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A potato molecular-function map for carbohydrate metabolism and transport

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Abstract Molecular-linkage maps based on functional gene markers (molecular-function maps) are the prerequisite for a candidate-gene approach to identify genes responsible for quantitative traits at the molecular level. Genetic linkage between a quantitative trait locus (QTL) and a candidate-gene locus is observed when there is a causal relationship between alleles of the candidate gene and the QTL effect. Functional gene markers can also be used for marker-assisted selection and as anchors for structural and functional comparisons between distantly related plant species sharing the same metabolic pathways. A first molecular-function map with 85 loci was constructed in potato based on 69 genes. Priority was given to genes operating in carbohydrate metabolism and transport. Public databases were searched for genes of interest from potato, tomato, or other plant species. DNA sequence information was used to develop PCR-based marker assays that allowed the localization of corresponding potato genes on existing RFLP linkage maps. Comparing the molecular-function map for genes operating in carbohydrate metabolism and transport with a QTL map for tuber starch content indicates a number of putative candidate genes for this important agronomic trait.

Keywords Molecular-function map · Carbohydrate metabolism · Tuber starch content · Candidate genes · Potato

Introduction

Carbohydrates are the major storage compounds of cereal and tuber crops. In potato, tuber starch content is an agronomically important quantitative character which is controlled by genetic factors and by environmental conditions. Genetic mapping using DNA markers allowed the dissection of genetic components of this quantitative trait into discrete quantitative trait loci (QTLs) and their localization on the potato molecular maps (Freyre and Douches 1994; Schäfer-Pregl et al. 1998). QTL mapping yields information on the minimal number and approximate genomic position of the factors controlling a quantitative trait, but does not identify their molecular basis. However, the existence of QTLs implies that the gene(s) responsible for the phenotypic effect must occur as molecular alleles with an impact on metabolic function.

The amount of starch accumulated in mature potato tubers is the net result of photosynthetic carbon fixation, the synthesis of transient starch and its conversion into sucrose in photosynthetically active source leaves, the vascular transport of sucrose from the leaves to the developing-sink tuber, and starch synthesis and degradation in the tuber during the growth period. The metabolism of carbohydrates and the partitioning of fixed carbon has been extensively studied in solanaceous plants at the physiological, biochemical and molecular levels (reviewed in Frommer and Sonnewald 1995). Enzymes and transport proteins participating in these processes have been characterized biochemically, and corresponding genes from potato or other plants have been cloned and characterized at the molecular level. When the expression of some of these genes was drastically altered in transgenic plants, either by antisense repression or overexpression, effects on the morphology and the carbohydrate partitioning affecting tuber starch content positively or negatively were observed in some cases and not in others (Frommer and Sonnewald 1995).

If natural allelic variants of genes with a functional role in carbohydrate metabolism and transport are responsible for the observed genetic variability of tuber starch content, genetic linkage will be observed between a QTL for tuber starch content and a candidate gene locus. To identify such linkages, in relation to the QTL map for tuber starch content, a molecular map of the candidate genes is required.

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Such a molecular map, based on genes with assigned functional roles in general plant metabolism, may also be useful for comparing the structure and function of distantly related plant genomes such as sugar beet and potato, important crop species which have in common a vegetative storage organ. The different end products, starch and sucrose, accumulated in tubers and beet roots, respectively, are the result of the same metabolic pathways.

We therefore, constructed a potato molecular map mainly for genes operating in carbohydrate metabolism and transport. CAPS (cleaved amplified polymorphic sequence), SCAR (sequence characterized amplified region) or RFLP (restriction fragment length polymorphism) marker assays for 69 genes were used to identify 85 genetic loci in mapping populations for which molecular maps were available (Schäfer-Pregl et al. 1998; Gebhardt et al. 1991). Potato or tomato DNA sequences available in public databases were used to design gene-specific PCR primers. When a gene of interest was not available from potato or tomato, sequence motifs conserved between homologues of different plant species were used for primer design (Schneider et al. 1999). Comparison between QTL maps for tuber starch content (Schäfer-Pregl et al. 1998) and the molecular-function map indicates a number of candidate genes having alleles that may differentially influence the starch content of potato tubers.

Materials and methods

Plant material

Parents and 90–150 F_1 hybrids of the diploid mapping population K31, and 49 F_1 hybrids of the diploid mapping population LH, were used for segregation analysis of PCR products showing a length polymorphism (SCAR) or of PCR products showing a length polymorphism after digestion with a restriction enzyme (CAPS). RFLP maps have been constructed for the 12 chromosome pairs of both parents of the two populations (Schäfer-Pregl et al. 1998). Mapping populations BC916[2] and F1840, used for gene mapping based on RFLP analysis, have been described previously (Gebhardt et al. 1991).

CAPS and SCAR markers

Forward and reverse primers were designed based on gene-specific DNA sequences selected from GenBank and EMBL databases (Table 1). The University of Wisconsin Genetics Computer Group software package [Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wis. 1999] was used for sequence analysis. PCR (Saiki et al. 1988) was performed with 20 ng of total genomic DNA of the parents and F_1 hybrids as templates in a 15-µl total volume of 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.4 mM each of dATP, dCTP, dGTP and dTTP, and 0.2 µM of primer, using 0.75 units of *Taq* DNA Polymerase (Gibco Life Technologies). A touchdown program was used for PCR with the following conditions:1×94°C for 3 min, five cycles with 1-min denaturation at 94°C, 1-min annealing, first at 60°C, then decreasing by 1°C in the subsequent cycles, and 2-min extension at 72°C, followed by 30 cycles of denaturation at 94°C for 50 s, annealing at 55°C for 60 s, and extension at 72°C for 90 s. After the last cycle, reactions were incubated for 5 min at 72°C. PCR products were size-separated on 1.5–2% agarose gels. Homomorphic PCR products were separated after digestion with one of the 4-bp cutter restriction enzymes *Taq*I, *Rsa*I, *Alu*I, *Dde*I, *Hpa*II and *Hae*III.

