Mutation induction in *Sucrose synthase 1* to study cold acclimation in winter wheat

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INTRODUCTION

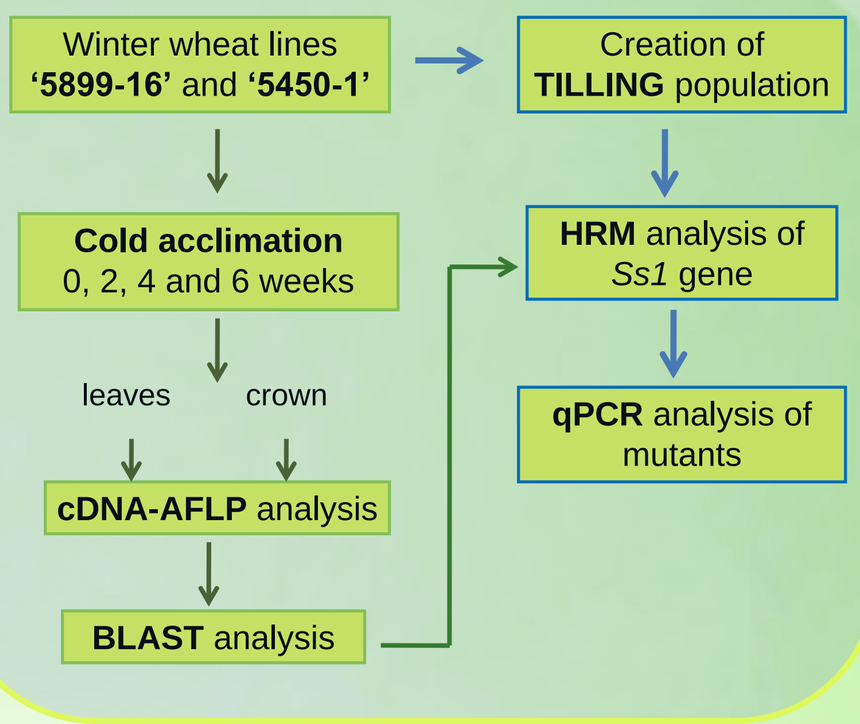
Freezing tolerance of winter wheat is one of the main factors governing winter survival. Freezing tolerance is associated with the occurrence of a cold acclimation which is triggered by the induction of cold responsive (*Cor*) genes after exposure of plants to low non-freezing temperatures for certain periods of time. In particular, *Cor* genes are transcriptionally activated during cold acclimation, and the accumulated COR proteins lead to protection of the integrity of cell structures from freezing damage (Kosová et al., 2010). Carbohydrates, in particular, are recognized as playing an important role in freezing tolerance and the accumulation of simple sugars such as trehalose, raffinose and sucrose has been shown to be correlated with enhanced freezing tolerance. Such accumulation of sucrose and other simple sugars during cold acclimation contribute to the stabilization of membranes and may play a key role in protecting the cells from freezing and dehydration. Sucrose synthase (Ss) (E.C. 2.4.1.13) catalyzes the reversible conversion of sucrose and a nucleoside diphosphate into the corresponding nucleoside diphosphate-glucose and fructose being one of the main enzymes of carbohydrate metabolism. Creation and testing of *Ss1* gene mutant forms would be one of the ways to determine the Ss role in cold acclimation process.

The objectives of this study were:

- to create a TILLING population in winter wheat;
- to identify new alleles of *Ss1* gene;
- to compare relative expression of *Ss1* in leaves and crowns of mutant *versus* wild type plants during cold acclimation.

Figure 1. Differential susceptibility to freezing of two winter wheat lines: WW-32 (A); WW-68DH (B).

