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Mapping QTLs for sucrose content, yield and quality in a sugar beet population fingerprinted by EST-related markers

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Abstract Seventy five expressed sequence tags (ESTs) that are associated with functions in carbohydrate and nitrogen metabolism were genotyped in 108 plants of an F2 population of sugar beet (*Beta vulgaris* L.) segregating for sugar quality and yield parameters. Supplemented by known RFLP and AFLP markers, the resulting map spans 446 cM of the 758-Mbp genome of sugar beet. F3 test-cross plants were analysed for corrected sugar yield, beet yield, ion balance and the content of sugar, amino nitrogen, potassium and sodium in six locations. Twenty one significant quantitative trait loci (QTLs) were detected using the composite interval mapping approach. Expressed genes flanking the QTLs were identified in all cases. Correlations between QTLs and potential candidate genes are discussed.

Keywords *Sugar beet* · Yield · Sucrose content · QTL · EST · Candidate gene

Introduction

Sugar beets account for 25% of the worldwide sucrose production. Thus the understanding of the genetic factors underlying sugar purification and yield with a view to improving these traits are of major economic importance. Measurable traits related to sugar production include sugar content (SC), beet yield (BY), content of amino nitrogen (AN), potassium (K) and sodium (NA). The latter three parameters influence the ion balance (IB), a complex trait which influences the industrial purification of sucrose and contributes to the actual sugar yield. The most important complex trait, depending on beet yield and sugar content, is corrected sugar yield (CSY).

Sugar-related traits of sugar beet are inherited in a complex manner and can be dissected into distinct genetic components (quantitative trait loci, QTLs; Geldermann 1975). In a given population, the allelic constitution at these loci and their interaction with the environment determine the phenotype of single plants. QTLs can be mapped in a population when they are linked with molecular markers whose chromosomal position is known. A QTL analysis for yield data of sugar beet based on two segregating populations grown in different environments has been published (Weber et al. 1999, 2000). In this study, a number of QTLs were determined; however, in the two populations they mapped to different chromosomal positions, and few were stably expressed in the same population across locations. This highlights both the role of different plant material used for the two populations and the strong genotype × environment interactions in shaping sucrose yield in sugar beet. The results pinpoint some pitfalls of QTL detection and analysis in applied breeding programs.

To identify the genes that underlie QTLs, the candidate-gene approach uses expressed genes with known functions, which are thought likely to be related to the phenotypic traits being studied, as molecular genetic markers. Genes to be anchored can be selected on the basis of the physiological and biochemical properties of their products, which are assumed to play a role in the expression of the trait under investigation. Anchored genes become candidates for QTLs if their map position coincides with those of significant QTLs. With respect to carbohydrate metabolism in maize, QTLs for enzyme activities have been correlated with genetic loci encoding those enzymes; moreover, for ADP-glucose pyrophosphorylase, an RFLP polymorphism has been correlated with a QTL for starch kernel content (Prioul et al. 1999). In tomato, a gene for apoplastic invertase was identified as a candidate for a QTL for sugar content, and has been proven to contribute to the accumulation of soluble sugar

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in the berry (Fridman et al. 2000). In a study aimed at mapping QTLs that influence quantitative levels of enzyme activity in primary and secondary metabolism of *Arabidopsis thaliana*, QTLs were mapped either very close to known enzyme-encoding loci, or overlapping QTLs for different enzymes hinted at the involvement of common regulatory genes (Mitchell-Olds and Pedersen 1998). The importance of both structural and regulatory loci emerges also from studies on QTLs for the resistance to the corn earworm in maize (McMullen et al. 1998). The role of regulatory loci is further underlined by the finding that in tomato an oncogene-related gene product affects fruit size (Frary et al. 2000) and a rice gene, which encodes an ortholog of the transcription factor CONSTANS of *Arabidopsis*, corresponds to a major QTL for photoperiod sensitivity in rice (Yano et al. 2000).

