Journal of Experimental Botany, Vol. 58, No. 3, pp. 699–715, 2007
doi:10.1093/jxb/erl245

Journal of Experimental Botany

RESEARCH PAPER

Transcript profiles at different growth stages and tap-root zones identify correlated developmental and metabolic pathways of sugar beet

Diana Bellin[1], Britta Schulz[2], Thomas Rosleff Soerensen[1,3], Francesco Salamini[1,4] and Katharina Schneider[1,5,*]

[1] *Max-Planck-Institute for Plant Breeding Research, Department of Plant Breeding and Yield Physiology, Carl-von-Linné Weg 10, D-50827 Köln, Germany*

[2] *KWS SAAT AG, Grimsehlstrasse 31, D-37555 Einbeck, Germany*

[3] *Institute of Genome Research, University of Bielefeld, D-33594 Bielefeld, Germany*

[4] *Fondazione Parco Tecnologico Padano, Via Albert Einstein c/o Polo Universitario, Cascina Codazza, I-26900 Lodi (Lo), Italy*

[5] *GSF – National Research Center for Environment and Health, Institute for Radiation Protection, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany*

Received 4 May 2006; Revised 24 October 2006; Accepted 25 October 2006

Abstract

Field-grown sugar beets were analysed for morphological characters, sucrose content, and reproducible transcript profiles by macroarray analyses with 11 520 unique sugar-beet cDNA targets in two different years. Seasonal differences were partly compensated by expressing sampling dates as thermal time. During early beet development the number of cambial rings, root length, and sucrose concentration had already achieved >40% of their final values. Sucrose levels rose from 10% to 17% over the thermal time of 1300–1400 °Cd with only small changes later when lower concentrations were restricted to the exterior zone at the minimum of the spatial sucrose gradient through the beet. The number of leaves and root diameter followed the same temporal growth pattern, but mass increased until beet maturity at around 2000 °Cd. Cluster analysis identified 543 transcripts with reproducible preferential expression between 1300–1400 °Cd, and 170 showing the highest transcript levels later. In maturing beets, 373 transcripts were over-represented in the inner zone and 148 in the outer zone. During early development, genes involved in cytoskeletal reorganization and transport processes showed the highest transcript levels. Cell wall biogenesis-, defence-, stress-, and degradation-related transcripts were identified in all samples, and associated with pathogen attack during late development and in the outer zone. Candidates with potential roles in carbohydrate metabolism appeared to serve anaplerotic functions by converting excess intermediates to sucrose production. Transcripts preferentially occurring in sucrose-accumulating young beet cells and newly generated peripheral cells of mature beets are discussed as potential breeding targets to improve sink strength and growth.

Key words: Candidate genes, macroarray analysis, sucrose accumulation, sugar beet, tap root formation.

Introduction

During the first year of its biennial life cycle, sugar beet (*Beta vulgaris* L.) develops a sucrose-rich tap root composed of 90% root tissue and 10% hypocotyl tissue (Elliott and Weston, 1993). The root contains up to 20% sucrose per fresh weight at maturity. Sugar beet breeding aims towards increasing the extractable sucrose and to lower the concentration of nitrate, potassium, and sodium

* To whom correspondence should be addressed. E-mail: katharina.schneider@gsf.de

which inhibit sucrose purification, as well as to increase resistance to biotic or abiotic stress factors.

After germination, dry biomass of sugar beet leaves exceeds that of roots (Elliott and Weston, 1993), but at the 8–10 leaf stage, leaves and roots start growing simultaneously, and, later on, roots take up the major part of dry weight. Sucrose accumulates during growth in the absence of a ripening phenomenon (Milford, 1973). The morphology of the tap root is conditioned by a central cambium produced between primary xylem and phloem, and by 12–15 supernumerary secondary cambium rings initiated centrifugally. During the first 8 weeks after emergence, eight cambium rings are laid down. Only four or five more rings are formed during later development when the focus is on rapid cell expansion and maximum cell division of cambial cells and their derivatives. Within a vascular ring, xylem and phloem are organized in bundles and separated by parenchymatous rays. The innermost six rings account for about 75% of the tap root; the rings outside contribute progressively less to the radial expansion. The most expanded sector of the beet is characterized by the highest cambial activity. The transport and distribution of sucrose follow the same pattern (Elliott and Weston, 1993). A correlation between cell size and sucrose concentration exists for cells up to $10–15\times10^{-8}$ cm^3, but in larger cells sucrose concentration decreases. There is evidence of apoplastic sucrose unloading from the phloem elements, followed by sucrose cleavage involving sucrose synthases and invertases, and by resynthesis of sucrose in storage cells (Fieuw and Willenbrink, 1990). Finally, sucrose is stored in the vacuoles of parenchymatous cells, and a proton–sucrose antiport system is involved in this process (Willenbrink *et al*., 1984).

Concerning all phenomena observed, the underlying molecular pathways and the genes involved have not yet been described. To enable a molecular genetic approach targeted to candidate genes with functions in sugar beet development and sucrose accumulation, a comprehensive unigene cDNA collection covering at least 40% of the sugar beet transcriptome is now available (Herwig *et al*., 2002). In addition, protocols for macroarray analysis of field-grown sugar beets, which allow transcripts to be detected in quantities as small as 0.01% of the poly(A)$^+$ RNA used for probe synthesis (Bellin *et al*., 2002), have been established. With this technique, signal intensities from spots of the same cDNA on different filters varied <2-fold for about 90% of all cDNAs tested, and the variability within one filter was just about 1% (Bellin *et al*., 2002). Here, data sets on morphology and sucrose accumulation are presented for the same field-grown sugar beet hybrid samples for which transcript profiling by macroarray analysis has been conducted. In this paper the focus is (i) on beets of six to seven developmental stages from a time-course study carried out in two consecutive years, and (ii) on two separate regions of maturing beets which are characterized by different sucrose concentrations. For beet development, two expression profile gene clusters with increased expression at the early or the late stage, respectively, were identified. Correlations between the data sets depicting the temporal and spatial sucrose gradients in beet identify molecular pathways and the underlying genes which may play a role in sugar beet development and which are potential targets for sugar-beet breeding.

Materials and methods

Plant material, cultivation, and harvesting of samples

Seeds of the sugar beet hybrid KWS86203 (KWS SAAT AG, Einbeck, Germany) were sown in field plots at the Max Planck Institute for Plant Breeding Research in Cologne (Germany) on 2 May 2001 and 4 April 2002. Plants within one row were 21 cm apart, and the distance between rows was 21 cm. Cultivation followed local agricultural practice.

Plants were harvested at seven time points each in two consecutive years. To account for seasonal differences the thermal time was calculated in growing degree days (°Cd) according to the formula thermal time=[(high+low temperature)/2]–T_0 (Thornley and Johnson, 1990) using temperatures measured at Cologne airport and T_0=6 °C. In the first year, 44 d after sowing (DAS) corresponded to 452 °Cd, 60 DAS to 657 °Cd, 76 DAS to 877 °Cd, 97 DAS to 1201 °Cd, 111 DAS to 1420 °Cd, 131 DAS to 1668 °Cd, and 168 DAS to 2019 °Cd. In the second year, sugar beet plants were harvested 62 DAS (514 °Cd), 82 DAS (807 °Cd), 99 DAS (996 °Cd), 120 DAS (1293 °Cd), 141 DAS (1599 °Cd), 166 DAS (1916 °Cd), and 188 DAS (2072 °Cd). At 1916 °Cd in the second year, additional beet material was harvested from the central 5-cm-wide region containing approximately the four innermost cambial rings and the most exterior non-green zone of 1 cm thickness excluding root tissue of at least three intermediate cambial rings.

Determination of morphological characters and sucrose content

At each time point of the kinetic study, 3–10 representative plants were harvested to measure beet diameter and length, to count the number of leaves and beet cambium rings in the region of the largest diameter, and to determine their mass. To estimate sucrose content, freeze-dried beet tissue was extracted, and sucrose was enzymatically determined (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany). For each sample, sucrose measurements were performed in triplicate.

Sequence analysis

A total of 11 520 sugar beet cDNA clones selected by oligo fingerprinting (Herwig *et al*., 2002) were sequenced. EST sequences are accessible at EMBL/GenBank under the accession numbers BQ605167, BQ582276–BQ595857, and CF542669–CF543782. Trace files were reprocessed by the base calling program Phred (Ewing and Green, 1998; Ewing *et al*., 1998). Vector sequences were clipped from the raw EST sequences with Crossmatch (http://bozeman.mbt.washington.edu/phrap.docs/phrap.html). High quality sequences were obtained by using a sliding window approach using at least 50 bp with a minimal average Phred score of 20 and filtering out slippage (Telles and da Silva, 2001). The trimmed sequences were compared with cDNA sequences from *Arabidopsis thaliana*

available from TIGR (ftp://ftp.tigr.org/pub/data/a_thaliana/ath1/SEQUENCES/ATH1.cdna, version 5.0) using NCBI-TBLASTX and to the non-redundant protein database from NCBI (ftp://ftp.ncbi.nlm.nih.gov/blast/db/nr, version 2005-01-13) using NCBI-BLASTX.

Macroarray generation and hybridization experiments

For macroarray generation, 11 520 unique sugar beet cDNAs were PCR-amplified and printed by the robotic system MicroGridII (Biorobotics, Carsen group, Ontario, Canada) onto 22 cm×22 cm nylon filters (Hybond N^+; Amersham Biosciences, Heidelberg, Germany). Based on the observation that PCR products had an average concentration of 50 ng μl^{-1}, *c*. 8 ng of DNA was immobilized per spot using pins 400 μm in size in eight repeated spotting steps. The printing pattern consisted of blocks comprising 4×4 spots with eight pairs of two identical spots each, and 384 blocks maximally containing eight different 384-well plates in duplicate constituted one sector. One 22 cm×22 cm filter accommodated six sectors with 23 800 spots including controls and additional empty positions in each of the six sectors for local adjustment of expression values (details available upon request). As controls for unspecific hybridization, vector sequences as well as the insert of pAW109 (derived from GeneAmplimer® pAW 109 RNA; Applied Biosystems, Weiterstadt, Germany) were spotted (Bellin *et al*., 2002). For sensitivity assessment, 0.8–32 ng of non-plant sequences such as a part of the human nebulin gene (Desprez *et al*., 1998) were immobilized, and the nebulin fragment was also used at a concentration of 50 ng μl^{-1} for local normalization of signal intensities.

