Figure 1

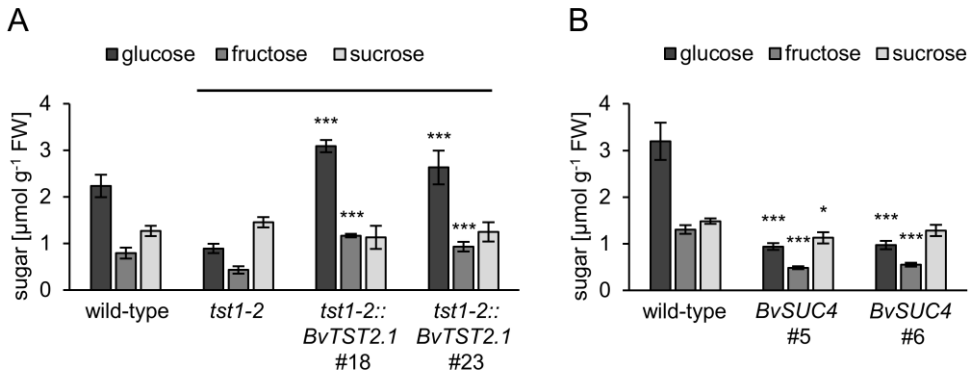

Fig. 1. Soluble sugar contents of 4-week old plants grown on soil. **A** Sugar levels of *tst1-2* mutant, *tst1-2::BvTST2.1* overexpressor line 18 and 23 and the corresponding wild-type. Data are presented as mean ±SE of at least 6 biological replicates. **B** Sugar levels of *BvSUC4* overexpressor line 5 and 6 and the corresponding wild-type. Data are presented as mean ±SE of at least 4 biological replicates. Asterisks indicate statistically significant differences between the *tst1-2* and the *tst1-2::BvTST2.1* lines or between the *BvSUC4* lines and the corresponding wild-type analyzed with Students t-test (* p≤0.05; *** P≤0.001).