RFLP markers

Potato cDNA clones coding for an apoplastic invertase (Hedley et al. 1994) and phosphoenolpyruvate carboxylase (Merkelbach et al. 1993) were provided by R. Waugh (Scottish Crop Research Institute, Invergowri, UK) and F. Kreuzaler (Technical University Aachen, Germany), respectively. cDNA clones for sucrose transporter 1 (Riesmeier et al. 1993) sucrose transporter 4, and a putative sucrose sensor (Barker et al. 2000), were provided by W. Frommer (Tübingen University, Germany). A genomic fragment of acidic β-1,3-glucanase was obtained from E. Kombrink (MPI für Züchtungsforschung, Köln, Germany). RFLP markers for α-amylase, sucrose phosphate synthase, hexokinase and lactate dehydrogenase 2 were EST clones present in a collection of approximately 1800 unselected tuber cDNA clones (unpublished results of this laboratory). Function assignment of the EST clones was based on a more than 90% sequence similarity to potato genes in the database. RFLP analysis was performed as described previously by hybridization of Southern blots to ^{32}P-labeled marker probes (Gebhardt et al. 1989). The stringency of the post-hybridization washes of filters (in 5 mM Sodium phosphate, 1 mM EDTA, 0.2% SDS, pH 7.0, at room temperature) allowed the detection of cross-hybridizing genomic restriction fragments from 100% to approximately 80% sequence identity with the probe.

Linkage analysis

Parents and progeny of the mapping population were scored for the presence or absence of segregating PCR or RFLP fragments. Linkage to the RFLP markers previously mapped using the same plants was determined as described before (Ritter et al.1990; Schäfer-Pregl et al. 1998).

DNA sequencing

PCR products were purified from agarose gels using the QiaEx gel-extraction kit (Qiagen, Hilden, Germany) and sequenced either directly or after cloning in the pGEM®-T vector (Promega). DNA sequences were determined by the MPIZ DNA core facility on PE Biosystems Abi Prism 377 and 3700 sequencers using BigDye-terminator chemistry.

Results

Fifty PCR primer pairs specific for the genes described in Table 1 were designed. Forty four primer pairs were derived from the DNA sequence of potato genes and three pairs from tomato genes (*Eno, Ndpk*, *Glo*, Table 2). For genes *Ant, Rca and* Pgk_{cp}, degenerate primers based on sequence motifs conserved between the homologous genes of four other plant species were designed (Schneider et al. 1999). Three PCR products obtained with potato gene primers, and seven PCR products obtained with tomato gene primers or with degenerate primer pairs, respectively, were sequenced. The DNA sequences were between 80% and 100% identical to the DNA sequences of corresponding genes in the database (Table 2). When using potato genomic DNA as a template, most primer pairs generated products larger than expected from the transcribed sequence, indicating the presence of introns.

Table 1 Genes placed onto the potato molecular-function map

Function assignment	EC number	Species[m] accession number	Gene	Linkage group	Primer, 5´ to 3´	Population[a]	PCR product, kb	Marker assay[b]
A: Starch synthesis and degradation (chloroplasts, amyloplasts)								
ADP-glucose pyrophosphorylase B[e]	2.7.7.27	St_x55155	AGPaseB	VII, XII	–	1,2,3,4	–	R, TaqI
ADP-glucose pyrophosphorylase S[e]	2.7.7.27	St_x61186	*AGPaseS*	I, IV, VIII	–	1,2,3,4	–	R, RsaI, TaqI
Granule-bound starch synthase I[e]	2.4.1.21	St_x58453	*GbssI (wx)*	VIII	–	1, 3, 4	–	R, RsaI
Soluble starch synthase I	2.4.1.21	St_y10416	*SssI*	III	f-ctgtcagtatccgatcagcaac r-cttcttactgcagacctggaac	3	2.4, 2.6	S
Granule-bound starch synthase II	2.4.1.21	St_x87988	*GbssII*	II	f-gctgcaagtgctgatgaatcga r-ttagaccatggagcgcattctg	3	1.8	C, TaqI
Soluble starch synthase III	2.4.1.21	St_x94400	*SssIII*	II	f-aacaaaagttcaggtcctctctc r-aaatcccaccatcttctctctc	3	1.3	C, AluI
Starch branching enzyme I[e]	2.4.1.18	St_y08786	*SbeI*	IV	–	1, 3, 4	–	R, TaqI
Starch branching enzyme II	2.4.1.18	St_aj000004	*SbeII*	IX	f-ctcgtagtgctacaggtatcac r-tgatggagtgtccatacgta	3	1.8	C, AluI
Disproportionating enzyme	2.4.1.25	St_x68664	*Dpe-P*	IV	f-cactacttttcaatctcctatccc r-gcatagtcacgaacttttttcc	3	3.0	C, TaqI
Starch-granule-bound protein R1	–	St_y09533	*Gb-R1*	V	f-tccatcctgagactggagatac r-acttgtactgcaggactggaag	4	1.7	C, RsaI
De-branching enzyme	3.2.1.41	St_a52190	*Dbe*	XI	f-ctgatgtcagcatctatgagct r-gatacgacaaggaccatttgca	3, 4	2.1	C, TaqI R, AluI
α-Amylase [e, g]	3.2.1.1	St_m79328	*AmyZ*	IV	–	1, 2	–	R, TaqI
α-Glucosidase	3.2.1.20	St_aj001374	*Agl*	IV	f-accaagctgtggttaaccagag r-gcagttgcgaataactgtggca	3	0.8	C, AluI
H-type starch phosphorylase	2.4.1.1	–[f]	*StpH*	IX	f-gcatactatgctgctactgctg r-gcacatcatatgcaagagcctg	3	1.8	C, RsaI
L-type starch phosphorylase	2.4.1.1	St_x73684	*Stp1*	V	f-acacactatgttctgcttctcttc r-actatcctccacctcaaccttc	4	0.8	C, TaqI, RsaI
α-Glucan phosphorylase	2.4.1.1	St_d00520	*Stp23*	III	f-atggcgactgcaaatggagca r-ccatacttgtacctaagtccatag	4	1.6	C, TaqI, RsaI, AluI
B: Sucrose metabolism (cytosol, apoplast)								
UDP-glucose pyrophosphorylase[c]	2.7.7.9	St_p19595	*UGPase*	XI	–	1, 2, 4	-	R, TaqI
Fructose-1,6-bisphosphatase	3.1.3.11	St_x76946	Fbp_{cy}	IV	f-tgaagaaccatcagcgggatac r-tgcagggagaagatcaaaagaaac	3	1.4	C, RsaI, TaqI
Pyrophosphate fructose-6-phosphate 1-phosphotransferase, α subunit	2.7.1.90	St_m55190	*Pfp-α*	IV	f-ccctcaggcaacaggataacac r-cggacaaaagatcagattagaacgac	3	2.5	C, AluI