For probe preparation, RNA was extracted from pooled tissue of three representative beets. For probe synthesis, poly(A)$^+$ RNA was purified from total RNA using the mRNA purification kit from Amersham Biosciences (Heidelberg, Germany), according to the instructions of the manufacturer. To confirm proportionality between signal intensity and the amount of labelled transcript, the *in vitro* transcript of the nebulin control was added to each probe at 0.01% which was previously established as a spiked control (Bernard *et al*., 1996) for normalization purposes. Probe synthesis, mock hybridization, oligohybridization, and complex probe hybridization were carried out as described in Bellin *et al*. (2002), with the exception that the DNA was precipitated with 5 μl of 3 M Na-acetate pH 5.3, 5 μl of 10 mg ml^{-1} yeast tRNA (Invitrogen, Groningen, The Netherlands), and 60 μl isopropanol for 1 h at –20 °C to remove unincorporated nucleotides. After centrifugation for 30 min at room temperature, the pellet was resuspended in 100 μl H_2O.

In total, 35 filters were used in one to four consecutive hybridization experiments.

Macroarray data analysis, statistics, and cluster formation

Hybridization signals were acquired by a phosphorimager (STORM; GE Healthcare, Heidelberg, Germany), quantified by the program Arrayvision (Imaging Research Inc., Haverhill, UK) and corrected by subtracting the local background. Based on the concept of spiked controls (Bernard *et al*., 1996), the median signal intensity of 96 spotted nebulin transcripts present in each of the six sectors of each filter was used as the normalization standard for the respective sector. The procedure of value normalization (Bellin *et al*., 2002) was automated by developing Excel macros.

Data analysis was based on four different hybridizations. As cDNAs were spotted in duplicate on each filter, eight independent data points per cDNA entered the evaluation in total. The small-sample procedure of the ArrayStat program package (Imaging Research Inc.) was selected to estimate the random error and to exclude outliers because of statistical reasons. Samples for which fewer than six reproducible values were left after outlier detection were excluded from the following analysis. An *F*-test in the same program was used to evaluate the significance of the differential expression during the time-course experiment.

To cluster profiles of differentially expressed transcripts the partition clustering algorithm *k*-means in the Genesis software (Sturn *et al*., 2002) was used for data of both years separately. In this procedure, *k* values from 2 to 15 were selected. Only expression values of transcripts with complete data sets for all time points evaluated were $\log_2$-transformed according to the Genesis software requirements, and included in the analysis. To avoid a bias by the very first, small sample at 452 °Cd and 514 °Cd in the first and second years, respectively, which revealed the highest variability between years, expression data sets only included samples collected later. The distance between expression patterns was measured using the Pearson correlation, and the median centre function was applied to highlight differential expression during the time course. For the data sets of either year, reproducible clusters were obtained with $k=2$ generating two clusters, cl1 and cl2. Best congruence was found between cluster members of either cl1 or cl2 in both years, and transcripts identified in the same cluster in the two years were selected as candidates. For each cluster member, the highest average expression value obtained at the time point of its strongest expression is referred to as the absolute value. The ratio of the highest and the lowest expression value within the time-course is the relative value which reflects the changes in abundance of the steady-state transcript levels during the time-course.

For the topological analysis, expression values for the inner and the outer beet zone were derived from four independent hybridizations to the same type of filters described above. Data of these eight technical replications were used to calculate average values for each tissue. Outliers were then detected by the ArrayStat program package (Imaging Research Inc.) using the pooled-curve fit procedure. Transcripts showing fewer than six reproducible values were eliminated for statistical reasons. Average values were tested for significant differences between the inner and outer beet using the *z*-test without threshold values. Finally, the candidates were ranked according to the ratios calculated from their expression values of the two beet regions.

Semi-quantitative RT-PCR analysis

First-strand cDNA was synthesized from 0.5 μg DNase-treated (Ambion Inc., Huntingdon, UK) poly(A)$^+$ RNA using Superscript II reverse transcriptase (Invitrogen, Groningen, The Netherlands). Equal amounts of cDNA samples were estimated based on housekeeping genes, and the transcript pattern for the 60S ribosomal protein (accession number BQ488925) is given as an example in Fig. 2. Information on sequence-specific primers, PCR conditions, and expected and obtained fragment sizes is listed in Supplementary data (see Supplementary Table 1 at *JXB* online). For each transcript, the number of cycles providing the most dynamic range of the transcript profile was experimentally determined. RT-PCR products were separated in 1.5% agarose gels, scanned using the same phosphorimager as for the macroarray data acquisition, and densitometrically analysed using ImageQuant software (GE Healthcare, Heidelberg, Germany). For each transcript, all densitometric values were summed up over the annual time-course, and the amount of transcript in an individual sample was expressed as a percentage of the total value of expression.

Results

Morphological and biochemical characterization of root development and sucrose accumulation

Sugar-beet development was analysed in field plots in two consecutive seasons. As temperature is a main factor determining seasonal effects on plant growth, cumulative

thermal time (Thornley and Johnson, 1990) was calculated for each sample. The concept of summing up temperature over time is based on the observation that plants need certain amounts of warmth to reach a developmental stage. To evaluate sugar-beet development in the two years, morphological as well as biochemical characters were plotted against thermal time, covering the entire growth period from early stages of development starting at 500 °Cd up to beet maturity at a little more than 2000 °Cd (Fig. 1A–F). With the exception of beet mass, the largest increases in measured characters were found during the early stages. The number of cambium rings and sucrose content increased most between 452 °Cd and 1420 °Cd in the first year and 514 °Cd and 1293 °Cd in the second year. The number of cambium rings was already about half of the final number in the first sample, with a maximum of 13 or 14 at the end of the vegetative season. After only 657 °Cd in the first year and 514 °Cd in the second year, the sucrose concentration was 10.2% and 7.5% of the fresh weight, respectively. These values are equal to nearly 60% and 45% of the sucrose percentage at the end of the first and second season, 17.4% and 17.1%, respectively. At 1916 °Cd in the second year, the spatial distribution of sucrose in the beet was analysed. The core of the tap root, which contains fully expanded cambial rings, had a sucrose concentration of 17.2±0.6% while the outermost exterior zone contained 12.4±0.1% sucrose per gram fresh weight.

With the exception of the fourth sample in the first year, root length increased from about 10 cm at the first measurement up to >20 cm at 1420 °Cd and 1293 °Cd in the first and second year and reached maximal length of 23 cm and 26 cm at 1668 °Cd and 1916 °Cd in the two seasons tested.

Leaf number and root diameter also showed the largest increase in the first part of the period monitored in the two years (Fig. 1D, E), but, considering their maximal values, the first measurements at around 500 °Cd were much smaller than the relative values of the previously mentioned traits, indicating a delay and a steep increase during the time monitored. The maximum leaf number of 43 was counted at 1668 °Cd in the first year, and in the second year this was at 1599 °Cd with 46 leaves. Root diameter reached the plateau phase at 1201 °Cd in the first year and, in the second year, a more linear increase in root diameter was observed until 1599 °Cd.

Mass increase appears to be further delayed in both years (Fig. 1F), and especially in the second year, the highest rates of increase were observed between the last time points reaching the maximum mass of about 1.1 kg at 1916 °Cd.

Tap root candidate transcripts differentially expressed during development and across the radial dimension of the beet

Macroarray results were obtained for 11 520 cDNA clones (Table 1A). In the time-course experiment, 3486 transcripts (30.26%) showed differential expression in the first year and 2052 (17.81%) in the second year. No differential expression was recorded for 7692 and 9076 transcripts, respectively. Data on 342 and 392 transcripts had to be discarded for statistical reasons in the two experiments.

Macroarray data analyses were performed for each year separately. Based on transcripts with complete sets of expression values, cluster analysis revealed two groups of differentially transcribed genes in both years. Transcripts of the first cluster, cl1, showed a higher expression during the first part of the development while transcripts of the second cluster, cl2, were characterized by higher expression values at the end of the season (Fig. 1G, H). The transition between high and low expression levels of cl1 genes was found at around 1420 °Cd in the first year and between 1293 °Cd and 1599 °Cd in the second year. Complementarily, the transition from low to high levels of cl2 transcripts occurred at around 1420 °Cd in the first year and between 1293 °Cd and 1599 °Cd in the second year. These time points, at which the expression profiles reverse, mark the transition from the early phase of rapid increase in most traits, apart from mass, to the plateau phase in which only minor changes in trait values were observed (Fig. 1A–F).

Crosswise comparisons between cl1 and cl2 members in both years revealed that 80.9% of cl1 transcripts in the first year were identical to 95% of the cl1 transcripts in the second. Concerning cl2, 91% of the transcripts identified in the first year were in common with 62% of the transcripts identified in the second. Considering both years, 543 putative genes revealed a reproducible preferential transcription during early stages of development, and 170 putative genes during late developmental stages (Table 1B). Profiles of selected transcripts were validated by semi-quantitative RT-PCR as shown for a representative set of six transcripts (Fig. 2).

The comparison of the two beet zones with differing sucrose content identified 373 transcripts with a higher expression in the inner beet region and 148 in the outer (Table 1B).

Classification of differentially expressed genes deduced from the time-course experiment

For 366 transcripts of cluster 1 and 102 transcripts of cluster 2, sequence analysis retrieved annotations with expected values below e^{-20} (Table 1B; see Supplementary Table 2 at *JXB* online). These transcripts were manually assigned to functional gene classes. The 40 transcripts with the highest absolute and relative expression (or both) are presented for both clusters (Table 2), with relative expression providing the factor by which transcript levels increase or decrease during the time-course. In cl1, 13 were among the 40 most expressed at both the absolute and relative scale, while in cl2, 16 of such transcripts were identified.

