

Table S1. Effects of 2,4-D over a 10 mg L⁻¹ – 50 mg L⁻¹ range on the frequency of mouldy germinating grains (a), root length (b) and grain germination rate (c) of barley *tw* mutants and the *WT*. (d) *P* values of differences between sterilized and unsterilized grains under different experimental conditions

(a) Frequency (%) of mouldy germinating grains – MGG assay, n=10 (100 grains)

Genotype	With grain sterilization				Without grain sterilization			
	Concentration of 2,4-D, mg L ⁻¹				Concentration of 2,4-D, mg L ⁻¹			
	0	10	30	50	0	10	30	50
<i>WT</i>	4 ± 2	3 ± 2	4 ± 2	8 ± 2	15 ± 2	13 ± 2	15 ± 3	14 ± 2
<i>tw</i>	5 ± 2	8 ± 2	8 ± 1	7 ± 2	16 ± 2	11 ± 1 ^a	11 ± 2	12 ± 2
<i>tw₁</i>	1 ± 1	2 ± 1	1 ± 1	3 ± 2	8 ± 2 ¹	7 ± 2 ¹	6 ± 2 ¹	7 ± 2 ¹
<i>tw₂</i>	3 ± 2	8 ± 1 ^{1a}	9 ± 2 ^a	5 ± 2	20 ± 2	13 ± 2 ^a	12 ± 2 ^a	15 ± 3
<i>twmk</i>	1 ± 1	0	0 ¹	1 ± 1 ²	5 ± 2 ¹	8 ± 1	4 ± 2 ²	4 ± 2 ¹
<i>twN18</i>	3 ± 2	0	0 ¹	1 ± 1 ²	8 ± 2 ¹	7 ± 2 ¹	8 ± 1 ¹	10 ± 1
<i>WT</i> 2016	6 ± 2	7 ± 2	7 ± 2	9 ± 2	24 ± 2	22 ± 2	18 ± 2 ^a	24 ± 3
<i>tw₂</i> 2016	20 ± 3 ²	23 ± 2 ³	25 ± 2 ³	22 ± 2 ³	35 ± 2 ²	39 ± 2 ³	42 ± 3 ³	47 ± 3 ^{3b}

(b) Root length (cm) after 5 days, n=30

Genotype	With grain sterilization				Without grain sterilization			
	Concentration of 2,4-D, mg L ⁻¹				Concentration of 2,4-D, mg L ⁻¹			
	0	10	30	50	0	10	30	50
<i>WT</i>	5.0 ± 0.1	4.5 ± 0.2 ^a	5.1 ± 0.2	4.6 ± 0.2	4.6 ± 0.2	4.4 ± 0.2	4.4 ± 0.2	4.1 ± 0.2
<i>tw</i>	4.5 ± 0.2 ²	4.4 ± 0.1	4.5 ± 0.2 ¹	4.4 ± 0.1	4.5 ± 0.2	3.8 ± 0.2 ^{1a}	3.8 ± 0.2 ^{1a}	3.3 ± 0.2 ^{2c}
<i>tw₁</i>	4.9 ± 0.2	4.7 ± 0.2	4.3 ± 0.1 ^{2a}	4.4 ± 0.2	4.6 ± 0.2	3.7 ± 0.2 ^{1b}	3.9 ± 0.1 ^{2a}	3.9 ± 0.1 ^a
<i>tw₂</i>	4.8 ± 0.2	4.6 ± 0.2	4.7 ± 0.2	4.4 ± 0.1	4.3 ± 0.2	4.1 ± 0.2	3.9 ± 0.2	3.6 ± 0.2 ^a
<i>twmk</i>	4.5 ± 0.1 ²	4.7 ± 0.2	4.2 ± 0.1 ^{2a}	3.7 ± 0.2 ^{2c}	4.9 ± 0.2	3.9 ± 0.2 ^c	4.1 ± 0.2 ^b	3.9 ± 0.2 ^c
<i>twN18</i>	4.7 ± 0.1	5.1 ± 0.1 ¹	4.7 ± 0.1	4.6 ± 0.2	5.0 ± 0.2	4.7 ± 0.2	3.6 ± 0.2 ^{2c}	4.2 ± 0.2 ^a
<i>WT</i> 2016	5.4 ± 0.2	5.5 ± 0.2	5.4 ± 0.2	5.1 ± 0.2	5.2 ± 0.2	5.3 ± 0.2	4.7 ± 0.2 ^a	4.5 ± 0.1 ^a
<i>tw₂</i> 2016	4.8 ± 0.3	4.8 ± 0.2 ²	4.3 ± 0.2 ³	4.0 ± 0.2 ^{3a}	5.1 ± 0.2	4.3 ± 0.2 ^{2a}	3.5 ± 0.2 ^{3c}	3.6 ± 0.2 ^{3c}

