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Isoenzymatic identification of quantitative traits in crosses between heterozygous parents: mapping tuber traits in diploid potato (*Solanum* spp.)

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Abstract. Eleven isozyme markers were utilized for quantitative trait loci (QTL) analysis in diploid potato. These markers are distributed among 7 of the 12 chromosomes and therefore give a representative, though sparse, survey of the potato genome. Tuber specific gravity and tuder dormancy were studied. Two segregating diploid populations were constructed from heterozygous self-incompatible parents. These two populations, TRP132 (127 individuals) and TRP133 (110 individuals), have a common maternal parent and combine genomes of *Solanum tuberosum* (haploid), *S. chacoense*, and *S. phureja*. The populations were planted at two locations in Michigan in 1990 using a randomized complete block design with three replications per location. After harvest they were characterized with the isozymes and evaluated for specific gravity and tuber dormancy. To test for QTLs, one-way analyses of variance were conducted for each locus by trait combination. Significant associations between markers and quantitative trait variation were identified, which accounted for a range from 4% to 15% of the phenotypic variation for specific gravity, and from 4.5% to 20.4% for tuber dormancy. Two-way analyses of variance between significant markers were used to identify epistatic interactions between markers. Multiple regression analyses were used to estimate the overall effect of the significant markers on the phenotypic variation for these traits. These values ranged from 15.3% and 32.3% for specific gravity. For dormancy, the significant loci accounted for 8.5% and 36.9% of the total phenotypic variation for each of the populations. We find that isozyme analysis is a useful tool for preliminary QTL studies in potato.

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Introduction

The concept of applying marker-assisted selection to the process of plant breeding has long been considered. Sax (1923) proposed identifying and selecting for "minor genes" of interest by linkage with "major genes", which could be scored more easily. Traditionally, the genetic markers used to develop maps in plants have been those affecting morphological characters. However, during recent years the use of isozymes and restriction fragment length polymorphisms (RFLPs) in plant breeding and their advantage over morphological markers has been reported (Tanksley and Rick 1980; Tanksley 1983; Beckmann and Soller 1983; Helentjaris et al. 1985; Tanksley et al. 1989). Genetic maps based upon these biochemical markers have been developed for a number of species, such as maize, tomato, pepper, potato, lettuce, rice, and *Brassica* (Helentjaris et al. 1986; Bernatsky and Tanksley 1986; Tanksley et al. 1988; Landry et al. 1987; Bonierbale et al. 1988; Gebhardt et al. 1989; McCouch et al. 1988; Slocum et al. 1990).

In addition to their use for the study and identification of monogenic traits, saturated genetic maps provide a means to estimate the number and genomic distribution of quantitative trait loci (QTLs) and to examine them as discrete Mendelian factors (Tanksley et al. 1982; Beckmann and Soller 1988; Lander and Botstein 1989; Stuber 1989; Paterson et al. 1991). The utilization of isozymes for the study of quantitative traits has been reported in maize (Stuber et al. 1980,

1982; Pollack et al. 1984; Frei et al. 1986a; Kahler and Wehrhahn 1986; Stuber et al. 1987) and tomato (Tanksley et al. 1982; Weller 1987; Weller et al. 1988).

Breeding of the cultivated potato, *Solanum tuberosum* subsp. *tuberosum* (2n = 4x = 48), is complicated by tetrasomic inheritance, the presence of cytoplasmic and nuclear sterilities (Grun et al. 1977), and inbreeding depression. In addition, it is generally acknowledged that the genetic base of cultivated tetraploid potato is narrow (Mendoza and Haynes 1974). One approach utilized to simplify the genetic system in potato is to breed at the diploid level using haploids of cultivated species and diploid wild and cultivated tuber-bearing species. These represent a large source of valuable germ plasm, which can broaden the genetic base of the cultivated potato and also provide specific desirable traits. The improved 2x germ plasm is then transferred into the 4x level using 2n gametes (Chase 1986; Iwanaga 1983; Peloquin et al. 1989). The efficiency of this approach could be greatly increased if the introgression of genes from the wild species could be closely monitored with molecular markers (Tanksley et al. 1989). Linkage of RFLPs with a major gene conferring resistance to cyst nematode (Barone et al. 1990) and two genes controlling resistance to PVX (Ritter et al. 1991) have been identified in diploid potato; however, linkages to quantitative traits have not yet been reported in this crop.