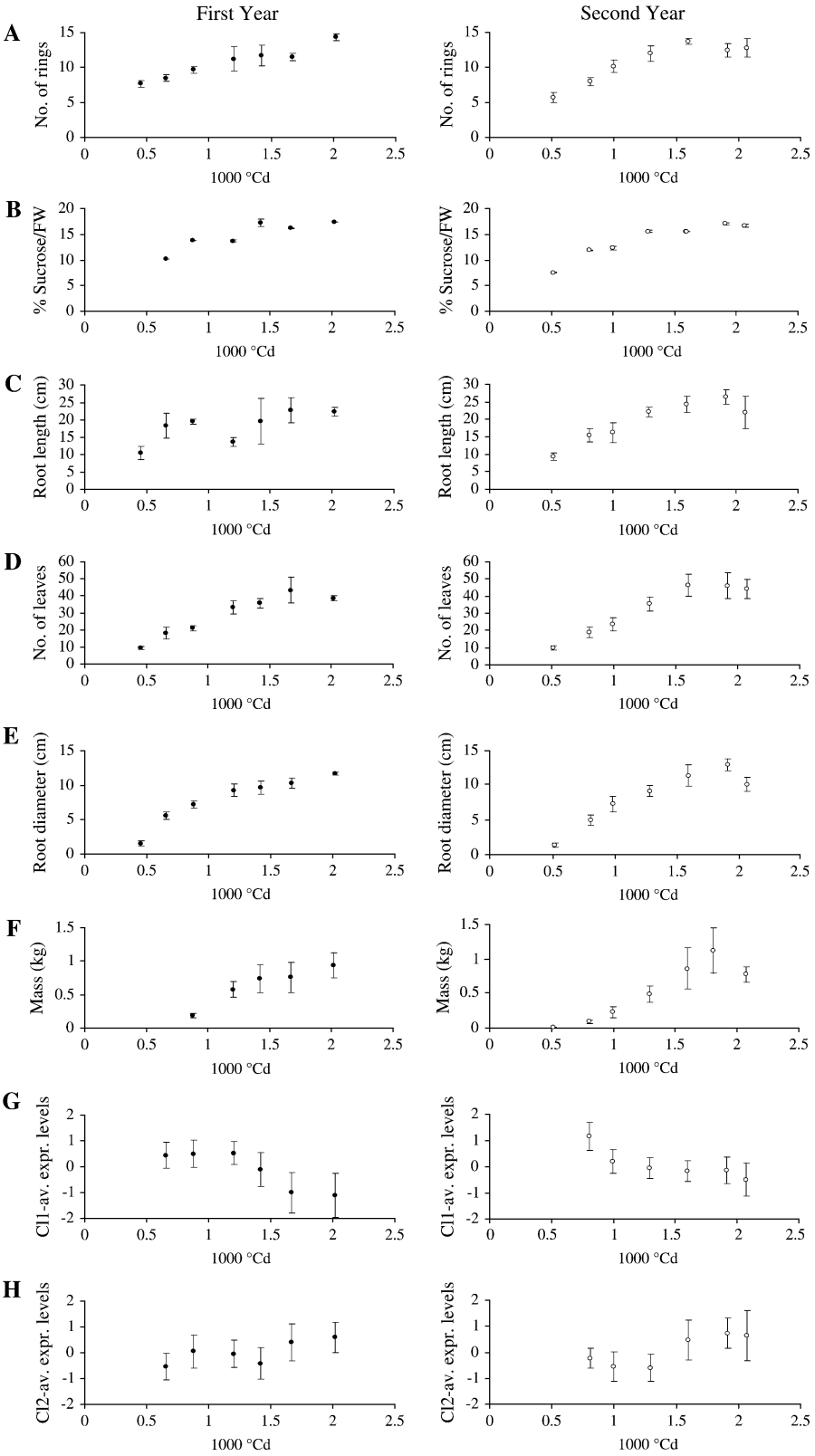

Fig. 1. Growth characters, sucrose accumulation, and expression profiles of transcript clusters during sugar beet development. Average values and standard deviations determined for (A) number of cambial rings, (B) sucrose content, (C) root length, (D) number of leaves, (E) root diameter, (F) beet mass, (G) $\log_2$-values of cluster 1 transcript levels, and (H) $\log_2$-values of cluster 2 transcript levels at seven time points expressed as thermal time (°Cd) in two years. Clusters 1 and 2 comprise 543 and 170 transcripts, respectively. Filled symbols represent values from the first year, open symbols those from the second year.

Table 1. *Statistics on macroarray analysis performed with sugar beet samples from a time-course experiment and a topological dissection of the tap root*

(A) *Summary of data analyses: numbers of clones are reported with the respective percentages in parentheses*

	Time-course, 1st year	Time-course, 2nd year	Beet topology
Total numbers of cDNA clones	11 520 (100%)	11 520 (100%)	11 520 (100%)
Differential expression	3 486 (30.26%)	2 052 (17.81%)	521 (4.52%)
No differential expression	7 692 (66.77%)	9 076 (78.78%)	8 746 (75.92%)
Discarded for statistical reasons	342 (2.97%)	392 (3.40%)	2 253 (19.56%)

(B) *Classes of preferentially expressed transcripts*

	Cluster 1 of time course	Cluster 2 of time course	Inner beet	Outer beet
Number of differentially expressed transcripts (annotation with e-value $<e^{-20}$)	543 (366)	170 (102)	373 (240)	148 (97)

Differentially expressed genes with strong absolute or relative transcription during early development are predicted to contribute to high activity of cytoskeletal reorganization, transport processes, cell wall biogenesis, and defence responses

Among cluster 1 transcripts (Table 2A), 11 gene products are present which function in cytoskeletal reorganization, the secretory pathway, and vesicular trafficking, a group of transcripts absent from cluster 2. Encoded gene products such as tubulins and a KNOLLE-related putative syntaxin (Müller *et al*., 2003) play essential roles during cell division and cytokinesis. A predicted signal recognition particle receptor (Pool *et al*., 2002), as well as SEC14 family members (Bankaitis *et al*., 1989) and two C2 domain-containing proteins with similarity to SRC2 (Oufattole *et al*., 2005), are associated with synthesis and export of secretory proteins.

A second group of transcripts over-represented in cl1 are putatively involved in transport processes with a predominant group of major intrinsic proteins which are part of the transport systems for water and small non-electrolytes (Johansson *et al*., 2000).

A group of transcripts abundantly expressed during early, but also late development is predicted to function in cell wall biogenesis and modification. In the first phase of development, among others transcripts for glycoside hydrolase, xyloglucan:xyloglucosyl transferase and expansin proteins were identified which are potentially involved in the reorganization of pectins, components of the primary cell wall (Xu *et al*., 2004; Cosgrove, 2005; http://afmb.cnrs-mrs.fr/CAZY/).

Activities in stress defence and pathogenesis response are indicated by the transcription of a major latex protein (Nessler *et al*., 1985), an osmotin- and a thaumatin-like protein, as well as Bet v I allergen. Preferential transcription of gene products such as an aspartyl protease, a nucellin member (Chen and Foolad, 1997), and two transcripts with deduced U-box domains characteristic of the ubiquitination machinery (Azevedo *et al*., 2001) suggest high protein degradation.

Among gene products involved in signal transduction, five transcripts for phosphate-responsive proteins were preferentially expressed in the early phase, and a similar gene product, EXO, has been associated with cell division in meristem development (Farrar *et al*., 2003).

Metabolism-related transcripts were represented by three which encode mutase family proteins catalysing the formation of C–P bonds in phosphonates (Anzai *et al*., 1987). Analogues are known from carnation, but their functions are as yet unclear (Wang *et al*., 1993). These transcripts also contain a signature of isocitrate lyase, an enzyme of the glyoxylate cycle. The transcript for isocitrate lyase correlated with seedling vigour in sugar beet (de los Reyes *et al*., 2003). In the study cited, germination-related transcripts participate in lipid metabolism. Also cluster 1 contains eight transcripts expressed at a relatively low level for enzymes predicted to be involved in fatty acid oxidation (see Supplementary Table 2 at *JXB* online). Additionally, the presence of three different transcripts for adenosylhomocysteinase, and 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase indicates the relevance of methionine biosynthesis in this developmental period. The amino acid regulates one-carbon metabolism and methylation capacity (Mudd and Datko, 1990; Ranocha *et al*., 2001; Kocsis *et al*., 2003). The importance of one-carbon metabolism is further supported by the high expression of the transcript encoding GTP cyclohydrolase, a key enzyme in the synthesis of the C1-unit transferring cofactor folate (Hanson and Gregory, 2002).

Preferential expression of genes during late development indicates activity in protein biosynthesis, plant growth factor metabolism, cell wall biogenesis, and defence responses

Transcripts for ribosomal proteins and translation initiation factors associated with ribosome and protein biosynthesis

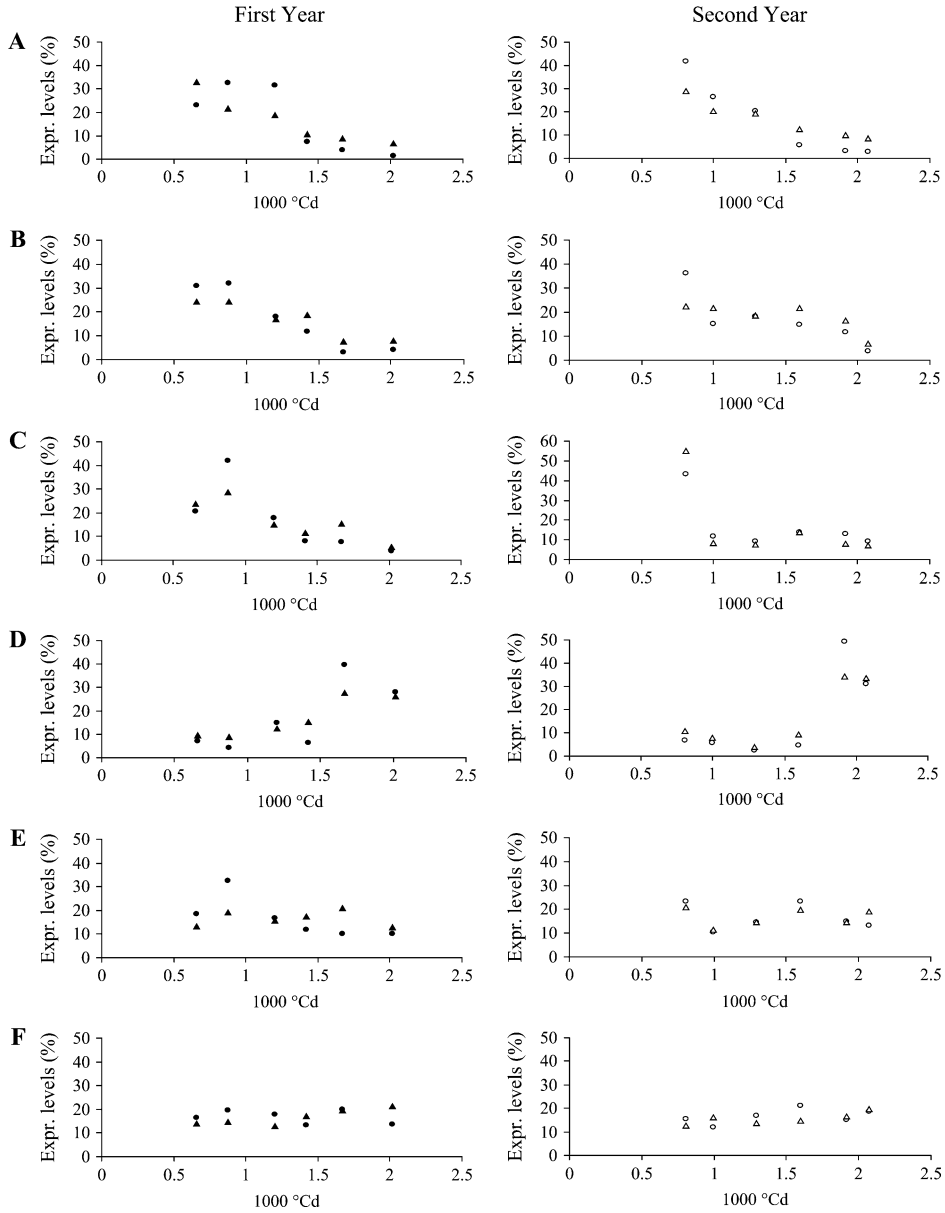

Fig. 2. Validation of macroarray expression values by semi-quantitative RT-PCR analysis for selected transcripts in the time-course experiment. Densitometric values of agarose gel-separated semi-quantitative RT-PCR products (triangles) and macroarray expression values (circles) were expressed as a percentage of the sum of all values in the annual time-course for a single transcript obtained with the respective technique. Cluster 1 transcripts for (A) gibberellin-responsive protein (BQ586825), (B) putative carboxyphosphonoenolpyruvate mutase (BQ588168), (C) sucrose synthase 2 (BQ490013), (D) the cluster 2 transcript for glycosyl hydrolase family 17 protein similar to beta-1, 3-glucanase (BQ591809), (E) the transcript encoding sucrose synthase 1, SBSS1 (X81974/BQ490130/BQ594479), and (F) the transcript for 60S ribosomal protein (BQ488925, control for equal amounts of cDNA in each sample). In all samples RNA populations used as templates for RT-PCR analysis were the same as those used for macroarray probe preparations. Filled symbols represent values from the first year, open symbols those from the second year.

were over-represented in cl2 (Table 2B). Also genes involved in plant growth-factor regulation such as gibberellin 20-oxidase (Fleet and Sun, 2005), zeaxanthin epoxidase catalysing a crucial step in ABA synthesis (Seo and Koshiba, 2002), and two ethylene-responsive transcriptional coactivators belong to the over-represented group.