Figure 2

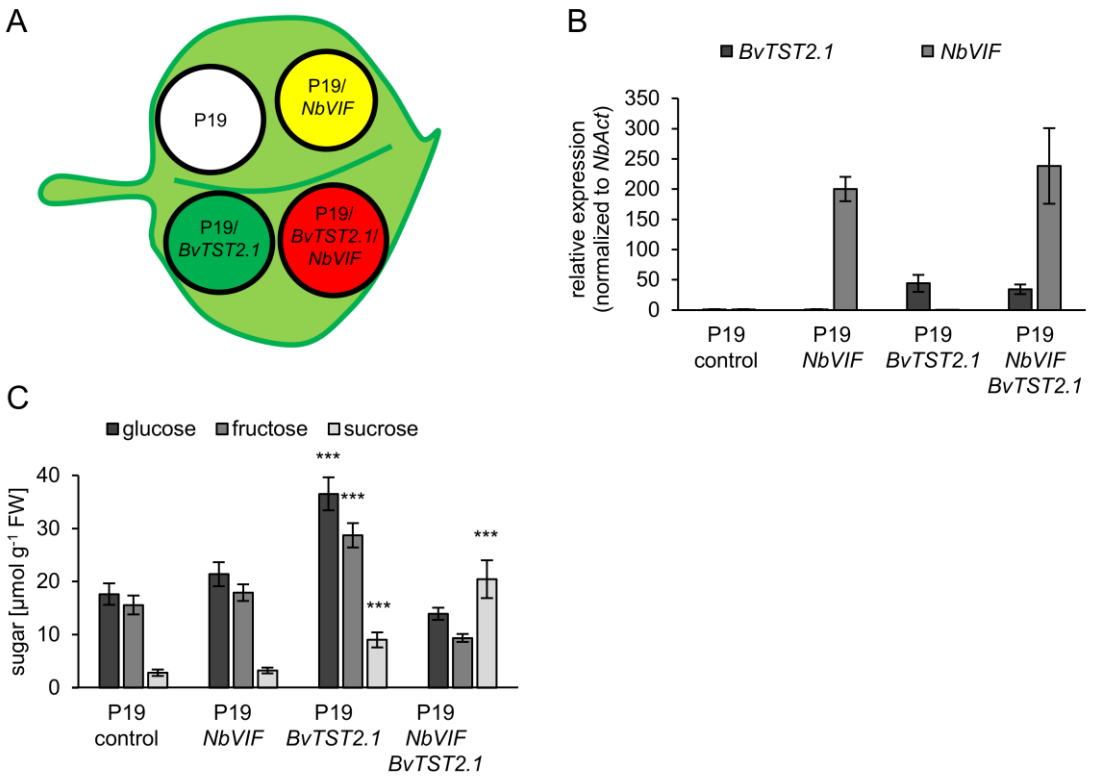

Fig. 2. Elucidation of *Bv*TST2.1's *in vivo* function using *N. benthamiana* infiltration assay. **A** Schematic drawing of a *N. benthamiana* leaf infiltrated with Agrobacteria harboring different expression constructs. **B** Normalized expression of *BvTST2.1* and *NbVIF* in infiltrated leaf tissue area. **C** Soluble sugar levels of leaf tissue harvested 4 days after infiltration. Data are presented as mean ±SE of at least 6 biological replicates. Asterisks indicate statistically significant differences analyzed with Students t-test (*** P≤0.001). P19 = P19 protein of *tomato bushy stunt virus*, a suppressor of gene silencing (Voinnet et al., 2003); *NbVIF* = inhibitor protein of *N. benthamiana* vacuolar invertase.

