5}$ generation were tested). A mean plot value did not represent inhomogeneous genotypes well. Scorings for homogeneity were based on a simplified model using a scaling from

1 to 9 with ‘1’ representing ‘very homogeneous’ and ‘9’ representing ‘very inhomogeneous’. Genotypes with a rating of ‘6’ or higher were not included in the statistical analysis. The scorings for homogeneity were carried out on June 30th, 2014, for both replications.

## 2.8.) Statistical analysis

The traits FHB severity (last visual scoring as %FHB, and AUDPC, see below), percentage of *Fusarium* damaged kernels (%FDK), flowering date (FLD), plant height (PLH), severity of powdery mildew infections (POM), and severity of yellow rust infections (YER) were all analyzed by one-way ANOVA (analysis of variance). As mentioned earlier, all inhomogeneous lines with a homogeneity scoring larger than six (i.e. 34 lines) had been excluded prior to analysis.

For the trait FHB severity, the area under the disease progress curve (AUDPC), calculated from five visual scorings, was used. The AUDPC represents the disease progress over time and therefore allows to detect genotypic differences better than single scorings. The area under the disease progress curve was calculated with the following simplified formula with increased emphasis on the last scoring:

$$\mathbf{AUDPC = S1 \times 7 + S2 \times 4 + S3 \times 4 + S4 \times 4 + S5 \times 4}$$

S1-S5 … scorings one to five carried out
10, 14, 18, 22, and 26 days after full anthesis

For all ANOVAs, the following linear model was utilized:

$$\mathbf{x_{ij} = \mu + \alpha_i + \beta_j + \varepsilon}$$

$x_{ij}$ … phenotypic value observed for a certain genotype
$\mu$ … overall mean
$\alpha_i$ … effect of genotype i
$\beta_j$ … effect of replication j
$\varepsilon$ … residual error

All data were tested for normal distribution before the ANOVA analysis with Shapiro-Wilk test for normality. Data for most traits were not normally distributed, but the deviation was usually small causing only light changes in significance. Therefore, ANOVAs were carried out without logarithmic transformation of data.

The least significant difference (LSD) was calculated to check at which level significant differences can be found among genotypes. The following formula was used:

$$\mathbf{LSD = t \times \sqrt{((2 \times MS_{residuals}) / n)}}$$

t … critical value from the t-distribution table with degrees of freedom from the ANOVA for the same trait

$MS_{residuals}$ … mean square of residuals (ANOVA)

n … number of replications

Calculations were performed in Microsoft Excel 2010 using data from ANOVAs carried out in R.

Correlations between all traits listed above were calculated with correlation coefficients via Pearson correlation. ANOVAs and correlation coefficients were calculated with the programs R (version 3.0.1) and R studio. Correlation graphs (scatterplots) were also drawn in R. Frequency distribution graphs (histograms) were drawn in Microsoft Excel 2010.

As the results in this master thesis only represent data from one environment (location Tulln in 2014), heritabilities cannot be estimated. Instead, the two replications were tested for repeatability with the following formula (adapted from Nyquist (1991)):

$$\mathbf{Repeatability\ (REP) = 1 - MS_{residuals}/MS_{genotypes}}$$

$MS_{residuals}$ … mean square of residuals (ANOVA)
$MS_{genotypes}$ … mean square of genotypes (ANOVA)

Values close to 1 represent good repeatability, meaning that scorings in replications one and two led to similar results. Calculations were performed in Microsoft Excel 2010 using data from ANOVAs carried out in R.

# 3.) Results

Results for all screenings performed for the master thesis at hand will be presented in the upcoming chapters.

## 3.1.) Data overview

In this chapter, the general statistic distribution parameters (i.e. minimum, maximum, median, mean) for FHB related traits and all other important traits investigated in this master thesis will be depicted. Results will include data for all populations together as well as for the four populations individually. In addition to the general statistic distribution parameters, the mean of the parental lines will also be shown for each population. As all parental lines were part of at least two populations (the respective RIL population and the diverse collection of lines in population 4), their overall mean was calculated and used for the analysis of all populations together. The parental mean depicted in tables will also be the overall mean over all populations containing the parental genotype. For the analysis of individual populations, the parental mean was calculated based only on the two replications of this specific population (as for all lines).

Parent one for populations 1 to 3 was the FHB resistant line *G8.06*. Parent two differs for all three populations (see also table 2, chapter 2.1.): For population 1 it was the variety *Tulus*. For population 2, the first filial generation ($F_1$) of the cross *Agostino* × *Grenado* was used as crossing partner for *G8.06*. Here, the two grandparents *Agostino* and *Grenado* were used as control in the field trial (and not the $F_1$ plant) and two values will be presented in all tables. Parent two for population 3 was the variety *Elpaso*. Population 4 was not a crossing population, but instead, a collection of 35 varieties currently registered in Austria and 19 breeding lines from former triticale FHB resistance trials carried out at IFA-Tulln (so there will be no information on parental values). All four populations differed in the number of genotypes tested. After removal of the 34 inhomogeneous lines, 407 genotypes were analyzed in total. Finally, population 1 contained 141 genotypes (3 previously removed), population 2 was built up of 117 genotypes (21 removed), and population 3 was comprised of 101 genotypes (10 removed). No inhomogeneous line was found in population 4, so all 54 genotypes were tested.

### 3.1.1.) FHB related traits

Symptoms of FHB in the field and on kernels were found on all genotypes in all populations. The percentage of infected spikelets per plot from the fifth and last scoring 26 days after inoculation (%FHB) and the area under the disease progress curve for five visual scorings (AUDPC) are used to represent the severity of FHB infections within the four triticale populations. The percentage of *Fusarium* damaged kernels (%FDK) is also related to FHB and therefore utilized for comparison with the other two traits. A list containing summarized data for all three traits linked to FHB (i.e. %FHB, AUDPC, and %FDK) can be seen in table 5.

**Table 5: General statistic distribution parameters for the FHB related traits percentage of infected spikelets per plot for the last scoring (%FHB), area under the disease progress curve for FHB severity (AUDPC), and percentage of *Fusarium* damaged kernels (%FDK). Results are depicted for all populations combined and for populations one to four individually. The mean performance of the parental lines is included for populations one to three.**

| Trait | Population(s) | Minimum | Median | Mean | Maximum | Parent 1 | Parent 2 |
|---|---|---|---|---|---|---|---|
| **%FHB** | *All populations* | 5,5 | 18,0 | 19,2 | 50,0 | - | - |
| | *Population 1* | 6,0 | 17,5 | 19,2 | 50,0 | 12,0 | 28,3 |
| | *Population 2* | 7,0 | 18,0 | 20,1 | 62,5 | 12,0 | 12,8 / 44,3 |
| | *Population 3* | 7,0 | 17,0 | 17,7 | 36,5 | 12,0 | 13,8 |
| | *Population 4* | 5,5 | 19,0 | 19,8 | 38,5 | - | - |
| **AUDPC** (scoring 1-5) | *All populations* | 47,4 | 150,6 | 163,6 | 489,5 | - | - |
| | *Population 1* | 55,1 | 136,7 | 154,3 | 391,5 | 101,4 | 243,9 |
| | *Population 2* | 47,7 | 163,4 | 183,9 | 696,0 | 101,4 | 94,1 / 489,5 |
| | *Population 3* | 52,4 | 144,7 | 154,5 | 433,8 | 101,4 | 121,0 |
| | *Population 4* | 47,4 | 154,2 | 163,0 | 351,5 | - | - |
| **%FDK** | *All populations* | 5,0 | 22,5 | 25,7 | 99,5 | - | - |
| | *Population 1* | 5,0 | 22,5 | 25,2 | 90,0 | 10,1 | 43,8 |
| | *Population 2* | 6,5 | 20,0 | 23,9 | 68,5 | 10,1 | 45,0 / 45,0 |
| | *Population 3* | 6,5 | 22,5 | 26,2 | 87,5 | 10,1 | 18,8 |
| | *Population 4* | 5,0 | 26,9 | 30,2 | 99,5 | - | - |

For the trait %FHB, all four populations showed quite similar results, except for the maximum values. The mean percentage of infected spikelets per plot was found to be around 19%. The lowest mean was found in population 3 (17,68%), the highest mean in population 2 (20,13%). Population 3 also had the narrowest range of response (7,0 – 36,5%), followed by population 4 (5,5 – 38,5 %) and population 1 (6,0 – 50,0%). The highest range was found in population 2 (7,0 – 62,5%). The maximum value in population 2 exceeded the overall maximum value because the parental line *Grenado* performed different in population 2 and population 4, so that the overall mean of this line was lower than its mean in population 2 (44,25% instead of 62,5% in population 2). The high maximum level in population 2 therefore results from *Grenado*, although its overall mean in the table is lower. The (grand)parental lines *Agostino* and *Elpaso* in populations 2 and 3 exhibited FHB symptoms on a similar level as the resistant parental line *G8.06*, while the (grand)parents *Grenado* and *Tulus* in populations 2 and 1 performed worse.

Same as for the trait %FHB, the four populations did not differ much for the trait AUDPC, except for their maximum values. The mean values for the trait FHB severity expressed as AUDPC were found to be around 160 units. Populations 4 (351,5) and 1 (391,5) had the lowest maximum AUDPC levels. Population 3 followed closely (433,8), while population 2 again had a very high level (696,0) due to the performance of *Grenado*. Nevertheless, these discrepancies did not lead to large differences in mean values.

More distinct differences could be seen for the trait %FDK. Astonishingly, population 2 had the lowest mean (23,89%) and maximum (68,5%) values of all populations. Additionally, *Grenado* was not representing the maximum value in population 2. The highest mean (30,18%) and maximum (99,5%) values were found in population 4. The other two populations had intermediate average levels of %FDK, coming close to the overall mean at approximately 26%.

Lines with slightly lower levels of %FHB, AUDPC, and %FDK than the resistant parental line *G8.06* were found in all three RIL populations. Nevertheless, no significant positive transgression could be observed (see also LSD values in histograms in chapter 3.2.1.). Significant negative transgressions were found for the traits %FHB and AUDPC in population 3, and for the trait %FDK in all three RIL populations.

A separate analysis for registered varieties and advanced breeding lines in population 4 revealed that the two groups differed considerably (see table 6). In fact, the breeding lines as such performed better than any other population. On average, the varieties performed worse than the other populations.

**Table 6: General statistic distribution parameters for different groups of population 4.**

| Trait | Population 4 | Minimum | Median | Mean | Maximum |
|---|---|---|---|---|---|
| **%FHB** | *Registered varieties* | 11,0 | 21,0 | 21,8 | 38,5 |
| | *Advanced breeding lines* | 5,5 | 15,5 | 16,1 | 23,5 |
| **AUDPC** | *Registered varieties* | 62,4 | 169,7 | 181,9 | 351,5 |
| | *Advanced breeding lines* | 47,4 | 121,7 | 128,1 | 228,4 |
| **%FDK** | *Registered varieties* | 15,0 | 35,0 | 38,0 | 99,5 |
| | *Advanced breeding lines* | 5,0 | 15,0 | 15,7 | 25,0 |

A complete list of all trait performances of the varieties and breeding lines of population 4 can be seen in the appendix. The ranking of all genotypes of population 4 from low to high level of %FDK (the FHB related trait with highest repeatability, see below) confirms the differences among the two groups in population 4: Among the top 20 genotypes with lowest %FDK ratings, 17 of the advanced breeding lines from IFA-Tulln and only three registered cultivars can be found, while the 20 genotypes with highest %FDK ratings are all currently registered cultivars. The least severely infected genotype was *TRIT_F7.06* (in population 4 as well as for all populations). The variety *Gringo* on the other hand, had the highest level of infection in population 4. Again, we want to stress that the parental line *Grenado* performed worse in population 2 and had the overall highest FHB severity field ratings (but not %FDK ratings). Remarkable differences in the ranking order were found for a number of genotypes for the traits %FHB/AUDPC and %FDK (e.g. for the genotype *Agostino*). The most widely grown triticale cultivars in Austria in 2014, *Agostino*, *Triamant*, and *Elpaso* (BAES, 2014), had low to medium rates for the FHB related traits (i.e. 12,8, 22,0, 13,8 %FHB, and 45, 30, 18,8 %FDK, respectively).

Figure 10 illustrates the mean disease progress expressed via percentage of infected spikelets per plot for all parental lines. In addition, the least severely infected genotype, line *TRIT_F7.06*, is also depicted. The overall most severely infected

genotype was the parental line *Grenado*, so this line represents the maximum infection level.

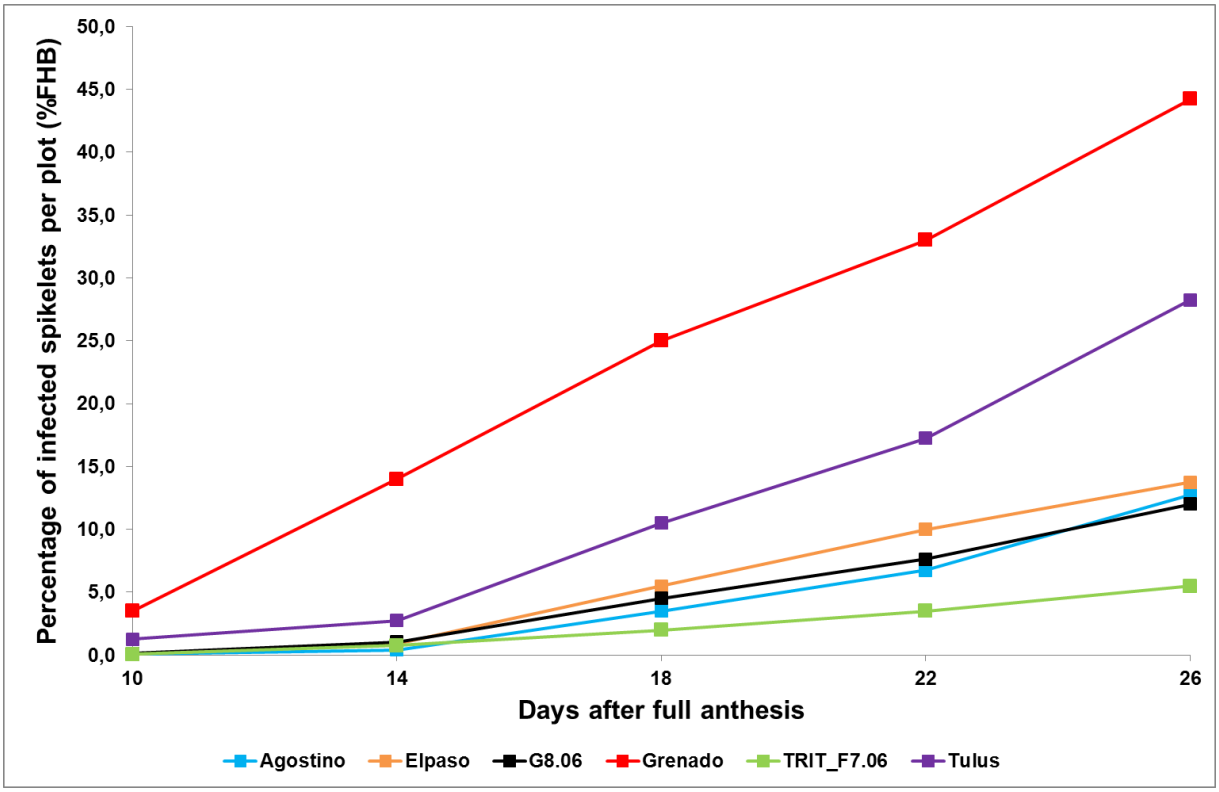



**Figure 10: Mean disease progress for specific lines (parental lines and lines with minimum/maximum level of FHB).**

The repeatability of scorings for FHB related traits between the two replications can be seen in table 7. Overall, a medium level of repeatability could be achieved. In populations 1 and 2, repeatability was rather high, but in populations 3 and 4, only low to medium repeatability levels were accomplished. The highest repeatability was found for the trait %FDK. The repeatability for the two traits %FHB and AUDPC was at a similar, but lower level.

**Table 7: Repeatability of scorings for FHB related traits between the two replications.**

| Repeatability (REP) of trait | REP %FHB | REP AUDPC | REP %FDK |
|---|---|---|---|
| **All populations** | 0,63 | 0,59 | 0,78 |
| **Population 1** | 0,81 | 0,83 | 0,90 |
| **Population 2** | 0,85 | 0,88 | 0,77 |
| **Population 3** | 0,55 | 0,49 | 0,71 |
| **Population 4** | 0,51 | 0,64 | 0,82 |

### 3.1.2.) Relevant traits other than FHB

In addition to the severity of FHB infections, the four traits flowering date (FLD), plant height (PLH), severity of powdery mildew infections (POM), and severity of yellow rust infections (YER) were investigated in this master thesis. A list containing summarized data for these four traits can be seen in table 8.

**Table 8: General statistic distribution parameters for the traits flowering date (FLD), plant height (PLH), severity of powdery mildew infections (POM), and severity of yellow rust infections (YER). Results are depicted for all populations combined and for populations one to four individually. The mean performance of the parental lines is included for populations one to three.**

| Trait | Population(s) | Minimum | Median | Mean | Maximum | Parent 1 | Parent 2 |
|---|---|---|---|---|---|---|---|
| **FLD** (days after the 30th of April) | *All populations* | 23,0 | 25,0 | 25,3 | 29,0 | - | - |
| | *Population 1* | 23,0 | 26,0 | 25,8 | 29,0 | 25,5 | 25,5 |
| | *Population 2* | 23,0 | 25,0 | 24,6 | 28,0 | 25,5 | 25,5 / 26,5 |
| | *Population 3* | 23,0 | 25,0 | 25,4 | 29,0 | 25,5 | 24,5 |
| | *Population 4* | 23,0 | 25,0 | 25,3 | 28,0 | - | - |
| **PLH** (cm) | *All populations* | 87,5 | 127,5 | 127,6 | 162,5 | - | - |
| | *Population 1* | 103,8 | 127,5 | 127,8 | 145,0 | 139,4 | 115,0 |
| | *Population 2* | 92,5 | 137,5 | 132,6 | 162,5 | 139,4 | 102,5 / 96,3 |
| | *Population 3* | 100,0 | 127,5 | 127,3 | 155,0 | 139,4 | 121,3 |
| | *Population 4* | 87,5 | 120,0 | 116,3 | 135,0 | - | - |
| **POM** (1-9) | *All populations* | 2,0 | 3,0 | 3,1 | 7,5 | - | - |
| | *Population 1* | 2,0 | 3,0 | 3,5 | 7,0 | 3,1 | 2,3 |
| | *Population 2* | 2,0 | 2,5 | 2,5 | 5,0 | 3,1 | 2,0 / 2,0 |
| | *Population 3* | 2,0 | 3,5 | 3,6 | 7,5 | 3,1 | 3,8 |
| | *Population 4* | 2,0 | 2,6 | 2,7 | 4,0 | - | - |
| **YER** (1-9) | *All populations* | 1,0 | 1,5 | 1,5 | 7,5 | - | - |
| | *Population 1* | 1,0 | 1,5 | 1,4 | 3,5 | 1,0 | 2,0 |
| | *Population 2* | 1,0 | 1,5 | 1,4 | 2,5 | 1,0 | 1,3 / 1,3 |
| | *Population 3* | 1,0 | 1,5 | 1,8 | 4,0 | 1,0 | 1,8 |
| | *Population 4* | 1,0 | 1,8 | 1,9 | 7,5 | - | - |

For the trait FLD, all four populations showed very similar behavior. In general, genotypes reached the stage of anthesis two to four days later in replication 2 (data not shown).

The highest range and mean for the trait PLH was found in population 2 (92,5 – 162,5 cm; 132,6 cm), the lowest mean in population 4 (116,3 cm). The other two populations had similar, intermediate levels of plant height. In a separate analysis of varieties and breeding lines, varieties were shown to be a bit shorter than the advanced breeding lines (data not shown).

On average, only light infections of powdery mildew (POM) could be observed in all four populations. The lowest severity was found in populations 2 and 4. In populations 1 and 3, slightly higher mean values and considerably higher maximum values occurred.

The severity of yellow rust infections (YER) was even lower than for powdery mildew. A high level of infection could only be found on a single variety in population 4 (*Mikado*).

The repeatability between the two replications was also calculated for the four traits FLD, PLH, POM, and YER, as can be seen in table 9. Repeatability was at a medium level for the trait FLD, high for the trait PLH and medium to quite high for the trait POM. For the trait YER on the contrary, repeatability values were very different among populations, ranging from low or rather low (populations 3, 2, and 1) to high (population 4).

**Table 9: Repeatability of scorings for relevant traits other than FHB between the two replications.**

| Repeatability (REP) of trait | REP FLD | REP PLH | REP POM | REP YER |
|---|---|---|---|---|
| **All populations** | 0,52 | 0,93 | 0,83 | 0,41 |
| **Population 1** | 0,59 | 0,79 | 0,79 | 0,42 |
| **Population 2** | 0,59 | 0,97 | 0,82 | 0,25 |
| **Population 3** | 0,73 | 0,88 | 0,82 | 0,19 |
| **Population 4** | 0,74 | 0,89 | 0,62 | 0,86 |

## 3.2.) Frequency distributions of the data

The frequency distributions (i.e. the number of genotypes representing certain levels of a trait) for the three FHB related traits and the other four traits will be illustrated with histograms. In all histograms presented in this section the four populations will be displayed separately. For histograms over all genotypes without separation into populations, please see the appendix. The LSD value, representing the minimum difference among genotypes for them to be significantly different from each other at the probability of error of 5%, will be included in all histograms.

### 3.2.1.) Histograms for FHB related traits

Figure 11 shows the frequency distribution for the trait %FHB. All four populations had similar median and mean values (see also table 5). Maximum values in turn were much higher in populations 1 and 2. Most genotypes reached low to medium levels of FHB infections until the fifth field scoring. Registered varieties and breeding lines in population 4 differed remarkably, as mentioned earlier.