Transcripts for gene products related to signal transduction, defence, stress, protein degradation, and cell-wall biogenesis were of similar abundance to cl1, even if the composition of the group was different. During late development, transcription of a presumed vacuolar processing enzyme, a cysteine proteinase known to be induced in senescing organs associated with programmed cell death (Rojo *et al*., 2004; Hara-Nishimura *et al*., 2005; Thompson and Vierstra, 2005), showed abundant expression. The same pattern was evident for a transcript encoding glutamate-cysteine ligase/gamma-glutamylcysteine synthetase which catalyses a rate-limiting step in the

Table 2. *Specification of the 40 transcripts with the highest absolute and relative expression values in (A) cluster 1 with preferential expression during early development, and (B) cluster 2 with preferential expression during late development*

In total 67 transcripts are presented for cluster 1 including 13 which belong to both the lists of the 40 transcripts with the highest absolute and relative expression (values in bold). For cluster 2, 64 transcripts are listed of which 16 belong to both the lists of the 40 transcripts with highest absolute and relative expression.
Within (A) and (B) the left part of the table is continued at the top of the right part.

(A) ***Cluster 1***

Absolute expression	Relative expression	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*
Not classified			
8.12	3.21	BQ586165	Expressed protein
45.38	**14.68**	BQ593946	Expressed protein
9.55	**21.11**	BQ587957	Nodulin MtN21 family protein
0.66	**16.63**	CF543143	Expressed protein
Gene products related to chloroplast or photosynthetic activity			
20.16	4.84	BQ585096	Thiazole biosynthetic enzyme, chloroplast
Signal transduction/DNA binding/protein modification			
27.41	6.87	BQ595587	Gravity-responsive protein
15.62	8.75	BQ591137	Phosphate-responsive 1 family protein
10.46	4.16	BQ589842	Phosphate-responsive protein
11.35	**69.30**	BQ593402	Phosphate-responsive protein
2.80	**38.14**	BQ593266	Phosphate-responsive protein
6.96	**15.92**	BQ586757	Phosphate-responsive protein
3.17°	**25.33**	BQ586692	Protein phosphatase 2C, putative
1.91	**13.06**	BQ594833	AP2 domain-containing transcription factor
Biosynthesis of cofactors			
9.42	**33.61**	BQ595003	GTP cyclohydrolase I
Nucleic acid metabolism/nuclear structure			
7.98	3.35	BQ582963	DNA-directed RNA polymerase
11.60	**15.21**	BQ594114	Ribonuclease 1
Protein biosynthesis/ribosomes			
7.82	8.73	BQ585145	Glycyl-tRNA synthetase
Cytoskeleton/secretory pathway/vesicular trafficking			
8.07	4.07	BQ585054	Tubulin alpha-2/alpha-4 chain
20.11	6.33	BQ589560	Tubulin alpha-2/alpha-4 chain
17.32	5.53	BQ595067	Tubulin alpha-2/alpha-4 chain
8.12	3.41	BQ585079	Tubulin beta-2/beta-3 chain
9.01	3.36	BQ592286	Tubulin beta-6 chain
11.11	4.04	BQ586251	SEC14 cytosolic factor family protein
8.30[i]	3.37	BQ590684	SEC14 cytosolic factor family protein
8.91°	6.69	BQ585628	Mutase family protein similar to carboxyvinyl-carboxyphosphonate phosphorylmutase, isocitrate lyase signature
7.43°	9.93	BQ588168	Mutase family protein similar to carboxyvinyl-carboxyphosphonate phosphorylmutase, isocitrate lyase signature
5.63°	**13.08**	BQ588409	Mutase family protein similar to carboxyvinyl-carboxyphosphonate phosphorylmutase, isocitrate lyase signature
Isoprenoid/terpene biosynthesis			
8.28	5.41	BQ583188	3-Hydroxy-3-methylglutaryl-CoA reductase 1
Plant growth factor regulation			
7.83°,[a]	**23.77**	BQ586825	Gibberellin-responsive protein
Cell wall biogenesis			
20.13	**22.22**	BQ588429	Xyloglucan:xyloglucosyl transferase, putative
7.72°	**38.94**	BQ592039	Xyloglucan:xyloglucosyl transferase, putative
8.87	**27.94**	BQ593230	Expansin family protein (EXPL2)
11.34	**64.61**	BQ593271	Expansin, putative (EXP1)
7.32	**22.25**		Glycosyl hydrolase family 1 protein
0.37	**13.67**	BQ591238	SSXT protein-related/glycine-rich protein
1.05	**14.31**	BQ593767	Quercetin 3-*O*-methyltransferase 1
3.71°	**21.49**	CF543708	Glycoside hydrolase family 28 protein
4.59°,[a]	**20.94**	BQ592105	Glycoside hydrolase family 28 protein
4.91°	**14.80**	BQ592288	Glycoside hydrolase family 28 protein
0.74	**18.36**	BQ594298	Invertase/pectin methylesterase inhibitor family protein
0.97	**24.45**	BQ593445	Expansin, putative (EXP10)
Defence/stress/degradation			
13.94	12.56	BQ593025	Aspartyl protease family protein
9.82	8.52	BQ594311	Bet v I allergen family protein
0.87	**13.83**	BQ586560	L-Ascorbate peroxidase 3 (APX3)
0.46	**16.72**	CF542734	Leucine-rich repeat transmembrane protein kinase
1.90°,[a]	**62.03**	BQ595076	Major latex protein-related
3.27	**17.63**	BQ594011	Nucellin protein
2.87°	**14.05**	BQ593801	Osmotin-like protein
3.02	**17.87**	BQ594555	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
1.37°,[a]	**28.21**	BQ593849	Thaumatin-like protein, putative
2.10	**19.74**	BQ589200	U-box domain-containing protein

Table 2. *Continued*

Absolute expression	Relative expression	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*
8.97	2.50	BQ586465	Signal recognition particle receptor alpha subunit family protein
4.68	**22.44**	BQ585515	C2 domain-containing protein /src2-like protein
3.42°	**19.35**	BQ587472	C2 domain-containing protein/src2-like protein
0.56	**15.14**	BQ595440	Syntaxin-related protein KNOLLE
Primary metabolism			
33.27	5.23	BQ593004	Adenosylhomocysteinase
12.82	3.47	BQ591034	Adenosylhomocysteinase
8.65	3.67	BQ585805	Adenosylhomocysteinase
28.14	4.27	BQ585967	5-Methyltetrahydropteroyltriglutamate–homocysteine methyltransferase
(B) *Cluster 2*			
Not classified			
35.29	5.71	BQ590073	Expressed protein
9.05	2.03	BQ586045	Expressed protein
5.29	3.23	BQ585691	Expressed protein
4.20	2.04	BQ583121	Expressed protein
2.98[i]	1.89	BQ583539	Membrane protein
5.23[i]	**7.18**	BQ587220	Expressed protein
1.59	**22.84**	BQ588522	Expressed protein
1.30	**9.09**	BQ595608	Expressed protein
1.02°	**5.77**	BQ585875	Expressed protein
1.96[i]	**7.94**	CF543465	Transmembrane protein, putative
Gene products related to chloroplast or photosynthetic activity			
1.66°	**17.11**	BQ584561	Chlorophyll A-B binding protein 2, chloroplast
1.48	**9.53**	BQ586209	Chlorophyll A-B binding protein 2, chloroplast
0.76	**18.24**	BQ593299	Chlorophyll A-B binding protein CP26, chloroplast
0.93	**6.92**	BQ586323	Chlorophyll A-B binding protein CP26, chloroplast
Signal transduction/DNA binding/protein modification			
4.90	3.03	BQ583122	MADS-box protein (AGL9)
15.57	5.15	BQ584567	DC1 domain-containing protein
14.88	**7.86**	BQ590895	No apical meristem (NAM) family protein
3.24	**6.18**	BQ588708	Zinc finger (CCCH-type) family protein
2.14	**12.61**	BQ591915	WD-40 repeat family protein
0.89	**6.37**	BQ595693	Serine/threonine protein kinase, putative
Biosynthesis of cofactor			
4.55	4.13	BQ591635	Thiamine biosynthesis family protein
Nucleic acid metabolism/nuclear structure			
3.94	3.28	BQ590069	Nucleosome assembly protein (NAP), putative
3.42	2.40	BQ590574	DEAD box RNA helicase (PRH75)
3.56	3.19	BQ589651	RNA recognition motif (RRM)-containing protein
2.78	**23.30**	BQ589446	Regulator of chromosome condensation (RCC1) family protein
3.27	**19.62**	BQ586501	U-box domain-containing protein
Transport			
17.02	11.91	BQ585425	Major intrinsic family protein
10.12	2.71	BQ583065	Plasma membrane intrinsic protein
19.69[i]	2.83	BQ590606	Plasma membrane intrinsic protein
8.84°	**23.43**	BQ588316	Mitochondrial substrate carrier family protein
20.11	**13.34**	BQ594584	Major intrinsic family protein
0.94	**30.39**	BQ593628	Amino acid permease 2 (AAP2)
2.22°,[a]	**18.61**	BQ593958	Delta tonoplast integral protein (delta-TIP)
Protein biosynthesis/ribosomes			
2.04	**16.28**	BQ589465	40S ribosomal protein S6 (RPS6B)
1.18	**6.05**	BQ588552	60S ribosomal protein L30
0.55	**6.52**	BQ585016	Eukaryotic translation initiation factor 3 subunit 8
Plant growth factor regulation			
21.35[i]	5.59	BQ590806	Gibberellin-regulated protein 1 (GASA1)
6.08	5.43	BQ590943	Gibberellin-regulated protein 1 (GASA1)
8.64[i]	**6.00**	BQ590935	Gibberellin-regulated protein 1 (GASA1)
1.00	**6.38**	BQ587858	Zeaxanthin epoxidase (ZEP) (ABA1)
0.53	**10.82**	BQ594558	Ethylene-responsive transcriptional coactivator, putative
0.62	**8.88**	BQ594572	Ethylene-responsive transcriptional coactivator, putative
1.59	**7.46**	BQ588816	Oxidoreductase, 2OG-Fe(II) oxygenase family protein similar to gibberellin 20-oxidase
Cell wall biogenesis			
4.21	3.19	BQ585777	Glycosyl hydrolase family 20 protein
6.21	2.55	BQ583709	Glycosyl hydrolase family 35 protein
12.24	**25.78**	BQ594510	Xyloglucan:xyloglucosyl transferase, putative
5.34	**18.95**	BQ590721	Peroxidase, putative
4.83	**20.68**	BQ590274	Peroxidase, putative
0.76	**6.10**	BQ588372	Hydroxyproline-rich glycoprotein family protein
1.69[i,a]	**20.85**	BQ591809	Glycosyl hydrolase family 17 protein
1.46°,[a]	**13.46**	BQ590768	Glycosyl hydrolase family 18 protein
Defence/stress/degradation			
24.29[i]	5.22	BQ590762	Plant defensin-fusion protein, putative
2.73	3.66	BQ594096	Heat shock protein 70, putative
5.26	3.07	BQ583096	Leucine-rich repeat protein, putative
21.69	**5.84**	BQ585213	Plant defensin-fusion protein, putative
3.80[i]	**35.97**	BQ582658	Osmotin-like protein (OSM34)
3.70	**14.79**	BQ591564	Galactinol synthase, putative
14.37	**7.37**	CF543356	Glutamate–cysteine ligase
19.07	**7.98**	BQ588953	Vacuolar processing enzyme gamma
2.84	**7.34**	BQ585930	Aspartyl protease family protein