In sugar beet, metabolic pathways that are expected to be relevant to both sugar quality and yield, and are localized in the chloroplast, include those for photosynthesis, metabolism of transient starch and the generation of assimilates for export (Elliott and Weston 1993). In the cytosol, catabolism of assimilates via glycolysis, together with the reactions that make up the citrate cycle, and the enzymes of sucrose synthesis, are important. A further relevant metabolic process is the transport of sucrose to the sink tissue, the expanded root. After hydrolysis and re-synthesis, sucrose is deposited in the vacuoles of the root parenchymatic tissue (Fieuw and Willenbrink 1990). The industrial process of sugar purification is influenced by the accumulation of ions like potassium and sodium, and amino-nitrogen compounds generated by nitrate reduction and transaminating reactions. Maps including the positions of functional genes that are involved in these pathways are available for the completely sequenced model plant *A. thaliana* (The *Arabidopsis* genome initiative 2000) and for maize, tomato and potato (Tanksley et al. 1992; Causse et al. 1995; Chen et al. 2001). Such maps also allow studies of chromosomal synteny between species and comparative analysis of QTL maps, as demonstrated for organ pigmentation loci in the Solanaceae (Thorup et al. 2000).

For sugar beet, only molecular genetic maps based on anonymous restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers have been produced (Barzen et al. 1992, 1995; Schondelmaier and Jung 1997; Schumacher et al. 1997). These maps cover 700 cM of the nine linkage groups which represent the 758-Mbp genome of the species (Arumuganathan and Earle 1991). Recently, 42 sugar beet genes have been assigned to linkage groups using DNA sequence information from the corresponding homo- and hetero logous ESTs (Schneider et al. 1999). Here we: (1) present the first genetic map incorporating functional genes which have an assigned position with reference to known RFLP and AFLP markers (Barzen et al. 1992, 1995; Schumacher et al. 1997); (2) identify QTLs for sugar yield and quality parameters as evaluated in six locations; and (3) discuss linkage between some candidate genes and QTLs.

Materials and methods

Plant material, field trials and statistical analysis

All breeding material was generated and evaluated by KWS SAAT AG (Einbeck, Germany). The population 618 of Schneider et al. (1999) formed the basis for this analysis. This population derives from a cross between diploid parents that differed significantly in their yield parameters. A total of 108 F2 plants were genotyped and 101 F3 families were crossed to a common tester to generate testcross hybrids. The hybrids were evaluated in field trials based on two lattice designs (10×5 and 8×8) at four locations in Germany, namely Einbeck (EIN), Kleinwanzleben (KWL), Seligenstadt (SEL) and Friemar (FRI), and at two locations in France, namely Monceu (MON) and Villerseau (VIS), in 1999. Seeds were sown in 3-row plots of 11 m^2 with 3–5 replications, in the first 2 weeks of April 1999. Beets were harvested between the end of September and the beginning of October 1999. Beet yield was determined and the content of sugar, potassium, sodium and α-amino nitrogen were measured as described in Burba and Puscz (1976). Corrected sugar yield was determined according to Burba and Schieweck (1993). Trait values for each plot were transformed to relative values based on four standard varieties common for all trials. Adjusted mean values of each lattice trial were used for the ANOVA analysis across locations. All trait values subjected to QTL analysis were tested for normal distribution using the one-sample Kolmogorov–Smirnov test (SPSS Inc 1999). None of them, except the trait NA at the location MOC, showed a significant deviation from normal distribution.

Heritabilities on a testcross progeny mean basis were calculated as described by Hallauer and Miranda (1981). Since the progenies of the population were split in two designs per location, the mean of the two estimates for heritability was used. Phenotypic trait correlations were calculated based on the corrected mean values of the entries.

Two additional populations, referred to as K2 and 704 (Schäfer-Pregl et al. 1999; Schneider et al. 1999), were used to assign map positions to fragments of expressed genes that turned out to be monomorphic in population 618.

Molecular analysis

Accession numbers of sugar beet gene fragments and ESTs used as molecular markers, primer sequences, amplification protocols and the detection of polymorphisms by cleaved amplified polymorphic sequence (CAPS), single-strand conformation polymorphism (SSCP) and Heteroduplex analysis have been described elsewhere (Schneider et al. 1999, http://www.mpiz-koeln.mpg.de/sugarbeet). Further data and information on amplification primers used for the 21 RFLP probes MP001, MP004, MP015, MP017, MP019, MP040, MP043, MP044, MP045, MP068, MP079, MP090, MP092, MP095, MP096, MP099, MP110, MP132, MP143, MP175 and MP180 of Barzen et al. (1992, 1995) are available upon request. AFLP analysis was performed according to standard protocols using 16 *Eco*RI-*Mse*I primer combinations.

