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QUANTITATIVE TRAIT LOCI ANALYSIS OF TUBER TRAITS IN DIPLOID POTATO (Solanum spp.)

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QUANTITATIVE TRAIT LOCI ANALYSIS OF TUBER TRAITS IN DIPLOID POTATO (*Solanum spp*)

By

Rosanna Freyre

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ABSTRACT

QUANTITATIVE TRAIT LOCI ANALYSIS OF TUBER TRAITS IN DIPLOID POTATO (*Solanum spp.*)

By

Rosanna Freyre

One breeding method for potato (*Solanum tuberosum* subspp. *tuberosum*) is using wild species. This method could be more efficient if the introgression of genes from these species were monitored with molecular markers. Furthermore, the use of molecular markers allows the dissection of quantitative traits into discrete genetic factors. The objective of this research was to perform quantitative trait loci (QTL) analysis on two tuber traits in potato: specific gravity and dormancy. Two 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*. A preliminary analysis using isozymes was performed. QTLs were determined by one-way analyses of variances for each locus by trait combination ($P < 0.05$). Epistatic interactions were detected through two-way analyses of variance. Further studies focused on TRP133, which was characterized for 10 isozyme loci, 44 RFLPs and 63 RAPDs. Eighty-seven loci segregating from the female parent were utilized to construct a linkage map comprising 10 of the 12 chromosomes in the genome. For dormancy, 6 QTLs were

identified that explained 57.5% of the phenotypic variation for the trait. Specific gravity was evaluated in 3 environments. QTLs were mapped separately for each location and in combination. A total of 10 QTLs on six chromosomes were identified. The numbers and effects of QTLs detected varied across environments, and they explained from 39% to 45% of the phenotypic variation for the trait. Using the average data a multilocus model was developed. This gives consistent results when tested across environments, and may be valuable for marker-assisted selection. This research developed the basic methodology for QTL analysis in potato which is now available for future studies with other traits and germplasms.

To my parents, Ana and Alfredo,

for all their love and support.

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Every step along this research was totally new for me so I needed much help and advice along the way. I would like to thank friends in the Potato Group that have helped me in many ways, and particularly during field work: Donna Kells, Karen Hokanson, Dave Maas, Maywa Blanco, Drs. Kazmierz Jastrzebski and Dick Chase. My appreciation also goes to Theresa Woods and Dr. Kazmierz Jastrzebski for their assistance during the evaluation of traits. Thanks go to Drs. Steve Tanksley and Christianne Gebhardt for supplying the tomato and potato RFLP probes, respectively.

Many thanks to Bryon Sosinski for optimizing the PCR protocol and screening of the parents with primers. The helpful advice of Jose Barbosa, Jorge da Silva and Susan McCouch at Cornell University during the construction of the map are greatly appreciated. My thanks go to Dr. Rodomiro Ortiz, Scott Warnke, Paul Fisher and Jeff Smeenk for their valuable help and advice during the statistical analysis and computer work. I am particularly grateful to Brian Diers for thoughtful discussion, letting me borrow his computer many times, and answering many questions during the last phase

of my research.

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Special thanks go to my parents, Alfredo and Ana, to Samira Daroub for her special friendship, and to Paul Fisher and many other family members and friends for all their love and support.

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LIST OF ABREVIATIONS

TRP132	2x population derived from cross of 84SD22 x 84S11
TRP133	2x population derived from cross of 84SD22 x 84S10
MES90	Montcalm Experimental Station, 1990 field trial
MES91	Montcalm Experimental Station, 1991 field trial
CHES90	Clarksville Horticulture Experimental Station, 1990 field trial
AVE	Average data, combined over 3 environments
Isozymes:	
Dia-1	Diaphorase, allele 1
Est-1	Esterase, allele 1
Got-1	Gluconate Oxaloacetate Transaminase, allele 1
Got-2	Gluconate Oxaloacetate Transaminase, allele 2
Idh-1	Isocitrate Dehydrogenase, allele 1
Mdh-1	Malate Dehydrogenase, allele 1
6-Pgdh-3	6-Phosphogluconate Dehydrogenase, allele 1
Pgi-1	Phosphogluconate Isomerase, allele 1
Pgm-1	Phosphoglucomutase, allele 1
Pgm-2	Phosphoglucomutase, allele 2
Prx-3	Peroxidase, allele 3
RFLPs	Restriction Fragment Length Polymorphisms
TG..	Tomato genomic probes, Cornell University
CD..	Tomato cDNA probes, Cornell University
GP..	Potato genomic probes, Max Planck Institut
CP..	Potato cDNA probes, Max Planck Institut
RAPDs	Random Amplified Polymorphic DNA

GENERAL INTRODUCTION

The cultivated potato, *Solanum tuberosum* subsp. *tuberosum*, is one of the most important world food crops. It is cultivated in 130 countries and ranks fourth in volume of production in the world with approximately 290 million tons annually (FAO, 1992). This crop is tetraploid (2n=4x=48) with tetrasomic inheritance. One methodology utilized to simplify its genetic system is to breed at the diploid level which takes advantage of simple disomic inheritance. Over 70% of the wild and cultivated tuber-bearing *Solanum* species are diploids (Hawkes, 1990). These species have agronomically important attributes and can be easily crossed with haploids extracted from the cultivated species (*Solanum tuberosum* subsp. *tuberosum* or *andigena*). The improved 2x germplasm is then transferred to the 4x level using sexual polyploidization through 2n gametes (Chase, 1968; Iwanaga, 1983, Peloquin et al., 1989). This ploidy manipulation approach has been applied in several institutions in the world and has led to new cultivars released in USA and the International Potato Center (CIP) (Peloquin et al., 1989; Ortiz, 1991). One drawback to the use of 2x germplasm in potato breeding is the slow progress due to linkage drag of undesirable traits from the wild species. However, it has been suggested that the efficiency of breeding could be largely increased if the introgression of genes from the wild species could be closely monitored with molecular markers (Tanksley et al., 1989). Furthermore, the use of molecular markers for the analysis of quantitative traits has been described (Tanksley et al., 1982; Beckmann and Soller, 1988;

Paterson et al., 1988; Lander and Botstein, 1989). In this study, two diploid populations combining genomes of haploid *S. tuberosum* and the wild species *S. chacoense* and *S. phureja* were utilized for quantitative trait loci (QTL) analysis of tuber traits using molecular markers. The traits studied were specific gravity, which is an indirect measurement for dry matter content, and tuber dormancy. Both these traits have importance for the potato industry in Michigan, and moreover they are relatively easily measured. These characteristics made them adequate to study the feasibility of applying QTL analysis in the Potato Breeding Program at Michigan State University.

The first molecular markers available for study were isozymes. QTL analysis using these markers has been reported in maize (Stuber et al., 1980; Stuber et al., 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). However, their use as markers is limited due to the small numbers available. In potato, 15 enzyme-coding loci are presently known to segregate (Douches and Quiros, 1988a) and they had been previously utilized mostly for variety fingerprinting (Douches and Ludlam, 1991b), half-tetrad analysis (Werner et al., 1992) and systematic studies (Spooner et al., 1992). The first chapter of this study, Isoenzymatic identification of quantitative traits in crosses between heterozygous parents: Mapping tuber traits in diploid potato (*Solanum spp*), refers to QTL analysis with isozymes utilizing two populations, evaluating specific gravity in two environments, and also tuber dormancy.

Another set of molecular markers with greater potential based on restriction fragment length polymorphisms (RFLPs) have the advantage that the number available is virtually unlimited (Beckmann and Soller, 1983; Helentjaris et al., 1985). RFLPs maps have been

constructed for many crops including potato. The first map in potato was developed using tomato RFLP probes on an interspecific 2x population involving three diploid species: *S. phureja*, haploid *S. tuberosum*, and *S. chacoense* (Bonierbale et al., 1988). This map was further saturated with markers using a population involving haploid *S. tuberosum* and *S. berthaultii* (Tanksley et al., 1992). Independently, another map was developed using a 2x *S. tuberosum* population and potato RFLP probes (Gebhardt et al., 1989b). This map was also aligned with the homoeologous tomato genome (Gebhardt et al., 1991). RFLP markers have since been used to fingerprint potato lines (Gebhardt et al., 1989a; Douches et al., 1991a), to determine the phylogeny of wild and cultivated species (Debener et al., 1990) and to determine the extent of genetic variability in cultivars (Powell et al., 1991). Linkage with two major genes conferring resistance to PVX (Ritter et al., 1991), a gene conferring resistance to cyst nematode (Barone et al., 1990; Gebhardt et al., 1993; Pineda et al., 1993), and three flower color loci (van Eck et al., 1993) have also been reported. Research involving genetic mapping of quantitative trichome-mediated insect resistance (Bonierbale et al., 1992) and loci affecting tuberization (van der Berg et al., 1992) are in progress.

Recently, polymerase chain reaction (PCR)-based genetic markers have become available. A novel technique that rapidly generates and screens random DNA segments for polymorphisms between different genotypes was developed simultaneously by Welsh and McClelland (1990) and Williams et al. (1990). This technique offers the advantages of less time and labor involved as compared with RFLP analysis. The randomly amplified polymorphic DNA (RAPD) markers have since been incorporated in linkage maps in tomato (Klein-Lankhorst et al., 1991) and *Brassica* (Quiros et al., 1991); have

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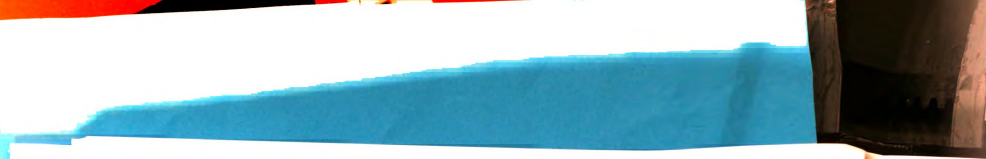
been utilized for characterization and cultivar identification in *Brassica* (Hu and Quiros, 1991; Boury et al., 1992; Kresovich et al., 1992), rice (Fukuoka et al., 1992), cocoa (Wilde et al., 1992), papaya (Stiles et al, 1993) and apple (Koller et al., 1993; Harada et al., 1993). Linkage to resistance genes have been identified in tomato (Martin et al., 1991), lettuce (Michelmore et al., 1991; Paran et al., 1991) bean (Miklas et al., 1993) and oats (Penner et al., 1993). In potato, RAPD markers have been utilized in evolutionary studies (Cisneros et al., 1991); to detect gene introgression (Waugh et al., 1992); their segregations in 2x and 4x families have been studied (Quiros et al., 1993); and they have been utilized to screen somatic hybrids (Xu et al., 1993).