Two tuber traits of economic importance in potato are dry matter content and tuber dormancy. High dry matter content is a particularly important trait in potato cultivars used in the potato chip industry because of its association with increased chip yield and lower oil absorption (Owings 1979). Tuber dormancy is the obligate period of non-sprouting after harvest even under conditions favorable for sprouting (Thompson et al. 1980), and is critical because long-term storage without sprout growth is an important aspect of potato marketing. Previous genetic studies have reported that these two tuber traits are polygenic (Ruttencutter et al. 1979; Landeo 1979; Thompson et al. 1980), and they have been identified in selections made from South American diploid tuber-bearing relatives of the potato.

In potato 15 enzyme-coding loci are presently known to segregate (Douches and Quiros 1987; 1988). Some of them have been mapped onto several chromosomes on existing potato RFLP maps (Bonierbale et al. 1988; Gebhardt et al. 1989, 1991). Both isozyme and RFLP markers can be used for QTL analysis in potato. Isozymes can be used for a preliminary study, and subsequently, a more detailed genome survey using RFLP markers would be conducted. The objectives of this study were to characterize two diploid populations with isozymes, to conduct field and storage studies to characterize them for two polygenic tuber traits, and to identify associations between the markers and quantitative trait variation.

Materials and methods

Plant material

Two F_1 2x populations designated as TRP132 and TRP133 were utilized in this study. Clone 84SD22, which is a hybrid between haploid *S. tuberosum* (2x) and *S. chacoense*, was the common female parent. The males used were *S. phureja* clones 84S11 and 84S10 in the cases of TRP132 and TRP133, respectively. These parents were chosen because of their isozyme diversity and divergent characteristics: 84SD22 has a high dry matter content and long dormancy, while the *S. phureja* clones have low dry matter content and short dormancy. A total of 127 and 110 genotypes were used in TRP132 and TRP133, respectively. The seed tubers for the 1990 field sutdies were obtained from 1989 field plots.

Measurement of traits

Parents and progenies were evaluated for dry matter content and tuber dormancy from field-grown tubers. Both populations along with two of the parents (84SD22 and 84S10) were planted in 1990 at two locations using a randomized complete block design (RCBD) with three replications per location. There were not enough tubers of 84S11 to be planted in the field. Each plot consisted of eight plants with a within row distance of approximately 0.3 m and between row distance of 0.9 m. The two locations used were the Montcalm Research Farm, Edmore, Michigan (MES), and the Clarksville Horticultural Experiment Station, Clarksville, Michigan (CHES). The MES location was planted on May 14, 1990 and harvested after 119 days, while CHES was planted on May 24, 1990, and harvested after 131 days.

Following harvest, the specific gravity was determined for each genotype for both locations using the weight in air/weight in water method: [air wt/(air wt – water wt)]. This value is used to estimate the dry matter content of the tubers (Wilson and Lindsay 1959). A digital scale with a ± 1 g accuracy and a minimum sample size of 1 kg/plot were used. The value of specific gravity for each genotype was obtained from the mean of the three values from each of the replications in the field. For dormancy, a total of four tubers per genotype were placed on trays in storage at 10 °C following harvest and evaluated weekly. The length of dormancy was determined as the average number of days required for 2-mm-long sprouts to be evident for each genotype.

Isozyme analysis

The progenies and the parents were characterized for 11 segregating isozyme loci (*Dia-1*, *Est-1*, *Got-2*, *Idh-1*, *Mdh-1*, *6-Pgdh-3*, *Pgi-1*, *Pgm-1*, *Pgm-2*, *Prx-3*) using both leaf and tuber tissue. Electrophoretic and enzyme staining procedures have been described elsewhere (Douches and Ludlam 1991). The yellow flesh locus (Y) segregating in TRP133 was also scored.