Table 1 (continued)

Function assignment	EC number	Species[m] accession number	Gene	Linkage group	Primer, 5´ to 3´	Population[a]	PCR product, kb	Marker assay[b]
Pyrophosphate fructose-6-phosphate 1-phosphotransferase, β subunit	2.7.1.90	St_m55191	*Pfp-β*	II	f-gtcatgatagatgctcgatcaac r-acatcagcaatgtagtccgtaac	4	2.5	C, RsaI
Sucrose phosphate synthase[g]	2.4.1.14	St_x73477	*Sps*	VII	f-caagtgagtgaccagtgaaaag r-gaaagaggtcgcagagaagcag	2, 4	1.3	C, TaqI, R, TaqI
Sucrose synthase 3	2.4.1.13	St_u24088	*Sus3*	VII	f-catgacaaggaaagcatgacccc r-gcaaagtaaatcttatacatghtgacc	4	1.2	C, TaqI
Sucrose synthase 4[k]	2.4.1.13	St_u24087	*Sus4*	XII	f-caagctgacctggacaccacagt r-accacattgaaaaccataggaattct	3	1.3	C, RsaI
Soluble acid invertase	3.2.1.26	St_x70368	*Pain-1*	III	f-gtcttcgataagacttttcgag r-cagtggtcgggtctctaaagt	3	2.0	C, DdeI, TaqI
Apoplastic invertase	3.2.1.26	St_z22645	Inv_{ap}	IX, X	–	1	–	R, TaqI
C: Transport (membranes)								
Sucrose transporter 1[e]	–	St_x69165	*Sut1*	XI	f-agcttccatagctgctggtgtt r-cggactaaggttaaggctatgg	1, 3	1.2	C, RsaI, R, TaqI
Sucrose transporter 4[e]	–	–	*Sut4*	IV	–	1	–	R, TaqI
Sucrose transporter-like[e], Sugar sensor?	–	St_af166498	*Sut2*	V	–	1	–	R, TaqI
Triose phosphate translocator[e]	–	St_x67045	*Tpt*	X	–	2	–	R, RsaI
Glucose-6-phosphate translocator	–	St_af020816	*Gpt*	V	f-ggctcacacaattggtcatgtg r-ccaagattgcaatagcagcacc	4	1.4	C, HaeIII
Plasma membrane H^+-ATPase 1[e]	3.6.1.34	St_x76536	*Pha1*	III, VI	f-tcctggagatggtgtctactct r-gcagtatcaatggcatcctggt	2, 3	0.8	C, TaqI, R, TaqI
Plasma membrane H^+-ATPase 2[e]	3.6.1.34	St_x76535	*Pha2*	VII	f-caaacatgtacccgtcagcatc r-agctatcaggcattggagatgg	1, 4	0.9	R, TaqI, C, TaqI
Adenylate transporter	–	St_x62123, At_x65549, Os_d12637, Tt_x95864	*Ant*	XI	f-tggagaggaaacactgcYaatgt[l] r-atgttRgcaccagcWcccttga[l]	4	1.1+0.9	C, TaqI
Inorganic phosphate transporter 1	–	St_x98890	*Pt1*	IX	f-tcagcagctgttaatggagtcg r-tgcaacaccttggacatgtcgt	4	0.6	C, RsaI
Inorganic phosphate transporter 2	–	St_x98891	*Pt2*	III	f-cgccacgatcatgtctgaatac r-taaacgccacggtgaaccagta	4	0.7	C, AluI
D: Calvin cycle, photorespiration (chloroplasts)								
Ribulose bisphosphate carboxylase activase	–	So_j03610, Lp_af037361, At_x14212, Md_z21794	*Rca*	X	f-acacYgtMaacaaccagatg[l] r-actctcttgacattctcttgc	4	0.7	C, TaqI

Table 1 (continued)

Function assignment	EC number	Species[m] accession number	Gene	Linkage group	Primer, 5´ to 3´	Population[a]	PCR product, kb	Marker assay[b]
Ribulose bisphosphate carboxylase small subunit[e]	4.1.1.39	–[i]	*rbcS*	II (2), III	–	1,3,4	–	R, TaqI, RsaI
Phosphoglycerate kinase	2.7.2.3	At_u37701, Nt_z48977, So_x68430, Ta_x15233	Pgk_{cp}	VII	f-aaYccWaagMgVccatttgc [l] r-aaRctRgcDccaccaccagt [l]	4	0.9	C, TaqI
Fructose-1,6-bisphosphatase	3.1.3.11	–[h]	Fbp_{cp}	IX	f-tactggagttcaaggtgctgtc r-tgcagagtaaggcttccgacta	3	1.6	C, TaqI
Transaldolase	2.2.1.2	St_u95923	*Tal1*	XI	f-attccttgtgtgtcaaatgctcc r-cgactaacgaagaatgaagcaac	4	1.5	C, TaqI
Transketolase	2.2.1.1	St_z50099	*Tk*	V	f-gagacacggaaattgctttcac r-tcagcactaccaccaaggaaac	4	0.6	R, TaqI
Pentose-5-phosphate 3-epimerase	5.1.3.1	St_z50098	*Ppe*	III, V, XII	f-tccgtccatcctttctgctaac r-aactccaccatcaacttcaatc	3	3.0	C, AluI, TaqI
Glycolate oxidase	1.1.3.15	Le_x92888	*Glo*	VII, X	f-cagcgttgaggaggttgcttca r-gacagccactcaatgccatagt	4	1.7	C, TaqI
E: Glycolysis, oxidative pentose phosphate pathway (cytosol)								
Fructokinase	2.7.1.4	St_z12823	*Fk*	VI	f-gctttggcgttcgtgactctac r-agtggtgtcaacagtcttcacg	3	2.0	C, TaqI
Hexokinase[g]	2.7.1.1	St_x94302	*Hxk*	III	f-catctgccgttgacagagtatg r-gattatattgcggctgagcttgc	2, 3	0.8	C, RsaI, R, TaqI
Glyceraldehyde 3-phosphate dehydrogenase[d]	1.2.1.12	St_U17005	*Gap C*	V	–	1	–	R, TaqI
Enolase	4.2.1.11	Le_x58108	*Eno*	IX	f-cttggtgcaaatgccatccttg r-cagcttcaatactctcggtcac	3	2.1+1.9	C, AluI, TaqI
Pyruvate kinase	2.7.1.40	St_x53688	*Pk*	IV	f-tcactgtatccacagactatacc r-cactctccccacttaacataac	3	0.9, 0.8	S
Lactate dehydrogenase 2[g]	1.1.1.27	St_af067859	*Ldh2*	VIII (3)	–	2	–	R, TaqI
Phosphoenol-pyruvate carboxylase	4.1.1.31	St_x67053	*Ppc*	X, XII	–	1	–	R, TaqI
Glucose-6-phosphate dehydrogenase	1.1.1.49	St_x74421	*G6pdh*	II	f-tcttctattttgctcttcctcc, r-actcgtatttctgcctttcttg	3	1.6	C, DdeI
F: TCA cycle (mitochondria)								
Pyruvate dehydrogenase, E1α	1.2.4.1	St_z26949	*Pdh-E1α*	V	f-tcaacaagccgagccattaac r-gattagcagcaccatcaccatac	3	0.6	C, RsaI
Citrate synthase	4.1.3.7	St_x75082	*Cis*	I	f-ctttcgcagaactccatgtcca r-ggtcacttggttgctagtgctt	3	1.5	C, TaqI
Aconitase	4.2.1.3	St_x97012	*Aco*	VII	f-catggctcctgaatatggtgca r-atacaagtggtgcagccatagc	4	1.3	C, AluI, HpaII

