Inducible Over-Expression of the CBF3 Abiotic Stress Regulon in Transgenic Bahiagrass (*Paspalum Notatum* Flugge)

V. A. James
University of Florida

F. Altpeter
University of Florida

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Inducible over-expression of the CBF3 abiotic stress regulon in transgenic bahiagrass (*Paspalum notatum* Flugge)

V.A. James and F. Altpeter
University of Florida - IFAS, Agronomy Department, PMCB, Laboratory of Molecular Plant Physiology, 2191 McCarty Hall, P.O. Box 110300, Gainesville FL 32611-0300, USA. Email: faltpeter@mail.ifas.ufl.edu

Keywords: bahiagrass, *Paspalum notatum* (Flugge), genetic transformation, abiotic stress tolerance, CBF3

Introduction Bahiagrass is an important turf and forage grass in the Southern US and in the subtropical regions around the world. The objective of this experiment was to further enhance the productivity and persistence of bahiagrass during seasonal periods of drought and / or freezing and in salt affected regions by over-expression of the stress inducible transcription factor CBF3. Transcription factors like CBF3 are capable of activating the expression of multiple genes involved in protection against environmental stresses (Kasuga *et al*., 1999).

Materials and methods The CBF3 gene, HVA1 or Dhn8 promoter candidates were isolated from genomic wild or cultivated barley DNA by PCR. Primers for isolation of target genes were designed according to the published cultivated barley sequences. Plant transformation vectors were constructed on basis of vector pJFnptII (Altpeter *et al*., 2000). Biolistic gene transfer was carried out 6 weeks after initiation of callus cultures from mature seeds. Transgenic plants expressing the selectable *nptII* gene were regenerated on paromomycin containing medium and confirmed with NPT II-ELISA (Agdia) (Altpeter and James, 2004). Transgenic plants over-expressing CBF3 are currently identified by real time RT-PCR and will be subjected to cold stress (-5º C) in a completely randomized block design in a controlled environment chamber. Leaf tissue damage will be visually scored 1 day after cold stress and biomass production will be evaluated four weeks after recovery from cold stress.

Results A DREB1A transcription factor ortholog (CBF3), the dehydrin 8 (Dhn8) and HVA1 promoters were isolated from genomic, wild or cultivated barley. Plant transformation vectors placing CBF3 or GUS under control of the stress-inducible barley HVA1 (Figure 1B) or Dhn8 promoter were constructed and introduced into bahiagrass (Figure 1A). An efficient protocol for gene transfer to bahiagrass was established and supported the stable integration of transcription factor CBF3 and analysis of stress inducible promoter candidates (Figure 1C).

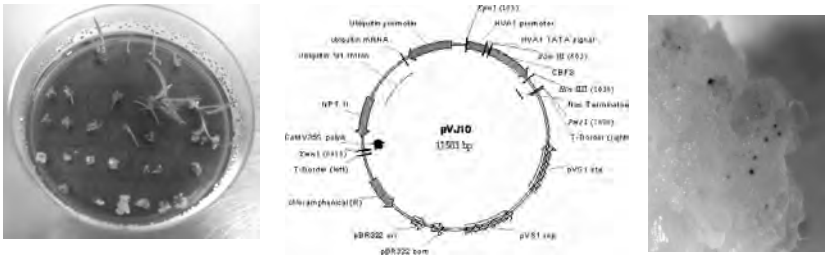

Figure 1 A: Regeneration of bahiagrass transformed with vector pJV10; B: plant transformation vector pVJ10 with CBF3 and selectable marker expression cassettes; C: Cold induced (4ºC for 16 h) Gus reporter gene expression under regulatory control of the HVA1 promoter.

Conclusions An efficient protocol for tissue culture and generation of transgenic plants of the commercially important bahiagrass cultivar 'Argentine' has been developed. The CBF3 transcription factor and promoter candidates for cold, salt and, or drought inducible expression of transgenes were isolated by PCR and subcloned to drive CBF3 transcription factor or GUS reporter gene expression respectively. Dhn8 and HVA1 promoters were able to drive stress inducible reporter gene expression in bahiagrass (Figure 1C). Data correlating CBF3 over-expression in transgenic bahiagrass with freezing stress response is being obtained.

References

Altpeter, F., J. Xu, & S. Ahmed. (2000). Generation of large numbers of independently transformed fertile perennial ryegrass (*Lolium perenne* L.) plants of forage- and turf-type cultivars. Mol. Breeding 6:519-528.

Altpeter, F & V. James (2004) Genetic transformation of turf-type bahiagrass (Paspalum notatum Flugge) by biolistic gene transfer. Intern. Turfgrass Soc. Res. J. (accepted for publication).

Kasuga, M., Q. Liu, S. Miura, K. Yamaguchi-Shinozaki, and K. Shinozaki (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotech. 29:287-291.