Statistical analysis

Statistical analyses were carried out for a RCBD at each location for both traits. In the case of tuber dormancy, the $\log_{10}$ transformation for the average number of days to sprouting was used in all of the analyses to improve normality. For specific gravity,

two-way ANOVAs combined over locations were conducted for each population, and broad sense heritability values were estimated by the variance component method. In the case of dormancy, two-way ANOVA over replications and genotypes at the one location was used for the estimation of heritability.

Single factor ANOVAs were conducted for each pairwise combination of quantitative trait and marker locus (GLM, Statistical Analysis Systems, Cary, N.C.). To detect linkage of a marker locus with a QTL, the segregation data was divided into genotypic classes (backcross, F_2 or triallelic segregations). *F*-tests were used to statistically test if the means of the genotypic classes were different ($P < 0.05$). A significant difference in means was interpreted as linkage of QTL to the marker locus.

Epistatic interactions between significant markers were tested by two-way analyses of variance (PROC GLM, SAS). The significant main effects and significant interactions were combined in a multivariate linear regression model to predict the total variation explained with the markers (Keim et al. 1990). To study the effect of heterozygosity, correlation analyses were performed between the number of heterozygous isozyme loci (expressed as percentage) and quantitative trait value for each genotype.

Results

The populations TRP133 and TRP132 were characterized for 11 and 10 segregating isozyme loci, respectively. The genotypes for the parents (84SD22, 84S10, and 84S11) are shown in Table 1. 84SD22 was heterozygous for 9 loci, while 84S10 and 84S11 were heterozygous for 3 and 2 loci, respectively. Segregation patterns in the populations could be of three types: test-cross, F_2, or triallelic (Table 1, Fig. 1). Chi-square analyses indicated that segregation of all the isozyme loci fit the expected ratios in both populations (data not shown). On the basis of combined data from both populations (237 individuals), a linkage between *Est-1* and *Got-1* (12.8 +/− 3.0 map units) was identified.

Heterozygosity was estimated as the percentage of heterozygous isozyme loci for each individual. In TRP133 heterozygosity values ranged from 9% to 100% with a mean of 53%, and the distribution of these values in the population was normal. For TRP132 heterozygosity values ranged from 0% to 90%, with a mean of 52%. Nevertheless, the distribution of values in this population was skewed since 42% of the individuals had heterozygosity values between 60% and 70% (data not shown).

Specific gravity

MES and CHES locations were harvested after 119 and 131 days from planting, respectively, The range of values and means for specific gravity for both populations and parents in both locations is shown in Table 2. A range

Table 1. Isozyme genotypes for the parents and two populations

Isozymes	84SD22 (♀)	84S10 (♂ for TRP133)	Segregation in TRP133	84S11 (♂ for TRP132)	Segregation in TRP132
Dia-1	12[a]	11	12:11 (BC)[c]	11	12:11 (BC)
Est-1	FS[b]	SS	FS:SS (BC)	SS	FS:SS (BC)
Got-1	35	33	35:33 (BC)	33	35:33 (BC)
Got-2	15	55	15:55 (BC)	55	15:55 (BC)
Idh-1	12	11	12:11 (BC)	11	12:11 (BC)
Mdh-1	22	12	22:12 (BC)	12	22:12 (BC)
6-Pdh-3	12	11	12:11 (BC)	12	11:12:22 (F_2)[c]
Pgi-1	22	12	22:12 (BC)	22	22 (no segregation)
Pgm-1	13	33	13:33 (BC)	33	13:33 (BC)
Pgm-2	23	12	12:22:13:23 (tri)[c]	22	23:22 (BC)
Prx-3	13	11	13:11 (BC)	11	13:11 (BC)

[a] 12 refers to the allelic designation *Dia-1*^{1}*1*^{2}
[b] FS refers to Fast and Slow alleles, respectively (Douches and Quiros 1988)
[c] BC, tri, and F_2 refer to backcross, triallelic and F_2-type segregations, respectively

