Cold Sweetening in Diploid Potato: Mapping Quantitative Trait Loci and Candidate Genes

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ABSTRACT

A candidate gene approach has been used as a first step to identify the molecular basis of quantitative trait variation in potato. Sugar content of tubers upon cold storage was the model trait chosen because the metabolic pathways involved in starch and sugar metabolism are well known and many of the genes have been cloned. Tubers of two F_1 populations of diploid potato grown in six environments were evaluated for sugar content after cold storage. The populations were genotyped with RFLP, AFLP, and candidate gene markers. QTL analysis revealed that QTL for glucose, fructose, and sucrose content were located on all potato chromosomes. Most QTL for glucose content mapped to the same positions as QTL for fructose content. QTL explaining >10% of the variability for reducing sugars were located on linkage groups I, III, VII, VIII, IX, and XI. QTL consistent across populations and/or environments were identified. QTL were linked to genes encoding invertase, sucrose synthase 3, sucrose phosphate synthase, ADP-glucose pyrophosphorylase, sucrose transporter 1, and a putative sucrose sensor. The results suggest that allelic variants of enzymes operating in carbohydrate metabolic pathways contribute to the genetic variation in cold sweetening.

THE accumulation of free sugars in plants exposed to low temperatures is a widespread phenomenon that has long been recognized (Müller-Thurgau 1882). The type of sugars accumulated is species dependent (Guy *et al.* 1992). Storage organs, such as potato tubers, accumulate both reducing sugars (glucose and fructose) and sucrose when subjected to chilling temperatures, a phenomenon known as cold sweetening (Burton 1969). Dormant potato tubers are metabolically inactive except for slow starch degradation and synthesis of sucrose with energy provided by glycolysis and respiration. Cold sweetening is explained as a shift in the balance between starch degradation and glycolysis, leading to the accumulation of sucrose (Isherwood 1973), which is then converted into glucose and fructose. Although the metabolic pathways and enzymes involved in starch-hexose interconversion are well characterized, little is known about their individual contribution to cold sweetening and *in vivo* regulation (Greiner *et al.* 1999).

Reducing sugar content in cold-stored potatoes is a major problem for the potato processing industry since the industry favors storing tubers at temperatures <10° to delay sprouting. The high frying temperature used for the production of potato chips and french fries causes a nonenzymatic Maillard reaction between free aldehyde groups of reducing sugars and free α-amino groups of amino acids and proteins (Talburt *et al.* 1975), which results in a dark and bitter product. The correlation between the reducing sugar content of tubers and the extent of browning during processing has been documented (Coffin *et al.* 1987; Scheffler *et al.* 1992).

The sugar content of potato tubers is a quantitative trait with heritability values ranging from very high (0.91; Grassert *et al.* 1984) to intermediate (0.47–0.63; Pereira *et al.* 1994). Quantitative traits can be genetically dissected using linkage maps that are based on molecular markers (reviewed by Tanksley 1993; Stuber 1995). Quantitative trait locus (QTL) analysis in plants is performed in segregating populations generated by experimental crosses. Because the cultivated potato is a tetraploid displaying tetrasomic inheritance, diploid potato has been used to generate several molecular linkage maps for the species (Bonierbale *et al.* 1988; Gebhardt *et al.* 1989, 2001; Jacobs *et al.* 1995; Van Eck *et al.* 1995; Milbourne *et al.* 1998). These molecular maps have been instrumental for locating a number of QTL for tuber-associated traits (Freyre *et al.* 1994; Van Eck *et al.* 1994; Van Den Berg *et al.* 1996; Schäfer-Pregl *et al.* 1998). One QTL study addressed the cold-sweetening phenomenon by measuring chip color (Douches and Freyre 1994).

Despite the importance of quantitative genetic variation in many areas of plant biology, there is little understanding of the molecular basis that controls this varia-

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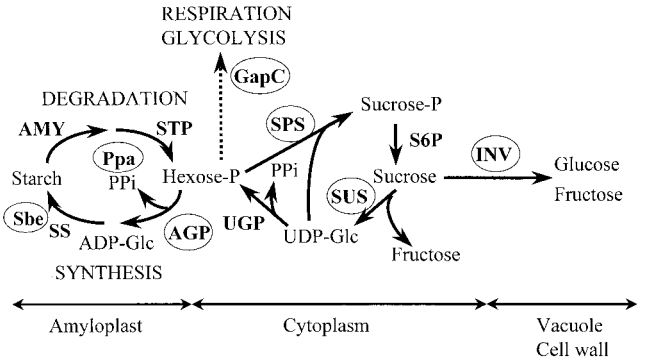


FIGURE 1.—Schematic diagram for sugar/starch metabolism in potato tubers (modified after SOWOKINOS 2001). Enzymes included are ADP-glucose pyrophosphorylase (*AGP*), starch synthase (*SS*), starch branching enzyme (*Sbe*), starch phosphorylase (*STP*), amylase (*AMY*), glyceraldehyde 3-phosphate dehydrogenase (*GapC*), sucrose phosphate synthase (*SPS*), sucrose 6-P phosphatase (*S6P*), UDP-glucose pyrophosphorylase (*UGP*), sucrose synthase (*SUS*), invertase (*INV*), and pyrophosphatase (*Ppa*). Circles indicate the enzymes for which encoding genes have been mapped in H94A or H94C. For map positions of other genes involved in carbohydrate metabolism and transport see CHEN *et al.* (2001).

tion (MITCHELL-OLDS and PEDERSEN 1998). QTL maps are the first step toward the identification of genes responsible for QTL, either by positional cloning (FRIDMAN *et al.* 2000; FRARY *et al.* 2000) or by a candidate gene approach. The candidate gene approach can follow two strategies (PFLIEGER *et al.* 2001). One strategy selects genes that are known to be functionally relevant for the trait of interest and tests their allelic polymorphisms for association with trait variation. Finding an association between a candidate gene and the trait may indicate a causal role in trait variation (LANDER and SCHORK 1994; PRIOUL *et al.* 1999). The second strategy compares map positions of candidate genes with positions of QTL for the trait of interest to determine if they map to the same genomic region. Further studies are then required such as linkage disequilibrium mapping (LANDER and SCHORK 1994; TALBOT *et al.* 1999; MEUWISSEN and GODDARD 2000), QTL analysis of transcript and protein levels (CAUSSE *et al.* 1995), and, finally, complementation analysis to validate the causal role of a candidate gene.

