Allele-Specific Differential Gene Expression In Two Barley Cultivars And Reciprocal F1 Hybrids

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SUMMARY

Uncovering the genetic variation underlying the phenotypic diversity in crop plants is a pre-requisite for understanding plant environment interactions and, consequently, is critical for development of novel adapted varieties. While DNA polymorphisms can affect function of a protein by altering amino acid sequence, they can also affect the level of gene expression through mutations in gene regulatory elements (*cis*-regulation) or interacting protein factors (*trans*-regulation). *Cis*- and *trans*-effects on gene expression can be distinguished by studying the expression of gene alleles in F1 hybrids. Affymetrix Barley1 GeneChip was recently used to compare gene expression between six-rowed North American malting barley cultivar Morex and 2-rowed UK cultivar Golden Promise. Expression level polymorphisms were found for ca. 10% of the assayed genes. Quantitative real-time PCR was used to study transcript abundance of 13 of these genes in parental lines and reciprocal F1 crosses in comparable tissues. Statistically significant differences ($p<0.05$) between parental lines were confirmed for 5 genes, while expression of 11 genes was significantly different among parents and reciprocal F1 lines. Various expression patterns were identified which could be explained by *cis*- and *trans*-effects. *Cis*-effects were observed in F1 hybrids as apparently intermediate amount of transcript compared to transcript abundance in parents. The expression of a gene in F1 hybrids at levels comparable to only one of the parents, on the other hand, indicated *trans*-effect. Pyrosequencing analysis of allele-specific transcribed SNPs was used to confirm *cis*-effects in F1 hybrids. *Cis*-regulation appeared to affect the majority of the studied genes, although *trans*-regulation was observed for most of the genes as well.

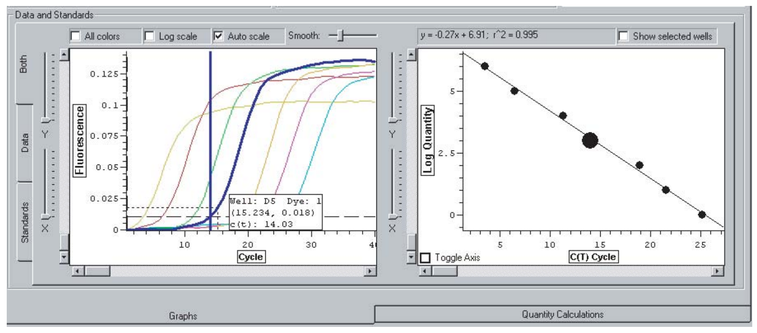
Experimental outline

1. Genes differentially expressed between Golden Promise and Morex in different plant organs were selected using Affymetrix Barley1 GeneChip (Druka et al., unpublished).