Figure 3

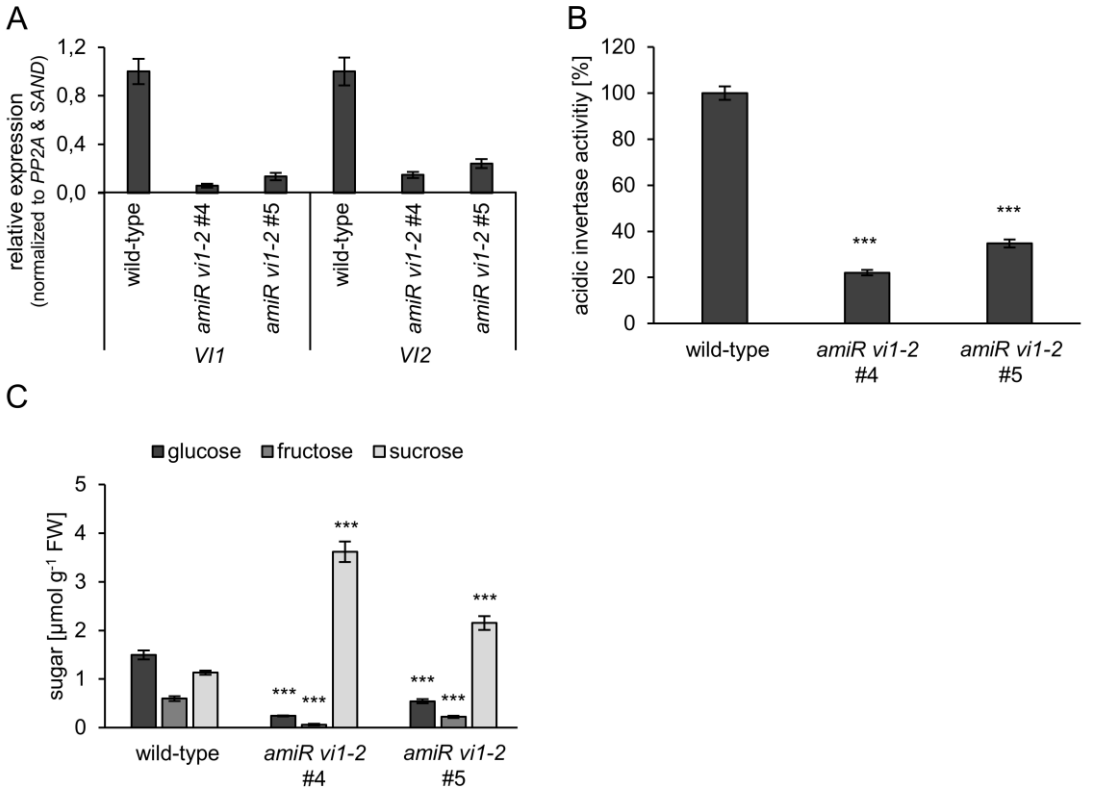

Fig. 3. Molecular, biochemical characterization and soluble sugar content of 4-week old wild-type plants and *amiR vi1-2* lines 4 and 5. **A** Normalized relative expression level of *vacuolar invertase* (*VI*) *1* and *2* in leaf samples Data are presented as mean ±SE of 4 biological replicates. **B** Acidic invertase activity in leaf samples. Data are presented as mean ±SE of 5 biological replicates. **C** Sugar levels in leaves of 4-week old plants grown on soil. Data are presented as mean ±SE of 6 biological replicates. Asterisks indicate statistically significant differences between the wild-type and the *amiR vi1-2* lines analyzed with Students t-test (*** P≤0.001).

Figure 4

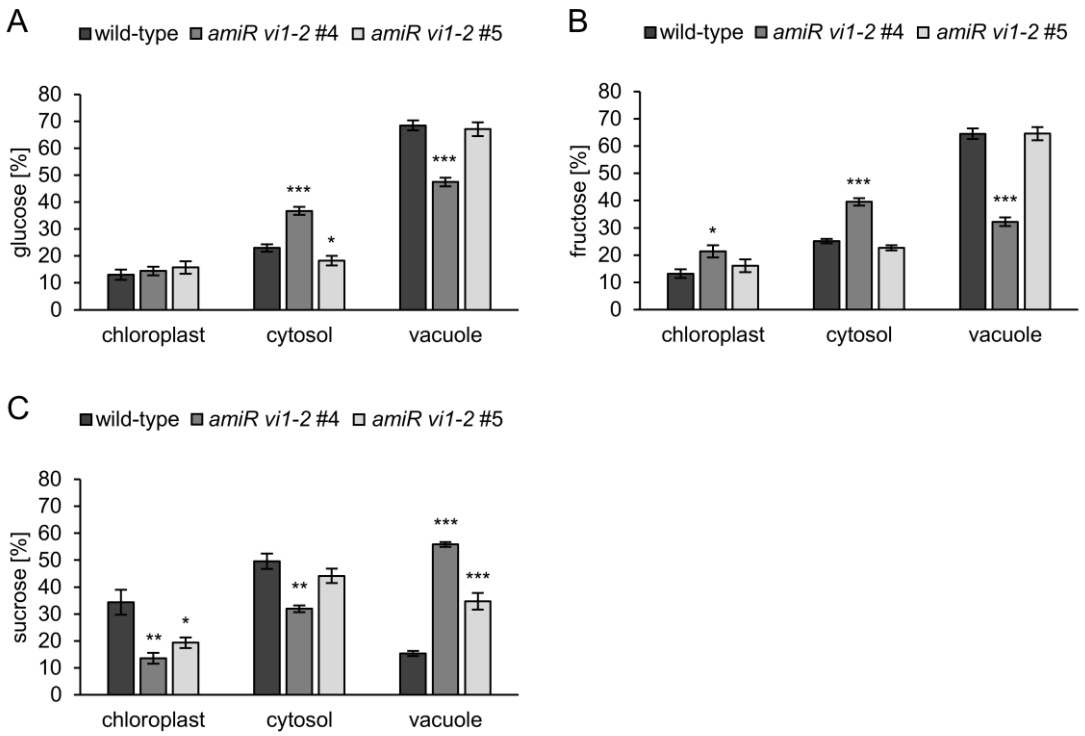

Fig. 4. Analysis of subcellular sugar distribution of 4-week old wild-type plants and *amiR vi1-2* lines 4 and 5 after non-aqueous fractionation. Subcellular distribution of glucose (**A**), fructose (**B**) and sucrose (**C**). Data are presented as mean ±SE of 4 biological replicates consisting of 3 plants each. Asterisks indicate statistically significant differences between the wild-type and the *amiR vi1-2* lines analyzed with Students t-test (* P≤0.05; ** P≤0.01; *** P≤0.001).