The RFLP and subsequent RAPD analyses in this research focused on one population. Chapter 2, Quantitative trait loci analysis of tuber dormancy in diploid potato (*Solanum spp*), refers to the development of a linkage map including isozyme, RFLP and RAPD loci and the identification of QTLs associated with dormancy. The third chapter, Quantitative trait loci analysis of specific gravity in diploid potato (*Solanum spp*) over environments: development of a model for marker-assisted selection, refers to QTL analysis performed on this trait separately for three environments, and the use of the average data to develop a multilocus model to be used for marker-assisted selection.



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CHAPTER ONE

ISOENZYMATIC IDENTIFICATION OF QUANTITATIVE TRAITS IN CROSSES BETWEEN HETEROZYGOUS PARENTS: MAPPING TUBER TRAITS IN DIPLOID POTATO (*Solanum spp.*)

ABSTRACT. Eleven isozyme markers were utilized for Quantitative Trait Loci (QTL) analysis in diploid potato. These markers are distributed among seven of the twelve chromosomes and therefore give a representative, though sparse, survey of the potato genome. Tuber specific gravity and tuber 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. Isozyme analysis has proved to be a useful tool for preliminary QTL studies in potato.

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 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 examine them as discrete Mendelian factors

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(Tanksley et al., 1982; Beckmann and Soller, 1988; Lander and Botstein, 1989; Stuber, 1989b; Paterson et al., 1991). The utilization of isozymes for the study of quantitative traits has been reported in maize (Stuber et al., 1980; Stuber et al., 1982; Pollack et al., 1984; Frei et al., 1986; 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 germplasm, which can broaden the genetic base of the cultivated potato and also provide specific desirable traits. The improved 2x germplasm is then transferred into the 4x level using 2n gametes (Chase, 1968; 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

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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, fifteen 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; Gebhardt et al., 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 the *S. phureja* clones 84S11 in the case of TRP132, and 84S10 for TRP133. 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 studies 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). 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, 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 4 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-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 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, NC). 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 1.1. 84SD22 was heterozygous for nine loci, while 84S10 and 84S11 were heterozygous for three and two loci, respectively. Segregation patterns

[a] 84S1
[b] 12 re
[c] FS re
[d] BC, t

Table 1.1. Isozyme genotypes for the parents and two populations

Isozymes	84SD22 (♀)	84S10 (♂)[a]	Segregation in TRP133	84S11 (♂)[a]	Segregation in TRP132
Dia-1	12[b]	11	12:11 (BC)[c]	11	12:11 (BC)
Est-1	FS[c]	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)
6Pgdh-3	12	11	12:11 (BC)	12	11:12:22(F_2)[c]
Pgi-1	22	12	22:12 (BC)	22	22 (no seg.)
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] 84S10 and 84S11 are male parents for populations TRP133 and TRP132, respectively
[b] 12 refers to the allelic designation Dia-$1^1 1^2$
[c] FS refers to Fast and Slow alleles, respectively (Douches and Quiros, 1988)
[d] BC, tri and F_2 refer to backcross, triallelic and F_2-type segregations, respectively

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in the populations could be of three types: testcross, F_2 or triallelic (Table 1.1). Chi-square analyses indicated that segregation of all the isozyme loci fit the expected ratios in both populations (data not shown). Based upon 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 1.2. A range 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 (Figure 1.1). 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

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Table 1.2. Values of specific gravity obtained for populations TRP133 and TRP132, 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.

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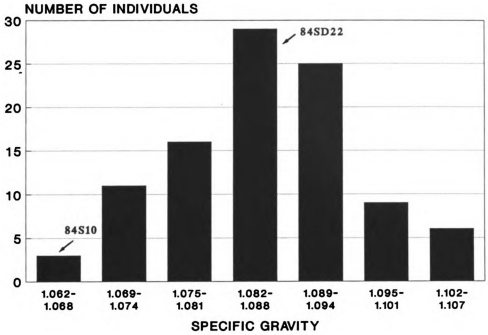

Figure 1.1. Frequency distribution of specific gravity values for TRP132 grown in Clarksville, Michigan 1990 (84SD22 and 84S10 are the female and male parents, respectively.)

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among these classes. In population TRP133, significant differences for genotypes were found for three 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 1.3). 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 3.1). 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 analysis of variance estimated that 16.7% of the total phenotypic variation for specific gravity could be explained by the effect of the three significant loci for TRP133 at MES. For CHES location, this value was 32.3% with the three 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 four significant loci at MES. For CHES, 17.5% of the variation could be explained by the two significant loci (Table 1.4).

Table 1.
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Table 1.3. Significant association between specific gravity and isozymes for populations TRP133 and TRP132

	Genotype	Mean Specific Gravity	Pr > F	R^2
TRP133				
MES				
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		
CHES				
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				
MES				
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		
CHES				
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[a]	
Dia-1	11	1.088	0.099	
	12	1.085	ns	

[a] **ns** = not significant at the 0.05 level

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Table 1.4. Values obtained from the multiple analyses of variance for specific gravity for populations TRP133 and TRP132

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

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). 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}. 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 (Figure 1.2).

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 110, with a mean of 18 days. The range of days to sprouting for population TRP132 was 10 to 120, with a mean of 34. The distributions of both populations were found to be highly skewed towards lack of dormancy 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 (Figure 1.3).

Broad sense heritability estimates for dormancy with data from the one location were 93.8% for 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 1.5).

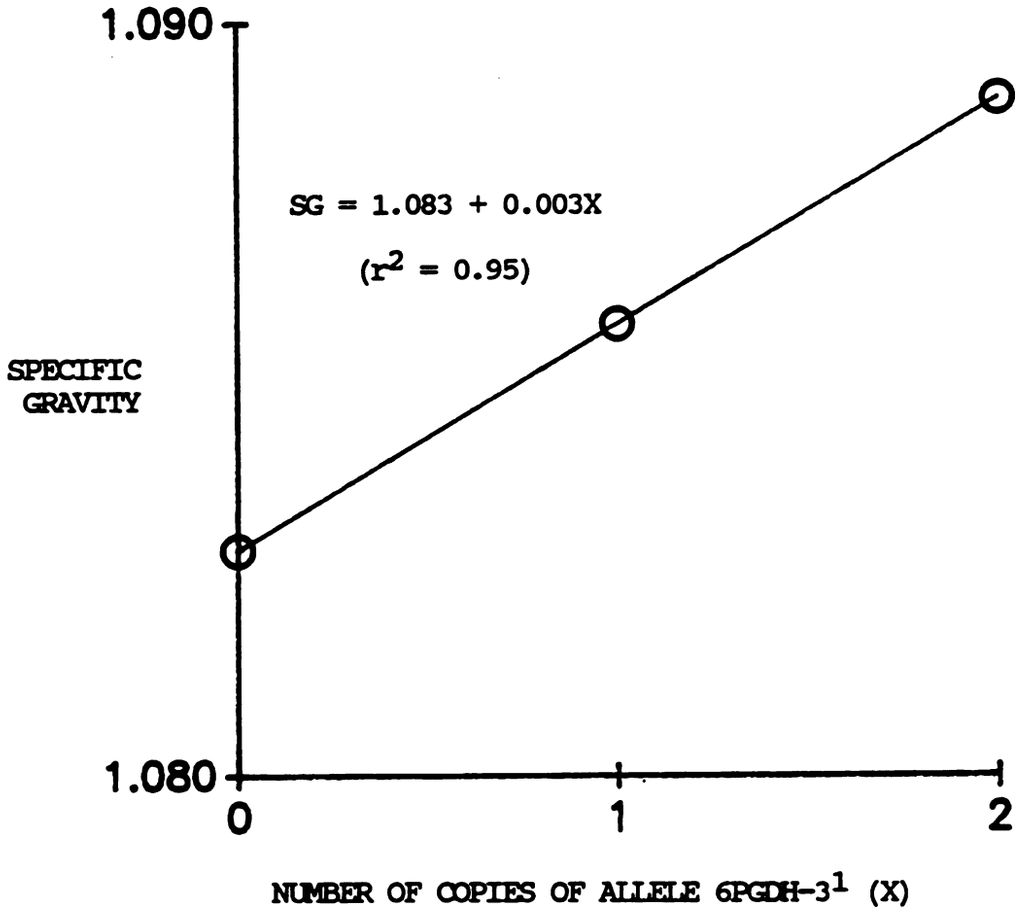


Figure 1.2. Regression on the means for each genotypic class of 6-Pgdh-3 in family TRP132 (averaged over both locations)

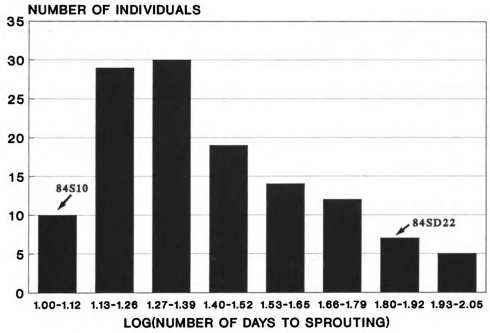


Figure 1.3. Frequency distribution of length of dormancy (log_{10}transformed) values for TRP132 (84SD22 and 84S10 are the female and male parents, respectively).

