Drought-Tolerance in Perennial Ryegrass: A Cisgenic® Approach

Kerry Templeton
Pastoral Genomics, New Zealand

C. Bryant
Pastoral Genomics, New Zealand

M. Biswas
Pastoral Genomics, New Zealand

G. Gill
Pastoral Genomics, New Zealand

S. Bajaj
Pastoral Genomics, New Zealand

See next page for additional authors

The XXI International Grassland Congress / VIII International Rangeland Congress took place in Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

Presenter Information

Kerry Templeton, C. Bryant, M. Biswas, G. Gill, S. Bajaj, P. Sathish, and C. Hanley

Drought-tolerance in perennial ryegrass : a cisgenic® approach

K . Templeton , C . Bryant , M . Biswas , G . Gill , S . Bajaj , P . Sathish , Z . Hanley
Pastoral Genomics , c/o ViaLactia Biosciences , PO Box 109185 , Newmarket , Auckland , New Zealand . E-mail : kerry . templeton@vialactia .com

Key words : water-deficit ,plant transformation ,dehydrin (*Dhn*) promoter ,*vacuolar pyrophosphatase* 1 (*VP*1)

Introduction We employed a SAGE™-protocol using perennial ryegrass tissues sourced from active New Zealand pastures during the peak of autumn ; winter ; spring and summer seasons to understand the seasonal changes in gene expression . Analysis of 14 ,559 redundant SAGE™ tags , which were present more than once in our SAGE™ library , revealed season-specific expression profiles for numerous genes that are likely to be involved in stress-tolerance and plant-growth and development (Sathish *et al* . 2007) . We observed the ryegrass ortholog of Arabidopsis *vacuolar pyrophosphatase* 1 , which has been demonstrated to confer drought-tolerance (Gaxiola *et al* . 2001) , was expressed at least two-fold greater in summer than in other seasons . We have decided to explore the options of enhancing this gene's expression in cisgenic® ryegrass plants to confer drought tolerance .

Materials and methods A total of 159 ,002 non-redundant 14-mer SAGE™ tags were sequenced and mapped to our perennial ryegrass DNA database , comprising methyl-filtered (GeneThresher®) and Expressed Sequence Tag (EST) sequences . We screened for ryegrass genes with high expression in summer and also validated in non-ryegrass systems to confer drought tolerance . The ryegrass ortholog of the Arabidopsis *vacuolar pyrophosphatase* 1 gene met our criteria and we selected perennial ryegrass dehydrin promoter for expression in accordance with our cisgenic® strategy to improve ryegrass . We transformed perennial ryegrass using an *Agrobacterium*-mediated transformation protocol (Bajaj *et al* . 2006) and evaluated the regenerated plants in a controlled environment .

Results The expression levels for *vacuolar pyrophosphatase* 1 in pasture grown perennial ryegrass is presented in Table 1 .

The *vp*1 gene with its 3′UTR intact was cloned downstream of a perennial ryegrass dehydrin (*dhn*) promoter that is induced under drought conditions (data not shown) . A semi-cisgenic® binary vector was constructed to enable *Agrobacterium*-mediated transformation of perennial ryegrass cultivar Tolosa (Bajaj *et al* . 2006) . More than 100 independent transformation events were obtained and randomly selected subset of plants were screened for drought tolerance in New Zealand Controlled Environment Laboratory (NZCEL) , Palmerston North , New Zealand . We monitored the expression of *vp*1 in these plants growing under drought and non-stressed conditions and observed enhanced levels of expression of the introduced cisgenic® *vp*1 in these plants under drought (Figure 1) .

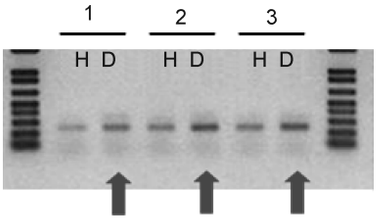

Figure 1 *Enhanced expression of* vp1 *during during drought . H = Hydrated ;D = Drought .* 1 ,2 ,3 = *randomly selected cisgenic® lines .*

Table 1 *Normalised expression level for vacuolar pyrophosphatase* 1 *gene in pasture grown perennial ryegrass (from Sathish et al* ,2007) .

SAGE™ Tag	Annotation	Autumn	Winter	Spring	Summer
		Tag counts per 100 000tags			
ATGACGACGA	*vacuolar pyrophosphatase*1	2	5	7	12

Conclusion Our results , which will be discussed in the poster , indicate it is possible to improve drought tolerance in perennial ryegrass using the cisgenic® approach .

References

Bajaj , S . , Ran , Y . , Phillips , J . , Kularajathevan , G . , Pal , S . , Cohen , D . , Elborough , K . , & Puthigae , S . (2006) A high throughput *Agrobacterium tumefaciens*-mediated transformation method for functional genomics of perennial ryegrass (*Lolium perenne* L .) *Plant Cell Reports* , 25 : 651-659 .

Gaxiola , R .A . , Li , J . , Undurraga , S . , Dang , L .M . , Allen , G J . , Alper , S .L . , & Fink G .R . (2001) Drought-and salt-tolerant plants result from overexpression of the AVP1 H1-pump . *Proceedings of the National Academy of Science , USA* , 98 , 11444 - 11449 .

Sathish , P . , Withana , N . , Biswas , M . , Bryant , C . , Templeton , K . , Al-Wahb , M . , Smith-Espinoza , C . , Roche , J . , Elborough , K .M . , & Phillips , J .R . (2007) : Transcriptome analysis reveals season-specific *rbcS* gene expression profiles in diploid perennial ryegrass *Lolium perenne* .*Plant Biotechnology Journal* 5 :146-161 .