Molecular characterisation of dormancy phase transition in raspberry (*Rubus idaeus* L.) buds

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Introduction

Endodormancy, a temporary arrest of meristem activity in the bud during the cold season, is a critical factor controlling the life cycle of woody perennials in temperate climates. The suppression of growth is controlled from within the dormant bud itself and its release in spring is characterised by a chilling requirement.

Understanding the molecular mechanisms underlying dormancy release will enable the development of markers for the proper timing of rest-breaking practices.
We used a cDNA microarray-based approach to identify genes differentially regulated during the dormancy transition in raspberry.

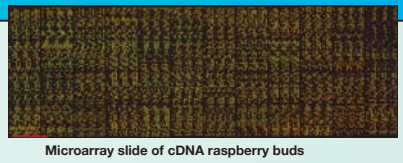
Plant material

Raspberry plants (cv. Glen Ample) were grown in a greenhouse, hardened outdoors for four weeks, and finally moved into cold storage (4°C) for accumulation of chilling. The dormancy status of the buds was determined over a timecourse of 15 weeks by forcing raspberry plants, which had accumulated a defined amount of chilling, in a growth-promoting environment (16°C, 12 h light).

Materials and Methods

Two cDNA libraries were obtained from buds sampled before (endodormant) and after endodormancy release (paradormant) and 2,600 clones from each library were printed onto microarray slides.

cDNA reverse transcribed from bud RNA sampled from cold-stored plants at weekly intervals were sequentially hybridised to the array, resulting in gene expression profiles with a high temporal resolution.



Microarray slide of cDNA raspberry buds

Results

Expression patterns

Differentially regulated transcripts were identified by principal component analysis and classified into 4 expression patterns (ExP) as shown in Fig. 1: The 100 most strongly differentially regulated clones from each category were selected for sequencing.

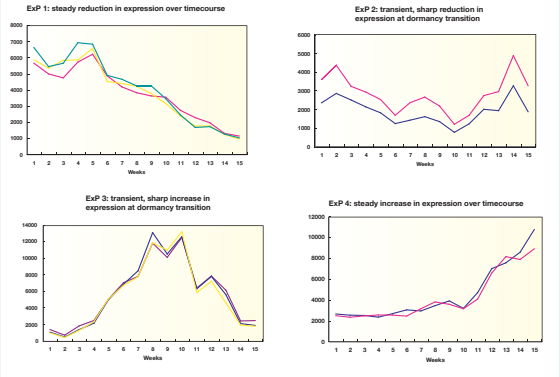


Fig. 1 Expression patterns

Bioinfomatic analyses of differentially regulated cDNAs

The potential functions of differentially regulated clones were analysed by BLAST searches against the NCBI database and the Arabidopsis genome. Clones were then classified into functional groups (Fig. 2) according to the MIPS Functional Catalog database (http://mips.gsf.de/proj/funcatDB/search_main_frame.html) Several genes identified which could be related to meristem dormancy are listed in table 1.

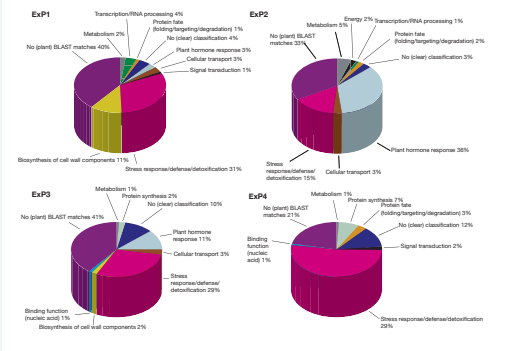


Fig. 2: Genes clustered in functional classes

Gene Name	Gene Family	Expression Pattern	Comments
Dehydrins	Stress-responsive	1	Interdependency of dormancy and freezing tolerance
Heat shock protein 18.2	Stress-responsive	3	Involved in developmental processes (Medina-Escobar et al., 1998)
Cytosolic ascorbate Peroxidase	Stress-responsive	3	Increase in oxidative stress was observed at the release of dormancy (Pacey-Miller et al., 2003)
Methallothionein	Stress-responsive	4	Adaptive response of the plant to environmental stress.
Gibberellic acid-regulated protein (GASA 1)	Hormone-responsive	3	Expressed in meristematic regions (Aubert et al., 1998) during cell division
Abscissic acid-inducible genes	Hormone-responsive	2	Commonly downregulated at the dormancy transition when rates of bud development increase
Polygalacturonase-inhibiting protein	Cell wall metabolism	1	Increase activity of polygalacturonase in loosening of cell wall prior to the resumption of cell division
Starch phosphorylase	Carbohydrate metabolism	2	Accumulation of soluble carbohydrates to resume growth
Translation initiation factors	Protein synthesis	4	Increase in protein synthesis toward the end of the dormancy process
MADS box	Transcription factor	3	Role in developmental processes

Table 1: Selected genes showing differential expression in raspberry buds during dormancy phase transitions

Conclusions and Future Work

- Raspberry plant represents a valid model system for studying dormancy in woody perennials.
- Using a microarray technology, several genes belonging to different families have been highlighted as potentially associated with dormancy phase transitions.
- Microarray analysis provides leads for further research into mechanisms of dormancy control e.g. MADS box, ATPase transporters and genes involved in carbohydrate metabolism.
- Integration with ongoing gene-mapping studies will provide confirmation of gene involvement if they can be shown to map to linkage groups that control bud dormancy-related traits.
- This information will be of practical value in breeding by providing genetic markers for dormancy-related traits.

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