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UDP-glucosyltransferase HvUGT13248 confers type II resistance to *Fusarium graminearum* in barley

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1 **Short title:** Function of UGT13248 in barley

2 **Title:** *HvUGT13248* Confers Type II Resistance to *Fusarium graminearum* in Barley

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24

25 **Abstract**

26 *Fusarium* head blight (FHB) of barley (*Hordeum vulgare*) causes yield losses and accumulation of  
27 trichothecene mycotoxins (e.g. deoxynivalenol (DON)) in grains. Glucosylation of DON to the  
28 nontoxic DON-3-*O*-glucoside (D3G) is catalyzed by UDP-glucosyltransferases (UGTs), e.g. barley  
29 UGT13248. We explored the natural diversity of *UGT13248* in 496 barley accessions and  
30 showed that all accessions tested likely carried functional alleles of *UGT13248*, as no genotypes  
31 tested showed **strongly** increased seedling sensitivity to DON. From a TILLING population, we  
32 identified two mutant alleles (T368I and H369Y) that, based on protein modeling, likely affect  
33 UDP-glucose binding of UGT13248. In DON feeding experiments, DON to D3G conversion was  
34 strongly reduced in spikes of these mutants compared to controls and plants overexpressing  
35 UGT13248 showed increased resistance to DON and increased DON to D3G conversion.  
36 Moreover, field grown plants carrying the T368I and H369Y mutations inoculated with *F.*  
37 *graminearum* showed increased FHB disease severity and reduced D3G production. Barley is  
38 generally considered to have type II resistance that limits the spread of *F. graminearum* from  
39 the infected spikelet to adjacent spikelets. Point inoculation experiments with *F. graminearum*  
40 showed increased spread of infection in T368I and H369Y across the spike compared to wild-  
41 type, while overexpression plants showed decreased spread of FHB symptoms. Confocal  
42 microscopy revealed that *F. graminearum* spread to distant rachis nodes in T368I and H369Y,  
43 but was arrested at the rachis node of the inoculated spikelet in wild-type plants. Taken  
44 together, UGT13248 confers type II resistance to FHB in barley via conjugation of DON to D3G.

45

## 46 INTRODUCTION

47 *Fusarium* Head Blight (FHB) is a devastating disease of small-grain cereals and can lead to  
48 severe crop losses by reducing crop yield and grain quality (Bai et al., 2018; Chen et al., 2019;  
49 Johns et al., 2022). It is caused by several species of the fungal genus *Fusarium*. *Fusarium*  
50 *graminearum* (teleomorph *Gibberella zeae*) is considered to be the primary cause of FHB in  
51 cereal crops globally, especially in wheat (Starkey et al., 2007; Xu and Nicholson, 2009).

52 *Fusarium spp.* produce several groups of mycotoxins, including trichothecenes, zearalenone,  
53 beauvericin, enniatins and fumonisins (Ferrigo et al., 2016; McCormick et al., 2011; Santini et  
54 al., 2012). Trichothecenes are a large family of sesquiterpenoids defined by their heterocyclic  
55 structure, which includes a 9,10-double bond and a 12,13-epoxide (Chen et al., 2019). Type B  
56 trichothecene mycotoxins, characterized by a keto group at C-8, are produced during *Fusarium*-  
57 plant interactions and include deoxynivalenol (DON), nivalenol (NIV), 15-*O*-acetyl-DON, and 3-  
58 *O*-acetyl-DON (McCormick et al., 2011; Varga et al., 2015), of which DON and NIV are among  
59 the most frequently detected mycotoxins in cereal grains worldwide (Lee and Ryu, 2017). The  
60 more recently discovered NX-toxins, such as the prominent NX-3 are structural analogs, but lack  
61 the C-8 keto-group (Varga et al., 2015). Trichothecene mycotoxins inhibit protein biosynthesis  
62 (McLaughlin et al., 1977) and their accumulation is coincident with the switch between  
63 biotrophic and necrotrophic growth of *F. graminearum*, indicating they play an important role  
64 in infection (Bushnell et al., 2003).

65 The biosynthetic enzymes for trichothecene production are encoded by 15 *TRI* genes in  
66 *F. graminearum* (Chen et al., 2019). The *TRI5* gene encodes trichodiene synthase, which is the  
67 first enzyme in the trichothecene biosynthetic pathway, and cyclizes farnesyl pyrophosphate to  
68 trichodiene (Hohn and Beremand, 1989). The ability of *F. graminearum* to produce  
69 trichothecenes is a prerequisite for full pathogenicity and fungal spread in wheat spikes, as *tri5*  
70 mutants can initially infect wheat, but the infection is restricted to the rachis node of the  
71 infected spikelet and does not spread throughout the spike (Bai et al., 2001; Jansen et al., 2005;  
72 Proctor et al., 1995). Hence, in wheat, tolerance to trichothecenes is required for the ability to  
73 limit spread of the infection in the spike (type II resistance), but not resistance to initial  
74 infection (type I resistance) (Jansen et al., 2005; Schroeder and Christensen, 1963). Conversion

75 of DON to DON-3-*O*-glucoside (D3G) reduced toxicity on wheat ribosomes (Poppenberger et al.,  
76 2003) and co-segregated with a major FHB resistance QTL (*Fhb1*) in wheat (Lemmens et al.,  
77 2005). UDP-glucosyl transferases (UGTs) catalyze DON to D3G conversion by transferring  
78 glucose from the UDP-glucose substrate to the hydroxyl group of carbon 3 of DON  
79 (Poppenberger et al., 2003). Heterologous expression of the barley *UGT13248* gene in wheat  
80 resulted in increased type II resistance to *F. graminearum*, underscoring the role of DON in  
81 overcoming type II resistance to FHB in wheat (Li et al., 2015).

82 In contrast to wheat, barley is generally considered to have innate type II resistance, and  
83 extensive spread of the disease throughout the spike is rare (Boddu et al., 2007; Steffenson,  
84 2003). Thus, FHB impacts the yield of wheat more than barley. However, because the lemma  
85 and palea are retained on barley kernels throughout harvest and processing, barley can  
86 accumulate high levels of mycotoxins, which are problematic for human and animal health  
87 (Steffenson, 2003). No major FHB resistance locus affecting plant immune signaling or  
88 biochemistry has been identified in barley to date. FHB resistance in barley is quantitatively  
89 inherited, and QTL associated with resistance are also associated with multiple agro-  
90 morphological traits that influence resistance, including plant height, heading date, spike  
91 morphology, including row type and spike density, as well as grain protein content (de la Peña  
92 et al., 1999; Huang et al., 2021; Massman et al., 2011; Mesfin et al., 2003; Zhu et al., 1999).  
93 Interestingly, while wild-type *F. graminearum* grew through the rachis node in the wheat cv.  
94 'Nandu' and a *tri5* mutant strain was restricted at the rachis node, both of these strains were  
95 arrested at the rachis node in the barley cv. 'Chevron' (Jansen et al., 2005). This demonstrates a  
96 strong correlation between trichothecene production and FHB disease progression in wheat,  
97 while the relationship between FHB disease progression and trichothecene production in barley  
98 remains unclear.

99 UDP-glucosyl transferases (UGTs) have been studied and functionally characterized in  
100 multiple plant species, and some of them have been shown to detoxify DON. In *Arabidopsis*  
101 *thaliana*, a cluster of six UGTs was identified, of which UGT73C5 (DOG1) and UGT73C4, were  
102 found to convert DON to D3G (Poppenberger et al., 2003; Schweiger et al., 2010). In barley,  
103 nine UGT genes were upregulated in response to *F. graminearum* (Boddu et al., 2006), three

104 UGT genes were expressed in response to a wild-type *F. graminearum* but not a *tri5* mutant  
105 strain (Boddu et al., 2007) and two UGTs were expressed in DON- but not water-treated barley  
106 plants (Gardiner et al., 2010). Four of these barley UGTs, UGT14077, UGT5876, UGT13248 and  
107 UGT19290, were expressed in yeast and grown on DON-containing media and only UGT13248  
108 was found to convert DON to D3G (Schweiger et al., 2010). Interestingly, the closest homolog to  
109 DOGT1 in barley, UGT5876, did not show activity to DON in yeast (Schweiger et al., 2010),  
110 suggesting that close protein homology does not necessarily predict substrate specificity in  
111 these proteins. Further, UGT14077 was found to glucosylate another *Fusarium* toxin,  
112 zearalenone (Michlmayr et al., 2017). Heterologous expression of UGT13248 in *Arabidopsis*  
113 *thaliana* and wheat resulted in DON-resistant seedlings and reduced FHB severity, respectively  
114 (Li et al., 2015; Shin et al., 2012). Both transgenic *Arabidopsis* and wheat rapidly conjugated  
115 DON to D3G. UGTs that glucosylated DON were identified in wheat (He et al., 2020; Kirana et  
116 al., 2022), *Aegilops tauschii* (Kirana et al., 2022), rice (Schweiger et al., 2013), sorghum  
117 (Schweiger et al., 2013) and *Brachypodium distachyon* (Schweiger et al., 2013). In *B. distachyon*,  
118 two UGTs Bradi5g03300, the closest *Brachypodium* homolog to barley UGT13248, and  
119 Bradi5g02780 were shown to catalyze DON to D3G conversion in yeast (Schweiger et al., 2013).  
120 Plants with mutations in Bradi5g03300 showed similar colonization upon point inoculation with  
121 *F. graminearum* as compared to wild-type *Brachypodium* plants. Moreover, they were more  
122 susceptible when spray inoculation was used, suggesting that Bradi5g03300 is involved in type  
123 I, but not type II resistance in *Brachypodium* (Pasquet et al., 2016). Bradi5g03300  
124 overexpression plants also showed increased root tolerance to DON (Pasquet et al., 2016).

125 Crystal structures for *Os79*, a rice UGT with high homology to UGT13248, complexed  
126 with the nonreactive co-substrate analog UDP-2-deoxy-2-fluoroglucose and trichothecene,  
127 which lacks the C3 hydroxyl group to which the glucose molecule is attached, as well as with  
128 UDP alone were reported (Wetterhorn et al., 2016). The *Os79* structure is similar to other  
129 known plant UGTs and contain the plant secondary product glycosyltransferase (PSPG) motif, a  
130 hallmark of UGT enzymes, which is considered part of the region interacting with the UDP-sugar  
131 co-substrate (Gachon et al., 2005; Vogt and Jones, 2000; Wetterhorn et al., 2016). The active  
132 site of *Os79* is located in a cleft between the C-terminal donor-binding and the N-terminal

133 acceptor domains (Wetterhorn et al., 2016). Using structural alignments, it was shown that  
134 *Os79* likely utilizes a catalytic mechanism similar to those of other plant UGTs, where His 27  
135 activates the trichothecene O3 hydroxyl for nucleophilic attack at C1 of the UDP-glucose donor  
136 molecule (Wetterhorn et al., 2016). Kinetic analysis of *Os79* mutants identified Thr 291 as  
137 required to position the UDP moiety during the nucleophilic attack or for catalysis as a catalytic  
138 acid (Wetterhorn et al., 2016). *Os79* conjugates multiple trichothecene substrates such as DON,  
139 nivalenol, isotrichodermol, and HT-2 toxin, but not T-2 toxin (Wetterhorn et al., 2016).  
140 Interestingly, three mutations in *Os79* yielded a H122A/L123A/Q202L *Os79* triple mutant that  
141 had an increased active site volume and showed activity on T-2 toxin (Wetterhorn et al., 2017).  
142 UGT13248 was able to conjugate nivalenol, whereas DOGT1 was not (Li et al., 2017).

143           DON is a virulence factor in wheat and detoxification of DON is clearly linked to  
144 increased type II resistance. However, it is unclear if this is true in other grasses, including *B.*  
145 *distachyon* and barley. We decided to study UGT13248 directly in barley to understand if  
146 detoxification of DON by conversion to D3G affects FHB disease progression in this crop. We  
147 first investigated natural variation in *UGT13248* to identify UGT13248 variants with altered  
148 function. In 496 barley accessions tested, no UGT13248 alleles that abolished function were  
149 identified, suggesting that UGT13248 function is highly conserved throughout the species. We  
150 next identified mutations with reduced UGT13248 function and plants overexpressing  
151 *UGT13248* to study the role of UGT13248 in barley-*Fusarium* interactions in detail. We found  
152 that UGT13248 confers type II resistance to *F. graminearum* in barley.

153

## 154 **RESULTS**

### 155 **Many barley accessions contain functional UGT13248 alleles**

156 Our previous research showed that barley UGT13248 converts DON to D3G in wheat,  
157 *Arabidopsis* and yeast (Li et al., 2015; Schweiger et al., 2010; Shin et al., 2012). Overexpression  
158 of UGT13248 in wheat resulted in rapid DON to D3G conversion and reduced FHB prevalence (Li  
159 et al., 2015). Here, we aimed to understand the function of UGT13248 directly in barley.  
160 Multiple groups have identified QTLs in barley that affect DON accumulation and FHB severity

161 (Huang et al., 2018; Massman et al., 2011); however, ~~the QTLs found generally affected agro-~~  
162 ~~morphological traits including plant height, heading date, or spike morphology (Massman et al.,~~  
163 ~~2011; Mesfin et al., 2003; Zhu et al., 1999).~~ *UGT13248* has not been identified as a contributor  
164 to FHB resistance in any of these QTL studies (Huang et al., 2018; Massman et al., 2011). We  
165 decided to screen a natural population of barley accessions for mutations in *UGT13248* that  
166 might affect *UGT13248* function. First, we compared the *UGT13248* protein sequences of eight  
167 susceptible and 18 resistant barley accessions (Supplemental Table 1; (Huang et al., 2013). We  
168 considered the *UGT13248* sequence of cv. 'Morex', used for the first barley whole genome  
169 sequencing project (Mayer et al., 2012), the wild-type allele, as *UGT13248* was identified  
170 initially in this cultivar (GU170355; (Schweiger et al., 2010). In these 26 accessions, two non-  
171 synonymous mutations were found. A104V was found in seven accessions and G213R in two  
172 accessions (Supplemental Figure 1A and Supplemental Table 1). Of these, only G213R was  
173 exclusively found in two accessions that are susceptible to FHB, PI383933, and ICB111809  
174 (Huang et al., 2013). We grew representative accessions that carried *UGT13248* alleles  
175 representing each mutation, and wild-type Morex plants on mock and DON-containing media  
176 and measured seedling root length for six days. While seedling root length on DON-containing  
177 media was reduced in the cv. 'Fredrickson' that carried the A104V mutations, other accessions  
178 carrying the same mutation did not show significantly reduced root growth (Supplemental  
179 Figure 1 B-D). None of the accessions containing mutations in *UGT13248* showed severely  
180 reduced root growth on DON-containing media (Supplemental Figure 1 B-D), suggesting that  
181 none of the mutations had a drastic effect on DON-induced root growth inhibition.

182 We next analyzed the *UGT13248* sequences of 19 additional barley accessions,  
183 sequenced as part of a barley pan-genome analysis (Jayakodi et al., 2020). We identified A104V  
184 in six additional accessions and G213R in seven additional accessions (Supplemental Figure 1A  
185 and Supplemental Table 1). We also identified an additional mutation in the accession FT 11/  
186 B1K-04-12, corresponding to R47H (Supplemental Figure 1A). No difference in seedling root  
187 growth was observed between mock and DON-containing media for FT11 (Supplemental Figure  
188 1E).