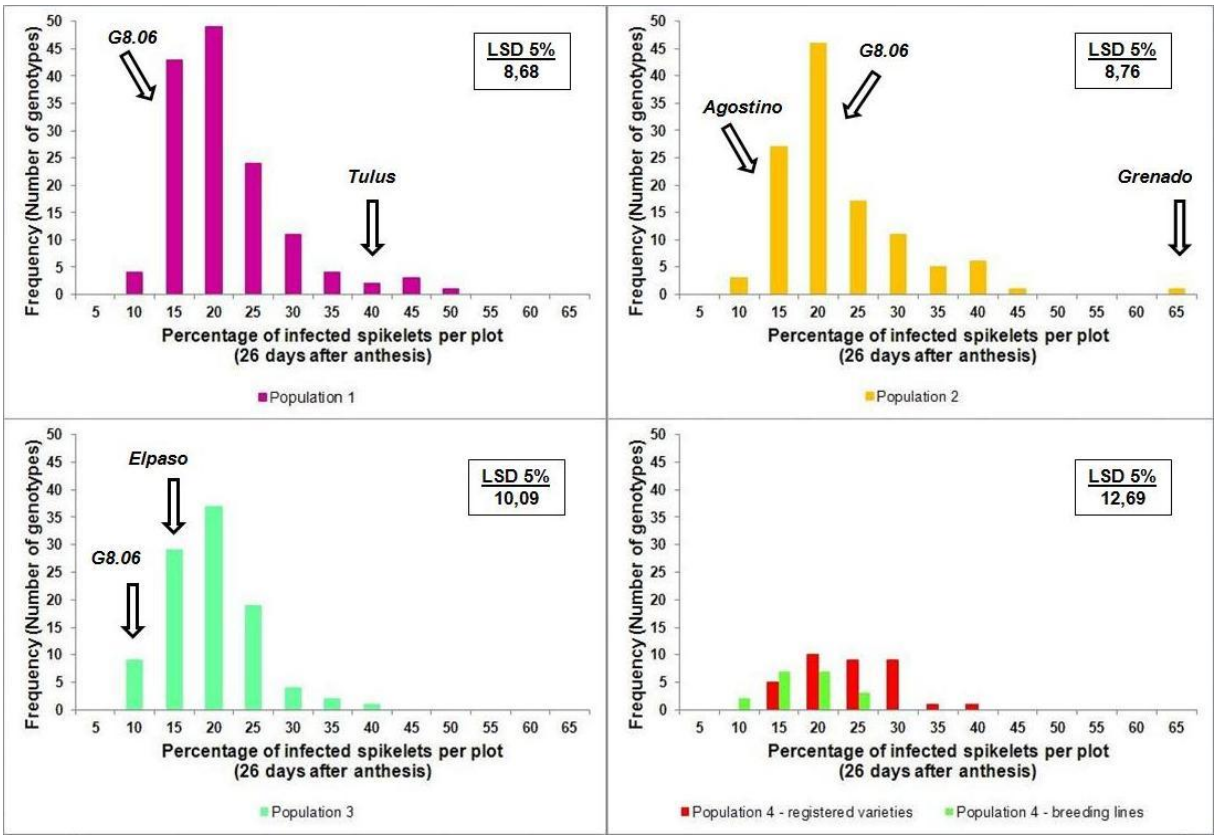



**Figure 11: Frequency distribution of genotypes for the trait %FHB in populations 1 to 4. Total number of genotypes was 141 (population 1), 117 (population 2), 101 (population 3), and 54 (population 4).**

Significant differences for the trait %FHB (figure 11) among the parental lines were found in populations 1 and 2, but not in population 3. In population 2, the parental line *Agostino* had an even lower rating than the resistant parent *G8.06*, while calculated over all four populations, the latter genotype performed better. Same as between *G8.06* and *Elpaso*, no significant differences were found for *G8.06* and *Agostino*. The parental line *Grenado* was by far rated highest among all genotypes, as shown in the histogram for population 2.

The frequency distribution for the trait AUDPC corresponds well to the histogram for %FHB, as the latter largely influences the level of AUDPC. Again, the four populations show a similar pattern of distribution, as can be seen in figure 12 (most histograms indicate a slight skewness in distribution towards resistance, except for population 4). Population 1 exhibits the narrowest range of distribution. Population 2 on the contrary, has the widest range with *Grenado* lying far outside. Once again, significant differences among the parental lines were detected between *G8.06* and *Tulus* (population 1), and *G8.06* and *Grenado* (population 2). *Agostino* and *Elpaso* did not differ significantly from the resistant parental line *G8.06*.

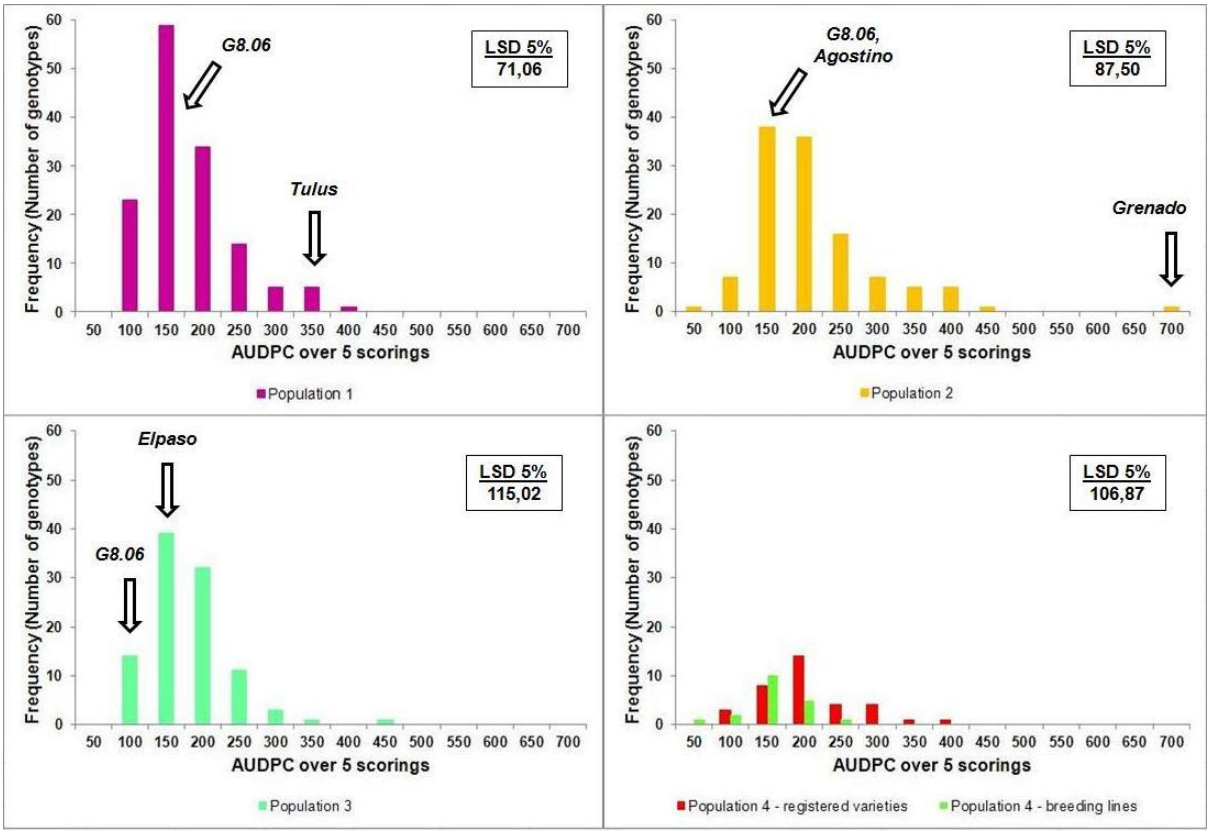



**Figure 12: Frequency distribution of genotypes for the trait AUDPC in populations 1 to 4. Total number of genotypes was 141 (population 1), 117 (population 2), 101 (population 3), and 54 (population 4).**

For the trait %FDK, all four populations had again a similar pattern of distribution, as depicted in figure 13. The most remarkable aspects are that population 2 does not represent the highest values and that population 4 has a wider and shallower distribution than for the other two traits. *Grenado* does not represent the maximum level of %FDK, but instead the variety *Gringo* in population 4 (99,5%). Among all FHB related traits, the trait %FDK differentiates best between registered cultivars and breeding lines in population 4. The means for these two groups differ significantly from each other according to the LSD analysis. Significant differences between the parental lines were found in populations 1 and 2. Only *Elpaso* in population 3 did not differ significantly from *G8.06*.

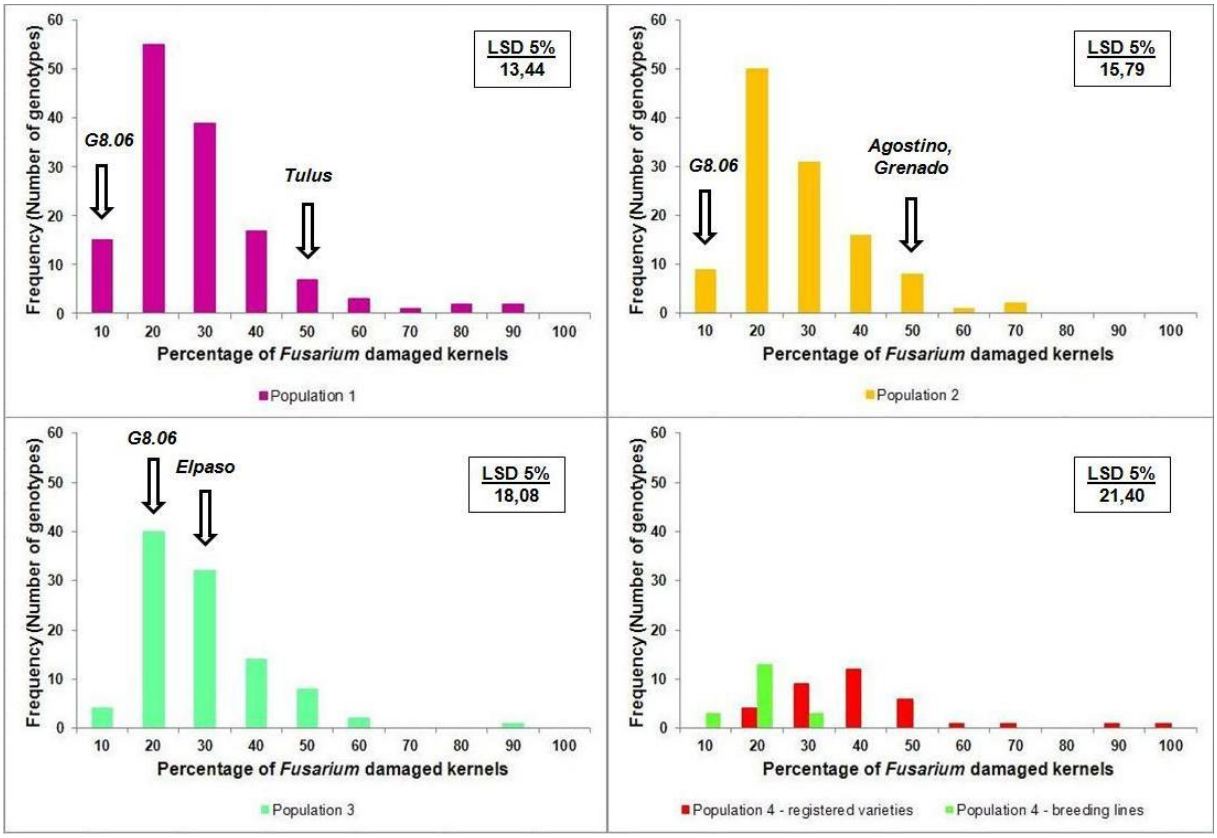



**Figure 13: Frequency distribution of genotypes for the trait %FDK in populations 1 to 4. Total number of genotypes was 141 (population 1), 117 (population 2), 101 (population 3), and 54 (population 4).**

### 3.2.2.) Histograms for relevant traits other than FHB

Although the standard statistic parameters were nearly the same for all populations for the trait flowering date (FLD), the distribution in population 1 differed slightly from the other three populations, as illustrated in figure 14. While the distributions in the populations 2, 3, and 4 appear to be close to normal distribution, the distribution in population 1 does not display the typical peak at the center. The mean flowering dates ranged from the 23rd of May, 2014, until the 29th of May. The real dates for replication 1 ranged from the 22nd of May until the 26th of May, and those for replication 2 from the 24th of May until the 1st of June. Breeding lines and varieties in population 4 did not differ remarkably for the trait FLD. Significant differences among parental lines could not be observed in any RIL population.

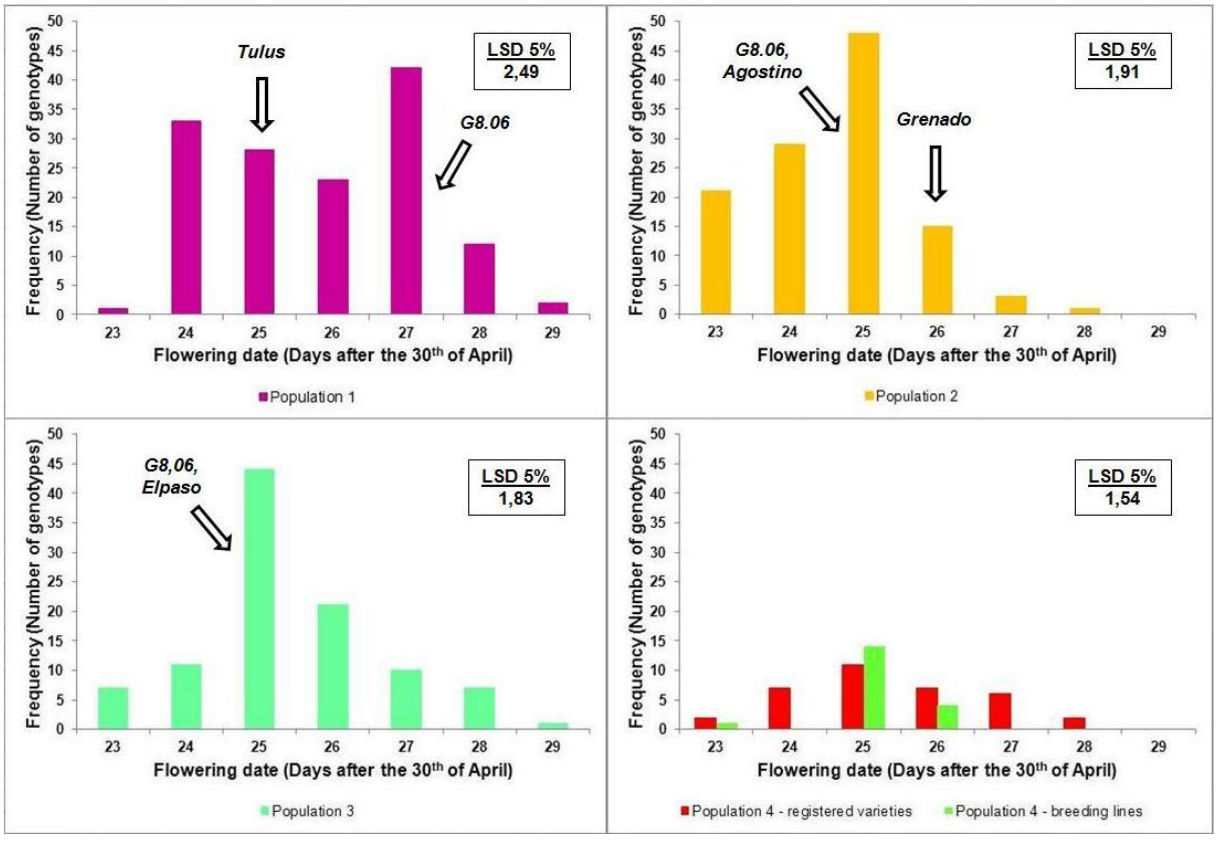



**Figure 14: Frequency distribution of genotypes for the trait FLD in populations 1 to 4. Total number of genotypes was 141 (population 1), 117 (population 2), 101 (population 3), and 54 (population 4).**

The frequency distribution for the trait plant height (PLH) can be seen in figure 15. The populations 1, 3, and 4 represent a similar distribution pattern resembling normal distribution, while population 2 shows a wider and shallower distribution. Although the pattern of distribution is nearly the same in most populations, the distributions do not occur at an equal level of plant height. Population 2 is mainly built up of larger genotypes. The two populations 1 and 3 contain a large number of genotypes with intermediate plant height. Population 4 on the other hand, is mainly comprised of genotypes with lower plant height, but varieties and breeding lines differ remarkably. Registered varieties are on average, a bit lower than the advanced breeding lines. In general, the mean plant height in population 4 (116,3 cm; Table 8) is significantly lower than the mean plant height in any of the three RIL populations (overall LSD value of 9,82 cm). The resistant parental line *G8.06* was significantly higher than all other parental lines.

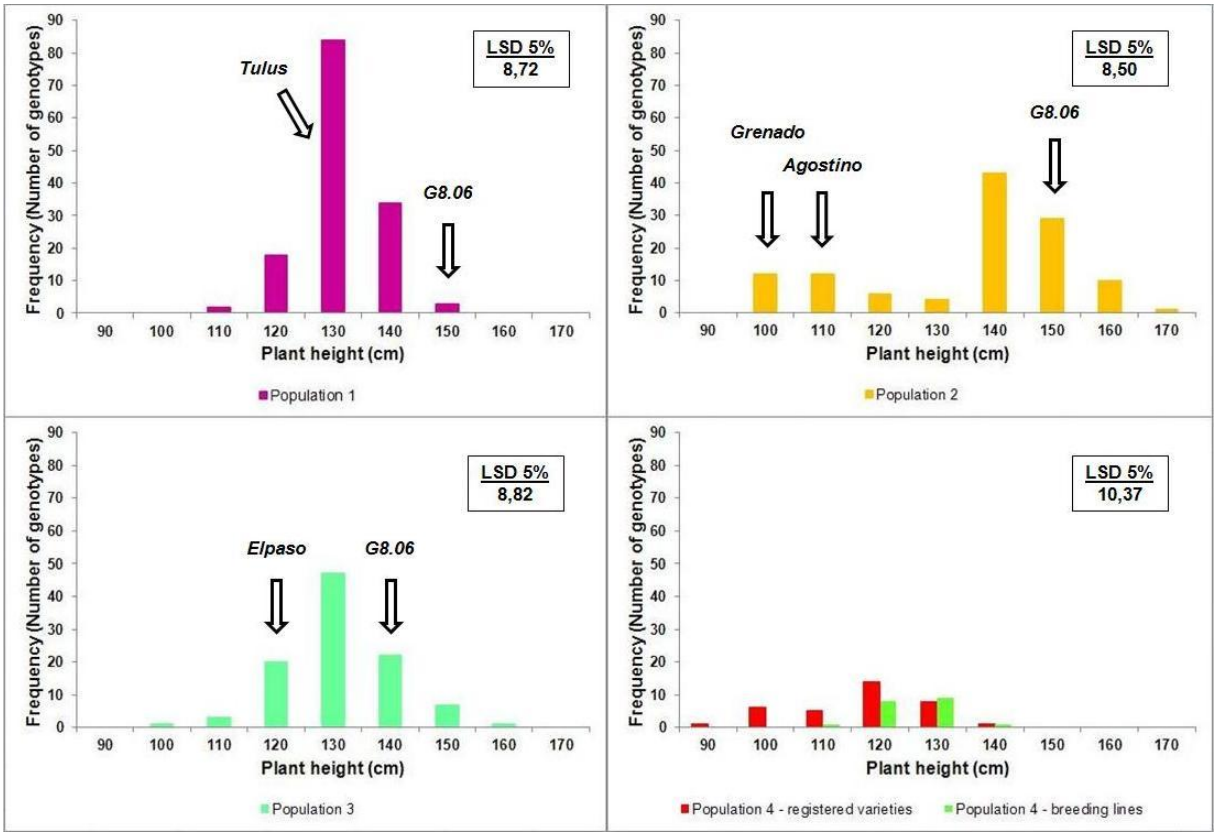



**Figure 15: Frequency distribution of genotypes for the trait PLH in populations 1 to 4. Total number of genotypes was 141 (population 1), 117 (population 2), 101 (population 3), and 54 (population 4).**

Overall, distributions for the trait POM were concentrated at low levels of infection, as depicted in figure 16. Only a few genotypes in populations 1 and 3 had higher levels of powdery mildew infections. On average, plants in population 2 were found to be the least infected (see also table 8). The breeding lines in population 4 showed slightly higher levels of powdery mildew infections than the registered cultivars. Significant differences among the parental lines were found in populations 2 and 3, but not in population 1. In population 2, the FHB resistant line *G8.06* was more heavily infected with powdery mildew than the two grandparental lines *Agostino* and *Grenado*. In population 3, *G8.06* had the same level of infection as in population 2, but *Elpaso* had an even higher level of infection (yet the level was similar to that of *G8.06* in population 1). Lines with significantly lower levels of infection than *G8.06* were detected in all three RIL populations.

