

A

Line 1					
Name	Tamlo-A1 P325L (medium)	Tamlo-B1 G319R (strong)	Tamlo-D1 P335L (very strong)	Mlo genotype	HCE [%]
Cadenza				AABBDD	84
Tamlo-aa BBDD line 1 (a)	X			aa BBDD	80
Tamlo-AA bb DD line 1 (a)		X		AA bb DD	75
Tamlo-AABB dd line 1 (a)			X	AABB dd	72
Tamlo-aabb DD line 1 (a)				aabb DD	57
Tamlo-aabb DD line 1 (b)	X	X		aabb DD	n.d.
Tamlo-aabb DD line 1 (c)				aabb DD	n.d.
Tamlo-aa BBdd line 1 (a)	X		X	aa BB dd	78
Tamlo-aa BBdd line 1 (b)				aa BB dd	n.d.
Tamlo-AAbb dd line 1 (a)		X		AA bb dd	23
Tamlo-AAbb dd line 1 (b)		X	X	AA bb dd	13
Tamlo-AAbb dd line 1 (c)				AA bb dd	26
Tamlo-aabbdd line 1 (a)				aabbdd	21
Tamlo-aabbdd line 1 (b)				aabbdd	27
Tamlo-aabbdd line 1 (c)	X	X	X	aabbdd	29
Tamlo-aabbdd line 1 (d)				aabbdd	17
Tamlo-aabbdd line 1 (e)				aabbdd	21

B

Line 2					
Name	Tamlo-A1 P325L (medium)	Tamlo-B1 G319R (strong)	Tamlo-D1 G319R (strong)	MLO genotype	HCE [%]
Cadenza				AABBDD	84
Tamlo-aa BBDD line 2 (a)	X			aa BBDD	80
Tamlo-AA bb DD line 2 (a)		X		AA bb DD	75
Tamlo-AABB dd line 2 (a)			X	AABB dd	69
Tamlo-aabb DD line 2 (a)	X	X		aabb DD	39
Tamlo-aa BBdd line 2 (a)	X		X	aa BB dd	72
Tamlo-AAbb dd line 2 (a)		X	X	AA bb dd	24
Tamlo-AAbb dd line 2 (b)				AA bb dd	25
Tamlo-aabbdd line 2 (a)				aabbdd	23
Tamlo-aabbdd line 2 (b)	X	X	X	aabbdd	34
Tamlo-aabbdd line 2 (c)				aabbdd	42

C

Line 3					
Name	Tamlo-A1 P325L (medium)	Tamlo-B1 G296E (strong)	Tamlo-D1 P321S (medium)	MLO genotype	HCE [%]
Cadenza				AABBDD	77
Tamlo-aa BBDD line 3 (a)	X			aa BBDD	76
Tamlo-AA bb DD line 3 (a)		X		AA bb DD	82
Tamlo-AABB dd line 3 (a)			X	AABB dd	79
Tamlo-aabb DD line 3 (a)	X	X		aabb DD	51
Tamlo-aabb DD line 3 (b)				aabb DD	n.d.
Tamlo-aa BBdd line 3 (a)				aa BB dd	41
Tamlo-aa BBdd line 3 (b)	X		X	aa BB dd	n.d.
Tamlo-aa BBdd line 3 (c)				aa BB dd	n.d.
Tamlo-aa BBdd line 3 (d)				aa BB dd	n.d.
Tamlo-AAbb dd line 3 (a)		X	X	AA bb dd	42
Tamlo-aabbdd line 3 (a)				aabbdd	39
Tamlo-aabbdd line 3 (b)	X	X	X	aabbdd	41
Tamlo-aabbdd line 3 (c)				aabbdd	n.d.