189 We next utilized protein modeling as a predictive tool to develop hypotheses if these  
190 mutations potentially affect UGT13248 function. SWISS MODEL blast identified the closest  
191 homolog to UGT13248 at 73 % amino acid sequence identity as rice *Os79*, for which a crystal  
192 structure has been resolved (Wetterhorn et al., 2016). We aligned the sequence used for the  
193 *Os79* crystal structure (PDB ID: 5TMD), the full-length *Os79* protein sequence, and the  
194 UGT13248 protein sequence (Supplemental Figure 2A). We used the *Os79* three-dimensional  
195 structure with trichothecene and the nonreactive co-substrate analog UDP-2-deoxy-2-  
196 fluoroglucose modeled within the active site cavity as a template for barley UGT13248 protein  
197 models. The resulting homology models of UGT13248 exhibited a similar Rossmann-fold type  
198 three-dimensional structure as *Os79*. In *Os79*, the proposed catalytic residues for initiating the  
199 glycosylation of trichothecene via deprotonation of the hydroxyl group are H27 and D120, while  
200 T291 is hypothesized to be involved in coordinating the UDP during the subsequent nucleophilic  
201 attack. Based on sequence alignments, the corresponding UGT13248 residues that could be  
202 hypothesized to act as the catalytic dyad are H38 and D130, and T299 could serve as the UDP-  
203 coordinating group (Supplemental Figure 2A). The homology models indicated that these three  
204 residues would likely be structurally positioned within an active site pocket similar to that of  
205 *Os79* and, therefore may be important for the correct function of UGT13248 (Supplemental  
206 Figure 2B). The structural positions of mutated residues in models of R47H, A104V, and G213R,  
207 indicated that these amino acids are not within close proximity with the UDP-glucose and  
208 trichothecene molecules (Supplemental Figure 2C-E). It was therefore considered less likely that  
209 these mutations would influence UGT13248 function.

210 To explore the genetic diversity of *UGT13248* in a larger natural population, we utilized  
211 **previously-generated** exome capture sequencing data (Chen et al., 2022; Hemshrot et al., 2019;  
212 Kono et al., 2019; Lei et al., 2019; Mascher et al., 2013; Nice et al., 2016; Russell et al., 2016) to  
213 screen for mutations in *UGT13248*. The data set included 83 elite lines, 188 wild barley  
214 accessions and 187 landraces. The exome capture sequencing data covered two regions of the  
215 Morex UGT13248 gene. One region covered a portion of exon 1 and intron 1, and the other  
216 region covered the majority of exon 2, intron 2, and exon 3 (Supplemental Figure 3A). The  
217 second region contains the PSPG box (Gachon et al., 2005; Vogt and Jones, 2000), thought to be

218 important for UDP-glucose binding, as well as the predicted residue for UDP positioning T299  
219 that had been identified during homology modeling (Supplemental Figure 2A). From this  
220 analysis, nine non-synonymous mutations were identified in 64 of these 458 accessions. These  
221 nine non-synonymous mutations were G213R, M281I, D282N, F385S, S403I, L419V, Y440L,  
222 S461P and F468L (Supplemental Figure 3A). G213R, which we identified in nine of the 45 barley  
223 accessions with full-length genomic DNA sequence data (Supplemental Table 1), was found in  
224 an additional 38 landraces and 3 elite cultivars using the exome capture sequencing approach  
225 (Supplemental Table 2). The other eight non-synonymous mutations were identified in seven  
226 landraces and 16 wild barley accessions (Supplemental Table 2). Analysis of the protein  
227 homology models of these mutants indicated that the mutated residues M281I, D282N, F385S,  
228 S403I, L419V, Y440L, S461P, and F468L were not structurally positioned within the catalytic site,  
229 leading us to hypothesize that none of these mutations would directly influence UGT13248  
230 catalytic function (Supplemental Figure 3B-I). To confirm, we screened 14 accessions,  
231 representing these other eight non-synonymous *UGT13248* mutations, and measured seedling  
232 root growth on mock and DON-containing media. Seedling root length on DON-containing  
233 media was slightly, but significantly reduced in FT222, which carries the M281I mutation, FT735  
234 that carries the F468L mutation, as well as two of six accessions that carry the D282N mutation,  
235 Hor2867, and PI57099 (Supplemental Figure 4 A, B, D and E). None of the accessions tested  
236 however exhibited a complete inhibition of root growth upon DON exposure (Supplemental  
237 Figure 4), suggesting that the mutations M281I, D282N, F385S, S403I, L419V, Y440L, S461P, and  
238 F468L did not have a deleterious effect on UGT13248-dependent DON to D3G conversion.  
239 Overall, none of the 11 mutations, identified in the 496 accessions analyzed, had a strong  
240 adverse effect on UGT13248 activity and all these lines likely contained functional UGT13248  
241 alleles. ~~Taken together, we conclude that mutations in UGT13248 are rare and likely do not~~  
242 ~~disrupt UGT 13248 protein function.~~

243

#### 244 **Mutations in UGT13248 or constitutive expression of UGT13248 affect root sensitivity to DON**

245 To further study the role of UGT13248 in barley, we decided to utilize plants that constitutively  
246 express UGT13248. We transformed *UGT13248* under the control of the constitutive *Zea mays*

247 *Ubiquitin (Ubi-1)* promotor into the barley cultivar 'Golden Promise' (Supplemental Figure 5A).  
248 We identified three independent insertion lines in Golden Promise (Supplemental Figure 5B). As  
249 Golden Promise does not reliably flower under our growth conditions, we crossed the  
250 transgenic lines with the cultivar 'Rasmusson'. We backcrossed the plants once with Rasmusson  
251 and then identified sets of sister lines that contained and expressed the *UGT13248* **transgene**  
252 **transcript and the UGT13248 protein** (UGT+) or did not contain **either** (UGT-) from two  
253 independent transgenic events (#39003 and #39009) (Supplemental Figure 5C **and E**). For both  
254 sets of sister lines the accession containing the transgene and expressing UGT13248 (UGT+) did  
255 not show significantly reduced seedling root growth two to six days after DON treatment, while  
256 the non-transgenic UGT- lines, **as well as Golden Promise and Rasmusson wild-type seedlings**  
257 did (Figure 1A and B). We conclude that overexpression of UGT13248 increases root resistance  
258 to DON application.

259 We also screened a TILLING population in the barley cultivar Morex (TILLMore; (Talame  
260 et al., 2008a) for UGT13248 mutations. We identified three lines that contained non-  
261 synonymous mutations T368I, H369Y, and S403N. Two of the three lines, T368I and H369Y,  
262 exhibited strongly reduced seedling root growth two to six days after transfer to DON-  
263 containing media when compared to wild-type Morex plants (Figure 1C and D). S403N plants  
264 were indistinguishable from Morex plants (Figure 1C). Additionally, we crossed the H369Y and  
265 T368I plants to Morex and analyzed the resulting F<sub>2</sub> population. Upon exposure to DON the  
266 roots of homozygous T368I and H369Y mutants were significantly shorter than roots of  
267 homozygous wild-type and heterozygous plants (Supplemental Figure 6A and B). We conclude  
268 that plants carrying the T368I and H369Y mutations of UGT13248, but not the S403N mutation,  
269 have reduced UGT13248 function.

270

### 271 **Protein models suggest that UGT13248 T368I and H369Y variants might have altered** 272 **substrate binding**

273 To further understand the effect of the T368I and H369Y mutations on UGT13248 function, we  
274 created protein models for UGT13248 carrying the T368I, H369Y, and S403N mutations and

275 compared these to the wild-type UGT13248 protein model (Figure 2). According to the protein  
276 models that were generated, amino acids T368 and H369 are located within 4 Å of the UDP-  
277 glucose molecule. Thus, we hypothesized that these two residues may contribute to the  
278 hydrogen bonding network for the stabilization of UDP at the active site (Figure 2). In the *Os79*  
279 crystal structure, residue T291 was proposed to be involved in either the positioning of the  
280 substrate, or in protonation (Wetterhorn et al., 2016). In the UGT13248 protein homology  
281 model, T368 is positioned within hydrogen bonding distance of H369, which in turn is  
282 positioned within hydrogen bonding distance of UDP-glucose. Therefore, T368 and H369 may  
283 be required for positioning of substrate within the active site. On the other hand, in the  
284 UGT13248 protein homology model, S403 is distant (>4 Å) from the active site and is therefore  
285 not expected to be involved in substrate binding. This may explain why the mutant S403N roots  
286 did not show increased susceptibility to DON.

287 Interestingly, we noted in the protein model that when the polar residue threonine is  
288 replaced by a hydrophobic isoleucine (T368I), the hydrogen bond between a hydroxyl side chain  
289 and the nitrogen atom of H369 is disrupted, which could potentially indicate an impact on the  
290 hydrogen bonding network surrounding the substrate. Furthermore, when the charged residue  
291 histidine is replaced by hydrophobic tyrosine (H369Y) in the protein model, the negatively  
292 charged hydroxyl group of the tyrosine side chain has the potential to cause steric hindrance of  
293 UDP, possibly inhibiting correct substrate binding within the active site (Figure 2).

294 Taken together, these differences in the protein models suggest that the T368I and  
295 H369Y mutations could affect UGT13248 function by influencing the binding of the UDP-  
296 glucose substrate, potentially impacting the enzyme function, which may explain the increased  
297 sensitivity to DON demonstrated in plants carrying these mutations.

298

### 299 **UGT13248 is required for DON to D3G conversion**

300 To test the effect of UGT13248 overexpression on DON to D3G conversion, we inoculated  
301 spikes of #39003\_UGT- and #39003\_UGT+ sister lines with DON at anthesis. We measured DON  
302 and D3G levels 0 h, 4 h, 24 h, 48 h, and 96 h after inoculation. UGT- plants that do not contain

303 the transgene, showed some increase in D3G / DON over time, while the D3G / DON ratio was  
304 significantly increased in UGT+ plants at 24 h, 48 h, and 96 h (Figure 3A). To better understand  
305 the dynamics of *UGT13248* expression in these genotypes, we used qRT-PCR to compare  
306 expression of the endogenous *UGT13248* and the *UGT13248-FLAG* transgene in mock- and  
307 DON-treated UGT- and UGT+ plants (Supplemental Figure 5D). UGT+ and UGT- plants showed  
308 similar levels of endogenous *UGT13248* in mock treated samples at 0 h, 4 h, and 24 h after  
309 inoculation. In UGT- plants a strong increase in endogenous *UGT13248* was detected at 4 h and  
310 24 h after DON inoculation. In UGT+ plants an increase of endogenous *UGT13248* expression  
311 was detected at 4 h, but not 24 h after DON application. In addition to the endogenous  
312 *UGT13248*, UGT+ plants expressed the *UGT13248-FLAG* transgene at a constitutive and  
313 elevated level compared to endogenous *UGT13248*. The total amount of *UGT13248* expression  
314 in UGT+ plants is higher compared to UGT- plants at 0 h, 4 h, and 24 h after mock treatment  
315 and at 0 h and 4 h after DON application. The combined level of *UGT13248-FLAG* and  
316 endogenous *UGT13248* expression in UGT+ plants is similar to the endogenous *UGT13248*  
317 expression in UGT- plants at 24 h after DON treatment. This suggests that *UGT13248* expression  
318 is upregulated upon DON application. It is possible that the level of *UGT13248* expression is  
319 regulated by a feedback mechanism that prohibits continued *UGT13248* expression in response  
320 to DON after a certain *UGT13248* expression level is reached or alternatively, DON to D3G  
321 conjugation might reduce available DON concentrations and thus reduce DON-induced  
322 *UGT13248* gene expression. Taken together, the data show that UGT13248 converts DON to  
323 D3G in barley and that earlier and higher levels of *UGT13248* lead to increased conversion of  
324 DON to D3G.

325 To understand the effect of altered UDP-glucose binding on UGT13248 function in more  
326 detail, T368I, H369Y, S403N, and Morex spikes were inoculated with DON at anthesis. DON and  
327 D3G were measured at 0 h, 12 h, 24 h, 48 h, and 96 h after inoculation. T368I and H369Y plants  
328 showed significantly reduced D3G / DON accumulation at 24 h, 48 h, and 96 h, compared to  
329 Morex and S403N plants (Figure 3B). This shows that the T368I and H369Y mutations inhibit  
330 UGT13248-dependent DON to D3G conversion.

331

### 332 **UGT13248 mutants have increased susceptibility to FHB in the field**

333 We performed field evaluations to investigate the effect of the reduced UGT13248 activity on  
334 FHB severity and DON and D3G accumulation upon plant infection with *F. graminearum*. Field  
335 evaluations of Morex, T368I, H369Y, and S403N plants were performed in two years, 2019 and  
336 2020, and in two different field locations in Minnesota, Saint Paul and Crookston. **No**  
337 **differences in plant height were observed between Morex and any of the mutants in three**  
338 **environments (Supplemental Figure 7A-C).** In Saint Paul, plants were spray-inoculated at  
339 anthesis and in Crookston grain spawn inoculation was used. In all four environments T368I  
340 plants showed significantly increased FHB severity compared to wild-type Morex, while S403N  
341 plants did not. In three out of four environments (Saint Paul, 2020, Crookston, 2019 and  
342 Crookston, 2020) H369Y plants showed significantly increased FHB severity compared to wild-  
343 type Morex (Figure 4A and B). FHB disease development is influenced by many factors including  
344 temperature and humidity thus it is not unusual to see variation of FHB severity in different  
345 environments (Steffenson, 2003). We conclude that H369Y and T368I plants, that have strongly  
346 reduced UGT13248 activity, are more susceptible to *F. graminearum*.

347 DON and D3G accumulation were measured in plants from the Saint Paul trial in 2019  
348 and 2020 (Supplemental Figure 7D-G). In both years, the ratio of D3G to DON was significantly  
349 reduced in T368I and H369Y plants compared to wild-type Morex and S403N plants from 14  
350 days after inoculation to harvest (30-32 days after inoculation) (Figure 4C and D). Thus, even in  
351 the environment that we did not detect any increased FHB severity for H369Y (Saint Paul 2019),  
352 we still detected a robust reduction in the conversion of DON to D3G in H369Y plants under  
353 field conditions.

354

### 355 **Point inoculation experiments suggest a role of UGT13248 in type II resistance in barley**

356 ~~It has previously been shown that *F. graminearum* produces DON during the infection process~~  
357 ~~(Evans et al., 2000) and that trichothecenes are virulence factors in wheat (Proctor et al., 1995).~~  
358 To gain a more detailed understanding of the effect of UGT13248 on FHB disease progression in  
359 barley, we used point inoculation experiments on UGT13248 mutants and overexpression

360 plants. We inoculated the two central spikelets in the middle of each spike on either side of the  
361 spike with *F. graminearum* strain PH-1 and monitored FHB severity over time. In both sets of  
362 UGT13248 overexpression plants (#39003 and #39009), the UGT+ individuals, carrying the  
363 UGT13248 transgene, showed significantly increased resistance to *F. graminearum* at 7 and 11  
364 days after inoculation compared to the UGT- sister lines (Figure 5B). The UGT+ (39009) plants  
365 also showed significantly decreased susceptibility at 14 and 21 days after infection compared to  
366 their UGT- (39009) sister line, while no significant difference could be detected between the  
367 second set of sister lines (39003) (Figure 5A and B). In the case of the 39003 sister lines, the  
368 UGT+ plants were shorter and showed increased spike density compared to the UGT-  
369 individuals (Supplemental Figure 8), which likely increased disease progression at the later time  
370 points in the UGT+ plants. We conclude that UGT13248 overexpression leads to increased type  
371 II resistance to *F. graminearum* at least early after infection with *F. graminearum*.

372 Next, point inoculation experiments were performed for Morex, T368I, H369Y, and  
373 S403N plants. While FHB symptoms were restricted to one to two rows of spikelets basipetal  
374 and acropetal each from the inoculated spikelets in Morex and S403N, symptoms spread much  
375 further in T368I and H369Y plants (Figure 5C). T368I and H369Y plants, which have strongly  
376 reduced UGT13248 activity showed significantly increased FHB severity at 7, 11, and 14 days  
377 after inoculation when compared to wild-type Morex and S403N plants (Figure 5D). We  
378 conclude that DON to D3G conversion by UGT13248 is a major contributor to type II resistance  
379 in barley.