Table 1.5. Significant association between tuber dormancy and isozymes for populations TRP133 and TRP132

	Genotype	Log_{10}Mean Days to Sprouting	Pr > F	R^2
TRP133				
6-Pgdh-3	11 12	1.267 1.155	0.006	0.068
Got-1	33 35	1.286 1.155	0.001	0.090
Got-2	15 55	1.325 1.128	0.000	0.204
Pgm-2	23 22	1.265 1.164	0.006	0.068
Prx-3	11 13	1.264 1.165	0.017	0.052
Est-1	SS FS	1.269 1.164	0.011	0.058
TRP132				
Est-1	SS FS	1.498 1.347	0.000	0.085
Got-1	33 35	1.478 1.368	0.016	0.045

No significant epistatic interactions between significant markers were found for this trait by two-way analysis of variance in either population. Multiple analysis of variance estimated that 36.9% of the total phenotypic variation for tuber dormancy was due to the effect of the six significant loci for TRP133, while for TRP132, 8.5% of the phenotypic variation could be explained by the effect of the two significant loci (Table 1.6).

Table 1.6. Values obtained from the multiple analyses of variance for tuber dormancy for populations TRP133 and TRP132

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

DISCUSSION

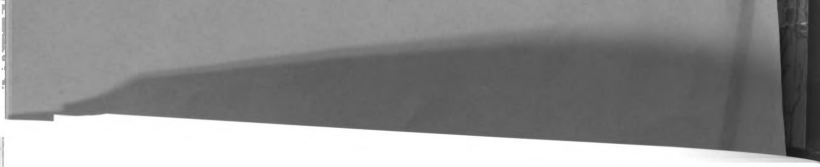
Ten and eleven isozyme loci were segregating in the two populations studied. Previous linkage analyses with RFLP markers indicate that these isozyme markers are distributed among seven of the twelve potato chromosomes (Bonierbale et al., 1987). In addition, gene-centromere map distance estimates (Douches 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 was continuous as 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 as compared with results obtained in similar studies with tomato where 56% of the comparisons were found to be significant (Tanksley et al., 1982) and in maize where these values ranged from 60% to 66% (Edwards et al., 1987). This may be due to the fact that in these 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 three isozyme loci (*6-Pgdh-3*, *Got-2* and *Pgm-1*) and specific gravity in population TRP133, and 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 x 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 six isozyme loci and dormancy. Two of these, *Est-1* and *Got-1* were also significant in TRP132, 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

be estimated by the R^2 value obtained in the analyses of variance. 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 was estimated through multiple analyses of variance. 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 are associated only 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, 1989a). In 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 could be underestimated due to loose linkages. Subsequent RFLP analysis to survey the whole potato genome should identify more and tighter linkages and therefore a greater percentage of variation for the trait may be explained.

All two-way combinations between 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 analysis of variance 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 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 is 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 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, it can be more confidently stated 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.

Isozyme analysis has proved to be a useful tool for QTL studies in potato. Isozyme characterization can be quickly completed in a large number of individuals, 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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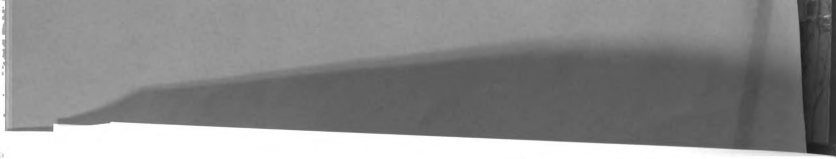


CHAPTER TWO

QUANTITATIVE TRAIT LOCI ANALYSIS OF TUBER DORMANCY IN DIPLOID POTATO (*Solanum spp.*)

ABSTRACT. Quantitative trait loci (QTL) analysis for tuber dormancy was performed in a diploid population of potato (TRP133) consisting of 110 individuals. This population was derived from the cross of a hybrid between haploid *S. tuberosum* (2x) and *S. chacoense*, with a *S. phureja* clone. The population was characterized for 10 isozyme loci, 44 RFLPs and 63 RAPDs. Eighty-seven of the loci segregating from the female parent were utilized to develop a linkage map that comprises 10 of the 12 chromosomes in the genome. The length of dormancy in the population ranged from 10 to 90 days to sprouting, with a mean of 19 days. QTLs for this trait were determined by conducting one-way analyses of variance for each locus by trait combination. Twenty-two markers had a significant association with dormancy, identifying 6 QTLs localized on each of chromosomes 2, 3, 4, 5, 7 and 8. The QTL with the strongest effect on the trait was detected on chromosome 7. A multilocus model was developed using the locus with highest R^2 value in each QTL. This model explained 57.5% of the phenotypic variation for dormancy. Seven per cent of the possible epistatic interactions tested through two-way analyses of variance were significant. When these were included in the main effects model, it explained 72.5% of the phenotypic variation for dormancy. QTL analysis in potato, the methodology to transfer traits and interactions into the 4x level, and QTLs of value for marker-assisted selection are discussed.

INTRODUCTION

Tuber dormancy is defined as the obligate period of non-sprouting after harvest even under conditions favorable for sprouting (Simmonds, 1964). Dormancy release involves changes in respiration and levels of enzymes, sucrose, nitrogenous compounds and endogenous hormones in the tuber, as reviewed by Hemberg (1985). Current literature supports an "inhibitor/promoter" hypothesis, with critical events associated with dormancy release involving a shift in the growth regulator ratio in favor of promoters, and subsequent establishment of positive feedback between the bud and mobilized food reserves (Coleman, 1987).

The length of dormancy is characteristic of different potato varieties (Burton, 1963; Simmonds, 1964; Bogucki and Nelson, 1980; Jeoung et al., 1983). This is an important trait in potato production, since long-term storage without sprout growth is critical for tuber marketing. One method used to prolong the length of tuber dormancy is storage under low temperatures (4°C), however this causes a conversion of non-reducing to reducing sugars which is undesirable for the processing industry. Various dormancy-inducing chemicals have also been tried (Burton, 1966), but questions concerning their toxicologies were raised (Vaughn and Spencer, 1991) and some have been banned from use. An alternative approach is to increase the length of dormancy through genetic means. Long dormant periods have been identified in selections made from South American diploid tuber-bearing relatives of the potato (Thompson et al., 1980; Hermundstad and Peloquin, 1985; Hermundstad, 1986) and this characteristic can be introgressed to the cultivated gene pool.

Tuber dormancy is under polygenic control (Simmonds, 1964), and more than three genes are involved (Flewelling, 1987). Quantitative trait loci (QTL) analysis using molecular markers (Lander and Botstein, 1988) provides a tool for a more detailed study of this trait. The numbers and genomic distribution of quantitative trait loci and their contribution to the variation of the trait can be estimated. This knowledge is necessary to be able to monitor the introgression of these genes and provide a framework for a more analytical breeding of the potato using wild relatives. Isozymes and RFLPs have

been used for dissecting quantitative traits in maize (Edwards et al., 1987; Stuber et al., 1987), tomato (Tanksley et al., 1982; Weller et al., 1988; Tanksley and Hewitt, 1988; Paterson et al., 1988), soybean (Keim et al, 1990; Diers et al., 1992), wheat (Miura et al., 1992) and barley (Hayes et al., 1992; Heun, 1992; Hackett et al., 1992). In a previous study we reported on the use of isozymes to identify QTLs for specific gravity and dormancy in potato (Chapter 1). In this study, QTL analysis of tuber dormancy has been complemented through the use of previously-mapped RFLPs and unmapped RAPD markers.

MATERIALS AND METHODS

Plant Material

One of the two populations previously utilized in Chapter 1 was chosen for this study. This population named TRP133, is a diploid F_1 consisting of 110 genotypes. The female parent used in the cross was clone 84SD22, a hybrid between haploid *S. tuberosum* (2x) and *S. chacoense*, while the male parent was *S. phureja* clone 84S10. The parents were chosen because of long and very short dormancy periods, respectively, and previously known isozyme diversity.

Measurement of dormancy

Four tubers of 3 to 5 cm diameter per genotype were selected after harvest from 1989 field plots at Montcalm Research Farm, Edmore, MI. These were placed on trays in storage at 10°C in the dark, which are the common storage conditions, 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.

Genotyping

The parents were characterized for the morphological marker yellow flesh (Y), isozymes, RFLPs, and RAPDs. Markers that were heterozygous in one of the parents

and homozygous in the other were used to characterize the progeny, where a BC_1-type segregation (1:1) was expected. For all markers, the presence of the unique allele from the heterozygous parent was scored as 1 and the homozygous as 0 in the segregating progeny.

a. Isozyme analysis:

10 segregating isozyme loci (*Dia-1, Est-1, Got-1, Got-2, Idh-1, 6-Pgdh-3, Pgi-1, Pgm-1 Pgm-2, Prx-3*) were utilized as described in Chapter 1.

b. RFLP analysis:

Tomato genomic and cDNA probes utilized were kindly provided by S. Tanksley, Cornell University, and potato genomic and cDNA probes by C. Gebhardt, Max Planck Institut, Germany. At least four markers per chromosome were selected based on their position on previous potato maps (Bonierbale et al., 1988; Tanksley et al., 1992; Gebhardt et al., 1991). DNA was extracted from leaf tissue for all genotypes following a procedure by Saghai-Maroof et al. (1984). The concentration was quantified using a fluorometer (Hoefer Scientific Instruments, Model TKO 100). Seven μgs of DNA was digested with the following endonucleases using 2 units of enzyme per μg of DNA: *EcoRI, HindIII, XbaI, DraI, EcoRV, BamHI.* The digested DNA samples were separated on 0.8% agarose gels. Southern transfer on Nytran nylon membranes, oligolabelling of probes with ^{32}P, filter hybridization using a Robbins Scientific Incubator (Model 310), and filter washes were all performed according to Sisco et al. (1990). Filters were wrapped in plastic wrap and placed in X-ray cassettes at -80°C for 2-10 days.

c. RAPD analysis:

The PCR protocol followed Williams et al. (1990) with minor modifications to optimize for potato DNA. Each reaction was composed of: 1x buffer (100 mM KCl, 100 mM Tris-HCl pH 8.3), 0.8 mM dNTPs, 5 mM $MgCl_2$, 1 U Stoeffel enzyme (Perkin Elmer Cetus), 12.5 ng of primer and 12.5 ng of potato DNA brought up to a final volume of 12.5 μl with ddH_2O. The thermocycler (Perkin Elmer Cetus DNA Thermocycler 480) was programmed for 3 cycles of 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C; followed by 34 cycles of 1 min at 94°C, 1 min at 40°C, and 2 min at 72°C; and 5 min extension at 72°C. On completion, the amplification products were

separated by electrophoresis on a 1.6% agarose gel in 1x TAE buffer. Lambda DNA cut with *EcoRI* and *HindIII* was used as fragment size marker.

