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

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(Article begins on next page)

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 F₂ 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 α -³²P 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 F₁ plants were backcrossed to Rasmusson. In the F₃ 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 BC₁F₆ 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 NaN₃ 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 BC₁F₂ generation was used. **Sister lines that contained the homozygous
622 mutant or wild-type allele, respectively, were selected for T368I and H369Y in the BC₄F₂
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). These (Quinlan and Hall, 2010)
657 “cap50x” have > 50X coverage in Morex capture data
658 (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).

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 µL 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 (µg / 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 NH₄NO₃, 1 g / L KH₂PO₄, 0.5 g / L MgSO₄ x 7 H₂O 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 x 10⁸ 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 µl *F. graminearum* (PH 1) at 1 x 10⁵
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 × 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 1×10^5 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 METTVTAVSGTTSSSVGHGAGGGAARVLLLPSPGAQGHNPMLQLGRRLAYHGLRPTLVATRYVLSTTPAPGAPFDV
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 10⁵ 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).

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

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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 μm . 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 μm . Blue color indicates autofluorescence, red color indicated fungal
1125 tissue.

1126

1127

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

Line designation	Type	Effect on <i>UGT13248</i>
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

1129

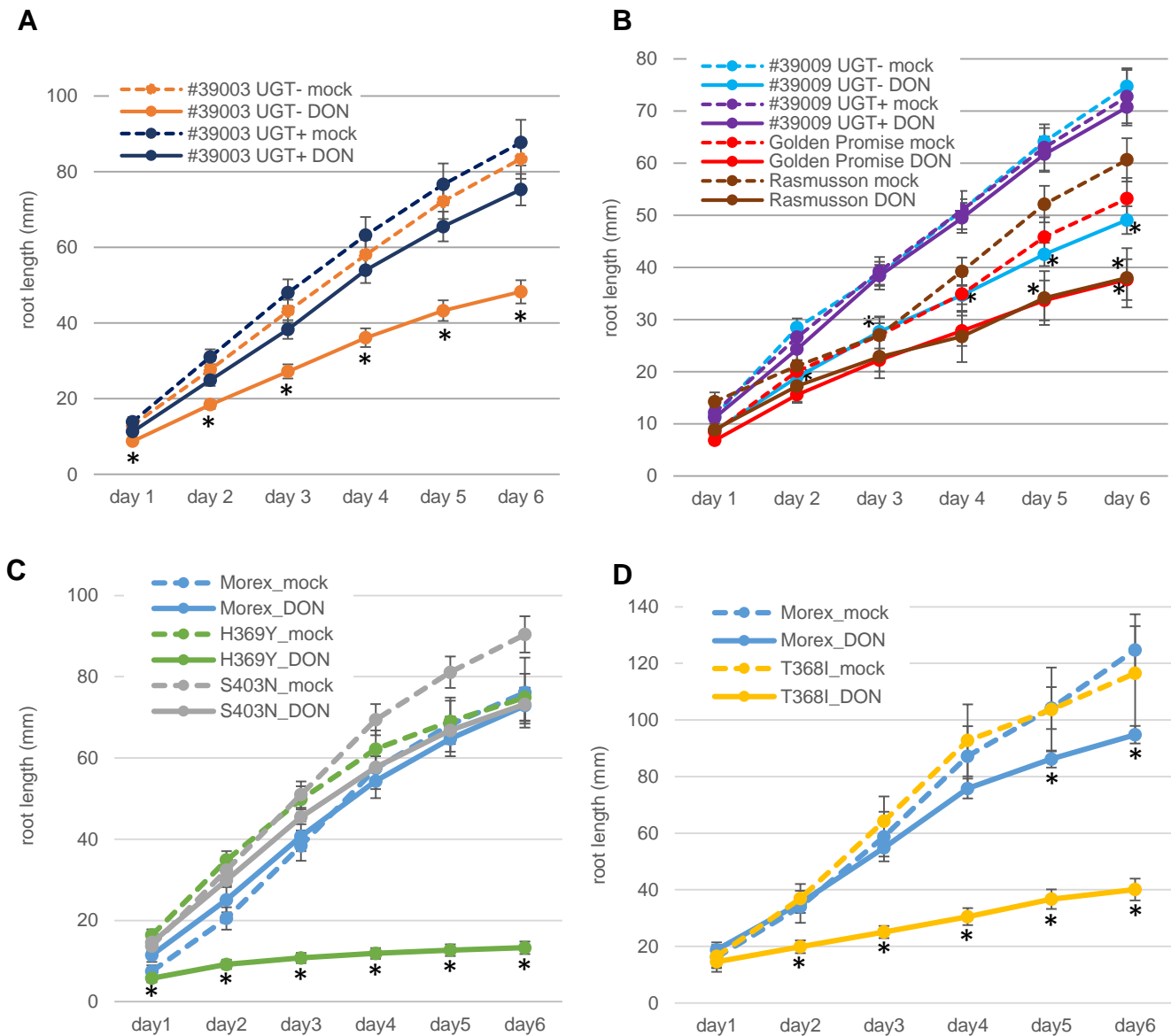


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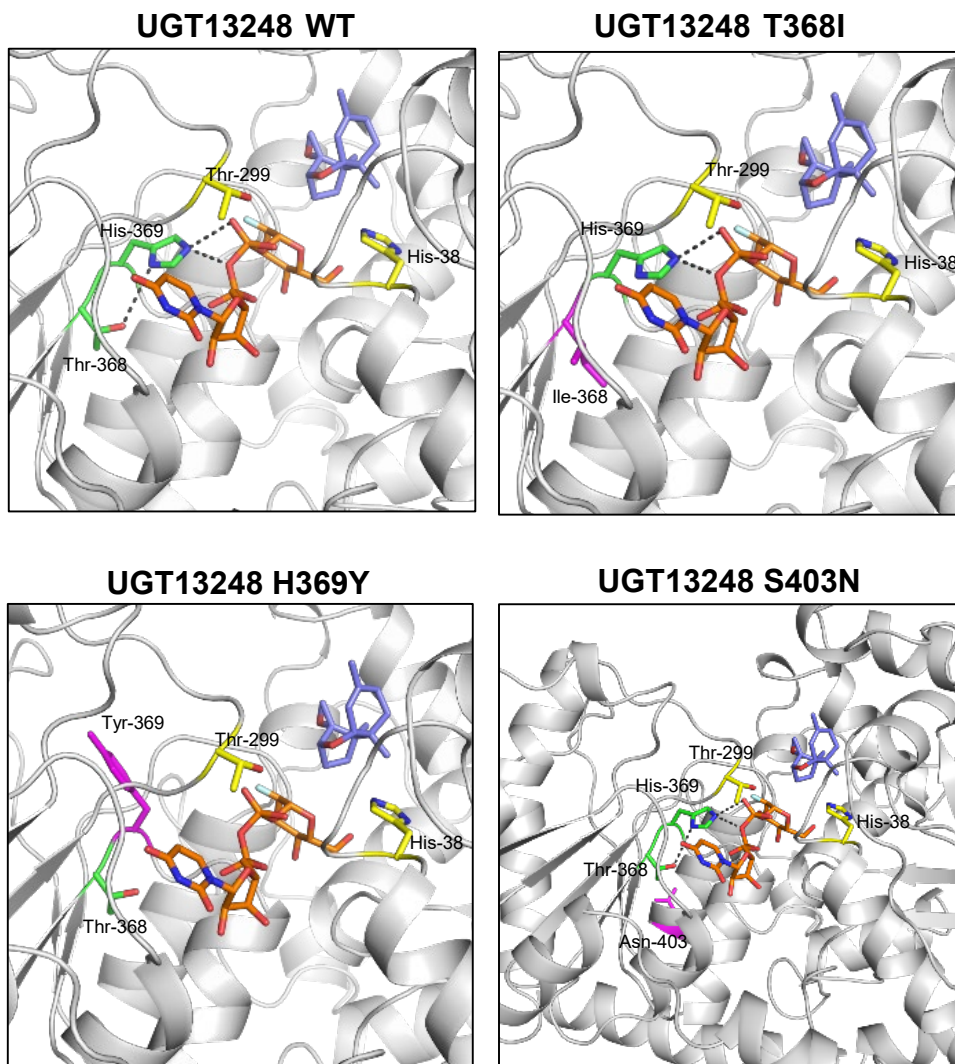