380

### 381 **Differences in susceptibility to *F. graminearum* in the field are not primarily dependent on** 382 **differences in type II resistance**

383 We utilized a set of barley genotypes to determine if point inoculation can reproduce  
384 differences in FHB severity previously described under field conditions. We inoculated the  
385 resistant cultivars Chevron, Quest, Stellar, and Frederickson and the susceptible accessions  
386 Morex, PI383933, ICB111809, and Stander, together with the H369Y plants. Only the highly  
387 susceptible accession PI383933 (Huang et al., 2013) and H369Y plants showed very high FHB

388 severity, while all other genotypes showed comparable FHB severity (Supplemental Figure 9A).  
389 We also plotted the average number of diseased spikelets in each genotype (Supplemental  
390 Figure 9B) as this may be a truer representation of fungal spread as different genotypes had  
391 different total numbers of spikelets or rachis nodes, and the tested genotypes were comprised  
392 of 2-row and 6-row accessions. The 2-row accessions Fredrickson and ICB111809 showed the  
393 lowest number of diseased spikelets, the 6-row accessions Chevron, Morex, Stander and Stellar  
394 showed slightly higher numbers of diseased spikelets, the 6-row accession PI383933 showed a  
395 higher number of diseased spikelets and the 6-row accession Quest showed an intermediate  
396 number of diseased spikelets compared to PI383933 and Chevron, Morex, Stander and Stellar.  
397 However, the number of diseased spikelets was significantly higher in H369Y plants compared  
398 to all other genotypes tested (Supplemental Figure 9B).

399 PI383933 plants are very short and have short, dense spikes ((Huang et al., 2018),  
400 Supplemental Figures 8 C-E). While PI383933 plants contain the G213R mutations of UGT13248,  
401 PI383933 seedlings did not show high sensitivity to DON (Supplemental Figure 1C), suggesting  
402 that they carry a functional UGT13248 allele. Additionally, in a mapping population of a cross  
403 between Rasmusson and PI383933 (Huang et al., 2018), taller plants were generally more  
404 resistant to FHB and shorter plants were generally more susceptible to FHB. This was  
405 independent of the Rasmusson or PI383933 *UGT13248* allele they carried, but dependent on  
406 plant height (Supplemental Figure 10 B). We also observed that the spikelets on the tip of the  
407 PI383933 spikes remained green in individuals with less than 100% FHB severity, while the  
408 terminal florets in H369Y plants were bleached (Supplemental Figure 10 A). Thus, susceptibility  
409 of PI383933 to *F. graminearum* is likely due to plant morphology rather than reduced type II  
410 resistance.

411 Taken together, this suggests that FHB susceptibility in the field is not likely  
412 predominantly dependent on differences in type II resistance, but rather type I resistance, row-  
413 type, and various agro-morphological traits that do not affect FHB development upon point  
414 inoculation under controlled growth conditions.

415



416 ***Fusarium graminearum* spreads through the rachis of T368I and H369Y but is restricted to the**  
417 **inoculated rachis node in wild-type barley plants**

418 Previously, Jansen *et al.* (Jansen et al., 2005) studied the spatiotemporal dynamics of *F.*  
419 *graminearum* infection in wheat and barley using a GFP-labeled wild-type *F. graminearum*  
420 strain as well as a strain lacking trichothecene production. The authors found that in barley  
421 both the wild-type and the trichothecene knockout strain were inhibited at the rachis node of  
422 the infected spikelet. We used the H369Y and Morex plants to study if UGT13248 was required  
423 for blocking *F. graminearum* invasion of the rachis. We inoculated ~~the~~ two central spikelets on  
424 either side ~~and~~ in the middle of each spike with *F. graminearum* strain PH-1 and 21 days later  
425 collected rachis and florets separately from ~~five equal sections of each~~ Morex and H369Y spikes  
426 (Figure 6A-C). Each spike was separated into five equal portions based on the total number of  
427 rachis nodes. Section 1 was on the base of the spike and section 5 at the tip. Section 3  
428 contained the inoculated central spikelets, the lateral spikelets adjacent to the inoculated  
429 spikelets, and all central and lateral spikelets of the rachis nodes directly acro- and basipetal of  
430 the inoculated spikelets. For example, if the spike contained 60 spikelets, there were 12  
431 spikelets and four rachis nodes in each section. For each section, ergosterol and DON  
432 accumulation was measured separately for floret and rachis tissue. Ergosterol is a major sterol  
433 found in fungal cell membranes and was used as a proxy for *F. graminearum* quantity.  
434 Ergosterol accumulation was significantly increased in section 4, located acropetal to the  
435 inoculated spikelets, of H369Y plants compared to wild-type Morex. H369Y plants also  
436 contained significantly more ergosterol in the rachis sections two and three compared to Morex  
437 (Figure 6A and C). DON levels were significantly higher in H369Y florets in sections two, three,  
438 and four as well as H369Y rachis in sections three and four (Figure 6B and C). We conclude that  
439 H369Y plants contain more DON and *F. graminearum* than wild-type Morex, specifically in the  
440 rachis near the inoculation site.

441 Next, we decided to study symptom development in H369Y and Morex plants upon  
442 inoculation with a *F. graminearum* reporter strain that constitutively expressed dsRed  
443 (*pgpdA::dsRed*) (Ilgen et al., 2009). For these experiments, we only inoculated one central floret  
444 in the middle of the spike and reduced the *F. graminearum* inoculum by half to reduce the

445 chance of mycelium development on the outside of the spike. Spread of FHB symptoms and  
446 overall FHB severity 14 days after inoculation were strongly increased in H369Y spikes  
447 compared to wild-type Morex (Supplemental Figure 11A-D). Increased FHB severity was  
448 observed after point inoculation of homozygous H369Y plants 7 and 21 days after inoculation,  
449 but not in corresponding sister lines (Supplemental Figure 11E). In T368I and H369Y mutant  
450 plants browning of the rachis was observed over the entire length of the rachis, but not in the  
451 corresponding wild-type sister lines. We used confocal laser scanning microscopy to visualize  
452 fungal mycelia at base of the spike and observed structures that resembled fungal hyphae in  
453 higher magnification images of both T368I and H369Y mutants plants, while no fungal signal  
454 was observed in the corresponding wild-type tissues (Figure 7A). Next confocal laser scanning  
455 microscopy was used to visualize the infection process in H369Y and Morex plants in more  
456 detail. While *F. graminearum* could be detected at the rachis nodes directly at the inoculated  
457 spikelets in both H369Y and Morex, *F. graminearum* was only detected at the fourth rachis  
458 nodes acro- and basipetal of the inoculated spikelets in H369Y, but not Morex (Figure 7B and  
459 Supplemental Figure 12). Additionally, *F. graminearum* was detected throughout the rachis  
460 node and all rachis tissue at the inoculated rachis node in H369Y. In contrast *F. graminearum*  
461 was detected only locally at the rachis node of the inoculated spikelet in wild-type rachis tissue  
462 and not in more distant parts of the rachis (Figure 7B and Supplemental Figure 12). This shows  
463 that *F. graminearum* does infect the rachis of H369Y plants, but is arrested at the inoculated  
464 rachis node in wild-type plants. Examination of the susceptible PI383933 plants showed a  
465 similar pattern to wild-type plants (Supplemental Figure 10C). We conclude, UGT13248-  
466 dependent detoxification of DON is required for type II resistance in barley.

467

## 468 DISCUSSION AND CONCLUSIONS

469

### 470 UGT13248 function in barley is highly conserved

471 Here, we screened 45 barley accessions for natural variation in full-length *UGT13248*  
472 (Supplemental Table 1) and 458 accessions for natural variation in two regions of *UGT13248*

473 covered by exome capture sequencing (Supplemental Table 2). The accessions tested include  
474 modern cultivars, landraces, and wild barleys. None of the 11 non-synonymous mutations  
475 identified, resulted in disruption of DON resistance in barley seedlings carrying these mutations  
476 (Supplemental Figures 1 and 4). Only one of these mutations, F385S, was located within the  
477 PSPG motif, thought to be required for UGT-UDP-sugar interaction (Supplemental Figure 3A).  
478 Further, protein modeling suggested that none of the mutations was likely to disrupt UGT13248  
479 protein function (Supplemental Figures 2 and 3). Interestingly, the only UGT13248 mutations  
480 with reduced UGT13248 function were H369Y and T368I, found in a sodium azide-mutagenized  
481 TILLING population of barley cultivar Morex (Figure 1). We conclude that UGT13248 gene  
482 function is highly conserved in barley.

483

#### 484 **UGT13248 converts DON to D3G in barley**

485 It has previously been shown that UGT13248 expression in yeast and wheat resulted in the  
486 conversion of DON to D3G (Li et al., 2015; Schweiger et al., 2010). Here, we observed that DON  
487 to D3G conversion in barley spikes that carried T368I and H369Y mutations was significantly  
488 reduced compared to wild-type barley (Figure 3B). We also demonstrated that constitutive  
489 expression of *UGT13248* under the control of the *Zea mays Ubiquitin* (*Ubi1*) promoter resulted  
490 in strongly increased DON to D3G conversion (Figure 3A). This further shows that UGT13248  
491 catalyzes DON to D3G conversion *in planta*. While *UGT13248* expression was induced by DON  
492 treatment in both barley spikes with wild-type and constitutive *UGT13248* expression, low-level  
493 constitutive *UGT13248* expression was observed even in plants that did not carry the *Ubi-*  
494 *1::UGT13248* transgene (Supplemental Figure 5D). This suggests that *UGT13248* expression is  
495 upregulated upon DON application and that the level of *UGT13248* expression might be  
496 regulated by a feedback mechanism that prohibits continued *UGT13248* expression in response  
497 to DON after a certain *UGT13248* expression level is reached.

498 UGT13248 has been shown to catalyze NIV to NIV3G conversion in wheat (Li et al.,  
499 2017). Interestingly, *Arabidopsis* DOGT1 (UGT73C5) showed activity on DON and the growth  
500 hormone brassinolide, but not NIV (Poppenberger et al., 2005). Another *Arabidopsis* UGT,

501 UGT73C6, converted zearalenone to ZON-4-O-Glc in yeast (Poppenberger et al., 2006). The  
502 closest homolog to DOGT1 in barley UGT5876, did not show activity to DON in yeast (Schweiger  
503 et al., 2010). This shows that UGTs are promiscuous and might have multiple substrates. It is  
504 possible that UGT13248 can glucosylate other trichothecenes in barley, as well as potentially  
505 unrelated compounds including growth regulators and mycotoxins produced by other fungal  
506 pathogens. However, constitutive expression of UGT13248 did not lead to any detectable  
507 pleiotropic effects. Hence, it is unlikely that UGT13248 drastically affects plant growth  
508 regulation, as opposed to DOGT1 in *Arabidopsis* where overexpression plants showed a dwarf  
509 phenotype (Poppenberger et al., 2005).

510

#### 511 **UGT13248 is a major resistance factor in barley to *Fusarium graminearum***

512 Barley carrying mutations in UGT13248 that caused reduced UGT13248 function, H369Y and  
513 T368I, are highly susceptible to *F. graminearum*, both in field studies and in point inoculation  
514 experiments (Figures 4 and 5). The difference in FHB severity is clearest with point inoculation  
515 experiments (Figure 5). We conclude that UGT13248 is required for type II resistance to *F.*  
516 *graminearum* in barley. Our experiments cannot distinguish between type I and type II  
517 resistance in the field and we cannot rule out an additional effect of UGT13248 on type I  
518 resistance in field studies. The conserved type II resistance observed for this species  
519 (Steffenson, 2003) might be due to conserved UGT13248 function.

520 Previously, the *B. distachyon* UGT Bradi5g03300, the closest *Brachypodium* homolog to  
521 barley UGT13248, was suggested to not contribute to type II resistance in that species (Pasquet  
522 et al., 2016). Mutations in Bradi5g03300 only caused increased colonization with *F.*  
523 *graminearum* when spray inoculation was used. In contrast, point inoculation experiments did  
524 not show any difference in FHB severity between plants with mutations in Bradi5g03300  
525 compared to wild-type plants (Pasquet et al., 2016). The mutations in Bradi5g03300 are located  
526 within the PSPG box similar to the H369Y and T368I mutations in barley UGT13248 studied here  
527 (Supplemental Figure 13). The observed differences may be due to differences in flower  
528 morphology in *B. distachyon* compared to barley. It is also possible that the study of

529 Bradi5g03300 was complicated by the presence of Bradi5g02780, another UGT that showed  
530 DON resistance in yeast (Schweiger et al., 2013), and that may be functionally redundant with  
531 Bradi5g03300. The differences observed in the function of UGTs in this study compared to *B.*  
532 *distachyon* show that studies in model organisms may not always be predictive of the  
533 mechanism observed in related crop species.

534 In wheat, a few major contributors to FHB resistance, including *Fhb1* and *Fhb7*, have  
535 been identified (Bai et al., 1999; Wang et al., 2020). However, in barley, FHB resistance has  
536 previously been found to be multifactorial and mainly dependent on agro-morphological traits  
537 (Massman et al., 2011). Hence, our identification of a major contributor to type II resistance in  
538 barley is notable. Interestingly, previous barley mapping studies have not identified a QTL  
539 associated with FHB resistance and coincident with *UGT13248*, which is likely due to functional  
540 conservation of *UGT13248*.

541 While barley *UGT13248* was found to play a critical role in plant immunity to *F.*  
542 *graminearum*, it is curious why *UGT13248* appears functionally conserved globally in barley, as  
543 not all growth regions are likely substantially affected by FHB outbreaks. However, various plant  
544 pathogenic fungi of the genera *Fusarium*, *Microcyclospora*, *Myrothecium*, *Peltaster*, *Spicellum*,  
545 *Stachybotrys*, *Trichoderma*, and *Trichothecium* produce trichothecene mycotoxins (Proctor et  
546 al., 2018). Up to 87% of tested food and feed samples from the Middle East and Africa  
547 contained type B trichothecenes (Rodrigues et al., 2011). It is possible that *UGT13248* can  
548 detoxify trichothecene mycotoxins from various fungal pathogens and might hence contribute  
549 to disease resistance in many different environments. It can also not be ruled out that  
550 *UGT13248* glucosylates other unknown compounds that are unrelated to trichothecenes.

551 Interestingly, in *Aegilops tauschii* nine of 147 accessions tested showed increased FHB  
552 susceptibility and increased FHB spread (Kirana et al., 2022). These nine accessions all carried a  
553 nonsense mutation just prior to the PSPG box in the UGT AET5Gv20385300 (Supplemental  
554 Figure 13), an ortholog of barley *UGT13248* (Kirana et al., 2022). *A. tauschii* is the D subgenome  
555 donor for bread wheat and the sequenced cultivar Chinese Spring did also contain a missense  
556 mutation in the wheat ortholog of *UGT13248* in the D genome (Kirana et al., 2022). This again

557 raises the question why UGT13248 is functionally conserved globally in barley but not in these  
558 related species.

559

### 560 **DON detoxification is required for type II resistance to FHB in barley**

561 Trichothecene production is known to be required for *Fusarium graminearum* spread in wheat  
562 spikes (Jansen et al., 2005; Schroeder and Christensen, 1963). In the barley cultivar  
563 ‘Chevron’, however, both wild-type and *tri5* mutant strains of *F. graminearum* were arrested at  
564 the rachis node of the inoculated spikelet (Jansen et al., 2005). Utilizing functional mutations in  
565 UGT13248, H369Y and T368I, we showed that functional UGT13248 is required for the arrest of  
566 *F. graminearum* at the inoculated rachis node in barley (Figure 7 and Supplemental Figures 11  
567 and 12). Hence, the functional conservation of UGT13248 across barley may explain the  
568 conserved type II resistance observed for this species (Steffenson, 2003).