Primers were commercial 10-mers from Operon Technologies (Alameda, California), specifically from Kits A, F, G, H and I. Primers were selected when they generated bands in one parent and not in the other. Because of complete dominance in these markers, the heterozygous and homozygous forms for the presence of an allele in a parent could not be distinguished until the band was observed either to segregate or be present in all the progeny, respectively.

Construction of linkage map

Markers which were in heterozygous form in the female parent 84SD22 were utilized to develop the linkage map. The LINKAGE-1 program (Suiter et al., 1983) was used to determine fit to expected Mendelian ratios for each marker, and the linkage phase between linked markers. Segregating data for markers linked in repulsion were reversed to allow adequate estimation of recombination distances. The map was then constructed with MAPMAKER (Lander et al., 1987) v.01 for Macintosh, using LOD scores exceeding 3.0. Linkage groups were assigned to specific chromosomes based on the isozyme and RFLP loci previously mapped (Bonierbale et al., 1988; Tanksley et al., 1992); RAPD markers were subsequently added.

Statistical Analyses

These have been previously described in Chapter 1. Briefly, the segregation data for each marker in the population was divided into the two genotypic classes. Single factor ANOVAs between each pairwise combination of dormancy data and marker locus were conducted (PROC GLM, Statistical Analysis Systems, Cary, NC). Markers with distorted segregation ratios were not used in the analyses. F-tests were used to statistically test if the means of the genotypic marker classes were different ($P < 0.05$). A significant difference in dormancy means was interpreted as linkage of a QTL to the marker locus. For linked markers, the phenotypic effect of the marker allele was estimated by the difference between the trait means of its genotypic classes. QTLs were

localized based on the position of the marker loci on the map. Significant markers in the same chromosome were considered as one QTL if the distance between them did not exceed 50 cM (Paterson et al., 1991). The loci with the highest R^2 value per QTL were then combined in a multiple analysis of variance model to predict the total variation for dormancy explained by the identified QTLs (Keim et al., 1990).

Epistatic interactions between significant loci were tested by two-way analyses of variance. Significant interactions were then included in the multiple analysis of variance to determine their contribution in the phenotypic variation for dormancy. When there were several interactions between the same pairs of QTLs, the one with the highest R^2 value was utilized. The main effect of the loci in the interactions were also included in the model if not already present.

RESULTS

The average number of days to sprouting for the parents was 80 and 10 days for the female and male parents, respectively. The distribution of values in the population was continuous and highly skewed towards short dormancy, having a range from 10 to 90 days and mean of 19. Therefore, the transformation $\log_{10}$ of the number of days to sprouting was used to improve normality. The frequency distribution of these values is shown in Figure 2.1. Two genotypes (designated as TRP133-1 and TRP133-215) showed transgressive segregation, with lengths of dormancy of 90 and 87 days, respectively ($\log_{10}$ of 1.954 and 1.940).

All isozyme loci fit the expected segregation ratio, as previously described in Chapter 1. From all RFLP probes evaluated in the parents, only one (TG83) was found to be heterozygous in both parents thus having an F_2-type segregation. Data from this probe was not included in the analysis. All other probes resolved loci heterozygous in one of the parents, and homozygous in the other. Thirty-four RFLP probes that segregated in a BC_1 fashion were scored. Eight of these resolved two loci and were designated with the addition of a T or B (for top and bottom locus), respectively.

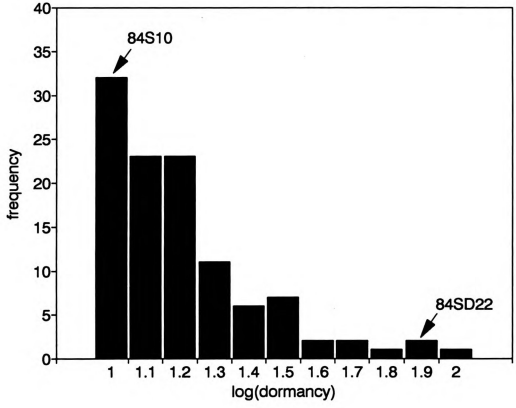
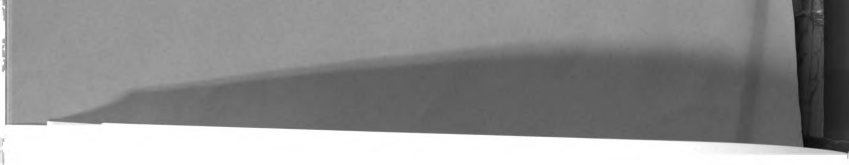


Figure 2.1. Frequency distribution of length of dormancy (log_{10} transformed) values in population TRP133 (84SD22 and 84S10 are the female and male parents, respectively).



Additionally, loci TG122T and TG152T showed triallelic segregations: both parents had one unique allele and the other in common, resulting in 4 genotypic classes in theprogeny. In these cases the presence or absence of the unique allele from each parent was scored independently. The total number of RFLP loci that were scored was 44. The expected segregation ratio was found for all loci except TG141, TG18, TG53 and CD31. The female and male parents were heterozygous for 32 and 12 loci, respectively. A total of 29 random primers were utilized in this study. These produced a range of one to five scorable loci, resulting in a total of 63 RAPDs scored in the progeny. Eight of the RAPDs had distorted segregation ratios (data not shown). The female and male parents were heterozygous for 50 and 13 loci, respectively.

The linkage map developed with isozyme, RFLP and RAPD loci which were in heterozygous form in the female parent is shown (Fig. 2.2). None of the RFLP probes selected by their known location on chromosomes 9 and 12 showed polymorphism or could be scored successfully, so no markers could be assigned to these chromosomes. Some RAPD loci could not be mapped, either because they showed linkage only to other RAPD loci, or no linkage to any other locus.

One-way ANOVAs were conducted between the tuber dormancy data and the two genotypic classes for each of the markers used. In addition to significant association found with the 6 isozyme loci previously described in Chapter 1, significant QTLs were found with 1 RFLP loci and 15 RAPDs (Table 2.1). Genotype TRP133-1 with a dormant period of 90 days and TRP-215 with 87 days, had 82% and 95% of the favorable alleles for the 22 significant markers, respectively. The significant RFLP locus (TG31B) and 4 of the RAPD loci were heterozygous in the male parent. They showed no linkage between each other and their chromosomal location has not been established. The position of the significant loci segregating from the female parent is shown (Fig. 2.2). These markers identify 6 QTLs, one on each of chromosomes 2, 3, 4, 5, 7 and 8.