Figure 5

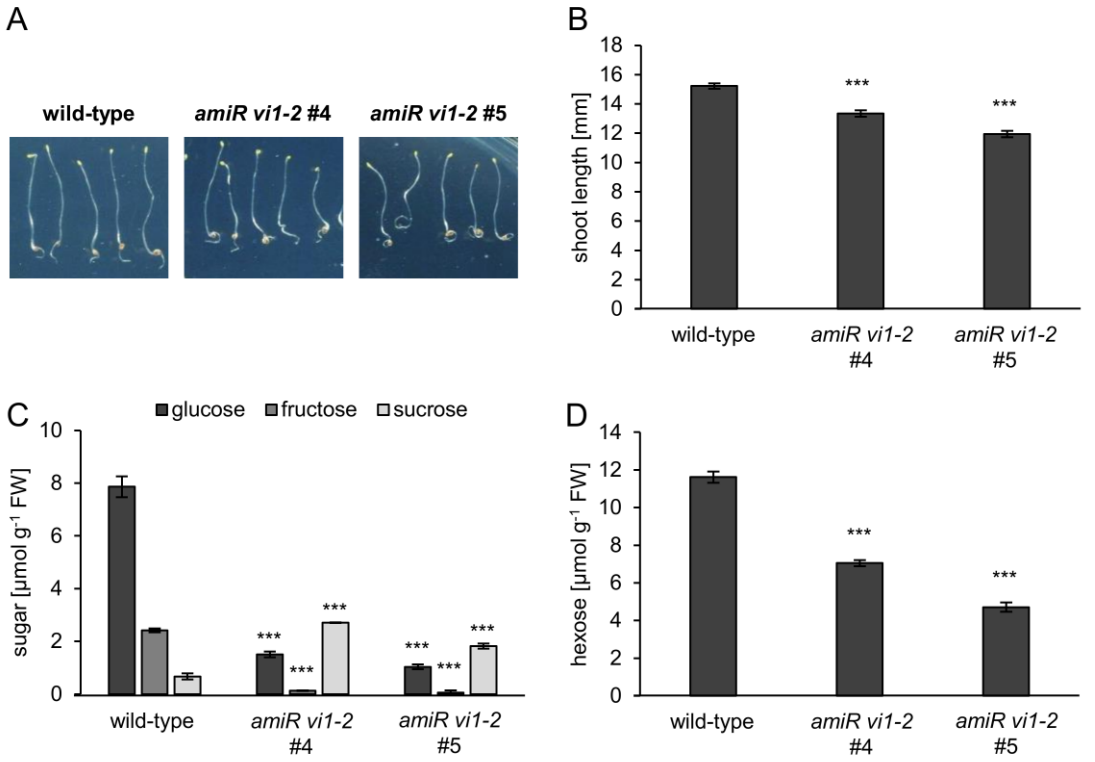

Fig. 5. Effects of dark treatment on plant phenotype and sugar levels of wild-type plants and *amiR vi1-2* lines 4 and 5 after germination for 7 days in darkness. **A** Etiolated seedlings. **B** Analysis of etiolated shoot lengths. Data are presented as mean ±SE of at least 30 biological replicates. **C** Sugar levels in etiolated seedlings. Data are presented as mean ±SE of 3 biological replicates. **D** Total hexose levels in etiolated seedlings. Data are presented as mean ±SE of 3 biological replicates. Asterisks indicate statistically significant differences between the wild-type and the *amiR vi1-2* lines analyzed with Students t-test (*** P≤0.001).