569

## 570 **MATERIALS AND METHODS**

### 571 **Plant Material and growth conditions**

572 Plants were grown at 20°C, 16 h daylight, and 8 h dark at 18°C in BM2 soil (Berger) with 3-4  
573 seedlings per five-inch square pot. After seedling emergence, plants were fertilized with  
574 Osmocote plus fertilizer (Scotts). **The plant genotypes used in this study are summarized in**  
575 **Table 1.**

576

### 577 **Transgenic barley plants**

578 The *UGT13248* gene was previously cloned with a C-terminal FLAG tag into pENTR TM/ D TOPO  
579 (Shin et al., 2012) and transferred into binary vector pIPKb002 (Himmelbach et al., 2007) using  
580 Gateway cloning technology (Invitrogen). Immature barley cultivar Golden Promise embryos  
581 were transformed using the *Agrobacterium tumefaciens* strain AGL1 and transformants were  
582 selected on 50 mg / L hygromycin containing media (Hensel et al., 2008). T1 plants were  
583 screened for the presence of the transgene using UGT13248\_fwd and FLAG\_rev primers

584 (Supplemental Table 3). For DNA gel blotting genomic DNA (10 µg) was digested with XbaI,  
585 separated on a 1 % agarose gel, and transferred onto Hybond N+ membranes (Amersham  
586 Biosciences). The *hpt* gene probe was derived from a PCR-amplified product (Supplemental  
587 Table 3). The probe was labeled with  $\alpha$ -<sup>32</sup>P CTP using the Prime-a-Gene labeling system  
588 (Promega), following the manufacturer's instructions. The radiolabeled *hpt* probe was used for  
589 the hybridization, and results were visualized using autoradiography.

590 One transgenic plant each from two independent transgenic events was crossed to  
591 barley cultivar Rasmusson and F<sub>1</sub> plants were backcrossed to Rasmusson. In the F<sub>3</sub> generation,  
592 individuals containing the UGT13248-FLAG (UGT+) and individuals not containing the transgene  
593 (UGT-) were identified. All experiments were conducted using grains that descended from a  
594 single individual plant and were used in the BC<sub>1</sub>F<sub>6</sub> generation.

595 For Western Blot analysis one individual seedling for each genotype was harvested and  
596 tissue was ground in liquid nitrogen. Plant tissue was mixed with equal volumes of 2 x Laemmli  
597 buffer, heated at 95°C for 5 minutes and supernatant was loaded onto a 10% polyacrylamide  
598 gel (Biorad, USA, 1610182). Stain-free technology was used to visualize equal loading of the gel.  
599 The proteins were transferred onto a PVDF membrane and Western Blot analysis was  
600 performed according to the manufacturer's instructions using an anti-FLAG HRP antibody  
601 (GenScript, USA, A01428).

602

### 603 **TILLING lines**

604 The TILLMore population (Talame et al., 2008a, b) of 4,906 M3 families in the background of  
605 the cv. Morex, mutagenized with NaN<sub>3</sub> was screened for mutations in *UGT13248* (Talame et al.,  
606 2008a, b). We identified three non-synonymous mutations in 2,432 families screened with four  
607 sets of primer pairs spanning exon 2 and the first 283 bp of exon 3. DNA samples of the  
608 TILLMore resource were organized in 8-fold pools and analysed by High Resolution Melting  
609 (HRM). With HRM, allelic differences are detected based on differences in melting temperature  
610 using a DNA intercalating dye. A 7500 FAST Real Time- HRM- ready instrument (Applied  
611 Biosystems) was used, along with Melt Doctor Amplification kit (Applied Biosystems). HRM

612 primers were designed based on the UGT13248 sequence (GenBank GU170355). Primers are  
613 listed in Supplemental Table 3. Putative mutants were confirmed by Sanger sequencing using a  
614 TILLING\_fwd and TILLING\_rev primers (Supplemental Table 3). Three lines carrying mutations in  
615 UGT13248 were identified. TILLMore #438 contained a C to T nucleotide substitution at base  
616 pair 1103 of the cDNA sequence that caused a T368I amino acid substitution of UGT13248.  
617 TILLMore #1624 carried a G to A nucleotide substitution at bp 1105 of the cDNA sequence,  
618 resulting in a S403N amino acid substitution. TILLMore #1683 carried a C to T nucleotide  
619 substitution at 1208 bp, resulting in a H369Y amino acid substitution. Homozygous plants were  
620 used in the M6/ M7 generation. For Supplemental Figure 6, M6 plants were crossed with Morex  
621 and progeny in the BC<sub>1</sub>F<sub>2</sub> generation was used. **Sister lines that contained the homozygous  
622 mutant or wild-type allele, respectively, were selected for T368I and H369Y in the BC<sub>4</sub>F<sub>2</sub>  
623 generation. We only selected lines with a 6-row morphology. These lines were used for Figure  
624 6A and Supplemental Figure 11E.**

625

#### 626 **Full-length sequencing of UGT13248**

627 Genomic DNA from 26 barley genotypes (Supplemental Table S1, all genotypes from Huang *et*  
628 *al.*, 2013) was extracted using the Plant/Fungi DNA Isolation Kit (Norgen Biotek). Sanger  
629 sequencing was performed with primer sets designed to cover 2,477 bp of *UGT13248* genomic  
630 sequence (Table S3). Genomic sequences of UGT13248 from 19 additional accessions were  
631 obtained from Jayakodi *et al.* (2020) (Jayakodi *et al.*, 2020) (Jayakodi *et al.*, 2020). Sequences  
632 were compared using BLAST (<https://blast.ncbi.nlm.nih.gov>).

633

#### 634 **Exome capture resequencing data collection and processing**

635 All resequencing data reported here are derived from previously published datasets, including  
636 wild, landrace, and elite barley lines reported in (Chen *et al.*, 2022; Hemshrot *et al.*, 2019; Kono  
637 *et al.*, 2019; Lei *et al.*, 2019; Mascher *et al.*, 2013; Nice *et al.*, 2016; Russell *et al.*, 2016). The  
638 sequence was generated after exome capture using the barley Roche NimbleGen (Madison, WI)  
639 SeqCap EZ Developer probe pools (Mascher *et al.*, 2013).



640 For sequence alignment and quality control, we used publicly available software  
641 integrated with bash scripts in the “sequence\_handling” workflow (Liu et al., 2022). Sequence  
642 quality assessment used FastQC (<http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/>).  
643 Adapters were trimmed using Scythe (<https://github.com/vsbuffalo/scythe>). Burrows-Wheeler  
644 Aligner (BWA-MEM) (Li et al., 2009) was used to align reads to the barley reference genome  
645 Morex\_v2 (Monat et al., 2019). Read mapping used default parameters for BWA-MEM except  
646 for the following: 16 threads, minimum seed length of 8, re-seed value of 1.0, gap penalty of 8,  
647 and a minimum threshold of 85 for 100 bp PE reads or a minimum threshold of 106 for 125 bp  
648 PE reads. Read mapping parameters were chosen to permit a ~2% mismatch between reads  
649 and the reference sequence; the highest estimated nucleotide diversity reported based on  
650 Sanger resequencing in wild and cultivated barley (Morrell et al., 2014; Morrell et al., 2003;  
651 Morrell et al., 2006). Picard v2.20.2 (<https://github.com/broadinstitute/picard>), was used for  
652 alignment sorting, de-duplicating, and adding read groups to the read-mapped files. Read  
653 depth, and coverage estimates were generated by ‘bedtools genomecov’ (Quinlan and Hall,  
654 2010). We used ~241X exome capture reads from a Morex sample (Mascher et al., 2013) to the  
655 Morex v2 reference genome empirically define regions are considered covered by exome  
656 capture ([https://github.com/MorrellLAB/captured\\_50x\\_BED](https://github.com/MorrellLAB/captured_50x_BED)). These (Quinlan and Hall, 2010)  
657 “cap50x” have > 50X coverage in Morex capture data  
658 ([https://github.com/MorrellLAB/captured\\_50x\\_BED](https://github.com/MorrellLAB/captured_50x_BED)).

659 Alignment processing followed the Genome Analysis Toolkit (GATK) best practices  
660 workflow (DePristo et al., 2011; McKenna et al., 2010). Individual sample genotype likelihoods  
661 were then calculated using GATK v4.1.2 HaplotypeCaller, with a value of 0.008 per base pair  
662 passed to the “heterozygosity” option. This value is the mean estimate of coding nucleotide  
663 sequence diversity, based on previous Sanger resequencing experiments (Caldwell et al., 2006;  
664 Morrell et al., 2014). Single sample GVCFs were merged using GenomicsDBImport, then called  
665 jointly from the genotype likelihoods with the GATK tool Genotype GVCFs (McKenna et al.,  
666 2010). GATK’s Variant Quality Score Recalibration (VQSR) was used for variant filtering. SNPs  
667 were also filtered to include sites that fall within the “cap50x” regions, are polymorphic and bi-  
668 allelic with genotype quality (GQ) > 3, and have per sample depth (DP) between 5 and 78

669 (extremely high depth is likely due high copy number genomic regions collapsed in the  
670 reference genome, so we take the 95th percentile of coverage as the upper cutoff).  
671 Heterozygous genotypes were set to missing if the allele balance deviated more than 0.1 from  
672 the expected allele balance of 0.5 (Muyas et al., 2019; Pedersen et al., 2021). Briefly, allele  
673 balance helps identify systematic or alignment errors that could result in false SNP calls. The  
674 filtered VCF was generated as part of a larger cohort of samples with 458 of the samples used  
675 for this study. Genotyping for the UGT13248 locus was extracted from the GATK-derived VCF  
676 using bcftools (Danecek et al., 2021) ([https://github.com/MorrellLAB/Locus\\_HvUGT13248](https://github.com/MorrellLAB/Locus_HvUGT13248)).

677 UGT13248 CDS according to Morex V2 starting position is Chr5 position 330626689.  
678

### 679 **Plant height, spike length and spike density**

680 Height was measured as the distance from the base of the plant to the top of the spike,  
681 excluding awns on eight plants each. Spike length was measured as the distance between the  
682 top and bottom spikelets on five spikes. Spike density was calculated as the number of rachis  
683 nodes per cm spike.

684

### 685 **DON plate assays, DON inoculation, and DON and D3G measurements**

686 Barley grains were surface-sterilized for 10 minutes in a 1 % sodium hypochlorite solution  
687 containing 0.01 % Tween 20. Grains were washed five times with sterile water and placed on ½  
688 x Murashige and Skoog (MS) medium with 0.8 % agar. Plates were kept at 4 °C for two days for  
689 stratification and then transferred to 16 h light / 8 h dark conditions at 22 °C for two to three  
690 days. Seedlings were transferred onto ½ x MS medium containing 1 mg / L DON or mock. DON  
691 was dissolved in 70 % ethanol at 5 mg / mL and stored at -20°C. Five mg / mL DON stock  
692 solution was added at 1:5000 ratio to medium for 1 mg / mL DON plates. Similarly, 70 %  
693 ethanol was added at 1:5000 ratio to medium for mock plates. Root length was measured daily  
694 for six days.

695 For inoculation experiments, DON was diluted to 0.2 µg / µL, and 10 µL of this solution  
696 were inoculated at anthesis between the palea and lemma of each floret. We inoculated three

697 central spikelets on each side of any spike and harvested the inoculated section of the spike  
698 (total of 18 spikelets). For each biological replicates spike sections from three separate spikes  
699 were combined. Samples were ground in liquid nitrogen and trichothecenes extracted with 4  
700 volumes per weight with a mixture of acetonitrile /water /acetic acid: 79 / 20 / 1 for 90 minutes  
701 at room temperature. 500  $\mu$ L per sample were dried down and resuspended in 1 mL 50 %  
702 aqueous methanol, centrifuged and transferred into HPLC vials and measured using a 1290  
703 Agilent UHPC system coupled to a Sciex 6500+ QTrap MS. Liquid chromatography and tandem  
704 mass spectrometry details were as previously described (Fiby et al., 2021).

705 For Figure 6A and B, DON was measured in parts per million ( $\mu$ g / g), by gas  
706 chromatography/mass spectrometry (Mirocha et al., 1998). Ergosterol was measured as  
707 described previously (Dong et al., 2006). Samples that were below the detection limit were set  
708 to 0 mg / L.

709

#### 710 **Plant inoculation with *F. graminearum***

711 *F. graminearum* was grown in liquid CMC medium (15g / L carboxymethylcellulose (CMC), 1 g /  
712 L  $\text{NH}_4\text{NO}_3$ , 1 g / L  $\text{KH}_2\text{PO}_4$ , 0.5 g / L  $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  and 1 g / L Yeast Extract) for five days at room  
713 temperature while shaking at 150 rpm. Fungal cultures were filtered through 1-fold Miracloth,  
714 and filtrate was collected by centrifugation for 10 minutes at 2500 x *g* and 4 °C. Fungal spores  
715 were washed with sterile water and the concentration was adjusted to  $1 \times 10^8$  macroconidia  
716 per mL.

717 For point inoculation experiments with *F. graminearum* strain PH-1, one central spikelet  
718 in the middle of each side of a spike was inoculated with 10  $\mu$ l *F. graminearum* (PH 1) at  $1 \times 10^5$   
719 macroconidia / mL at anthesis in a controlled environment chamber. Inoculated spikes were  
720 covered with a plastic bag for 48 h. FHB severity was scored at the indicated time points by  
721 calculating the ratio of the number of infected spikelets versus the total number of spikelets.

722 For field experiments each genotype was planted in a 1.52 m  $\times$  0.31 m single-row plot  
723 and inoculation experiments were performed as previously described (Huang et al., 2021). In  
724 brief Morex, T368I, H369Y, and S403N genotypes were planted in six replicates for each

725 location (Saint Paul and Crookston) and year (2019 and 2020). The susceptible check Stander  
726 and the resistant check Chevron were planted in 3 replicates for each location and year. In Saint  
727 Paul, plants were spray-inoculated twice with  $1 \times 10^5$  macroconidia / mL *F. graminearum* within  
728 three to four days. The first application was performed two days after heading. In Crookston,  
729 plants were inoculated by spreading *F. graminearum*-colonized maize seed (grain spawn) onto  
730 the soil surface at the four–five leaf stage and at flag-leaf emergence. Mist irrigation was used  
731 from before the first inoculation and maintained until FHB severity was recorded to facilitate  
732 disease development. FHB severity was scored by calculating the ratio of the counted number  
733 of infected kernels versus the total number of kernels within a spike from ten randomly  
734 selected spikes per plot. For each plot, the ratios were averaged to obtain FHB severity for each  
735 biological replicate. DON was measured on grain samples harvested from FHB nurseries in Saint  
736 Paul after threshing and cleaning. A sample of cleaned grains from each plot was measured for  
737 DON accumulation, in parts per million ( $\mu\text{g} / \text{g}$ ), by gas chromatography/mass spectrometry as  
738 previously described (Mirocha et al., 1998).

739

#### 740 **Protein modeling**

741 Structural models of UGT13248 were created using SWISS-MODEL (Waterhouse et al., 2018).  
742 Protein BLAST searches were conducted within the SWISS-MODEL server. The protein sequence  
743 submitted for BLAST searches was  
744 METTVTAVSGTTSSSVGHGAGGGAARVLLLPSPGAQGHNTNMLQLGRRLAYHGLRPTLVATRYVLSTTPAPGAPFDV  
745 AAISDGF DAGGMALCPDPAEYFSRLEAVGSETLRELLSEARAGRPVRVLYDAHLAWARRVAQASGVAAAAFFSQPC  
746 SVDVYVY GELWAGRLALPATDGRALLARGVLGVELGLEDMPFPAAVPESQPAFLQVSVGQFEGLDYADDVLVNSFRDIE  
747 PKEVEYMELTWRAKMGVPTLPSYLLGDGRLPSNKS YGLDLFNSEVECMDWLEKQMNSSVVLVSYGTVSNYDATQLEE  
748 LGNGLCNSSKPFLLWVRSNEEHKLSEELKEKCGKIGLIVSWCPQLEVL A HRAIGCFVTHCGWNSTLEALVNGVPFVGIPH  
749 WADQPTIAKYVESAWGMGVRARKNKNGLCKKEEVERCIREVMDGERKDEYKKNAMNWMQAKEAMQEGGSSDK  
750 HVAEFATKYSSI. The template with the highest sequence identity was selected for modeling (73  
751 % sequence identity). The model was built using the three-dimensional crystal structure of Os79  
752 in complex with the non-reactive co-substrate UDP-2-fluoro-2-deoxy-D-glucose and  
753 trichothecene (PDB ID: 5TMD) (Wetterhorn et al., 2016) as the search template. Structural

754 models were visualized in the PyMOL Molecular Graphics System, Version 1.2r3pre  
755 (Schrödinger, LLC) to determine putative active site motifs. Putative ligand non-reactive mimic  
756 UDP-2-fluoro-2-deoxy-D-glucose and trichothecene were docked in the active site by structural  
757 alignment. Structural models of UGT13248 mutants were generated and compared in PyMOL  
758 (Barber, 2021).

