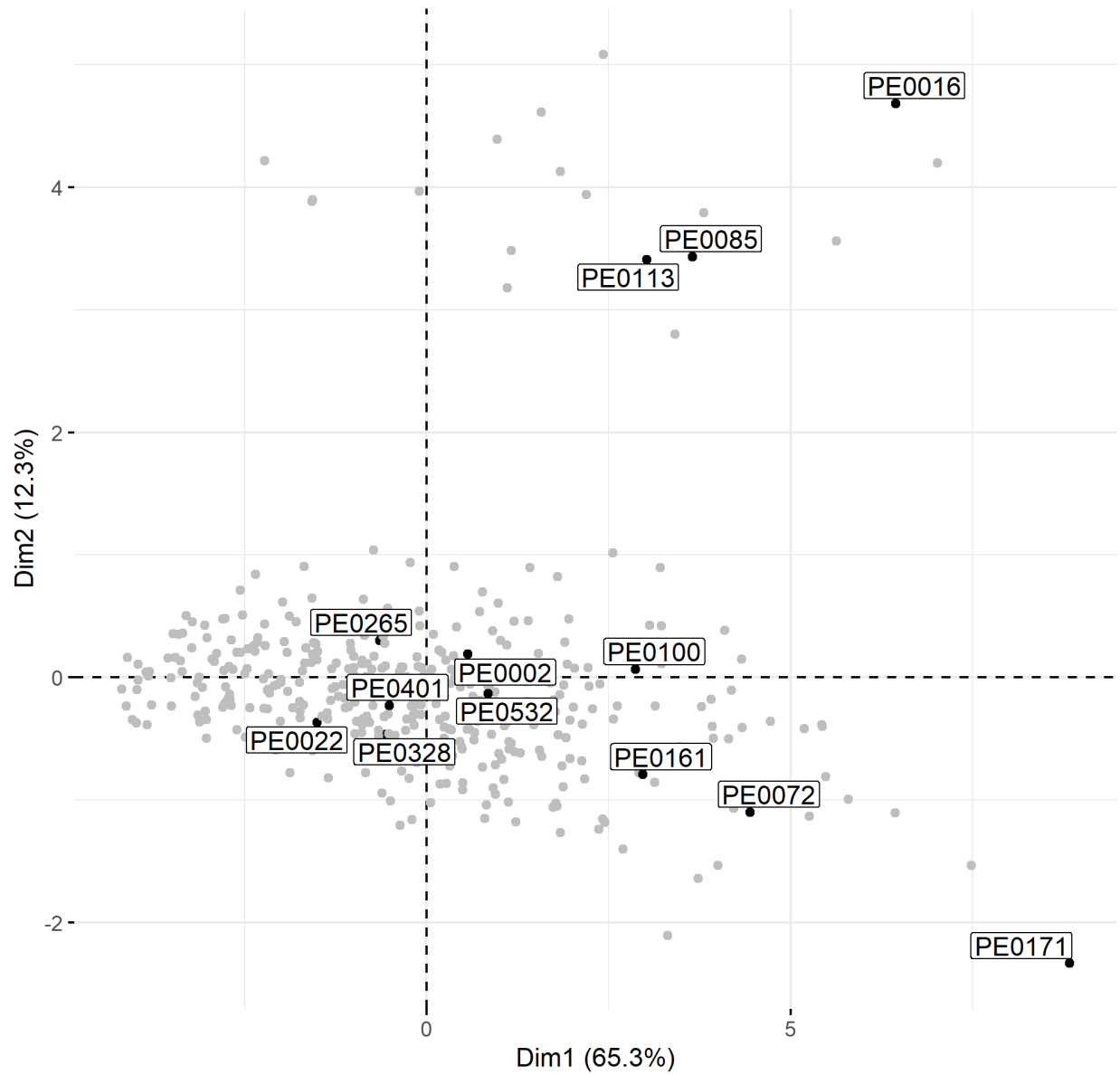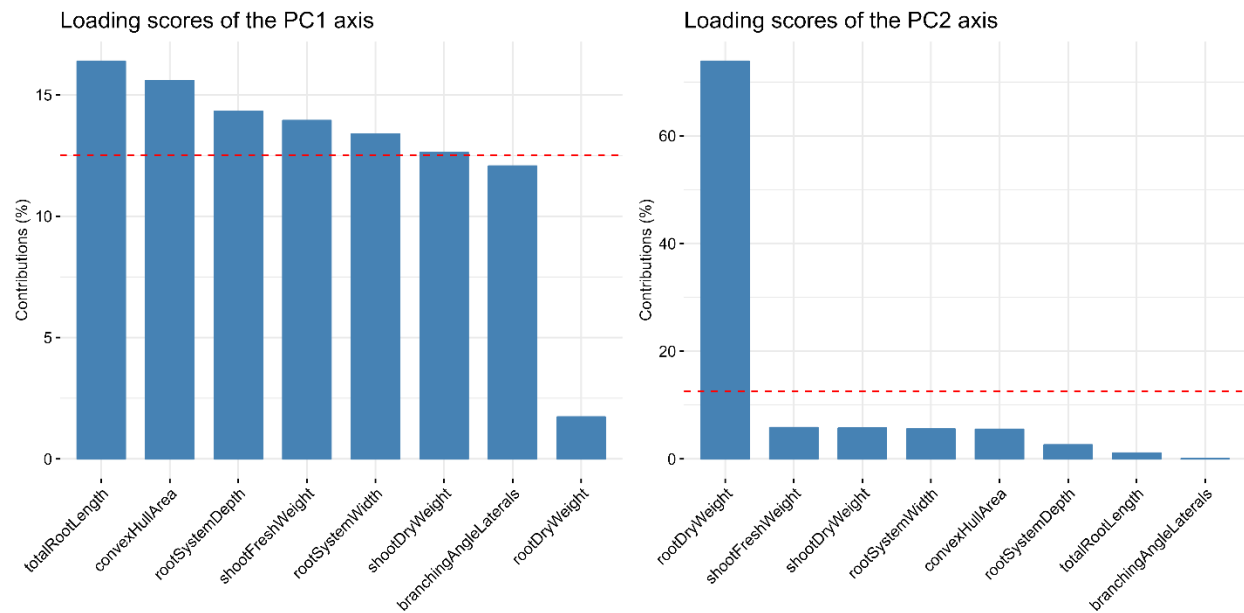# Supplementary material

A) Principal component analysis of genotypes based on all the phenotypic traits

B) Loading scores of the first two axes of the PCA

Loading scores of the PC1 axis

Loading scores of the PC2 axis

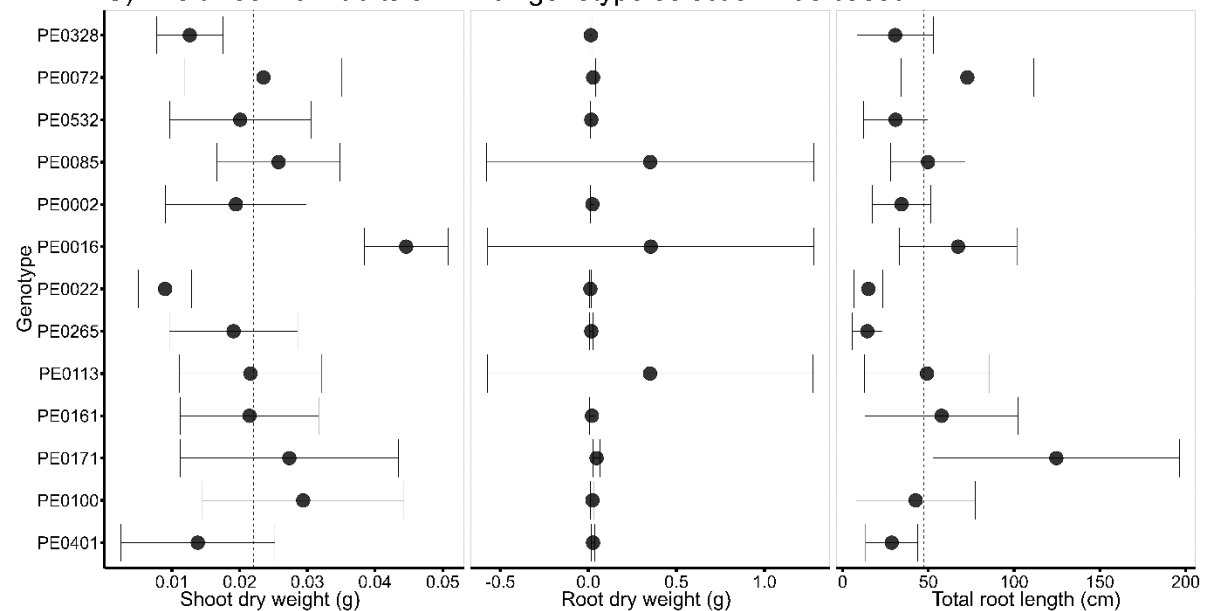C) The three main traits on which genotype selection was based

Figure S1: Root and shoot traits from a phenotyping experiment of 423 DH lines. Seeds were germinated at 24-22 °C (day-night) on filter paper and were transferred after 2 days to the germination paper-based phenotyping system GrowScreen-PaGe (description of the system and plant growth conditions in [38]). The seedlings were exposed for 7 days to 24-22 °C (day-night), before the temperature was reduced for another 7 days to 16-12 °C (day-night). Plant traits shown

here were measured 14 days after transfer to the phenotyping system (for trait description see [34]). (A) Principal component analysis (PCA) of genotypes based on all the phenotypic traits. (B) Loading scores of the first two axes the PCA. (C) The three main traits on which we based the genotype selection for our study. Dots and error bars represent mean values standard deviation from the mean, respectively.