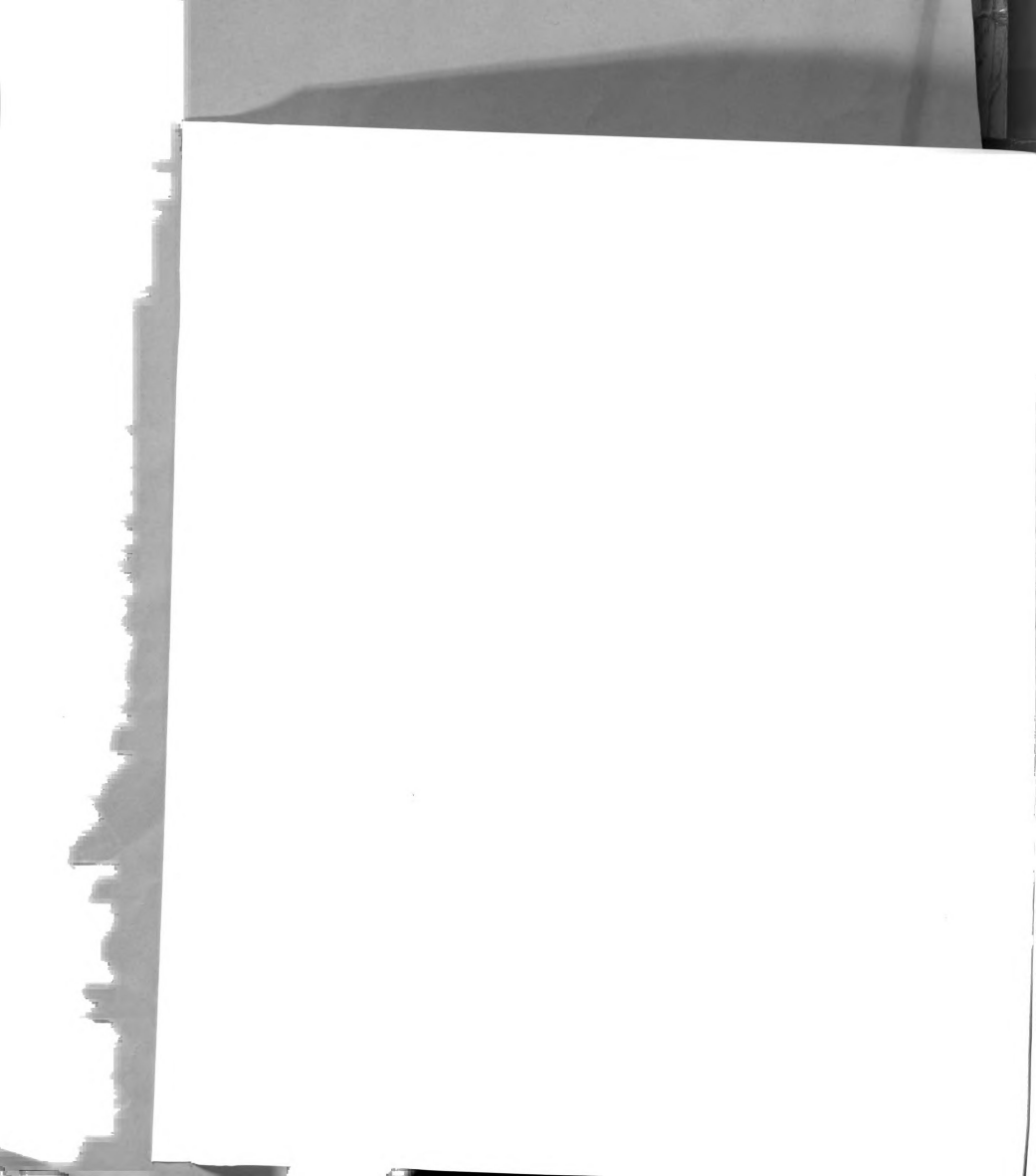


Figure 2.2. Molecular linkage map and localization of QTLs for tuber dormancy [a].

[a] significant markers are indicated by asterisks on the right side of their name; *, **, *** indicate 0.05, 0.01 and 0.001 probability levels, respectively.

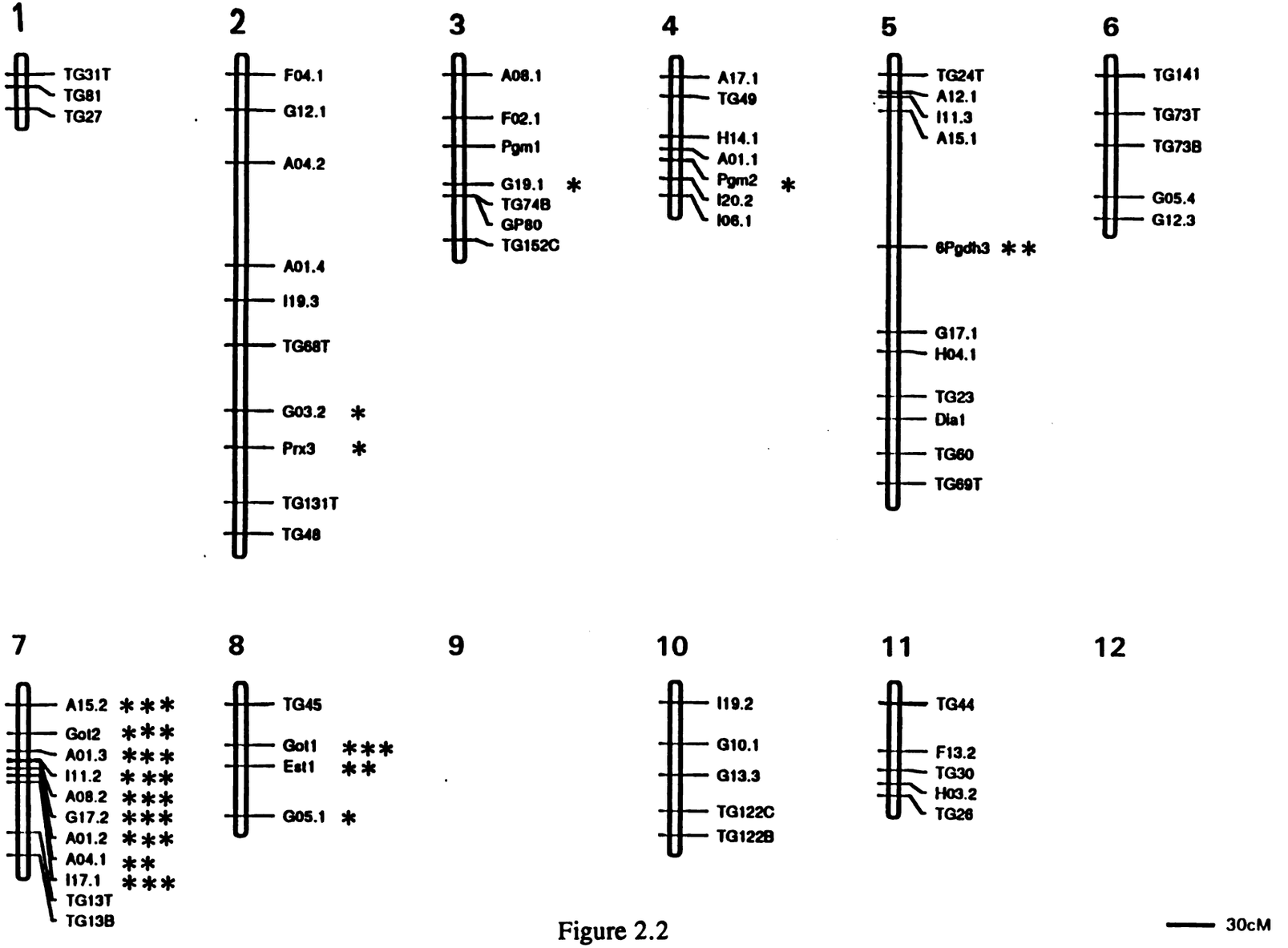

1
TG31T
TG81
TG27
2
F04.1
G12.1
A04.2
A01.4
I19.3
TG68T
G03.2 *
Prx3 *
TG131T
TG48
3
A08.1
F02.1
Pgm1
G19.1 *
TG74B
GP80
TG152C
4
A17.1
TG49
H14.1
A01.1
Pgm2 *
I20.2
I06.1
5
TG24T
A12.1
I11.3
A15.1
6Pgdh3 **
G17.1
H04.1
TG23
Dia1
TG60
TG69T
6
TG141
TG73T
TG73B
G05.4
G12.3
7
A15.2 ***
Got2 ***
A01.3 ***
I11.2 ***
A08.2 ***
G17.2 ***
A01.2 ***
A04.1 **
I17.1 ***
TG13T
TG13B
8
TG45
Got1 ***
Est1 **
G05.1 *
9
10
I19.2
G10.1
G13.3
TG122C
TG122B
11
TG44
F13.2
TG30
H03.2
TG26
12
30cM

Figure 2.2

Table 2.1. Significant association between markers and tuber dormancy

Marker[a]	Chrom.[b]	R^2 (%)	Phenotypic effect (days)[c]
Heteroz. in ♀:			
Prx-3	2	5.2 *	3.8
G03.2	2	5.4 *	4.3
G19.1	3	4.3 *	3.4
Pgm-2	4	5.5 *	3.8
6-Pgdh-3	5	6.9 **	4.2
A15.2	7	14.4 ***	6.3
Got-2	7	20.4 ***	7.7
A01.3	7	14.6 ***	6.5
I11.2	7	15.5 ***	6.9
A08.2	7	13.4 ***	5.9
G17.2	7	15.7 ***	6.7
A01.2	7	15.0 ***	6.4
A04.1	7	12.4 **	5.8
I17.1	7	13.7 ***	6.1
Got-1	8	9.0 ***	5.0
Est-1	8	5.8 **	4.0
G05.1	8	4.2 *	3.6
Heteroz. in ♂:			
F01.2	-	10.5 **	5.7
F05.1	-	8.4 **	4.8
G12.2	-	5.2 *	3.7
G13.1	-	4.6 *	3.5
TG31B	-	7.2 **	4.6

[a] isozyme loci are italicized; TG31B is a RFLP locus; others are RAPD markers.
[b] chromosomal location for markers heterozygous in the male parent have not been identified.
*, **, *** indicate significance at the 0.05, 0.01 and 0.001 probability levels, respectively.
[c] indicates phenotypic difference between the trait means of the markers classes, in days to sprouting.

The amount of phenotypic variation for tuber dormancy explained by each significant marker, as determined by its R^2 value, ranged from 4.2% to 20.4%, This represents a difference of 3.6 and 7.7 days to sprouting between the means of the genotypic classes of the markers, respectively (Table 2.1). The most frequent R^2 values were between 4% and 6% (36% of all markers) and between 14% to 16% (22%). On average the loci on the QTL on chromosome 7 had the highest R^2 values, all of them being above 12%. This represented a difference of more than 5 days to sprouting between the means of the genotypic classes. The isozyme marker *Got-2* on this same QTL had markedly the highest effect of all loci, explaining 20.4% of the phenotypic variation.

Seventeen out of 231 possible epistatic interactions were significant (7%) (Table 2.2). Most of these interactions involve one marker that was heterozygous in the male parent with others heterozygous in the female parent, so the two chromosomal locations involved could not be identified. In the cases where the interaction was between two loci segregating from the female parent, it involved loci on chromosomes 3 and 7 (G19.1**Got-2*), and 5 and 7 (G05.1 with others). The phenotypic variation of dormancy explained by each of these interactions ranged between 3.5% and 7.1%, and more than half of them (53%) explained between 4% and 6% of the variation.

The marker with the highest R^2 value per QTL was chosen to develop a multilocus model. All significant markers heterozygous in the male parent were also included. Accordingly, a model with the markers *Pgm-2*, *6-Pgdh-3*, *Got-2*, *Got-1*, *Est-1*, F01.2, F05.1, G03.2, G12.2, G13.1, G19.1 and TG31B was developed, which explained 57.5% of the phenotypic variation for dormancy. This value increased to 72.1% when the significant interactions were included (Table 2.3).