759

## 760 **Confocal Microscopy**

761 *A. F. graminearum* reporter strain 8/1 with constitutive dsRed expression (*pgpdA::dsRed*) and  
762 inducible GFP expression (*pTRIS::GFP*) (Ilgen et al., 2009) was used to monitor fungal growth  
763 and trichothecene biosynthesis *in vivo*. Five µl of a 1 x 10<sup>5</sup> macroconidia / mL suspension was  
764 applied to one central floret on one side of the spike and covered with clear plastic bag for 24  
765 hours to maintain high humidity. At 14 dpi, rachises of infected barley spikes were hand-  
766 sectioned longitudinally and observed using confocal laser scanning microscopy (Nikon A1si,  
767 UMN Imaging Center). Optical configuration for fluorescence detection was as previously  
768 described (Boenisch and Schäfer, 2011). Plant cell wall autofluorescence (DAPI) was excited at  
769 406 nm and detected in the 425-475 nm range. DsRed was excited at 562 nm and detected in  
770 the 575-625 nm range. Z-stacking was used to collect images at multiple focal planes. Image  
771 processing and data generation were performed using the Nikon NIS-Elements platform  
772 ([www.microscope.healthcare.nikon.com/products/software/nis-elements](http://www.microscope.healthcare.nikon.com/products/software/nis-elements)).

773

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789

#### 790 **AUTHOR CONTRIBUTIONS**

791

792 G.B. and G.J.M. designed the research. G.B., Y.H., G.H., S.H., X.L., M.Q., Y.D., and F.B. performed  
793 research. S.M., S.S. and J.K. provided tools. G.B., Y.H., C.L., S.R.W., and P.L.M. analyzed data.  
794 G.B. wrote the paper with input from all authors.

795

#### 796 **SUPPLEMENTAL MATERIALS**

797 Supplemental Table 1: List of accessions used for full-length sequencing of *UGT13248*.

798 Supplemental Table 2: List of accessions used for exome capture sequencing analysis.

799 Supplemental Table 3: List of primers.

800 Supplemental Figure 1: Non-synonymous mutations identified in *UGT13248* in 45 barley  
801 accessions.

802 Supplemental Figure 2: Comparison of rice Os79 and barley UGT13248.

803 Supplemental Figure 3: Non-synonymous mutations identified using exome capture sequencing  
804 of 458 barley accessions.

805 Supplemental Figure 4: **Seedling root growth upon treatment with DON.**

806 Supplemental Figure 5: UGT13248 overexpression lines.

807 Supplemental Figure 6: The wild-type *UGT13248* gene is a single dominant gene that confers  
808 seedling root resistance to DON.

809 Supplemental Figure 7: Plant height, **DON and D3G measurements** in field experiments.

810 Supplemental Figure 8: Plant Height and spike density for *UGT13248* overexpression lines.

811 Supplemental Figure 9: Only H369Y and PI383933 plants show increased FHB severity in point  
812 inoculation experiments.

813 Supplemental Figure 10: Susceptibility of PI383933 is not due to mutations in *UGT13248*.

814 Supplemental Figure 11: Disease symptoms after point inoculation with *F. graminearum*  
815 reporter strain.

816 Supplemental Figure 12: *F. graminearum* reporter strain is detected in H369Y rachis tissue.

817 Supplemental Figure 13: Alignment of orthologous UGT sequences in rice (*Os79*), barley  
818 (*UGT13248*), *B. distachyon* (Bradi5g03300), *Aegilops tauschii* (AET5Gv20385300) and wheat  
819 (*TaUGT5D*).

820

## 821 REFERENCES

822 Bai, G.H., Kolb, F.L., Shaner, G., and Domier, L.L. (1999). Amplified fragment length polymorphism  
823 markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology*  
824 *89*, 343-348.

825 Bai, G.H., Plattner, R., Desjardins, A., and Kolb, F. (2001). Resistance to Fusarium head blight and  
826 deoxynivalenol accumulation in wheat. *Plant Breeding* *120*, 1-6.

827 Bai, G.H., Su, Z.Q., and Cai, J. (2018). Wheat resistance to Fusarium head blight. *Can J Plant Pathol* *40*,  
828 336-346.

829 Barber, R.D. (2021). Software to Visualize Proteins and Perform Structural Alignments. *Curr Protoc* *1*,  
830 e292.

831 Boddu, J., Cho, S., Kruger, W.M., and Muehlbauer, G.J. (2006). Transcriptome analysis of the barley-  
832 *Fusarium graminearum* interaction. *Mol Plant Microbe In* *19*, 407-417.

833 Boddu, J., Cho, S.H., and Muehlbauer, G.J. (2007). Transcriptome analysis of trichothecene-induced gene  
834 expression in barley. *Mol Plant Microbe In* *20*, 1364-1375.

835 Boenisch, M.J., and Schäfer, W. (2011). *Fusarium graminearum* forms mycotoxin producing infection  
836 structures on wheat. *Bmc Plant Biol* *11*.

837 Bushnell, W.R., Hayen, B.E., and Pritsch, C. (2003). Histology and Physiology of Fusarium head blight. In  
838 *Fusarium head blight of wheat and barley*, K.J. Leonard, and W.R. Bushnell, eds. (St. Paul, MN, USA,  
839 American Phytopathological Society Press), pp. 44-83.

840 Caldwell, K.S., Russell, J., Langridge, P., and Powell, W. (2006). Extreme population-dependent linkage  
841 disequilibrium detected in an inbreeding plant species, *Hordeum vulgare*. *Genetics* 172, 557-567.

842 Chen, Y., Kistler, H.C., and Ma, Z.H. (2019). *Fusarium graminearum* Trichothecene Mycotoxins:  
843 Biosynthesis, Regulation, and Management. *Annual Review of Phytopathology*, Vol 57, 2019 57, 15-39.

844 Chen, Y.Y., Schreiber, M., Bayer, M.M., Dawson, I.K., Hedley, P.E., Lei, L., Akhunova, A., Liu, C.C., Smith,  
845 K.P., Fay, J.C., *et al.* (2022). The evolutionary patterns of barley pericentromeric chromosome regions, as  
846 shaped by linkage disequilibrium and domestication. *Plant J* 111, 1580-1594.

847 Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T.,  
848 McCarthy, S.A., Davies, R.M., *et al.* (2021). Twelve years of SAMtools and BCFtools. *Gigascience* 10.

849 de la Peña, R.C., Smith, K.P., Capettini, F., Muehlbauer, G.J., Gallo-Meagher, M., Dill-Macky, R., Somers,  
850 D.A., and Rasmusson, D.C. (1999). Quantitative trait loci associated with resistance to *Fusarium* head  
851 blight and kernel discoloration in barley. *Theor Appl Genet* 99, 561-569.

852 DePristo, M.A., Banks, E., Poplin, R., Garimella, K.V., Maguire, J.R., Hartl, C., Philippakis, A.A., del Angel,  
853 G., Rivas, M.A., Hanna, M., *et al.* (2011). A framework for variation discovery and genotyping using next-  
854 generation DNA sequencing data. *Nat Genet* 43, 491-+.

855 Dong, Y., Steffenson, B.J., and Mirocha, C.J. (2006). Analysis of ergosterol in single kernel and ground  
856 grain by gas chromatography-mass spectrometry. *J Agric Food Chem* 54, 4121-4125.

857 Evans, C.K., Xie, W., Dill-Macky, R., and Mirocha, C.J. (2000). Biosynthesis of deoxynivalenol in spikelets  
858 of barley inoculated with macroconidia of *Fusarium graminearum*. *Plant Disease* 84, 654-660.

859 Ferrigo, D., Raiola, A., and Causin, R. (2016). *Fusarium* Toxins in Cereals: Occurrence, Legislation, Factors  
860 Promoting the Appearance and Their Management. *Molecules* 21.

861 Fiby, I., Soppel, M.M., Michlmayr, H., Adam, G., and Berthiller, F. (2021). Development and Validation of  
862 an LC-MS/MS Based Method for the Determination of Deoxynivalenol and Its Modified Forms in Maize.  
863 *Toxins* 13.

864 Gachon, C.M.M., Langlois-Meurinne, M., and Saindrenan, P. (2005). Plant secondary metabolism  
865 glycosyltransferases: the emerging functional analysis. *Trends in Plant Science* 10, 542-549.

866 Gardiner, S.A., Boddu, J., Berthiller, F., Hametner, C., Stupar, R.M., Adam, G., and Muehlbauer, G.J.  
867 (2010). Transcriptome Analysis of the Barley-Deoxynivalenol Interaction: Evidence for a Role of  
868 Glutathione in Deoxynivalenol Detoxification. *Mol Plant Microbe In* 23, 962-976.

869 He, Y., Wu, L., Liu, X., Jiang, P., Yu, L.X., Qiu, J.B., Wang, G., Zhang, X., and Ma, H.X. (2020). TaUGT6, a  
870 Novel UDP-Glycosyltransferase Gene Enhances the Resistance to FHB and DON Accumulation in Wheat.  
871 *Front Plant Sci* 11.

872 Hemshrot, A., Poets, A.M., Tyagi, P., Lei, L., Carter, C.K., Hirsch, C.N., Li, L., Brown-Guedira, G., Morrell,  
873 P.L., Muehlbauer, G.J., *et al.* (2019). Development of a Multiparent Population for Genetic Mapping and  
874 Allele Discovery in Six-Row Barley. *Genetics* 213, 595-613.

875 Hensel, G., Valkov, V., Middlefell-Williams, J., and Kumlehn, J. (2008). Efficient generation of transgenic  
876 barley: the way forward to modulate plant-microbe interactions. *J Plant Physiol* 165, 71-82.

877 Himmelbach, A., Zierold, U., Hensel, G., Riechen, J., Douchkov, D., Schweizer, P., and Kumlehn, J. (2007).  
878 A set of modular binary vectors for transformation of cereals. *Plant Physiol* 145, 1192-1200.

879 Hohn, T.M., and Beremand, P.D. (1989). Isolation and Nucleotide-Sequence of a Sesquiterpene Cyclase  
880 Gene from the Trichothecene-Producing Fungus *Fusarium-Sporotrichioides*. *Gene* 79, 131-138.

881 Huang, Y., Haas, M., Heinen, S., Steffenson, B.J., Smith, K.P., and Muehlbauer, G.J. (2018). QTL Mapping  
882 of *Fusarium* Head Blight and Correlated Agromorphological Traits in an Elite Barley Cultivar Rasmusson.  
883 *Front Plant Sci* 9, 1260.

884 Huang, Y.D., Millett, B.P., Beaubien, K.A., Dahl, S.K., Steffenson, B.J., Smith, K.P., and Muehlbauer, G.J.  
885 (2013). Haplotype diversity and population structure in cultivated and wild barley evaluated for  
886 *Fusarium* head blight responses. *Theor Appl Genet* 126, 619-636.



887 Huang, Y.D., Yin, L., Sallam, A.H., Heinen, S., Li, L., Beaubien, K., Dill-Macky, R., Dong, Y.H., Steffenson,  
888 B.J., Smith, K.P., *et al.* (2021). Genetic dissection of a pericentromeric region of barley chromosome 6H  
889 associated with Fusarium head blight resistance, grain protein content and agronomic traits. *Theor Appl*  
890 *Genet* *134*, 3963-3981.

891 Ilgen, P., Hadelar, B., Maier, F.J., and Schafer, W. (2009). Developing kernel and rachis node induce the  
892 trichothecene pathway of Fusarium graminearum during wheat head infection. *Mol Plant Microbe*  
893 *Interact* *22*, 899-908.

894 Jansen, C., von Wettstein, D., Schafer, W., Kogel, K.H., Felk, A., and Maier, F.J. (2005). Infection patterns  
895 in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted Fusarium  
896 graminearum. *Proc Natl Acad Sci U S A* *102*, 16892-16897.

897 Jayakodi, M., Padmarasu, S., Haberer, G., Bonthala, V.S., Gundlach, H., Monat, C., Lux, T., Kamal, N.,  
898 Lang, D., Himmelbach, A., *et al.* (2020). The barley pan-genome reveals the hidden legacy of mutation  
899 breeding. *Nature* *588*, 284-289.

900 Johns, L.E., Bebbler, D.P., Gurr, S.J., and Brown, N.A. (2022). Emerging health threat and cost of Fusarium  
901 mycotoxins in European wheat. *Nat Food* *3*, 1014-+.

902 Kirana, R.P., Gaurav, K., Arora, S., Wiesenberger, G., Doppler, M., Michel, S., Zimmerl, S., Matic, M., Eze,  
903 C.E., Kumar, M., *et al.* (2022). Identification of a UDP-glucosyltransferase conferring deoxynivalenol  
904 resistance in Aegilops tauschii and wheat. *Plant Biotechnology Journal*.

905 Kono, T.J.Y., Liu, C., Vonderharr, E.E., Koenig, D., Fay, J.C., Smith, K.P., and Morrell, P.L. (2019). The Fate  
906 of Deleterious Variants in a Barley Genomic Prediction Population. *Genetics* *213*, 1531-1544.

907 Lee, H.J., and Ryu, D. (2017). Worldwide Occurrence of Mycotoxins in Cereals and Cereal-Derived Food  
908 Products: Public Health Perspectives of Their Co-occurrence. *J Agric Food Chem* *65*, 7034-7051.

909 Lei, L., Poets, A.M., Liu, C.C., Wyant, S.R., Hoffman, P.J., Carter, C.K., Shaw, B.G., Li, X., Muehlbauer, G.J.,  
910 Katagiri, F., *et al.* (2019). Environmental Association Identifies Candidates for Tolerance to Low  
911 Temperature and Drought. *G3-Genes Genom Genet* *9*, 3423-3438.

912 Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Buerstmayr,  
913 H., Mesterhazy, A., Krska, R., *et al.* (2005). The ability to detoxify the mycotoxin deoxynivalenol  
914 colocalizes with a major quantitative trait locus for fusarium head blight resistance in wheat. *Mol Plant*  
915 *Microbe In* *18*, 1318-1324.

916 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R.,  
917 and Proc, G.P.D. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* *25*, 2078-  
918 2079.

919 Li, X., Michlmayr, H., Schweiger, W., Malachova, A., Shin, S., Huang, Y.D., Dong, Y.H., Wiesenberger, G.,  
920 McCormick, S., Lemmens, M., *et al.* (2017). A barley UDP-glucosyltransferase inactivates nivalenol and  
921 provides Fusarium Head Blight resistance in transgenic wheat. *Journal of Experimental Botany* *68*, 2187-  
922 2197.

923 Li, X., Shin, S., Heinen, S., Dill-Macky, R., Berthiller, F., Nersesian, N., Clemente, T., McCormick, S., and  
924 Muehlbauer, G.J. (2015). Transgenic Wheat Expressing a Barley UDP-Glucosyltransferase Detoxifies  
925 Deoxynivalenol and Provides High Levels of Resistance to Fusarium graminearum. *Mol Plant Microbe In*  
926 *28*, 1237-1246.