Table 2. *Continued*

Absolute expression	Relative expression	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*	Absolute expression	Relative expression	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*
Protein biosynthesis/ribosomes							
7.11[i]	5.71	BQ594109	Ribosomal protein L7Ae	**4.56**	**12.89**	BQ582378	Basic endochitinase
5.52[i]	3.65	BQ593917	Ribosomal protein L7Ae	**3.72**[o]	**23.10**	BQ588674	Basic endochitinase
4.70	2.32	BQ584144	40S ribosomal protein S23	2.37	**6.44**	BQ588071	Osmotin-like protein (OSM34)
4.11	2.77	BQ590090	40S ribosomal protein S4	1.22	**6.63**	BQ588698	FAD-binding domain-containing protein
4.76	2.62	BQ583875	40S ribosomal protein S9	2.00	**7.00**	BQ590418	Aspartyl protease family protein
10.20	2.25	BQ583695	Eukaryotic translation initiation factor SUI1, putative	Transport 0.50	**6.12**	BQ595431	Ammonium transporter 2

[a] Expression values among the top 40 in either the inner or outer zone of the root.
[o,i] Preferential expression in the outer (o) or inner (i) parts, respectively, of the beet at maturity.

biosynthesis of the antioxidant glutathione (Noctor *et al*., 2002). A transcript for a putative galactinol synthase involved in the heat stress-dependent synthesis of the trisaccharide raffinose (Panikulangara *et al*., 2004) showed high absolute and relative expression in cl2.

Four of the most overrepresented transcripts during late development encode chlorophyll A-B binding proteins.

Transcription profile of the inner tap-root zone reflects high protein turnover whereas transcriptional activity in the outer zone is more in line with exposure to pathogens and light

Among preferentially core-expressed transcripts, 240 were annotated with expected values below e^{-20}; among transcripts with preferential expression in the outer beet, 97 fulfilled this criterion (Table 1B). Transcripts with differential expression in the inner and outer beet region were ranked according to their expression value ratios, and the 40 most extreme were grouped according to their predicted functions in Table 3.

Both beet regions shared gene products in most functional categories, but there were a few exceptions. The category of proteolysis including a cullin and an F-box protein, as well as an ubiquitin-conjugating enzyme and an ATP-dependent Clp protease, is unique to the inner zone. The associated protein turnover may be refilling substrate pools required for reorganization of metabolic activity also involving transcription and translation. Transcriptional activity is indicated by the presence of transcription-factor domains such as zinc finger- or MADS-box elements in signal transduction-related gene products preferentially expressed in the inner zone. Transcripts encoding ribosomal proteins were also over-represented at moderate levels (see Supplementary Table 2 at *JXB* online), and a putative transcript for 1-phosphatidylinositol-4-phosphate 5-kinase regulating development of procambial cells (Elge *et al*., 2001) was found preferentially in the inner region.

Among the most prominent gene products in the outer beet region there are some related to chloroplast or photosynthetic activity and plant growth factor regulation. Transcripts encoding gibberellin 2-oxidase and a gibberellin-responsive protein, both members of cluster 1, point to a particular role for this growth factor. Further signal transduction-related transcripts in the outer region comprise a group of phosphatases with functions at the post-translational level.

Transcripts encoding transporters are present in both zones. However, those encoding multi-drug transporters such as MATE efflux (Brown *et al*., 1999; Eckardt, 2001) and ABC1 proteins (Martinoia *et al*., 2002) possibly involved in detoxification, were only found in the external root zone, whereas a sugar transporter was identified in the inner sucrose-rich region.

In both the inner and the outer part of the beet, transcripts involved in cytoskeleton formation, vesicular

Table 3. *Specification of the 40 transcripts with the most preferential expression in inner and outer regions of the mature sugar beet tap root*

Ratio in/out: ratio of average expression values deduced from macroarray analysis.

Preferential expression in the inner region of beet			Preferential expression in the outer region of beet		
Ratio in/out	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*	Ratio in/out	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*
Not classified			Not classified		
14.17	BQ591224	Expressed protein	0.26	BQ589783	Hypothetical protein
5.52	BQ593608	Expressed protein	0.27	BQ587565	Expressed protein
4.00	BQ583771	Expressed protein	0.30	BQ595659	Bacterial transferase hexapeptide repeat-containing protein
3.99	BQ592114	Expressed protein	0.30	BQ586471	AMP-dependent synthetase and ligase family protein
3.73	BQ593391	Expressed protein	Gene products related to chloroplast or photosynthetic activity		
Signal transduction			0.27	BQ587635	Ribulose bisphosphate carboxylase small chain 1A
5.17	BQ588649	Zinc finger (FYVE type) family protein	0.35^{cl2}	BQ586661	Ribulose bisphosphate carboxylase small chain 1A
3.81	CF542926	Zinc finger homeobox family protein	$0.36^{cl2,a}$	BQ584561	Chlorophyll A-B binding protein 2, chloroplast
4.37	BQ593260	CwfJ-like family protein/zinc finger (CCCH-type) family protein	0.35	BQ587083	Chloroplast nucleoid DNA-binding protein-related
4.03	BQ588471	MADS-box family protein	Signal transduction		
3.43	BQ585182	Protein phosphatase 2C. putative	$0.34^{cl1,a}$	BQ582478	Protein kinase, putative
3.63	BQ589454	1-Phosphatidylinositol-4-phosphate 5-kinase, putative	0.33^{cl1}	BQ586692	Protein phosphatase 2C, putative
3.74	BQ589847	Gigantea protein (GI)	0.21^{cl1}	BQ594060	Expressed protein similar to phosphatase
3.82	BQ593125	Armadillo/beta-catenin repeat family protein	0.31	BQ588158	Expressed protein similar to phosphatase
3.65	BQ594577	Tetratricopeptide repeat (TPR)-containing protein	0.36	BQ589615	MAP kinase phosphatase (MKP1)
			0.28	BQ588951	Transducin family protein/WD-40 repeat family protein
			0.31	BQ591951	Leucine carboxyl methyltransferase family protein
Nucleic acid metabolism					
3.68^{cl1}	BQ594594	Adenine phosphoribosyltransferase, putative	Protein biosynthesis		
6.77	BQ588780	Nucleotidyltransferase family protein	0.35	BQ595769	60S ribosomal protein L32
Cytoskeleton/vesicular trafficking/protein secretion			Cytoskeleton/vesicular trafficking/protein secretion		
3.52	BQ582665	Actin 11	0.29	BQ593766	Pattern formation protein (EMB30) (GNOM)
4.13	CF543631	Vacuoleless1 (VCL1)	0.22	BQ595258	F-actin capping protein alpha subunit family protein
3.78	BQ583992	Signal recognition particle-related	0.16	BQ592217	Kinesin motor family protein
			0.26	BQ591141	Syntaxin 71
Energy					
4.14	BQ595308	Oxidoreductase, zinc-binding dehydrogenase family protein	Energy		
14.83	BQ591790	Short-chain dehydrogenase/reductase	0.25	BQ583013	Cytochrome B561 family protein
Primary metabolism			Primary metabolism		
4.67	BQ583056	Glyceraldehyde 3-phosphate dehydrogenase, cytosolic, putative	0.29	BQ586302	Dihydrolipoamide dehydrogenase 2, plastidic
6.76^{cl1}	BQ583755	Sugar isomerase (SIS) domain-containing protein	0.26	BQ590837	Sucrase-related
3.75	BQ586452	Acyl-CoA dehydrogenase-related	0.21^{cl2}	CF543502	Glucosamine-6-phosphate isomerase/6-phosphogluconolactonase
Cell wall biogenesis					
5.33	BQ592679	Hydroxyproline-rich glycoprotein family protein	Plant growth factor regulation		
$6.10^{cl2,a}$	BQ591809	Glycosyl hydrolase family 17 protein	0.35	BQ585530	1-Aminocyclopropane-1-carboxylate oxidase
4.92	BQ593381	Glycosyltransferase family protein 47	0.35^{cl1}	BQ587958	Gibberellin 2-oxidase, putative
5.69^{cl2}	BQ589116	Eukaryotic phosphomannomutase family protein	$0.22^{cl1,a}$	BQ586825	Gibberellin-responsive protein, putative
3.73	BQ592303	Cinnamoyl-CoA reductase family			
3.28	BQ588778	Alpha-*N*-acetylglucosaminidase family	Cell wall biogenesis		
			$0.36^{cl1,a}$	BQ592105	Glycoside hydrolase family 28 protein
Stress/defence			$0.28^{cl2,a}$	BQ590768	Glycosyl hydrolase family 18 protein
5.33	BQ590759	Peroxidase 3 (PER3) (P3)	0.33	BQ592049	Extracellular dermal glycoprotein, putative