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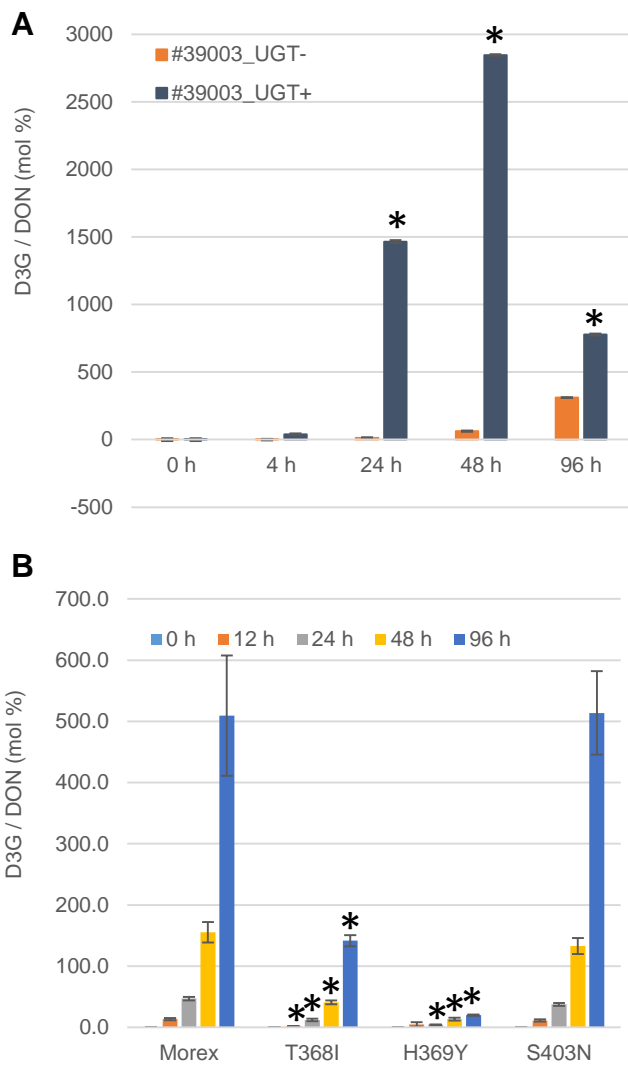


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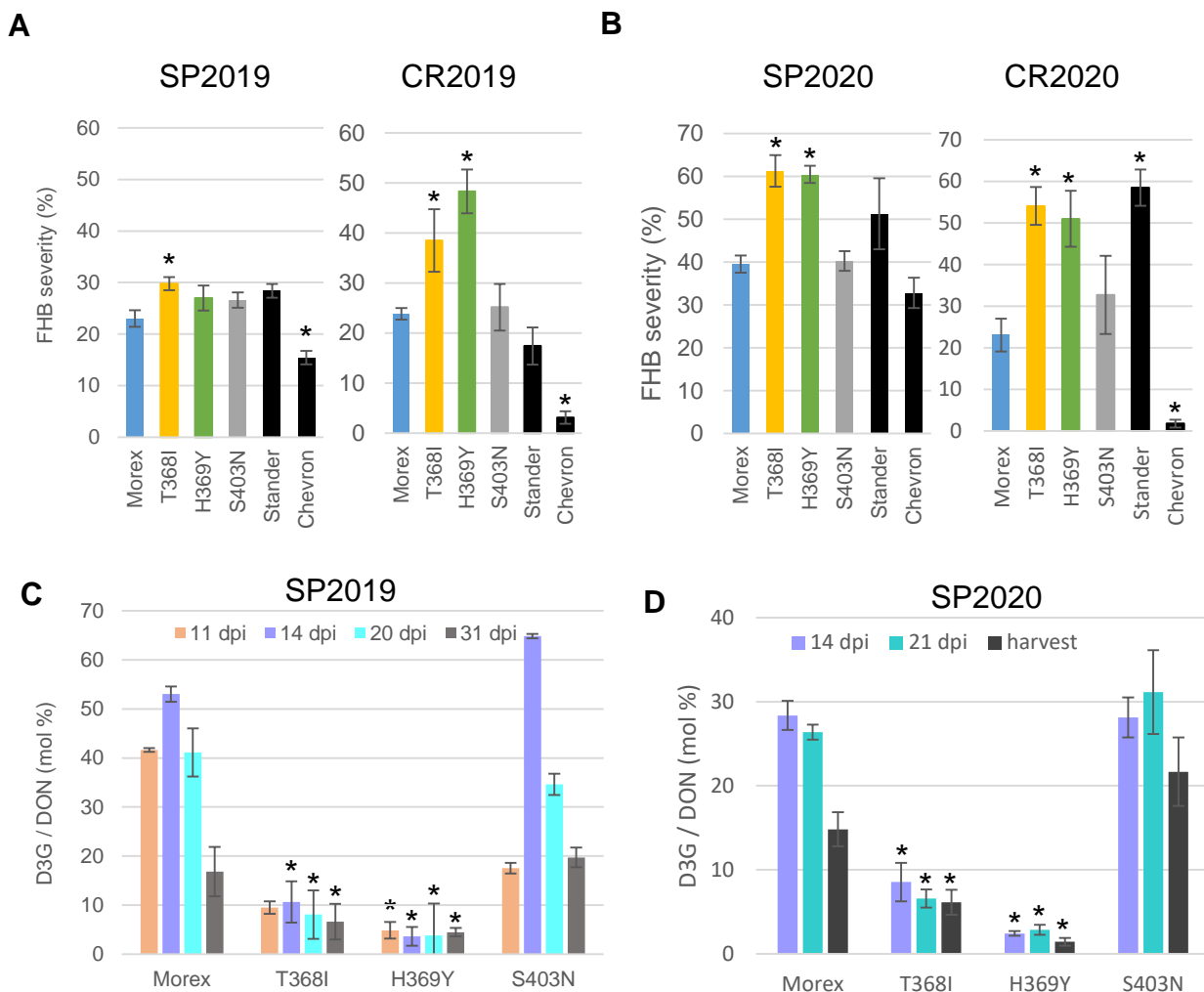