927 Liu, C., Hoffman, P.J., Wyant, S.R., Dittmar, E.L., Takebasahi, N., Hamann, S., Lei, L., and Morrell, P.L.  
928 (2022). MorrellLAB/sequence\_handling: Release v3.0: SNP calling with GATK 4.1 and Slurm  
929 compatibility.

930 Mascher, M., Richmond, T.A., Gerhardt, D.J., Himmelbach, A., Clissold, L., Sampath, D., Ayling, S.,  
931 Steuernagel, B., Pfeifer, M., D'Ascenzo, M., *et al.* (2013). Barley whole exome capture: a tool for genomic  
932 research in the genus Hordeum and beyond. *Plant J* *76*, 494-505.

933 Massman, J., Cooper, B., Horsley, R., Neate, S., Dill-Macky, R., Chao, S., Dong, Y., Schwarz, P.,  
934 Muehlbauer, G.J., and Smith, K.P. (2011). Genome-wide association mapping of Fusarium head blight  
935 resistance in contemporary barley breeding germplasm. *Molecular Breeding* 27, 439-454.

936 Mayer, K.F.X., Waugh, R., Langridge, P., Close, T.J., Wise, R.P., Graner, A., Matsumoto, T., Sato, K.,  
937 Schulman, A., Muehlbauer, G.J., *et al.* (2012). A physical, genetic and functional sequence assembly of  
938 the barley genome. *Nature* 491, 711-+.

939 McCormick, S.P., Stanley, A.M., Stover, N.A., and Alexander, N.J. (2011). Trichothecenes: From Simple to  
940 Complex Mycotoxins. *Toxins* 3, 802-814.

941 McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytzky, A., Garimella, K., Altshuler,  
942 D., Gabriel, S., Daly, M., *et al.* (2010). The Genome Analysis Toolkit: A MapReduce framework for  
943 analyzing next-generation DNA sequencing data. *Genome Res* 20, 1297-1303.

944 McLaughlin, C.S., Vaughn, M.H., Campbell, J.M., Wei, C.M., Stafford, M.E., and Hansin, B.S., eds. (1977).  
945 Inhibition of protein synthesis by trichothecenes. (Park Forest South, IL, U.S.A., Pathotoxin Publishers).

946 Mesfin, A., Smith, K.P., Dill-Macky, R., Evans, C.K., Waugh, R., Gustus, C.D., and Muehlbauer, G.J. (2003).  
947 Quantitative trait loci for fusarium head blight resistance in barley detected in a two-rowed by six-rowed  
948 population. *Crop Sci* 43, 307-318.

949 Michlmayr, H., Varga, E., Lupi, F., Malachova, A., Hametner, C., Berthiller, F., and Adam, G. (2017).  
950 Synthesis of Mono- and Di-Glucosides of Zearalenone and alpha-/beta-Zearalenol by Recombinant  
951 Barley Glucosyltransferase HvUGT14077. *Toxins* 9.

952 Mirocha, C.J., Kolaczowski, E., Xie, W.P., Yu, H., and Jelen, H. (1998). Analysis of deoxynivalenol and its  
953 derivatives (batch and single kernel) using gas chromatography mass spectrometry. *J Agr Food Chem* 46,  
954 1414-1418.

955 Monat, C., Padmarasu, S., Lux, T., Wicker, T., Gundlach, H., Himmelbach, A., Ens, J., Li, C.D., Muehlbauer,  
956 G.J., Schulman, A.H., *et al.* (2019). TRITEX: chromosome-scale sequence assembly of Triticeae genomes  
957 with open-source tools. *Genome Biology* 20.

958 Morrell, P.L., Gonzales, A.M., Meyer, K.K.T., and Clegg, M.T. (2014). Resequencing Data Indicate a  
959 Modest Effect of Domestication on Diversity in Barley: A Cultigen With Multiple Origins. *J Hered* 105,  
960 253-264.

961 Morrell, P.L., Lundy, K.E., and Clegg, M.T. (2003). Distinct geographic patterns of genetic diversity are  
962 maintained in wild barley (*Hordeum vulgare* ssp *spontaneum*) despite migration. *P Natl Acad Sci USA*  
963 100, 10812-10817.

964 Morrell, P.L., Toleno, D.M., Lundy, K.E., and Clegg, M.T. (2006). Estimating the contribution of mutation,  
965 recombination and gene conversion in the generation of haplotypic diversity. *Genetics* 173, 1705-1723.

966 Muyas, F., Bosio, M., Puig, A., Susak, H., Domenech, L., Escaramis, G., Zapata, L., Demidov, G., Estivill, X.,  
967 Rabionet, R., *et al.* (2019). Allele balance bias identifies systematic genotyping errors and false disease  
968 associations. *Hum Mutat* 40, 115-126.

969 Nice, L.M., Steffenson, B.J., Brown-Guedira, G.L., Akhunov, E.D., Liu, C., Kono, T.J.Y., Morrell, P.L., Blake,  
970 T.K., Horsley, R.D., Smith, K.P., *et al.* (2016). Development and Genetic Characterization of an Advanced  
971 Backcross-Nested Association Mapping (AB-NAM) Population of Wild x Cultivated Barley. *Genetics* 203,  
972 1453-+.

973 Pasquet, J.C., Changenet, V., Macadre, C., Boex-Fontvieille, E., Soulhat, C., Bouchabke-Coussa, O.,  
974 Dalmais, M., Atanasova-Penichon, V., Bendahmane, A., Saindrenan, P., *et al.* (2016). A Brachypodium  
975 UDP-Glycosyltransferase Confers Root Tolerance to Deoxynivalenol and Resistance to Fusarium  
976 Infection. *Plant Physiol* 172, 559-574.

977 Pedersen, B.S., Brown, J.M., Dashnow, H., Wallace, A.D., Velinder, M., Tristani-Firouzi, M., Schiffman,  
978 J.D., Tvrdik, T., Mao, R., Best, D.H., *et al.* (2021). Effective variant filtering and expected candidate  
979 variant yield in studies of rare human disease. *Npj Genom Med* 6.

980 Poppenberger, B., Berthiller, F., Bachmann, H., Lucyshyn, D., Peterbauer, C., Mitterbauer, R.,  
981 Schuhmacher, R., Krska, R., Glossl, J., and Adam, G. (2006). Heterologous expression of Arabidopsis UDP-  
982 glucosyltransferases in *Saccharomyces cerevisiae* for production of zearalenone-4-O-glucoside. *Appl*  
983 *Environ Microb* 72, 4404-4410.

984 Poppenberger, B., Berthiller, F., Lucyshyn, D., Sieberer, T., Schuhmacher, R., Krska, R., Kuchler, K., Glossl,  
985 J., Luschnig, C., and Adam, G. (2003). Detoxification of the *Fusarium* mycotoxin deoxynivalenol by a UDP-  
986 glucosyltransferase from *Arabidopsis thaliana*. *J Biol Chem* 278, 47905-47914.

987 Poppenberger, B., Fujioka, S., Soeno, K., George, G.L., Vaistij, F.E., Hiranuma, S., Seto, H., Takatsuto, S.,  
988 Adam, G., Yoshida, S., *et al.* (2005). The UGT73C5 of *Arabidopsis thaliana* glucosylates brassinosteroids.  
989 *P Natl Acad Sci USA* 102, 15253-15258.

990 Proctor, R.H., Hohn, T.M., and McCormick, S.P. (1995). Reduced virulence of *Gibberella zeae* caused by  
991 disruption of a trichothecene toxin biosynthetic gene. *Mol Plant Microbe Interact* 8, 593-601.

992 Proctor, R.H., McCormick, S.P., Kim, H.S., Cardoza, R.E., Stanley, A.M., Lindo, L., Kelly, A., Brown, D.W.,  
993 Lee, T., Vaughan, M.M., *et al.* (2018). Evolution of structural diversity of trichothecenes, a family of  
994 toxins produced by plant pathogenic and entomopathogenic fungi. *Plos Pathog* 14.

995 Quinlan, A.R., and Hall, I.M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features.  
996 *Bioinformatics* 26, 841-842.

997 Rodrigues, I., Handl, J., and Binder, E.M. (2011). Mycotoxin occurrence in commodities, feeds and feed  
998 ingredients sourced in the Middle East and Africa. *Food Addit Contam B* 4, 168-179.

999 Russell, J., Mascher, M., Dawson, I.K., Kyriakidis, S., Calixto, C., Freund, F., Bayer, M., Milne, I., Marshall-  
1000 Griffiths, T., Heinen, S., *et al.* (2016). Exome sequencing of geographically diverse barley landraces and  
1001 wild relatives gives insights into environmental adaptation. *Nat Genet* 48, 1024-+.

1002 Santini, A., Meca, G., Uhlig, S., and Ritieni, A. (2012). Fusaproliferin, beauvericin and enniatins:  
1003 occurrence in food - a review. *World Mycotoxin J* 5, 71-81.

1004 Schroeder, H.W., and Christensen, J.J. (1963). Factors Affecting Resistance of Wheat to Scab Caused by  
1005 *Gibberella Zeae*. *Phytopathology* 53, 831-&.

1006 Schweiger, W., Boddu, J., Shin, S., Poppenberger, B., Berthiller, F., Lemmens, M., Muehlbauer, G.J., and  
1007 Adam, G. (2010). Validation of a candidate deoxynivalenol-inactivating UDP-glucosyltransferase from  
1008 barley by heterologous expression in yeast. *Mol Plant Microbe Interact* 23, 977-986.

1009 Schweiger, W., Steiner, B., Ametz, C., Siegwart, G., Wiesenberger, G., Berthiller, F., Lemmens, M., Jia,  
1010 H.Y., Adam, G., Muehlbauer, G.J., *et al.* (2013). Transcriptomic characterization of two major *Fusarium*  
1011 resistance quantitative trait loci (QTLs), *Fhb1* and *Qfhs.ifa-5A*, identifies novel candidate genes. *Mol*  
1012 *Plant Pathol* 14, 772-785.

1013 Shin, S., Torres-Acosta, J.A., Heinen, S.J., McCormick, S., Lemmens, M., Paris, M.P., Berthiller, F., Adam,  
1014 G., and Muehlbauer, G.J. (2012). Transgenic *Arabidopsis thaliana* expressing a barley UDP-  
1015 glucosyltransferase exhibit resistance to the mycotoxin deoxynivalenol. *J Exp Bot* 63, 4731-4740.

1016 Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J., and  
1017 O'Donnell, K. (2007). Global molecular surveillance reveals novel *Fusarium* head blight species and  
1018 trichothecene toxin diversity. *Fungal Genet Biol* 44, 1191-1204.

1019 Steffenson, B.J. (2003). *Fusarium* head blight of barley: Impact, epidemics, management, and strategies  
1020 for identifying and utilizing genetic resistance. In *Fusarium head blight of wheat and barley*, K.J. Leonard,  
1021 and W.R. Bushnell, eds. (St. Paul, MN, USA, American Phytopathological Society Press), pp. 241-295.

1022 Talame, V., Bovina, R., Sanguineti, M.C., Tuberosa, R., Lundqvist, U., and Salvi, S. (2008a). TILLMore, a  
1023 resource for the discovery of chemically induced mutants in barley. *Plant Biotechnology Journal* 6, 477-  
1024 485.

1025 Talame, V., Bovina, R., Sanguineti, M.C., Tuberosa, R., Lundqvist, U., and Salvi, S. (2008b). TILLMore, a  
1026 resource for the discovery of chemically induced mutants in barley. *Plant Biotechnol J* 6, 477-485.

1027 Varga, E., Wiesenberger, G., Hametner, C., Ward, T.J., Dong, Y.H., Schofbeck, D., McCormick, S., Broz, K.,  
1028 Stuckler, R., Schuhmacher, R., *et al.* (2015). New tricks of an old enemy: isolates of *Fusarium*  
1029 *graminearum* produce a type A trichothecene mycotoxin. *Environ Microbiol* *17*, 2588-2600.  
1030 Vogt, T., and Jones, P. (2000). Glycosyltransferases in plant natural product synthesis: characterization of  
1031 a supergene family. *Trends in Plant Science* *5*, 380-386.  
1032 Wang, H., Sun, S., Ge, W., Zhao, L., Hou, B., Wang, K., Lyu, Z., Chen, L., Xu, S., Guo, J., *et al.* (2020).  
1033 Horizontal gene transfer of *Fhb7* from fungus underlies *Fusarium* head blight resistance in wheat.  
1034 *Science* *368*.  
1035 Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer,  
1036 T.A.P., Rempfer, C., Bordoli, L., *et al.* (2018). SWISS-MODEL: homology modelling of protein structures  
1037 and complexes. *Nucleic Acids Research* *46*, W296-W303.  
1038 Wetterhorn, K.M., Gabardi, K., Michlmayr, H., Malachova, A., Busman, M., McCormick, S.P., Berthiller,  
1039 F., Adam, G., and Rayment, I. (2017). Determinants and Expansion of Specificity in a Trichothecene UDP-  
1040 Glucosyltransferase from *Oryza sativa*. *Biochemistry* *56*, 6585-6596.  
1041 Wetterhorn, K.M., Newmister, S.A., Caniza, R.K., Busman, M., McCormick, S.P., Berthiller, F., Adam, G.,  
1042 and Rayment, I. (2016). Crystal Structure of Os79 (Os04g0206600) from *Oryza sativa*: A UDP-  
1043 glucosyltransferase Involved in the Detoxification of Deoxynivalenol. *Biochemistry* *55*, 6175-6186.  
1044 Xu, X.M., and Nicholson, P. (2009). Community Ecology of Fungal Pathogens Causing Wheat Head Blight.  
1045 *Annual Review of Phytopathology* *47*, 83-103.  
1046 Zhu, H., Gilchrist, L., Hayes, P., Kleinhofs, A., Kudrna, D., Liu, Z., Prom, L., Steffenson, B., Toojinda, T., and  
1047 Vivar, H. (1999). Does function follow form? Principal QTLs for *Fusarium* head blight (FHB) resistance are  
1048 coincident with QTLs for inflorescence traits and plant height in a doubled-haploid population of barley.  
1049 *Theor Appl Genet* *99*, 1221-1232.

1050

1051 FIGURE LEGENDS:

1052 **Figure 1: UGT13248 can alleviate DON induced root growth inhibition in barley seedlings.**

1053 A) – D) Two- to three-day old barley seedlings were transferred onto 0.5 x MS medium  
1054 containing 1 mg / L DON (DON) or equal volumes of 70 % ethanol (mock). Root length was  
1055 measured one day later (day1) and daily thereafter for 5 additional days. Data are from two to  
1056 four independent experiments with 4-6 seedlings each combined. Means and SEM are shown.  
1057 Asterisks indicated significant differences at  $p < 0.01$  using Student's T-test comparing mock  
1058 and DON samples for each time point within each genotype. A) and B) show results for two  
1059 independent transgenic lines that express UGT13248 under control of *Zea mays* Ubi-1 promoter  
1060 (UGT+), non-transgenic sister lines (UGT-), **wild-type Golden Promise and Rasmusson**. C) and D)  
1061 show plants with mutations in UGT13248 T368I, H369Y and S403N as well as wild-type Morex  
1062 seedlings.

1063

1064 **Figure 2: Protein models of UGT13248.**

1065 Protein models based on the rice Os79 crystal structure are shown. Trichothecene is shown in  
1066 purple, the non-reactive substrate UDP-2-fluoro-2-deoxy-D-glucose in orange, the conserved  
1067 Thr299 and His38 residues in yellow and His369 and Thr368 in green. For T368I Thr at position  
1068 368 was replaced with Ile, for H369Y His at position 369 was replaced by Tyr and for S403N Ser  
1069 at position 403 was replaced by Asn. Mutated residues are shown in pink.