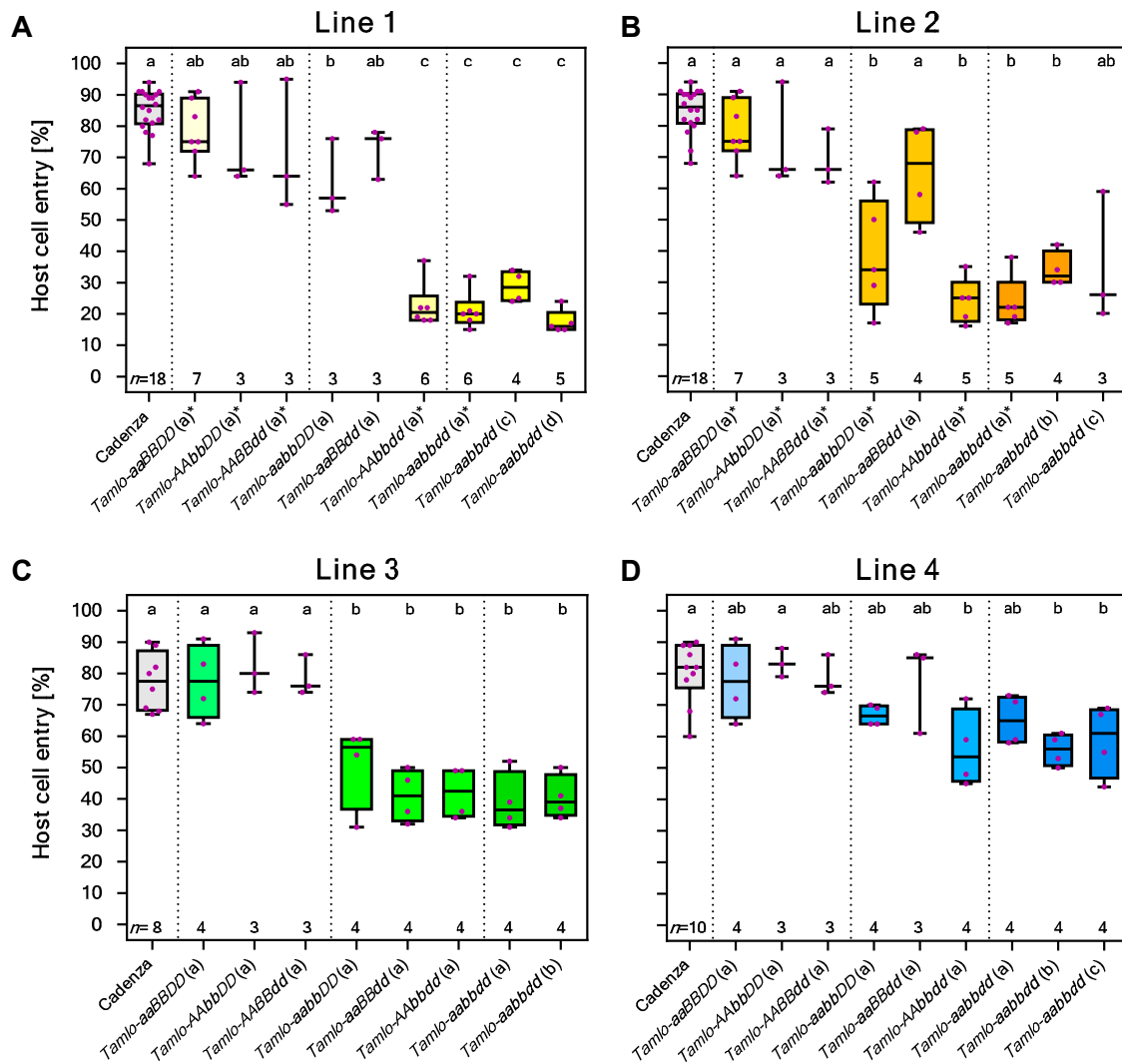
D

Line 4					
Name	Tamlo-A1 P325L (medium)	Tamlo-B1 T297I (strong)	Tamlo-D1 P321S (medium)	MLO genotype	HCE [%]
Cadenza				AABBDD	78
Tamlo-aa BBDD line 4 (a)	X			aa BBDD	76
Tamlo-AA bb DD line 4 (a)		X		AA bb DD	83
Tamlo-AABB dd line 4 (a)			X	AABB dd	79
Tamlo-aabb DD line 4 (a)	X	X		aabb DD	66
Tamlo-aabb DD line 4 (b)				aabb DD	n.d.
Tamlo-aa BBdd line 4 (a)	X		X	aa BB dd	86
Tamlo-AAbb dd line 4 (a)		X	X	AA bb dd	51
Tamlo-AAbb dd line 4 (b)				AA bb dd	n.d.
Tamlo-AAbb dd line 4 (c)				AA bb dd	n.d.
Tamlo-aabbdd line 4 (a)				aabbdd	63
Tamlo-aabbdd line 4 (b)	X	X	X	aabbdd	55
Tamlo-aabbdd line 4 (c)				aabbdd	56

HCE: host cell entry; mean percentage value from several experimental repetitions  
n.d.: not determined

**Supplemental Figure 1. Summary of all generated wheat TILLING *Tamlo* mutants and their respective allele combinations.**





























The Figure summarizes features of single-, double-, and triple-mutant combinations of the four TILLING *Tamlo* lines, line 1 (yellow, **A**), line 2 (orange, **B**), line 3 (green, **C**), and line 4 (blue, **D**), generated by stacking of different mutant alleles of the wheat *Mlo* homoeologs *TaMlo-A1*, *TaMlo-B1*, and *TaMlo-D1*. For each of the four lines, the available single-, double- and triple mutants are given (column “Name”) and their respective genotypes are indicated (column “MLO genotype”). Each mutant is characterized by distinctive *Tamlo* alleles, leading to particular amino acid substitutions, whose presence is indicated by crosses in the columns “*TaMlo-A1*”, “*TaMlo-B1*”, and “*TaMlo-D1*” and specifically quoted for each line in the same column head (second line). These alleles were assessed previously for their resistance-conferring capacity (medium, strong, or very strong, as given in brackets below the amino acid substitutions; [18]). Note that some mutant combinations are represented by several independent segregants, distinguished by lower-case letters (a, b, c ...) following the mutant name. The column HCE indicates the mean host cell entry rate, as experimentally determined with *Bgt* isolate JA82. Data of TILLING *Tamlo* genotypes that are shaded in grey were published before [18].