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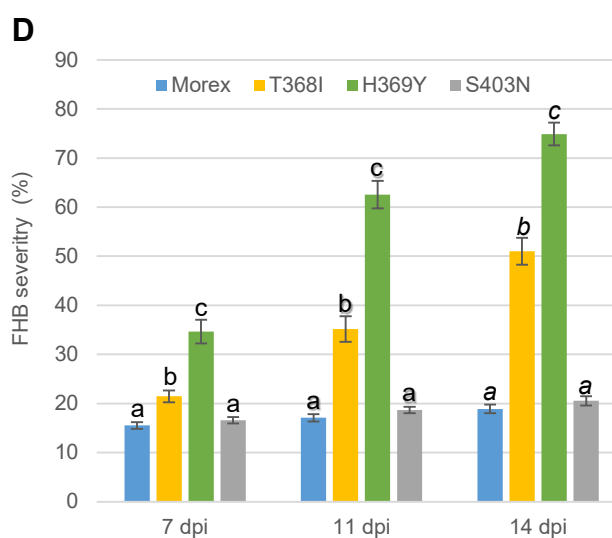
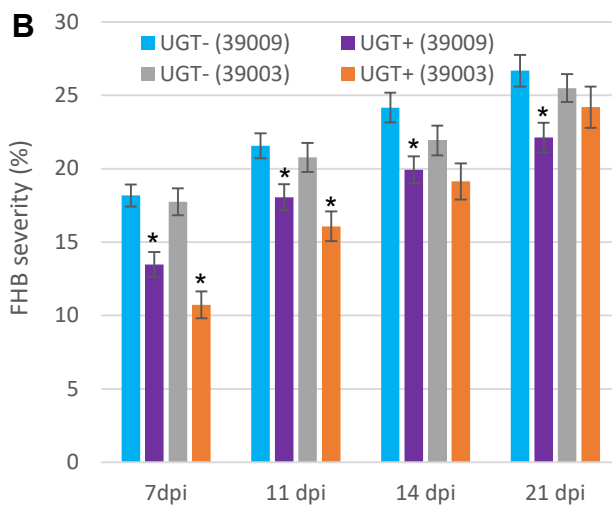
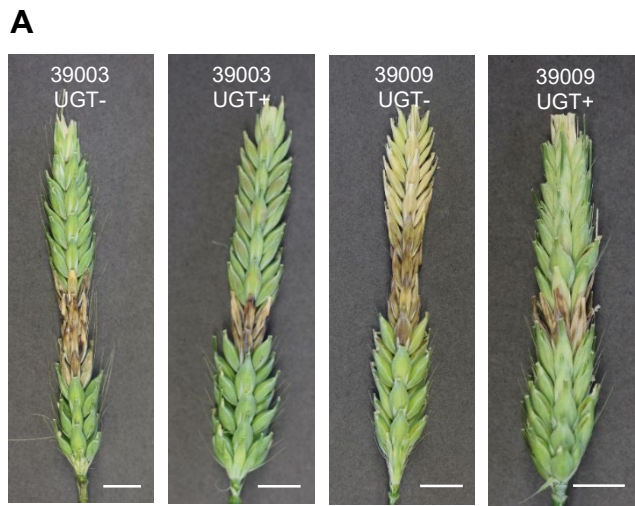