Map construction and QTL analysis

The construction of a genetic map based on segregation data was approached using JOINMAP (version 2.0; Stam 1993; Stam and Ooijen 1995) and MAPMAKER 3.0b (Lander et al. 1987).

Analysis of variance (GLM procedure) was performed with programs written for SAS software (SAS Institute Inc. 1989). Scheffe's multiple comparison procedure was used to analyse differences among the four marker-class means. For each marker locus, the effects of the two different alleles on the phenotype were determined.

A QTL analysis was performed with PLABQTL (Utz and Melchinger 1995), using the composite-interval mapping approach of Zeng (1993, 1994), which combines the interval mapping (Lander and Botstein 1989) with multiple regression analysis

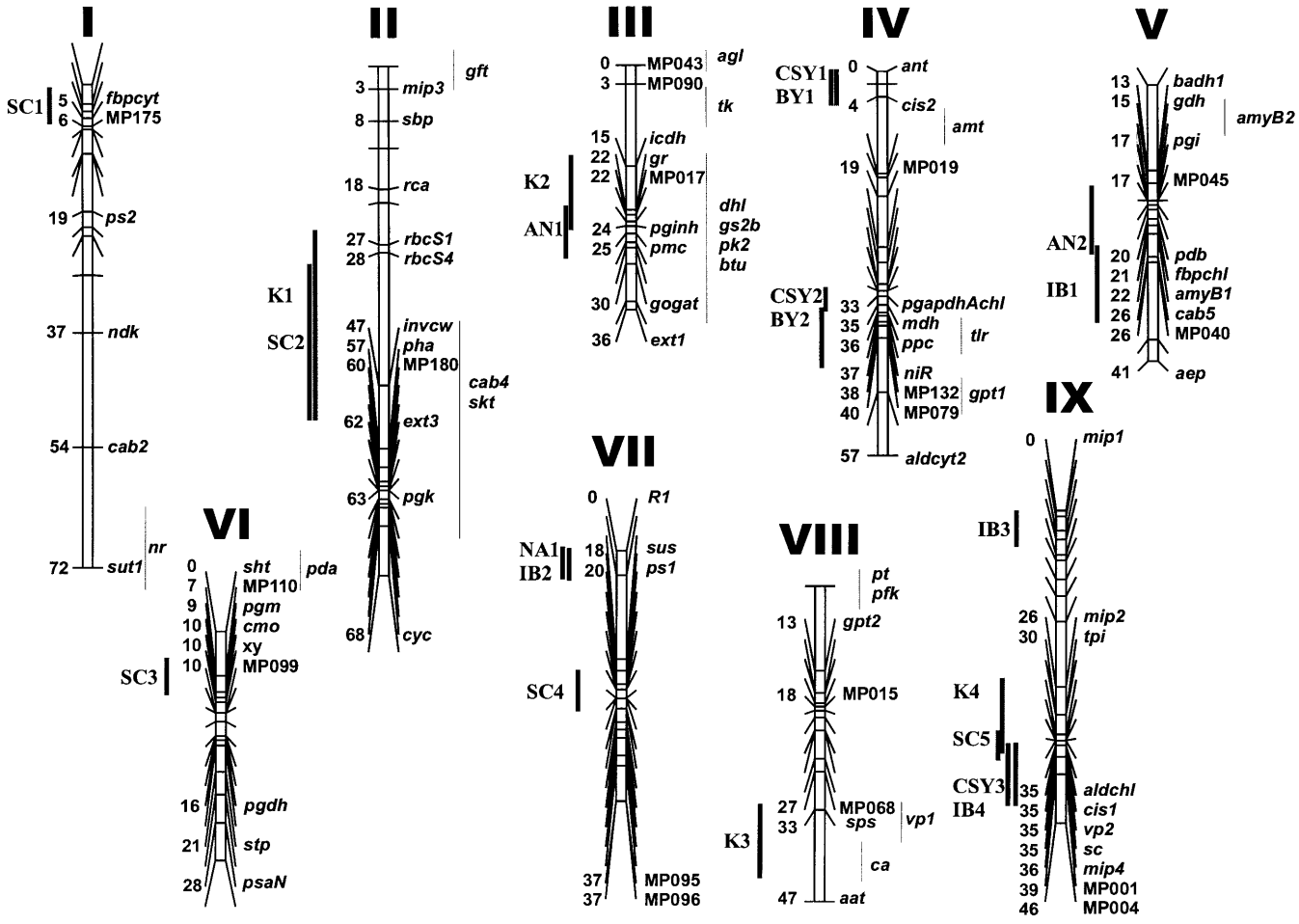

Fig. 1 Genetic map of sugar beet with chromosomal positions of functional genes and QTLs for sugar yield and quality traits. The genetic map comprises 75 genes for which symbols and names are reported on http://www.mpiz-koeln.mpg.de/sugarbeet. For 56 of them, the chromosomal position is given in Centimorgans (cM) to the left of each chromosome. For other 19 gene fragments (see text) the chromosomal region in which they are most likely to map is indicated to the right of each chromosome by a *bar*. Eighteen RFLP markers are also shown by their respective numbers as well as the anonymous genomic fragment xy. The positions of 99 AFLP markers are indicated by *dashes*, details are available upon request. QTL regions for the traits CSY, BY, SC, AN, K, NA and IB are numbered according to Table 3 and indicated by *bars* in the figure

(Cowen 1989; Stam 1991). In order to achieve empirical significance thresholds for the models used, a permutation analysis was performed with PLABQTL. After 1,000 permutations, LOD thresholds for QTL significance were set to 3.8. R^2 values of QTL-linked markers were taken from the simultaneous fit function of PLABQTL.

Table 1 Classification of functional genes mapped in sugar beet according to metabolic pathways

Metabolic pathway	Number of gene markers
Photosynthetic reactions	10
Calvin cycle	10
Oxidative pentose phosphate cycle	1
Glycolysis	11
Citric acid cycle	2