The cold-sweetening trait is particularly suitable for testing the feasibility of the candidate gene approach in plants. The metabolic pathways involved in sugar metabolism are limited and well known. Carbohydrate metabolism has been thoroughly studied in potato and many genes have been cloned and characterized (reviewed in FROMMER and SONNEWALD 1995; STITT and SONNEWALD 1995). Major enzymatic reactions involved in the formation of glucose, fructose, and sucrose in potato tubers are outlined in Figure 1. Moreover, a potato molecular function map including genes for carbohydrate metabolism and transport has been constructed (CHEN *et al.* 2001). This map includes genes relevant to sugar production, such as those encoding invertases, sucrose phosphate synthase, and sucrose synthases.

The objective of this work was to identify QTL for cold sweetening in the potato genome and to evaluate specific metabolic genes as candidate genes, as a first step toward identifying genes controlling this economically important trait.

MATERIALS AND METHODS

Plant material: Two diploid F_1 populations, H94A and H94C, were used. Population H94A resulted from crossing *Solanum tuberosum* line H81.839/1, selected for its low reducing sugar content (RSC; P_A = P54, seed parent; RSC = 0.09% ± 0.01), with line H80.696/4 (P_B = P40, pollen parent; RSC = 0.26% ± 0.05). The H94A mapping population consisted of 146 F_1 genotypes. Population H94C was derived from crossing the *S. tuberosum* line H82.337/49 (P_A = P18, seed parent; RSC = 0.51% ± 0.19) with line H82.2032/1 (P_B = P50, pollen parent; RSC = 1.83% ± 0.59). Both parents were unselected for reducing sugar content. A total of 189 F_1 hybrids were genotyped in this population. The parental lines of both mapping populations were highly heterozygous (GEBHARDT *et al.* 1989). The first tuber generation was obtained from seedling plants grown in pots in the greenhouse. Tubers were multiplied in pots under Saran cover, to obtain virus-free seed tubers for field trials.

Field trials and experimental design: In 1996, populations H94A and H94C were grown at two locations in Germany: Carolinensiel, an experimental field close to the North Sea coast, and Scharnhorst, the Max-Planck-Institute's field station. At Carolinensiel, 10 tubers per clone were planted in a row in three replications. This location was also used for seed tuber propagation due to low aphid pressure in this region. At Scharnhorst, 10 tubers per clone were planted in a row without replication (Table 1). Spacing was 75 cm between rows and 40 cm between plants. Parental clones and eight commercial cultivars were included as standards in each trial. To minimize border effects, the first and last plants in each row were excluded from phenotypic analysis and the trials were surrounded by a guard row of potato plants.

In the 1997 trials, both populations were grown at three different locations: Carolinensiel and Cologne in Germany and Vitoria in Spain. Seed tubers came from the Carolinensiel field in 1996. One-row plots with six tubers per genotype were planted in a completely randomized block design with three replications at all locations (Table 1). Other than that, field trials were conducted as in 1996. Depending on year and location, between 109 and 144 genotypes of population H94A and between 126 and 171 genotypes of the H94C population were evaluated in the field (Table 2).

Tuber storage and measurement of glucose, fructose, and sucrose content: Potato tubers were harvested and stored at 4° for 3 months. After cold storage, three to four random tubers per genotype were washed, peeled, freeze dried, and ground to a fine powder. Sugars were extracted from 100 mg dry powder according to VIOLA and DAVIES (1992) with minor modifications. Glucose (G), fructose (F), and sucrose (S) content were measured by a coupled enzymatic assay (Boehringer Mannheim, Mannheim, Germany) following the supplier's instructions. Sugar content of samples was determined by measuring NAPD reduction spectrophotometrically at 340 nm using a microtiter plate reader (Labsystems, Germany) and the SCA4 software (Merlin, Germany) on the basis of standard curves. Sugar content was obtained as percentage of dry tuber weight (μg/100 μg dry weight).

TABLE 1

Summary of experiments conducted for populations H94A and H94C

Environment	Environment code[a]	No. of replications	No. of plants per clone and replication	Sugar content measured after cold storage[b]	
				H94A	H94C
Carolinensiel 96	1	3	10	✓	✓
Carolinensiel 97	2	3	6	✓	✓
Cologne 97	3	3	6	✓	✓
Vitoria 97	4	3	6	✓	✓
Greenhouse 95	5	1	10	✓	✓
Scharnhorst 96	6	1	10	✓	✓

[a] The codes assigned to each environment are used in Tables 2–4 and in the Supplementary Table.
[b] Sugar content corresponds to individual measurements of glucose, fructose, and sucrose content.

DNA markers and map construction: Restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) markers were used for genotyping populations H94A and H94C. RFLP anchor markers were chosen from previous potato maps (Gebhardt *et al.* 1989, 2001) on the basis of polymorphism between parents and map position. Populations H94A and H94C were genotyped with 42 and 29 RFLP anchor markers, respectively, 23 of which were mapped in both populations. The RFLP markers included the following genes related to starch and hexose metabolism or transport: ADP-glucose pyrophosphorylase S and B (*AGPaseS*, *AGPaseB*), starch branching enzyme I (*SbeI*), glyceraldehyde 3-phosphate dehydrogenase (*GapC*), apoplastic invertase (Inv_{ap}), soluble inorganic pyrophosphatase 1 (*Ppa1*), sucrose transporter 1 (*Sut1*), and a putative sucrose sensor (*Sut2*). In addition, population H94A was genotyped with cleaved amplified polymorphic sequence (CAPS) markers for sucrose phosphate synthase (*Sps*) and sucrose synthase 3 (*Sus3*; Chen *et al.* 2001). RFLP analysis was done as described before (Gebhardt *et al.* 1989).

To increase genome coverage, AFLP analysis was performed according to Vos *et al.* (1995) with *Hin*dIII/*Mse*I and *Eco*RI/*Mse*I adaptors. The same nine primer combinations with extensions of three nucleotides (E + 3, H + 3, and M + 3 primers) were used for the selective amplification of fragments in both populations. The *Hin*dIII/*Mse*I primer combinations were H + AAT combined with either M + ACA (HM1) or M + ACC (HM2) and H + ACC combined with M + AAT (HM3) or M + ACA (HM4) or M + ACT (HM5). The *Eco*RI/*Mse*I primer combinations were E + AAC combined with M + CAG (EM1), E + ACA with M + CAT (EM2), and E + ACT combined with either M + CAA (EM3) or M + CAT (EM4). Designation of AFLP markers was based on the primer combination and on an arbitrary identification number assigned to each individual AFLP fragment. AFLP markers segregating in populations H94A and H94C were numbered independent from each other. No attempt was made to identify identical AFLP fragments in the two populations.