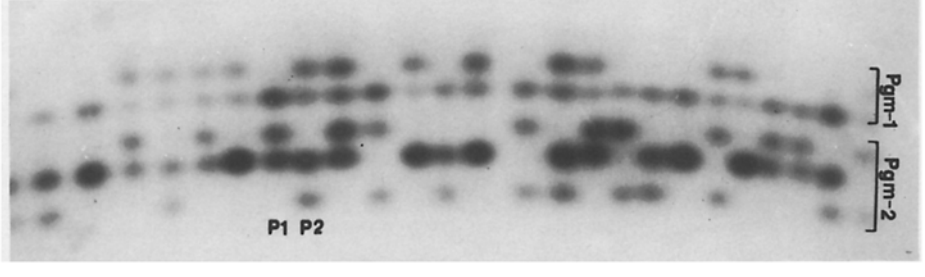

Fig. 1. *Pgm* loci in F_1 progeny TRP133. *P1* corresponds to parent 84S10 with genotype *Pgm-1*^{3}*1*^{3}, *Pgm-2*^{1}*2*^{2}; *P2* corresponds to parent 84SD22 with genotype *Pgm-1*^{1}*1*^{3}, *Pgm-2*^{2}*2*^{3}

of specific gravity values between 1.062 and 1.107 corresponds to a dry matter content between 17.43% and 24.98% in the tubers. The distribution pattern for both populations and locations is represented by population TRP132 at CHES (Fig. 2). Broad-sense heritability estimates for specific gravity combined over both locations were 89.2% and 86.1% for TRP133 and TRP132, respectively.

Values of specific gravity were averaged over each genotypic class for each isozyme locus, and one-way ANOVAs were conducted to test for significant differences among classes. In population TRP133, significant differences for genotypes were found for 3 unlinked loci, *6-Pgdh-3*, *Got-2*, and *Pgm-1*, and results were consistent over both locations. The amount of phenotypic variation (R^2) for specific gravity explained by individual markers ranged from 4.5% to 7% for MES and from 8.2% to 15% for CHES (Table 3). In TRP132, significant differences were also found for *6-Pgdh-3* and *Got-2* over both locations, however *Pgm-1* and *Dia-1* were significant only at the MES location. The amount of phenotypic variation for specific gravity explained by the markers at MES ranged from 4% to 6.8%, while for CHES it ranged from 6.6% to 10% (Table 3). No correlation was found between the number of heterozygous loci per genotype and specific gravity for either population grown at either location.

Two-way combinations of significant markers were tested for epistatic interactions at the 0.05 level. The only significant interaction found was *6-Pgdh-3*Got-2* for TRP133 at CHES. Multiple linear regression estimated that 16.7% of the total phenotypic variation for specific gravity could be explained by the effect of the 3 significant loci for TRP133 at MES. For the CHES location, this value was 32.3% with the 3 significant loci and 35.7% when the significant epistatic interaction was included in the model. For population TRP132, 19.6% of the phenotypic variation could be explained by the effect of the 4 significant loci at MES. For CHES, 17.5% of the variation could be explained by the 2 significant loci (Table 4).

In population TRP132, the *6-Pgdh-3* locus segregated in a F_2 manner and was found to have a significant association with specific gravity. This locus provided the only opportunity to examine gene action in this study (Edwards et al. 1987; Nienhuis et al. 1987).

Table 2. Values of specific gravity obtained for TRP133 and TRP132 populations, and the two parents[a]

	Montcalm		Clarksville	
	Range	Mean ± SE	Range	Mean ± SE
TRP133	1.046–1.099	1.079 ± 0.001	1.057–1.110	1.083 ± 0.001
TRP132	1.061–1.105	1.086 ± 0.001	1.062–1.115	1.086 ± 0.001
84SD22	1.078–1.082	1.080 ± 0.003	1.076–1.092	1.084 ± 0.011
84S10	1.065–1.068	1.067 ± 0.002	1.062–1.065	1.064 ± 0.002

[a] For the populations, the range and mean are obtained from the mean values for each genotype from the three replications. For the parents, the range and mean are obtained from the mean value from the three replications in each of the population's fields