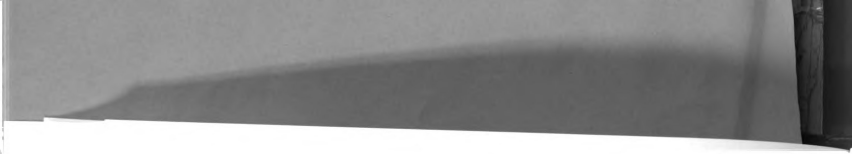


Table 2.2. Significant epistatic interactions between significant markers

Interaction	R^2 (%)
Heterozygous in ♀:	
G05.1 * G17.2	3.9 *
G05.1 * I11.2	5.5 *
G05.1 * I17.1	3.8 *
G05.1 * A01.3	6.4 **
G05.1 * A01.2	4.0 *
G05.1 * A04.1	3.5 *
G05.1 * A08.2	3.6 *
G19.1 * *Got-2*	4.2 *
Heterozygous in ♂[a]:	
F01.2 * F05.1	4.6 *
F01.2 * G12.2	5.4 *
F01.2 * TG31B	7.1 **
F05.1 * TG31B	6.2 *
F05.1 * *Prx-3*	5.7 *
G12.2 * G19.1	5.5 *
G12.2 * *6-Pgdh-3*	5.5 *
TG31B * *Prx-3*	6.4 **

[a] one or both of the loci in the interaction are heterozygous in the male parent.
*, ** indicate significance at the 0.05, and 0.01 probability levels, respectively.

Table 2.3. Models used to determine the amount of phenotypic variation for tuber dormancy explained by QTLs and epistatic interactions

Model	R^2 (%)
Main Effects: *Pgm-2* *6-Pgdh-3* *Got-2* *Got-1* G03.2 G19.1 F01.2 F05.1 G12.2 G13.1 TG31B	57.5 ***
With Interactions: *Prx-3* *Pgm-2* *6-Pgdh-3* *Got-2* *Got-1* G03.2 G19.1 G05.1 A01.3 F01.2 F05.1 G12.2 G13.1 TG31B F01.2 * F05.1 F01.2 * G12.2 F01.2 * TG31B F05.1 * TG31B F05.1 * *Prx-3* G05.1 * A01.3 G12.2 * G19.1 G12.2 * *6-Pgdh-3* G19.1 * *Got-2* TG31B * *Prx-3*	72.1 ***

*** indicates significance at the 0.001 probability level

DISCUSSION

The distribution of number of days to sprouting for population TRP133 was highly skewed towards short dormancy, which may be explained by dominant genes coming from the *S. phureja* male parent. Although a normal distribution of values is preferred for QTL analysis, studies have been previously performed in traits with skewed distributions in tomato (Nienhuis et al., 1987; Paterson et al., 1991). Some degree of transgressive segregation was also observed in the population, since two genotypes have even longer dormant periods than the female parent.

In previous cases of mapping with heterozygous parents in potato, loci segregating from both parents were combined in one map by linkage to markers for which both of them were heterozygous (Bonierbale et al., 1988), and formation of what has been designed as "allelic bridges" (Ritter et al., 1990). In the present case, all isozyme and RFLP markers were heterozygous in either one of the parents, with the exception of only one RFLP locus, heterozygous in both. Most markers were heterozygous in the female parent, which is an interspecific hybrid between *S. tuberosum* and *S. chacoense*. Therefore, a linkage map with the markers segregating from this parent was constructed. This map consisted of a total of 87 loci when the RAPDs were included. The linear order of the isozyme and RFLP markers the same as in the previous potato maps (Bonierbale et al., 1988, Tanksley et al., 1992), although recombination distances differ. This may be due to the utilization of different species in the mapping population.

In the map developed in this study, a total of 46 RAPDs were incorporated. These markers show high polymorphism and repeatable results in potato. While the number of isozyme loci is limited, there is an immense number of random primers available for use, many of which resolve several segregating loci. The PCR technique is relatively simple, and the time necessary to obtain results is short as compared with RFLP analysis. These characteristics make RAPD markers a valuable addition to QTL analysis and marker-assisted techniques, particularly when using backcross-type populations where there are only two genotypic classes.

Significant association was found between tuber dormancy and 22 markers. Seventeen of these markers were heterozygous in the female parent and identified 6 QTLs, one on each of chromosomes 2, 3, 4, 5, 7 and 8. Additionally, 5 markers segregating from the male parent also showed significant association with the trait. These loci were not linked to each other, and their chromosomal location could not be established. The two genotypes with longest dormant periods in the population had 82% and 95% of the favorable alleles of the significant markers, thus corroborating their effects on the trait.

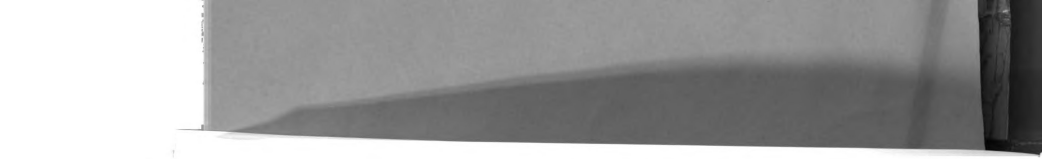
On each of chromosomes 3, 4 and 5, only one significant marker was identified. On chromosome 5, this may be due to the fact that *6-Pgdh-3* is not closely linked to any other marker. For *Pgm-2* on chromosome 4, the effect of this QTL might be too small to be detected with other linked markers, while G19.1 on chromosome 3 has a high P value (P=0.049) and might be a false positive. The highest number of significant markers was identified on chromosome 7. On this chromosome nine markers are clustered on a region spanning 49 cM, eight of which are RAPDs.

The values of R^2 for individual markers ranged between 4.2% and 20.4% which represents a difference of 3.3 and 7.7 days to sprouting between the means of the genotypic classes for the markers, respectively. On average, the highest effect on the trait is by the markers on chromosome 7, each of which contributes with more than 12% of the phenotypic variation of the trait. The isozyme marker *Got-2* has markedly the highest effect, explaining 20.4% of the variation. All significant loci on this QTL were segregating from the female parent. This QTL constitutes an important region of the genome associated with long dormancy and could be tagged in future generations utilizing the markers identified.

A total of 7% of the possible epistatic interactions between significant markers were also significant in this study, as compared with 3% and 1% in two different studies in maize (Edwards et al., 1987; Stuber et al., 1992). When these were included in the multiple analysis model, the amount of phenotypic variation of dormancy explained by the markers was 72.1%, giving an increase of 15% over the main effects model. Therefore, epistatic interactions seem to be contibuting significantly on the variation of

this trait. To maintain these interactions in future generations, transfer of intact portions of the genome might be necessary. In potato breeding improved 2x germplasm is transferred into the cultivated 4x level using 2n gametes (Chase, 1968; Iwanaga 1983; Peloquin et al., 1989). These gametes are produced either by genetically equivalent FDR (First Division Restitution) or SDR (Second Division Restitution) mechanisms. 4x-2x crosses with FDR gametes might be more appropriate in breeding for this trait since they transfer approximately 80% of the genome intact from parent to offspring, thus maintaining a large fraction of the epistatic interactions (Peloquin and Ortiz, 1991).

This study in potato differs from traditional QTL studies performed on inbreeding crops. In potato the generation of inbred parents is not practical due to self-incompatibility and inbreeding depression at the 2x level. Therefore, heterozygous parents must be used to develop the mapping population, and both parents can contribute markers associated to the trait. Secondly, utilization of 0.01 or 0.001 significance levels have been suggested to avoid identification of false positives (Lander and Botstein, 1989). In this first study on this trait, the less stringent level of 0.05 was utilized as indicated by Soller and Brody (1976). RAPD markers have also been incorporated in the QTL analysis, which has not been previously reported. The markers utilized are not evenly spaced in the map and information for two chromosomes is missing. Although it is recognized that the potato genome was not completely surveyed, 6 QTLs were identified which explain 57.5% of the phenotypic variation for tuber dormancy, a value that is similar to that found in studies with quantitative traits in inbreeding crops. Furthermore, the QTL identified on chromosome 7 which has a significant effect upon tuber dormancy may indicate major gene control of this complex trait, and could be used in marker-assisted selection.



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CHAPTER THREE

QUANTITATIVE TRAIT LOCI ANALYSIS OF SPECIFIC GRAVITY IN DIPLOID POTATO (*Solanum spp.*) OVER ENVIRONMENTS: DEVELOPMENT OF A MODEL FOR MARKER-ASSISTED SELECTION

ABSTRACT. Dry matter content in potato, which is an important factor in potato processing, is estimated through specific gravity. In this study we performed quantitative trait loci (QTL) analysis for this trait in 2x potato over 3 environments. The population used in this study consisted of 110 individuals and was derived from the cross of a hybrid of haploid *S. tuberosum* (2x) and *S. chacoense*, with a *S. phureja* clone. This population was characterized for 10 isozyme loci, 44 RFLPs and 63 RAPDs, and 87 of these loci segregating from the female parent used for mapping. Field trials were conducted in two locations in Michigan in 1990 using 3 replications, and a third field trial was conducted in 1991 with 90 individuals and 2 replications. At each location, specific gravity was determined through the weight in air/weight in water method. QTLs were mapped separately for each location and for their average over environments by one-way analyses of variance for each marker locus by trait combination. A total of 10 QTLs were identified over environments and they were localized on chromosomes 1, 2, 3, 4, 7, and 11. The numbers and effects of QTLs detected varied across environments. The locus with highest R^2 value per QTL in each location was chosen to develop multilocus models. Each of these was used in a multiple analysis of variance with data from the location it was developed. The models explained from 39% to 45% of the phenotypic variation for the trait. Each model was also tested with data from the other environments, and in general the predictive value across locations was low. Meanwhile, using the average data a model that gave consistent results when tested across environments was developed, which is comparable to the best model developed for each case. This model may be valuable for marker-assisted selection at the seedling stage in a potato breeding program.