Photorespiration	5
Starch metabolism	9
Sucrose synthesis	1
Transport processes of assimilates and ions	18
Sucrose degradation	3
Protection against oxidative stress	1
Cell wall synthesis	3
Nitrogen metabolism	6
Betaine synthesis	2
Cell cycle and cytoskeleton assembly	4
Others	4

Results

Construction of a map incorporating genes involved in specific metabolic pathways

Based on the DNA sequence information of the corresponding ESTs, 100 genes with functions in metabolic pathways related to sugar quality and metabolism were analysed for the presence of polymorphisms in the parents of mapping populations. Ninety such loci (Table 1) were found to be polymorphic in at least one mapping population and assigned to the linkage groups of the sugar beet genome (Schneider et al. 1999, http://www.mpiz-

Table 2 Phenotypic trait correlations. Phenotypic trait correlations were calculated on the basis of corrected mean values of the entries

Trait correlations	CSY	SC	BY	K	NA	AN	IB
CSY	–	0.47	0.95	–0.27	0.23	–0.44	–0.04
SC		–	0.17	–0.38	–0.41	–0.23	–0.42
BY			–	–0.16	0.40	–0.39	0.11
K				–	0.13	0.67	0.88
NA					–	–0.13	0.49
AN						–	0.33
IB							–

content. The QTLs on chromosomes VI and VII were specific for sugar content only. For α-amino nitrogen, two significant QTLs were identified on chromosomes III and V. The QTL on chromosome III also gave high LOD scores for potassium content, while one QTL specific for this trait was mapped on chromosome VIII. For sodium content, a single QTL was detected at the top of chromosome VII, which coincided with a QTL for ion balance. Two more unique QTLs for ion balance were found, one at the top of chromosome IX and the other in the centre of chromosome V. For all QTL regions, linked expressed gene fragments were identified (Table 3). In order to assess the contribution of those markers to the phenotype, R^2 values for all QTL-linked loci are summarized in Table 4. The QTLs named CSY1, BY1, SC5 and K1 had R^2 values higher than 15% in at least five locations, indicating their relevance irrespective of the environment. For all QTLs, except SC3, K1, K3, K4, NA1, IB2, IB3 and IB4, the high value allele was contributed by the high-yield parent of the cross (Table 3).