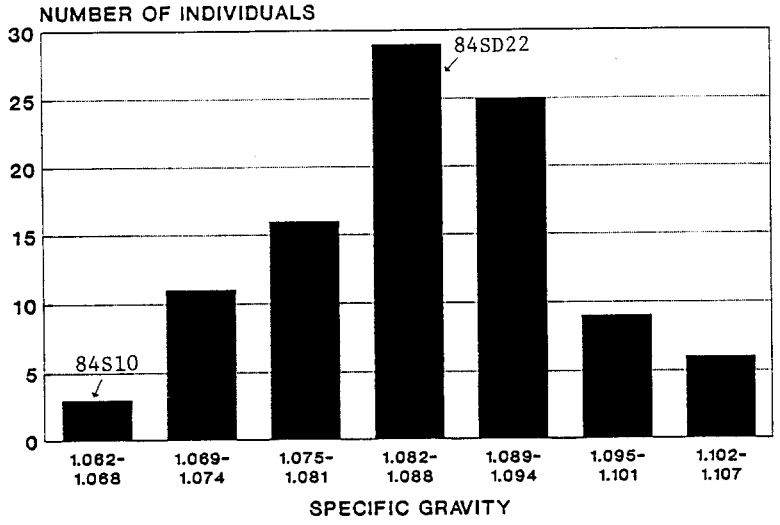

Fig. 2. Distribution of specific gravity values for population TRP132 grown in Clarksville, Michigan 1990

Table 3. Significant association between specific gravity and isozymes for populations TRP133 and TRP132

	Genotype[a]	Mean specific gravity	Pr > F	R^2
TRP133				
Montcalm				
6-Pgdh-3	11	1.081	0.019	0.050
	12	1.077		
Got-2	15	1.081	0.027	0.045
	55	1.077		
Pgm-1	13	1.081	0.005	0.070
	33	1.076		
Clarksville				
6-Pgdh-3	11	1.086	0.000	0.150
	12	1.080		
Got-2	15	1.086	0.001	0.100
	55	1.081		
Pgm-1	13	1.085	0.003	0.082
	33	1.080		
TRP132				
Montcalm				
6-Pgdh-3	11	1.090	0.013	0.068
	12	1.085		
	22	1.084		
Got-2	15	1.088	0.003	0.068
	55	1.084		
Pgm-1	13	1.088	0.024	0.040
	33	1.084		
Dia-1	11	1.088	0.003	0.068
	12	1.084		
Clarksville				
6-Pgdh-3	11	1.089	0.015	0.066
	12	1.086		
	22	1.083		
Got-2	15	1.089	0.000	0.100
	55	1.083		
Pgm-1	13	1.088	0.077	
	33	1.085	ns	
Dia-1	11	1.088	0.099	
	12	1.085	ns	

ns, Not significant at the 0.05 level
[a] See footnote to Table 1 for definition of genotypes

Table 4. Multiple regression values for specific gravity for populations TRP133 and TRP132

Model	Pr > F	R^2
TRP133		
MES *6-Pgdh-3*	0.000	0.167
Got-2		
Pgm-1		
CHES *6-Pgdh-3*	0.000	0.323
Got-2		
Pgm-1		
6-Pgdh-3	0.000	0.357
Got-2		
Pgm-1		
*6-Pgdh-3*Got-2*		
TRP132		
MES *6-Pgdh-3*	0.000	0.196
Got-2		
Pgm-1		
Dia-1		
CHES *6-Pgdh-3*	0.000	0.175
Got-2		
Pgm-1		
Dia-1		

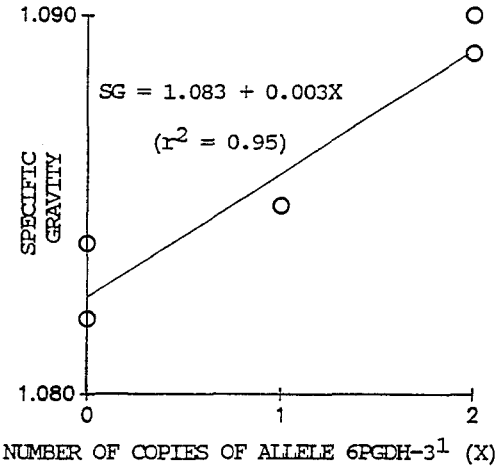

Fig. 3. Regression on the means for each genotypic class of *6-Pgdh-3* in family TRP132 (averaged over both locations)