Linkage maps for the 24 chromosomes of diploid potato ($2n = 2x = 24$) were constructed for each mapping population as previously described (Ritter *et al.* 1990; Schäfer-Pregl *et al.* 1998).

Statistical and QTL analyses: A subset of 171 and 188 marker fragments was selected for QTL mapping, covering most of the genetic maps of populations H94A and H94C, respectively. The phenotypic values for the traits glucose, fructose, and sucrose content per line in six environments (years and locations, Table 1) were obtained as means of three to four tubers per replication. These mean values were used in the QTL analyses. All traits were analyzed separately in each of the six environments.

The association between phenotype and marker genotype was investigated with both a *t*-test and interval analysis using SAS software (SAS Institute 1990). Results from both methods were in good agreement and, therefore, only results from the single marker analysis are reported. $P < 0.01$ was the exclusion threshold for declaring the presence of a QTL linked to a marker locus. In most cases, a QTL was detected at several, closely linked markers. To account for the variability of QTL position due to mapping uncertainty, putative QTL were allocated to map sections ("bins") on the basis of the two most distal significant marker loci when considering all environments. The size of the bins is shown in Table 3 and in Figures 2 and 3.

Analysis of variance was performed at single-marker loci among the two or four phenotypic means, depending on the number of marker genotypic classes distinguishable at each marker locus (Ritter *et al.* 1990; Schäfer-Pregl *et al.* 1998), using the GLM procedure of SAS. The proportion of the observed phenotypic variance attributable to a particular QTL was estimated by the coefficient of determination (R^2) from a linear model analysis.

Chi-square goodness-of-fit tests were used to test single-marker segregation against the expected 1:1 or 3:1 ratios.

A statistical test for overlapping by chance between QTL for the same trait in different environments was conducted following the procedure of Grube *et al.* (2000). We assumed that for each parental map and each environment, the QTL were independent and occupied single map bins. On the basis of an average length of 750 cM of the four parental maps and the average size of the map section covered by a QTL (Table 3), the map was divided into 50 equal bins of 15 cM length. The probability that QTL for one trait are found by chance in the same bins in any two environments was calculated, using the hypergeometric probability distribution (Bain and Engelhardt 1992). Probabilities associated with coincidences in three or more environments were computed in the same way, taking into account only conditional probability rules. In an analogous manner, probabilities of overlaps by chance between QTL for different traits across environments and between QTL and candidate genes were estimated.

RESULTS

Evaluation of sugar content: Tuber sugar content after cold storage was evaluated in populations H94A

TABLE 2

Statistical parameters of sugar content after cold storage and number of clones (*N*) analyzed for populations H94A and H94C

		H94A			H94C		
Trait	Environment code[a]	*N*	Sugar content[b]	Range of variation	*N*	Sugar content[b]	Range of variation
Glucose	1	144	1.56 (0.81)	0.20–4.39	147	0.94 (0.51)	0.11–2.45
	2	109	1.08 (0.63)	0.09–3.03	126	1.16 (0.52)	0.25–2.50
	3	132	0.97 (0.55)	0.07–3.33	137	0.84 (0.45)	0.07–2.10
	4	117	0.80 (0.58)	0.01–2.79	153	0.88 (0.43)	0.02–2.45
	5	132	0.39 (0.39)	0.01–2.22	169	0.42 (0.37)	0.01–1.93
	6	125	0.90 (0.73)	0.06–3.75	153	0.55 (0.48)	0.05–3.60
Fructose	1	144	1.48 (0.69)	0.24–3.84	147	1.02 (0.49)	0.04–2.54
	2	109	1.17 (0.57)	0.22–2.92	126	1.35 (0.47)	0.28–2.53
	3	132	1.28 (0.62)	0.24–3.29	137	1.30 (0.55)	0.20–3.01
	4	117	1.14 (0.58)	0.07–3.16	153	1.17 (0.46)	0.06–2.40
	5	132	0.54 (0.45)	0.01–2.45	169	0.62 (0.41)	0.03–1.79
	6	125	1.25 (0.78)	0.13–4.15	153	0.82 (0.56)	0.14–3.55
Sucrose	1	143	1.67 (0.81)	0.44–4.51	147	1.29 (0.64)	0.52–4.16
	2	109	2.84 (1.62)	0.63–7.21	126	1.89 (1.02)	0.61–6.41
	3	132	1.33 (0.81)	0.32–4.56	137	1.17 (0.51)	0.5–3.88
	4	117	1.30 (0.81)	0.41–7.22	153	1.99 (1.06)	0.51–7.05
	5	126	0.97 (0.57)	0.23–4.47	169	0.73 (0.25)	0.25–2.60
	6	122	0.91 (0.49)	0.20–2.51	153	0.79 (0.33)	0.05–2.29

