Improvement of Forage Grass Quality for Future Climate and Agricultural Demands

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Introduction Climate changes, human environmental impact and increased pressure on global agricultural productivity constantly put new demands on high quality, environmental benign forage production. DLF-TRIFOLIUM is focusing on developing next generation forage grasses with significantly improved stress tolerance, disease and insect resistance as well as improved performance under low fertilizer input. In order to meet these challenges we combine traditional breeding with genetic engineering, molecular marker systems, and advanced screenings for salt and drought tolerance and improved nitrogen use efficiency. Fructans are important for the quality of forage grasses, but the fructan content is known to decrease significantly during the growth season. The aim of this study was to analyze genetically engineered high fructan grasses for seasonal variation in fructan levels and to characterize the plants under drought stress.

Materials and methods Transgenic perennial ryegrass (*Lolium perenne*) plants were generated via particle bombardment with plasmids or linear fragments containing coding regions of sucrose: sucrose 1-fructosyltransferase (*Ac1-SST*) and fructan: fructan 6G-fructosyltransferease (*Ac6G-FFT*) genes from onion (*Allium cepa*) driven by the maize Ubiquitin promoter. Transformants showing transgene transcription by Q-RT-PCR were analyzed for water soluble carbohydrate (WSC) content according to Gadegaard *et al*. (2007) over a 4 month period in the greenhouse. Samples were harvested in two fractions: leaves 1-6 cm above soil and leaves above 6 cm; all samples were taken at the same time of the day. For drought tolerance assay selected transgenic and control lines were grown together with three elite varieties in well watered pots in three replicates, before plants were cut down to 6 cm and subjected to twenty days of drought. After re-watering and 30 days of recovery, regrowth was measured as the increase in leaf length.

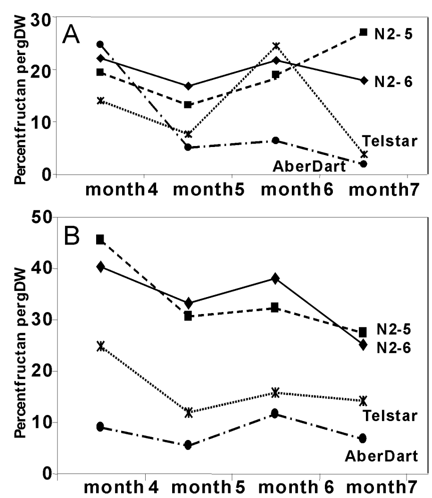

Figure 1 *Fructan levels in two transgenic lines and two commercial varieties over a four month cutting period. A) Grass fraction > 6 cm above soil; B) grass fraction 1-6 cm.*

Results More than 200 individual transgenic lines were produced, which showed transcription of at least one of the two fructan biosynthesis genes *Ac1-SST* and/or *Ac6G-FFT*. These plants were analyzed for WSC content and in total 34 lines were identified, which produced significantly higher fructan levels than the control lines (on average more than 19% /DW). A comparison between two selected lines (N2-5 and N2-6) and two commercial varieties (Telstar and Aberdart) over a 4 month period in the greenhouse demonstrated higher and more stable fructan levels in the transgenic lines (Figure 1). Especially in the lower grass part (1-6 cm) the fructan level was significantly higher in the transgenic lines at all cuts (Figure 1B). High fructan lines subjected to a twenty days drought test in the greenhouse displayed markedly better regrowth than control plants upon rehydration (Figure 2). Only transgenic lines with improved fructan content (min. 19%) showed regrowth, whereas none of the nine control plants or elite varieties recovered.

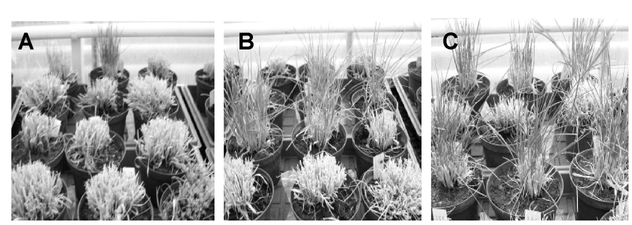

Figure 2 *Drought test of nine perennial ryegrass elite variety plants (A, row 1-3) and 24 high-fructan transgenic lines (A, row 4, B and C, all rows). Plants were subjected to twenty days of drought and rehydrated for 30 days before the assessment of regrowth.*

Conclusions In commercial varieties the fructan content decreased after the first cut, whereas transgenic ryegrass plants expressing fructan biosynthesis genes Ac1-SST and/or Ac6G-FFT in general showed significantly higher fructan content, and the level of fructan remained fairly constant through the growth period. Several high-fructan transgenic lines also showed improved drought tolerance. First results from an ongoing field trial demonstrate that the transgenic lines also accumulate higher fructan levels under field conditions.