Table 1 (continued)

Function assignment	EC number	Species[m] accession number	Gene	Linkage group	Primer, 5´ to 3´	Population[a]	PCR product, kb	Marker assay[b]
Isocitrate dehydrogenase 1	1.1.1.42	St_75638	*Icdh-1*	I	f-aaaatatccccagacttgtccc r-aacagcgtcaatgaactcttcc	3	1.8	C, AluI
Fumarase	4.2.1.2	St_x91615	*Fum1*	VII, IX	f-gaagcagccagggtatttaagg r-catgtgcatccaaatgaccatgt	4	1.9	C, RsaI
Malate dehydrogenase	1.1.1.39	St_z23023	*Mdh*	VIII	f-gtcaagccgatgatcttgcagt r-acagtggattgttcagacgtcc	4	3.0	C, AluI
NAD^+-dependent malic enzyme	1.1.1.39	St_z23002	*Me*	III, V	f-ccttggttcaataaggacacgg r-caacgagcatgaacagcttcca	4	1.1, 1.2	S
G: Miscellaneous								
Soluble inorganic pyrophosphatase[e]	3.6.1.1	St_z36894	*Ppa1*	VIII, XII	–	1	–	R, TaqI
Soluble inorganic pyrophosphatase[e]	3.6.1.1	St_aj225172	*Ppa2*	IX	–	1	–	R, TaqI
GDP-mannose pyrophosphorylase	2.7.7.13	St_af022716	*GMPase*	III	f-gaaactgaaccacttggcactg r-ccctctccaatcttggcagatt	4	0.6	C, AluI
Uncoupling protein	–	St_y11220	*Ucp*	IX	f-gtatcgtacctgggttacatcg r-acattccaagatcccaagcgtc	3	2.5	C, TaqI
Pectin methyl esterase	3.1.1.11	St_s66607	*Pme*	VII	f-acttgctgaagcagttgctgca r-ttagccacagtgaacggcatag	4	1.5, 1.2, 1.0	C, TaqI
3-deoxy-D-arabino-heptulosonate 7-phosphate synthase	4.1.2.15	St_m95201	*ShkB*	XI	f-catcttctccataacccctttacc r-gctcacagttctccacaaaatc	3	1.2	C, RsaI
Nucleoside diphosphate kinase	2.7.4.6	Le_x75324	*Ndpk*	IX	f-atgatcaagcctgatggtgtcca r-aagagtgaaggctgctctgcca	3	1.1, 0.4	S
β-1,3-glucanase, basic[e]	3.2.1.39	St_aj009932	*Glu B*	I	f-cagcctatcggagtatgctatg r-cattgaatccacaagggcatcg	3, 1	0.7	C, RsaI, R, TaqI
β-1,3-glucanase, acidic	3.2.1.39	–	*Glu A*	I	–	1	–	R, TaqI
Chitinase, class I, basic[e]	3.2.1.14	–	*Cht B*	X	–	1, 3, 4	–	R, TaqI
Protein kinase	2.7.1.37	St_x90990	*Prk*	II	f-ccatctgagtcaggaatcacct r-acattgatcgagcactcgttgg	3	1.6	C, RsaI

[a] Four populations were used for mapping: 1=BC916[2], 2=F1840 (Gebhardt et al. 1991, 1994), 3=K31, 4=LH (Schäfer-Pregl et al. 1998)

[b] C=CAPS, S=SCAR, R=RFLP

[c] The sequence of RFLP marker *CP58* originating from leaf cDNA (Gebhardt et al. 1991) is highly similar (80–100%) to the 3' end of potato UGPase

[d] The sequence of the cold-regulated partial cDNA CI13 isolated from cold-stored tubers (van Berkel et al. 1994) is highly similar (86%) to GAP C of *Petunia hybrida* (Acc. No. x60346) (Schneider 1995)

[e] Map position of this gene has been reported elsewhere (Gebhardt et al. 1994; du Jardin et al. 1995; Rojas-Beltran et al. 1999; Barker et al. 2000)

[f] Potato sequence from Mori et al. (1991)

[g] Potato EST clone (unpublished results from this laboratory) with >90% similarity to the accession number was used as a probe for RFLP mapping

[h] Potato sequence from Koßmann et al. (1992)

[i] Potato sequence from Eckes et al. (1985), and Wolter et al. (1988)