INTRODUCTION

The proportion of dry matter and water in potato tubers determines to a great extent its food value and culinary quality. A good mealy potato will consist of about 25% dry matter and 75% water, and as dry matter decreases potato sogginess will increase (Chase et al., 1990). Dry matter content can be measured directly by oven drying but this is time consuming and involves a loss of sampling material. A more common practice is the estimation of dry matter from specific gravity. These two characters have a simple linear relationship which is highly correlated; regression equations have been developed for 4x and 2x potatoes (Wilson and Lindsay, 1969; Schippers, 1976; Simmonds, 1977; Wannamaker et al., 1992). High specific gravity is particularly important in the potato chip industry because it is associated with increased chip yield and superior quality product. Chips produced from high specific gravity potatoes absorb less oil during the frying process and are therefore more desirable and cheaper to produce. A specific gravity greater than 1.080 which is equivalent to 21.2% dry matter content is preferred by the chip industry (Gould, 1989).

The cultivated potato, *Solanum tuberosum* spp. *tuberosum* is tetraploid (2n=4x=48), however, over 70% of the tuber-bearing *Solanums* are diploid (Hawkes, 1990). These species represent a valuable source of germplasm that can be used to broaden the genetic base of the potato, and provide specific desirable traits. For example, high specific gravity levels have been found in selections of South American diploid species, and furthermore, progress in selection for high specific gravity among 2x populations has been attained (Ruttencutter et al., 1979). These diploid wild and cultivated tuber-bearing species can be used in potato breeding at the diploid level using haploids of cultivated species, and then the improved 2x germplasm can be transferred to the cultivated 4x level through 2n gametes (Chase, 1968; Iwanaga, 1983; Peloquin et al., 1989).

Little is known about the genetic control of specific gravity but it is generally treated as a quantitative character in breeding (Haynes and Haynes, 1983). Other quantitative traits in crops such as maize (Edwards et al., 1987; Stuber et al., 1987),

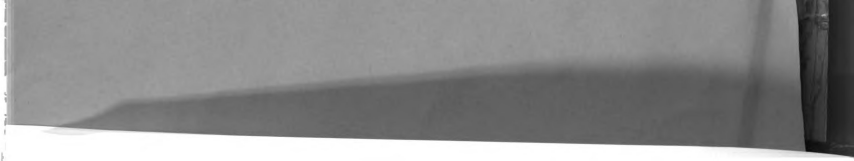
tomato (Weller et al., 1982; Tanksley et al., 1982, Tanksley and Hewitt, 1988; Paterson et al., 1988), soybean (Keim et al, 1990; Diers et al., 1992), wheat (Miura et al., 1992) and barley (Hayes et al., 1992; Heun, 1992; Hackett et al., 1992) have been studied using molecular markers. The availability of saturated linkage maps makes it possible to dissect quantitative traits into discrete genetic factors (QTLs) and their phenotypic effects and genetic position can be estimated (Paterson et al., 1988; Lander and Botstein, 1989). Recently, the effect of environment on QTLs was studied in F_2 and F_3 populations in tomato (Paterson et al., 1991) and in F_3 lines backcrossed to the parents in maize (Stuber et al., 1992). The potato is a clonally propagated crop, and therefore offers a particular advantage for this type of analysis. Genotyping with molecular markers and evaluation of traits can be performed on exactly the same plant material in the first generation, allowing the study of effects of QTLs across different environments and years.

The use of isozymes for QTL analysis of specific gravity was described in Chapter 1. Here that research is complemented through the addition of RFLP and RAPD markers. Furthermore, the effect of environment on QTL expression for this trait at three locations has been analized. Multilocus models with markers representing the QTLs were developed for each location to determine the contribution of the QTLs to the phenotypic variation of the trait. Additionally, the data from the average of the three locations was used to develop a more stable model than the ones developed at each one of the environments. This model could be used for marker-assisted selection in future generations using this material.

MATERIALS AND METHODS

Plant Material

A diploid F_1 population named TRP133 and consisting of 110 genotypes was utilized in this study (as in Chapters 1 and 2). This population is derived from the cross of clones 84SD22 (a hybrid between haploid *Solanum tuberosum* and *S. chacoense*), and 84S10 (*S. phureja*) as female and male parents, respectively.



Field Trials

The clonal material was planted in three environments during two years: at Montcalm Experiment Station, Edmore, MI in 1990 and 1991 (MES90 and MES91), and at Clarksville Horticulture Experiment Station, Clarksville, Michigan, in 1990 (CHES90). A randomized complete block design (RCBD) was utilized, with 8 plants per plot, and spacing of approximately 0.3 m and 0.9 m within and between rows, respectively. In 1990, the 110 genotypes were planted with three replications, while in 1991 due to availability of material, only two replications and 90 of the genotypes were used.

Measurement of specific gravity

After harvest at each location, specific gravity was determined for all genotypes using the weight in air/weight in water method: [air wt./(air wt. - water wt.)]. A minimum sample size of 1 kg/plot was used. The value of specific gravity for each genotype was obtained from the mean of either the three or two values from each of the replications in the field.

Genotyping

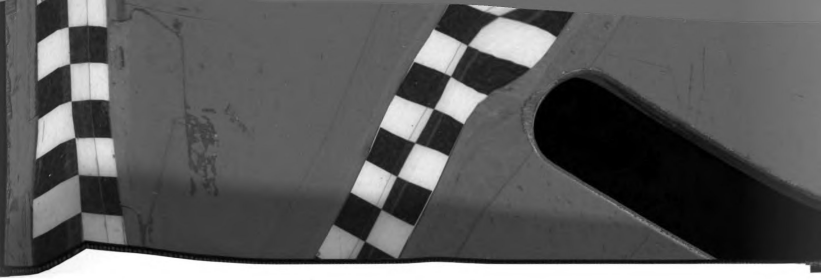
The genotypes in the population were characterized for the morphological marker yellow flesh (*Y*), 10 isozyme loci, 44 RFLPs, and 63 RAPDs. RFLP probes were kindly provided by S. Tanksley at Cornell University, and C. Gebhardt at Max Planck Institut, Germany. RAPDs were resolved using commercial 10-mer primers (Operon Technologies). All the markers used for the analysis were heterozygous in one of the parents and homozygous in the other, thus segregating as a BC_1 (1:1) in the progeny. Most of the markers were segregating from the female parent 84SD22, and these were used for construction of the linkage map with MAPMAKER (Lander et al., 1987) v.01 for Macintosh as described in Chapter 2.

Statistical Analyses

Data from the three locations was tested through an ANOVA which partitioned

the effects of location, replications/locations, genotypes, and genotype x location. The methodology utilized for QTL analyses has been previously described for another trait, tuber dormancy (Chapter 2). Briefly, linkage of QTL to a marker locus was determined with F-tests in single factor ANOVAs between each pairwise combination of specific gravity data and genotypic classes for the marker locus (PROC GLM, Statistical Analysis Systems, Cary, NC). A significant difference in means ($P < 0.05$) was interpreted as linkage of the QTL to the marker locus. When two or more significant markers were found on the same linkage group, they were considered to be linked to independent QTLs if they were separated by more than 50 cM (Paterson et al., 1991). In this study, QTLs were identified for each environment, and also for the mean of specific gravity from the three environments (from now on described as AVE). AVE data was obtained from the 90 individuals for which there were values at the 3 locations. Epistatic interactions between significant markers at each location were tested by two-way analyses of variance.

For each environment, a model with the markers with the highest R^2 value per QTL was developed. This was used in a multiple analysis of variance to predict the total variation for specific gravity explained by the identified QTLs. As a matter of comparison, the model obtained at each location was tested with data from the other environments. A model was also developed with data from AVE and tested separately in each one of the locations, using 110 individuals for MES90 and CHES90, and 90 individuals for MES91 and AVE. Furthermore, the significant epistatic interactions at each location were included in the main effects models to determine their contribution to the phenotypic variation for the trait. When there were several interactions between markers linked to the same pairs of QTLs, the interaction with the highest R^2 value was utilized. The main effects of the markers in the interactions were also included in the model if not already present.



RESULTS

The dates of harvest were 119, 131 and 120 days from planting for MES90, CHES90 and MES91, respectively. The frequency distributions (Figure 3.1), and the range of values and mean for the genotypes and parents at the three locations are shown (Table 3.1). On average, values of specific gravity were higher at CHES90, and MES90 had higher values than MES91. Both MES90 and CHES90 had ranges of 0.053 specific gravity units, equivalent to 10.3% dry matter content, and at MES91 the range was 0.043 or 8.4% dry matter. For the AVE data, the mean was in between that of CHES90 and MES90, and the range was 0.037 or 7.2% dry matter. Phenotypic correlation of specific gravity data between MES90 and CHES90 was 0.81; between MES90 and MES91 it was 0.72; and between CHES90 and MES91 it was 0.71. The results from the combined analysis of variance over the three locations are shown in Table 3.2. Locations, genotype, and genotype x locations effects were significant.