Discussion

A genetic map of sugar beet, based on population 618, includes the positions of 75 expressed genes related to sugar metabolism and transport (Fig. 1). To provide a link to previously published genetic maps of sugar beet (Barzen et al. 1992, 1995; Schondelmaier and Jung 1997; Schumacher et al. 1997), 18 RFLP anchors were added to the map. To increase marker density, 99 AFLP markers were also integrated. It is noteworthy that the map length increased only marginally from 417 cM without AFLP to 446 cM with AFLP markers.

In the same population, a total of 21 QTLs were mapped for the traits CSY, BY, SC, AN, K, NA and IB (Table 3). The QTLs identified in our study did not coincide with the QTLs reported earlier for the same traits in

Table 3 QTL data for seven quality traits in sugar beet: CSY (corrected sugar yield), BY (beet yield), SC (sugar content), AN (α-amino nitrogen content), K (potassium content), NA (sodium content) and IB (ion balance) based on PLABQTL analysis. ^QTLs for which the high value allele was derived from the low yield parent

Chromosome	Position (cM)	Average LOD	Name of QTL	Nearest markers/genes
Corrected sugar yield/sugar yield, CSY				
IV	0–5	28	CSY1	*ant*, *cis2*
	32–36	10	CSY2	*mdh*, *pgapdhAchl*, *ppc*, *tlr*
IX	35–45	4	CSY3	*aldchl*, *cis1*, *vp2*, *sc*, *mip4*, M1
Beet yield, BY				
IV	0–5	22	BY1	*ant*, *cis2*
	35–45	8	BY2	*mdh*, *ppc*, *tlr*, M79
Sugar content, SC				
I	0–6	7	SC1	*fbpcyt*, M175
II	30–55	5	SC2	*invcw*
VI	5–10	6	SC3^	M110, *pgm*, *cmo*, xy, M99
VII	20–25	4	SC4	*psI*
IX	32–36	4	SC5	*aldchl*, *cis1*, *vp2*, *sc*, *mip4*
α-Amino-Nitrogen, AN				
III	22–28	6	AN1	*gr*, *gs2b*, *dhl*, M17, *pginh*, *pmc*
V	15–20	4	AN2	*gdh*, *pgi*, M45, *pdb*
Potassium, K				
II	25–55	14	K1^	*rbcS1*, *rbcS4*, *invcw*, *skt*
III	14–25	8	K2	*icdh*, *gr*, M17, *pginh*, *pmc*
VIII	32–45	6	K3^	*sps*, ca
IX	25–35	10	K4^	*mip2*, *tpi*, *aldchl*, *cis1*, *vp2*, *sc*
Sodium, NA				
VII	0–5	4	NA1^	R1
Ion balance, IB				
V	24–35	6	IB1	*cab5*, M40
VII	0–5	13	IB2^	R1
IX	0–5	6	IB3^	*mip1*
IX	35–45	6	IB4^	*aldchl*, *cis1*, *vp2*, *sc*, *mip4*, M1

Table 4 R^2 values of markers linked to significant QTLs. R^2 values were taken from the simultaneous fit function of the PLABQTL program. The mean value refers to the measurement across all locations. The minus score indicates non-significant LOD scores in the respective single location. *For CSY3 the mean value in this analysis was just below the threshold of significance