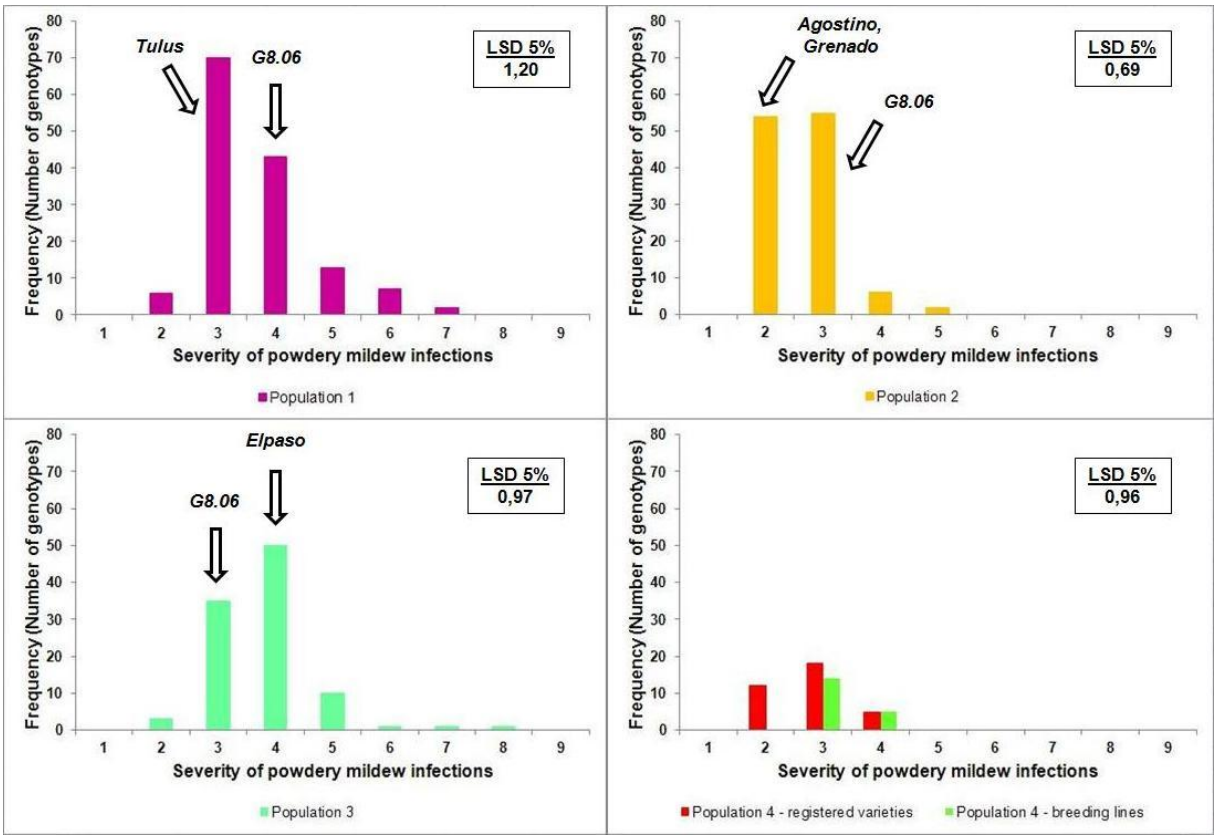



**Figure 16: Frequency distribution of genotypes for the trait POM in populations 1 to 4. Total number of genotypes was 141 (population 1), 117 (population 2), 101 (population 3), and 54 (population 4).**

The great majority of all genotypes showed either no or only light symptoms of yellow rust infections, as shown in figure 17. Only the variety *Mikado* (population 4) suffered from strong yellow rust infections. The FHB resistant line *G8.06* was not found to be infected in any population. Significant differences between parental lines were only found for *G8.06* and *Tulus* in population 1.

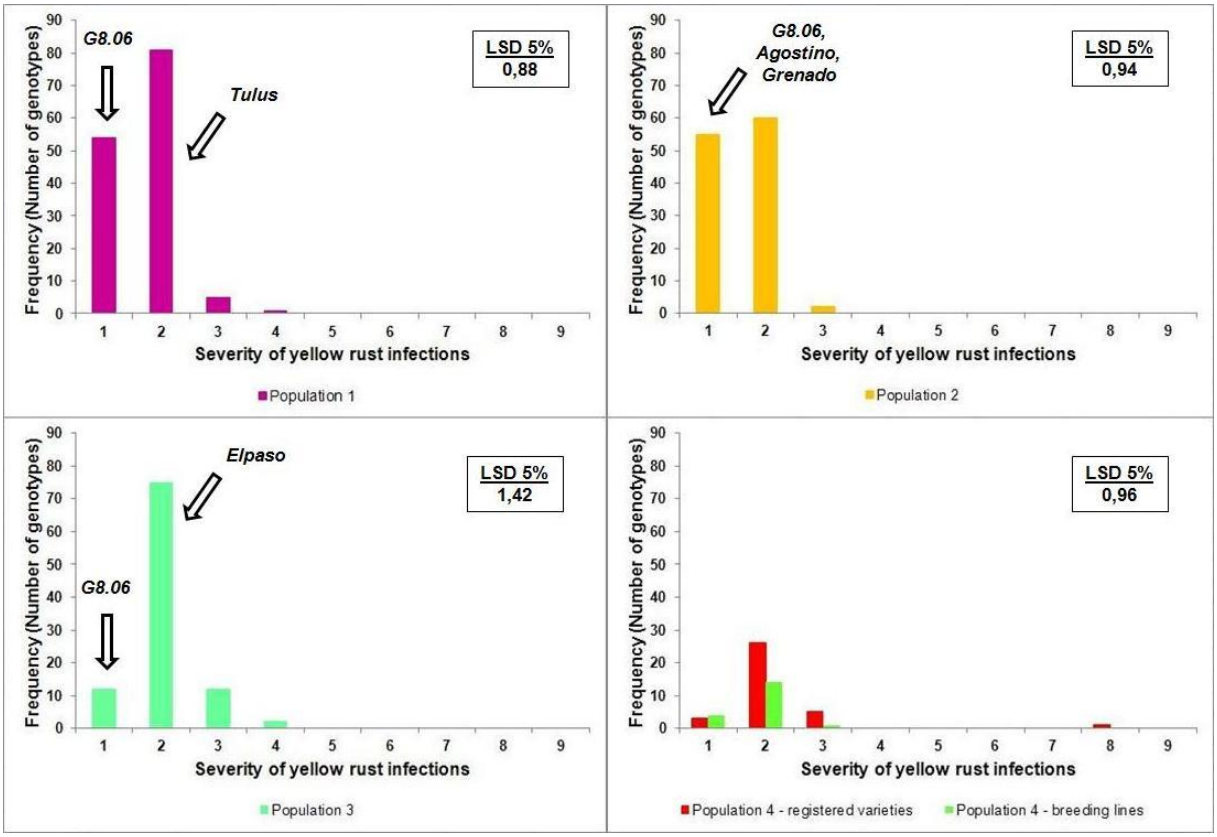



**Figure 17: Frequency distribution of genotypes for the trait YER in populations 1 to 4. Total number of genotypes was 141 (population 1), 117 (population 2), 101 (population 3), and 54 (population 4).**

## 3.3.) Analysis of variance (ANOVA)

The three FHB related traits and the other four important traits were analyzed via one-way ANOVA with the two factors genotype and replication, as mentioned earlier. Data were always tested for all populations together as well as for individual populations. Deviations from the general behavior in single populations will be mentioned in the text, the according tables can be seen in the appendix.

### 3.3.1.) ANOVAs for FHB related traits

Results for the ANOVA for the trait %FHB can be seen in table 10. Significant differences were found for both genotypes and replications. In populations 3 and 4, the factor replication was not significant.

**Table 10: Results for the ANOVA for the trait %FHB over all four populations.**

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 406 | 38593 | 95,057 | 2,7203 | <0,001 |
| **Replication** | 1 | 195 | 194,766 | 5,5738 | 0,019 |
| **Residuals** | 415 | 14501 | 34,943 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

Significant differences for genotypes and replications were also determined for the trait AUDPC, as depicted in table 11. Same as for the trait %FHB, there were no significant differences among replications in populations 3 and 4.

**Table 11: Results for the ANOVA for the trait AUDPC over all four populations.**

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 406 | 3882917 | 9564 | 2,4141 | <0,001 |
| **Replication** | 1 | 76613 | 76613 | 19,3388 | <0,001 |
| **Residuals** | 415 | 1644063 | 3962 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

The analysis of variance for the trait %FDK revealed that there were overall significant differences among genotypes, but not between replications, as shown in table 12. The same results were only found for population 3. In all other populations, significant differences occurred between replications.

Table 12: Results for the ANOVA for the trait %FDK over all four populations.

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 406 | 162287 | 399,72 | 4,5991 | <0,001 |
| **Replication** | 1 | 89 | 89,48 | 1,0296 | 0,311 |
| **Residuals** | 415 | 36069 | 86,91 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

### 3.3.2.) ANOVAs for relevant traits other than FHB

For the trait FLD, significant differences could be identified among genotypes as well as replications, as can be seen in table 13. This was true for all populations combined as well as for individual populations.

Table 13: Results for the ANOVA for the trait FLD over all four populations.

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 406 | 1419,59 | 3,5 | 2,0964 | <0,001 |
| **Replication** | 1 | 2025,82 | 2025,82 | 1214,5918 | <0,001 |
| **Residuals** | 415 | 692,18 | 1,67 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

Table 14 demonstrates the results for the ANOVA for the trait PLH for all populations. Significant differences were detected for both genotypes and replications. The factor replication was not significant in populations 3 and 4.

Table 14: Results for the ANOVA for the trait PLH over all four populations.

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 406 | 137283 | 338,14 | 14,13 | <0,001 |
| **Replication** | 1 | 124 | 123,74 | 4,9554 | 0,027 |
| **Residuals** | 415 | 10363 | 24,97 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

Significant differences among genotypes and replications were also found for the trait POM, as shown in table 15. In population 1, there were no significant differences between replications.

**Table 15: Results for the ANOVA for the trait POM over all four populations.**

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 406 | 670,76 | 1,6521 | 5,7458 | <0,001 |
| **Replication** | 1 | 10,92 | 10,9232 | 37,9892 | <0,001 |
| **Residuals** | 415 | 119,33 | 0,2875 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

For the trait YER, overall significant differences could be determined among genotypes, but not between replications, as depicted in table 16. Nevertheless, the factor replication was significant in populations 1, 3, and 4. In populations 2 and 3, there were no significant differences among genotypes.

**Table 16: Results for the ANOVA for the trait YER over all four populations.**

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 406 | 266,887 | 0,65736 | 1,6973 | <0,001 |
| **Replication** | 1 | 0,688 | 0,68753 | 1,7752 | 0,184 |
| **Residuals** | 415 | 160,729 | 0,3873 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

## 3.4.) Correlation analysis

Linear correlations among traits were calculated with Pearson correlation, as explained above. Again, all populations were analyzed together and also individually. Correlation coefficients for all populations can be seen in table 17, those for single populations in tables 18 to 21. For a number of trait combinations, no significant linear correlation was found.

Overall, medium to high correlations were found among FHB related traits. Low to medium negative correlation coefficients were identified for FHB related traits and the

trait plant height. Correlations between FHB related traits and the traits flowering date or severity of powdery mildew or yellow rust infections were low (to medium) and not stable across populations.

**Table 17: Pearson correlation coefficients analyzed for all trait combinations for all four populations.**

| | %FHB | AUDPC | %FDK | FLD | PLH | POM |
|---|---|---|---|---|---|---|
| **AUDPC** | 0,94*** | | | | | |
| **%FDK** | 0,64*** | 0,57*** | | | | |
| **FLD** | ns | ns | 0,12* | | | |
| **PLH** | -0,44*** | -0,49*** | -0,37*** | ns | | |
| **POM** | -0,15** | -0,16** | -0,11* | ns | ns | |
| **YER** | 0,15** | 0,18*** | 0,11* | ns | -0,19*** | ns |

* = <0,05; ** = <0,01; *** = <0,001; ns = no significant linear correlation

Correlations among the two FHB severity parameters %FHB and AUDPC were high in all four populations. The correlations between the trait %FDK and the other two FHB traits were rather low in population 3, medium in populations 2 and 4, and rather high in population 1, leading to a medium overall correlation. Correlation coefficients for the relationship of FHB related traits with the trait plant height were low in populations 1 and 3, and medium in populations 2 and 4. There was no linear correlation for the traits %FDK and plant height in population 3. The trait flowering date was only sporadically correlated with other traits (at low levels), especially in population 2. Correlations of the trait severity of powdery mildew with other traits either had low coefficients or were not significant at all. The same was true for the trait severity of yellow rust infections. Here, correlations with FHB severity traits were only found in populations 3 and 4 at low to medium levels.

**Table 18: Pearson correlation coefficients analyzed for all trait combinations for population 1.**

| | %FHB | AUDPC | %FDK | FLD | PLH | POM |
|---|---|---|---|---|---|---|
| **AUDPC** | 0,95*** | | | | | |
| **%FDK** | 0,83*** | 0,81*** | | | | |
| **FLD** | ns | ns | ns | | | |
| **PLH** | -0,34*** | -0,39*** | -0,39*** | ns | | |
| **POM** | ns | ns | ns | -0,26** | ns | |
| **YER** | ns | ns | ns | ns | ns | ns |

* = <0,05; ** = <0,01; *** = <0,001; ns = no significant linear correlation

**Table 19: Pearson correlation coefficients analyzed for all trait combinations for population 2.**

| | %FHB | AUDPC | %FDK | FLD | PLH | POM |
|---|---|---|---|---|---|---|
| **AUDPC** | 0,98*** | | | | | |
| **%FDK** | 0,63*** | 0,61*** | | | | |
| **FLD** | 0,22* | 0,26** | 0,34*** | | | |
| **PLH** | -0,65*** | -0,69*** | -0,56*** | -0,30** | | |
| **POM** | -0,23* | -0,22* | -0,28** | ns | ns | |
| **YER** | ns | ns | ns | ns | ns | ns |

* = <0,05; ** = <0,01; *** = <0,001; ns = no significant linear correlation

Table 20: Pearson correlation coefficients analyzed for all trait combinations for population 3.

| | %FHB | AUDPC | %FDK | FLD | PLH | POM |
|---|---|---|---|---|---|---|
| **AUDPC** | 0,95*** | | | | | |
| **%FDK** | 0,39*** | 0,33*** | | | | |
| **FLD** | ns | ns | ns | | | |
| **PLH** | -0,30*** | -0,39*** | ns | 0,23* | | |
| **POM** | ns | ns | ns | ns | ns | |
| **YER** | 0,42*** | 0,50*** | ns | ns | ns | ns |

* = <0,05; ** = <0,01; *** = <0,001; ns = no significant linear correlation

Table 21: Pearson correlation coefficients analyzed for all trait combinations for population 4.

| | %FHB | AUDPC | %FDK | FLD | PLH | POM |
|---|---|---|---|---|---|---|
| **AUDPC** | 0,94*** | | | | | |
| **%FDK** | 0,56*** | 0,51*** | | | | |
| **FLD** | ns | ns | ns | | | |
| **PLH** | -0,47*** | -0,61*** | -0,48*** | ns | | |
| **POM** | ns | ns | -0,48*** | ns | 0,45*** | |
| **YER** | 0,39*** | 0,45*** | ns | ns | -0,32* | ns |

* = <0,05; ** = <0,01; *** = <0,001; ns = no significant linear correlation

Figures 18 to 21 illustrate scatterplots for important trait combinations. The highly positive correlation among the traits %FHB and AUDPC can be seen in figure 18. Figure 19 shows the overall medium correlation between the trait FHB severity expressed as AUDPC and the trait %FDK. The third scatterplot (i.e. figure 20) demonstrates that there is no linear correlation between the trait FLD and AUDPC. Higher levels of FHB severity were found for all flowering dates. The medium negative correlation of the two traits PLH and AUDPC is finally depicted in figure 21.

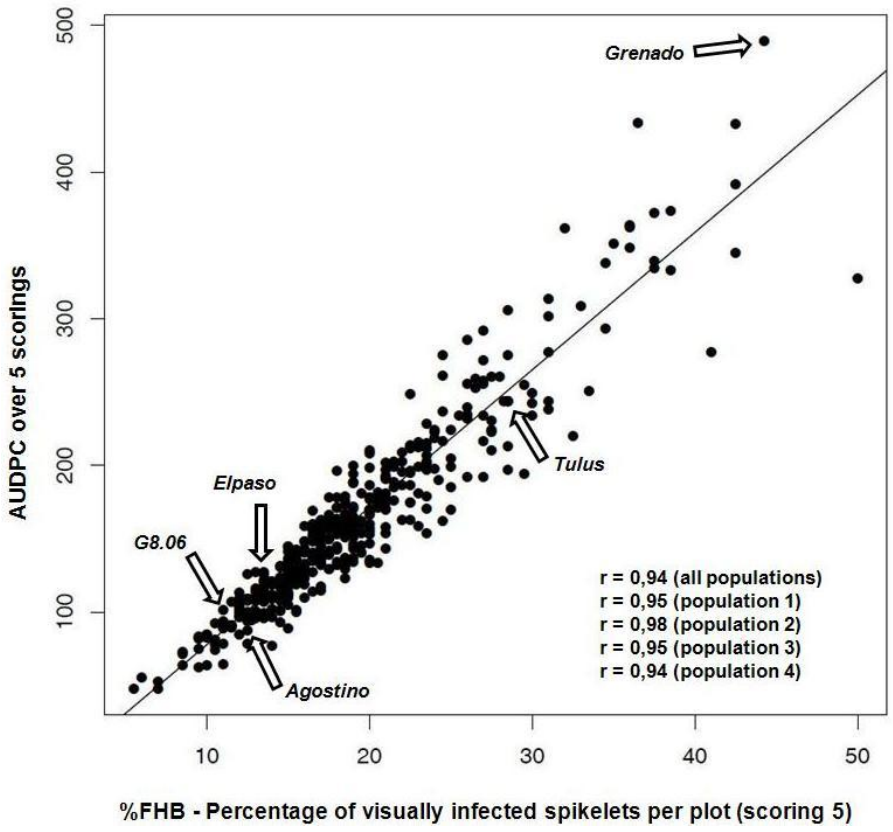



Figure 18: Scatterplot for the two traits %FHB and AUDPC for all populations.

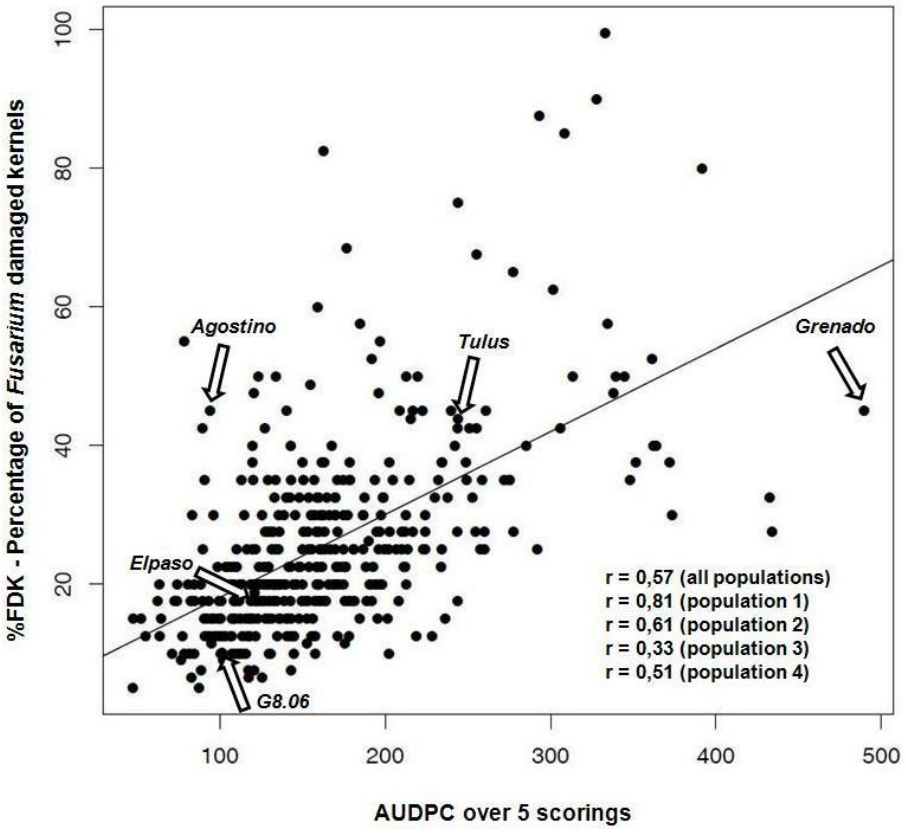



Figure 19: Scatterplot for the two traits AUDPC and %FDK for all populations.

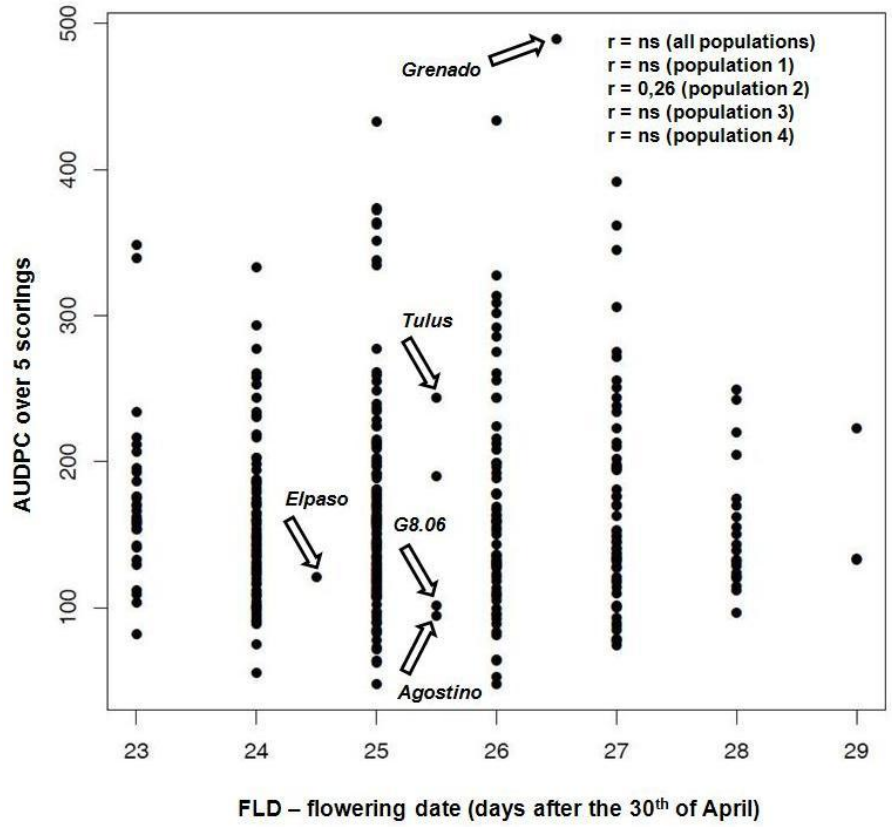



Figure 20: Scatterplot for the two traits FLD and AUDPC for all populations.