A) Environmental conditions during the temperature treatment in the growth chambers
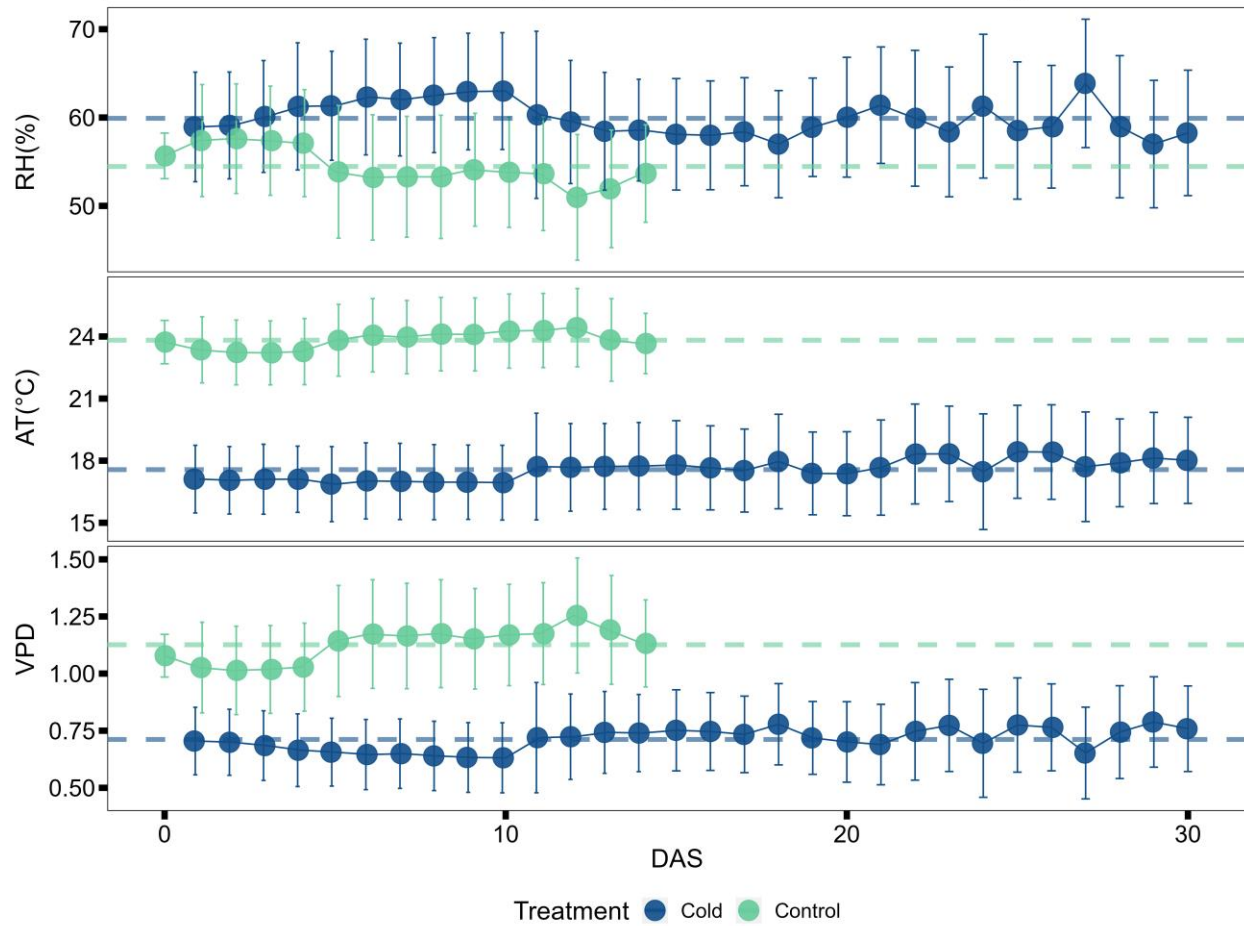
B) Diurnal course of the Photosynthetic Photon Flux Density (PPFD) in the growth chamber

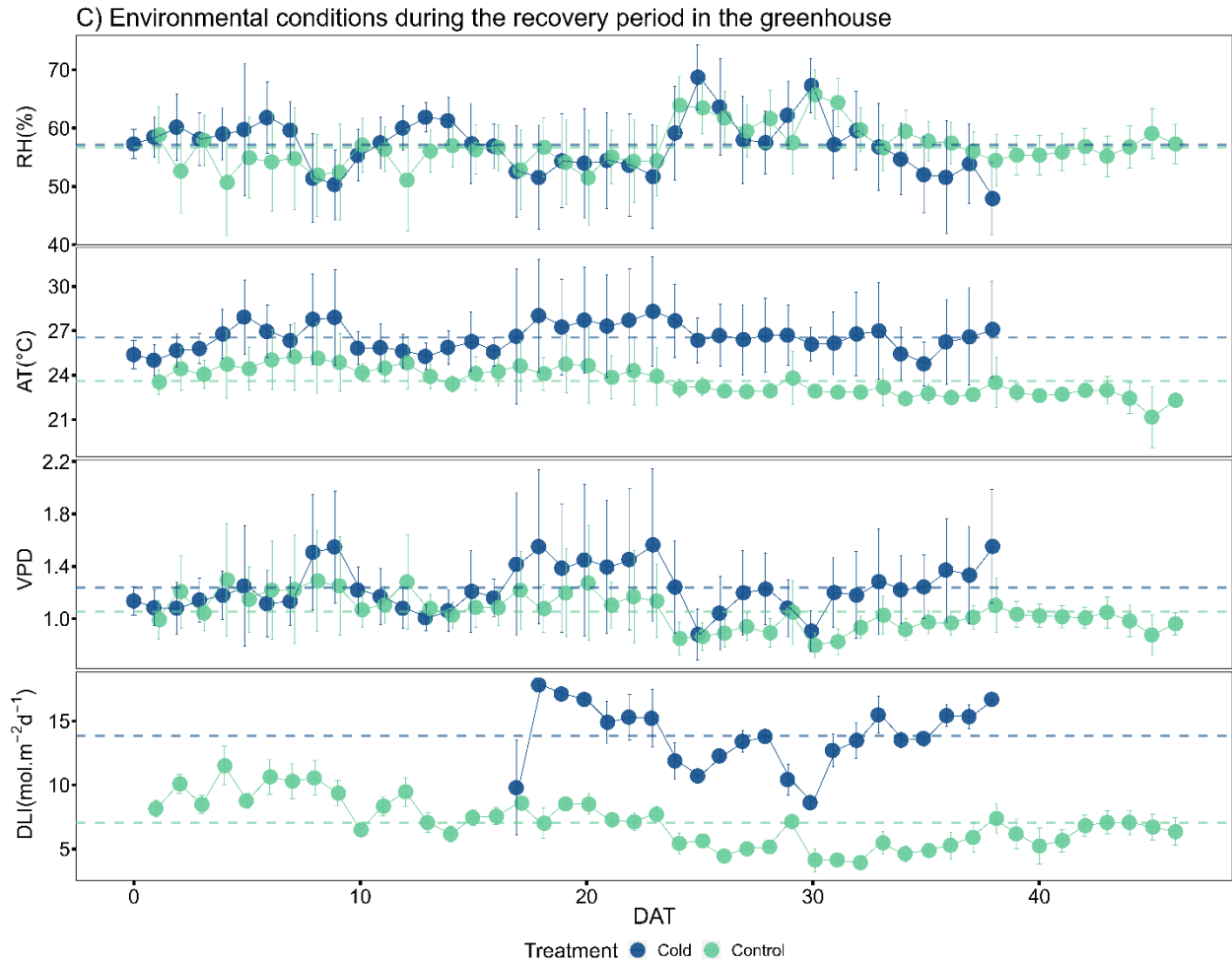C) Environmental conditions during the recovery period in the greenhouse
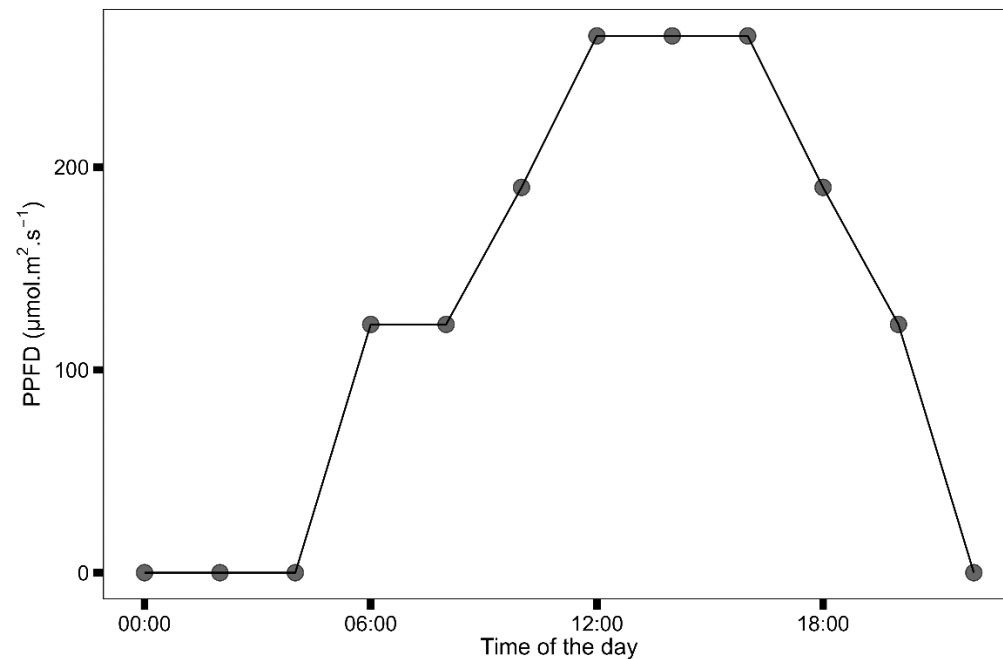
Figure S2: Environmental conditions recorded during the treatment in the growth chamber and the recovery in the greenhouse: relative humidity (RH), air temperature (AT), vapor pressure deficit (VPD) and daily light integral (DLI). The latter two were calculated based on relative humidity and air temperature, and photosynthetic photon flux density and daylength (PPFD) acquired from the same environmental stations, respectively. (A) Measured environmental conditions during the temperature treatment in the growth chambers; (B) Diurnal course of Photosynthetic Photon Flux Density (PPFD) during the temperature treatment in the growth chamber for both the cold treatment and the control treatment; (C) Daily daytime environmental data during the recovery period in the greenhouse, after transplantation from the temperature treatment in the growth chamber. Average and standard deviation values were calculated from three environmental stations placed at plant level in the greenhouse. The PPFD was not measured from the start for the cold treatment which explains the missing values for DLI. All values are presented in function of