Table 3. *Continued*

Preferential expression in the inner region of beet			Preferential expression in the outer region of beet		
Ratio in/out	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*	Ratio in/out	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*
3.71	BQ592954	Osmotin-like protein (OSM34)	Stress/defence		
7.37	BQ582517	Leucine-rich repeat family protein	0.18[cl1,a]	BQ595076	Major latex protein-related
4.36	CF543301	Leucine-rich repeat transmembrane protein kinase, putative	0.25[cl1,a]	BQ593849	Thaumatin-like protein, putative
			0.18	BQ595730	Protease inhibitor/seed storage/lipid transfer protein
Proteolysis					
3.37	BQ591821	Cullin family protein	0.34	BQ589139	Peroxidase 42
4.01	BQ582359	F-box family protein (FBW2)	0.27	BQ583818	Aldo/keto reductase, putative
4.32	BQ585021	Ubiquitin-conjugating enzyme 16	0.34	BQ590397	Carbonic anhydrase family protein
3.27	BQ584114	ATP-dependent Clp protease ATP-binding subunit (ClpC)			
			Transport		
			0.31	BQ583477	MATE efflux protein-related
Transport			0.34	BQ590158	Mitochondrial substrate carrier family protein
4.24	BQ586017	Mitochondrial phosphate transporter	0.28	BQ586828	ABC1 family protein
3.58	BQ593484	Sugar transporter family protein	0.26[cl1,a]	BQ593958	Delta tonoplast integral protein

[a] Expression values among the top 40 in either cl1 or cl2.
[cl1, cl2]Gene products with expression patterns belonging to cluster 1 and cluster 2, respectively.

trafficking, and protein secretion, as well as those related to defence and stress, were identified. Gene products involved in cell-wall biogenesis showed a similar distribution, but the annotations point to different functional contexts: whereas in the inner region annotations for phosphomannomutase or cinnamoyl-CoA reductase indicated biosynthetic activities in cell-wall differentiation, the outer root zone contained transcripts for a glycoside hydrolase family 28 protein with polygalacturonase activity, and a glycosyl hydrolase family 18 protein with chitinase activity, both associated with processes such as degradation, reorganization, and possibly defence.

It is noticeable that, among the genes preferentially expressed in the outer part of the mature beet, more cl1 members than cl2 members were present (Table 3). In the complete set of genes transcriptionally monitored (see Supplementary Table 2 at *JXB* online), the ratio of cl1:cl2 transcripts in the outer region was 22:5, whereas the ratio for the inner region was nearly even with 14 cl1 and 13 cl2 transcripts.

Genes involved in carbohydrate metabolism reveal preferential transcription during early beet development and in the core of the tap root

Based on their predicted function, 32 differentially expressed genes were assigned to glycolysis, the oxidative pentose phosphate cycle, the reductive tricarboxylic acid cycle, or to the biosynthesis pathway of sugars (Table 4).

Transcripts encoding hexokinase 1, two types of phosphofructokinase, fructose-*bis*phosphate aldolase, and glyceraldehyde 3-phosphate dehydrogenase were found co-regulated in cluster 1 with moderate absolute and relative expression values. Transcripts for dihydrolipoamide *S*-acetyltransferase, a component of the pyruvate dehydrogenase complex, and ATP-citrate synthase, aconitate hydratase, and malate dehydrogenase, followed a similar transcription pattern. The latter three enzymes act in the reductive tricarboxylic acid cycle, and aconitate hydratase is also a key enzyme of the glyoxylate pathway. Preferential expression during early development was also found for the transcripts encoding five different members of a mutase family characterized by the isocitrate lyase signature. Isocitrate lyase is a further key enzyme of the glyoxylate pathway but, contrary to the aconitate hydratase transcript, most of the mutase transcripts were about 2.5-fold more strongly expressed in the outer region of maturing beets than in the core.

Cl1-transcription patterns were found for an alcohol dehydrogenase, indicating alcoholic fermentation in young beet tissue, 6-phosphogluconate dehydrogenase, an enzyme typical of the oxidative pentose phosphate cycle, and sucrose synthase 2 (Bellin *et al*., 2002; BQ490013), a candidate not contained in the unique cDNA set, but added to the macroarray analysis whose expression pattern was confirmed by semi-quantitative RT-PCR (Fig. 2C). With additional transcripts for two sugar transporters and a sugar isomerase domain-containing gene product, cluster 1 included the majority of putatively carbohydrate metabolism-related transcripts, whereby the transcripts for the sugar isomerase domain-containing protein and one transporter were also preferentially expressed in the core

Table 4. *Putative pathway assignment of differentially expressed beet transcripts encoding enzymes involved in primary carbohydrate metabolism*

Expression pattern[a]	Absolute expression[b]	Relative expression[b]	Ratio in/out[c]	GenBank accession no.	Similarity to gene product from *Arabidopsis thaliana*
Glycolysis					
cl1	2.67	1.78	ns	BQ589814	Hexokinase 1
cl1	2.61	2.57	ns	BQ584553	Pyrophosphate-dependent 6-phosphofructose-1-kinase-related
cl1	4.50	2.76	na	BQ585449	pfkB-type carbohydrate kinase family protein
cl1	1.04	2.53	ns	BQ586182	Fructose-bisphosphate aldolase
cl1	0.42	9.23	na	BQ592452	Glyceraldehyde 3-phosphate dehydrogenase, cytosolic, putative
in	–	–	4.67	BQ583056	Glyceraldehyde 3-phosphate dehydrogenase, cytosolic, putative
in	–	–	2.12	BQ593325	Enolase
Pyruvate dehydrogenase complex, generation of acetyl-CoA					
cl1	1.51	3.29	na	BQ592960	Dihydrolipoamide *S*-acetyltransferase
out	–	–	0.29	BQ586302	Dihydrolipoamide dehydrogenase 2, plastidic
Alcoholic fermentation					
in	–	–	2.62	BQ594209	Pyruvate decarboxylase, putative
cl1	0.52	6.45	ns	CF543014	Alcohol dehydrogenase, putative
Reductive tricarboxylic acid cycle/glyoxylate cycle					
cl1	1.50	7.12	ns	BQ583510	ATP-citrate synthase (ATP-citrate (pro-S-)-lyase/citrate cleavage enzyme), putative
cl1	4.82	2.61	ns	BQ585474	Aconitate hydratase, cytoplasmic, putative
cl1	1.15	2.53	ns	BQ584388	Malate dehydrogenase [NAD], plastidic
in	–	–	2.31	BQ593041	Malate dehydrogenase [NAD], plastidic
in	–	–	2.49	CF542670	Succinyl-CoA ligase [GDP-forming] alpha-chain, mitochondrial, putative
cl1/out	8.91	6.69	0.44	BQ585628	Mutase family protein similar to carboxyvinyl-carboxy-phosphonate phosphorylmutase, isocitrate lyase signature
cl1/out	7.43	9.93	0.36	BQ588168	Mutase family protein similar to carboxyvinyl-carboxy-phosphonate phosphorylmutase, isocitrate lyase signature
cl1/out	3.08	7.11	0.40	BQ583774	Mutase family protein similar to carboxyvinyl-carboxy-phosphonate phosphorylmutase, isocitrate lyase signature
cl1	3.03	5.21	ns	BQ586481	Mutase family protein similar to carboxyvinyl-carboxy-phosphonate phosphorylmutase. isocitrate lyase signature
cl1/out	5.63	13.08	0.40	BQ588409	Mutase family protein similar to carboxyvinyl-carboxy-phosphonate phosphorylmutase, isocitrate lyase signature
Oxidative pentose phosphate pathway					
cl2/**out**	1.83	4.39	0.21	CF543502	Glucosamine-6-phosphate isomerase/6-phosphogluconolactonase
cl1	1.21	6.52	ns	BQ594275	6-Phosphogluconate dehydrogenase
cl2	0.76	2.19	ns	BQ587653	Transketolase, putative
Sucrose (re-)synthesis/degradation					
in	–	–	2.05	BQ594479	Sucrose synthase, putative
out	–	–	0.26	BQ590837	Sucrase-related
in	–	–	2.52	BQ588354	Fructose-1, 6-bisphosphatase, putative
in	–	–	2.31	BQ587727	Fructose-6-phosphate 2-kinase/fructose-2, 6-bisphosphatase
Others					
cl1/**in**	0.85	3.46	6.76	BQ583755	Sugar isomerase (SIS) domain-containing protein
cl1	0.75	4.97	ns	CF543258	Sugar transporter family protein
in	–	–	3.58	BQ593484	Sugar transporter family protein
cl1/in	0.80	2.45	3.06	BQ582657	Transporter-related low similarity to GDP-mannose transporter

[a] Preferential expression in cl1, cluster 1 of the-time-course experiment; cl2, cluster 2 of the time-course experiment; in, inner beet zone; out, outer beet zone; bold characters indicate expression values among the top 40 in the respective category (Tables 2, 3).
[b] Absolute and relative expression values of cl1 and cl2 members.
[c] The values for Ratio in/out describe how much detected transcript levels in the inner region of the beet exceed those of the outer region (Table 3). ns, Not significant; na, not analysable.

of the beet. Among transcripts with preferential expression in the inner part of the beet, a second putative glyceraldehyde 3-phosphate dehydrogenase and an enolase were identified. Transcripts for a further malate dehydrogenase and succinyl-CoA ligase followed a similar profile, as did those for the preferentially beet-expressed sucrose synthase SBSS1 (Hesse and Willmitzer, 1996; Bellin *et al*., 2002), a fructose-1, 6-*bis*phosphatase and a fructose-6-phosphate 2-kinase/fructose-2, 6-*bis*phosphatase, key enzymes involved in sucrose catabolism and glucose (re)synthesis important in regulating sink strength (Nielsen *et al*., 2004). A transcript for a possible pyruvate

decarboxylase involved in fermentation and a further sugar transporter showed a similar regulation.

Concerning invertases regulating sink strength (Sturm and Tang, 1999), three neutral isoforms and a further beta-fructofuranosidase produced data which could be analysed, but no differential expression was found. The same was true for two transcripts encoding sucrose phosphate synthase catalysing the final step in sucrose biosynthesis.

In addition to genes with a role in carbohydrate metabolism, the majority of differentially expressed genes with predicted functions in the metabolism of amino and fatty acids, as well as in lipid degradation, were identified in cl1 (see Supplementary Table 2 at *JXB* online). Among the 40 most expressed transcripts there were two for a 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, an essential enzyme in methionine biosynthesis, and a putative 3-hydroxy-3-methylglutaryl-CoA reductase 1 catalysing a key step in the synthesis of the isoprenoid precursor mevalonic acid (Table 2A).