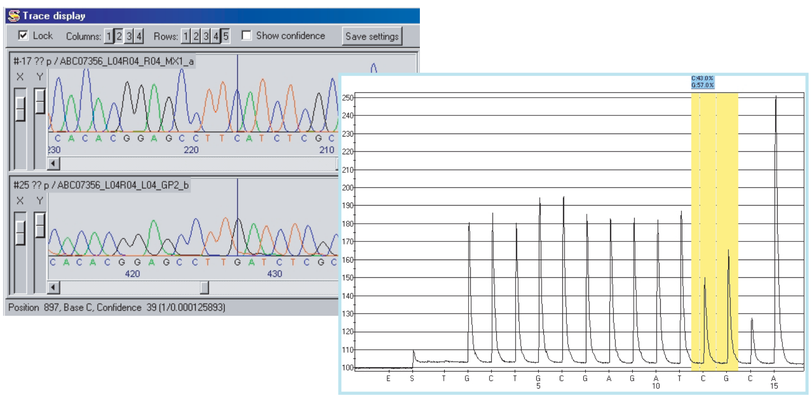
Upregulated genes

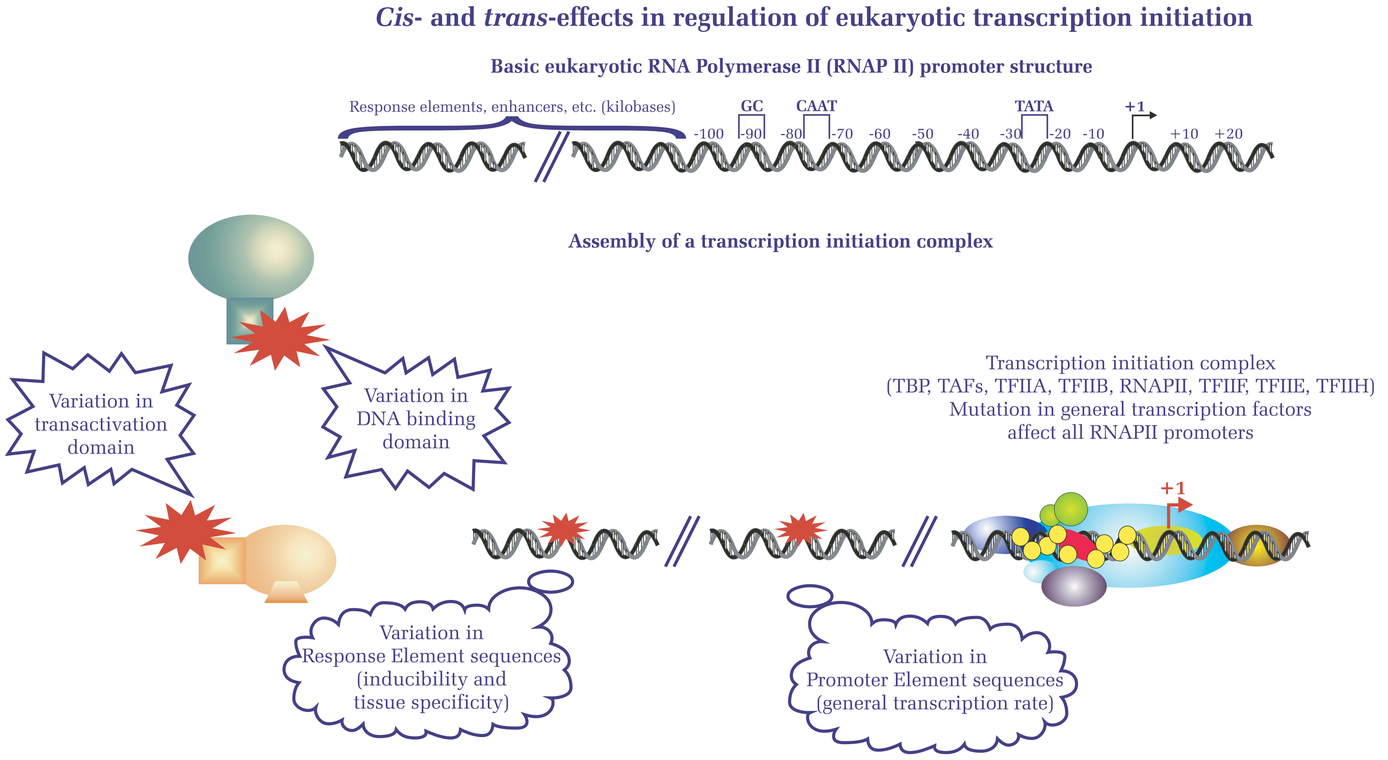
Tissue \ Genotype	GP	MX
Crown	1695	2313
Leaf	2667	831

2. Analyze mRNA abundance of a sub set of genes in parents and reciprocal F1 hybrids using quantitative real-time PCR.



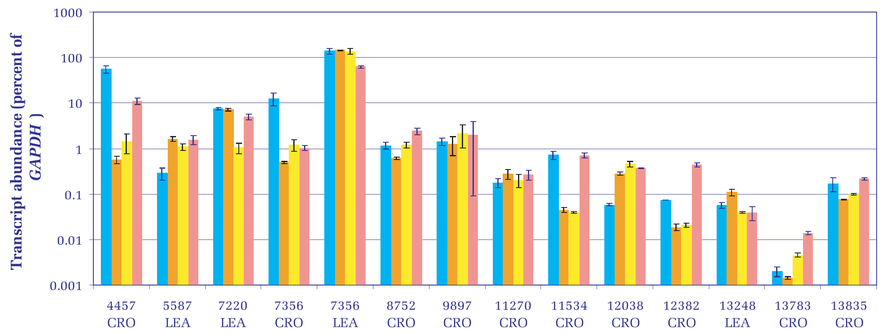
3. Identify SNP polymorphisms in transcribed regions of selected genes. Use SNP as allele-specific markers and pyrosequencing to determine ratios of Golden Promise and Morex alleles.