days after transplantation (DAT). Blue: recovery conditions in the greenhouse after the cold temperature treatment in the growth chamber (20-12 °C day-night); green: recovery conditions in the greenhouse after the control temperature treatment in the growth chamber (25-18 °C day-night).
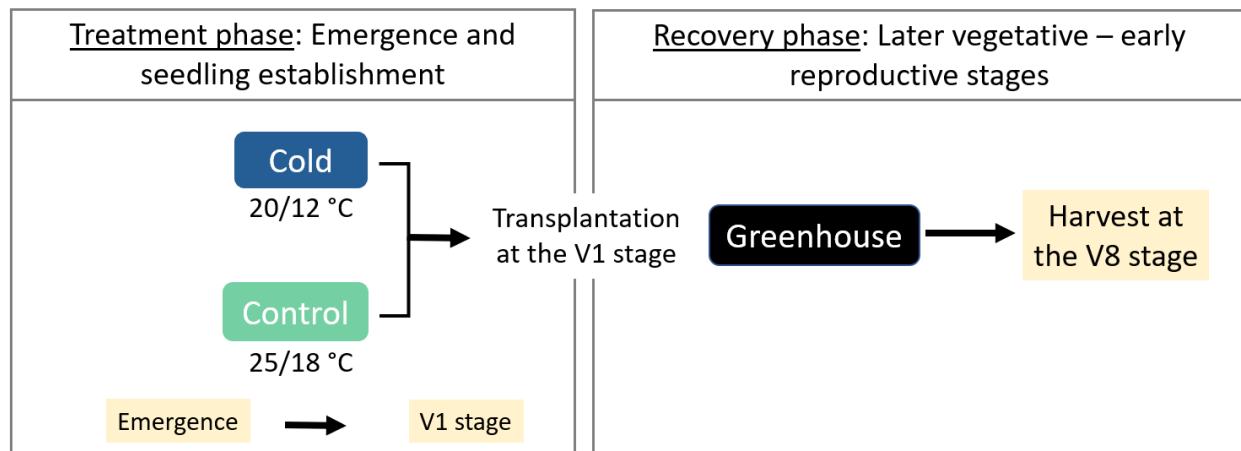

Figure S3: Description of the experimental steps of the cold and the control treatments
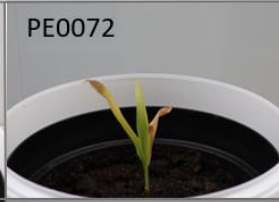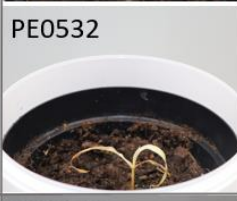
Survival rates	Color score	Seedling appearance		Cold tolerance level
< 50%	1-2	B73	PE0072	Low
[50-80%]	2-3	PE0532	PE0016	Medium
[80-90%]	3-5	PE0113	PE0161	High
100%	3-5	PE0100	PE0401	Very high