[k] The *Sus4* gene of potato corresponds to *sh1* of maize (Werr et al. 1985; Gebhardt et al. 1991)

[l] Y=C+T, W=A+T, M=A+C, R=A+G, V=A+C+G, D=A+G+T

[m] St=*Solanum tuberosum*, Le=*Lycopersicon esculentum*, Lp=*Lycopersicon pennellii*, Nt=*Nicotiana tabacum*, At=*Arabidopsis thaliana*, So=*Spinacia oleracea*, Md=*Malus domesticus*, Ta=*Triticum aestivum*, Tt=*Triticum turgidum*, Os=*Oryza sativa*

Table 2 Sequence similarity between PCR products of potato and corresponding reference sequences

Reference cDNA	Accession no.	Number of nucleotides compared	% similarity at nucleotide level
STP23 of potato[a]	D00520	660	100
SUS3 of potato[b]	U24088	710	98.5
SSSIII of potato[c]	X94400	333	100
GLO of tomato[d]	X92888	118	96.6
ENO of tomato[e]	X58108	334	97.0
NDPK of tomato[f]	X75324	390	94.4
PGK of tobacco[g]	Z48977	428	95.6
RCA of tomato[h]	AF037361	278	98.5
ANT of Arabidopsis[i] I	X65549	428	81.5
II		549	82.7

[a] STP23=α-Glucan phosphorylase; [b] SUS3=sucrose synthase 3; [c] SSSIII=soluble starch synthase III; [d] GLO=glycolate oxidase; [e] ENO=enolase; [f] NDPK=nucleoside diphosphate kinase; [g] PGK=phosphoglycerate kinase; [h] RCA=Rubisco activase; [i] ANT=adenylate transporter (two PCR fragments I and II were sequenced)

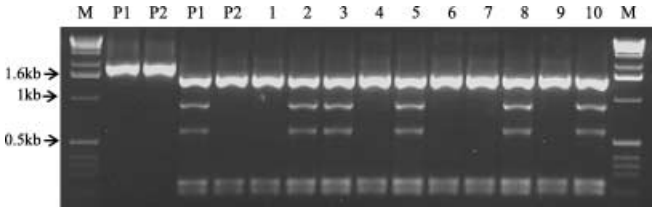


Fig. 1 CAPS marker for the α-glucan phosphorylase gene *Stp23* on potato chromosome III. The uniform 1.6-kb PCR products, shown for the parents P1 and P2 of the LH mapping population (lanes 2 and 3 from the left), were polymorphic after digestion with *Taq*I (lanes 4 and 5) and segregated in the F_1 plants (lanes 6–15 from the left). *M*=size marker

In the majority of cases (45 genes), PCR products had to be digested with a 4-bp cutter restriction enzyme to generate segregating DNA fragments (CAPS markers, Fig. 1). The most-informative restriction enzyme was *Taq*I, followed by *Rsa*I and *Alu*I (Table 1). Forty three of the forty five genes were mapped as CAPS markers using one of these three enzymes. The remaining two genes were mapped using *Hae*III and *Dde*I, respectively. Four primer pairs generated directly polymorphic PCR products (SCAR markers, Table 1). The PCR product of only one gene (*Tk*) did not segregate either as a SCAR or as a CAPS marker and was mapped based on RFLP analysis (Table 1).

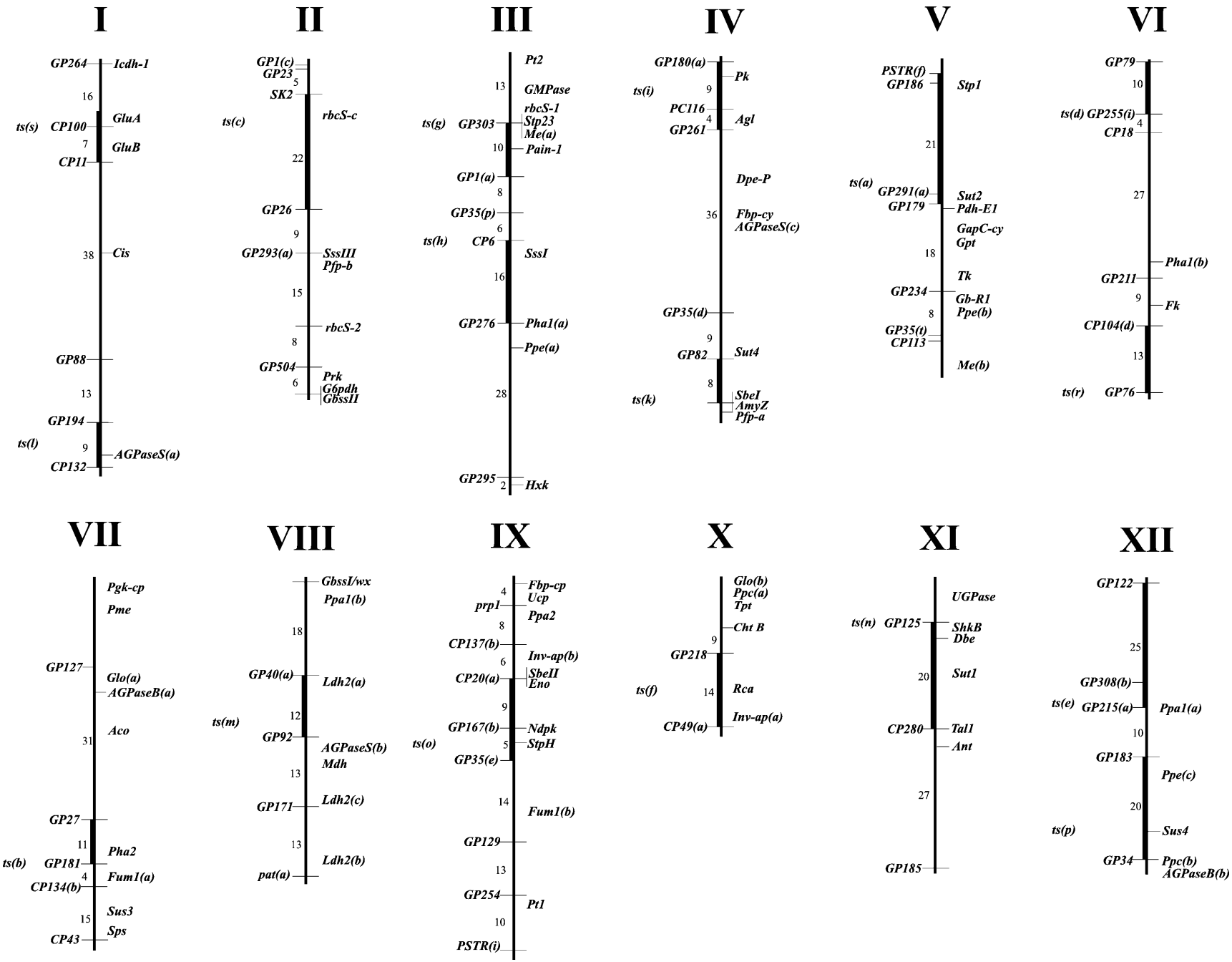
Twenty six genes were mapped by RFLP analysis using cloned cDNAs, ESTs, or genomic fragments as probes (Table 1). The map positions of 16 of these genes have been reported elsewhere (Gebhardt et al. 1994; du Jardin et al. 1995; Rojas-Beltran et al. 1999; Barker et al. 2000). They have been included in Table 1 and Fig. 2 based on the important role assigned to them in carbohydrate metabolism or transport (Frommer and Sonnewald 1995).