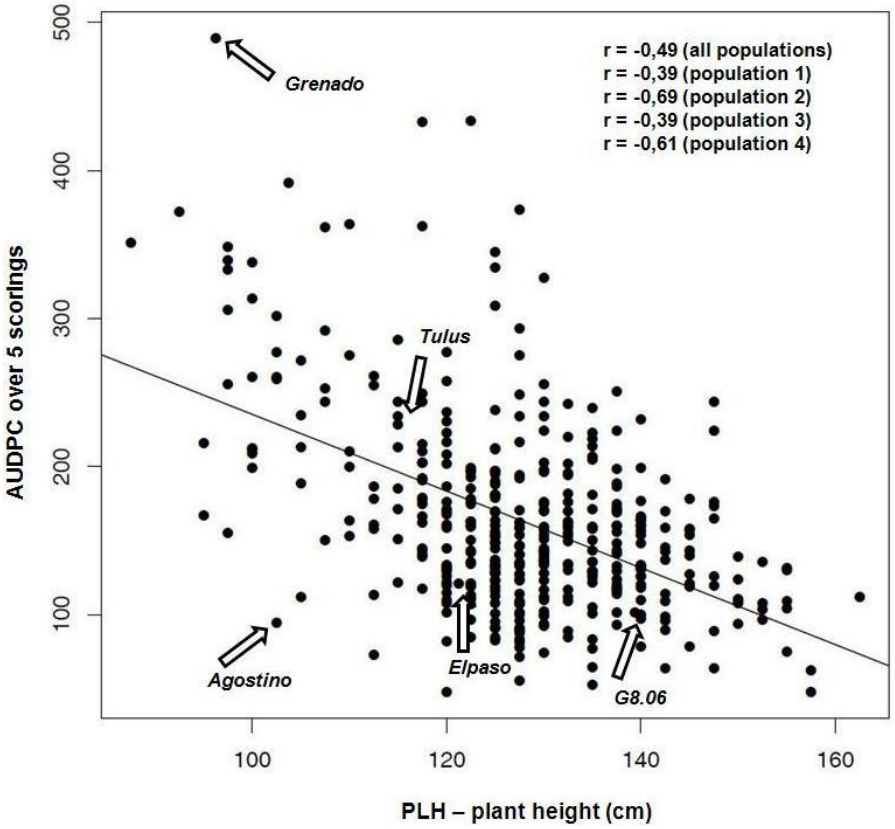



Figure 21: Scatterplot for the two traits PLH and AUDPC for all populations.

A separate analysis for population 4 for the two traits PLH and %FDK is shown in the scatterplot in figure 22. In population 4, a medium negative correlation is found between these two traits. Registered varieties are on average lower in plant height, but more severely infected with FHB than the advanced breeding lines from IFA-Tulln. The line *F7.06* has slightly lower levels of %FDK than *G8.06*, but is also significantly lower in plant height.

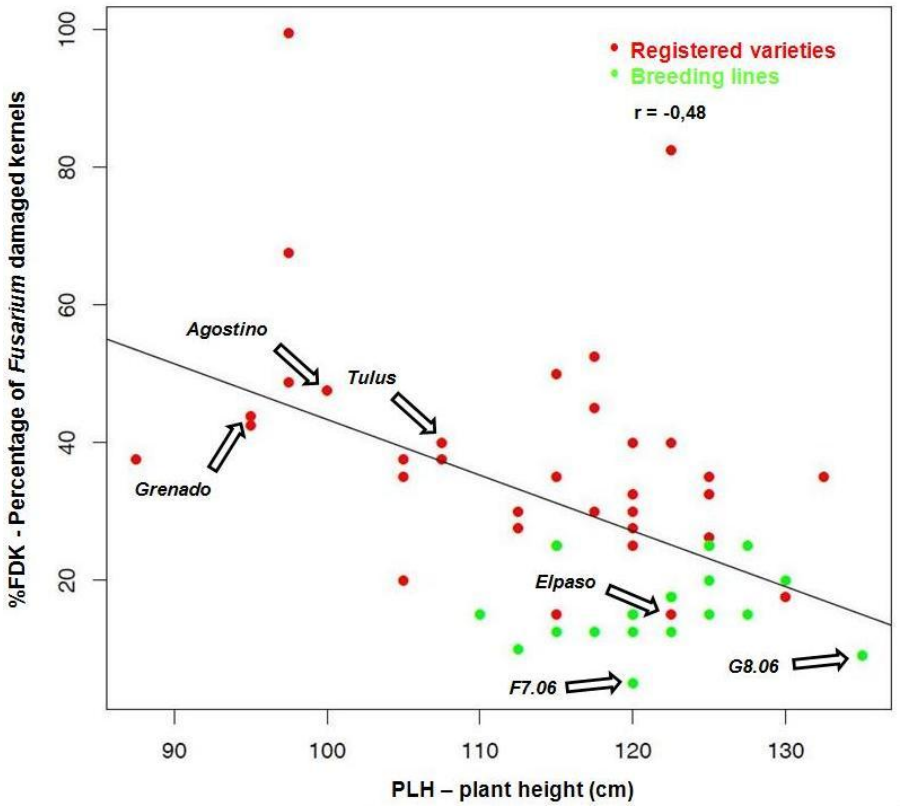



**Figure 22: Scatterplot for the two traits PLH and %FDK for population 4.**

# 4.) Discussion

## 4.1.) FHB resistance assessments

Visual assessments in the field and on kernels revealed that all lines and cultivars in the four populations showed symptoms of FHB after artificial inoculation with *Fusarium culmorum* IFA 104. Hence, no line was found to be completely resistant. The levels of FHB infections differed widely among genotypes and showed continuous variation. The same has been reported for other FHB resistance field studies with larger numbers of triticale lines (Miedaner et al., 2006a; Oettler and Wahle, 2001). Due to the horizontal or non-specific type of the FHB resistance (van Eeuwijk et al., 1995), a single aggressive isolate of an FHB pathogen is enough to differentiate among host genotypes, as has been proposed by Mesterházy (2003a).

In general, literature about FHB resistance in triticale is scarce. To our knowledge, this is the first report about FHB resistance in RIL populations of triticale with lines in more advanced ($F_5$) generation. Oettler and Wahle (2001) investigated 100 triticale genotypes for their FHB resistance for further breeding purposes. Miedaner et al. (2006a) and Oettler et al. (2004) also tested larger crossing populations, but earlier in the $F_1$ or $F_2$ generation. In other studies (e.g. Arseniuk et al. (1999), Miedaner et al. (2001a), Góral et al. (2013)) only more advanced lines for cultivar registration or registered cultivars were analyzed. All these surveys usually handled far less genotypes than in the study at hand.

In the present thesis, the FHB resistance of 441 genotypes was investigated and highly FHB resistant as well as susceptible lines were found in all four populations. Significant differences among genotypes were detected with ANOVAs in all four populations for all three traits associated with FHB resistance (i.e. %FHB; AUDPC, %FDK). The data were collected from one environment (location IFA-Tulln in 2014) only, as mentioned above. Therefore, heritabilities of quantitative traits indicating the genotypic influence on phenotypic appearance (Becker, 2011) could not be calculated. Instead, the repeatability of the results between the two replications was estimated, showing high values (0,90 – 0,71) for the trait %FDK for all four populations. For populations 1 and 2, high values (0,81 – 0,88) were also calculated for the traits %FHB and AUDPC. For populations 3 and 4 instead, the data for the

FHB severity parameters %FHB and AUDPC were only at a medium (0,49 – 0,64) repeatability level.

Various factors could have influenced the visual assessments in the field, leading to lower repeatability. First of all, as any other quantitative plant trait, FHB resistance in triticale and other small grain cereals is largely influenced by environment and genotype × environment interactions (Mesterházy, 1995; Miedaner et al., 2001a; Oettler and Wahle, 2001; Xu, 2003). Although artificial inoculations and a mist irrigation system were applied and several disease ratings were conducted, some microclimatic differences might still have occurred and caused changes in the performance of certain genotypes. The presence of a neighboring shelter belt may have influenced the reaction of plots close to the belt (higher degree of humidity due to the absence of wind). Hence, genotypes that were grown in differing distances to the shelter belt within the two replications, might have suffered from FHB differently. A wider range of flowering dates and a higher number of inoculations in replication 2 might have had an additional impact on the reproducibility of the data in all populations. The mean air temperature during the first days of inoculation was between 15°C and 20°C, but in the last few days of inoculation (at the end of May and beginning of June) the average daily temperature was at or below 15°C. The lower temperatures could have led to decreased FHB infection and development in a small number of genotypes with late flowering in replication 2 (Andersen, 1948; Parry et al., 1995). An additional relevant factor influencing the repeatability of FHB assessments could be the difficulty of visual FHB scorings in triticale *per se*. Wide variations in spike type, awn length, glume and awn color, and head shape occur in triticale, making FHB ratings much more difficult than in wheat (Miedaner et al., 2004; Oettler and Wahle, 2001). No single specific explanation for the lower repeatabilities especially for populations 3 and 4 can be found. In addition to the above mentioned factors, the smaller population size in comparison to populations 1 and 2, and the smaller variation for FHB severity field traits in population 3 might also have influenced the repeatability values.

The high repeatability values calculated for the trait %FDK suggest that, apparently, scorings for this trait were much less error-prone and possibly reflect the actual resistance level of the genotypes better, especially for population 3. Still, the high level of naturally shriveled triticale kernels made visual scorings challenging.

The mean percentage of infected spikelets per plot evaluated at the fifth and last scoring (%FHB) was at about 19% in all four populations. So, on average, approximately one fifth of all spikelets exhibited symptoms of FHB in the field, yet, single genotypes differed considerably (5,5 – 62,5%). Similar results as for the trait %FHB were found for the trait AUDPC calculated over five visual scorings. This can easily be explained by the fact that the former trait is part of the formula for the latter trait. Even more so, as in the work at hand more emphasis was put on the last scoring in the calculation of the AUDPC due to the fact that the last scoring generally better discriminates among genotypes (Buerstmayr, personal communication). Hence, the two FHB severity traits %FHB and AUDPC were highly correlated in all four populations (0,94 – 0,98). Nevertheless, as the AUDPC values represent the disease progress over time instead of a single scoring alone, this trait seems to be more reliable. Therefore, we still recommend relying on a number of FHB ratings during disease development.

Due to the onset of ripening processes and natural yellowing, the last scoring was performed 26 days after full anthesis so as not to confound effects of FHB and ripening. Afterwards, approximately three weeks passed by until harvesting. During this period, further fungal growth and disease expansion could have occurred. The higher ratings for the trait %FDK (varying from 5,0% to 99,5%) than for the trait %FHB could be seen as an indicator of increasing disease levels even at the late stages of plant development. The correlations between both FHB severity traits and the trait %FDK were moderate (0,33 for population 3) to high (0,83 for population 1). Medium to high correlations between FHB field scorings and the percentage of *Fusarium* damaged triticale kernels were also found by Arseniuk et al. (1999) and Miedaner et al. (2001a). Moderate correlations for the same traits were found by Góral et al. (2013). Again, we want to point at the earlier mentioned large environmental influence and the difficulty of visual FHB assessments in triticale as explanations for such differing results. Therefore, assessments on threshed kernels and mycotoxin analyses seem to be especially important in triticale.

## 4.2.) FHB resistance in the three RIL populations

Among all parental lines, the line *G8.06*, carrying the two resistance QTL *Fhb1* and *Qfhs.ifa-5A*, was least severely infected and had the lowest %FDK over all populations. The second parent in population 3, *Elpaso*, was following close behind

with slightly higher levels of infection. *Agostino*, the grandparental line of population 2, had even better disease ratings than *Elpaso*, but its percentage of *Fusarium* damaged kernels was much higher. *Tulus*, the second parental line in population 1, could be classified as being moderately resistant to FHB infections with overall approximately 28% of infected spikelets per plot for the fifth scoring. Nevertheless, it had a higher level of %FHB in population 1 (i.e. 36%) and similar %FDK scoring as *Agostino* and *Grenado* at around 44%. *Grenado* showed the highest level of FHB infections in the field.

The severity of FHB infections at the fifth and last rating ranged from 5,5% to 62,5%. The populations 1 and 2 had a similar pattern of distribution with significant differences among the parental lines. Interestingly, population 2 showed controversial results for the two traits representing FHB severity (i.e. %FHB and AUDPC) and the trait %FDK. For the FHB severity traits, population 2 had the highest mean and maximum levels, while for the trait %FDK the lowest values were found in this population. The grandparental line *Grenado* well represents this discrepancy. It was rated highest among all genotypes for the traits %FHB and AUDPC, but only had a moderate level of *Fusarium* damaged kernels. Góral et al. (2013) described *Grenado* as being medium susceptible to FHB, so the high levels of infection found in population 2 could be a deviation from the general behavior or the result of an overrated field infection level of *Grenado* in population 2. Alternatively, *Grenado* could contain FHB resistance genes against kernel infection, leading to lower kernel infections than assumed from the field ratings, a resistance mechanism that has been described by Mesterházy (1995). Another divergence in population 2 was found for the second grandparental line *Agostino* which showed low levels of FHB severity, but had the same level of %FDK as *Grenado* (i.e. 45%). All in all, population 2 showed similar mean infection levels as the other three populations. Due to the preponderance of additive gene effects for FHB resistance in triticale (Miedaner et al., 2006a; Oettler et al., 2004; Oettler and Wahle, 2001), the $F_1$ of the cross *Agostino* × *Grenado* (used as crossing partner for *G8.06*) might have had an intermediate FHB resistance level between its two parental lines. This intermediate level might rather resemble the degree of resistance found in the parental line *Tulus* in population 1, probably explaining why the distribution of FHB data was pretty similar in the two populations. The performance of the two grandparental lines might not be as closely

linked to the resistance level of the whole population as the performance of the $F_1$ parental line (that could not be included in the test).

The lowest mean and maximum levels for the FHB severity traits were found in population 3 (*G8.06* × *Elpaso*). For the trait %FDK on the other hand, intermediate levels were found with much better repeatability than for the traits %FHB and AUDPC. Hence, the data for the trait %FDK might best represent the FHB resistance level in population 3. Overall, the two parental lines showed low levels of FHB infections and did not differ significantly for any of the traits associated with FHB resistance. The frequency distributions are skewed towards resistance with some lines showing negative transgressions.

Altogether, a number of lines within the three RIL populations had lower levels of FHB infections than *G8.06*, but no transgressive segregants with significantly higher level of FHB resistance than *G8.06* were identified. The present data suggest that the triticale varieties *Tulus*, *Agostino*, *Grenado*, and *Elpaso* do not contribute other FHB resistance QTL than present in *G8.06* to the RIL populations. Nevertheless, selection of lines with low FHB levels seems possible within the three RIL populations.

## 4.3.) Variation for FHB resistance in the collection of registered cultivars and advanced breeding lines

A separate analysis of advanced breeding lines and registered varieties in population 4 revealed that the group of breeding lines from former FHB resistance trials at IFA-Tulln as such exhibited the best resistance among all populations tested. The registered cultivars in turn were more susceptible to FHB than any other population. Hence, an improvement in FHB resistance in triticale seems to be feasible.

In a number of previously conducted field trials in various environments, the resistant parental line *G8.06* had continuously displayed the lowest levels of FHB infections among all advanced triticale breeding lines from IFA-Tulln (Buerstmayr, personal communication). In our field trial in turn, the performance of *G8.06* was slightly surpassed by the line *F7.06*, selected from the same back-cross population of the highly resistant wheat line *CM-82036* (CIMMYT, Mexico) with the German triticale cultivar *Santop*. Line *F7.06* could especially be of interest as it was significantly lower in plant height than *G8.06*. In our field study, both lines had remarkably lower FHB

levels than their parental line *Santop*, which surprisingly exhibited already low to medium FHB resistance. For the two traits %FHB and AUDPC, significant differences were found between the line *F7.06* and *Santop*, but not for the trait %FDK. Calculated over all data, *G8.06* did not differ significantly from *Santop* for any of the FHB related traits. Considering the data for *G8.06* in population 4 only, significant differences to *Santop* could be found for the trait AUDPC (and almost for the trait %FHB). Further evaluations will be needed to determine the effect of the two resistance QTL *Fhb1* and *Qfhs.ifa-5A* in triticale and possible improvements of FHB resistance in the two breeding lines in comparison with their parental line.

Overall the three most widely grown cultivars in Austria in 2014, *Agostino*, *Triamant*, and *Elpaso* (BAES, 2014), showed low to medium levels of FHB infections in the field, as mentioned earlier. Yet, *Agostino* and *Triamant* exhibited higher levels of %FDK that might not be acceptable (i.e. 45% and 30%, respectively). Lines from FHB resistance breeding programs for future cultivar registration will have to surpass the FHB resistance level of these three important cultivars while showing good agronomic performance. Regarding the good performance and widespread cultivation of *Elpaso*, this task seems to remain challenging even for the lines with highest FHB resistance levels.

## 4.4.) Flowering date and its association with FHB resistance

The four populations tested showed very similar ranges of mean flowering dates, but differing distributions. Anthesis occurred over a period of two weeks from 20$^{th}$ of May until 3$^{rd}$ of June, 2014. Highly significant differences were found for both genotypes and replications in all four populations. Distinct deviations between the two replications could be expected, as the two replications were purposely sown several weeks apart, as mentioned earlier. Due to the inoculation technique (the whole replication was inoculated at once), the genotypes that reached anthesis last were inoculated only twice, while the genotypes flowering first were sprayed six or even seven times after they reached the stage of full anthesis. Although this procedure might dramatically increase the inoculum load for early flowering genotypes, pre-tests carried out at the IFA-Tulln clearly showed that FHB infection levels do not differ significantly whether there are only two inoculations or more – as long as all genotypes are inoculated at least once at full anthesis (the most susceptible growth stage) and FHB scorings are carried out in accordance to the specific flowering date

(Buerstmayr, personal communication). Similar results have been reported by Miedaner et al. (2006b). The trait flowering date was not stably correlated with any other plant trait. Low as well as high levels of FHB infections were found for early, intermediate, and late flowering genotypes. This indicates that the inoculation technique and the mist irrigation system used were adequate for FHB resistance assessments and that the weather conditions during inoculation were pretty similar for all genotypes.

## 4.5.) Plant height and its association with FHB resistance

Considerable differences among populations were found for the trait plant height. Repeatability values were high in all four populations. Overall, the triticale lines were much higher than most wheat and barley lines tested in our field nursery. The highest plants were found in population 2, while the registered cultivars in population 4 represented the smallest plants, as expected. Significant differences among genotypes were detected in all four populations. In populations 1 and 2, the factor replication was also significant. Although the triticale lines were very tall (up to 170 cm in single plots), lodging has never been observed. Apparently, triticale is a very stable, but elastic cereal crop. Therefore, the use of plant growth regulators was not necessary and cannot be recommended for future trials.

All three traits linked to FHB (i.e. %FHB, AUDPC, %FDK) showed a significant negative association with the trait plant height. In general, higher plants were less infected than smaller plants. The same has been manifold reported for natural conditions and for spray inoculated field trials (Buerstmayr et al., 2012; Couture, 1982; Hilton et al., 1999; Lu et al., 2013; Oettler and Wahle, 2001; Ransom, 2008). Plant height is considered to have an influence on FHB infection *per se*. Short plants tend to be exposed to higher humidity and are closer to the natural inoculum source (crop debris at the soil surface). Both factors promote the infection pressure. In published mapping studies of wheat only some FHB resistance QTL co-localize with plant height QTL, clearly showing that plant height *per se* does not seem to be the major factor involved in this association due to disease escape (Buerstmayr et al., 2009).

In all three RIL populations, smaller lines with good or even better FHB resistance than *G8.06* could be detected. Hence, for stability reasons (lodging could occur

under unfavorable conditions even if we did not observe it in 2014), the selection of smaller lines than *G8.06* with high FHB resistance seems to be feasible/reasonable.

## 4.6.) Powdery mildew and yellow rust and their associations with FHB resistance

Recent reports have indicated that triticale is becoming more susceptible to powdery mildew infections (Troch et al., 2012; Troch et al., 2013; Walker et al., 2011). In our study, higher levels of infection only occurred on individual lines. The mean infection levels were similar in all four populations and repeatability values were medium to high within individual populations. Genotypes differed significantly in all four populations. The lines *G8.06* and *Elpaso* seem to be more susceptible to powdery mildew than the other parental lines. The high variation for the traits FHB resistance and severity of powdery mildew infections might allow to select for lines with similar or better FHB resistance and higher powdery mildew resistance than line *G8.06*.

Although increasing levels of stripe rust have been reported for triticale in the last few years (Hovmøller and Justesen, 2007; Randhawa et al., 2012; Tian et al., 2004), we could observe higher levels of infection only on the registered variety *Mikado*. All other lines just showed low infection levels. Significant differences among genotypes were only detected in populations 1 and 4. While a large number of wheat lines in our nursery showed heavy symptoms of yellow rust infections, making fungicide applications necessary to prevent excessive yield losses, no such measurements seem to be required in triticale yet.