Trait	QTL	R^2 for QTL-linked loci						
		Mean value	Single locations					
			EIN	FRI	KWL	MOC	SEL	VIS
Corrected sugar yield	CSY1	56.4	56.2	46.1	31.4	50.0	33.6	62.4
	CSY2	16.0	–	–	7.6	–	17.2	19.4
	CSY3	*	–	18.2	13.2	–	–	–
Beet yield	BY1	57.4	39.6	50.2	42.1	40.8	27.3	45.9
	BY2	19.3	–	12.7	–	–	14.7	–
Sugar content	SC1	6.2	–	–	–	–	6.1	–
	SC2	24.2	–	–	–	–	–	–
	SC3	7.2	–	–	–	–	2.7	–
	SC4	24.3	–	–	21.7	21.9	–	–
	SC5	38.6	–	31.6	26.7	26.2	22.5	15.6
Amino nitrogen	AN1	13.5	–	11.0	–	4.7	9.5	–
	AN2	5.2	–	2.6	–	2.7	–	–
Potassium	K1	30.7	19.8	28.0	19.5	–	24.3	30.5
	K2	26.5	18.5	32.0	–	3.1	–	–
	K3	11.0	25.1	–	20.5	–	–	–
	K4	12.6	–	16.4	–	–	–	–
Sodium	NA1	16.4	–	–	–	–	–	20.1
Ion balance	IB1	20.9	–	–	–	–	30.2	–
	IB2	12.8	28.3	–	21.3	–	29.9	–
	IB3	7.3	–	–	19.8	–	18.2	–
	IB4	12.9	13.5	11.5	–	–	–	12.1

koeln.mpg.de/sugarbeet). For 74 genes, polymorphisms were detected by the SSCP technique, for eight loci by Heteroduplex analysis, for five genes by CAPS analysis, and in three cases RFLP hybridization succeeded in revealing polymorphisms. Sixty six genes were assigned to linkage groups in the population 618, and 56 gene markers were integrated into the map in the first and second round of Joinmap (Fig. 1). Twenty nine of these genes were also mapped in the K2 population, and served as anchors between the two populations. A further 19 genes were polymorphic only in the K2 or 704 population and were anchored to linkage groups there. The map positions of these genes were integrated into the map of population 618 as indicated in Fig. 1. Twenty one RFLP markers, which mapped at known positions on linkage groups, but showed no homology to known genes, and one genomic PCR fragment were converted to PCR-based CAPS or SSCP markers and also used as anchors (Schneider et al. 1999, and this report). Apart from three RFLP markers, all were integrated into the map of population 618, leaving a number of gaps larger than 15 cM. An additional 282 dominant AFLP markers were generated, of which 99 could be mapped in population 618 in the first or second round of JOINMAP analysis. Although clustering of the AFLP markers was observed, some gaps in the map could be reduced in size (Fig. 1). The map of population 618 presented here comprises 174 markers, and covers 446 cM of the 758-Mbp genome of sugar beet.

Detection of QTLs

For seven traits, phenotypic data from six locations were evaluated in the testcross progeny of population 618. The traits SC, BY, K, NA and AN, as well as the combined traits CSY and IB, all showed heritabilities higher than 0.8 providing the basis for the QTL mapping. To assess the relationships between the traits, the correlation coefficients were calculated from the trait values (Table 2). The strongest correlations were found between CSY and BY with a value of 0.95, and between IB and K with a value of 0.88.

Significant QTLs were detected for all traits when analysed across all locations (Table 3). For beet yield, one major QTL was identified at the top of chromosome IV, a position which coincides with a major QTL for corrected sugar yield. The latter QTL has a LOD score of 28, which is the most significant found in this study. A second QTL for beet yield was identified on chromosome IV, 35 cM distal from the first one. The position of this QTL partly overlaps that of a QTL for CSY. A third QTL for CSY, whose mean value across locations is on the border of significance, but which is significant in two locations (Table 4), was found on chromosome IX. A QTL for sugar content was found to map to this position as well, and additional QTLs for K and IB overlap in the same region. For SC four more significant QTLs were found on chromosomes I, II, VI and VII. The one on chromosome II overlapped with a QTL for potassium

sugar beet (Weber et al. 1999, 2000). One reason for this is that in the two experiments different populations based on unrelated plant material were used. Moreover, previously mapped QTLs often failed to show consistency across test locations (Weber et al. 1999, 2000), a phenomenon observed for a number of the QTLs in this study also (Table 4). This reveals the strong influence of the environment. A number of QTL regions, especially those mapped on chromosomes II and VIII, extend for more than 10 cM although the QTLs themselves are limited to very small genomic fragments. This is in part due to the low marker density in these regions. The number of plants genotyped and analysed at the phenotypic level, 108 genotypes in this report, may also have restricted the QTL analysis to the most prominent effects (Beavis et al. 1994; Melchinger et al. 1998). A second problem concerns the influence of the tester used to produce the hybrid progeny that were evaluated phenotypically (Melchinger et al. 1998). In fact, alleles derived from the tester, which may vary in the testcross progeny, could obscure QTL effects.