A subset of seven genes (*Sps*,*Dbe*, *Sut1*, *Pha1*,*Pha2*, *Hxk* and *GluB*) was mapped by both CAPS and RFLP analysis in different mapping populations (Table 1). In all seven cases both marker assays identified similar loci.

Eighty five loci were identified by the 69 gene markers (Table 1, Fig. 2). Eight gene markers identified two loci each (*AGPaseB*, Inv_{ap}, *Pha1*, *Glo*, *Ppc*, *Fum1*, *Me* and *Ppa1*) and four gene markers detected three loci each (*AGPaseS*, *rbcS*, *Ppe* and *Ldh2*).

Positions of the potato genes were analyzed for synteny with tomato EST markers, to which the same functions have been assigned based on sequence similarity (Ganal et al. 1998; Tanksley et al. 1992). CAPS markers for potato genes *Icdh-1* and *Aco* mapped to positions on linkage groups I and VII, respectively, which are in good agreement with isozyme loci *Idh-1* and *Aco-2* on the tomato molecular map. Potato genes *UGPase* on linkage group XI, as well as Pgk_{cp} and *Pha2*, both on linkage group VII, occupy similar map positions to tomato markers *CT182*, *CT114* and *CD54*, respectively, which code for the same enzymes (Ganal et al. 1998; Tanksley et al. 1992). In four cases, the potato gene marker identified a single locus, whereas two or more loci were identified by the corresponding tomato EST marker. The four loci were *AmyZ* (LG IV), *Stp1*, *Tk* (both LG V) and *Ndpk* (LG IX), which correspond to tomato marker loci *CT224B*, *CD31A*, *CD38B* and *CT225A*, respectively, and to which the same function has been assigned based on sequence similarity (Ganal et al. 1998; Tanksley et al. 1992). In one instance, the CAPS marker for the potato *Ppe* gene identified three loci in potato on linkage groups III, V and

Fig. 2 Molecular function map of potato. The 12 potato chromosomes are shown as a composite map based on the K31 mapping population. Genetic distances, indicated in Centimorgans on the left side of linkage groups, are the arithmetic means of similar marker intervals on maternal and paternal linkage groups (Schäfer-Pregl et al. 1998). Framework RFLP markers (Gebhardt et al. 1994) are positioned to the left side of the linkage groups, and gene markers are positioned on the right side (for gene identification see Table 1). The relative positions of genes mapped in populations other than K31 (not connected to the vertical linkage group with a horizontal bar, see Table 1 for details) were inferred from anchor RFLP markers mapped in several mapping populations. Small letters in *parenthesis* indicate that more than one locus was identified with the same probe or PCR primer pair. Map segments having QTLs for tuber starch content are shown as *black bars*. QTL positions *ts(a)* to *ts(s)*, as identified by interval mapping (Schäfer-Pregl et al. 1998), are shown on the left side of linkage groups


I
GP264
Icdh-1
16
ts(s)
CP100
GluA
7
GluB
CP11
38
Cis
GP88
13
GP194
ts(l)
9
AGPaseS(a)
CP132
II
GP1(c)
GP23
5
SK2
rbcS-c
ts(c)
22
GP26
9
GP293(a)
SssIII
Pfp-b
15
rbcS-2
8
GP504
Prk
6
G6pdh
GbssII
III
Pt2
13
GMPase
rbcS-1
ts(g)
GP303
Stp23
Me(a)
10
Pain-1
GP1(a)
8
GP35(p)
6
ts(h)
CP6
SssI
16
GP276
Pha1(a)
Ppe(a)
28
GP295
2
Hxk
IV
GP180(a)
Pk
ts(i)
9
PC116
4
GP261
Agl
Dpe-P
36
Fbp-cy
AGPaseS(c)
GP35(d)
9
GP82
Sut4
8
ts(k)
SbeI
AmyZ
Pfp-a
V
PSTR(f)
GP186
Stp1
21
ts(a)
GP291(a)
Sut2
GP179
Pdh-E1
GapC-cy
Gpt
18
Tk
GP234
Gb-R1
8
Ppe(b)
GP35(t)
CP113
Me(b)
VI
GP79
10
ts(d)
GP255(i)
4
CP18
27
Pha1(b)
GP211
9
Fk
CP104(d)
13
ts(r)
GP76
VII
Pgk-cp
Pme
GP127
Glo(a)
AGPaseB(a)
Aco
31
GP27
11
Pha2
ts(b)
GP181
4
Fum1(a)
CP134(b)
15
Sus3
Sps
CP43
VIII
GbssI/wx
Ppa1(b)
18
GP40(a)
Ldh2(a)
ts(m)
12
GP92
AGPaseS(b)
Mdh
13
GP171
Ldh2(c)
13
Ldh2(b)
pat(a)
IX
Fbp-cp
4
Ucp
prp1
Ppa2
8
CP137(b)
Inv-ap(b)
6
SbeII
CP20(a)
Eno
9
GP167(b)
Ndpk
ts(o)
5
StpH
GP35(e)
14
Fum1(b)
GP129
13
GP254
Pt1
10
PSTR(i)
X
Glo(b)
Ppc(a)
Tpt
Cht B
9
GP218
ts(f)
14
Rca
CP49(a)
Inv-ap(a)
XI
UGPase
ts(n)
GP125
ShkB
Dbe
20
Sut1
CP280
Tal1
Ant
27
GP185
XII
GP122
25
GP308(b)
ts(e)
GP215(a)
Ppa1(a)
10
GP183
Ppe(c)
20
ts(p)
Sus4
GP34
Ppc(b)
AGPaseB(b)