The homozygous class *6-Pgdh-3*^{1}*3*^{1} had higher values than either of the heterozygote *6-Pgdh-3*^{1}*3*^{2} or the other homozygote *6-Pgdh-3*^{2}*3*^{2} classes. Regression analysis was performed using the means for each genotypic class averaged over both locations. The data fit an additive model for gene action, and the effect of allele substitution in this locus could be determined (Fig. 3).

Tuber dormancy

The average number of days to sprouting at 10 °C for the parents was 10 and 80 days for 84S10 and 84SD22, respectively. In population TRP133, the average number of days to sprouting ranged from 10 to 112, with a mean of 20.2 days. The range of days to sprouting for population TRP132 was 10 to 120, with a mean of 33.9. The distributions of both populations were found to be highly skewed towards lack of dormancy, which was imparted by the *S. phureja* parent. The transformation $\log_{10}$ of the average number of days to sprouting was used in all the analyses. The distribution of transformed values is shown for TRP132 (Fig. 4).

Broad-sense heritability estimates for dormancy with data from the one location were 93.8% for

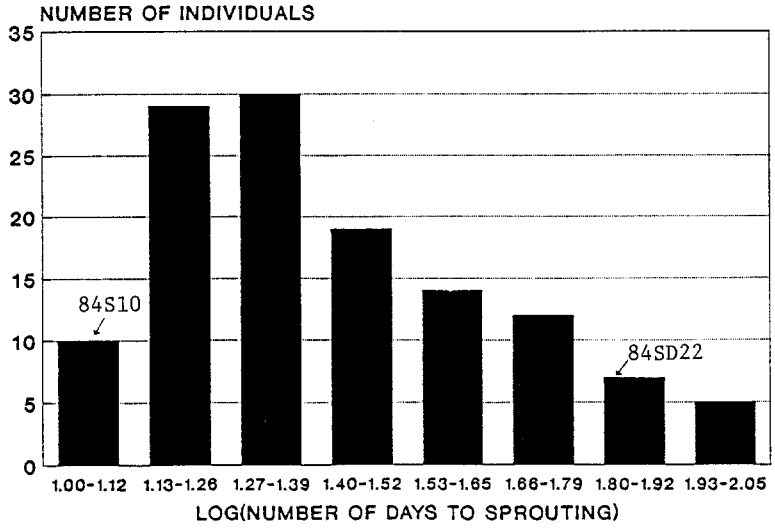

Fig. 4. Distribution of $\log_{10}$ days to sprouting values for population TRP132

Table 5. Significant association between tuber dormancy and isozymes for populations TRP133 and TRP132

	Genotype[a]	Log_{10} mean days sprouting	Pr > *F*	R^2
Trp133				
6-Pgdh-3	11	1.267	0.006	0.068
	12	1.155		
Got-1	33	1.286	0.001	0.090
	35	1.155		
Got-2	15	1.325	0.000	0.204
	55	1.128		
Pgm-2	23	1.265	0.006	0.068
	22	1.164		
Prx-3	11	1.264	0.017	0.052
	13	1.165		
Est-1	SS	1.269	0.011	0.058
	FS	1.164		
TRP132				
Est-1	SS	1.498	0.000	0.085
	FS	1.347		
Got-1	33	1.478	0.016	0.045
	35	1.368		

[a] See footnote to Table 1 for definition of genotypes

Table 6. Multiple regression values for tuber dormancy for populations TRP133 and TRP132

Model	Pr > *F*	R^2
TRP133		
6-Pgdh-3	0.001	0.369
Got-1		
Got-2		
Pgm-1		
Prx-3		
Est-1		
TRP132		
Est-1	0.004	0.085
Got-1		

TRP133 and 92.6% for TRP132. Correlation between the two tuber traits for each location and population was found to be significant only for TRP133 and specific gravity data from CHES. This showed a weak correlation of $r = 0.236$ ($P = 0.013$). The effect of number of heterozygous isozyme loci per genotype and the length of dormancy was also studied in each population, and no correlation was found.