(c) Germination (%), n=10 (100 grains)

Genotype	With grain sterilization				Without grain sterilization			
	Concentration of 2,4-D, mg L ⁻¹				Concentration of 2,4-D, mg L ⁻¹			
	0	10	30	50	0	10	30	50
WT	91 ± 2	94 ± 2	90 ± 2	94 ± 2	89 ± 2	89 ± 2	82 ± 2	89 ± 3
tw	89 ± 2	90 ± 3	92 ± 2	91 ± 3	84 ± 2	81 ± 2 ¹	84 ± 3	85 ± 3
tw ₁	95 ± 2	96 ± 2	93 ± 2	92 ± 2	92 ± 2	93 ± 2	95 ± 2 ²	93 ± 2
tw ₂	88 ± 2	85 ± 3 ¹	83 ± 3 ¹	86 ± 3	85 ± 2	86 ± 3	85 ± 3	84 ± 3
twmk	93 ± 2	92 ± 1	91 ± 2	89 ± 2	84 ± 2	88 ± 2	85 ± 2	83 ± 3
twN18	92 ± 1	93 ± 2	90 ± 2	91 ± 2	83 ± 2	82 ± 2	81 ± 2	84 ± 3
WT 2016	94 ± 2	91 ± 1	91 ± 1	92 ± 2	90 ± 3	88 ± 2	89 ± 3	87 ± 3
tw ₂ 2016	86 ± 3 ¹	84 ± 2 ²	83 ± 3 ¹	78 ± 2 ²	79 ± 2 ²	76 ± 2 ³	77 ± 4 ¹	76 ± 2 ²

(d) *P* values. Statistically significant *P* values (< 0.05) are presented in bold

Genotype	Concentration of 2,4-D, mg L ⁻¹			
	0	10	30	50
<i>Mouldy germinating grains</i>				
WT	0.0012	0.0011	0.0050	0.0614
<i>tw</i>	0.0012	0.2454	0.1951	0.1110
<i>tw</i> ₁	0.0075	0.0285	0.0223	0.0812
<i>tw</i> ₂	0.0002	0.0283	0.2641	0.0092
<i>twmk</i>	0.0571	0.0004	0.0293	0.1311
<i>twN18</i>	0.1262	0.0014	0.0004	0.0006
WT 2016	0.0001	0.0002	0.0012	0.0006
<i>tw</i> ₂ 2016	0.0008	0.0003	0.0008	0.0002
<i>Root length</i>				
WT	0.0939	0.9109	0.0129	0.0667
<i>tw</i>	0.6688	0.0195	0.0099	1E-05
<i>tw</i> ₁	0.3446	0.0017	0.1535	0.0571
<i>tw</i> ₂	0.0736	0.0813	0.0036	0.0048
<i>twmk</i>	0.0516	0.0010	0.8269	0.5035
<i>twN18</i>	0.0812	0.1075	8E-06	0.0971
WT 2016	0.5711	0.2161	0.0047	0.0144
<i>tw</i> ₂ 2016	0.5968	0.1558	0.0058	0.2037
<i>Germination rate</i>				
WT	0.4907	0.1095	0.0171	0.1718

<i>tw</i>	0.1718	0.0154	0.0504	0.1621
<i>tw₁</i>	0.2594	0.1888	0.5224	0.8050
<i>tw₂</i>	0.2594	0.6620	0.5114	0.6347
<i>twmk</i>	0.0024	0.1681	0.0299	0.1007
<i>twN18</i>	0.0034	0.0012	0.0067	0.0400
<i>WT 2016</i>	0.3508	0.1784	0.4907	0.2047
<i>tw₂ 2016</i>	0.0503	0.0137	0.2135	0.5645

^{1,2,3}Comparison with the respective concentration of the *WT*: ¹*P*<0.05; ²*P*<0.01; ³*P*<0.001. ^{a,b,c}Comparison with the respective control (0): ^a*P*<0.05; ^b*P*<0.01; ^c*P*<0.001. Other material from 2013 reproduction, MGG assay in 2017