Figure S4: Classification of genotype cold tolerance level based on seedling appearance after exposure to cold temperatures in the growth chamber and survival rates of seedlings upon two weeks under recovery conditions in the greenhouse. Two representative genotypes are shown for each cold tolerance category. We evaluated seedling appearance by assigning color scores to the genotypes based on the presence and the intensity range of cold damage in the leaves from 1, presence of advanced leaf necrosis or bleaching, to 5, least presence of leaf cold damage signs and maintained leaf greenness.
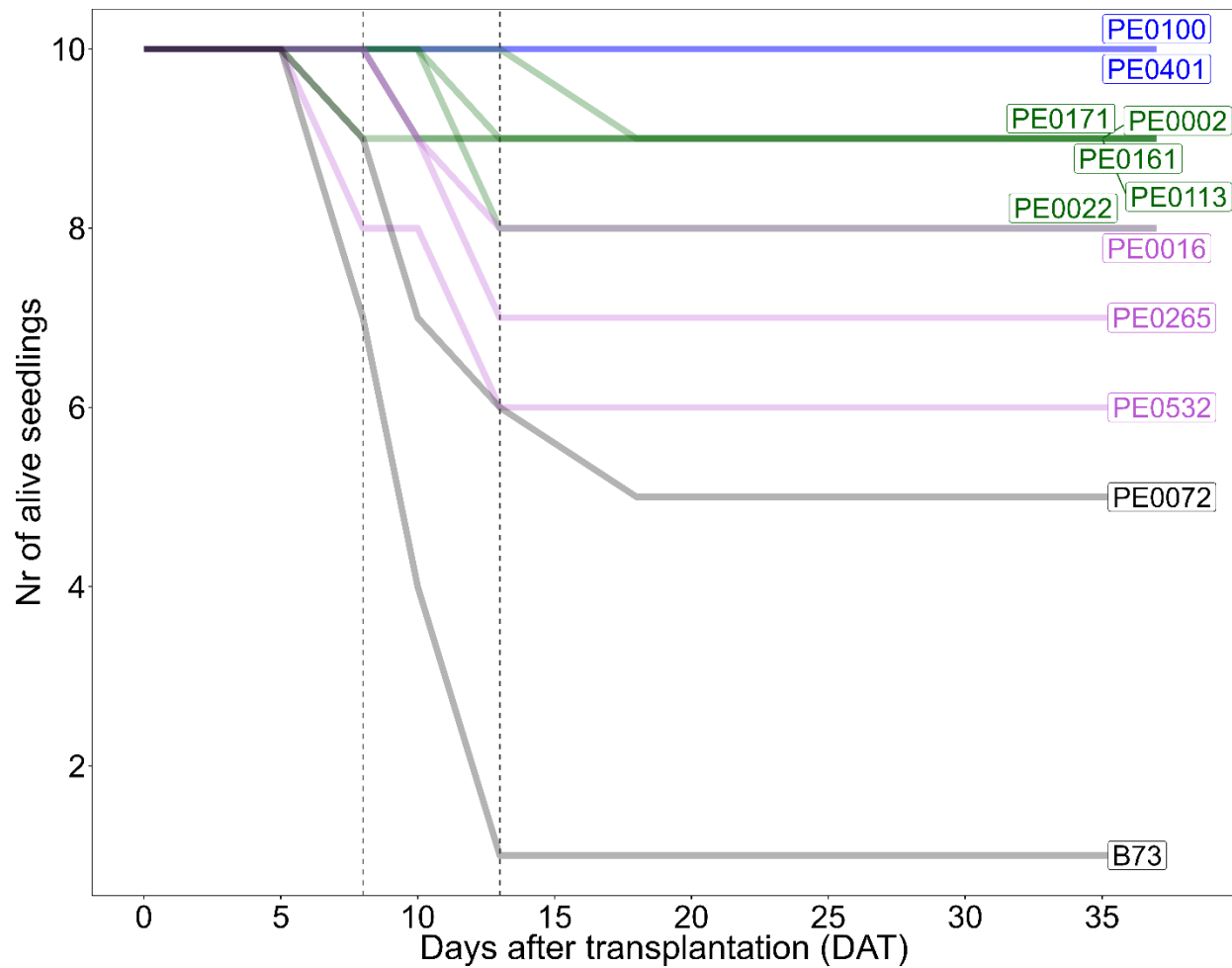
Figure S5: Timeline of seedling death during the recovery phase after the cold stress treatment. The number of alive seedlings for each genotype is shown in function of the number of days after transplantation (DAT) to the greenhouse. Cold tolerance categories are color-coded: blue, very high cold tolerance; green, high cold tolerance; magenta, medium cold tolerance; gray, low cold tolerance.
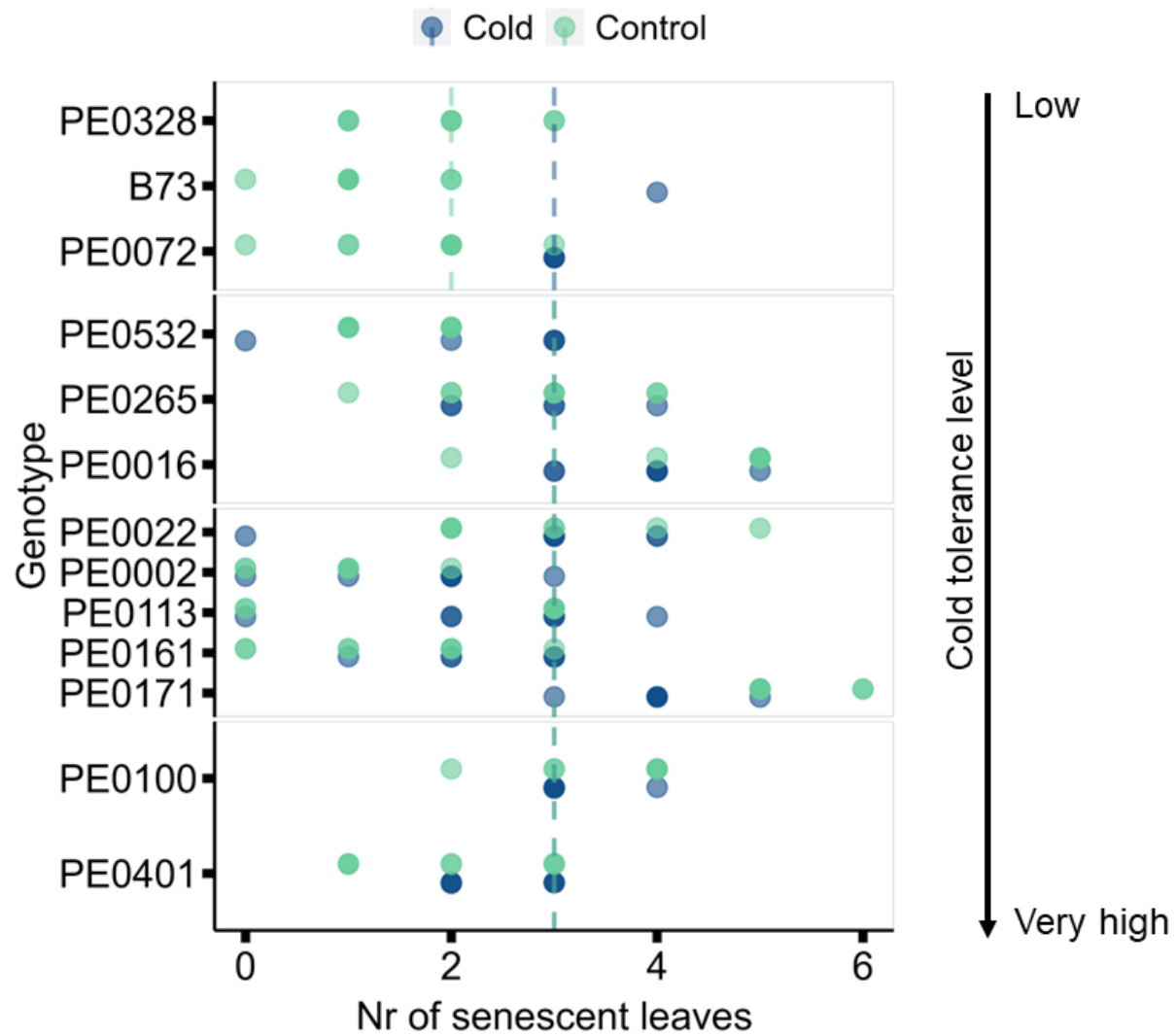
Figure S6: Number of completely senesced leaves by the time of harvest (V8) for the cold treatment (blue; n=1-9) and the control treatment (green; n=8). Genotypes are arranged according to their cold tolerance category. Vertical lines represent weighted means for each cold tolerance category.
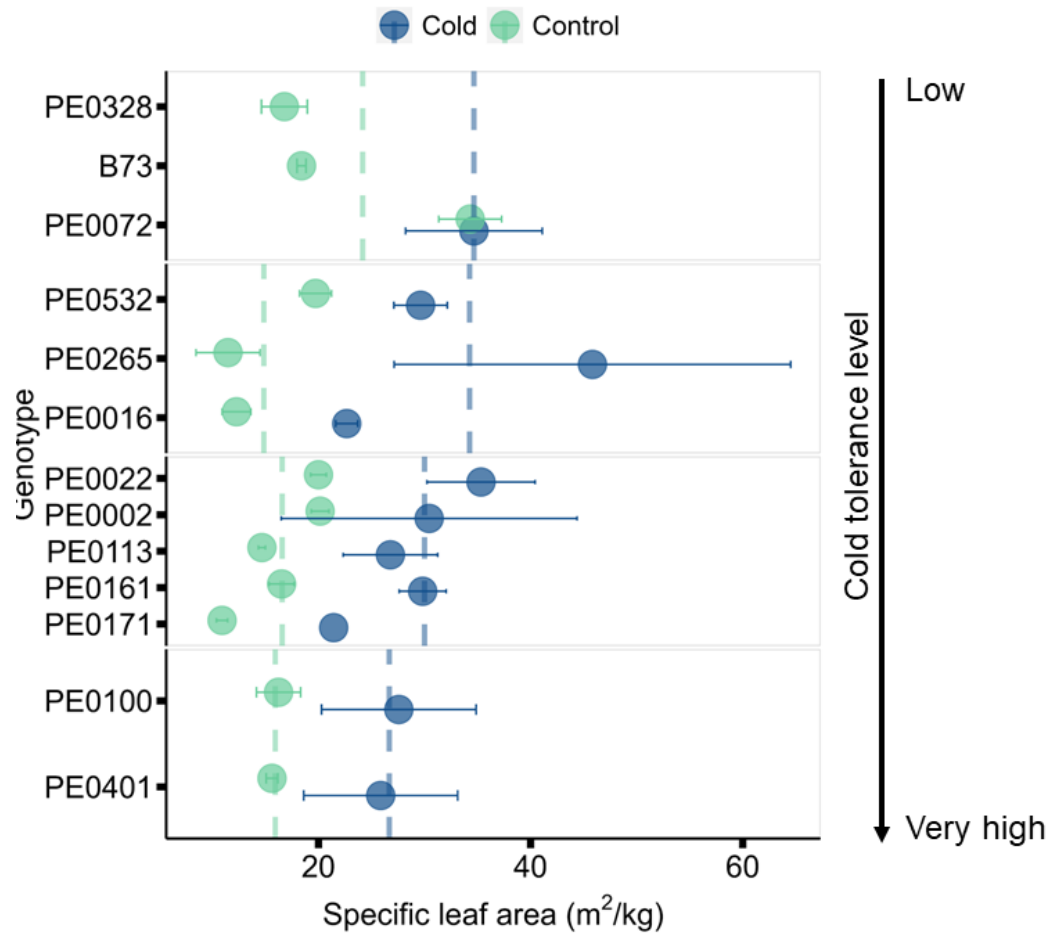
Figure S7: Specific leaf area of the first eight fully developed leaves calculated at the V8 stage. for the cold treatment (blue; n=1-9) and the control treatment (green; n=8). Genotypes are arranged according to their cold tolerance category. Vertical lines represent weighted means for each cold tolerance category.