1070

1071 **Figure 3: UGT13248 catalyzes DON to D3G conversion in barley spikes.**

1072 A) Barley spikes of two sister lines one containing a *ZmUBi-1::UGT13248* transgene (UGT+) and  
1073 one without (UGT-), were inoculated with 2 µg DON into 8 spikelets per spike at anthesis.  
1074 Samples from two separate spikes were collected at the indicated time point and combined for each  
1075 biological replicate. DON and D3G were measured. Data are shown as the mean ratio of D3G to DON of  
1076 3 independent biological replicates each and SEM. Asterisks indicate significant differences between  
1077 UGT- and UGT+ at each time point at  $p < 0.01$  using Student's T-test. B) Samples were treated and  
1078 collected as described in A) for the indicated genotypes.

1079

1080 **Figure 4: Mutations that reduce UGT13248 function result in increased susceptibility to**

1081 ***Fusarium graminearum* in the field.** A) and B) Plants of the indicated genotypes were grown in  
1082 the field, inoculated with *F. graminearum* at anthesis and scored 14 days later. For Morex,  
1083 T368I, H369Y and S403N six independent rows and for Chevron and Stander three rows each  
1084 per year (2019 or 2020) and environment (Saint Paul (SP) or Crookston (CR)) were used. For  
1085 each biological replicate 10 spikes per row were evaluated for FHB severity and combined.  
1086 Mean and SEM for three to six biological replicates each are shown. Asterisks indicate  
1087 statistically significant differences from wild-type Morex at  $p < 0.05$  using Student's T-test. C) to  
1088 D) Three spikes per row for each year were collected in Saint Paul at the indicated time points  
1089 and combined. DON and D3G were measured for four biological replicates each. Mean and SEM  
1090 for D3G to DON ratio are shown. Asterisks indicate significant differences to wild-type Morex at  
1091 each time point at  $p < 0.01$  using Student's T-test.

1092

1093 **Figure 5: UGT13248 is required for type II resistance.** A) and C) photographs show  
1094 representative images of spikes for each indicated genotype 14 (A) or 21 (C) days after point  
1095 inoculation with *F. graminearum* PH-1. Scale bars are 1 cm. B) and D) Plants of the indicated  
1096 genotypes were point inoculated in one central spikelet in the middle of the spike on each side  
1097 the spike at anthesis. FHB severity was assessed at the indicated time points. 16-24 spikes each  
1098 were assessed in two independent experiments and data were combined. Mean and SEM are  
1099 plotted. Asterisks indicate statistically significant differences at  $p < 0.05$  using Student's T-test.

1100

1101 **Figure 6: *F. graminearum* spread and DON accumulation in the rachis was increased in H369Y**  
1102 **compared to wild-type Morex plants.** A)-C) Inoculated spikes at 14 days after inoculation with  
1103 *F. graminearum* PH-1 were separated into five equal portions and rachis and spikelets were  
1104 collected separately. The sections were labelled section 1 to section 5 from the basipetal  
1105 portion of the spike to the most acropetal portion. Section 3 contained the inoculated spikelets.  
1106 Samples from three spikes each were combined and four independent samples each were  
1107 collected. A) Ergosterol as a proxy for fungal growth and B) DON was measured in each sample.  
1108 Means and SEM are shown. Asterisks indicate significant differences between Morex and  
1109 H369Y within each section and tissue at  $p < 0.05$  using Student's T-test. C) Graphic depiction of  
1110 data shown in A) and B). Colors indicate concentration of ergosterol and DON measured.

1111

1112 **Figure 7: *F. graminearum* was observed within the rachis of T368I and H369Y but not wild-**  
1113 **type Morex plants.**

1114 A) The junction between the most basipetal part of the spike and the peduncle of H369Y, T368I  
1115 and wild-type sister lines (WT) for each allele, was imaged with confocal laser scanning  
1116 microscopy 14 days after inoculation with *F. graminearum* (strain 8/1 (gpdAp::DsRed)). Scale  
1117 bars are 200  $\mu\text{m}$ . Blue color indicated autofluorescence, red color indicated fungal tissue. A 4x  
1118 objective lens was used for both wild-type and mutant plants. A zoomed in image with a 10x  
1119 objective lens was taken for both mutants. White squares indicate the region shown in higher

1120 magnification at 10x. B) Rachis nodes of the indicated genotypes were imaged with confocal  
1121 laser scanning microscopy 14 days after inoculation with *F. graminearum* (strain 8/1  
1122 (gpdAp::DsRed)). Rachis node at inoculated spikelet (inoculated) and four rachis nodes  
1123 basipetal (basipetal) and four rachis nodes acropetal (acropetal) of the inoculated spikelet are  
1124 shown. Scale bars are 200  $\mu\text{m}$ . Blue color indicates autofluorescence, red color indicated fungal  
1125 tissue.

1126

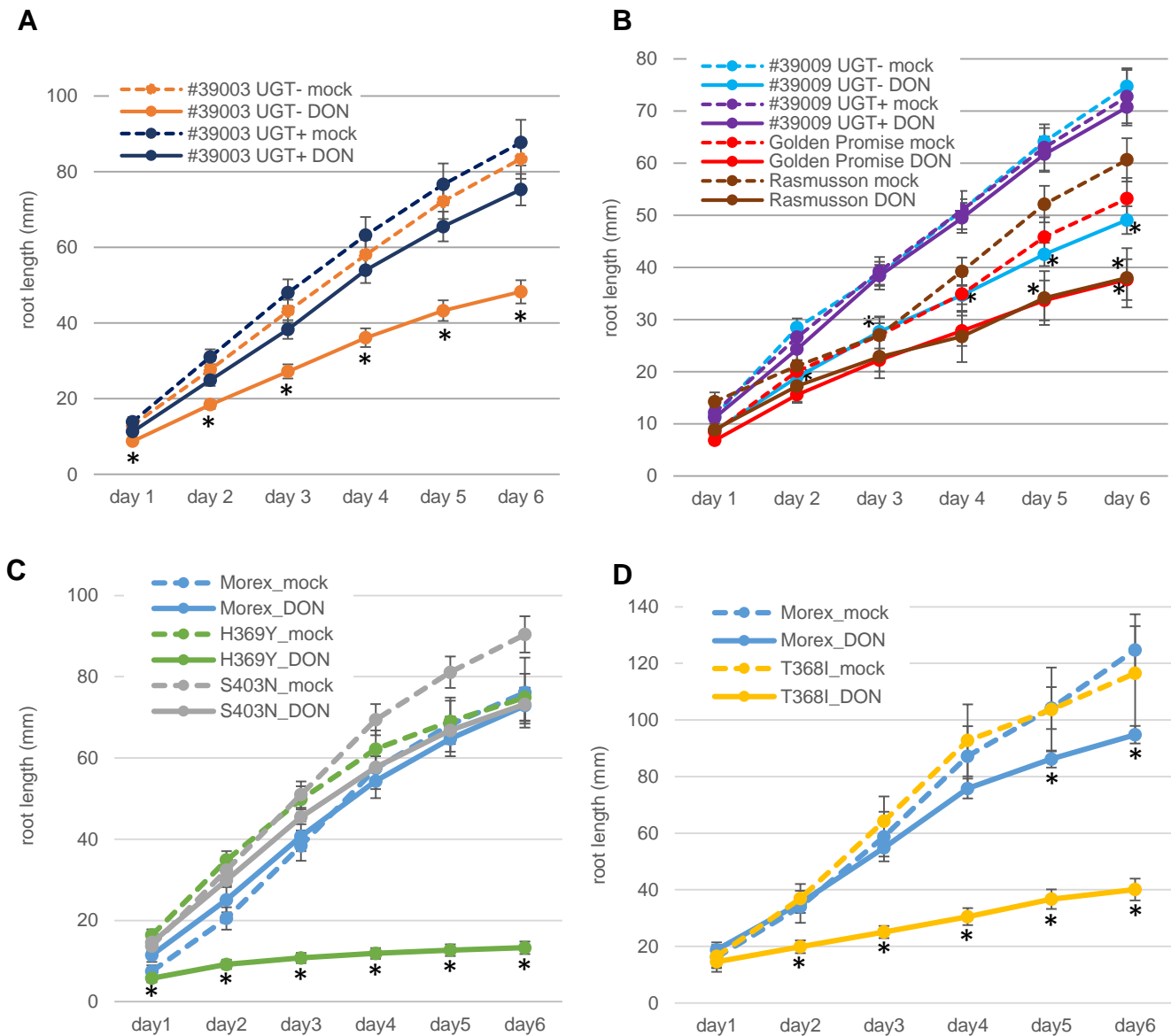
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1128 Table 1: Mutant and transgenic barley lines used in functional studies.

<b>Line designation</b>	<b>Type</b>	<b>Effect on <i>UGT13248</i></b>
Morex	TILLING background	none
T368I	TILLING mutant	nonsynonymous mutation
H369Y	TILLING mutant	nonsynonymous mutation
S403N	TILLING mutant	nonsynonymous mutation
Golden Promise	transformation background	none
#39003-UGT+	transformant	constitutive expression of <i>UGT13248</i>
#39009-UGT+	transformant	constitutive expression of <i>UGT13248</i>
#39003-UGT-	Non-transgenic sister line	none
#39009-UGT-	Non-transgenic sister line	none

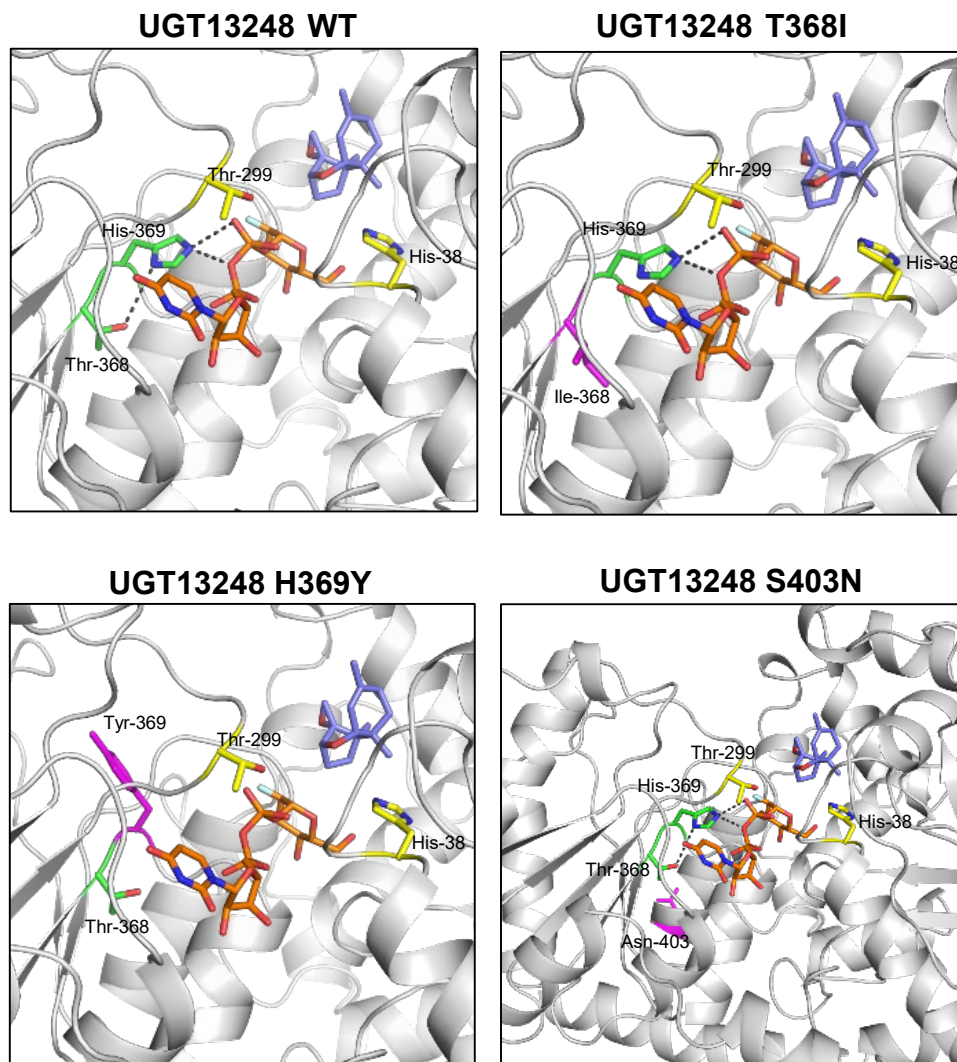
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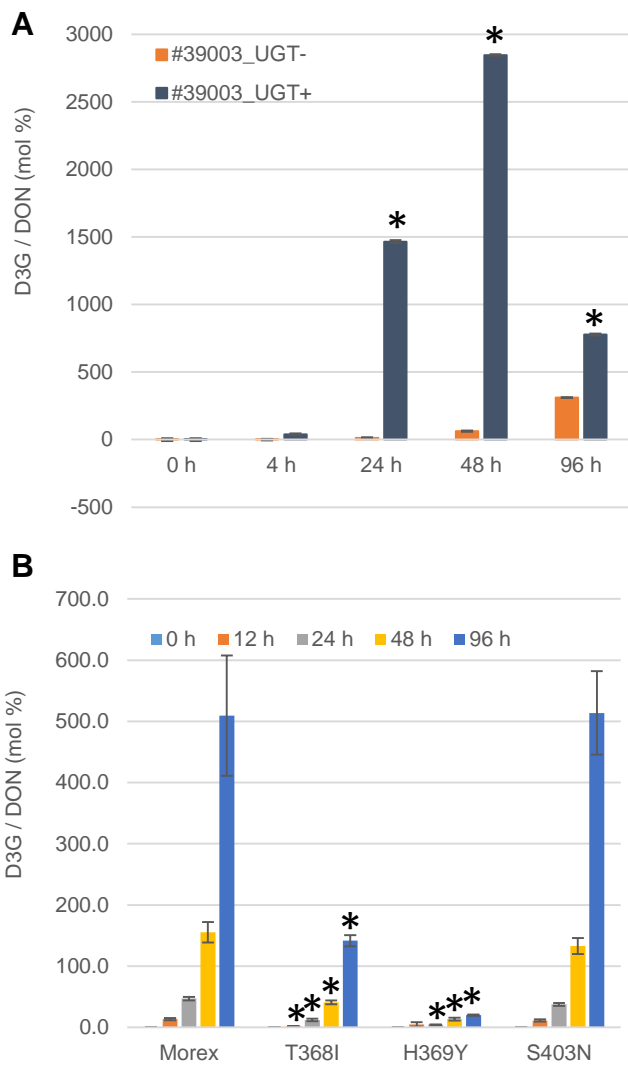
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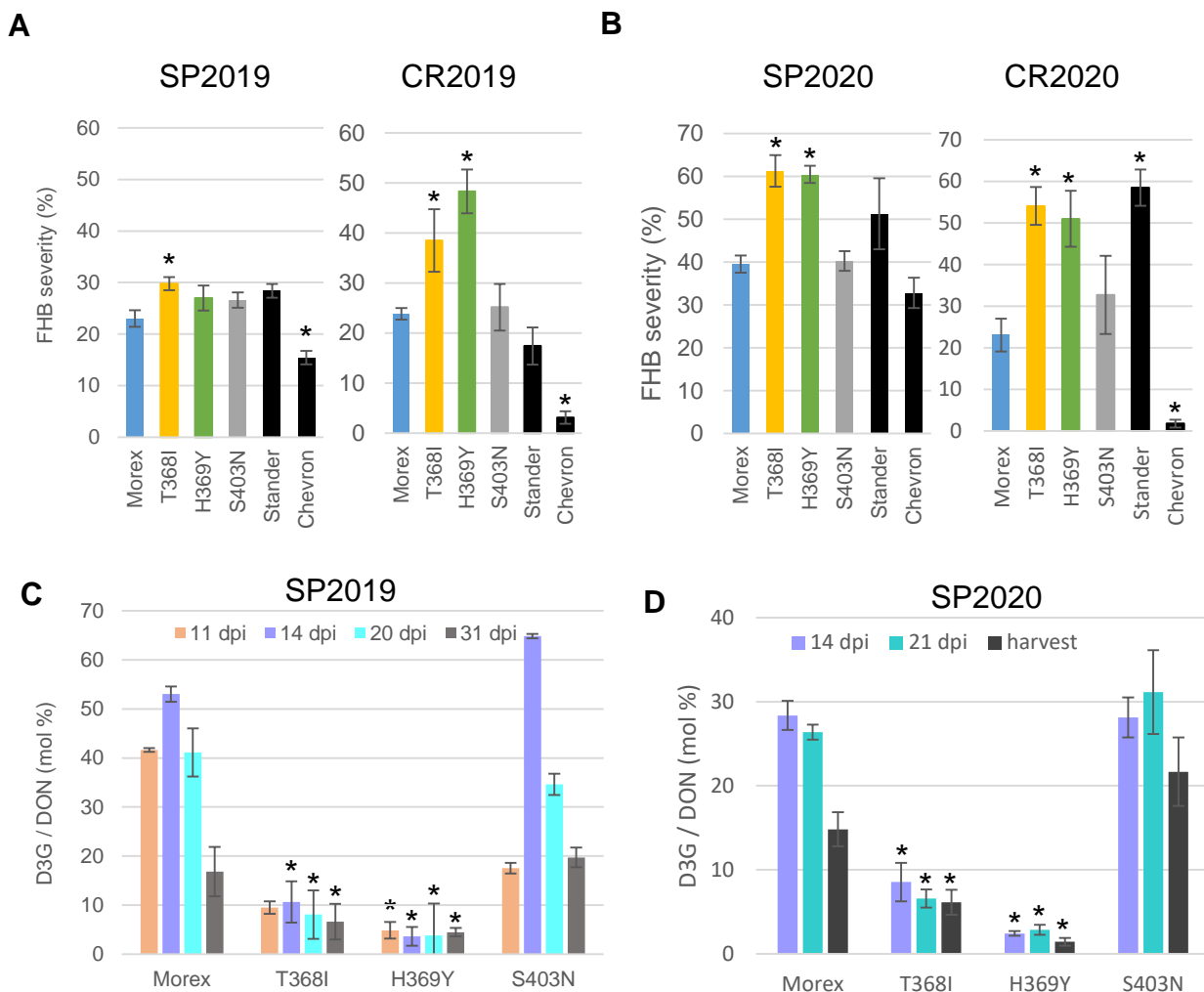
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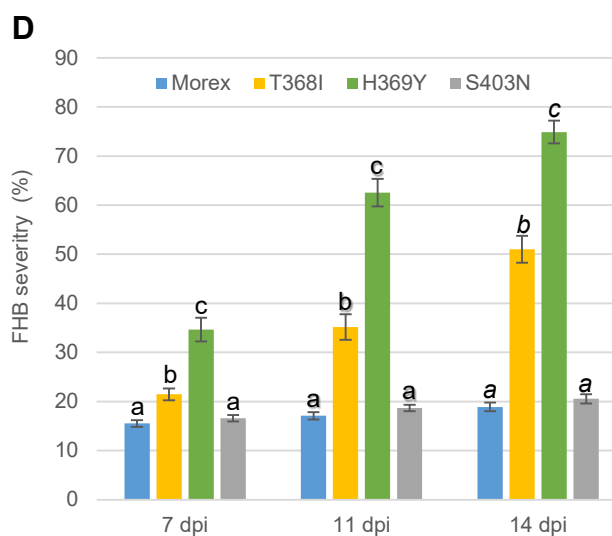
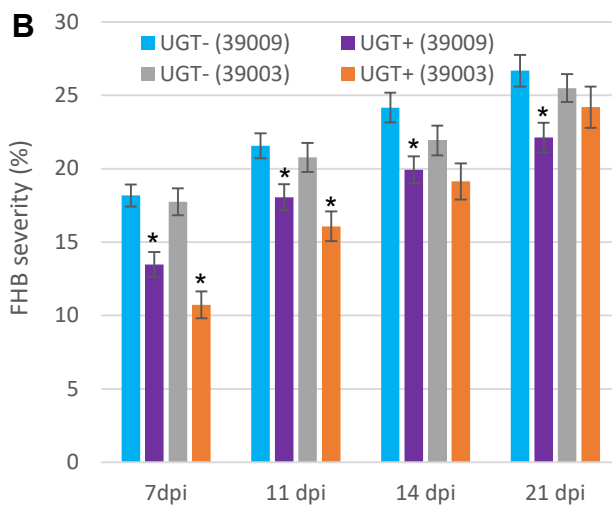
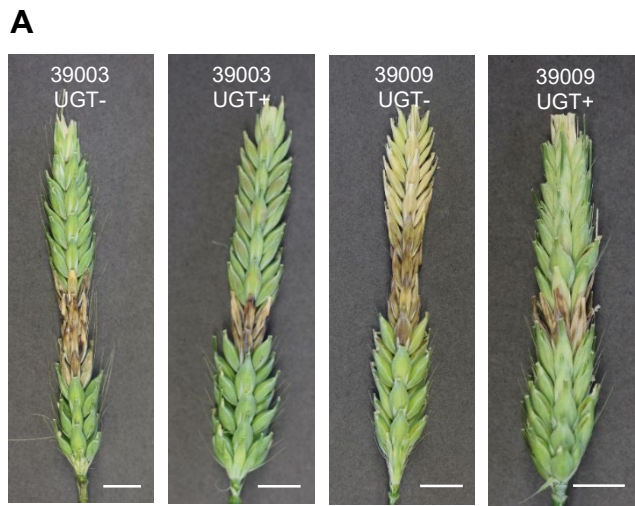