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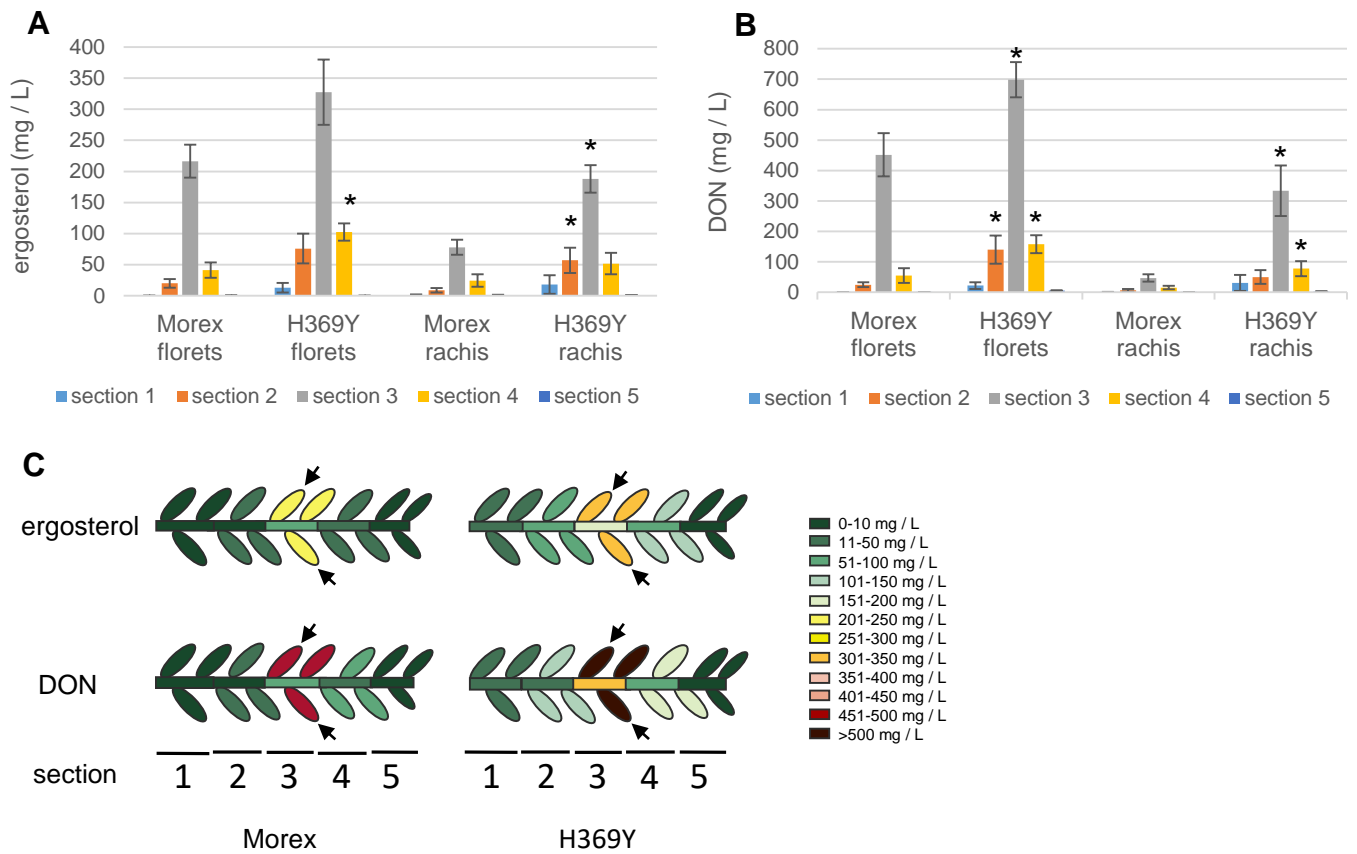