MATERIALS AND METHODS



RESULTS

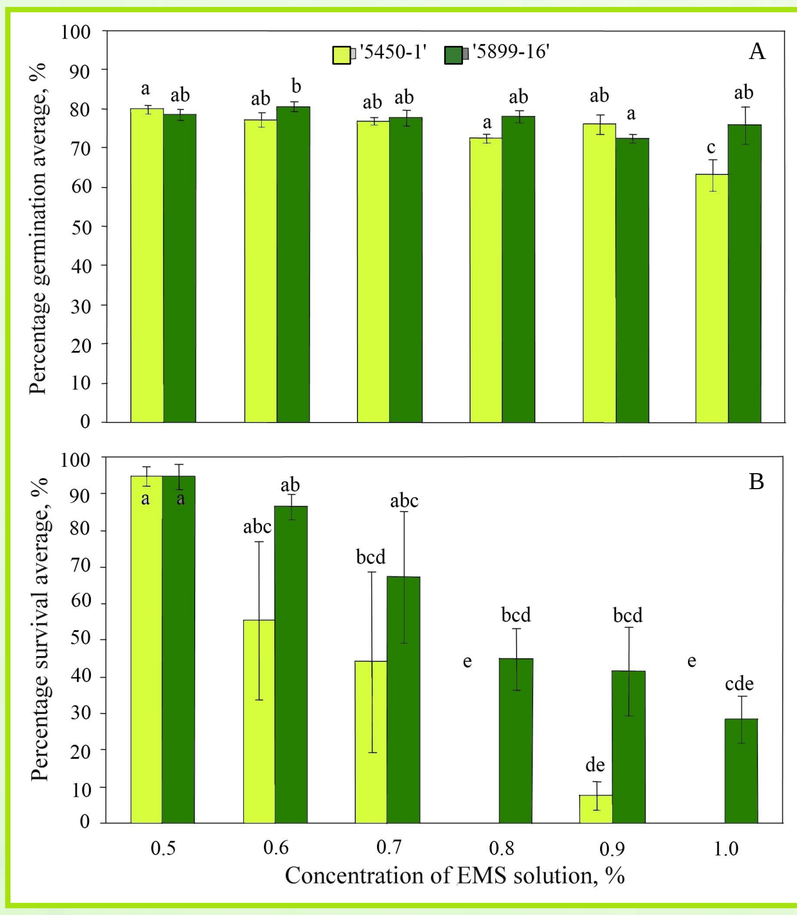


Figure 2. Dose optimization of a mutagen EMS. (A) Percentage germination average at 15th day after sowing; (B) Percentage survival average at two months after sowing of wheat genotypes '5450-1' and '5899-16' exposed to various concentrations of EMS solution.

Note. Vertical bars represent Standard Deviation (SD) of the mean. The letters above the boxes indicate statistically significant ($p<0.05$) differences between treatments.

cDNA-AFLP was performed using 48 primer pair combinations, which generated 522 transcript-derived fragments (TDFs) with an average of 11 fragments per primer pair. The length of the TDFs were in the range of 200–2000 bp. Twenty TDFs (3.8%) were identified as being differentially expressed (presence/absence) between cold acclimated and non-acclimated wheat. A total of fourteen TDFs revealed significant (E value < 1e-10) sequence similarities in a BLASTX search against the non-redundant (nr) protein database of GenBank.

Concentration of the mutagen EMS had an effect on both germination and development of wheat seedlings (Fig. 2). Two TILLING populations originating from the two winter wheat lines were further developed (1386 for '5899-16' and 761 for '5450-1').