One-way ANOVAs were conducted between the tuber dormancy data and isozyme locus genotypes. Significant differences were found for *6-Pgdh-3*, *Got-1*, *Got-2*, *Pgm-2*, *Prx-3*, and *Est-1* in TRP133. The amount of phenotypic variation for this trait explained by each marker ranged from 5.2% to 20.4%. In population TRP132, significant differences were found for *Est-1* and *Got-1*, which explained 8.5% and 4.5% of the phenotypic variation, respectively (Table 5).

No significant epistatic interactions between significant markers were found for this trait by two-way analysis of variance in either population. Multiple linear regression estimated that 36.9% of the total phenotypic variation for tuber dormancy was due to the effect of the 6 significant loci for TRP133, while for TRP132, 8.5% of the phenotypic variation could be explained by the effect of the 2 significant loci (Table 6).

Discussion

Ten and 11 isozyme loci were segregating the two populations studied. Previous linkage analyses with

RFLP markers indicate the these isozyme markers are distributed among 7 of the twelve potato chromosomes (Bonierbale et al. 1987). In addition, gene-centromere map distance estimates (Douches and Quiros 1987, 1988) indicate random distribution of these markers along the chromosome arms. This is confirmed by our current linkage analyses of data where only *Est-1* and *Got-1* were found to be linked. These facts lead us to believe that the isozyme markers used in this study give a representative, though sparse, survey of the potato genome.

Two quantitative traits were examined in each of the two populations, and one of the traits was examined in two locations. Data for these traits in the two populations were continuous, as would be expected for a polygenic trait. *F*-tests for each pairwise combination of quantitative trait and isozyme locus were used to determine whether significant differences in trait expression were associated with genotypes at each of the segregating isozyme loci. Significant ($P < 0.05$) associations were found for 12 of 33 comparisons in TRP133 (36%) and 8 of 30 comparisons in TRP132 (27%). These values are low when compared with results obtained in similar studies with tomato, where 56% of the comparisons were found to be significant (Tanksley et al. 1982), and maize, where these values ranged from 60% to 66% (Edwards et al. 1987). This may be due to the fact that in these latter two cases larger population sizes were used, which should detect smaller phenotypic effects, and also some of the markers were linked, thus reflecting the effect of common quantitative trait loci.

Significant association was found between 3 isozyme loci (*6-Pgdh-3*, *Got-2* and *Pgm-1*) and specific gravity in population TRP133, and the results were consistent in both locations. In TRP132, significant differences were also found for *6-Pgdh-3* and *Got-2* over both locations, and *Pgm-1* and *Dia-1* were significant at only one location. We conclude that isozyme loci *6-Pgdh-3* and *Got-2* show a strong, stable association with this trait, whereas *Pgm-1* and *Dia-1* may have a G × E interaction such as found with QTLs for fruit traits in tomato (Paterson et al. 1991). In the case of dormancy, the distribution of the average number of days to sprouting for both populations was highly skewed towards lack of dormancy. This could be explained by dominance effects from the *S. phureja* parents. For TRP133, significant association was found with 6 isozyme loci and dormancy. Two of these, *Est-1* and *Got-1*, were also significant in TRP132, thereby showing a stable association with this trait. Loci *6-Pgdh-3* and *Got-2* were found to be associated both with specific gravity and dormancy in TRP133. Nevertheless, only a weak correlation between both traits was found with specific gravity data from one of the locations in this population.