The level of transcription of carbohydrate metabolism-related genes was in agreement with the expression of 11 genes involved in fatty and amino acid metabolism in the inner beet region (see Supplementary Table 2 at *JXB* online). The high preferential transcription of a putative acyl-CoA dehydrogenase-related gene product in the core of the beet indicated the importance of beta-oxidation of fatty acids in this tissue (Table 3).

Only two transcripts encoding gene products potentially involved in the oxidative pentose phosphate cycle, a transketolase and a glucosamine-6-phosphate isomerase/6-phosphogluconolactonase, showed preferential expression during the late developmental stages. The latter was strongly overexpressed in the outer beet region, in which also a plastidic dihydrolipoamide dehydrogenase 2, a sucrase, and the above-mentioned mutase family proteins were preferentially transcribed. During late beet development and in the peripheral, sucrose-poor region, no fatty or amino acid metabolism-related transcripts were identified, apart from two with predicted functions in lipid oxidation.

Discussion

In the present work, sugar beet samples of different developmental stages and topological zones were analysed with respect to growth characters, sucrose content, and typical transcript profiles. It was the aim to correlate changes in phenotypic characters, and, in particular, in sucrose content as a molecular phenotype, with alterations in transcript levels ultimately to identify the underlying candidate genes for the breeding process.

For better comparability of beet development between years which are subject to climatic differences, sampling dates were expressed as thermal time which accounts for the accumulated warmth as a main growth promoter. The rates of increase in shoot and root growth during the vegetation period (Fig. 1A–F) are in line with previous reports (Elliott and Weston, 1993). With the exception of mass, all measured characters rapidly increased in the first phase, indicating the immediate start of ring initiation and sucrose accumulation in beet development. Mass accumulated mainly at a later stage. Largely in line with these findings, no more than two expression clusters of transcripts with almost complementary expression patterns were identified in the time-course experiment (Fig. 1G, H). The transition from high to low expression or vice versa was around 1420 °Cd in the first year and 1293–1599 °Cd in the second year, when maximal values for most morphological characters, as well as for sucrose percentage, had almost been achieved.

As the beet sucrose concentration remains nearly constant from 1300 °Cd to 1400 °Cd onwards, the net increase in sugar appears to be due to growth and mass increase in the late phase and sucrose accumulation of the newly generated cells in the outer zone. Thus beet sink strength is maintained, and plant metabolism may be adjusted to further sucrose accumulation and to the imminent winter period at the end of the first season reflected by high protein turnover and biosynthesis activity, especially in the inner zone in late development. Plant growth factors, as indicated by the respective class in cl2, may assume regulatory functions of the developmental programme (Table 2B), and candidates encoding gene products involved in pathogen defence and senescence which were over-represented among the cl2 members indicate their increasing importance in maturing, sucrose-rich beets.

The notion that there is a spatial gradient in cell expansion and sucrose accumulation throughout the beet from inner to outer zones during development is complemented by the finding that transcripts belonging to cl1 are over-represented in the outer beet zone. This implies that this region may be developmentally delayed, a condition which is also indicated by the low sucrose content and as yet unexpanded cambial rings. In both young beets and the outer region of more mature beets, cells derived from the cambia are considered small and undifferentiated, but preparing for sucrose accumulation and expansion, a situation arguing for the expression of related genes. Following this rationale, the temporal gradient of transcript abundance in beet development may be partly superimposed on the spatial gradient. As a result, maturing beets contain transcripts typical of the innermost sucrose-rich cells and typical of differentiating sucrose-poor cells in the outer parts simultaneously. Based on this reasoning, selected processes and the gene products involved can be presented according to their transcript levels as follows.

An example of a factor typical of expanding cells, both during early development and in the maturing outer zone, appears to be gibberellin. This is indicated by preferential

expression of two transcripts related to gibberellin synthesis and activity in both cl1 and the outer region (Tables 2, 3). Gibberellin-like activity also correlated well with the period of rapid cell expansion at the temporal scale (Elliott and Weston, 1993).

The nearly 9-fold increase in beet diameter between 500 °Cd and 1500 °Cd indicates a high growth rate and biosynthetic activity. This is reflected at the transcript level with high expression values for cl1 transcripts encoding gene products with functions in cytoskeletal reorganization, which is seen in the context of cell division and differentiation, and transport processes, which serve directed distribution of metabolites and cellular homeostasis in the case of membrane intrinsic proteins (Table 2A).

The largest number of differentially expressed beet transcripts encoding enzymes involved in primary carbohydrate metabolism is predicted to function in either the reductive tricarboxylic acid or the glyoxylate cycle, and shows moderate expression levels (Table 4). Both cycles are anaplerotic pathways refilling pools of substrates for biosynthetic reactions typical of actively growing tissue. Correspondingly, preferential expression is found in the young beets and/or the inner region, with the exception of four transcripts, showing similarity to mutase family proteins with an isocitrate lyase domain, which are preferentially expressed in the outer region of the root. This spatial type of expression makes it unlikely that the predicted enzymes participate in the same glyoxylate cycle. Either the identified gene products act in locally separated, but functionally similar pathways, or the mutase family proteins assume different functions (Anzai *et al*., 1987; Wang *et al*., 1993).

According to the model for sucrose accumulation in sugar-beet roots (Fieuw and Willenbrink, 1989), sucrose phosphate synthase and sucrose synthase play major roles in the regulation of sink strength; invertases are considered further candidates in this respect (Sturm and Tang, 1999). None of the two isoforms of sucrose phosphate synthase (Hesse *et al*., 1995) or the invertases showed preferential expression in the time-course analysis, and the possibility exists that regulation takes place at a different level in these cases. However, the sucrose synthase isoform which is preferentially transcribed in roots and inflorescences (accession number BQ490013; Bellin *et al*., 2002), showed the highest expression level during early beet development (Fig. 2C), correlating with the period of highest sucrose accumulation. In addition, a transcript for a sucrase-related protein, which is predicted to increase the sucrose flow by cleaving sucrose at the sink, is preferentially expressed in the outer region of the mature beet (Table 3). The preferentially root-expressed sucrose synthase isoform SBSS1 (accession numbers X81974/BQ490130/BQ594479; Hesse and Willmitzer, 1996; Bellin *et al*., 2002) was over-represented in the inner sucrose-rich region at maturity (Fig. 2, Table 3). Its pattern of expression was shared by transcripts encoding fructose-1, 6-bisphosphatase and fructose-6-phosphate 2-kinase/fructose-2, 6-bisphosphatase, two further enzymes mediating sink strength (Nielsen *et al*., 2004).

With only low levels of transcripts related to carbohydrate metabolism, but high transcript levels for fibre biogenesis- and stress defence-related gene products, a similar situation was found in sugar-cane stems (Casu *et al*., 2003, 2004). Just one sugar transporter, PST2, showed high abundance in the maturing sucrose-rich stem and was associated with maintaining sugar fluxes into mature-stem sink tissues and regulating the osmotic equilibrium, an important function in tissues with high sucrose levels. Interestingly, three transcripts for sugar transporters have been found preferentially expressed in cluster 1 and/or the inner beet region, in agreement with the highest sucrose increase rate and/or the highest absolute sucrose content (Table 4). The particular relevance of the putative gene products identified in the present analysis, however, cannot be supported without their detailed functional analysis carried out at the cellular level.

Cluster-1 transcripts preferentially expressed in the outer zone of maturing beets like those encoding the cell wall proteins xyloglucan:xyloglucosyl transferase and three glycoside hydrolase family 28 proteins (Tables 2A, 3) may alternatively be boundary-specific with constant expression during development, but due to the decreasing proportion of the boundary zone in the growing beet, which means a low ratio of surface area to total volume, lower transcript levels may have been detected in whole mature beets used for the kinetic study. In line with this hypothesis, most of the cl1 genes in the outer zone are not characterized by high absolute expression, but by high relative transcript levels (Table 2A), indicating a strong decrease in measured transcripts caused by dilution from early to late stages.

The few transcripts common to the outer beet region and cl2 are assumed to be regulated based on the spatial exposition of the maturing beet to exogenous pathogens and to light. Apart from transcripts for two chitinases implicated in pathogen defence, transcripts were found for a subunit of ribulose-*bis*phosphate carboxylase and a chlorophyll A-B binding protein, both being induced by light in leaves (Lu *et al*., 2002).

The present work shows that monitoring molecular phenotypes such as the metabolite sucrose in combination with gene expression profiling can be an effective tool to associate genes with functions and to identify key genes in metabolic processes which may be relevant in the plant breeding process. In natural or experimental populations, transcript levels of individual genes can be treated as quantitative factors to be mapped as genetic loci, a concept first introduced as 'genetical genomics' (Jansen and Nap, 2001). If the expression level is indicative of an agronomically important phenotype trait, selection in the

breeding process may be complemented by transcript profiling. Such effects were recently demonstrated for transcriptionally regulated genes in barley (Potokina *et al*., 2004, 2006). Quantitative trait analyses for sugar quality and yield are feasible (Weber *et al*., 1999, 2000; Schneider *et al*., 2002), and linkage analyses of the genes identified in the present work should determine their genetic relevance for the traits considered.

Supplementary data

Supplementary data can be found at *JXB* online. Table 1: PCR conditions and fragment sizes of candidate transcripts used for validation on macro array results in semi-quantitative RT-PCR experiments shown in Fig. 2. Table 2: Complete data set on cDNA sequence annotations and macroarray expression values for 11 520 unique sugar beet cDNAs.

Acknowledgements

The work was financially supported by the BMBF-funded SWEET GABI project (grant number 0312283E) which also funded DB and KS. We would like to thank Silke Möhring for providing the primers to amplify the candidate with the accession number BQ588168 and Susanne Horstmann-Schwarz for technical support.

References

Anzai H, Murakami T, Imai S, Satoh A, Nagaoka K, Thompson CJ. 1987. Transcriptional regulation of bialaphos biosynthesis in *Streptomyces hygroscopicus*. *Journal of Bacteriology* **169,** 3482–3488.

Azevedo C, Santos-Rosa MJ, Shirasu K. 2001. The U-box protein family in plants. *Trends in Plant Science* **6,** 354–358.

Bankaitis VA, Malehorn DE, Emr SD, Greene R. 1989. The *Saccharomyces cerevisiae* SEC14 gene encodes a cytosolic factor that is required for transport of secretory proteins from the yeast Golgi complex. *Journal of Cell Biology* **108,** 1271–1281.

Bellin D, Werber M, Theis T, Schulz B, Weisshaar B, Schneider K. 2002. EST sequencing, annotation and macroarray transcriptome analysis identify preferentially root-expressed genes in sugar beet. *Plant Biology* **4,** 700–710.