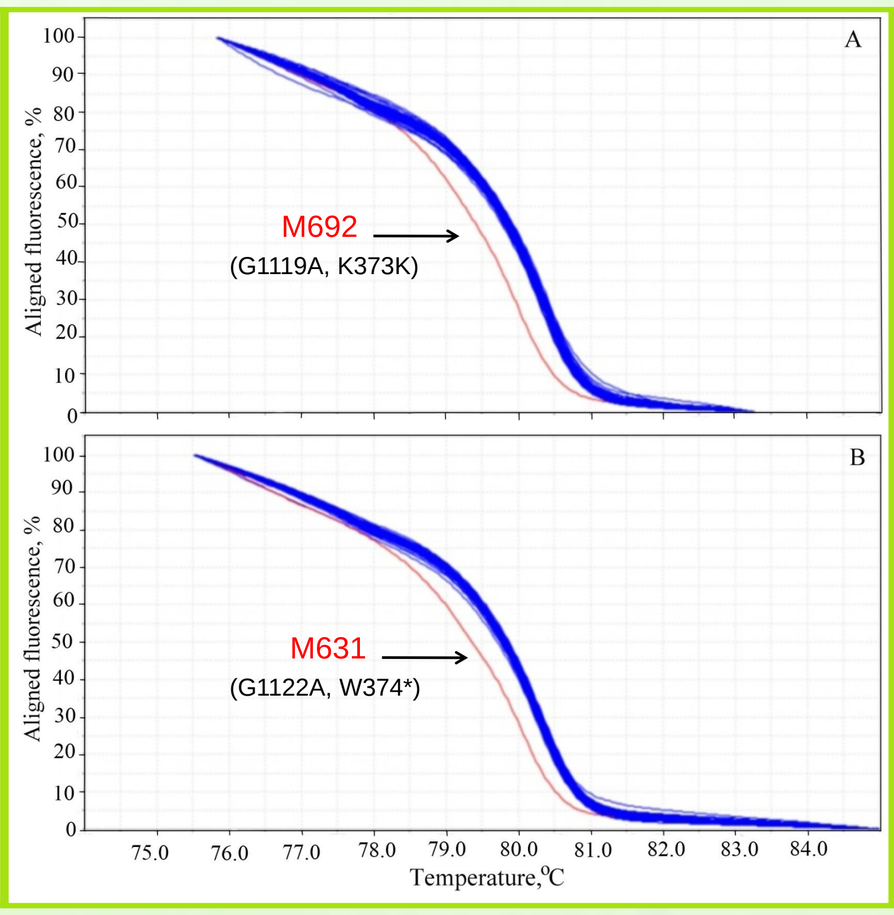


Figure 3. HRM analysis of 96 2-fold pooled samples. Aligned curves of fluorescence *vs* temperature showing wild type (blue) and mutant (red) samples: A) M692; B) M631.

Exon 8 of differentially expressed S*s1* gene was chosen for mutation screening by HRM analysis in wheat TILLING M_2 population. A total of 75.68 kb of DNA were tested and two putative mutants were identified. These 2-fold pooled samples of putative mutants had peaks shifted towards a lower temperature compared with the wild type genotype (Fig. 3). Sequencing results revealed two novel alleles of *Ss1* gene. Mutant No. 692 was found to have silent mutation (G1119A, K373K) and mutant No. 631 had nonsense mutation (G1122A, W374*).

qPCR analysis showed that premature stop codon mutation has a strong effect on *Ss1* gene expression (Fig. 4). Putative knock-out mutant M631 had significantly lower relative expression of *Ss1* gene in non-acclimated leaves as well as in crowns and leaves collected at 2, 4 and 6 weeks of cold acclimation compared with the wild type winter wheat line '5899-16' (control) and M692 mutant (silent mutation).

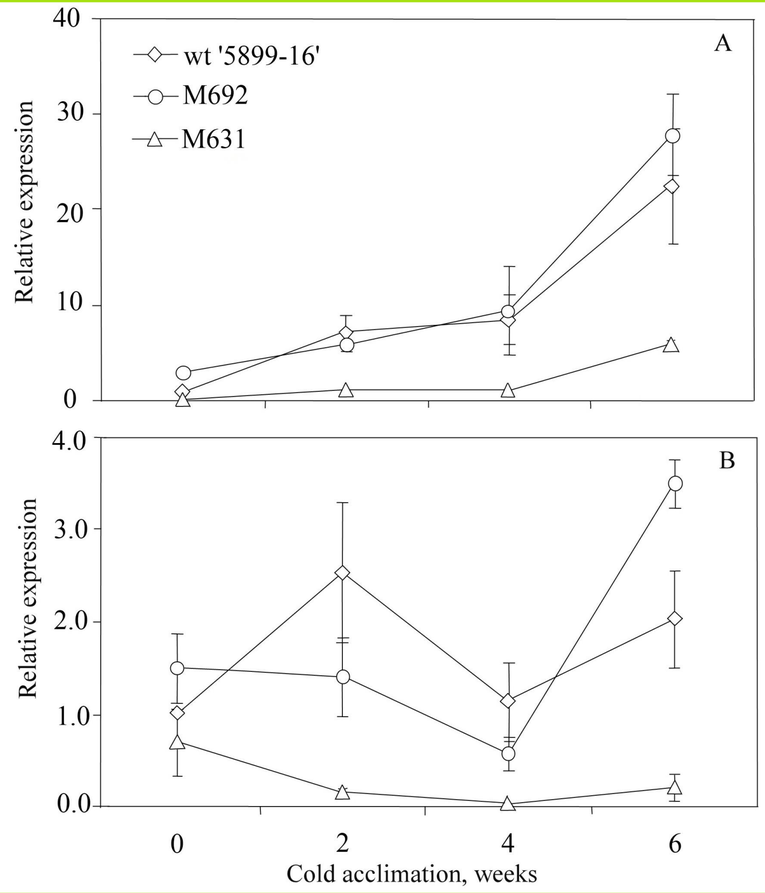


Figure 4. Relative expression of *Ss1* gene during cold acclimation in (A) leaves and (B) crowns of wild type genotype '5899-16' and two mutant genotypes M692 and M631 as revealed by qPCR. Total RNA was extracted from seedling leaves acclimated for 0 – 6 weeks at +5 °C. *ADP–ribosylation factor* gene (*Ta2991*) was used as endogenous control.

CONCLUSION

TILLING is a powerful tool for the development of new alleles for cold responsive genes in bread wheat. We have found new allele of *Ss1* gene showing decreased expression during cold-acclimation in both leaves and crowns.

PROSPECTS

Further work will reveal the effect of the mutation on cold tolerance of winter wheat and will enable the assessment of *Ss1* role in cold-acclimation process.