The proportion of phenotypic variation explained by individual marker loci can be estimated by the R^2 value obtained by the regression of trait values on marker genotypes. This study detected effects as small as 4% of the total phenotypic variation, while in maize factors contributing as little as 0.2% of the phenotypic variation in yield-related traits could be detected using isozyme markers and large populations (more than 1500 plants) (Stuber et al. 1987). For specific gravity, the phenotypic variation explained by individual markers ranged between 4.0% and 15%, whereas for tuber dormancy it was between 4.5% and 20.4%. The cumulative effects of all significant marker loci on the traits were estimated through multiple linear regressions. In this case, the amount of phenotypic variation that was explained by significant markers ranged between 16.7% and 32.3% for specific gravity, and for dormancy it was 8.5% and 36.9% for TRP132 and TRP133, respectively. At present it is not known whether the isozymes per se have a direct influence on the trait or whether there is only an association through linkage. It is generally assumed that these enzymes are nearly-neutral genetic markers, and alleles at most isozyme loci probably do not directly affect the phenotypic expression of the quantitative trait evaluated (Stuber 1989). In their studies in maize, Pollack et al. (1984) indicate that the *Acp-1* locus may be associated with yield either directly or through linkage; in tomato, Tanksley et al. (1982) and Weller et al. (1988) indicate that the effect of significant enzyme loci is due to linkage to the QTLs. The level of variation explained by individual marker loci is thus affected by its genetic linkage to the QTL. In our study the effect may have been underestimated due to loose linkages. Subsequent RFLP analysis to survey the whole potato genome should identify more and tighter linkages, and a greater percentage of variantion for the trait may be explained.

All two-way combinations of significant markers were tested to detect significant epistatic interactions affecting the traits. For specific gravity, the only significant interaction found was between *6-Pgdh-3* and *Got-2* for TRP133 at CHES. These two markers have been previously located in chromosomes 5 and 7, respectively (Bonierbale et al. 1987). The inclusion of this interaction in the multiple regression model resulted in an R^2 of 35.7% which represents a gain of 3.4% from the main-effects model. For dormancy, no significant epistatic interactions between markers were found. This is similar to results found in tomato, where several traits did not show any significant interactions (Weller et al. 1988).

There is no apparent effect of heterozygosity in either of the traits studied, as demonstrated by the lack of correlation between the number of heterozygous loci and the value of the trait for each individual. This contrasts with results found in maize where the level of heterozygosity plays a very large role in the expression

of grain yield (Edwards et al. 1987). Also, there is no association between the highest value for the quantitative trait and the heterozygous genotype of the isozyme loci showing significant linkage. Therefore, an additive model for the traits has been assumed. This is supported by the regression analysis with *6-Pgdh-3* in TRP132, which provided the only opportunity to examine gene action at a locus in this study. The effect of allele substitution can be estimated, and specific gravity can be explained by the regression formula Specific Gravity = 1.083 + 0.003X, where X equals the number of copies of the allele *6-Pgdh-3*[1], which corresponds to the favorable allele coming from 84SD22.

The potato poses challenges to QTL analysis. Generation of inbred potato lines in not practical due to self-incompatibility and inbreeding depression at the diploid level. F_1 populations constructed from heterozygous diploid parents can have backcross, F_2, and multiple-allelic segregation patterns occurring in a single cross, thus limiting our ability to examine intralocus effects of QTLs. However, we have identified associations between the isozyme markers and the traits by using one-way ANOVAs and *F*-tests using a significance level of $P < 0.05$. This level of significance has been considered to give a great risk of identifying false positives (Lander and Botstein 1989). Nevertheless, in this study, QTL analysis is strengthened by basing the results on two populations over two locations. Since significant linkages were determined across genetic backgrounds and locations, we can more confidently state that a QTL has been correctly identified. One advantage of potato over other crops is that since it is clonally propagated, enough seed tubers can be available of the same genotypes to conduct replicated studies, which is not feasible in most seed-propagated crops such as maize or tomato.

We find that isozyme analysis is a useful tool for QTL studies in potato. Isozyme characterization can be quickly completed in a large number of individuals, thereby providing a preliminary identification of putative linkages to quantitative traits. RFLP analysis is being used to further localize and fine-map QTLs with markers and to strategically survey the potato genome for other QTLs not revealed with isozymes.

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