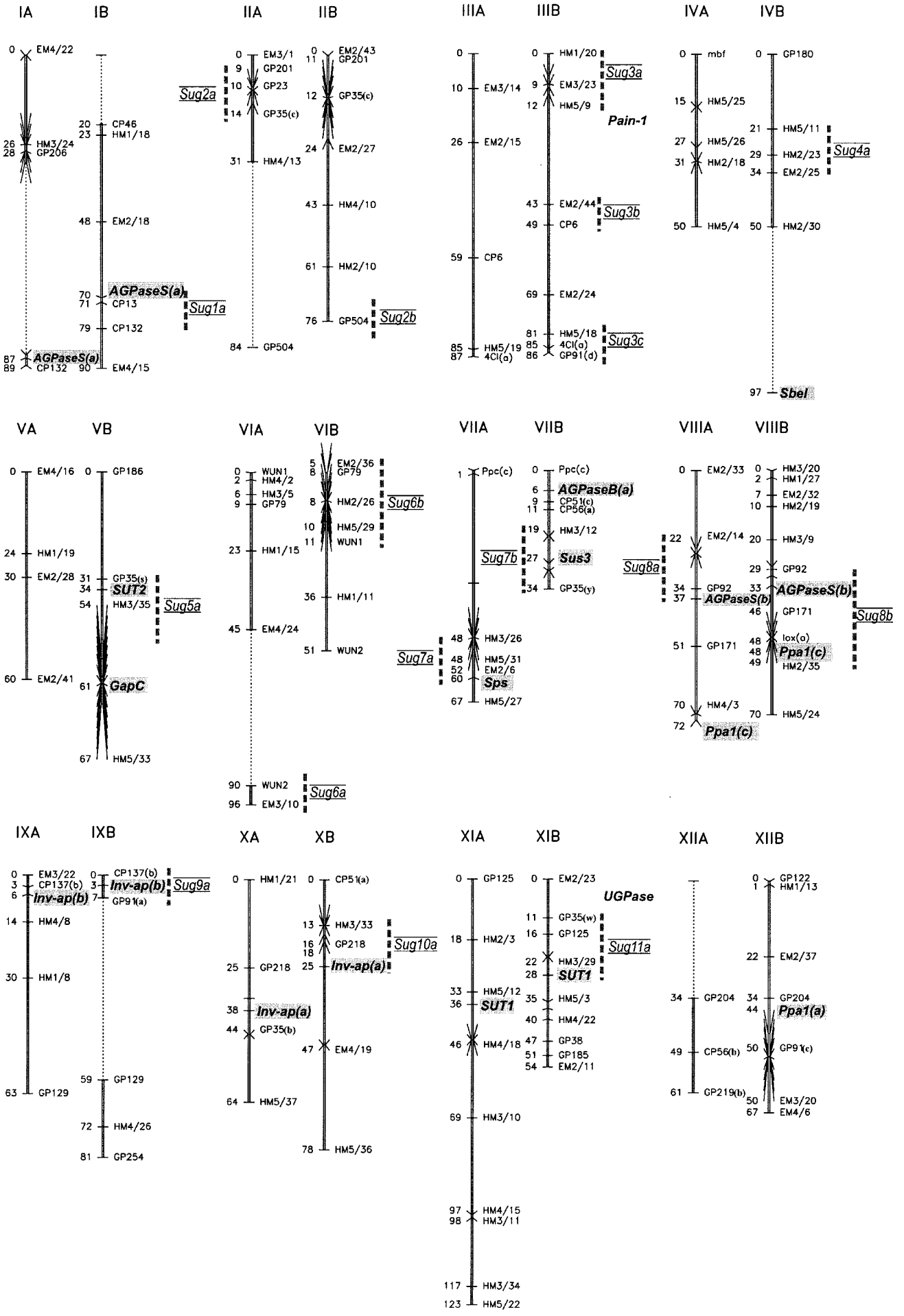
[a] Environment codes are as shown in Table 1.
[b] Values correspond to average sugar content (percentage dry weight). Standard deviations are in parentheses.

and H94C over 3 years at four locations, resulting in six environments (Table 1). Table 2 shows population means, standard deviations, and ranges of sugar content (percentage dry weight) of glucose, fructose, and sucrose. Reducing sugar content was lower in greenhouse-grown tubers than in the field (Table 2, environment 5).

Sugar contents were approximately normally distributed in the populations and showed transgressive segregation in all environments (not shown). Based on the ranges observed for sugar contents, less phenotypic variability was present in population H94C when compared to H94A (Table 2). Glucose and fructose contents of tubers after cold storage were highly correlated in all environments, with phenotypic correlations ranging from 0.89 to 0.93. Correlations in sugar content of tubers grown in different field environments were lower, but still highly significant with values ranging from 0.50 to 0.77. Correlations in sugar content of tubers grown in pots and in the field varied from 0.53 to 0.70 for H94A and between 0.19 and 0.35 for H94C.

Maps of populations H94A and H94C: Twenty-four linkage groups, 12 for each parent, were constructed for populations H94A and H94C on the basis of 433 and 447 RFLP and AFLP marker fragments, respectively (Figures 2 and 3). The level of heterozygosity was high in the parents. Only clone P54 (P_A of population H94A) appeared to be less heterozygous than the other parental lines, on the basis of the smaller number of segregating fragments descending from that parent. Marker distribution on the linkage groups was uneven, mainly due to clustering of AFLP markers. Genome coverage was incomplete in the H94A map in regions of linkage groups IA, IIA, VIA, and XIIA of parent P54 (P_A) and IB, IVB, and IXB of parent P40 (P_B). In the H94C map, gaps