A) Tassel fresh weight at the V8 stage
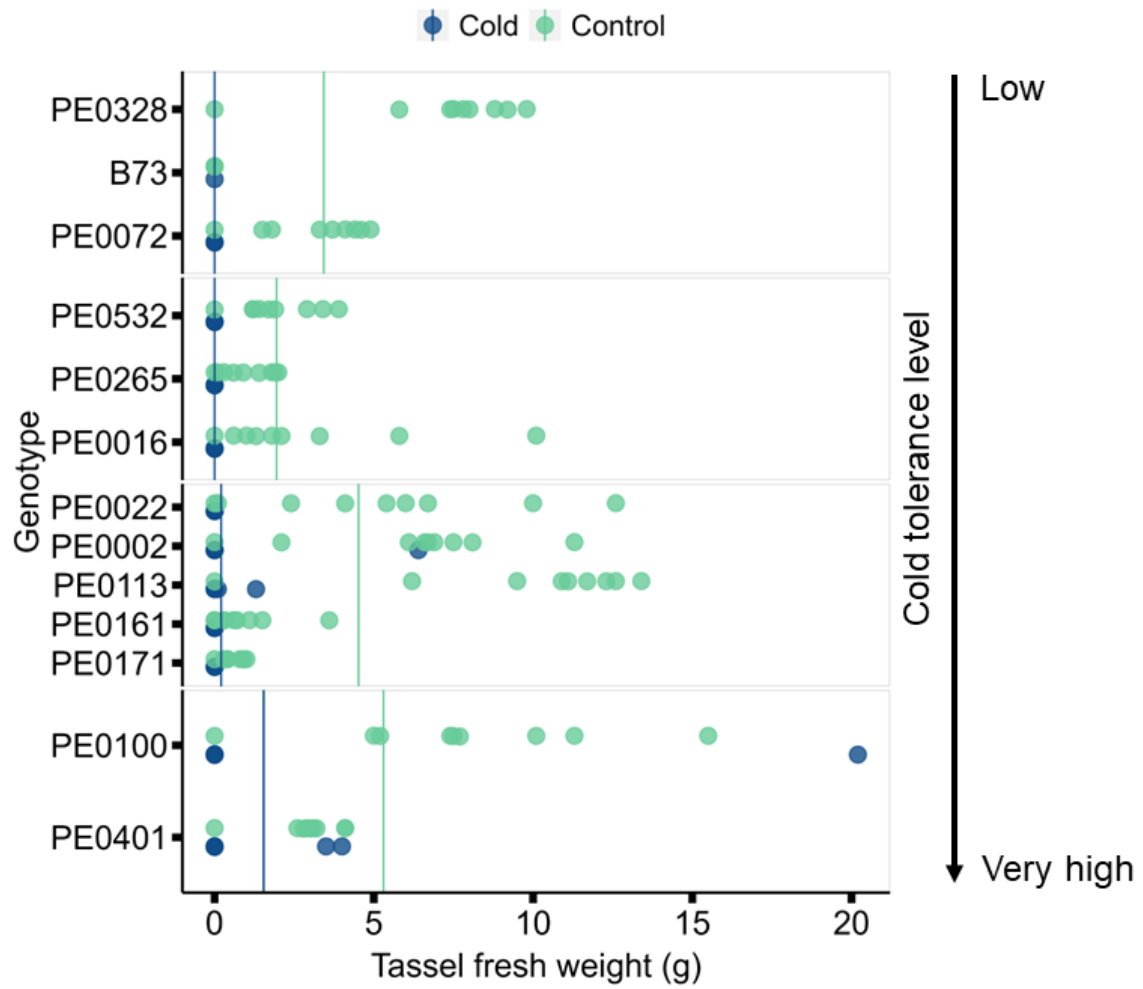
B)
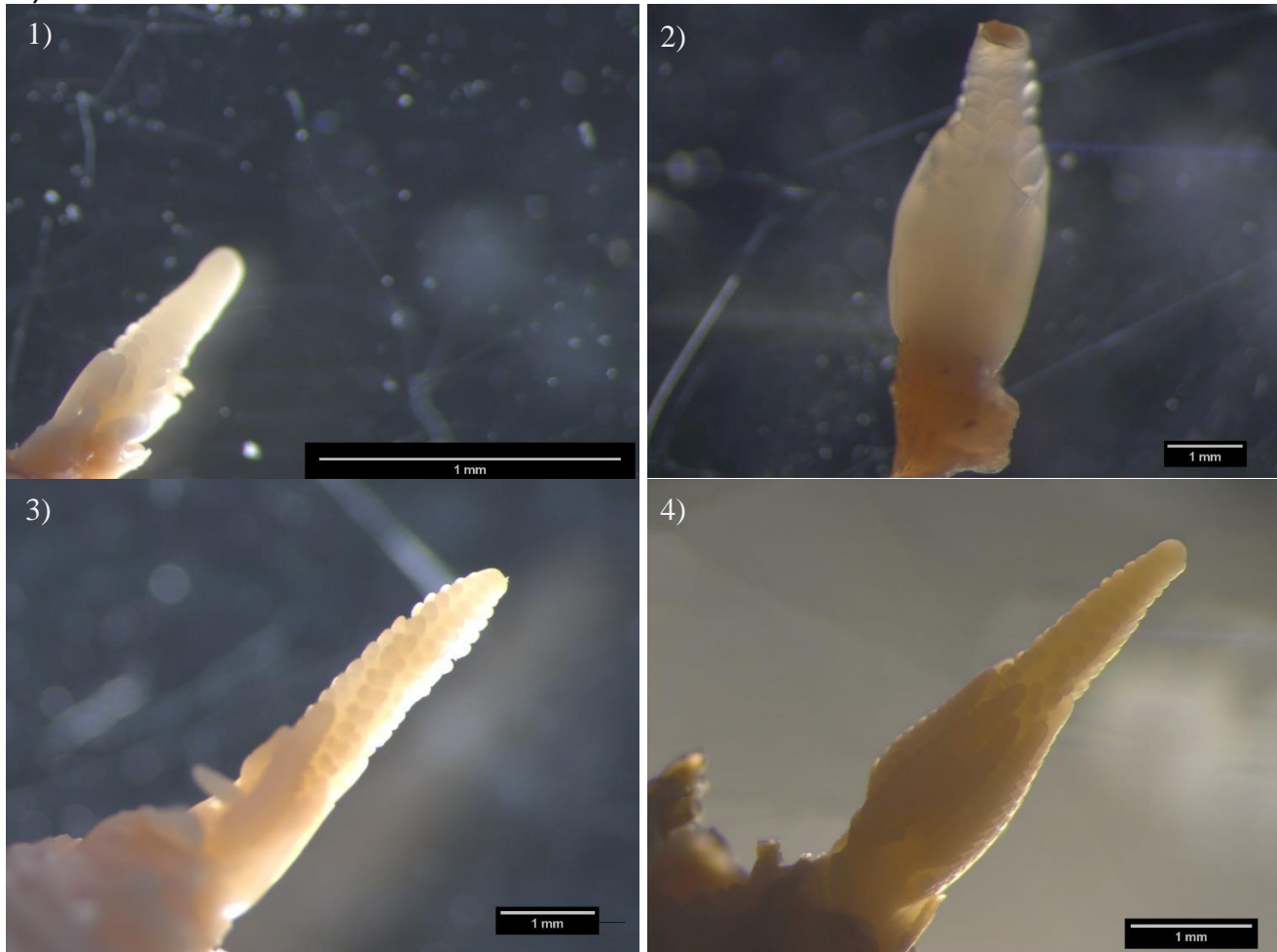
Figure S8: Tasseling differs among genotypes and between environmental conditions. (A) Tassel fresh weight at the eight fully developed leaves stage (V8) for cold treated plants (n=1-9) and control treated plants (n=8). A zero weight means that the tassel was not visible yet or that it was too small for accurate measurement on the balanced used during the measurements. Vertical lines represent weighted means for each cold tolerance category. (B) Tassel size of a selection of genotypes and vegetative stages observed in an independent experiment. Plants were grown in the greenhouse and were dissected at every V stage: The meristem was then observed under a stereomicroscope (MX12.5, Leica Camera, Wetzlar, Germany). 1) PE0100 at the V4 stage (x4 magnification); 2) PE0161 at the V4 stage (x5 magnification); 3) PE0401 at the V5 stage (x2.5 magnification); 4) PE0016 at the V5 stage (x5 magnification). The scale bar corresponds to 1 mm.
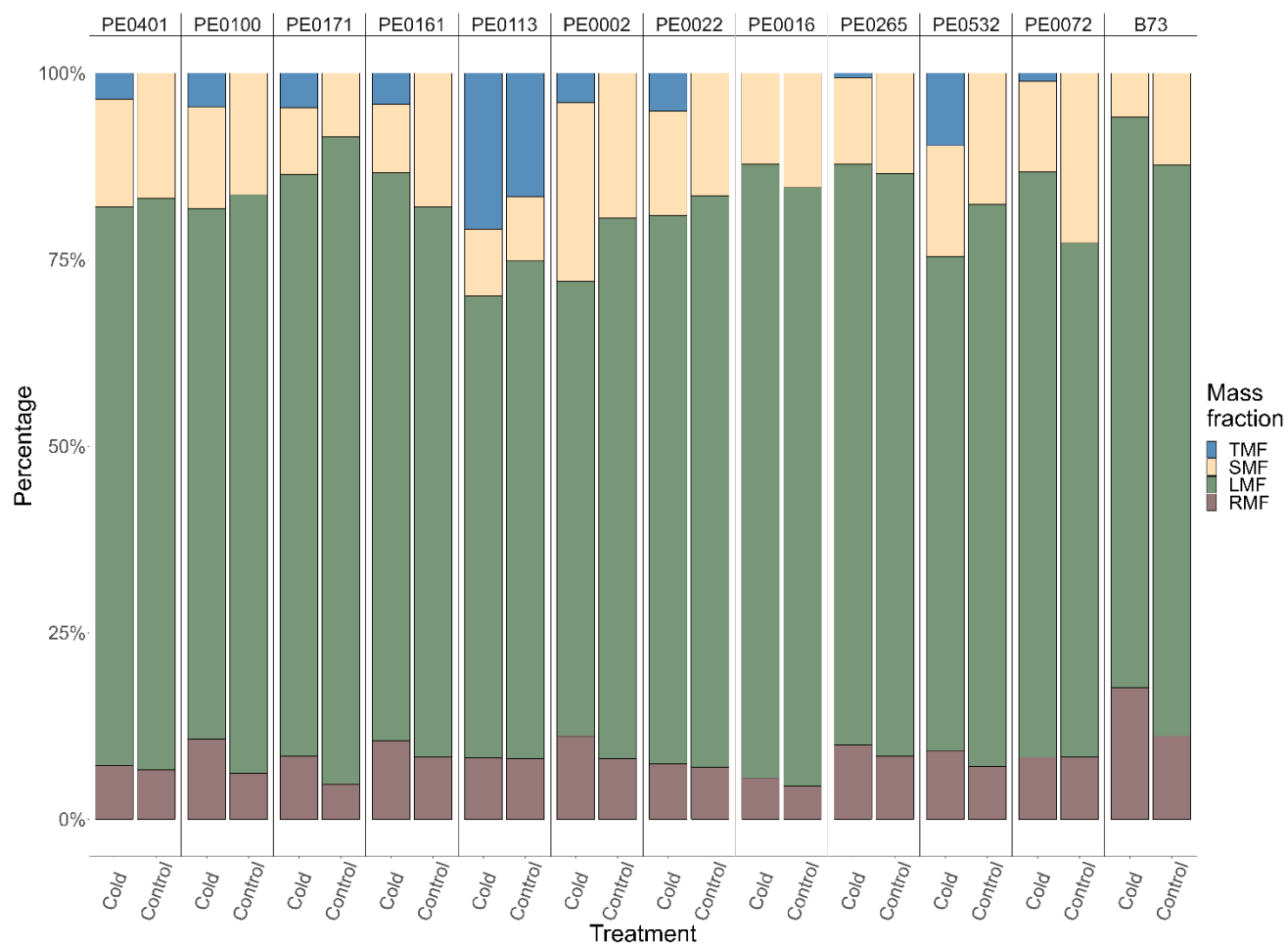
Figure S9: Mass fractions in percentage of total plant dry weight for different plant parts (TMF: tiller, SMF: stem, LMF: leaf, RMF: root) from the cold treatment(n=1-9) and control treatment (n=8).
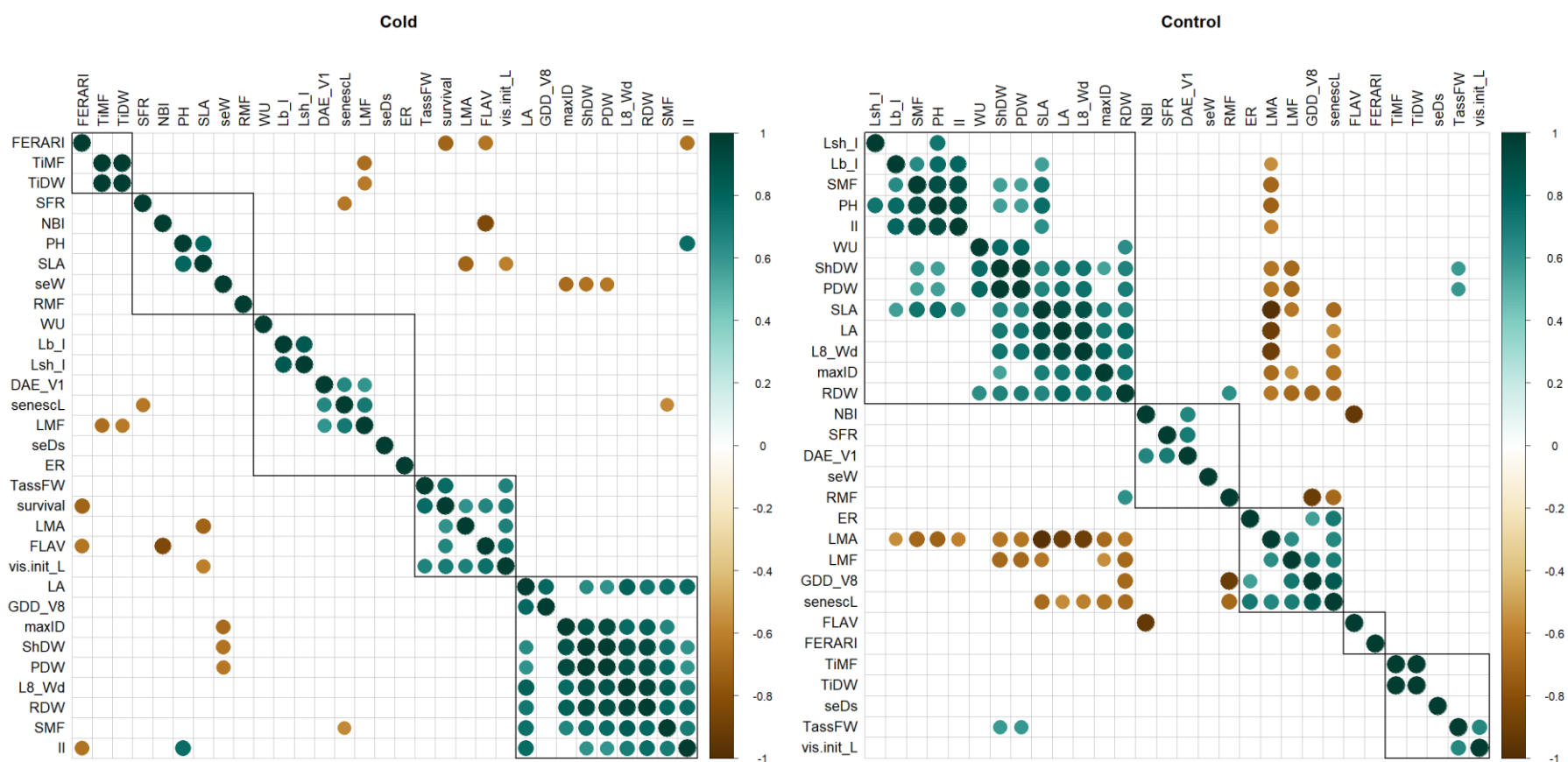
Figure S10: Correlation plots of plant traits under cold treatment (*left*) and control treatment (*right*). Traits measured at the seedling stage are DAE_V1 (days after emergence to reach V1 stage) and survival. Survival was not included in the control treatment correlation plot because survival rate was 100% for all genotypes. All other variables were measured at harvest. Non-significant correlations (p>0.05) were discarded from the plots. The color bar indicates the correlation coefficient from 1 (positive, green) to -1 (negative, brown). Trait abbreviations can be found in Figure S11 caption.