Correlations between the two foliar diseases powdery mildew and yellow rust and the traits related to FHB were low and not stable across populations. The average light levels of infections with the two leaf diseases might be responsible for these weak correlations. If higher disease severities of these two foliar diseases or comparable diseases occurred, FHB infection levels might be influenced significantly. Hence, for breeding purposes for FHB resistance, only low levels of other diseases are acceptable and appropriate control measurements have to be applied (Dill-Macky, 2003; Mesterházy, 2003a).

## 4.7.) Further research needed

The final goal of the project that the thesis at hand is involved in, is to find FHB resistant triticale lines with low mycotoxin accumulation in combination with good trait values for other important agronomic characteristics and high yield performance. In the first project year described in the present master thesis, neither mycotoxin analyses nor yield trials could be included due to labor and cost intensity of the mentioned tests. Both surveys will be carried out in later project years with pre-selected, promising triticale lines with low FHB scorings. Medium to high correlations of FHB ratings and/or %FDK with mycotoxin levels (especially deoxynivalenol) have been widely found in triticale FHB screenings (Arseniuk et al., 1999; Góral et al., 2013; Mesterházy, 2002; Miedaner et al., 2004; Perkowski et al., 1991; Veitch et al., 2008). Such associations indicate that pre-selection for lines with low FHB infection levels might lead to reduced mycotoxin contents in later generations. The same has been reported by Wilde and Miedaner (2006) who found that selection for FHB resistance in early generations reduced deoxynivalenol content in grain after inoculation. Veitch et al. (2008) also proposed to screen for low levels of FHB in early generations and add costly mycotoxin analyses later for pre-selected genotypes. Although high correlations between visual assessments of FHB and kernel mycotoxin contamination have often been observed, it is not always possible to predict mycotoxin levels from FHB scorings. On the one hand, high mycotoxin levels were also found in healthy looking grain (Miller et al., 1985; Perkowski et al., 1988), while on the other hand, lower mycotoxin levels than expected from visual scoring were detected in kernels due to degradation, detoxification or inhibited translocation (Boutigny et al., 2008; Lemmens et al., 2005; Mesterházy, 1995; Miller and Arnison, 1986; Miller et al., 1985; Snijders and Krechting, 1992). Therefore, mycotoxin analyses will have to be included if reliable data about mycotoxin accumulation in specific genotypes shall be obtained.

Significant positive correlations between FHB scorings and yield reductions were also noticed in triticale (Arseniuk et al., 1999; Oettler and Wahle, 2001). Hence, selection for more resistant triticale lines should also help in preventing higher yield losses. In the future, promising candidates should also be tested for their agronomic performance, as tests for yield components (e.g. kernel weight per spike, number of

kernels per spike, thousand kernel weight, test weight, etc.) (Oettler and Wahle, 2001) could not be included in the thesis at hand.

Repeated field trials in the upcoming years will hopefully help to improve the informative value of the present data. Again, we want to stress that because of the high level of genotype × environment interactions for FHB and other quantitative traits such as yield, genotypes will have to be tested in several environments (i.e. years, locations) to obtain reliable data about genotypic differentiation (Buerstmayr et al., 2008; Miedaner, 1997; Miedaner et al., 2001a; Oettler et al., 2004; Oettler and Wahle, 2001). Tests in multiple environments will lead to the selection of stably high yielding FHB resistant lines.

In addition to further phenotypic screenings, molecular based analyses (i.e. DNA extraction, marker analysis, QTL identification and mapping) will be conducted for selected triticale lines. The use of three RIL populations with the same resistance donor but three different rather susceptible cultivars will allow to develop genetic tools for FHB resistance improvement and their application in triticale breeding to select resistant triticale lines more efficiently.

# 5.) Conclusion

In the master thesis at hand, results of a field trial for Fusarium head blight (FHB) resistance in triticale conducted at the IFA-Tulln in 2014 have been presented. Three distinct inbred line populations and a fourth population of currently registered cultivars and advanced breeding lines were examined for several FHB related traits and other important parameters. Artificial inoculation techniques and a mist irrigation system were used to guarantee controlled environmental conditions for all plots.

Significant genotypic differences for traits linked to FHB resistance were found in all four populations. Among the parental lines, the FHB resistant line *G8.06*, donor of two major FHB resistance QTL, showed the highest level of resistance. On the other hand, *G8.06* also exhibited detrimental characteristics (e.g. larger plant height or higher levels of powdery mildew infections than FHB susceptible parental lines). In all three RIL populations, overall significant negative correlations between the traits FHB severity and plant height were found, indicating that the selection of smaller plants with high levels of FHB resistance is difficult. Nevertheless, several lines with similar or even better performance in FHB resistance than *G8.06* in combination with desirable agronomic features (e.g. smaller plant height) were observed in all three inbred line populations. These lines are promising candidates allowing the selection of highly resistant triticale lines in the future. On average, the group of currently registered varieties was characterized as being more susceptible than the other populations. These findings suggest that with the current breeding program significant progress can be made in regard to FHB resistance improvement in triticale.

Future investigations about mycotoxin accumulation and yield potential as well as molecular based analyses for selected lines will help to increase our knowledge about FHB resistance mechanisms in triticale and hopefully result in the selection of high yielding FHB resistant triticale lines for cultivar registration.

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# 7.) Appendix

## 7.1.) List of genotypes tested

Genotypes in red were still very inhomogeneous (homogeneity scoring of 6 or higher) and therefore excluded from statistical analysis. Abbreviations 'IFA-Tulln' and 'SZ Donau' in tables refer to the Institute of Biotechnology in Plant Production, Department of Agrobiotechnology, University of Natural Resources and Life Sciences (BOKU) and the Austrian breeding company Saatzucht Donau GesmbH. & CoKG, respectively.

| Population 1 $F_{4:5}$ (*G8.06* × *Tulus*) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Genotype** | **Breeder** | **Genotype** | **Breeder** | **Genotype** | **Breeder** | **Genotype** | **Breeder** |
| *G8.06* | IFA-Tulln | K07_53 | IFA-Tulln/SZ Donau | K07_107 | IFA-Tulln/SZ Donau | K07_152 | IFA-Tulln/SZ Donau |
| *Tulus* | Nordsaat Saatzucht GmbH | K07_54 | IFA-Tulln/SZ Donau | K07_109 | IFA-Tulln/SZ Donau | K07_154 | IFA-Tulln/SZ Donau |
| K07_5 | IFA-Tulln/SZ Donau | K07_55 | IFA-Tulln/SZ Donau | K07_110 | IFA-Tulln/SZ Donau | K07_155 | IFA-Tulln/SZ Donau |
| K07_7 | IFA-Tulln/SZ Donau | K07_56 | IFA-Tulln/SZ Donau | K07_111 | IFA-Tulln/SZ Donau | K07_156 | IFA-Tulln/SZ Donau |
| K07_8 | IFA-Tulln/SZ Donau | K07_57 | IFA-Tulln/SZ Donau | K07_112 | IFA-Tulln/SZ Donau | K07_157 | IFA-Tulln/SZ Donau |
| K07_10 | IFA-Tulln/SZ Donau | K07_58 | IFA-Tulln/SZ Donau | K07_113 | IFA-Tulln/SZ Donau | K07_158 | IFA-Tulln/SZ Donau |
| K07_13 | IFA-Tulln/SZ Donau | K07_59 | IFA-Tulln/SZ Donau | K07_115 | IFA-Tulln/SZ Donau | K07_159 | IFA-Tulln/SZ Donau |
| K07_15 | IFA-Tulln/SZ Donau | K07_60 | IFA-Tulln/SZ Donau | K07_116 | IFA-Tulln/SZ Donau | K07_160 | IFA-Tulln/SZ Donau |
| K07_16 | IFA-Tulln/SZ Donau | K07_67 | IFA-Tulln/SZ Donau | K07_117 | IFA-Tulln/SZ Donau | K07_161 | IFA-Tulln/SZ Donau |
| K07_17 | IFA-Tulln/SZ Donau | K07_71 | IFA-Tulln/SZ Donau | K07_118 | IFA-Tulln/SZ Donau | K07_162 | IFA-Tulln/SZ Donau |
| K07_18 | IFA-Tulln/SZ Donau | K07_72 | IFA-Tulln/SZ Donau | K07_120 | IFA-Tulln/SZ Donau | K07_163 | IFA-Tulln/SZ Donau |
| K07_19 | IFA-Tulln/SZ Donau | K07_73 | IFA-Tulln/SZ Donau | K07_121 | IFA-Tulln/SZ Donau | K07_164 | IFA-Tulln/SZ Donau |
| K07_20 | IFA-Tulln/SZ Donau | K07_74 | IFA-Tulln/SZ Donau | K07_123 | IFA-Tulln/SZ Donau | K07_165 | IFA-Tulln/SZ Donau |
| K07_21 | IFA-Tulln/SZ Donau | K07_75 | IFA-Tulln/SZ Donau | K07_125 | IFA-Tulln/SZ Donau | K07_166 | IFA-Tulln/SZ Donau |
| K07_22 | IFA-Tulln/SZ Donau | K07_76 | IFA-Tulln/SZ Donau | K07_126 | IFA-Tulln/SZ Donau | K07_168 | IFA-Tulln/SZ Donau |
| K07_23 | IFA-Tulln/SZ Donau | K07_77 | IFA-Tulln/SZ Donau | K07_127 | IFA-Tulln/SZ Donau | K07_169 | IFA-Tulln/SZ Donau |
| K07_26 | IFA-Tulln/SZ Donau | K07_78 | IFA-Tulln/SZ Donau | K07_128 | IFA-Tulln/SZ Donau | K07_170 | IFA-Tulln/SZ Donau |
| K07_28 | IFA-Tulln/SZ Donau | K07_79 | IFA-Tulln/SZ Donau | K07_129 | IFA-Tulln/SZ Donau | K07_171 | IFA-Tulln/SZ Donau |
| K07_31 | IFA-Tulln/SZ Donau | K07_80 | IFA-Tulln/SZ Donau | K07_131 | IFA-Tulln/SZ Donau | K07_173 | IFA-Tulln/SZ Donau |
| K07_32 | IFA-Tulln/SZ Donau | K07_82 | IFA-Tulln/SZ Donau | K07_132 | IFA-Tulln/SZ Donau | K07_174 | IFA-Tulln/SZ Donau |
| K07_33 | IFA-Tulln/SZ Donau | K07_83 | IFA-Tulln/SZ Donau | K07_134 | IFA-Tulln/SZ Donau | K07_175 | IFA-Tulln/SZ Donau |
| K07_34 | IFA-Tulln/SZ Donau | K07_84 | IFA-Tulln/SZ Donau | K07_137 | IFA-Tulln/SZ Donau | K07_176 | IFA-Tulln/SZ Donau |
| K07_35 | IFA-Tulln/SZ Donau | K07_85 | IFA-Tulln/SZ Donau | K07_138 | IFA-Tulln/SZ Donau | K07_177 | IFA-Tulln/SZ Donau |
| K07_36 | IFA-Tulln/SZ Donau | K07_88 | IFA-Tulln/SZ Donau | K07_139 | IFA-Tulln/SZ Donau | K07_178 | IFA-Tulln/SZ Donau |
| K07_37 | IFA-Tulln/SZ Donau | K07_91 | IFA-Tulln/SZ Donau | K07_140 | IFA-Tulln/SZ Donau | K07_180 | IFA-Tulln/SZ Donau |
| K07_38 | IFA-Tulln/SZ Donau | K07_92 | IFA-Tulln/SZ Donau | K07_141 | IFA-Tulln/SZ Donau | K07_181 | IFA-Tulln/SZ Donau |
| K07_39 | IFA-Tulln/SZ Donau | K07_93 | IFA-Tulln/SZ Donau | K07_142 | IFA-Tulln/SZ Donau | K07_184 | IFA-Tulln/SZ Donau |
| K07_41 | IFA-Tulln/SZ Donau | K07_94 | IFA-Tulln/SZ Donau | K07_143 | IFA-Tulln/SZ Donau | K07_185 | IFA-Tulln/SZ Donau |
| K07_43 | IFA-Tulln/SZ Donau | K07_95 | IFA-Tulln/SZ Donau | K07_144 | IFA-Tulln/SZ Donau | K07_186 | IFA-Tulln/SZ Donau |
| K07_44 | IFA-Tulln/SZ Donau | K07_97 | IFA-Tulln/SZ Donau | K07_145 | IFA-Tulln/SZ Donau | K07_187 | IFA-Tulln/SZ Donau |
| K07_45 | IFA-Tulln/SZ Donau | K07_98 | IFA-Tulln/SZ Donau | K07_146 | IFA-Tulln/SZ Donau | K07_190 | IFA-Tulln/SZ Donau |
| K07_46 | IFA-Tulln/SZ Donau | K07_99 | IFA-Tulln/SZ Donau | K07_147 | IFA-Tulln/SZ Donau | K07_191 | IFA-Tulln/SZ Donau |
| K07_48 | IFA-Tulln/SZ Donau | K07_101 | IFA-Tulln/SZ Donau | K07_148 | IFA-Tulln/SZ Donau | K07_192 | IFA-Tulln/SZ Donau |
| K07_49 | IFA-Tulln/SZ Donau | K07_103 | IFA-Tulln/SZ Donau | K07_149 | IFA-Tulln/SZ Donau | K07_193 | IFA-Tulln/SZ Donau |
| K07_50 | IFA-Tulln/SZ Donau | K07_104 | IFA-Tulln/SZ Donau | K07_150 | IFA-Tulln/SZ Donau | K07_196 | IFA-Tulln/SZ Donau |
| K07_52 | IFA-Tulln/SZ Donau | K07_105 | IFA-Tulln/SZ Donau | K07_151 | IFA-Tulln/SZ Donau | K07_198 | IFA-Tulln/SZ Donau |

| Population 2 $F_{4:5}$ (*G8.06* × ($F_1$ of *Agostino* × *Grenado*)) | | | | | |
|---|---|---|---|---|---|
| **Genotype** | **Breeder** | **Genotype** | **Breeder** | **Genotype** | **Breeder** |
| *G8.06* | IFA-Tulln | K08_69 | IFA-Tulln/SZ Donau | K08_132 | IFA-Tulln/SZ Donau |
| *Agostino* | Syngenta Cereals GmbH (Lantmännen SW Seed) | K08_70 | IFA-Tulln/SZ Donau | K08_135 | IFA-Tulln/SZ Donau |
| *Grenado* | Danko Plant Breeders LTD | K08_71 | IFA-Tulln/SZ Donau | K08_136 | IFA-Tulln/SZ Donau |
| K08_1 | IFA-Tulln/SZ Donau | K08_72 | IFA-Tulln/SZ Donau | K08_137 | IFA-Tulln/SZ Donau |
| K08_2 | IFA-Tulln/SZ Donau | K08_73 | IFA-Tulln/SZ Donau | K08_138 | IFA-Tulln/SZ Donau |
| K08_8 | IFA-Tulln/SZ Donau | K08_74 | IFA-Tulln/SZ Donau | K08_139 | IFA-Tulln/SZ Donau |
| K08_9 | IFA-Tulln/SZ Donau | K08_75 | IFA-Tulln/SZ Donau | K08_141 | IFA-Tulln/SZ Donau |
| K08_10 | IFA-Tulln/SZ Donau | K08_76 | IFA-Tulln/SZ Donau | K08_142 | IFA-Tulln/SZ Donau |
| K08_14 | IFA-Tulln/SZ Donau | K08_79 | IFA-Tulln/SZ Donau | K08_143 | IFA-Tulln/SZ Donau |
| K08_15 | IFA-Tulln/SZ Donau | K08_80 | IFA-Tulln/SZ Donau | K08_145 | IFA-Tulln/SZ Donau |
| K08_17 | IFA-Tulln/SZ Donau | K08_81 | IFA-Tulln/SZ Donau | K08_146 | IFA-Tulln/SZ Donau |
| K08_19 | IFA-Tulln/SZ Donau | K08_82 | IFA-Tulln/SZ Donau | K08_147 | IFA-Tulln/SZ Donau |
| K08_22 | IFA-Tulln/SZ Donau | K08_83 | IFA-Tulln/SZ Donau | K08_148 | IFA-Tulln/SZ Donau |
| K08_25 | IFA-Tulln/SZ Donau | K08_84 | IFA-Tulln/SZ Donau | K08_149 | IFA-Tulln/SZ Donau |
| K08_26 | IFA-Tulln/SZ Donau | K08_85 | IFA-Tulln/SZ Donau | K08_150 | IFA-Tulln/SZ Donau |
| K08_27 | IFA-Tulln/SZ Donau | K08_86 | IFA-Tulln/SZ Donau | K08_151 | IFA-Tulln/SZ Donau |
| K08_28 | IFA-Tulln/SZ Donau | K08_90 | IFA-Tulln/SZ Donau | K08_152 | IFA-Tulln/SZ Donau |
| K08_29 | IFA-Tulln/SZ Donau | K08_91 | IFA-Tulln/SZ Donau | K08_153 | IFA-Tulln/SZ Donau |
| K08_30 | IFA-Tulln/SZ Donau | K08_93 | IFA-Tulln/SZ Donau | K08_154 | IFA-Tulln/SZ Donau |
| K08_32 | IFA-Tulln/SZ Donau | K08_95 | IFA-Tulln/SZ Donau | K08_155 | IFA-Tulln/SZ Donau |
| K08_35 | IFA-Tulln/SZ Donau | K08_96 | IFA-Tulln/SZ Donau | K08_156 | IFA-Tulln/SZ Donau |
| K08_37 | IFA-Tulln/SZ Donau | K08_97 | IFA-Tulln/SZ Donau | K08_157 | IFA-Tulln/SZ Donau |
| K08_38 | IFA-Tulln/SZ Donau | K08_99 | IFA-Tulln/SZ Donau | K08_159 | IFA-Tulln/SZ Donau |
| K08_40 | IFA-Tulln/SZ Donau | K08_102 | IFA-Tulln/SZ Donau | K08_160 | IFA-Tulln/SZ Donau |
| K08_41 | IFA-Tulln/SZ Donau | K08_103 | IFA-Tulln/SZ Donau | K08_161 | IFA-Tulln/SZ Donau |
| K08_42 | IFA-Tulln/SZ Donau | K08_104 | IFA-Tulln/SZ Donau | K08_164 | IFA-Tulln/SZ Donau |
| K08_44 | IFA-Tulln/SZ Donau | K08_105 | IFA-Tulln/SZ Donau | K08_170 | IFA-Tulln/SZ Donau |
| K08_45 | IFA-Tulln/SZ Donau | K08_111 | IFA-Tulln/SZ Donau | K08_171 | IFA-Tulln/SZ Donau |
| K08_46 | IFA-Tulln/SZ Donau | K08_112 | IFA-Tulln/SZ Donau | K08_174 | IFA-Tulln/SZ Donau |
| K08_47 | IFA-Tulln/SZ Donau | K08_113 | IFA-Tulln/SZ Donau | K08_175 | IFA-Tulln/SZ Donau |
| K08_48 | IFA-Tulln/SZ Donau | K08_114 | IFA-Tulln/SZ Donau | K08_176 | IFA-Tulln/SZ Donau |
| K08_50 | IFA-Tulln/SZ Donau | K08_116 | IFA-Tulln/SZ Donau | K08_179 | IFA-Tulln/SZ Donau |
| K08_51 | IFA-Tulln/SZ Donau | K08_117 | IFA-Tulln/SZ Donau | K08_181 | IFA-Tulln/SZ Donau |
| K08_52 | IFA-Tulln/SZ Donau | K08_118 | IFA-Tulln/SZ Donau | K08_182 | IFA-Tulln/SZ Donau |
| K08_53 | IFA-Tulln/SZ Donau | K08_119 | IFA-Tulln/SZ Donau | K08_184 | IFA-Tulln/SZ Donau |
| K08_54 | IFA-Tulln/SZ Donau | K08_120 | IFA-Tulln/SZ Donau | K08_186 | IFA-Tulln/SZ Donau |
| K08_55 | IFA-Tulln/SZ Donau | K08_121 | IFA-Tulln/SZ Donau | K08_187 | IFA-Tulln/SZ Donau |
| K08_56 | IFA-Tulln/SZ Donau | K08_122 | IFA-Tulln/SZ Donau | K08_188 | IFA-Tulln/SZ Donau |
| K08_57 | IFA-Tulln/SZ Donau | K08_124 | IFA-Tulln/SZ Donau | K08_189 | IFA-Tulln/SZ Donau |
| K08_58 | IFA-Tulln/SZ Donau | K08_125 | IFA-Tulln/SZ Donau | K08_190 | IFA-Tulln/SZ Donau |
| K08_61 | IFA-Tulln/SZ Donau | K08_126 | IFA-Tulln/SZ Donau | K08_191 | IFA-Tulln/SZ Donau |
| K08_62 | IFA-Tulln/SZ Donau | K08_127 | IFA-Tulln/SZ Donau | K08_194 | IFA-Tulln/SZ Donau |
| K08_65 | IFA-Tulln/SZ Donau | K08_128 | IFA-Tulln/SZ Donau | K08_195 | IFA-Tulln/SZ Donau |
| K08_66 | IFA-Tulln/SZ Donau | K08_129 | IFA-Tulln/SZ Donau | K08_197 | IFA-Tulln/SZ Donau |
| K08_67 | IFA-Tulln/SZ Donau | K08_130 | IFA-Tulln/SZ Donau | K08_200 | IFA-Tulln/SZ Donau |
| K08_68 | IFA-Tulln/SZ Donau | K08_131 | IFA-Tulln/SZ Donau | K08_201 | IFA-Tulln/SZ Donau |