**Supplemental Figure 2. *Bgt* infection phenotypes of wheat TILLING *Tamlo* mutants in different allele combinations.**

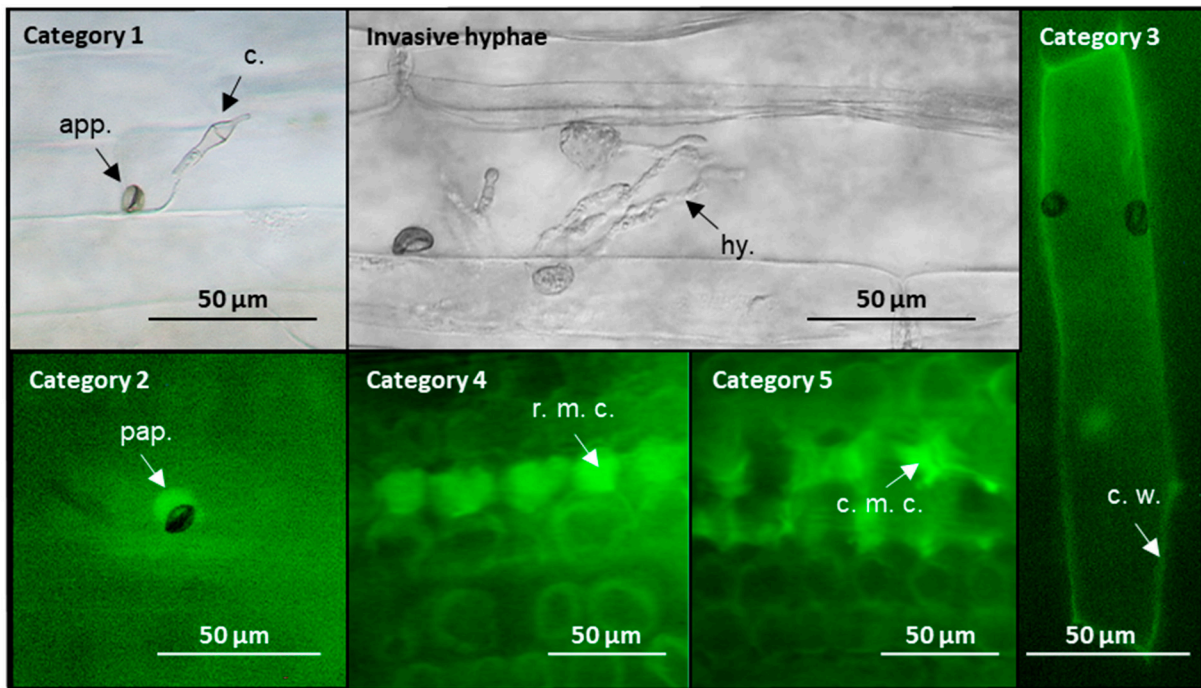
Primary leaves of 10-day-old wheat TILLING *Tamlo* single-, double-, and triple mutants (indicated by their respective genotype) of the four *Tamlo* lines described in Supplemental Figure 1 and the corresponding cv. Cadenza wild-type were fixed on a polycarbonate platform and inoculated with conidiophores of *Bgt* isolate JA82. Host cell entry rates for the indicated wheat genotypes were assessed microscopically at 3 dpi and are represented as boxplots. Data shown relate to line 1 (yellow, **A**), line 2 (orange, **B**), line 3 (green, **C**) and line 4 (blue, **D**). Data of individual biological replicates per genotype are displayed as colored dots, and the total number of biological replicates is given as n above the x-axis. Each biological replicate represents the mean of three or four evaluated leaves per genotype, with at least 200 *Bgt* interaction sites scored per leaf, i.e., a minimum of 600 inspected interaction sites per

genotype and replicate. Letters above the boxplots denote grouping according to a multi-paired ANOVA test ( $p < 0.05$ ). Data of TILLING *Tamlo* genotypes marked with an asterisk were published before (Acevedo-Garcia et al., 2017b).

	Mock	Zt05	Zt244	Zt153	Mock	Zt05	Zt244	Zt153
<b>Cadenza</b>								
<b>TILLING Tamlo-aabbdd</b>								
<b>TILLING Tamlo-aabbdd</b>								
<b>TILLING Tamlo-aabbdd</b>								
<b>TILLING Tamlo-aabbdd</b>								

**Supplemental Figure 3. Macroscopic infection phenotypes of *Tamlo* mutants treated with the three *Z. tritici* isolates Zt05, Zt153 and Zt244.**

Three representative leaves are shown for each plant genotype and fungal isolate at the time point 21 dpi. Disease symptoms are visible as necrosis and pycnidia produced by *Z. tritici*.



**Supplemental Figure 4. Categorization of cellular interaction phenotypes of wheat plants challenged with *MoT*.**

Primary leaves of wheat lines infected with *MoT* were harvested at different time points after inoculation. After extraction of leaf pigments, samples were evaluated by bright-field (category 1 and detection of invasive hyphae) or epi-fluorescence microscopy (categories 3 to 5). The micrographs shown illustrate different types of plant responses expressed by cells beneath fungal appressoria. Category 1, cell without any autofluorescence; category 2, local accumulation of autofluorescent material close to the appressorium; category 3, autofluorescence of cell walls of attacked epidermal cell; category 4, autofluorescence of round-shaped mesophyll cells; and category 5, autofluorescence of collapsed mesophyll cells. In addition, the occurrence of hyphae within epidermal cells (invasive hyphae) was recorded. Conidium (c.), appressorium (app.), papilla (pap.), bulbous hyphae (hy.), round-shaped mesophyll cell (r. m. c.), collapsed mesophyll cell (c. m. c.) and cell wall (c. w.) are marked with arrows.