PCA of both treatments

Loading scores of the first two axes of the PCA
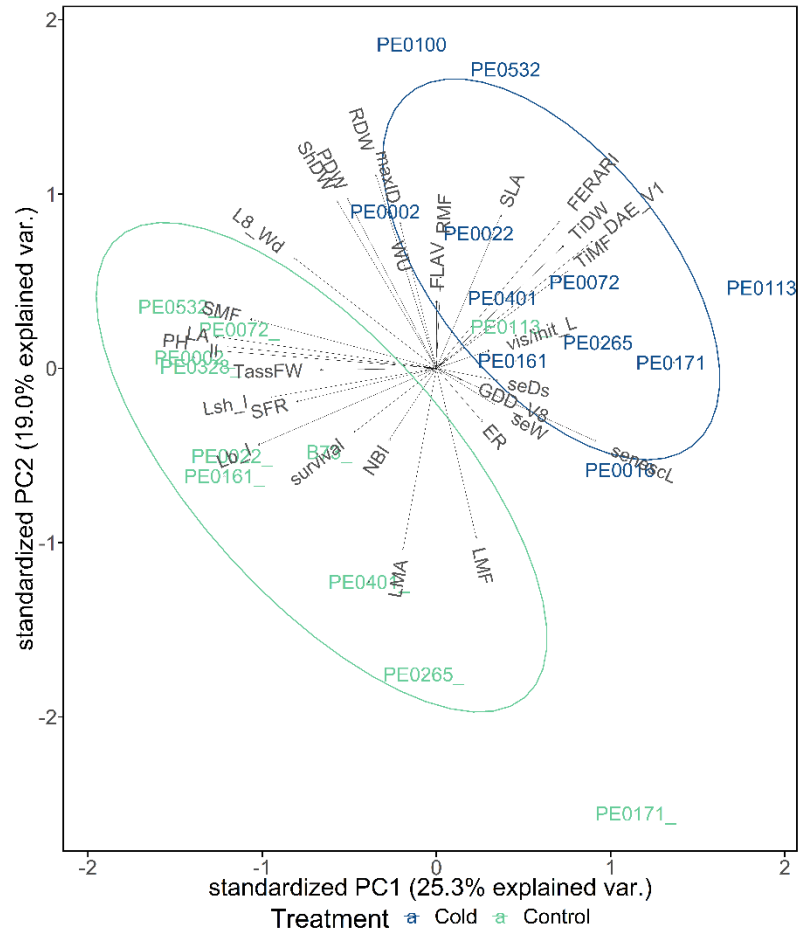
Loading scores of the PC1 axis