FIGURE 2.—QTL and linkage maps of population H94A. Linkage groups A and B are derived from the parents P54 and P40, respectively. Allelic bridges (RITTER *et al.* 1990) linking the parental linkage groups are not shown. HM*/* and EM*/* are AFLP markers obtained with *Hin*dIII/*Mse*I and *Eco*RI/*Mse*I primer combinations, respectively. CP* and GP* are RFLP markers. Gene markers (further details in CHEN *et al.* 2001; GEBHARDT *et al.* 2001) included are ADP-glucose pyrophosphorylase S and B (*AGPaseS*, *AGPaseB*), 4-coumarate: CoA ligase (*4Cl*), *ocs*-like bZIP-binding element (*mbf*), starch-branching enzyme I (*SbeI*), putative sucrose sensor (*Sut2*), glyceraldehyde 3-phosphate dehydrogenase (*GapC*), wound-induced genes 1 and 2 (*WUN1*, *WUN2*), phosphoenolpyruvate carboxylase (*Ppc*), sucrose phosphate synthase (*Sps*), sucrose synthase 3 (*Sus3*), pyrophosphatase 1 (*Ppa1*), lipoxygenase (*Lox*), apoplastic invertase (*Inv-ap*), and sucrose transporter 1 (*Sut1*). Loci detected by genes functional in starch and hexose metabolism or transport are shaded gray. Candidate genes *Pain-1* and *UGPase* were mapped in a different population (CHEN *et al.* 2001) and are shown at their approximate positions. Lowercase letters in parentheses indicate that more than one locus was detected with the same marker probe. Linkage group regions where markers were significant in the *t*-test are indicated by dotted lines and labeled with QTL names according to Table 3.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Sug11a
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AGPaseS(b)
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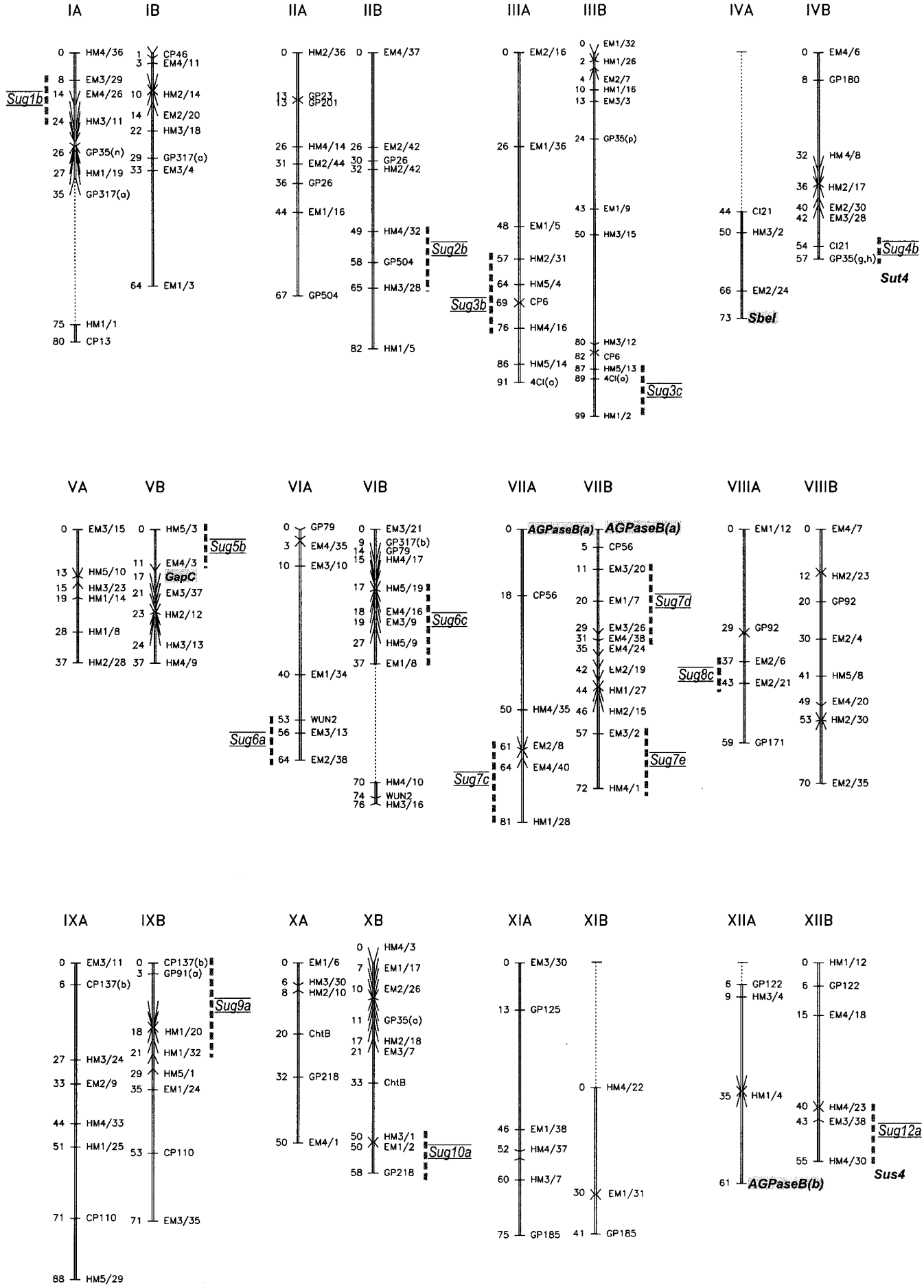
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Sug3c
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Sut4
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Sug7a
Sug7b
Sug7c
Sug7d
Sug7e
Sug8c
Sug9a
Sug10a
Sug12a
Sus4
AGPaseB(a)
AGPaseB(b)

were present on linkage groups IA and IVA of parent P18 (P_A) and on VIB and XIB of parent P50 (P_B; dotted lines in Figures 2 and 3). Deviations from the expected segregation ratios were observed on several linkage groups (not shown).

QTL for sugar content: Glucose, fructose, and sucrose contents were analyzed separately in each of the six environments. QTL significant at $P < 0.01$ are shown in Table 3. The sugar QTL were named according to the chromosome to which they mapped followed by a lowercase letter. Different letters indicate putatively different QTL located on the same chromosome. QTL linked to the same RFLP anchor marker on different linkage groups of the same chromosome were given identical names.

QTL analysis of glucose content of cold-stored tubers detected seven QTL in three to six environments (*Sug3b*, *Sug3c*, *Sug5b*, *Sug6c*, *Sug7b*, *Sug8a*, *Sug8b*) in populations H94A and H94C (Table 3). The most significant QTL were *Sug3b*, *Sug7b*, and *Sug8b*, which exhibited R^2 values of up to 24.5, 15.6, and 16.1%, respectively (data shown in Supplementary Table). On the basis of linkage to anchor RFLP loci, QTL in corresponding genomic positions in both populations were identified on linkage groups III [*Sug3c*, linked to *4Cl(a)*] and X (*Sug10a*, linked to *GP218*; Figures 2 and 3, Table 3).

Eight QTL for fructose content of cold-stored tubers were identified in three or more environments in the two populations. Six of these eight QTL were coincidental with QTL detected for glucose content (*Sug3b*, *Sug5b*, *Sug6c*, *Sug7b*, *Sug8a*, and *Sug8b*). The two additional QTL were found on chromosomes I and IX (*Sug1a* and *Sug9a*, respectively; Table 3). The most significant QTL mapped to chromosomes III, VII, VIII, and IX: *Sug3b*, *Sug7b*, *Sug8b*, and *Sug9a* explained up to 26, 15.8, 14.1, and 14.5% of the phenotypic variance, respectively (Supplementary Table). QTL *Sug9a* was found in corresponding bins of the maps of both populations on the basis of the closely linked anchor markers *CP137(b)* and *GP91(a)*.

The linkage observed between most QTL for fructose and glucose content in populations H94A and H94C (Table 3 and Figures 2 and 3) corroborated the high phenotypic correlations found between both reducing sugars.