Probeset	Tissue	GP / MX expression	Annotation	Barley map
Contig4457_at	CRO	+5.6	Similar to protodermal factor-like protein (*Oryza sativa*)	7H bin07
HVSMEf0016E23r2_s_at	LEA	-9.4	Similar to anthranilate N-hydroxycinnamoyl benzoyltransferase-like protein (*Oryza sativa*)	7H bin03
Contig7221_s_at	LEA	+67.4	Similar to ice recrystallization inhibition protein 2 (*Triticum aestivum*)	5H bin13-14
Contig7356_at	CRO	+31.0	*Hordeum vulgare* naringenin-chalcone synthase	2H bin15
Contig7356_at	LEA	+13.7	*Hordeum vulgare* naringenin-chalcone synthase	2H bin15
Contig8752_at	CRO	+5.8	Similar to putative lateral organ boundaries (LOB) domain protein 37 (*Oryza sativa*)	2H bin06-08
Contig9897_at	CRO	+207.0	Similar to putative anthranilate N-benzoyltransferase (*Oryza sativa*)	2H bin02
Contig11270_at	CRO	-6.1	Similar to CCAAT box transcription factor-like protein (*Oryza sativa*)	5H bin11
Contig11534_at	CRO	+7.4	*Hordeum vulgare* root-specific agglutinin isolectin 1 protein	1H bin11-12
Contig12038_at	CRO	-6.6	Similar to putative protein phosphatase-2C (*Arabidopsis*)	5H bin06
Contig12382_at	CRO	+9.3	Similar to putative xylanase inhibitor (*Oryza sativa*)	3H bin14
Contig13248_at	LEA	+6.4	Similar to predicted protein OSJNBa0052O21.15 (*Oryza sativa*)	5H bin05
Contig13783_at	CRO	+19.8	Similar to unknown protein (*Oryza sativa*)	3H bin02
Contig13835_at	CRO	+21.7	Similar to putative Myb-like DNA-binding domain protein (*Oryza sativa*)	5H bin11

Quantitative real-time PCR analysis of gene expression in Golden Promise, Morex and reciprocal F1 hybrids

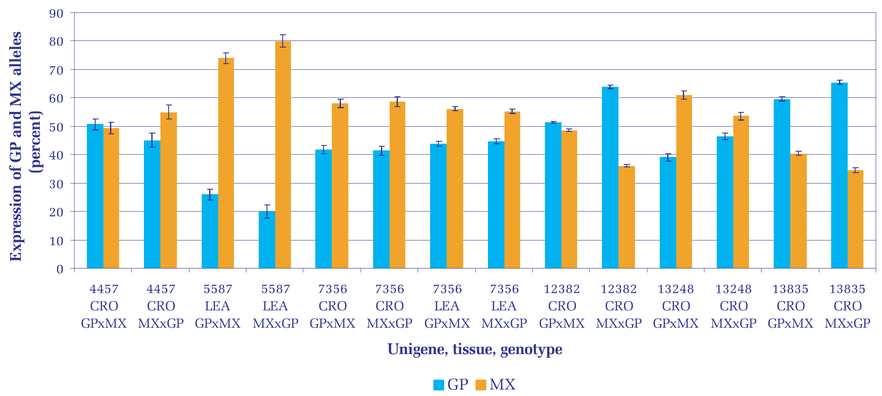


Conclusions

1. Of the 6 genes investigated by pyrosequencing, 5 showed pronounced allele-specific expression differences in F1 hybrids suggesting that variation in *cis*-elements in gene expression regulation is wide-spread in barley.

2. However, even allowing for different sensitivity of detection methods (quantitative real-time PCR and pyrosequencing), the *cis*-effects can not explain the overall variation in gene expression observed between parents and F1 hybrids. Thus, *trans*-effects account for an important source of variation in gene expression in barley.

Quantification of Golden Promise and Morex alleles in reciprocal F1 hybrids by pyrosequencing



Acknowledgments

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