Figure 6

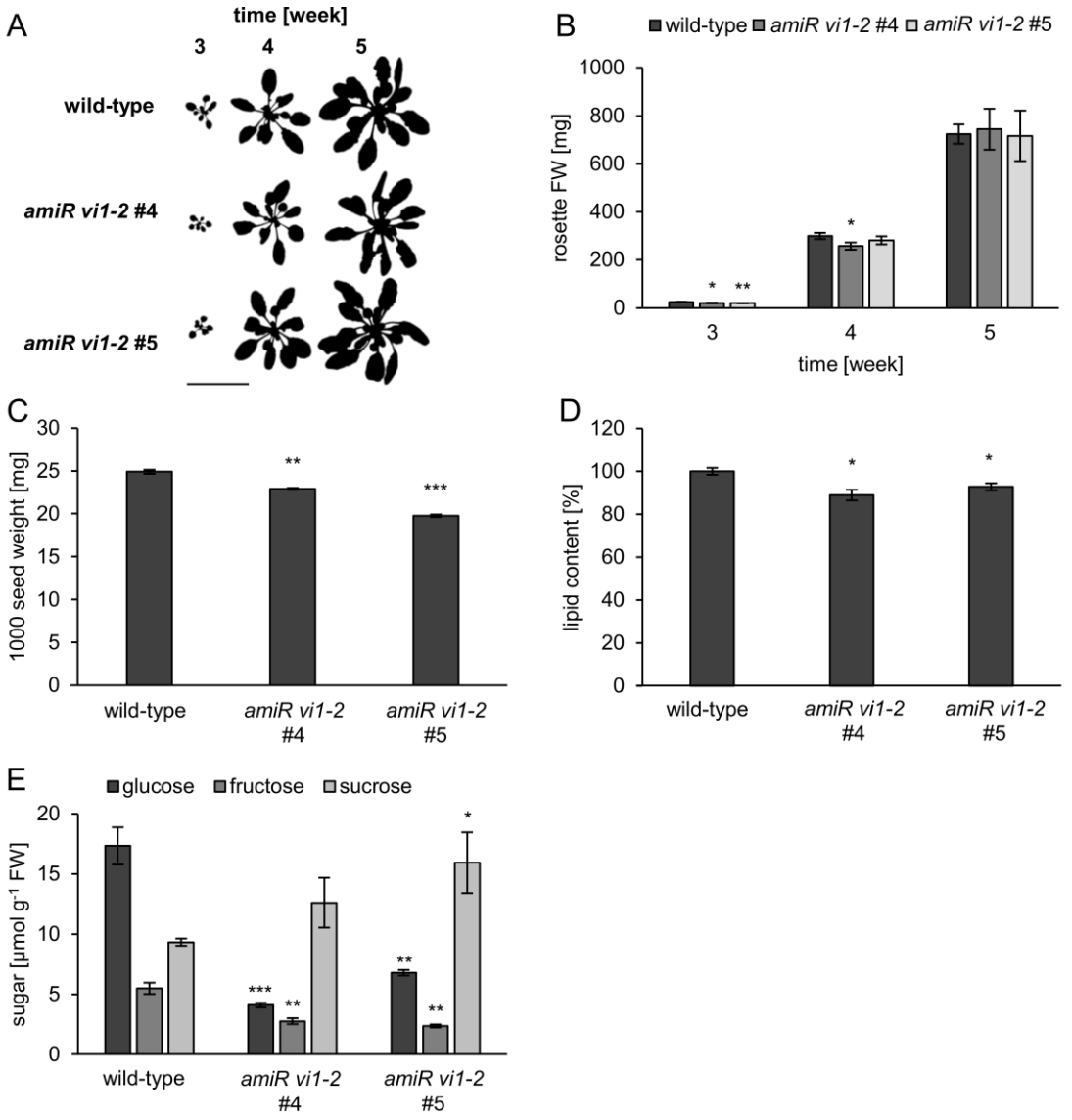

Fig. 6. Analysis of rosettes, seeds and siliques of wild-type plants and *amiR vi1-2* lines 4 and 5. **A** Rosette size of 3- to 5-week old plants. Bar = 5 cm. **B** Analysis of rosette fresh weight of 3- to 5-week old plants. Data are presented as mean ±SE of at least 6 biological replicates. **C** 1000 seed weight. Data are presented as mean ±SE of 3 biological replicates (deriving from the same harvest). **D** Lipid content of mutant seeds was normalized to lipid content of wild-type seeds. Data are presented as mean ±SE of 4 biological replicates. **E** Sugar content in siliques. Data are presented as mean ±SE of 3 biological replicates. Asterisks indicate statistically significant differences between the wild-type and the *amiR vi1-2* lines analyzed with Students t-test (* P≤0.05; ** P≤0.01; *** P≤0.001).