XII, whereas the tomato marker *CT248* for *Ppe* detected only the locus on linkage group III. No correspondence between map positions was found in the case of the potato *Me* gene for the NAD^+-dependent malic enzyme (LGs III and V) and the tomato marker *CT201*, which is similar to the $NADP^+$-dependent malic enzyme. The DNA sequences of the two markers shared only 56% similarity. The map positions of potato genes for fructose-1,6-bisphosphatase (Fbp_{cp} on LG IX and Fbp_{cy} on LG IV) were both different from the position of tomato marker *CD5* (LG 10) which has sequence similarity with the same enzyme. The potato homologue of a tomato glycolate oxidase gene (*Glo*), although 96.6% identical with the tomato sequence from which it was derived (Table 2), detected two loci on potato linkage groups VII and X, which were in disagreement with the position on tomato linkage group 2 of tomato marker *CD79* which is identical to the tomato *Glo* gene sequence used for primer design (M. Ganal, personal communication).

Discussion

PCR-based markers for genetic analysis and marker-assisted selection

Public databases were systematically searched for sequences of plant genes to which a functional role in carbohydrate metabolism and transport could be assigned. Sequence information was used to develop PCR-based marker assays that allowed the localization of potato genes with high sequence similarity on existing RFLP linkage maps. With this approach, an initial potato molecular-function map based on the RFLP mapping of cloned genes (Gebhardt et al. 1994) was expanded about four-fold. The gene markers as defined in this study, together with another set of sequence-based anchor markers (Oberhagemann et al. 1999), are a valuable tool for genetic analysis and marker-assisted selection in potato. They: (1) cover a considerable proportion of the potato genome, (2) are available as locus specific sequence information, and (3) can be analysed by PCR with small amounts of genomic DNA as a template. Marker assays will have to be verified and adapted for being informative, however, when applied to germ plasm other than the material used for gene mapping in this study.

Molecular-function maps for comparative studies of plant genomes

Most of the genes for which map positions are reported in this paper share high sequence similarity with housekeeping genes functioning in general plant metabolism. Even distantly related plant species have these genes in common. In different plant species, molecular maps based on conserved gene sequences may not only be used for comparative studies of plant genome evolution, but also for functional comparisons. This is based on the widely accepted assumption that sequence similarity implicates functional similarity. Few molecular maps are currently available in plants for specific sets of genes of known biochemical and physiological effect. Similar studies have been carried out in maize and sugar beet (Causse et al. 1995; Schneider et al. 1999). The sequencing of whole-plant genomes as done for *Arabidopsis* (Bevan et al. 1998; Lin et al. 1999), or the construction of linkage maps based on expressed sequence tags as done in rice (Kurata et al. 1994) and maize (Chao et al. 1994), makes available information on the genomic positions of functionally and structurally similar genes which can also be used for genomic comparisons. For the potato and tomato genomes, extensive colinearity has been demonstrated (Bonierbale et al. 1988). In tomato, a genetic map including isozyme loci and more than 300 randomly selected EST markers has been constructed (Tanksley et al. 1992). Based on DNA-sequence similarity to genes in the database, putative functions have been assigned to tomato EST markers (Ganal et al. 1998). The validity of genome comparisons using molecular-function maps was tested by comparing the positions of 13 potato gene markers relative to the positions of tomato EST markers with the same functional assignment. In potato and tomato, orthologous genes with conserved sequences and the same functional assignment are expected to occupy similar map positions. This was, in fact, observed for most of the genes examined, when also taking into account the different numbers of loci detected in several cases in the two species. The difference in the number of loci identified with the same gene marker might be due to the nature of the marker assays used for mapping. Depending on the hybridization stringency used, the RFLP assay also identifies loci with less than 100% sequence identity with the probe, whereas the PCR-based assays require almost complete identity between the primer and the target sequence and are, therefore, more gene-specific. Alternatively, the potato and tomato genomes may have indeed different numbers of copies of certain genes (Gebhardt et al. 1991). Three gene markers, however, did not occupy syntenic positions on the potato/tomato maps. First, this might be due to erroneous functional assignment when the sequence similarity is not extremely high, as in the case of malic enzyme (*Me*) where the potato gene shared only 56% similarity with the tomato EST. Second, in the case of gene families with members that are unlinked, members at different loci might be polymorphic in the two species compared. Third, members of gene families with high interspecific sequence conservation, compared to other members of the same family, may not always be truly orthologous. Differential selection pressure acting on members of a gene family after speciation may have resulted in a higher sequence divergence between orthologous members then between paralogous members of the same family. This might explain why a glycolate oxidase (*Glo*) gene marker, whose sequence is highly con-

served between potato and tomato, was located on different chromosomes in the two genomes.

Candidate genes for tuber starch content

Quantitative trait loci (QTLs) for tuber starch content and tuber yield have been mapped in our K31 and LH populations (Schäfer-Pregl et al. 1998). The marker intervals with the approximate positions of the tuber starch QTLs as previously identified are indicated in Fig. 2. The same populations were used for PCR-based mapping of genes involved in carbohydrate metabolism and transport. Comparison of the QTL map with the molecular-function map reveals a number of correlations between the map positions of the QTLs for tuber starch content and function-related loci. This was expected, due to the map coverage by both the QTL and the function related loci. Overlapping chromosomal positions are observed, either when the gene(s) responsible for a QTL effect are linked, but functionally unrelated to a marker gene, or when the marker gene itself is responsible for the QTL effect. Evaluation of the latter possibility has to be knowledge-based, considering the physiological, biochemical and molecular role of the candidate genes.