Our study showed that QTLs for different traits frequently overlapped in map position. There are three possible reasons for this. A single gene with pleiotropic effects could account for QTLs for unrelated traits. Alternatively, several tightly linked genes, which due to the small size and therefore limited resolution of the population are detected as only one locus, may each affect a single trait. A third possible explanation is that complex traits, such as corrected sugar yield and ion balance, depend on primary traits and thus reflect the same type of gene action. This possibility is supported by a strong positive trait correlation of 0.95 between CSY and BY (Table 2). It explains the co-localization of the QTL region with the largest effect, CSY1, which was found at the top of chromosome IV, with a region in which a large QTL for beet yield also maps. Similar reasoning applies to a second QTL on chromosome IV, which again affects CSY and BY. The second component which contributes to CSY, sugar content, is most probably responsible for the CSY QTL on chromosome IX. It is noteworthy that, in our study, beet yield and sugar content values were weakly positively correlated with a coefficient of 0.47; this permits the conclusion that in this study CSY can be improved by both an increase in BY and in SC.

It is expected that for traits that positively influence sugar quality and yield, like CSY, SC and BY, high-value alleles derive from the high yield parent and vice versa for traits with negative effects on sugar quality and yield like AN, KA, NA and IB. Some exceptions to this were observed including K2 and SC3 (Table 3). In such cases, the identification and the analysis of recombinants and the generation of plants with only high- or low-value alleles for particular traits may alter our interpretation of the parental origin of positive QTL alleles.

The following discussion concerning potential candidate genes for QTLs related to sugar accumulation is based on consideration of biochemical and physiological data from the literature. For beet yield, a limited set of genes related to energy metabolism like *ant* (adenylate transporter), *cis2* (citrate synthase), *mdh* (malate dehydrogenase) or *ppc* (phosphoenolpyruvate carboxylase) appear to be promising candidates for QTLs because of their linkage to BY and CSY (Table 3). With regard to sugar content, a promising candidate gene is *invcw* (cell wall-bound invertase), which has already been shown to contribute to the content of soluble sugar in tomato (Fridman et al. 2000). A second interesting candidate gene for the same trait is *sc* (sucrose carrier) located on chromosome IX. With regard to α-amino nitrogen, the QTL on chromosome III could be related to the linked *gs2b* locus which encodes chloroplastic glutamine synthetase, a key enzyme in nitrogen metabolism. For other traits, like K and IB, gene products involved in water homoeostasis and transport processes, such as those of the *mip* (major intrinsic protein) family, *vp2* (vacuolar pyrophosphatase) and *skt* (putative potassium channel) may be proposed as candidates.

We emphasize that our genetic map is not at all saturated with EST markers. Moreover, a fraction of the genes selected for the study were found to be monomorphic and could not be mapped. Sequence variation in regions of these genes not yet scanned, such as the promoter regions, could still contribute to QTL effects. In addition, all genes currently mapped participate in primary metabolic pathways, and while some of them can indeed contribute to phenotypic differences (Causse et al. 1995; Fridman et al. 2000), gene products involved in regulatory pathways should also influence agronomic traits (McMullen et al. 1998; Mitchell-Olds and Pedersen 1998).

For our candidate genes, validation experiments at the physiological, genetic and expression level are planned. The logic behind this approach is that if a gene product is present in the organ in which the trait is measured, and if the allelic variant of one parent out-performs the other allelic variant, that gene is much more likely actually to represent the QTL in question. Final proof can then be provided by the analysis of contrasting alleles in near-isogenic lines or complementation experiments.

The recent discovery that allelic haplotypes exist for expressed genes of sugar beet (Schneider et al. 2001), raises the possibility of mapping QTL candidate genes by association studies, as has been done in *Drosophila* (Stam and Laurie 1996) and applied in human genetics (Schork et al. 1998; McCarthy and Hilfiker 2000; Taillon-Miller et al. 2000). This approach, based on discontinuous SNP scanning, may allow an in-depth analysis of the role of the potential candidate genes identified in this study.

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