| Population 3 $F_{4:5}$ (*G8.06* × *Elpaso*) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Genotype** | **Breeder** | **Genotype** | **Breeder** | **Genotype** | **Breeder** | **Genotype** | **Breeder** |
| *G8.06* | IFA-Tulln | K09_32 | IFA-Tulln/SZ Donau | K09_61 | IFA-Tulln/SZ Donau | K09_93 | IFA-Tulln/SZ Donau |
| *Elpaso* | Danko Plant Breeders LTD | K09_33 | IFA-Tulln/SZ Donau | K09_62 | IFA-Tulln/SZ Donau | K09_94 | IFA-Tulln/SZ Donau |
| K09_1 | IFA-Tulln/SZ Donau | K09_34 | IFA-Tulln/SZ Donau | K09_63 | IFA-Tulln/SZ Donau | K09_95 | IFA-Tulln/SZ Donau |
| K09_3 | IFA-Tulln/SZ Donau | K09_36 | IFA-Tulln/SZ Donau | K09_64 | IFA-Tulln/SZ Donau | K09_96 | IFA-Tulln/SZ Donau |
| K09_4 | IFA-Tulln/SZ Donau | K09_37 | IFA-Tulln/SZ Donau | K09_65 | IFA-Tulln/SZ Donau | K09_97 | IFA-Tulln/SZ Donau |
| K09_5 | IFA-Tulln/SZ Donau | K09_38 | IFA-Tulln/SZ Donau | K09_66 | IFA-Tulln/SZ Donau | K09_98 | IFA-Tulln/SZ Donau |
| K09_6 | IFA-Tulln/SZ Donau | K09_39 | IFA-Tulln/SZ Donau | K09_67 | IFA-Tulln/SZ Donau | K09_99 | IFA-Tulln/SZ Donau |
| K09_8 | IFA-Tulln/SZ Donau | K09_40 | IFA-Tulln/SZ Donau | K09_68 | IFA-Tulln/SZ Donau | K09_100 | IFA-Tulln/SZ Donau |
| K09_9 | IFA-Tulln/SZ Donau | K09_41 | IFA-Tulln/SZ Donau | K09_69 | IFA-Tulln/SZ Donau | K09_101 | IFA-Tulln/SZ Donau |
| K09_10 | IFA-Tulln/SZ Donau | K09_42 | IFA-Tulln/SZ Donau | K09_70 | IFA-Tulln/SZ Donau | K09_102 | IFA-Tulln/SZ Donau |
| K09_11 | IFA-Tulln/SZ Donau | K09_43 | IFA-Tulln/SZ Donau | K09_71 | IFA-Tulln/SZ Donau | K09_103 | IFA-Tulln/SZ Donau |
| K09_12 | IFA-Tulln/SZ Donau | K09_44 | IFA-Tulln/SZ Donau | K09_73 | IFA-Tulln/SZ Donau | K09_104 | IFA-Tulln/SZ Donau |
| K09_13 | IFA-Tulln/SZ Donau | K09_45 | IFA-Tulln/SZ Donau | K09_74 | IFA-Tulln/SZ Donau | K09_105 | IFA-Tulln/SZ Donau |
| K09_14 | IFA-Tulln/SZ Donau | K09_46 | IFA-Tulln/SZ Donau | K09_75 | IFA-Tulln/SZ Donau | K09_106 | IFA-Tulln/SZ Donau |
| K09_16 | IFA-Tulln/SZ Donau | K09_47 | IFA-Tulln/SZ Donau | K09_76 | IFA-Tulln/SZ Donau | K09_107 | IFA-Tulln/SZ Donau |
| K09_17 | IFA-Tulln/SZ Donau | K09_48 | IFA-Tulln/SZ Donau | K09_77 | IFA-Tulln/SZ Donau | K09_108 | IFA-Tulln/SZ Donau |
| K09_18 | IFA-Tulln/SZ Donau | K09_49 | IFA-Tulln/SZ Donau | K09_78 | IFA-Tulln/SZ Donau | K09_110 | IFA-Tulln/SZ Donau |
| K09_20 | IFA-Tulln/SZ Donau | K09_50 | IFA-Tulln/SZ Donau | K09_79 | IFA-Tulln/SZ Donau | K09_111 | IFA-Tulln/SZ Donau |
| K09_21 | IFA-Tulln/SZ Donau | K09_51 | IFA-Tulln/SZ Donau | K09_80 | IFA-Tulln/SZ Donau | K09_113 | IFA-Tulln/SZ Donau |
| K09_22 | IFA-Tulln/SZ Donau | K09_52 | IFA-Tulln/SZ Donau | K09_81 | IFA-Tulln/SZ Donau | K09_114 | IFA-Tulln/SZ Donau |
| K09_23 | IFA-Tulln/SZ Donau | K09_53 | IFA-Tulln/SZ Donau | K09_82 | IFA-Tulln/SZ Donau | K09_115 | IFA-Tulln/SZ Donau |
| K09_24 | IFA-Tulln/SZ Donau | K09_54 | IFA-Tulln/SZ Donau | K09_83 | IFA-Tulln/SZ Donau | K09_116 | IFA-Tulln/SZ Donau |
| K09_26 | IFA-Tulln/SZ Donau | K09_55 | IFA-Tulln/SZ Donau | K09_85 | IFA-Tulln/SZ Donau | K09_117 | IFA-Tulln/SZ Donau |
| K09_27 | IFA-Tulln/SZ Donau | K09_56 | IFA-Tulln/SZ Donau | K09_86 | IFA-Tulln/SZ Donau | K09_119 | IFA-Tulln/SZ Donau |
| K09_28 | IFA-Tulln/SZ Donau | K09_57 | IFA-Tulln/SZ Donau | K09_88 | IFA-Tulln/SZ Donau | K09_120 | IFA-Tulln/SZ Donau |
| K09_29 | IFA-Tulln/SZ Donau | K09_58 | IFA-Tulln/SZ Donau | K09_89 | IFA-Tulln/SZ Donau | K09_121 | IFA-Tulln/SZ Donau |
| K09_30 | IFA-Tulln/SZ Donau | K09_59 | IFA-Tulln/SZ Donau | K09_90 | IFA-Tulln/SZ Donau | K09_122 | IFA-Tulln/SZ Donau |
| K09_31 | IFA-Tulln/SZ Donau | K09_60 | IFA-Tulln/SZ Donau | K09_91 | IFA-Tulln/SZ Donau | | |

| Population 4 (varieties and advanced breeding lines) | | | | | |
|---|---|---|---|---|---|
| **Genotype** | **Breeder** | **Genotype** | **Breeder** | **Genotype** | **Breeder** |
| *Agostino* | Syngenta Cereals GmbH (Lantmännen SW Seed) | *Mikado* | Danko Plant Breeders LTD | TRIT_B5.06 | IFA-Tulln |
| *Agrano* | Saka Pflanzenzucht GmbH & Co KG | *Mungis* | KWS Lochow GmbH | TRIT_B5.07 | IFA-Tulln |
| *Alekto* | Danko Plant Breeders LTD | *Palermo* | Danko Plant Breeders LTD | TRIT_B6.06 | IFA-Tulln |
| *Algoso* | Danko Plant Breeders LTD | *Polego* | Syngenta Cereals GmbH (Lantmännen SW Seed) | TRIT_B7.07 | IFA-Tulln |
| *Atletico* | Danko Plant Breeders LTD | *Presto (=Alamo)* | Probstdorfer Saatzucht GesmbH & CoKG | TRIT_B8.07 | IFA-Tulln |
| *Baltiko* | Danko Plant Breeders LTD | *Santop* | Saatzucht Dr. Hege GbRmbH | TRIT_C11.07 | IFA-Tulln |
| *Calorius* | Saatzucht Donau GesmbH & Co KG | *Securo* | Saatzucht Streng-Engelen GmbH & Co. KG | TRIT_C5.07 | IFA-Tulln |
| *Cosinus* | KWS Lochow GmbH | *Talentro* | Syngenta Cereals GmbH (Lantmännen SW Seed) | TRIT_C8.07 | IFA-Tulln |
| *Elpaso* | Danko Plant Breeders LTD | *Torino* | Danko Plant Breeders LTD | TRIT_D5.06 | IFA-Tulln |
| *Fredro* | Danko Plant Breeders LTD | *Triamant* | KWS Lochow GmbH | TRIT_D7.06 | IFA-Tulln |
| *Grenado* | Danko Plant Breeders LTD | *Tricanto* | Saatzucht Donau GesmbH & Co KG | TRIT_E1.07 | IFA-Tulln |
| *Gringo* | Danko Plant Breeders LTD | *Trimmer* | KWS Lochow GmbH | TRIT_E6.07 | IFA-Tulln |
| *Kaulos* | Syngenta Cereals GmbH (Lantmännen SW Seed) | *Trinidad* | Saatzucht Dr. Hege GbRmbH | TRIT_F3.07 | IFA-Tulln |
| *Kitaro* | Danko Plant Breeders LTD | *Trisidan* | Florimond Desprez Créations Variétales | TRIT_F7.06 | IFA-Tulln |
| *Koral* | Danko Plant Breeders LTD | *Trismart* | Danko Plant Breeders LTD | TRIT_F7.07 | IFA-Tulln |
| *Lasko* | Danko Plant Breeders LTD | *Tulus* | Nordsaat Saatzucht GmbH | TRIT_G8.06 | IFA-Tulln |
| *Madilo* | Danko Plant Breeders LTD | TRIT_A7.07 | IFA-Tulln | TRIT_H1.07 | IFA-Tulln |
| *Maestozo* | Danko Plant Breeders LTD | TRIT_A8.07 | IFA-Tulln | | |

## 7.2.) Preparation of inoculum

| | | |
|---|---|---|
| BOKU | Page: 1 von 7<br>Valid per: 20.05.2007 | Standard operating procedure<br>SOP 3-04 |
| | **Revision: 0** | **Production of Inoculum of *F. culmorum*** |

**Contents:**

## 1. Purpose / Principle

This SOP regulates the production of inoculum for plant inoculation experiments with *F. culmorum*. This SOP also regulates the handling of the inoculum and determination plus calculation of conidial concentrations, the area of application and the responsibility.

## 2. Area of application

Laboratory Fusarium Resistance Breeding, growth medium kitchen
IFA Tulln, Institut for Plant Production
responsible person for execution and calculation: Ing. Andrea Koutnik

## 3. Measurement principle/Basics

Seed a wheat-oat-mixture, swollen in babyfood-jars, with *F. culmorum*, grown on agar before, and leave it to grow for app. 2 weeks in diffuse daylight at room temperature. For better aeration and complete colonisation of the medium, vigorously shake the jars daily. Thereafter, store the jars in a refrigerator at 4 - 8°C. If needed, wash the kernels, count and calculate the conidia in the suspension.

## 4. Procedure data / Validating

Not available

## 5. Equipment, Equipment settings and Material

### *5.1. Equipment*

Autoclave Varioklav
LaminAir Heraeus
Microscope Nikon Labophot 2

### *5.2. Equipment settings*

Autoclave Varioklav: 121°C, 20 min, „forciert"
LaminAir: switch on 20 min before use, disinfect with alcohol directly before use
Microscope: Objective : 10 / 0,25 (yellow) and 40 / 0,55 (light blue)

### 5.3. Material

Wheat- oat- mixture (1 part oat and 2 parts wheat, volume/volume)
Baby food jars
plastic lid Magenta B-Cup, Sigma, part number: B86486.
Tea-filter
Collecting container (glass-flask, beaker)
Buerker Tuerk counting chamber

## 6. Chemicals

Not available

## 7. Analysis Procedure

### 7.1. Sample collection

Take the required Fusarium culture from the existing master culture collection at the IFA Tulln (laboratory/resistance breeding room: BP/E/24 B) and scatter it on SNA (special Nirenberg agar). After app. 72 h the grown Fusarium shall be used for the inoculation of the wheat - oat - mixture.

### 7.2. Sample Preparation

Put app. 50 g wheat - oat - mixture (1 part oat, 2 parts wheat) into baby food-jars, fill up with osmose water and leave it over night.. After app. 24 hours decant the surplus water and lock the glasses with plastic lids. Then autoclave at 121°C for 20 min, after cooling inoculate in the LaminAir with *F. culmorum* (one small piece of Fusarium-overgrown agar/jar).

Keep the inoculated, locked glasses for two weeks in diffuse daylight at room temperature, until the grains are well through-grown (brown-orange discoloration of the kernels). For better aeration and colonisation shake the jars (app. once per day). Subsequently the jars are stored in the refrigerator at 4-8°C.

For the production of inoculum of *F. culmorum* the glasses are filled with osmose water, shaken well and poured through a filter into a collecting container. Repeat the procedure again if necessary.

The conidia in this solution are counted and processed further accordingly.

### 7.3. Calibration

Not available

### 7.4. Measurement

Counting of the conidia takes place in a Buerker - Tuerk - chamber.

App. 10 small chambers are counted out and the average value/chamber is calculated. A small chamber corresponds to $25x10^{-5}$ mm³ = $25x10^{-8}$ ml

x conidia are in $25x10^{-8}$ ml
in 1 ml → y

Example: on average 6 conidia are contained in 25 x $10^{-8}$ ml

6 / 25 x $10^{-8}$ ml = 0,24 x $10^{8}$ conidia / ml =

C = 24 Mio. conidia/ml

### 7.5. Evaluation

The dilution factor is calculated with the following formula:

$$C1 \times V1 = C2 \times V2$$

C1 = known concentration of conidia-suspension
V1 = unknown volume of conidia-suspension
C2 = known concentration of inoculum
V2 = known volume of inoculum

Example:
From the example mentioned in Pt. 7.4 10 litres of inoculum in a concentration of 50.000 conidia/ml should be prepared.

$$V1 = \frac{50.000 \text{ con/ml} \times 10000 \text{ ml}}{24 \times 1.000.000 \text{ con/ml}} = 20{,}8 \text{ ml}$$

Production of the desired inoculum:
20,8 ml from the counted conidia-suspension are filled up to 10 L.

### 7.6. Handling

The conidia-suspension is labelled as follows in the desired concentrations in appropriate PP-tubes and frozen (in our example 20,8 ml of conidia-suspension is frozen):

| |
|---|
| Fusarium xy / IFA-Nr<br>Dissolved in xx L<br>Is equivalent to yy.yyy K/ml |

Example:

| |
|---|
| F.culmorum / IFA104<br>Dissolved in 10 L<br>Is equivalent to 50.000 K/ml |

The necessary quantity of frozen conidia-suspension tubes are thawed and vibrated in handwarm water and diluted accordingly to the data on the label.

### 7.7. Units of the results

The unit of concentration is K/ml (conidiens/ml).

The exact dilution data are, as described in Pt. 7.6., on the label.

## 8. Applicable Documents

Production of growth media (Test- SOP 3 - 03)
Manufacturing of master cultures
Laboratory order from 06 December 2005

## 9. Literature

Not available

## 10. Attachments

SOP-Validity proof
SOP-mailing list

**Contents:**

**Page**

## 1. Purpose / Principle

This SOP regulates the concentration measurement and further the calculation of conidia suspensions for inoculation.

Likewise the handling and calculation, the area of application and the responsibility.

## 2. Area of application

Laboratory Fusarium Resistance Breeding
IFA Tulln, Institute for Plant Production
responsible person for execution and calculation: Ing. Andrea Koutnik

## 3. Measurement principle/Basics

By laying a small quantity of conidia suspension on the Buerker Tuerk chamber and counting the conidia within exactly defined ranges (size and volumes of the individual counting squares are well-known) the concentration can be calculated and furthermore the desired inoculum concentration can be adjusted.

## 4. Procedure data / Validating

Not available

## 5. Equipment, Equipment settings and Material

### *5.1. Equipment*

Microscope Nikon Labophot 2

### *5.2. Equipment settings*

Microscope: Transmitted light, lamp middle strength
Objectives: PH1 10 / 0,25 (yellow) und PH3 40 / 0,65 (light blue)

### 5.3. Material

Bürker-Türk counting chamber (particularly partitioned slide)
Cover glasses for Haemacytometer 20 x 26 mm # 4
Pasteurpipettes (plastics or glass)

## 6. Chemicals

Not available

## 7. Analysis Procedure

### 7.1. Sample collection

Take a small quantity from a well mixed conidial suspension with a Pasteur pipette and apply app. 1-2 drops on the Buerker Tuerk chamber.

### 7.2. Sample Preparation

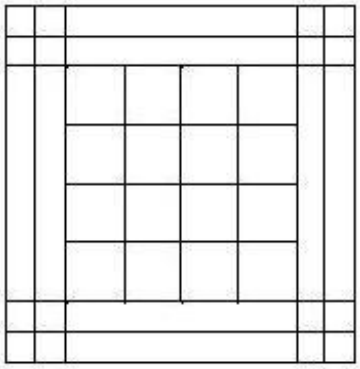
Apply the drop of the conidia suspension evenly on the chamber, so that the cover glass locks closely. By means of the objective PH1 10/0.25 examine if counting is possible and if the chamber is correctly filled, without air bubbles. If there are too many conidia in the suspension, dilute accordingly.

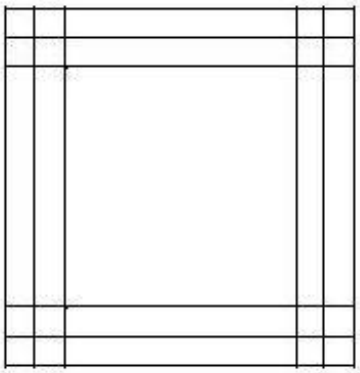
### 7.3. Calibration

Not available

### 7.4. Measurement

View of a counting unit:





Small squares V = 25 x $10^{-8}$ ml

Large squares V = 4 x $10^{-6}$ ml

Focus a counting unit with the objective PH3 40/0.65 exactly and count the conidia.
Proceed as follows:

**Small squares:** 10 squares are counted (in "z-form" = count 4 upper, 2 diagonal and 4 lower squares). Conidia, lying only partially within the squares are also counted partially! e.g.: if conidia only partially rise up into a small square which can be counted, they are counted as 0.5, or supplement with another conidium together as 1 conidium!

Several counting units in different places are counted and the average values of all countings per small square are calculated = x

A small square is $25x10^{-5}$ mm³ = $25x10^{-8}$ ml

x conidia are in $25x10^{-8}$ ml
in 1 ml → C1

Example: on the average 6 conidia are contained in 25 x $10^{-8}$ ml

6 / 25 x $10^{-8}$ ml = 0,24 x $10^{8}$ conidia / ml =

C1 = 24 Mio conidia/ml

**Large squares:** Several large squares in different places are counted, and again

(as explained above) conidia, lying only partially in the square, are counted also only partially! e.g.: if conidia only partially rise up into a large square which can be counted, they are counted as 0.5, or together supplement with another conidium as 1 conidium!

The average value of all squares is calculated = X

A large square corresponds to $4x10^{-3}$ mm³ = $4x10^{-6}$ ml

Example: 18 conidia are contained on the average in $4 \times 10^{-6}$ ml

$18 / 4 \times 10^{-6}$ ml = $4,5 \times 10^{6}$ conidia / ml =

C1 = 4,5 Mio conidia/ml

## 7.5. Evaluation

The dilution factor is calculated with following formula:

$$C1 \times V1 = C2 \times V2$$

C1 = known concentration of conidia suspension
V1 = unknown volume of conidia suspension
C2 = known concentration of inoculum
V2 = known volume of inoculum

Example:
From the example mentioned in Pt. 7.4 (Large squares) 2 litres of inoculum in a concentration of 100.000 conidia/ml should be produced.

$$V1 = \frac{100.000 \text{ Con/ml} \times 2000 \text{ ml}}{4,5 \times 1.000.000 \text{ Kon/ml}} = 44,4 \text{ml}$$

Production of the desired inoculum:
44,4 ml from the counted conidia suspension are diluted to 2 L.