Loading scores of the PC2 axis
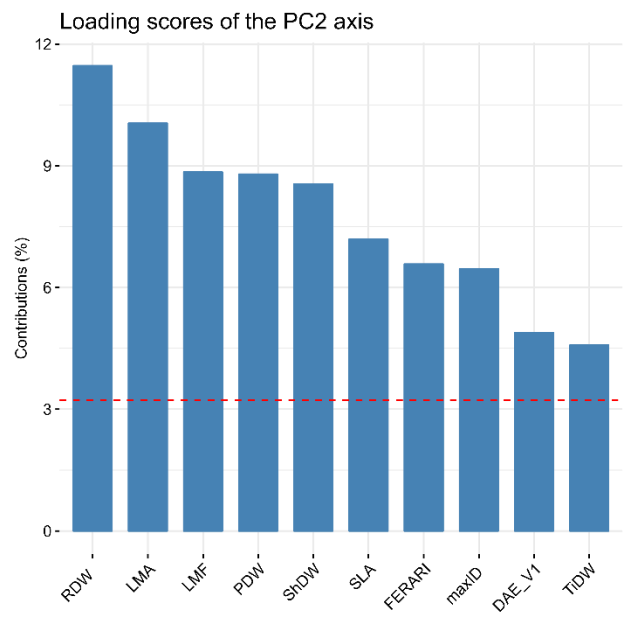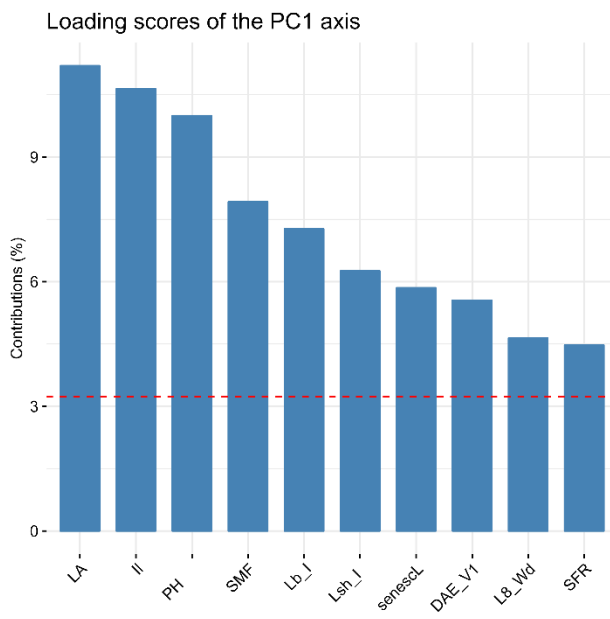
Figure S11: Principal component analysis and loading scores of its first two axes of measured plant traits under the cold and the control treatments. A) the principal component analysis of plant traits and genotypes. Principal components 1 (Dim-1) and 2 (Dim-2) explain 25.3% and 19.0 % of variability, respectively. B) Loading scores of Dim-1 and Dim-2. Included traits, measured before harvest: DAE_V1, days after emergence until the V1 stage; ER, emergence rates; seDs, seed density (g/cm^3); seW, seed weight (g): survival, survival rate (%). Included traits measured during and after harvest at the V8 stage: FERARI, Fluorescence Excitation Ratio Anthocyanin Relative Index; FLAV, Flavonol index; GDD_V8, Growing degree days until the V8 stage; Il, internode length (cm);, L8_Wd, leaf 8 width (cm); LA, leaf area (cm^2); Lb_l, leaf blade length (cm); LMA, leaf dry mass per unit area (g/cm^2); LMF, leaf mass fraction (%); Lsh_l, leaf sheath length (in cm); maxID, maximum internode diameter (cm); NBI, Nitrogen Balance Index; PDW, plant dry weight (g); PH, plant height (cm); RDW, root dry weight (g); RMF, root mass fraction (%); senesc_L, number of senescent leaves; SFR, Simple chlorophyll Fluorescence Ratio; ShDW, shoot dry weight (g); SLA, specific leaf area (cm^2/g); SMF, stem mass fraction (%); TassFW, tassel fresh weight (g); TiDW, tiller dry weight (g); TiMF, tiller mass fraction (%); vis/init_l, ratio of visible by initiated leaves; WU, water use (g/l).
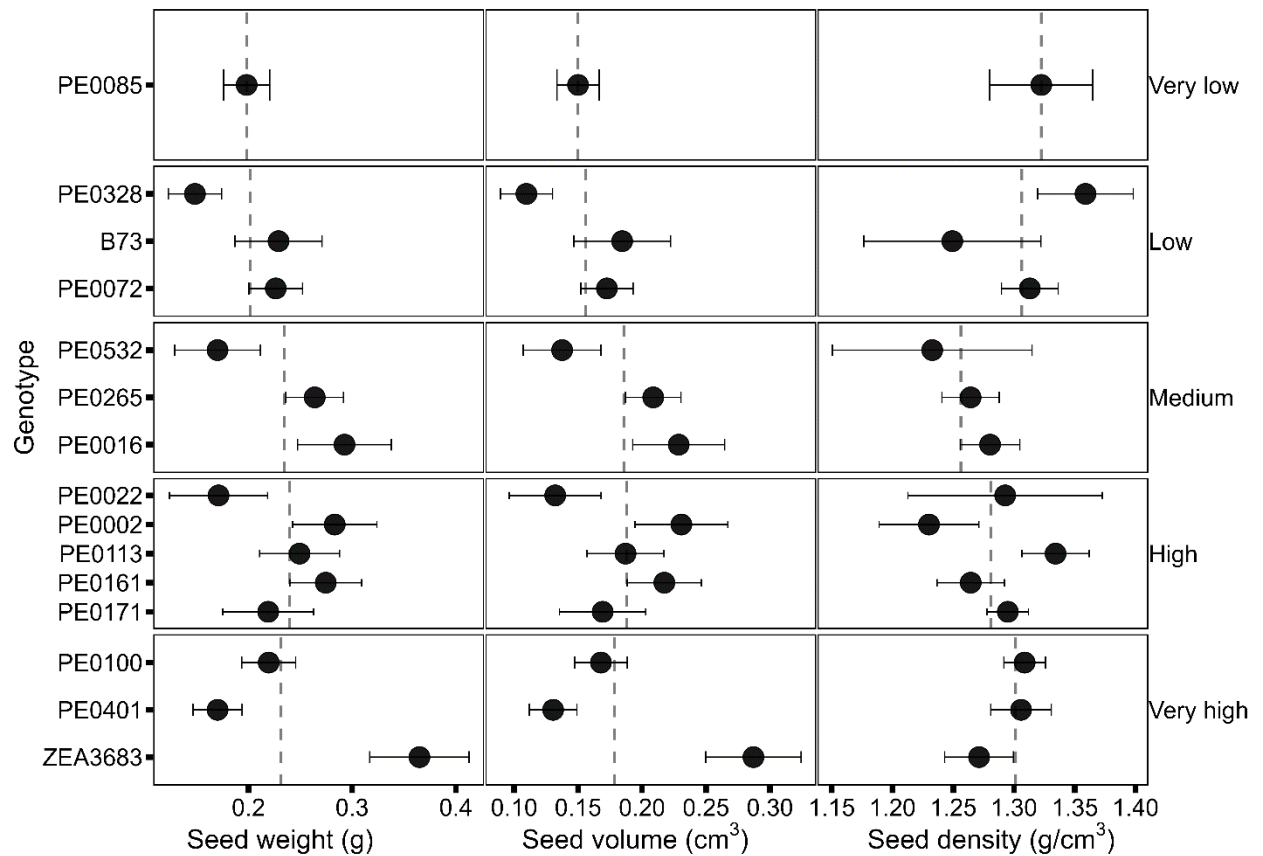
Figure S12: Seed traits of different genotypes used in this study, in addition to ZEA3683, a Petkuser Ferdinand Rot landrace (Germany). The landrace showed high cold tolerance levels and is therefore included in the "very high" cold tolerance category. Seedlings of PE0085 were not fit for transplantation under both the cold and control treatment. The genotype was included in the "very low" cold tolerance category (Table 1) for convenience but was further discarded from the experiments. The number of replicates is at least 28 seeds per genotype. Vertical lines represent weighted means for each cold tolerance category.