Only 3 (*Sug2b*, *Sug7b*, and *Sug9a*) of 17 putative QTL for sucrose content mapped in populations H94A and H94C were reproducible in more than two environments (Table 3). The phenotypic variances explained by single markers at these QTL were up to 10.2, 9.4, and 7%, respectively (Supplementary Table). In addition, QTL *Sug2b* (linked to *GP504*) and *Sug9a* [linked to *CP137(b)*] were located in similar positions in both populations (Table 3, Figures 2 and 3).

Six QTL for sucrose content (*Sug7b*, *Sug7c*, *Sug7e*, *Sug8a*, *Sug9a*, and *Sug12a*) overlapped with QTL for glucose or fructose content (Table 3).

Test for overlaps by chance between QTL across environments, traits, and candidate genes: Overlaps were observed between QTL for the same trait in different environments, between QTL for different traits across environments, and between QTL and candidate gene loci. The number of QTL detected per parental map, trait, and environment varied from one to six. The number of QTL for glucose, fructose, or sucrose per parental map across all six environments was between two and eight (Figures 2 and 3, Table 3).

The probabilities for overlaps by chance between QTL for one trait in two environments ranged from 0.15 for sucrose QTL *Sug6b* to 6.3×10^{-8} for fructose QTL *Sug3a* and *Sug5a*. The probabilities for overlaps between QTL for two traits ranged from 0.16 for fructose and sucrose content in population H94A to 5.1×10^{-5} for glucose and fructose content in H94C. All overlaps between QTL for glucose and fructose content had probabilities of having occurred by chance of <0.0008.

Probabilities that QTL were detected by chance in the same map bin in three environments ranged from 0.019 for sucrose QTL *Sug9a* to 1.2×10^{-7} for glucose QTL *Sug3c*. The probability for overlaps between QTL in four or more environments was $<10^{-5}$. Probabilities calculated for overlaps by chance between the three traits considering all environments ranged from 0.019 for P_A in population H94C to 0.0013 for P_A in population H94A.

Similarly, probabilities for overlaps by chance between sugar QTL and candidate gene loci in population H94A were calculated, on the basis of 13 candidate gene loci detected by 10 gene markers (see below and Figure 2). The probabilities for coincidence by chance between glucose and fructose QTL and candidate gene loci were 0.002 and 0.0006, respectively, and 0.3 for sucrose QTL and candidate gene loci.

Candidate genes: Ten potato genes with known map position (Chen *et al.* 2001) and known function in carbon metabolism or transport were mapped. At least 4 genes (for invertase, AGPase subunits S and B, and pyrophosphatase) are encoded by small gene families. Markers for these genes detected, therefore, more than one locus in the potato genome (Chen *et al.* 2001). The

Figure 3.—QTL and linkage maps of population H94C. Linkage groups A and B are derived from the parents P18 and P50, respectively. Other nomenclature is similar to Figure 2. Gene markers included are ADP-glucose pyrophosphorylase B (*AGPaseB*), 4-coumarate: CoA ligase (*4Cl*), cold-induced gene (*Ci21*), glyceraldehyde 3-phosphate dehydrogenase (*GapC*), wound-induced gene 2 (*WUN2*), basic class I chitinase (*ChtB*). Candidate genes *Sut4* and *Sus4* were mapped in a different population (Chen *et al.* 2001) and are shown at their approximate positions.

TABLE 3

Map position and reproducibility of QTL for sugar content detected in populations H94A and H94C

Linkage groups	QTL[a]	Population	Map position[b]	Significant for trait and environment[c]		
				Glucose	Fructose	Sucrose
I	*Sug1a*	H94A	IB 70–79	3	1, 2, 3, 5	
	Sug1b	H94C	IA 8–24			4, 5
II	*Sug2a*	H94A	IIA 9–14			5
	Sug2b	H94A	IIB 76	1		5
	Sug2b	H94C	IIB 49–65			2, 3, 4
III	*Sug3a*	H94A	IIIB 0–12		3, 5	
	Sug3b	H94A	IIIB 43–49			2, 4
	Sug3b	H94C	IIIA 57–76	2, 3, 4	1, 2, 3, 4, 5	
	Sug3c	H94A	IIIB 81–86	2, 4, 5		
	Sug3c	H94C	IIIB 87–99	2		2, 4
IV	*Sug4a*	H94A	IVB 21–34			3
	Sug4b	H94C	IVB 54–57		6	
V	*Sug5a*	H94A	VB 31–54		3, 5	
	Sug5b	H94C	VB 0–11	3, 4, 5	1, 4, 5	
VI	*Sug6a*	H94A	VIA 90–96			1
	Sug6a	H94C	VIA 53–64	5	5	
	Sug6b	H94A	VIB 5–11			4, 5
	Sug6c	H94C	VIB 17–37	1, 2, 4	1, 4, 5	
VII	*Sug7a*	H94A	VIIA 48–60	1	1, 2	
	Sug7b	H94A	VIIB 19–34	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	1, 3, 5
	Sug7c	H94C	VIIA 61–81	3, 5	2, 3	5
	Sug7d	H94C	VIIB 11–31	4	1, 4	
	Sug7e	H94C	VIIB 57–72	1, 6	1	4
VIII	*Sug8a*	H94A	VIIIA 22–37	1, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	3, 5
	Sug8b	H94A	VIIIB 29–49	1, 2, 3, 4, 5	1, 2, 3, 4, 5, 6	
	Sug8c	H94C	VIIIA 37–43			4
IX	*Sug9a*	H94A	IXB 0–7	1, 5	1, 3, 4, 5, 6	2, 3, 5
	Sug9a	H94C	IXB 0–21		3	3
X	*Sug10a*	H94A	XB 13–25	1, 3		
	Sug10a	H94C	XB 50–58	3		
XI	*Sug11a*	H94A	XIB 11–28	1, 2	1	
XII	*Sug12a*	H94C	XIIB 40–55	4	4, 5	5

[a] QTL are numbered on the basis of chromosome number. Different lowercase letters indicate putatively different QTL located on the same chromosome. QTL linked to the same RFLP anchor marker on different linkage groups of the same chromosome have identical names.

[b] Uppercase letters A and B indicate linkage groups derived from the parents P54 and P40 for population H94A and P18 and P50 for population H94C, respectively. QTL position is given as the map section in centimorgans that contains marker loci with significant effect.

[c] Numbers refer to environments in which QTL were detected for a given trait with $P \leq 0.01$ (for codes see Table 1).