Figure 7

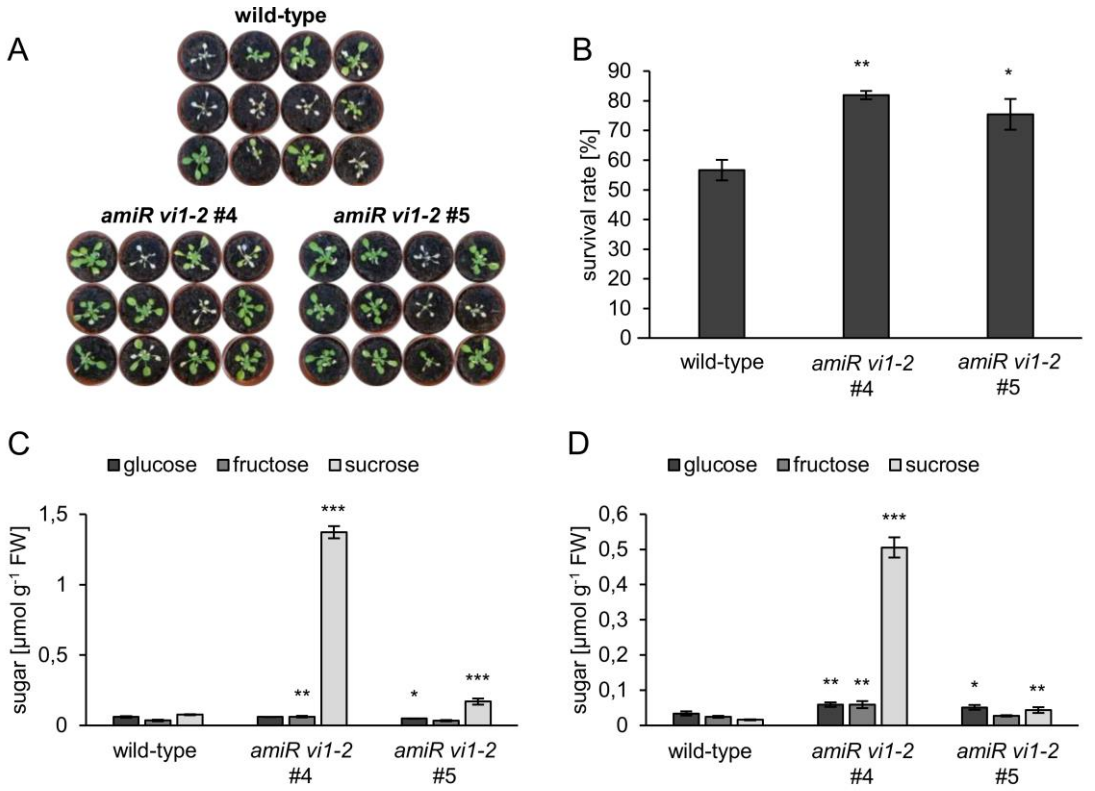

Fig. 7. Effects of dark treatment on wild-type plants and *amiR vi1-2* lines 4 and 5. Plants were cultivated for 4 weeks under standard conditions on soil, kept for 5 days in the dark and then recovered for 7 days under standard conditions. **A** Plants after dark recovery. **B** Quantification of survivors after dark recovery. Data are presented as mean ±SE of 3 independent experiments with each 12 plants per line. Sugar levels in leaves after 24 hours (**C**) and after 72 hours (**D**) of dark treatment. Data are presented as mean ±SE of 4 biological replicates. Asterisks indicate statistically significant differences between the wild-type and the *amiR vi1-2* lines analyzed with Students t-test (* P≤0.05; ** P≤0.01; *** P≤0.001).