## 7.3.) R commands

```
#Analysis of triticale field trial traits
#Analysis for homogeneous lines only (lines with homogeneity > 6 are
excluded)

#Read in all necessary data sets
#Set with all homogeneous lines and all traits
triticale<-read.table("Triticalehomogen.csv", header=T, sep=";", dec=",")
triticale$Plot<-as.factor(triticale$Plot)
triticale$Replication<-as.factor(triticale$Replication)
triticale$Name<-as.factor(triticale$Name)
triticale$Population<-as.factor(triticale$Population)
triticale$B1<-as.numeric(triticale$B1)
triticale$B2<-as.numeric(triticale$B2)
triticale$B3<-as.numeric(triticale$B3)
triticale$B4<-as.numeric(triticale$B4)
triticale$B5<-as.numeric(triticale$B5)
triticale$AUDPC5<-as.numeric(triticale$AUDPC5)
triticale$AUDPC4<-as.numeric(triticale$AUDPC4)
triticale$Heightmean<-as.numeric(triticale$Heightmean)
str(triticale)

#Set with means of all traits for genotypes (only homogeneous lines)
meangenotype<-read.table("Triticalemeangenotype.csv", header=T, sep=";", dec=",")
meangenotype$Name<-as.factor(meangenotype$Name)
meangenotype$Population<-as.factor(meangenotype$Population)
meangenotype$D30Aprilmean<-as.numeric(meangenotype$D30Aprilmean)
meangenotype$B1mean<-as.numeric(meangenotype$B1mean)
meangenotype$B2mean<-as.numeric(meangenotype$B2mean)
meangenotype$B3mean<-as.numeric(meangenotype$B3mean)
meangenotype$B4mean<-as.numeric(meangenotype$B4mean)
meangenotype$B5mean<-as.numeric(meangenotype$B5mean)
meangenotype$AUDPC5mean<-as.numeric(meangenotype$AUDPC5mean)
meangenotype$AUDPC4mean<-as.numeric(meangenotype$AUDPC4mean)
meangenotype$PercentFDKmean<-as.numeric(meangenotype$PercentFDKmean)
meangenotype$Heightmean<-as.numeric(meangenotype$Heightmean)
meangenotype$Powderymildewmean<-as.numeric(meangenotype$Powderymildewmean)
meangenotype$Homogeneitymean<-as.numeric(meangenotype$Homogeneitymean)
meangenotype$Yellowrustmean<-as.numeric(meangenotype$Yellowrustmean)
str(meangenotype)

#Set with means of all traits for genotypes within populations individually (only
homogeneous lines)
meangenotypepopulation<-read.table("Triticalemeangenotypepopulation.csv",
header=T, sep=";", dec=",")
meangenotypepopulation$Name<-as.factor(meangenotypepopulation$Name)
meangenotypepopulation$Population<-as.factor(meangenotypepopulation$Population)
meangenotypepopulation$D30Aprilmean<-
as.numeric(meangenotypepopulation$D30Aprilmean)
meangenotypepopulation$B1mean<-as.numeric(meangenotypepopulation$B1mean)
meangenotypepopulation$B2mean<-as.numeric(meangenotypepopulation$B2mean)
meangenotypepopulation$B3mean<-as.numeric(meangenotypepopulation$B3mean)
meangenotypepopulation$B4mean<-as.numeric(meangenotypepopulation$B4mean)
meangenotypepopulation$B5mean<-as.numeric(meangenotypepopulation$B5mean)
meangenotypepopulation$AUDPC5mean<-as.numeric(meangenotypepopulation$AUDPC5mean)
meangenotypepopulation$AUDPC4mean<-as.numeric(meangenotypepopulation$AUDPC4mean)
meangenotypepopulation$PercentFDKmean<-
as.numeric(meangenotypepopulation$PercentFDKmean)
```

```
meangenotypepopulation$Heightmean<-as.numeric(meangenotypepopulation$Heightmean)
meangenotypepopulation$Powderymildewmean<-
as.numeric(meangenotypepopulation$Powderymildewmean)
meangenotypepopulation$Homogeneitymean<-
as.numeric(meangenotypepopulation$Homogeneitymean)
meangenotypepopulation$Yellowrustmean<-
as.numeric(meangenotypepopulation$Yellowrustmean)
str(meangenotypepopulation)


#Remove missing data
triticalecorrect<-triticale[complete.cases(triticale),]
str(triticalecorrect)
homogeneitycorrect<-homogeneity[complete.cases(homogeneity),]
str(homogeneitycorrect)

#Attach data set triticalecorrect (without missing data)
attach(triticalecorrect)


#Create subsets with single populations
#All subsets do also include the parents of each population!
#Population 1
population1<-subset(triticalecorrect, Population=="1")
str(population1)
#Population 2
population2<-subset(triticalecorrect, Population=="2")
str(population2)
#Population 3
population3<-subset(triticalecorrect, Population=="3")
str(population3)
#Population 4
population4<-subset(triticalecorrect, Population=="4")
str(population4)
#Population 1 mean genotype
meangenotypepop1<-subset(meangenotypepopulation, Population=="1")
str(meangenotypepop1)
#Population 2 mean genotype
meangenotypepop2<-subset(meangenotypepopulation, Population=="2")
str(meangenotypepop2)
#Population 3 mean genotype
meangenotypepop3<-subset(meangenotypepopulation, Population=="3")
str(meangenotypepop3)
#Population 4 mean genotype
meangenotypepop4<-subset(meangenotypepopulation, Population=="4")
str(meangenotypepop4)
```

**For the following commands, there will only be a general form with 'TRAIT' representing all possible traits to be tested (i.e. scorings 1-5, AUDPC4, AUDPC5, %FDK, flowering date, plant height, powdery mildew, yellow rust).**

```
#Get general statistical parameters for each trait
#Analysis for all populations combined
summary(meangenotype$TRAIT)
#Analysis for single populations
#Population 1
summary(meangenotypepop1$TRAIT)
#Population 2
summary(meangenotypepop2$TRAIT)
#Population 3
summary(meangenotypepop3$TRAIT)
#Population 4
summary(meangenotypepop4$TRAIT)


#ANOVA
#All traits will be analyzed with the linear model
lm(TRAIT ~ Name + Replication)

#For specific TRAIT
modelTRAIT<-lm(TRAIT ~ Name + Replication)
pdf("ModelTRAIT_plots.pdf")
plot(modelTRAIT)
dev.off()
#Test for normal distribution of residuals (essential for ANOVA)
shapiro.test(modelTRAIT$residuals)
#ANOVA for TRAIT
anova(modelTRAIT)
summary(modelTRAIT)$r.squared
#ANOVAs for individual populations for specific TRAIT
anova(lm(population1$TRAIT ~ population1$Name + population1$Replication))
anova(lm(population2$TRAIT ~ population2$Name + population2$Replication))
anova(lm(population3$TRAIT ~ population3$Name + population3$Replication))
anova(lm(population4$TRAIT ~ population4$Name + population4$Replication))


#Correlation analysis of various traits (means of genotypes)
#For all populations
#TRAIT1 ~ TRAIT2
cor.test(meangenotype$TRAIT1mean, meangenotype$TRAIT2mean)

#For population 1
#TRAIT1 ~ TRAIT2
cor.test(meangenotypepop1$TRAIT1mean,meangenotypepop1$TRAIT2mean)

#For population 2
#TRAIT1 ~ TRAIT2
cor.test(meangenotypepop2$TRAIT1mean,meangenotypepop2$TRAIT2mean)

#For population 3
#TRAIT1 ~ TRAIT2
cor.test(meangenotypepop3$TRAIT1mean,meangenotypepop3$TRAIT2mean)

#For population 4
#TRAIT1 ~ TRAIT2
cor.test(meangenotypepop4$TRAIT1mean,meangenotypepop4$TRAIT2mean)
```

```
#Correlation analysis with scatterplots
#For all populations
#TRAIT1 ~ TRAIT2
plot(meangenotype$TRAIT1mean,meangenotype$TRAIT2mean, main="Scatterplot
TRAIT1/TRAIT2", xlab=" TRAIT1", ylab="TRAIT2", pch=16)
abline(lm(meangenotype$TRAIT2mean~meangenotype$TRAIT1mean))

#For population 1
#TRAIT1 ~ TRAIT2
plot(meangenotypepop1$TRAIT1mean,meangenotypepop1$TRAIT2mean, main="Scatterplot
TRAIT1/TRAIT2 Population 1", xlab=" TRAIT1", ylab="TRAIT2", pch=16)
abline(lm(meangenotypepop1$TRAIT2mean~meangenotypepop1$TRAIT1mean))

#For population 2
#TRAIT1 ~ TRAIT2
plot(meangenotypepop2$TRAIT1mean,meangenotypepop2$TRAIT2mean, main="Scatterplot
TRAIT1/TRAIT2 Population 2", xlab=" TRAIT1", ylab="TRAIT2", pch=16)
abline(lm(meangenotypepop2$TRAIT2mean~meangenotypepop2$TRAIT1mean))

#For population 3
#TRAIT1 ~ TRAIT2
plot(meangenotypepop3$TRAIT1mean,meangenotypepop3$TRAIT2mean, main="Scatterplot
TRAIT1/TRAIT2 Population 3", xlab=" TRAIT1", ylab="TRAIT2", pch=16)
abline(lm(meangenotypepop3$TRAIT2mean~meangenotypepop3$TRAIT1mean))

#For population 4
#TRAIT1 ~ TRAIT2
plot(meangenotypepop4$TRAIT1mean,meangenotypepop4$TRAIT2mean, main="Scatterplot
TRAIT1/TRAIT2 Population 4", xlab=" TRAIT1", ylab="TRAIT2", pch=16)
abline(lm(meangenotypepop4$TRAIT2mean~meangenotypepop4$TRAIT1mean))
```

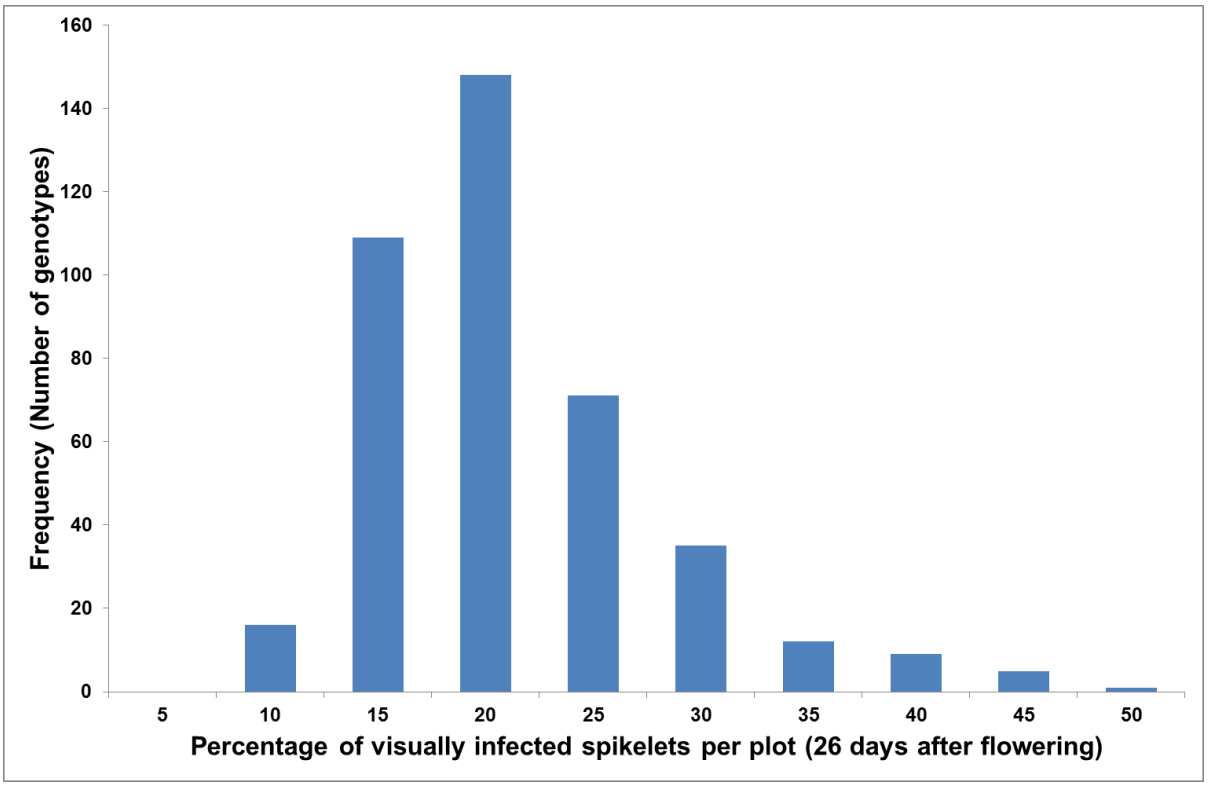
## 7.4.) Population 4 – Results for individual genotypes

The ranking of all genotypes of population 4 in the following table is depicted in order from low to high levels of %FDK (highest repeatability values among FHB traits).

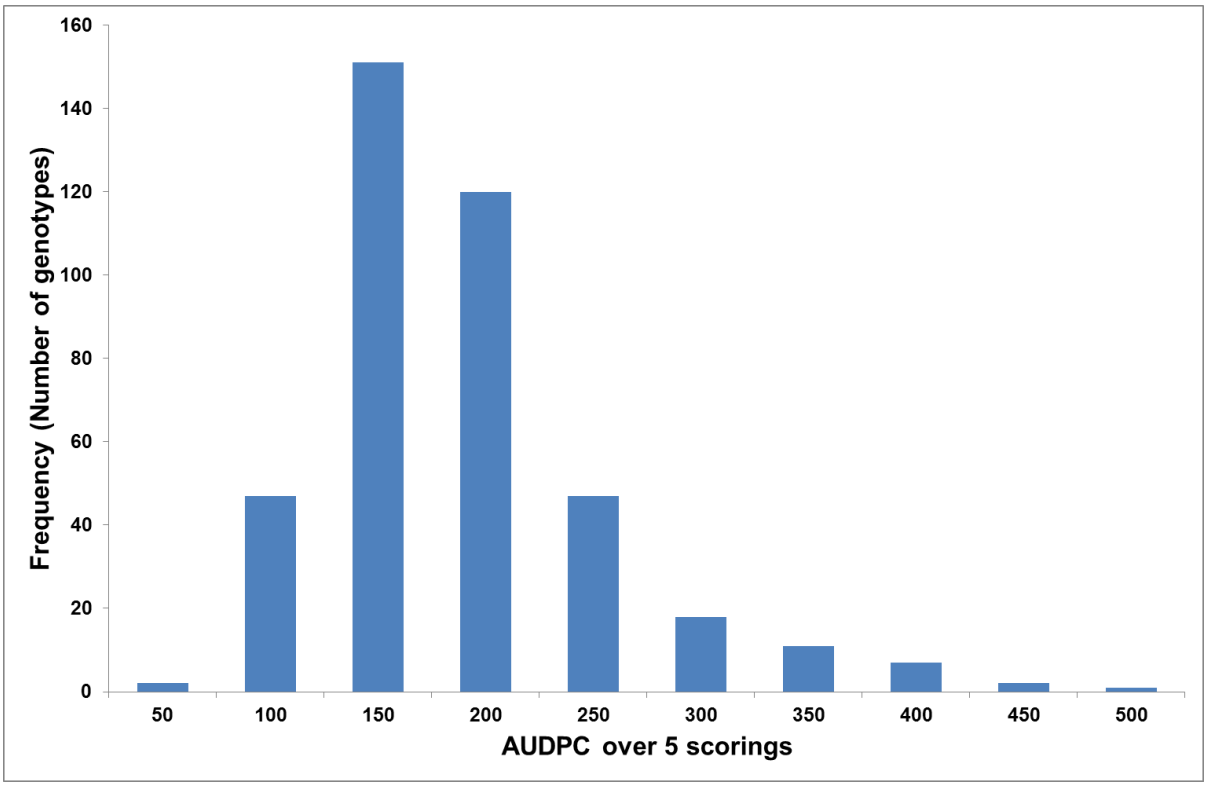
| Genotype | %FHB | AUDPC | %FDK | FLD | PLH | POM | YER |
|---|---|---|---|---|---|---|---|
| *TRIT_F7.06* | 5,5 | 47,4 | 5,0 | 25,0 | 120,0 | 3,5 | 1,5 |
| *G8.06* | 9,0 | 61,6 | 9,0 | 25,0 | 135,0 | 3,0 | 1,0 |
| *TRIT_B6.06* | 21,0 | 157,7 | 10,0 | 23,0 | 112,5 | 3,0 | 2,5 |
| *TRIT_C5.07* | 13,5 | 106,6 | 12,5 | 25,0 | 122,5 | 3,0 | 1,5 |
| *TRIT_F3.07* | 13,5 | 121,7 | 12,5 | 25,0 | 120,0 | 3,0 | 1,5 |
| *TRIT_F7.07* | 17,0 | 144,7 | 12,5 | 25,0 | 117,5 | 3,5 | 1,5 |
| *TRIT_D5.06* | 23,5 | 228,4 | 12,5 | 25,0 | 115,0 | 4,0 | 1,5 |
| *TRIT_B8.07* | 13,5 | 109,4 | 15,0 | 25,0 | 120,0 | 3,0 | 1,5 |
| *TRIT_H1.07* | 13,5 | 95,4 | 15,0 | 26,0 | 127,5 | 3,0 | 1,5 |
| *Elpaso* | 15,0 | 122,7 | 15,0 | 24,0 | 122,5 | 3,5 | 2,0 |
| *TRIT_A8.07* | 15,0 | 119,7 | 15,0 | 25,0 | 120,0 | 3,5 | 1,5 |
| *TRIT_B5.07* | 15,0 | 112,6 | 15,0 | 25,0 | 125,0 | 3,0 | 1,5 |
| *TRIT_C8.07* | 20,0 | 152,7 | 15,0 | 26,0 | 110,0 | 3,0 | 1,0 |
| *Santop* | 21,5 | 184,7 | 15,0 | 24,0 | 115,0 | 2,5 | 2,0 |
| *Tricanto* | 12,5 | 93,4 | 17,5 | 25,0 | 130,0 | 2,5 | 1,0 |
| *TRIT_B7.07* | 14,0 | 118,7 | 17,5 | 25,0 | 122,5 | 3,0 | 2,0 |
| *TRIT_A7.07* | 18,0 | 134,4 | 17,5 | 25,0 | 122,5 | 3,0 | 2,0 |
| *Palermo* | 15,0 | 112,0 | 20,0 | 28,0 | 105,0 | 2,0 | 2,0 |
| *TRIT_E1.07* | 16,0 | 122,7 | 20,0 | 26,0 | 130,0 | 3,0 | 1,0 |
| *TRIT_C11.07* | 20,0 | 168,7 | 20,0 | 26,0 | 125,0 | 3,0 | 1,0 |
| *TRIT_D7.06* | 15,5 | 121,7 | 25,0 | 25,0 | 115,0 | 2,5 | 2,0 |
| *Cosinus* | 19,0 | 160,4 | 25,0 | 25,0 | 120,0 | 3,0 | 2,0 |
| *TRIT_E6.07* | 19,0 | 155,7 | 25,0 | 25,0 | 125,0 | 3,5 | 1,5 |
| *Trinidad* | 21,0 | 181,1 | 25,0 | 25,0 | 125,0 | 3,5 | 2,0 |
| *TRIT_B5.06* | 23,5 | 153,7 | 25,0 | 25,0 | 127,5 | 2,5 | 2,0 |
| *Torino* | 30,0 | 234,1 | 25,0 | 24,0 | 115,0 | 2,5 | 2,0 |
| *Presto* | 24,3 | 190,1 | 26,3 | 25,5 | 125,0 | 2,8 | 1,8 |
| *Mungis* | 18,0 | 132,2 | 27,5 | 26,0 | 120,0 | 3,0 | 1,5 |
| *Kitaro* | 29,5 | 254,7 | 27,5 | 25,0 | 112,5 | 3,0 | 2,0 |
| *Capo* | 17,0 | 114,7 | 30,0 | 28,0 | 120,0 | 4,0 | 1,0 |
| *Trimmer* | 21,0 | 174,7 | 30,0 | 23,0 | 117,5 | 3,5 | 2,5 |
| *Triamant* | 22,0 | 186,7 | 30,0 | 24,0 | 112,5 | 2,0 | 2,0 |
| *Securo* | 16,0 | 148,1 | 32,5 | 27,0 | 125,0 | 2,0 | 1,5 |
| *Polego* | 21,0 | 169,7 | 32,5 | 23,0 | 120,0 | 2,5 | 1,0 |
| *Calorius* | 11,5 | 90,7 | 35,0 | 25,0 | 125,0 | 2,0 | 1,5 |
| *Trisidan* | 19,0 | 150,6 | 35,0 | 24,0 | 115,0 | 2,5 | 1,5 |
| *Mikado* | 27,0 | 271,9 | 35,0 | 27,0 | 105,0 | 2,0 | 7,5 |
| *Lasko* | 29,5 | 193,9 | 35,0 | 27,0 | 132,5 | 2,5 | 2,0 |
| *Fredro* | 17,5 | 150,0 | 37,5 | 25,0 | 107,5 | 2,5 | 2,0 |
| *Kaulos* | 26,0 | 234,4 | 37,5 | 25,0 | 105,0 | 2,5 | 2,0 |
| *Baltiko* | 35,0 | 351,5 | 37,5 | 25,0 | 87,5 | 2,5 | 3,0 |
| *Agrano* | 15,5 | 119,7 | 40,0 | 24,0 | 122,5 | 2,0 | 1,5 |
| *Maestozo* | 20,0 | 167,7 | 40,0 | 25,0 | 120,0 | 2,5 | 2,5 |
| *Tulus* | 20,5 | 166,0 | 40,0 | 26,0 | 107,5 | 2,0 | 2,0 |
| *Grenado* | 26,0 | 283,0 | 42,5 | 27,0 | 95,0 | 2,0 | 1,5 |
| *Alekto* | 23,0 | 215,4 | 43,8 | 26,0 | 95,0 | 2,3 | 1,5 |
| *Koral* | 16,5 | 140,4 | 45,0 | 25,0 | 117,5 | 2,5 | 2,0 |
| *Agostino* | 11,0 | 62,4 | 47,5 | 26,0 | 100,0 | 2,0 | 1,5 |
| *Talentro* | 18,0 | 154,7 | 48,8 | 26,0 | 97,5 | 2,0 | 1,8 |
| *Algoso* | 28,5 | 212,7 | 50,0 | 27,0 | 115,0 | 3,5 | 2,0 |
| *Madilo* | 27,0 | 192,0 | 52,5 | 25,0 | 117,5 | 2,5 | 1,5 |
| *Atletico* | 26,0 | 255,3 | 67,5 | 26,0 | 97,5 | 2,0 | 2,0 |
| *Trismart* | 22,5 | 162,7 | 82,5 | 27,0 | 122,5 | 3,0 | 2,5 |
| *Gringo* | 38,5 | 333,1 | 99,5 | 24,0 | 97,5 | 2,0 | 2,5 |

