

TRANSCRIPT PROFILING OF THE SUGAR BEET RESPONSE TO LOW TEMPERATURES

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sugar beet, hydroponics, cold stress, adaptation, cDNA-AFLP

Sugar beet (*Beta vulgaris* L.) is an important root crop in Europe. Its area of cultivation is subjected to possible cold or even freezing temperatures when the plantlets are in the early stages of development after emergence. During these cold periods, the sugar beet plantlets commonly experience a temperature differential between the roots and the aerial portion of the plantlets. Even when survive the stress, the cold damages can lead to yield and root quality losses, with consequent decrease of industrial value. For these reasons, cold stress is one of the most industrially relevant for sugar beet.

In the present work, two different experimental systems were set up and compared in a controlled environment simulating the occurrence of low temperatures stress on sugar beet plantlets. Plants were grown in hydroponic culture (22-17°C, 14:10 photoperiod, 100 µE/m²sec. light intensity) for three weeks, after seed emergence. The stress was applied for three hours in the dark to beet leaflets detached from plants and, alternatively, to the whole plant moved to the hydroponic medium pre-equilibrated at the stressing temperature (0°, -2°, -4°C air temperature). Besides, as an "hardening" treatment, control and stressed plants were exposed, during the two nights before the stress, to 5°C for 12 hrs.

The damage was estimated either as plant's survival rate, and as electrolyte leakage of leaf and root tissue. On detached leaves, the damage was 38% at -2°C and 97% at -5°C. In the whole plantlets experiments, the rate of survival ranged from 100% at 0°C down to 1.5% at -4°C. The use of an hydroponic culture system allowed separate studies on the aerial portion and the root system, as they were exposed at different temperatures, and different molecular events are expected to occur in the two cases. Total RNA was extracted from pooled leaves, roots and cotyledons collected from about 20 plants per experiment, after the stress treatments. mRNA was captured by means of paramagnetic beads and used as template for double stranded cDNA synthesis and AFLP analysis. In order to obtain just one TDF (differential transcription fragment) per transcript, the captured cDNA was digested by the 6 bp cutter enzyme *Ase I*, and released from the beads by the frequent cutter enzyme *Taq I*. Several combinations of selective primers were tested and the TDFs separated on sequencing gels were isolated for further analysis.