Figure 8

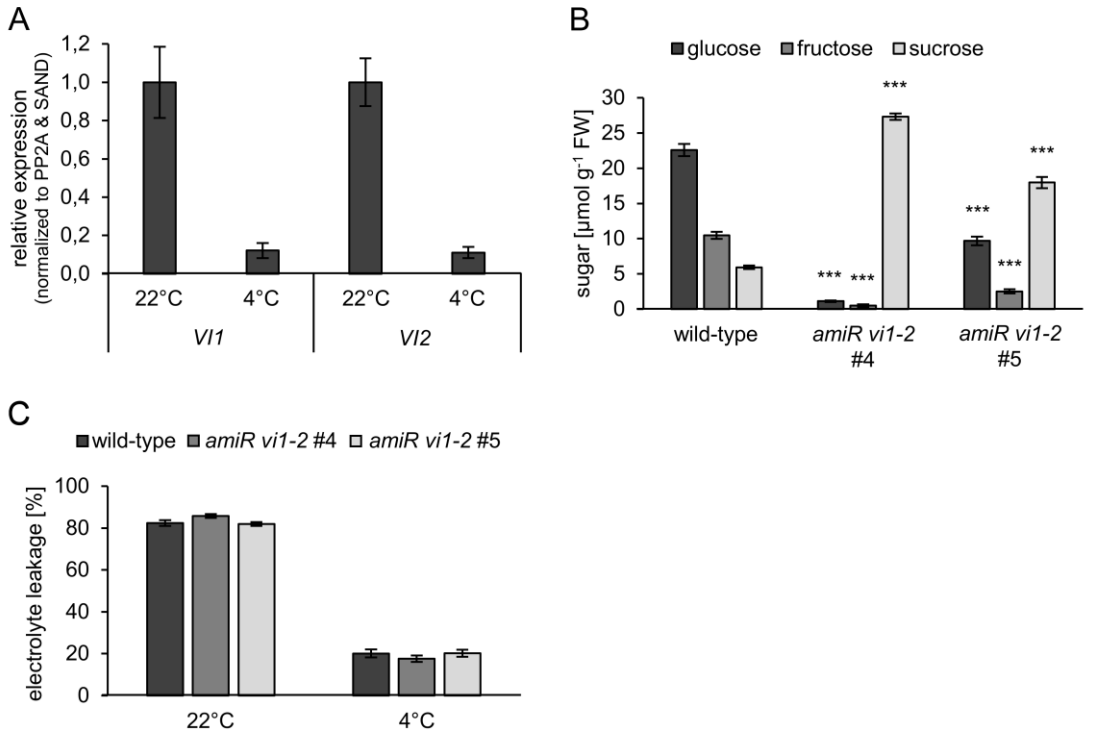

Fig. 8. Effects of cold treatment on wild-type plants and *amiR vi1-2* lines 4 and 5. Plants were cultivated for 4 weeks under standard conditions on soil and then transferred for 3 days to 4°C. **A** Relative expression level of *vacuolar invertase* (*VI*) *1* and *2* in cold acclimated wild-type leaf samples. Data are presented as mean ±SE of 4 biological replicates. **B** Sugar levels in cold acclimated leaves. Data are presented as mean ±SE of 6 biological replicates. **C** Analysis of electrolyte leakage of leaves kept in cold (4°C) for 4 days. Data are presented as mean ±SE of at least 8 biological replicates. Asterisks indicate statistically significant differences between the wild-type and the *amiR vi1-2* lines analyzed with Students t-test (*** P≤0.001).