The most-direct candidates controlling tuber starch content are genes coding for starch-metabolizing enzymes. Sixteen genes of this class were mapped yielding 19 loci on the potato molecular-function map (Gebhardt et al. 1994, and this paper). ADP-glucose pyrophosphorylase is a key enzyme of starch biosynthesis (reviewed in Frommer and Sonnewald 1995). Markers for the two subunits, S and B, of potato AGPase identified five loci one of which, *AGPaseS(a)* on linkage group I, was positioned in the same 9-cM marker interval as the QTL *ts(l)* (Fig. 2) having a small effect on tuber starch content (Schäfer-Pregl et al. 1998). One of four mapped starch synthase genes, *SssI* on linkage group III, is linked to QTL *ts(h)*. Genes *SbeI* for the starch branching enzme I and *Dbe* for the de-branching enzyme are linked to QTLs *ts(k)* and *ts(n)* on linkage groups IV and XI, respectively. Five genes coding for starch-degrading enzymes were mapped: *Stp23* on linkage group III, *AmyZ* and *Agl* both on linkage group IV, *Stp1* on linkage group V, and *StpH* on linkage group IX. All five loci are linked to QTLs for tuber starch content (Fig. 2). The clearest positional correlation was between *Stp23* and QTL *ts(g)* on linkage group III because both the QTL and the marker gene are most-closely linked to the same anchor RFLP marker *GP303* (Fig. 2 and Schäfer-Pregl et al 1998). This suggests that natural allelic variants of genes controlling starch synthesis and degradation may contribute to the genetic variability of tuber starch content.

The accumulation of tuber starch also depends on the availability of a substrate for starch synthesis and, therefore, more indirectly on photosynthesis, sugar metabolism, transport and energy supply (reviewed in Frommer and Sonnewald 1995). Allelic variants of genes operating in these metabolic pathways may be responsible for the variability of tuber starch content. Genes functionally linked to these pathways were positioned, therefore, on the potato molecular map. The list of the mapped genes is by no means complete. QTLs for tuber starch content were linked, among others, to loci for the ribulose bisphosphate carboxylase, small subunit [*rbcS-c* and *ts(c)* on LG II, *rbcS-1* and *ts(g)* on LG III], ribulose bisphosphate carboxylase activase [*Rca* and *ts(f)* on LG X], sucrose synthase 4 [*Sus4* and *ts(p)* on LG XII], pyruvate kinase [*Pk* and *ts(i)* on LG IV], pyrophosphate fructose-6-phosphate 1-phosphotransferase α subunit [*Pfp-α* and *ts(k)* on LG IV], malic enzyme [*Me(a)* and *ts(g)* on LG III], plasma membrane H^+-ATPase 2 [*Pha2* and *ts(b)* on LG VII], and soluble inorganic pyrophosphatase [*Ppa1(a)* and *ts(e)* on LG XII]. Particularly intriguing is the *Sut2* locus on linkage group V. This locus was identified by using as an RFLP probe a sucrose transporter-like gene which may function as a sucrose sensor (Barker et al. 2000). *Sut2* is tightly linked to the anchor marker *GP179* which itself is most closely linked to QTLs for tuber starch content [*ts(a)*], tuber yield, plant maturity and vigor (Schäfer-Pregl et al. 1998; Oberhagemann et al. 1999). It is conceivable that alleles of either a sucrose transporter or a sucrose sensor may affect the efficiency by which sucrose is translocated from source to sink tissues and have, therefore, an effect on the above traits.

In many cases, the same QTL was linked to several candidate gene loci. For example, at least three loci, *rbcS-1, Stp23* and *Me(a)*, overlap with QTL *ts(g)* on linkage group III, and three others, *SbeI*, *AmyZ* and *Pfp-α*, overlap with QTL *ts(k)* on linkage group IV. Further studies, for example QTL analysis based on activity measurements of the candidate enzymes (performed in maize, Causse et al. 1995), expression analysis, and functional analysis of molecular variants, are required for supporting the role of a putative candidate gene.

Three of the seventeen QTLs for tuber starch content shown in Fig. 2 were not linked to any known candidate-gene locus. QTL *ts(s)* on linkage group I was linked to acidic and basic β-1,3-glucanase genes (*GluA* and *GluB*) which, like chitinases (*ChtB* on LG X), are related to pathogenesis and defense (Kombrink and Somssich 1997) rather than to starch accumulation in tubers. Despite their linkage to QTLs for tuber starch content, they are not considered, therefore, as candidate genes for this trait.

The information on the total number of genes relevant for tuber starch content is incomplete and will remain so for the near future, despite the fact that the physiology, biochemistry and molecular biology of carbohydrate metabolism and transport in plants has been extensively studied. Genes controlling transcription, translation and post-translational modification of the starch metabolic enzymes have not yet been described. There are few lines of evidence, however, which suggest that the molecular-function map for the genes currently known to control carbohydrate metabolism and transport is a good basis for explaining, at the molecular level, QTLs for starch and sugar-related agronomic traits in plants. First,

in maize, all mutations affecting the starch content of the kernel that have been identified at the molecular level (Neuffer et al. 1997) affect the enzymes of carbohydrate metabolism, as are included in our map. Transcriptional regulators as candidate genes for QTLs, as described, for example, in the anthocyanin metabolic pathway (McMullen et al. 1998) have not been reported for starch metabolism. Second, the QTLs for the sugar content of kernels and other traits of maize were linked, for example, to the QTL for the enzymatic activity of sucrose phosphate synthase measured in maize kernels (Causse et al. 1995). Third, a first QTL for a trait related to carbohydrate metabolism, i.e., the sugar content of tomato fruits, has recently been characterized at the molecular level and was shown to be controlled by molecular variants of an apoplastic invertase gene. This gene is located on tomato chromosome 9 and is tightly linked to the potato anchor marker *GP263* (Fridman et al. 2000). On the map of potato chromosome IX, the marker *GP263* identifies a locus in the interval *CP137(b)–CP20 (a)* (Gebhardt et al. 1994). The same marker loci flank $Inv_{ap}(b)$ (Fig. 2), which is one of two loci detected with an apoplastic invertase probe of potato (Hedley et al. 1994). This demonstrates the validity of the molecular function map and of the candidate-gene approach.

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