Table S1: Summary of the analysis of variance (ANOVA) test results. Seed weight and density underwent a one-way ANOVA test with genotype as a factor. Otherwise, all traits were tested with a two-way ANOVA test, with treatment and genotype as factors, while excluding the genotypes B73 and PE0328 due to a single individual surviving, and no plants being transplanted, respectively, in the control treatment.

Trait	Effect	DFn	F	p	significance level	ges
DAE_V1	(Intercept)	1	4944.324	1.81E-210	***	0.933
	treatment	1	527.439	3.50E-72	***	0.598
	genotype	12	2.432	5.00E-03	**	0.076
	treatment:genotype	12	1.932	3.00E-02	*	0.061
dead_leaves_nr	(Intercept)	1	1329.622	2.12E-72	***	0.907
	treatment	1	2.134	0.146	ns	0.015
	genotype	10	17.783	1.99E-20	***	0.565
	treatment:genotype	10	2.405	0.012	*	0.149
FERARI	(Intercept)	1	19242.3	4.28E-97	***	0.996
	treatment	1	82.558	5.95E-14	***	0.508
	genotype	8	7.148	4.24E-07	***	0.417
	treatment:genotype	8	3.802	0.000793	***	0.275
FLAV	(Intercept)	1	1500.017	1.37E-53	***	0.949
	treatment	1	0.637	0.427	ns	0.008
	genotype	8	11.696	7.22E-11	***	0.539
	treatment:genotype	8	1.733	0.103	ns	0.148
GDD_V8	(Intercept)	1	118376.8	4.4E-203	***	0.999
	treatment	1	8.537	0.004	**	0.059
	genotype	10	28.931	3.91E-29	***	0.679
	treatment:genotype	10	8.122	3.18E-10	***	0.372
IL	(Intercept)	1	583.062	9.88E-55	***	0.788
	treatment	1	37.645	6.63E-09	***	0.193
	genotype	10	7.7	5.79E-10	***	0.329
	treatment:genotype	10	1.448	0.164	ns	0.084
L8_Wd	(Intercept)	1	7089.788	7.3E-120	***	0.981
	treatment	1	8.116	0.005	**	0.056
	genotype	10	36.378	8.29E-34	***	0.726
	treatment:genotype	10	5.501	7.5E-07	***	0.287
LA	(Intercept)	1	2803.363	4.83E-77	***	0.964
	treatment	1	122.446	2.82E-19	***	0.541
	genotype	10	24.066	7.88E-23	***	0.698
	treatment:genotype	10	7.066	2.16E-08	***	0.405
Lbl	(Intercept)	1	1129.071	1.36E-73	***	0.878
	treatment	1	32.696	5.29E-08	***	0.172
	genotype	10	0.842	0.589	ns	0.051
	treatment:genotype	10	1.028	0.422	ns	0.061
LDW	(Intercept)	1	1491.299	1.63E-75	***	0.916
	treatment	1	7.1501	8.00E-03	**	0.05
	genotype	10	6.4664	4.00E-08	***	0.321
	treatment:genotype	10	2.9279	2.00E-03	**	0.176

Trait	Effect	DFn	F	p	significance level	ges
LMA	(Intercept)	1	880.585	1.42E-52	***	0.894
	treatment	1	58.69	1E-11	***	0.361
	genotype	10	3.365	0.000783	***	0.244
	treatment:genotype	10	3.086	0.002	**	0.229
LMF	(Intercept)	1	29271.71	6.4E-155	***	0.996
	treatment	1	6.378	0.013	*	0.047
	genotype	10	14.554	4.43E-17	***	0.528
	treatment:genotype	10	3.569	0.000333	***	0.215
Lshl	(Intercept)	1	1092.055	1.34E-72	***	0.874
	treatment	1	18.008	3.75E-05	***	0.103
	genotype	10	2.784	0.003	**	0.151
	treatment:genotype	10	1.023	0.426	ns	0.061
maxID	(Intercept)	1	32170.83	0.00E+00	***	0.986
	treatment	1	266.668	1.88E-47	***	0.371
	genotype	10	87.095	8.42E-99	***	0.658
	treatment:genotype	10	23.27	2.71E-35	***	0.34
NBI	(Intercept)	1	6012.077	4.84E-77	***	0.987
	treatment	1	5.011	0.028	*	0.059
	genotype	8	13.4	4.17E-12	***	0.573
	treatment:genotype	8	1.693	0.113	ns	0.145
PDW	(Intercept)	1	1790.5	6.97E-78	***	0.932
	treatment	1	9.35	0.003	**	0.067
	genotype	10	10.463	7.92E-13	***	0.446
	treatment:genotype	10	5.018	3.73E-06	***	0.279
PH	(Intercept)	1	9103.891	6.2E-123	***	0.986
	treatment	1	147.041	3.72E-23	***	0.529
	genotype	10	71.743	3.31E-48	***	0.846
	treatment:genotype	10	3.813	0.000154	***	0.225
RDW	(Intercept)	1	903.182	2.09E-61	***	0.871
	treatment	1	27.305	6.49E-07	***	0.169
	genotype	10	9.917	2.62E-12	***	0.425
	treatment:genotype	10	5.33	1.34E-06	***	0.285
RMF	(Intercept)	1	1982.66	1.41E-80	***	0.938
	treatment	1	33.181	5.76E-08	***	0.203
	genotype	10	6.767	1.98E-08	***	0.342
	treatment:genotype	10	1.586	0.118	ns	0.109
SDW	(Intercept)	1	1104.967	9.78E-68	***	0.889
	treatment	1	1.057	3.06E-01	ns	0.008
	genotype	10	18.649	2.97E-21	***	0.575
	treatment:genotype	10	3.987	8.39E-05	***	0.224
SeDS	(Intercept)	1	459932.2	0.00E+00	***	0.999
	Genotype	14	26.951	4.61E-54	***	0.399
SeW	(Intercept)	1	23118.66	0.00E+00	***	0.976
	Genotype	14	93.387	3.37E-137	***	0.697

Trait	Effect	DFn	F	p	significance level	ges
SFR	(Intercept)	1	17241.3	3.39E-95	***	0.995
	treatment	1	7.251	0.009	**	0.083
	genotype	8	6.85	7.97E-07	***	0.407
	treatment:genotype	8	1.364	0.225	ns	0.12
ShDW	(Intercept)	1	1677.191	5.26E-78	***	0.926
	treatment	1	6.011	0.015	*	0.043
	genotype	10	9.255	1.48E-11	***	0.407
	treatment:genotype	10	3.736	0.00019	***	0.217
SLA	(Intercept)	1	1704.899	2.54E-66	***	0.943
	treatment	1	119.414	5.71E-19	***	0.534
	genotype	10	6.979	2.72E-08	***	0.402
	treatment:genotype	10	4.356	4.23E-05	***	0.295
SMF	(Intercept)	1	3105.741	1.29E-92	***	0.96
	treatment	1	23.192	4.01E-06	***	0.151
	genotype	10	20.159	4.54E-22	***	0.608
	treatment:genotype	10	6.165	1.17E-07	***	0.322
TassFW	(Intercept)	1	109.997	7.75E-20	***	0.412
	treatment	1	74.823	5.71E-15	***	0.323
	genotype	10	6.983	5.17E-09	***	0.308
	treatment:genotype	10	4.364	2.11E-05	***	0.218
TiDW	(Intercept)	1	34.951	3.78E-09	***	0.012
	treatment	1	10.344	0.001	**	0.004
	genotype	10	7.02	5.66E-11	***	0.024
	treatment:genotype	10	1.092	0.364	ns	0.004
TMF	(Intercept)	1	19.545	0.000017	***	0.098
	treatment	1	5.034	0.026	*	0.027
	genotype	10	8.346	4.36E-11	***	0.317
	treatment:genotype	10	0.297	0.981	ns	0.016
vis/init_leaves	(Intercept)	1	40946.28	2.5E-163	***	0.997
	treatment	1	0.208	0.649	ns	0.002
	genotype	10	17.466	1.01E-19	***	0.575
	treatment:genotype	10	2.482	0.009	**	0.161
WU	(Intercept)	1	2535.034	1.3E-81	***	0.956
	treatment	1	3.037	0.084	ns	0.025
	genotype	10	1.923	0.048	*	0.14
	treatment:genotype	10	3.085	0.002	**	0.207

The abbreviation 'ges' stands for generalized eta squared effect size. Significance level: *** p< 0.001; ** p< 0.01; * p< 0.05; ns p> 0.05 (non-significant).