10 gene markers detected 14 loci in the H94A and H94C maps (Figures 2 and 3). The positions were consistent with published function maps (Chen *et al.* 2001; Gebhardt *et al.* 2001), with the exception of an additional locus detected for *Ppa1* on linkage group VIII [*Ppa1(c)*].

The candidate gene loci were tested for their effects on sugar content after cold storage. Eight loci [*AGPaseS (a)*, *Sut2*, *Sus3*, *Sps*, *AGPaseS(b)*, *Inv-ap(b)*, *Inv-ap(a)*, and *Sut1*] showed QTL effects on two or even all three sugar traits at different significance levels in population H94A (Table 4). The phenotypic variance explained by individual candidate loci linked to the more stable QTL (significant in three or more environments) was >10%. For example, *AGPaseS(a)* explained up to 12.5 and 13.4% of the phenotypic variance for glucose and fructose, respectively, at *Sug1a*. *Sus3* explained up to 15.1 and 13.3% of the variance of the same sugars at *Sug7b*. Candidate loci *AGPaseS(b)* and *Inv-ap(b)* accounted for up to 13.3 and 7.5% of the variance for glucose and up to 13.9 and 14.5% for fructose at *Sug8b* and *Sug9a*, respectively (Table 4). The candidate genes were the best predictors of phenotypic variance at QTL on chromosomes I [*AGPaseS(a)*], V (*Sut2*), IX [*Inv-apo(b)*], X [*Inv-apo(a)*], and XI (*Sut1*).

DISCUSSION

Factors controlling cold sweetening are located on all potato chromosomes: QTL analysis revealed the polygenic nature of the cold-sweetening phenomenon in

TABLE 4

Analysis of variance for sugar content at candidate gene loci linked to QTL in population H94A

Linkage groups	QTL	Candidate gene locus	Significant for trait[a]			R^{2b}
			$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.001$	
I	*Sug1a*	*AGPaseS(a)*	G 1, 2, 4, 5, 6		G 3	13.4
	Sug1a	*AGPaseS(a)*	F 4, 6	F 2	F 1, 3, 5	12.5
V	*Sug5a*	*Sut2*		F 3, 5		5.8
	Sug5a	*Sut2*	S 5			4.0
VII	*Sug7a*	*Sps*	G 3	G 1		5.1
	Sug7a	*Sps*	F 1, 3			5.0
	Sug7a	*Sps*	S 5			4.2
	Sug7b	*Sus3*	G 6	G 2, 3, 4	G 1, 5	15.1
	Sug7b	*Sus3*	F 3	F 1, 2, 6	F 4, 5	13.3
	Sug7b	*Sus3*			S 3	12.0
VIII	*Sug8b*	*AGPaseS(b)*	G 1, 3	G 2, 4		13.3
	Sug8b	*AGPaseS(b)*	F 1, 3, 5	F 2, 4		13.9
IX	*Sug9a*	*Inv-ap(b)*	G 3, 4, 6	G 1, 5		7.5
	Sug9a	*Inv-ap(b)*	F 6	F 1, 3, 4	F 5	14.5
	Sug9a	*Inv-ap(b)*	S 2, 3, 5, 6			5.7
X	*Sug10a*	*Inv-ap(a)*	G 3, 6	G 1		5.0
	Sug10a	*Inv-ap(a)*	F 1, 6			3.0
XI	*Sug11a*	*Sut1*	G 1	G 2		7.5
	Sug11a	*Sut1*	F 2	F 1		15.0

[a] Traits refer to glucose (G), fructose (F), and sucrose (S) content in specific environments (for codes see Table 1) with the indicated levels of significance.

[b] R^2 is the amount of phenotypic variance explained by the candidate gene. When the marker was significant for more than one environment, only data for the underlined environment are shown.

potato. Between 1 and 3 putative QTL regions were identified per chromosome and, in total, 24 putative QTL for sugar content were found in the two F_1 diploid populations. The large number of QTL is in part explained by the fact that, in contrast to QTL mapping in progeny descending from inbred lines, up to four alleles per QTL are compared in an F_1 family descending from heterozygous parents (Leonards-Schippers *et al.* 1994). Because of this, the populations H94A and H94C had an allelic diversity comparable to four-way hybrids from inbred crossing. This situation increased the chance of QTL detection, while sacrificing part of the potential to analyze single-allele effects. Complex genetic control of sugar content has also been reported for sugarcane where 14 and 18 QTL were detected in two F_1 interspecific populations (Ming *et al.* 2001). Taking into consideration the autopolyploidy of this species, those QTL were estimated to correspond to 8 nonoverlapping regions of the sugarcane genome.

A QTL mapping experiment for chip color, which is strongly correlated with reducing sugar content (Schefler *et al.* 1992), was conducted in an interspecific cross of diploid potatoes and six QTL were reported (Douches and Freyre 1994). The markers used did not cover, however, all linkage groups. A strict comparison of the QTL positions found in our study with the QTL mapped by Douches and Freyre (1994) cannot be done due to the lack of shared DNA markers. Nevertheless, similarities between both studies can be tentatively identified on putative homologous regions of linkage groups V and X.

Simko *et al.* (1999) mapped QTL for glucose, fructose, and sucrose concentrations in leaf exudates in diploid potato. One of three major QTL reported, *suc8.1*, for sucrose concentration on chromosome VIII, can be clearly anchored to the major QTL region *Sug8b* on linkage group VIIIB of population H94A by means of RFLP marker *GP130*. The *GP130* marker is, at the same time, tightly linked to *suc8.1* and to the *AGPaseS(b)* locus (Gebhardt *et al.* 2001), which itself is linked to *Sug8b* (Figure 2, Table 4). This suggests that one or more genes located on potato chromosome VIII near or at the candidate locus *AGPaseS(b)* have pleiotropic effects on sucrose content in leaves and on cold sweetening in tubers.

Stability of QTL for sugar content across populations and environments: Despite the different genetic background of the two mapping populations, six QTL (20%) for sugar content were linked in both populations to the same RFLP markers, suggesting that allelic variation at the same loci may be responsible for the effects. The precision of the comparison between the four linkage groups for each chromosome was, however, limited by the number of anchor markers and the size of the populations. It may be possible, therefore, that effects associated with markers mapping to similar regions on homologous linkage groups (for example, *Sug7a* and *Sug7c*, *Sug7b* and *Sug7d*) resulted from the same QTL. Conversely, the possibility that effects linked to the same

marker may result from different, closely linked QTL cannot be ruled out.