## 7.5.) Frequency distribution of data for all populations combined

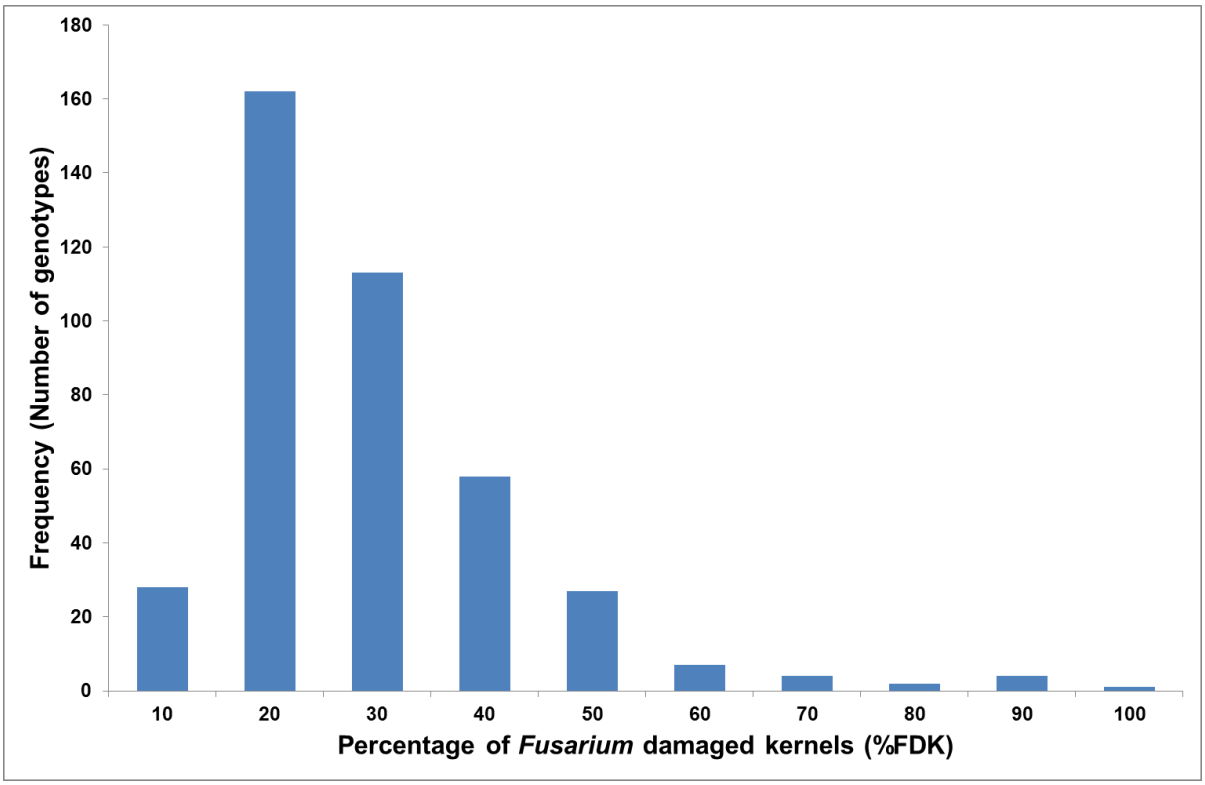
**For the trait %FHB:**



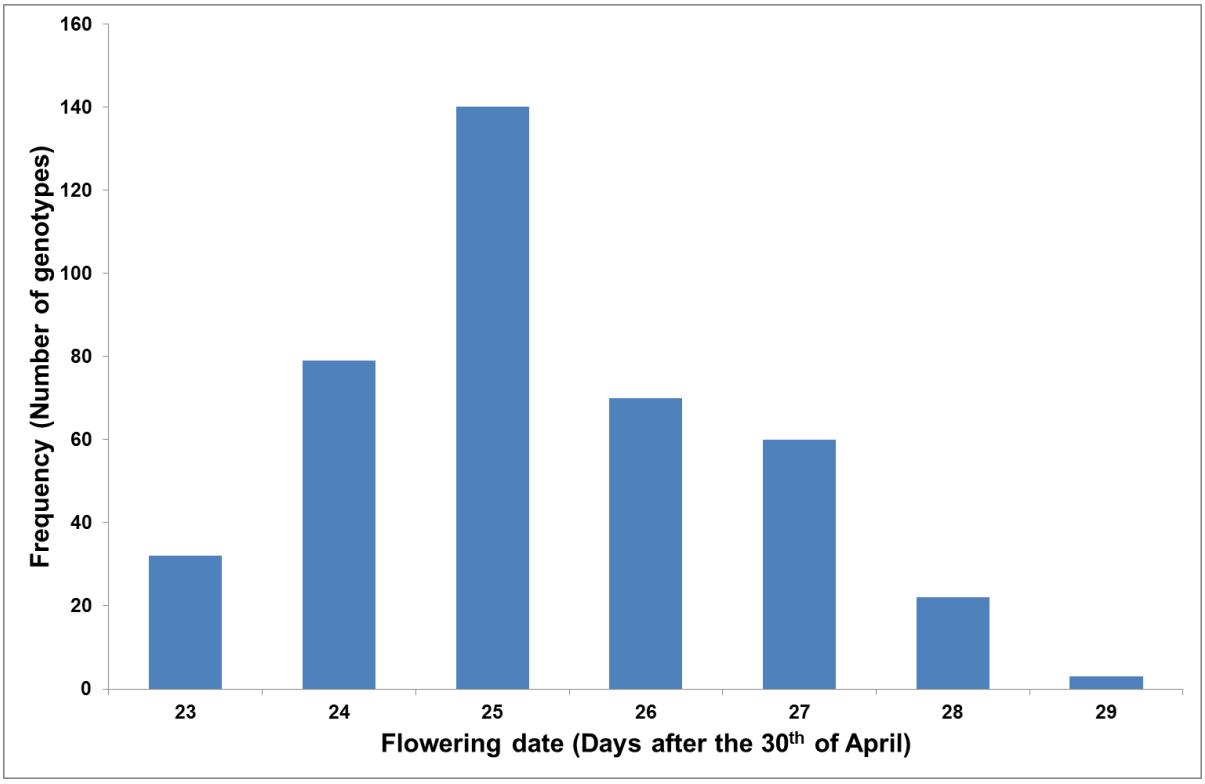

**For the trait AUDPC:**



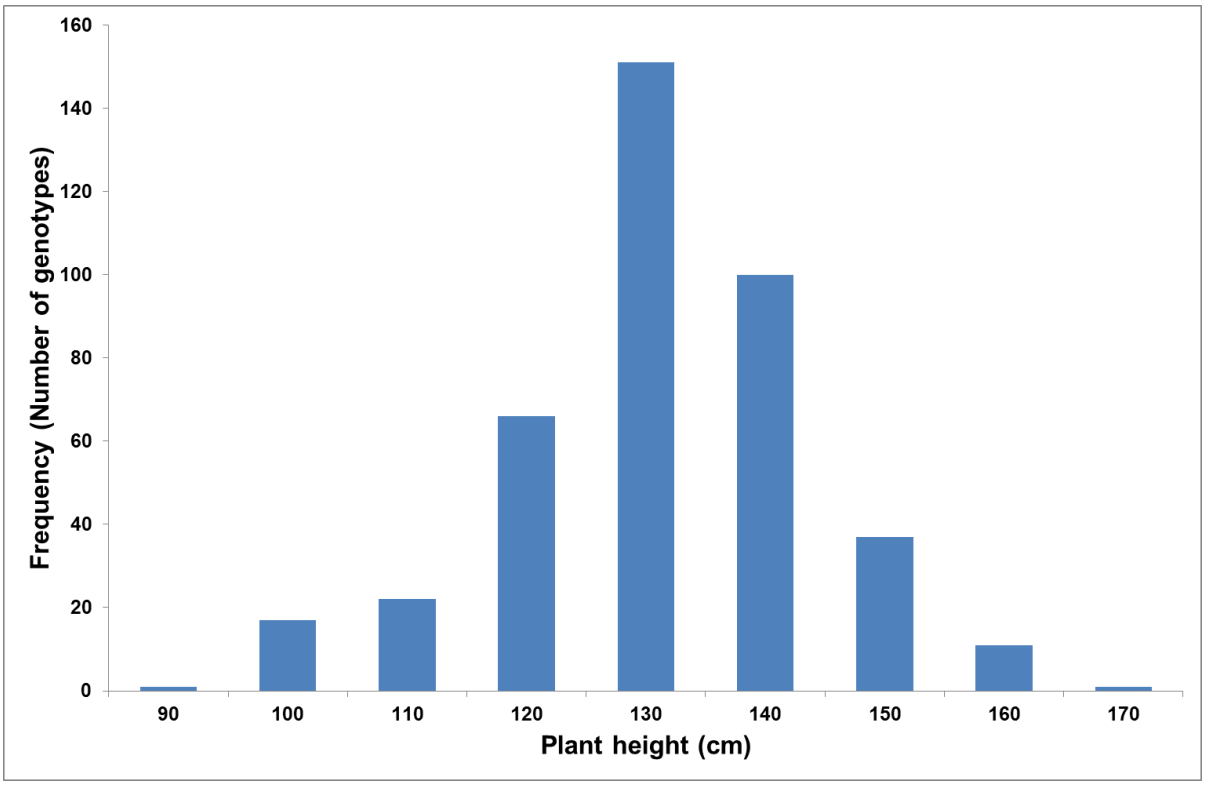
**For the trait %FDK:**



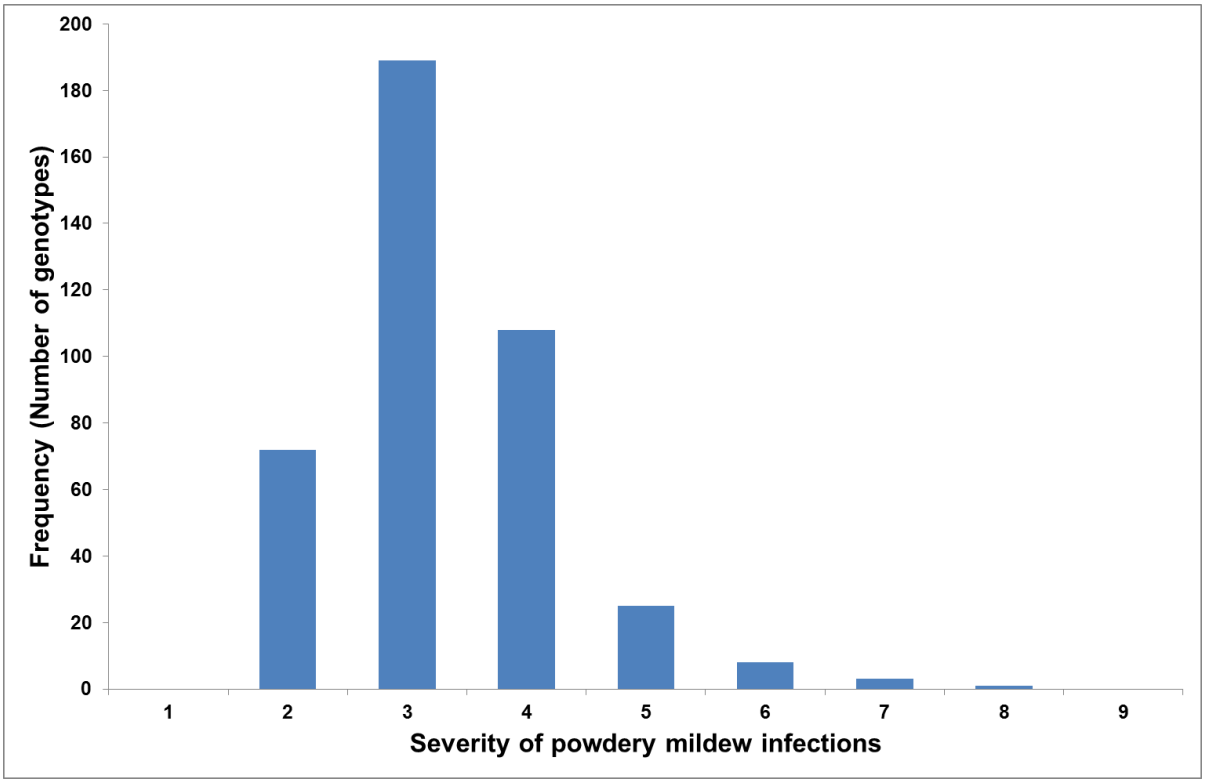

**For the trait FLD:**



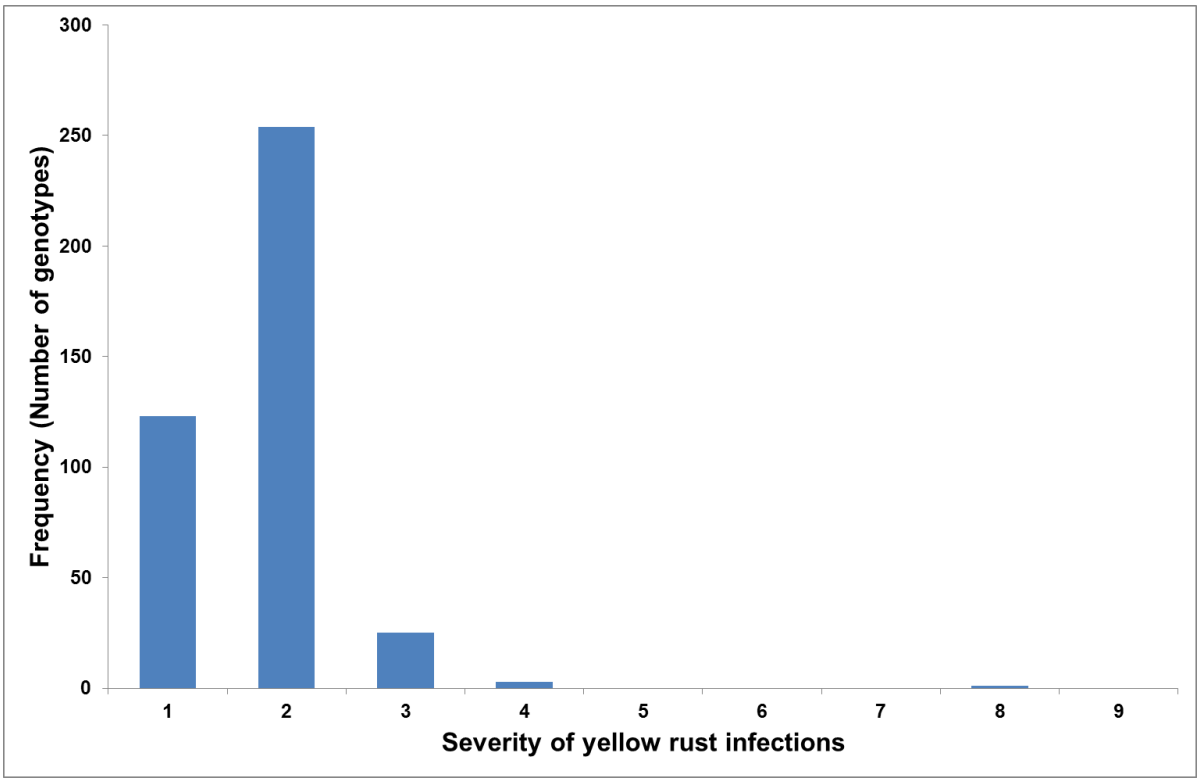
**For the trait PLH:**




**For the trait POM:**



**For the trait YER:**



## 7.6.) ANOVA results for individual populations

**For the trait %FHB:**

*Population 1*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 140 | 14518 | 103,7 | 5,38 | <0,001 |
| **Replication** | 1 | 2715 | 2714,98 | 140,85 | <0,001 |
| **Residuals** | 140 | 2698,5 | 19,28 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 2*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 116 | 14942,4 | 128,81 | 6,5836 | <0,001 |
| **Replication** | 1 | 626,9 | 626,88 | 32,0395 | <0,001 |
| **Residuals** | 116 | 2269,6 | 19,57 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 3*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 100 | 5748,4 | 57,484 | 2,2243 | <0,001 |
| **Replication** | 1 | 2,7 | 2,699 | 0,1044 | 0,747 |
| **Residuals** | 97 | 2506,8 | 25,843 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 4*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 53 | 4300,4 | 81,140 | 2,0265 | 0,005 |
| **Replication** | 1 | 132,6 | 132,576 | 3,3111 | 0,074 |
| **Residuals** | 53 | 2122,1 | 40,039 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

**For the trait AUDPC:**

*Population 1*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 140 | 1063324 | 7595 | 5,8779 | <0,001 |
| **Replication** | 1 | 533841 | 533841 | 413,1372 | <0,001 |
| **Residuals** | 140 | 180903 | 1292 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 2*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 116 | 1811949 | 15620 | 8,0044 | <0,001 |
| **Replication** | 1 | 92082 | 92082 | 47,1863 | <0,001 |
| **Residuals** | 116 | 226369 | 1951 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 3*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 100 | 656382 | 6563,8 | 1,9531 | <0,001 |
| **Replication** | 1 | 4529 | 4529,3 | 1,3477 | 0,249 |
| **Residuals** | 97 | 325994 | 3360,8 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 4*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 53 | 417193 | 7871,6 | 2,7731 | <0,001 |
| **Replication** | 1 | 4368 | 4368,1 | 1,5388 | 0,220 |
| **Residuals** | 53 | 150442 | 2838,5 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

**For the trait %FDK:**

*Population 1*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 140 | 63371 | 452,6 | 9,7895 | <0,001 |
| **Replication** | 1 | 5013 | 5013,2 | 108,4218 | <0,001 |
| **Residuals** | 140 | 6473 | 46,2 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 2*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 116 | 32405 | 279,35 | 4,3979 | <0,001 |
| **Replication** | 1 | 506 | 505,71 | 7,9615 | 0,005 |
| **Residuals** | 116 | 7368 | 63,52 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 3*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 100 | 28300,4 | 283,004 | 3,4094 | <0,001 |
| **Replication** | 1 | 22,9 | 22,903 | 0,2759 | 0,601 |
| **Residuals** | 97 | 8051,6 | 83,006 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 4*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 53 | 34305 | 647,26 | 5,6853 | <0,001 |
| **Replication** | 1 | 2514 | 2513,61 | 22,0789 | <0,001 |
| **Residuals** | 53 | 6034 | 113,85 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

**For the trait FLD:**

*Population 1*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 140 | 545,13 | 3,89 | 2,4636 | <0,001 |
| **Replication** | 1 | 1516,72 | 1516,72 | 959,6192 | <0,001 |
| **Residuals** | 140 | 221,28 | 1,58 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 2*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 116 | 260,24 | 2,243 | 2,4134 | <0,001 |
| **Replication** | 1 | 316,17 | 317,171 | 340,1294 | <0,001 |
| **Residuals** | 116 | 107,83 | 0,930 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 3*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 100 | 310,734 | 3,107 | 3,6666 | <0,001 |
| **Replication** | 1 | 313,796 | 313,796 | 370,2761 | <0,001 |
| **Residuals** | 97 | 82,204 | 0,847 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 4*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 53 | 119,074 | 2,247 | 3,795 | <0,001 |
| **Replication** | 1 | 119,290 | 119,290 | 201,499 | <0,001 |
| **Residuals** | 53 | 31,377 | 0,592 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

**For the trait PLH:**

*Population 1*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 140 | 12729 | 90,92 | 4,675 | <0,001 |
| **Replication** | 1 | 380,3 | 380,34 | 19,556 | <0,001 |
| **Residuals** | 140 | 2722,8 | 19,45 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 2*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 116 | 74373 | 641,15 | 34,810 | <0,001 |
| **Replication** | 1 | 1538 | 1538,46 | 83,528 | <0,001 |
| **Residuals** | 116 | 2137 | 18,42 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 3*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 100 | 16157,4 | 161,574 | 8,1763 | <0,001 |
| **Replication** | 1 | 8,2 | 8,163 | 0,4131 | 0,522 |
| **Residuals** | 97 | 1916,8 | 19,761 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 4*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 53 | 12829,2 | 242,06 | 9,0529 | <0,001 |
| **Replication** | 1 | 70,4 | 70,373 | 2,6319 | 0,111 |
| **Residuals** | 53 | 1417,1 | 26,738 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

**For the trait POM:**

*Population 1*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 140 | 247,901 | 1,77072 | 4,8146 | <0,001 |
| **Replication** | 1 | 0,511 | 0,51064 | 0,13884 | 0,241 |
| **Residuals** | 140 | 51,489 | 0,36778 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 2*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 116 | 78,983 | 0,68089 | 5,6589 | <0,001 |
| **Replication** | 1 | 1,543 | 1,54274 | 12,8218 | <0,001 |
| **Residuals** | 116 | 13,957 | 0,12032 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 3*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 100 | 133,8 | 1,338 | 5,582 | <0,001 |
| **Replication** | 1 | 12,25 | 12,25 | 51,108 | <0,001 |
| **Residuals** | 97 | 23,25 | 0,2397 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 4*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 53 | 32,102 | 0,6057 | 2,6522 | <0,001 |
| **Replication** | 1 | 14,313 | 14,3128 | 62,6721 | <0,001 |
| **Residuals** | 53 | 12,104 | 0,2284 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

**For the trait YER:**

*Population 1*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 140 | 47,518 | 0,3394 | 1,7295 | <0,001 |
| **Replication** | 1 | 14,525 | 14,5248 | 74,0115 | <0,001 |
| **Residuals** | 140 | 27,475 | 0,1963 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 2*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 116 | 35,06 | 0,30224 | 1,3336 | 0,061 |
| **Replication** | 1 | 0,209 | 0,2094 | 0,9239 | 0,338 |
| **Residuals** | 116 | 26,291 | 0,22664 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 3*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 100 | 63,636 | 0,6364 | 1,241 | 0,143 |
| **Replication** | 1 | 17,76 | 17,7602 | 34,635 | <0,001 |
| **Residuals** | 97 | 49,74 | 0,5128 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares

*Population 4*

| Source of variance | Df | SS | MS | F-value | P-value |
|---|---|---|---|---|---|
| **Genotype** | 53 | 83,769 | 1,5805 | 6,9656 | <0,001 |
| **Replication** | 1 | 12,391 | 12,3907 | 54,6074 | <0,001 |
| **Residuals** | 53 | 12,026 | 0,2269 | | |

Df = degrees of freedom; SS = sum squares; MS = mean squares