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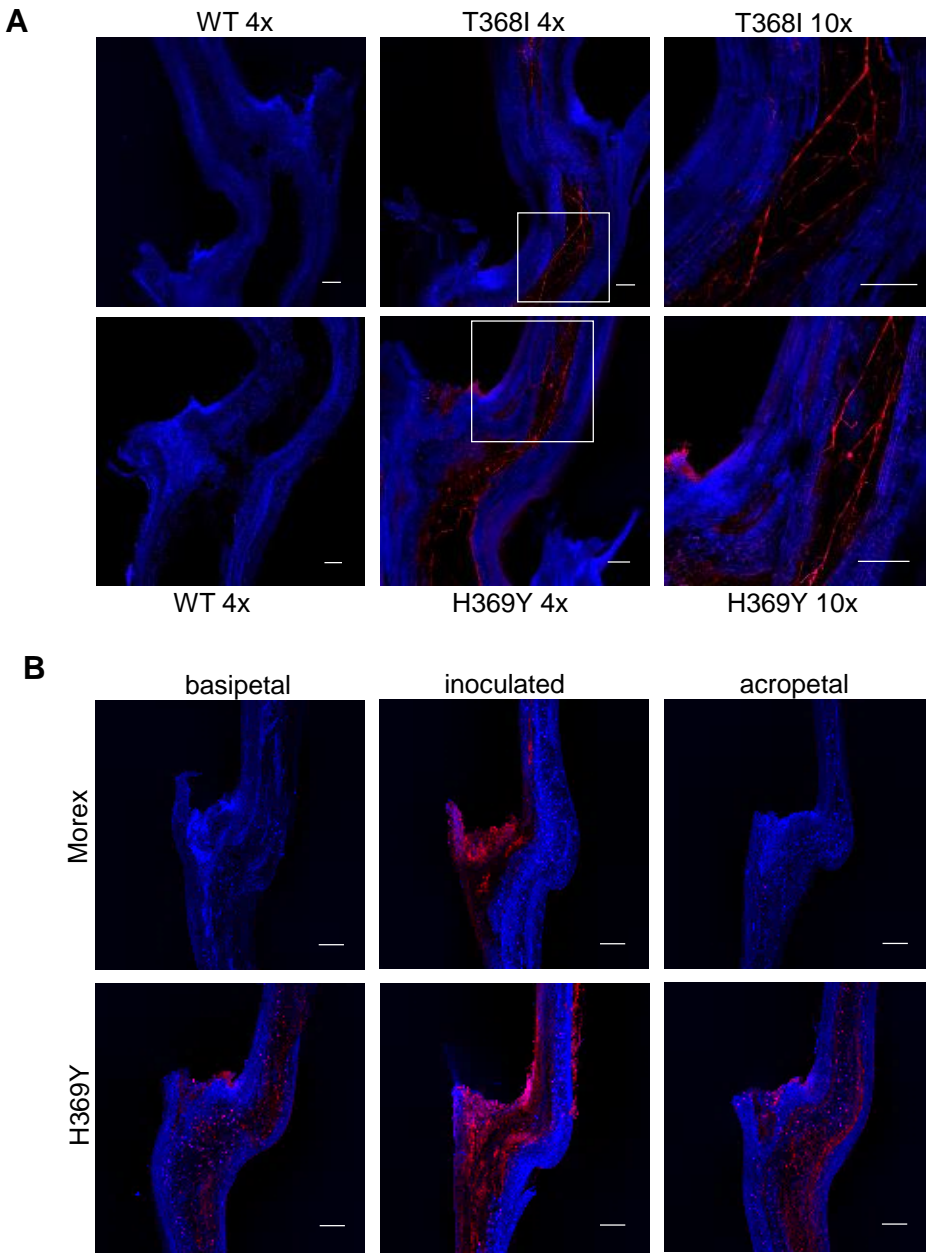


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