Populations H94A and H94C were propagated in six different environments, including plants grown in the greenhouse and in the field both in northern and southern European climates. Due to environmental differences, sizes of QTL effects and QTL positions were "moving targets," also showing variability. Nevertheless, five (H94A) and one (H94C) QTL for sugar content were localized in the same map bin in four to six environments. The probability of coincidence between these QTL by chance was $<10^{-5}$ and was considered negligible, suggesting that the same gene or group of genes is responsible for the QTL effects observed in different environments. Moreover, all these six QTL showed major effects on sugar content with an R^2 of 10% or higher. Small-effect QTL were less reproducible because they are prone to type 2 error.

Linkage of candidate genes to QTL for cold sweetening: The most consistent QTL effects in population H94A on chromosomes I, VII, VIII, and IX were all linked to candidate gene loci. In population H94C, reproducible QTL effects on sugar content across environments and/or traits were located on chromosomes II, III, V, VI, VII, and XII. These QTL have not yet been tested directly for linkage to candidate genes.

Allelic diversity of genes coding for metabolic enzymes, transporters, and regulatory proteins operating in carbohydrate metabolism could be responsible for genetic variability of cold sweetening. Differences may be related to cold sensitivity, catalytic properties, binding affinities to substrates or regulatory molecules, or to the efficiency and developmental regulation of transcription, translation, or degradation. In maize, where natural mutants affecting the starch or sugar content of the kernels have been characterized at the molecular level, mutant alleles of enzymes involved in carbohydrate metabolism, and not regulatory genes, were found to be responsible for the mutant phenotype (Hannah 2000).

The main pathways and some of the key enzymes involved in starch and sugar metabolism in plants are outlined in Figure 1. Some of the genes known to control sugar metabolism or transport were tested directly for linkage to cold-sweetening QTL in population H94A.

Sug9a, one of the most highly reproducible QTL, overlapped with the *Inv-ap(b)* locus on chromosome IX. Interestingly, the same locus is syntenic to a tomato invertase gene (Chen *et al.* 2001) that was recently shown to be a QTL for sugar content of tomato fruits (Fridman *et al.* 2000). Two additional invertase loci have been mapped to potato chromosomes III and X (Chen *et al.* 2001). The locus on chromosome X, *Inv-ap(a)*, is linked to QTL *Sug10a* and the third locus *Pain-1* on chromosome III (not mapped in H94A or H94C) may be a candidate for the QTL *Sug3a*. Invertase activity in tubers was found positively correlated with reducing sugar content (Pressey and Shaw 1966) and inhibition of invertase activity by a transgene encoding a proteinaceous inhibitor reduced cold-induced sweetening in potato (Greiner *et al.* 1999).

Major QTL *Sug7a* and *Sug7b* in population H94A were linked to CAPS markers for sucrose synthase (*Sus3*) and sucrose phosphate synthase (*Sps*), respectively. Another sucrose synthase locus *Sus4* maps to chromosome XII and may be a candidate for QTL *Sug12a* in H94C. Both enzymes play a crucial role in sucrose metabolism of plants (Zrenner *et al.* 1995; Sturm and Tang 1999; Figure 1).

The map positions of QTL *Sug1a* and *Sug8b* overlap with the *AGPaseS(a)* and *AGPaseS(b)* loci on chromosomes I and VIII, respectively. AGPase is a key enzyme in starch biosynthesis: Antisense repression of this enzyme resulted in reduced starch and increased sugar contents (Müller-Röber *et al.* 1992). Genes for subunits S and B of potato AGPase (*AGPaseS* and *AGPaseB*) mapped in populations H94A and H94C to chromosomes I, VII, VIII, and XII (Figures 2 and 3). Locus *AGPaseB(b)* on linkage group XII is, as *Sus4*, a candidate for QTL *Sug12a* in population H94C.

Two further QTL, *Sug11a* and *Sug5a*, were linked to *Sut1* and *Sut2*, a sugar transporter and a putative sucrose sensor (Barker *et al.* 2000). UDP-glucose pyrophosphorylase (UGPase), an enzyme that limits the synthesis of sucrose, maps distal to marker *GP125* on chromosome XI (Chen *et al.* 2001) in the QTL region containing *Sug11a*. Another transporter, *Sut4*, has been mapped to chromosome IV (Barker *et al.* 2000) and may be a candidate for *Sug4b*.

The probabilities for observing overlaps by chance between the candidate gene loci tested in H94A and QTL for reducing sugars were <0.01, supporting the possibility of a causal relationship between candidate gene allelic variants and QTL for cold sweetening. This observation provides, however, only indirect evidence for the involvement of the candidate locus in control of the trait. Another gene, closely linked to the candidate gene, could as well be the factor that controls the QTL. Further studies are required, therefore, to confirm the causal role of a candidate gene. This may be achieved by linkage disequilibrium mapping to fine map the QTL to smaller genetic intervals (Lander and Schork 1994; Talbot *et al.* 1999; Meuwissen and Goddard 2000) that are then accessible to candidate gene identification by genomic sequencing or by QTL analysis of transcript and protein levels or of enzyme activities (Causse *et al.* 1995). Final proof is provided by complementation analysis with specific candidate gene alleles in transgenic plants.

Several QTL, such as those on linkage groups VI, do not overlap with known candidate genes. This could be explained by the fact that, on the basis of their known function, only the most obvious candidate genes were tested in our experiment. Other genes besides those

functioning in carbohydrate metabolism may also be causal for cold-sweetening QTL. Candidate gene loci may have also escaped detection due to lack of polymorphism in RFLP- or PCR-based marker analysis.

Conclusion: The results of the QTL study in this article, together with the function map for carbohydrate metabolism and transport (Chen *et al.* 2001), provide the first steps toward unraveling the molecular basis of QTL for cold sweetening of potato tubers by a candidate gene approach. The candidate genes linked to cold-sweetening QTL in potato may also be relevant for QTL related to sugar metabolism in other plant species.

Our results provide a basis for performing marker-assisted selection using allelic variants of candidate genes in the Solanum gene pool, including wild relatives of cultivated potatoes (allele mining), and for analyzing possible associations of candidate gene alleles with resistance to cold sweetening. Overexpression, silencing, or controlled expression of specific allelic variants in transgenic plants could also be used for engineering cultivars suitable for potato processing industries.

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