Bernard K, Auphan N, Granjeaud S, Victorero G, Schmitt-Verhulst AM, Jordan BR, Nguyen C. 1996. Multiplex messenger assay: simultaneous, quantitative measurement of expression of many genes in the context of T cell activation. *Nucleic Acids Research* **24,** 1435–1442.

Brown MH, Paulsen IT, Skurray RA. 1999. The multidrug efflux protein NorM is a prototype of a new family of transporters. *Molecular Microbiology* **31,** 394–395.

Casu RE, Dimmock CM, Chapman SC, Grof CP, McIntyre CL, Bonnett GD, Manners JM. 2004. Identification of differentially expressed transcripts from maturing stem of sugarcane by *in silico* analysis of stem expressed sequence tags and gene expression profiling. *Plant Molecular Biology* **54,** 503–517.

Casu RE, Grof CP, Rae AL, McIntyre CL, Dimmock CM, Manners JM. 2003. Identification of a novel sugar transporter homologue strongly expressed in maturing stem vascular tissues of sugarcane by expressed sequence tag and microarray analysis. *Plant Molecular Biology* **52,** 371–386.

Chen F, Foolad MR. 1997. Molecular organization of a gene in barley which encodes a protein similar to aspartic protease and its specific expression in nucellar cells during degeneration. *Plant Molecular Biology* **35,** 821–831.

Cosgrove DJ. 2005. Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology* **6,** 850–861.

de los Reyes BG, Myers SJ, McGrath JM. 2003. Differential induction of glyoxylate cycle enzymes by stress as a marker for seedling vigor in sugar beet (*Beta vulgaris*). *Molecular Genetics and Genomics* **269,** 692–698.

Desprez T, Amselem J, Caboche M, Hofte H. 1998. Differential gene expression in Arabidopsis monitored using cDNA arrays. *The Plant Journal* **14,** 643–652.

Eckardt NA. 2001. Move it on out with MATEs. *The Plant Cell* **13,** 1477–1480.

Elge S, Brearley C, Xia HJ, Kehr J, Xue HW, Mueller-Roeber B. 2001. An Arabidopsis inositol phospholipid kinase strongly expressed in procambial cells: synthesis of PtdIns (4, 5)P_2 and PtdIns(3, 4, 5)P_3 in insect cells by 5-phosphorylation of precursors. *The Plant Journal* **26,** 561–571.

Elliott MC, Weston GD. 1993. Biology and physiology of the sugar-beet plant. In: Cooke DA, Scott RK, eds. *The sugar beet crop: science into practice*. London: Chapman and Hall, 37–66.

Ewing B, Green P. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Research* **8,** 186–194.

Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Research* **8,** 175–185.

Farrar K, Evans IM, Topping JF, Souter MA, Nielsen JE, Lindsey K. 2003. EXORDIUM: a gene expressed in proliferating cells and with a role in meristem function, identified by promoter trapping in Arabidopsis. *The Plant Journal* **33,** 61–73.

Fieuw S, Willenbrink J. 1989. Sucrose synthase and sucrose phosphate synthase in sugar beet plants (*Beta vulgaris* L. ssp *altissima*). *Journal of Plant Physiology* **131,** 153–162.

Fieuw S, Willenbrink J. 1990. Sugar transport and sugar-metabolizing enzymes in sugar storage roots (*Beta vulgaris* ssp. *altissima*). *Journal of Plant Physiology* **137,** 216–223.

Fleet CM, Sun TP. 2005. A DELLAcate balance: the role of gibberellin in plant morphogenesis. *Current Opinion in Plant Biology* **8,** 77–85.

Hanson AD, Gregory JF. 2002. Synthesis and turnover of folates in plants. *Current Opinion in Plant Biology* **5,** 244–249.

Hara-Nishimura I, Hatsugai N, Nakaune S, Kuroyanagi M, Nishimura M. 2005. Vacuolar processing enzyme: an executor of plant cell death. *Current Opinion in Plant Biology* **8,** 404–408.

Herwig R, Schulz B, Weisshaar B, *et al*. 2002. Construction of a 'unigene' cDNA clone set by oligonucleotide fingerprinting allows access to 25 000 potential sugar beet genes. *The Plant Journal* **32,** 845–857.

Hesse H, Sonnewald U, Willmitzer L. 1995. Cloning and expression analysis of sucrose-phosphate synthase from sugar beet (*Beta vulgaris* L.). *Molecular and General Genetics* **247,** 515–520.

Hesse H, Willmitzer L. 1996. Expression analysis of a sucrose synthase gene from sugar beet (*Beta vulgaris* L.). *Plant Molecular Biology* **30,** 863–872.

Jansen RC, Nap JP. 2001. Genetical genomics: the added value from segregation. *Trends in Genetics* **17,** 388–391.

Johansson I, Karlsson M, Johanson U, Larsson C, Kjellbom P. 2000. The role of aquaporins in cellular and whole plant water balance. *Biochimica et Biophysica Acta* **1465,** 324–342.

Kocsis MG, Ranocha P, Gage DA, *et al.* 2003. Insertional inactivation of the methionine *S*-methyltransferase gene eliminates the *S*-methylmethionine cycle and increases the methylation ratio. *Plant Physiology* **131,** 1808–1815.

Lu C, Koroleva OA, Farrar JF, Gallagher J, Pollock CJ, Tomos AD. 2002. Rubisco small subunit, chlorophyll a/b-binding protein and sucrose:fructan-6-fructosyl transferase gene expression and sugar status in single barley leaf cells *in situ*: cell type specificity and induction by light. *Plant Physiology* **130,** 1335–1348.

Martinoia E, Klein M, Geisler M, Bovet L, Forestier C, Kolukisaoglu U, Muller-Rober B, Schulz B. 2002. Multifunctionality of plant ABC transporters: more than just detoxifiers. *Planta* **214,** 345–355.

Milford GFJ. 1973. The growth and development of the storage root of sugar beet. *Annals of Applied Biology* **75,** 427–438.

Mudd SH, Datko AH. 1990. The *S*-methylmethionine cycle in *Lemna paucicostata*. *Plant Physiology* **93,** 623–630.

Müller I, Wagner W, Volker A, Schellmann S, Nacry P, Kuttner F, Schwarz-Sommer Z, Mayer U, Jurgens G. 2003. Syntaxin specificity of cytokinesis in Arabidopsis. *Nature Cell Biology* **5,** 531–534.

Nessler CL, Allen RD, Galewsky S. 1985. Identification and characterization of latex-specific proteins in opium poppy. *Plant Physiology* **79,** 499–504.

Nielsen TH, Rung JH, Villadsen D. 2004. Fructose-2, 6-*bis*phosphate: a traffic signal in plant metabolism. *Trends in Plant Science* **9,** 556–563.

Noctor G, Gomez L, Vanacker H, Foyer CH. 2002. Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. *Journal of Experimental Botany* **53,** 1283–1304.

Oufattole M, Park JH, Poxleitner M, Jiang L, Rogers JC. 2005. Selective membrane protein internalization accompanies movement from the endoplasmic reticulum to the protein storage vacuole pathway in Arabidopsis. *The Plant Cell* **17,** 3066–3080.

Panikulangara TJ, Eggers-Schumacher G, Wunderlich M, Stransky H, Schoffl F. 2004. Galactinol synthase1: a novel heat shock factor target gene responsible for heat-induced synthesis of raffinose family oligosaccharides in Arabidopsis. *Plant Physiology* **136,** 3148–3158.

Pool MR, Stumm J, Fulga TA, Sinning I, Dobberstein B. 2002. Distinct modes of signal recognition particle interaction with the ribosome. *Science* **297,** 1345–1348.

Potokina E, Caspers M, Prasad M, Kota R, Zhang J, Sreenivasulu Wang M, Graner A. 2004. Functional association between malting quality trait components and cDNA array based expression patterns in barley (*Hordeum vulgare* L.). *Molecular Breeding* **14,** 153–170.

Potokina E, Prasad M, Malysheva L, Roder MS, Graner A. 2006. Expression genetics and haplotype analysis reveal *cis* regulation of serine carboxypeptidase I *(Cxp1)*, a candidate gene for malting quality in barley (*Hordeum vulgare* L. *Functional and Integrative Genomics* **6,** 25–35.

Ranocha P, McNeil SD, Ziemak MJ, Li C, Tarczynski MC, Hanson AD. 2001. The *S*-methylmethionine cycle in angiosperms: ubiquity, antiquity and activity. *The Plant Journal* **25,** 575–584.

Rojo E, Martin R, Carter C, *et al.* 2004. VPEgamma exhibits a caspase-like activity that contributes to defense against pathogens. *Current Biology* **14,** 1897–1906.

Schneider K, Schäfer-Pregl R, Borchardt DC, Salamini F. 2002. Mapping QTLs for sucrose content, yield and quality in a sugar beet population fingerprinted by EST-related markers. *Theoretical and Applied Genetics* **104,** 1107–1113.

Seo M, Koshiba T. 2002. Complex regulation of ABA biosynthesis in plants. *Trends in Plant Science* **7,** 41–48.

Sturm A, Tang GQ. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends in Plant Science* **4,** 401–407.

Sturn A, Quackenbush J, Trajanoski Z. 2002. Genesis: cluster analysis of microarray data. *Bioinformatics* **18,** 207–208.

Telles G, da Silva F. 2001. Trimming and clustering sugarcane ESTs. *Genetics and Molecular Biology* **24,** 17–23.

Thompson AR, Vierstra RD. 2005. Autophagic recycling: lessons from yeast help define the process in plants. *Current Opinion in Plant Biology* **8,** 165–173.

Thornley JHM, Johnson IR. 1990. Empirical threshold models of developmental rate: the temperature-sum rule. In: *Plant and crop modelling: a mathematical approach to plant and crop physiology*. Oxford: Clarendon Press, 166–172.

Wang H, Brandt AS, Woodson WR. 1993. A flower senescence-related mRNA from carnation encodes a novel protein related to enzymes involved in phosphonate biosynthesis. *Plant Molecular Biology* **22,** 719–724.

Weber WE, Borchardt DC, Koch G. 1999. Combined linkage maps and QTLs in sugar beet (*Beta vulgaris* L.) from different populations. *Plant Breeding* **118,** 193–204.

Weber WE, Borchardt DC, Koch G. 2000. Marker analysis for quantitative traits in sugar beet. *Plant Breeding* **119,** 97–106.

Willenbrink J, Doll S, Getz HP, Meyer S. 1984. Zuckeraufnahme in isolierten Vakuolen und Protoplasten aus dem Speichergewebe von Beta-Rüben. *Berichte der Deutschen Botanischen Gesellschaft* **97,** 27–39.

Xu Z, Escamilla-Trevino L, Zeng L, *et al.* 2004. Functional genomic analysis of *Arabidopsis thaliana* glycoside hydrolase family 1. *Plant Molecular Biology* **55,** 343–367.