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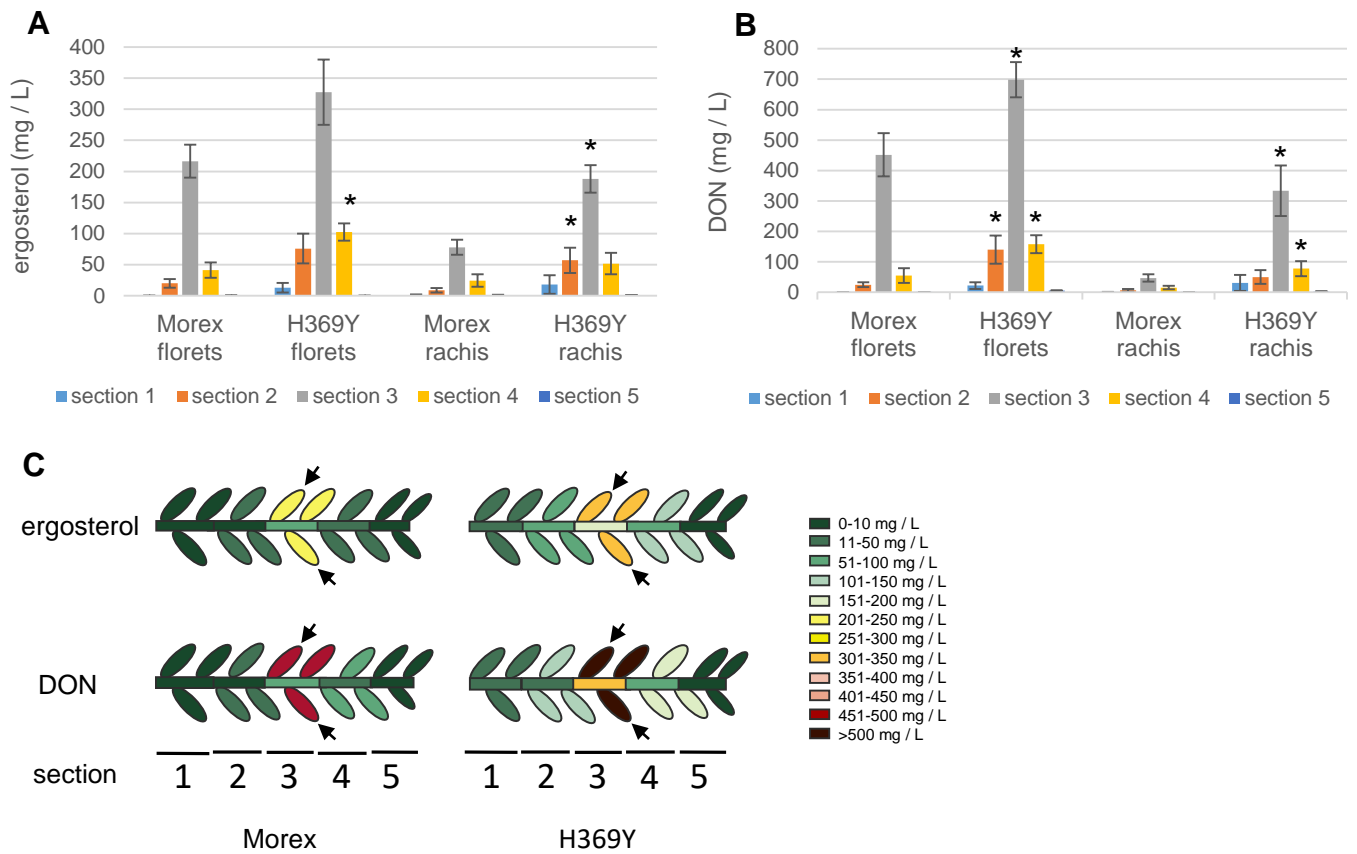
A) Barley spikes of two sister lines one containing a *ZmUBi-1::UGT13248* transgene (UGT+) and one without (UGT-), were inoculated with 2  $\mu$ g DON into 8 spikelets per spike at anthesis. Samples from two separate spikes were collected at the indicated time point and combined for each biological replicate. DON and D3G were measured. Data are shown as the mean ratio of D3G to DON of 3 independent biological replicates each and SEM. Asterisks indicate significant differences between UGT- and UGT+ at each time point at  $p < 0.01$  using Student's T-test. B) Samples were treated and collected as described in A) for the indicated genotypes.



**Figure 4: Mutations that reduce UGT13248 function result in increased susceptibility to *Fusarium graminearum* in the field.** A) and B) Plants of the indicated genotypes were grown in the field, inoculated with *F. graminearum* at anthesis and scored 14 days later. For Morex, T368I, H369Y and S403N six independent rows and for Chevron and Stander three rows each per year (2019 or 2020) and environment (Saint Paul (SP) or Crookston (CR)) were used. For each biological replicate 10 spikes per row were evaluated for FHB severity and combined. Mean and SEM for three to six biological replicates each are shown. Asterisks indicate statistically significant differences from wild-type Morex at  $p < 0.05$  using Student's T-test. C) to D) Three spikes per row for each year were collected in Saint Paul at the indicated time points and combined. DON and D3G were measured for four biological replicates each. Mean and SEM for D3G to DON ratio are shown. Asterisks indicate significant differences to wild-type Morex at each time point at  $p < 0.01$  using Student's T-test.

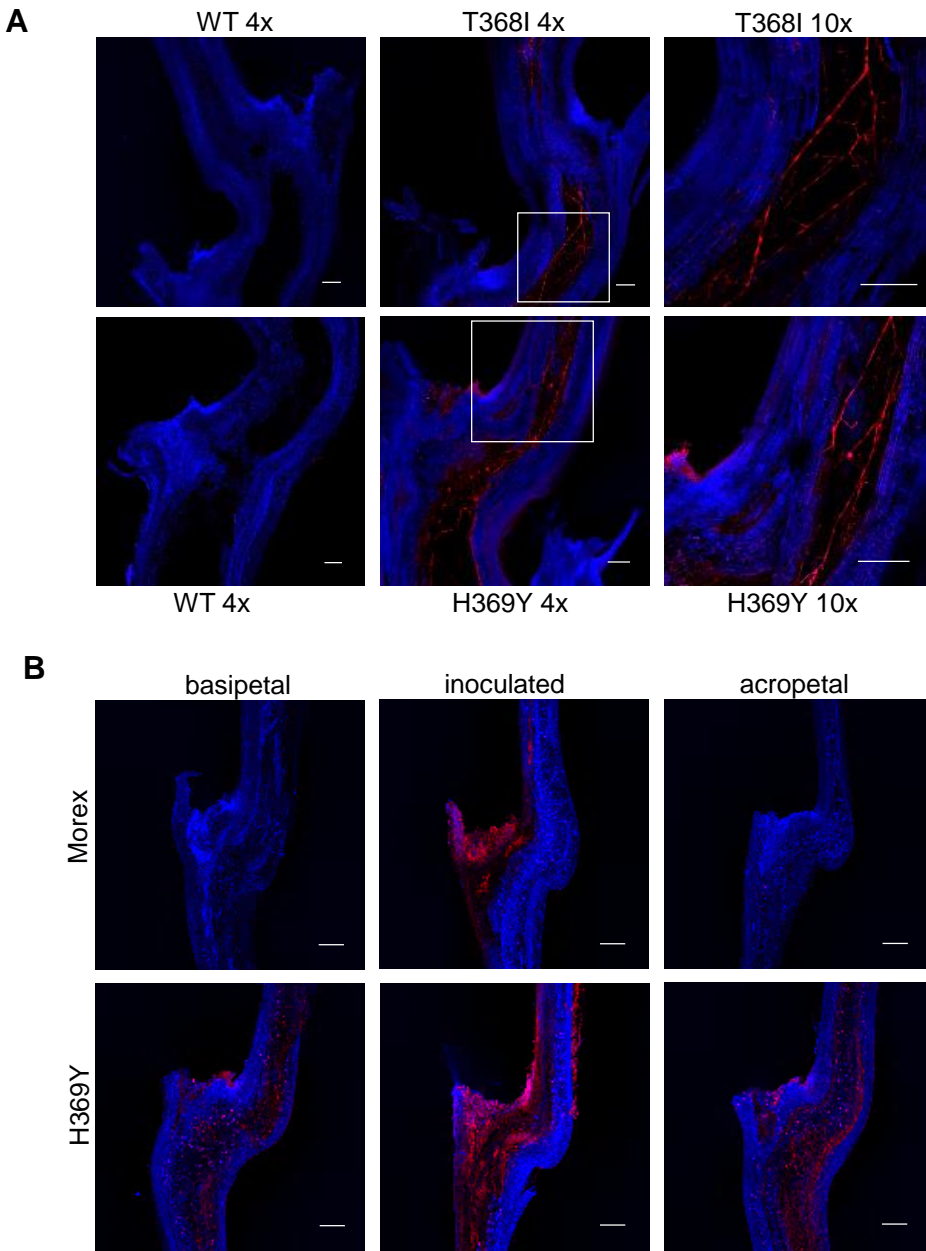


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**Figure 7: *F. graminearum* was observed within the rachis of T368I and H369Y but not wild-type Morex plants.** A) The junction between the most basipetal part of the spike and the peduncle of H369Y, T368I and wild-type sister lines (WT) for each allele, was imaged with confocal laser scanning microscopy 14 days after inoculation with *F. graminearum* (strain 8/1 (gpdAp::DsRed)). Scale bars are 200  $\mu$ m. Blue color indicated autofluorescence, red color indicated fungal tissue. A 4x objective lens was used for both wild-type and mutant plants. A zoomed in image with a 10x objective lens was taken for both mutants. White squares indicate the region shown in higher magnification at 10x. B) Rachis nodes of the indicated genotypes were imaged with confocal laser scanning microscopy 14 days after inoculation with *F. graminearum* (strain 8/1 (gpdAp::DsRed)) using a 4x objective lens. The rachis node at the inoculated spikelet (inoculated) and four rachis nodes basipetal (basipetal) and four rachis nodes acropetal (acropetal) of the inoculated spikelet are shown. Scale bars are 200  $\mu$ m. Blue color indicated autofluorescence, red color indicated fungal tissue.