Table 3.1. Values of specific gravity for parents and population TRP133 at each one of the three environments and the average

Environment	Range	Mean ± SE
MES90:		
Population	1.046 - 1.099	1.079 ± 0.001
84SD22		1.080 ± 0.003
84S10		1.067 ± 0.002
CHES90:		
Population	1.057 - 1.110	1.083 ± 0.001
84SD22		1.084 ± 0.011
84S10		1.064 ± 0.002
MES91:		
Population	1.052 - 1.095	1.075 ± 0.001
84SD22		1.077 ± 0.003
84S10		1.060 ± 0.001
AVE:		
Population	1.063 - 1.100	1.080 ± 0.001

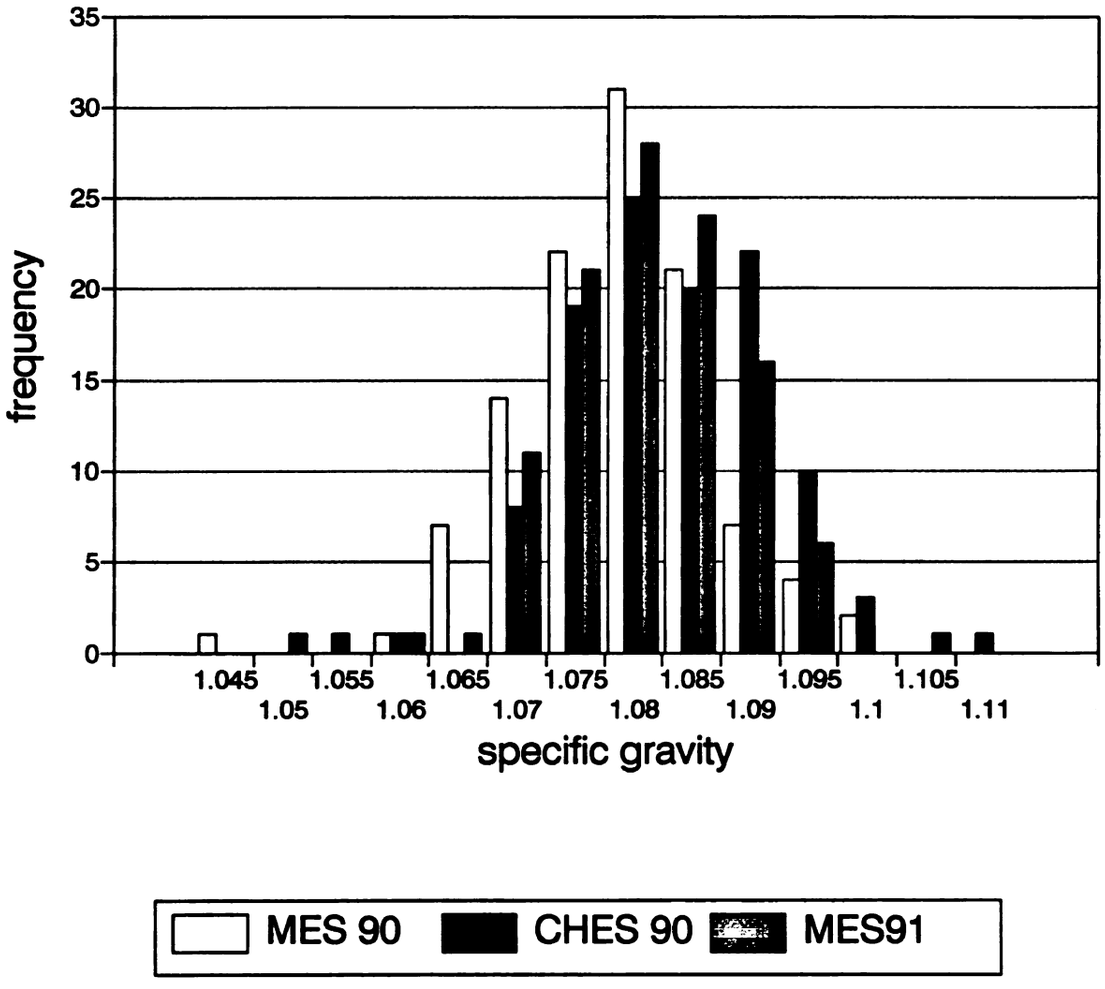


Figure 3.1. Frequency distributions of specific gravity values at the three environments

Table 3.2. Mean squares (MS) from the combined analysis of variance of specific gravity for the three environments

Source	df	MS x 10^6
Location	2	2385 *
Replications(Locations)	5	194
Genotype	109	1310 **
Genotype x Location	198	824 ***
Error	524	408
Total	838	

*, **, *** indicate significance at the 0.05, 0.01 and 0.001 probability levels, respectively.

Results from the one-way ANOVAs between marker loci and specific gravity data for each location and AVE are shown (Table 3.3). A total of 28 loci were significant in at least one environment or AVE. Twenty-four loci were segregating from the female parent identifying 10 QTLs, and their positions were localized on chromosomes 1, 2, 3, 5, 7 and 11 (Figure 3.2). Since the loci segregating from the male parent were not mapped, the position of the three loci segregating from this parent was not identified. Two loci which were not significant in any of the 3 locations (TG24T and TG14) were significant in AVE. None of the RFLP probes selected by their previously known positions on chromosomes 9 and 12 (Bonierbale et al., 1988; Tanksley et al., 1992) could be scored successfully due to lack of polymorphism in this population or technical problems, so no loci could be assigned to these chromosomes.

The number of significant loci identified in each one of the locations was: 19 loci in MES90, 18 in CHES90, 16 in MES91, and 19 in AVE, representing 7, 7, 5 and 7 QTLs, respectively. Two of 10 QTLs were identified in the three locations and also in AVE; 5 were identified in 2 locations, and 3 of these also in AVE; other 4 were identified in only one of the locations, and 2 of these also in AVE. The number of QTLs in common between locations was: 3 QTLs between MES90 and CHES90; 4 QTLs between MES90 and MES91; and 3 QTLs between CHES90 and MES91. From the QTLs identified in two of the locations: 2 were at both MES90 and MES91; 1 at MES90 and CHES90; and 1 at CHES90 and MES91. The values of phenotypic variation for the

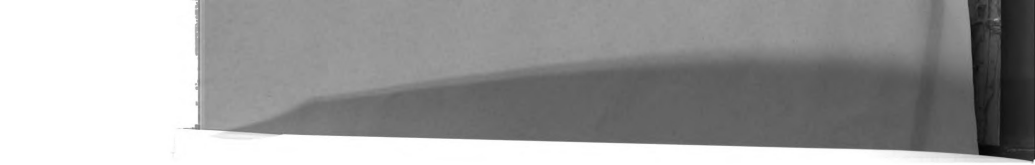


Table 3.3. Significant loci, chromosome locations and R^2 values for the three environments and the average

Locus	Chrom	R^2 (%)			
		MES90	CHES90	MES91	AVE
Het. in ♀[a]:					
TG27	1	ns[b]	7.5 **	5.7 *	6.7 *
F04.1	2	5.9 *	ns	ns	5.7 *
G12.1	2	5.8 *	ns	5.5 *	6.6 *
F02.1	3	ns	4.8 *	ns	ns
Pgm-1	3	7.0 *	8.2 **	ns	5.8 *
TG152C	3	4.6 *	ns	ns	ns
TG24T	5	ns	ns	ns	6.0 *
A15.1	5	ns	4.5 *	ns	ns
6-Pgdh-3	5	5.0 *	15.0 ***	6.7 *	12.2 ***
H04.1	5	ns	4.0 *	ns	ns
A15.2	7	4.7 *	7.8 *	6.6 *	7.7 *
Got-2	7	4.5 *	10.1 **	10.1 **	13.9 ***
A01.3	7	6.1 *	10.5 **	ns	7.3 *
I11.2	7	5.8 *	13.4 ***	5.4 *	10.3 **
A08.2	7	7.7 *	15.8 ***	6.8 *	13.1 ***
G17.2	7	6.0 *	14.9 ***	4.9 *	10.7 **
A01.2	7	5.8 *	11.3 **	5.4 *	9.1 **
A04.1	7	6.2 *	11.7 **	6.5 *	10.2 **
I17.1	7	9.7 **	14.8 ***	5.8 *	12.7 ***
TG13T	7	ns	5.5 *	ns	4.9 *
TG13B	7	5.2 *	5.2 *	ns	ns
F13.2	11	6.3 *	ns	ns	ns
TG30	11	7.2 **	ns	6.9 *	ns
H03.2	11	5.7 *	ns	5.3 *	ns
TG26	11	9.5 **	ns	9.6 *	ns
Het. in ♂[a]:					
I20.1	-[c]	ns	7.1 *	8.1 **	10.4 **
TG152B	-	ns	ns	6.4 *	5.9 *
TG14	-	ns	ns	ns	5.0 *

[a] indicates the loci that are heterozygous in the female and male parents, respectively
[b] ns indicates not significant
[c] the positions of markers segregating from the male parent have not been identified
*, **, *** indicate significance at 0.05, 0.01, 0.001 probability levels, respectively

Figure 3.2. Molecular linkage map and localization of QTLs for specific gravity for each environment and AVE [a]

[a] QTLs are indicated by bars which define the position on the chromosome, not necessarily the significant markers

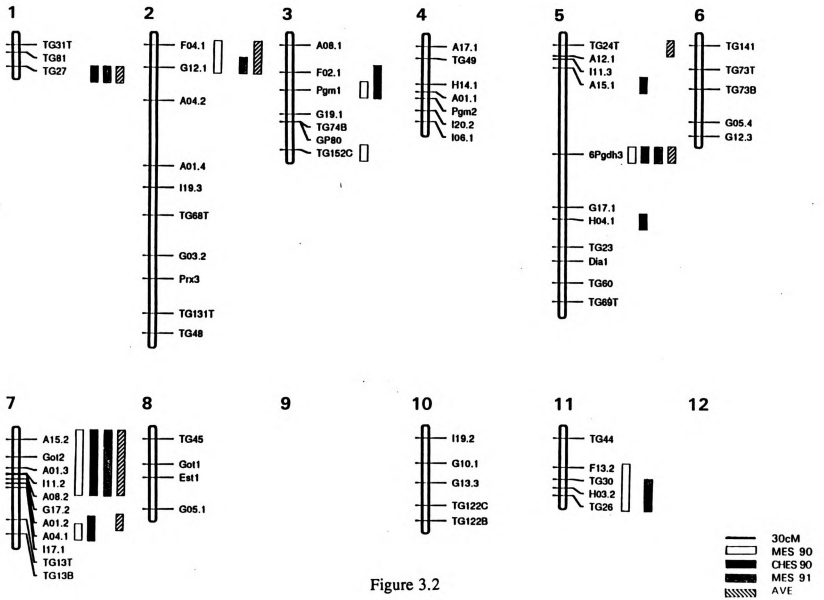
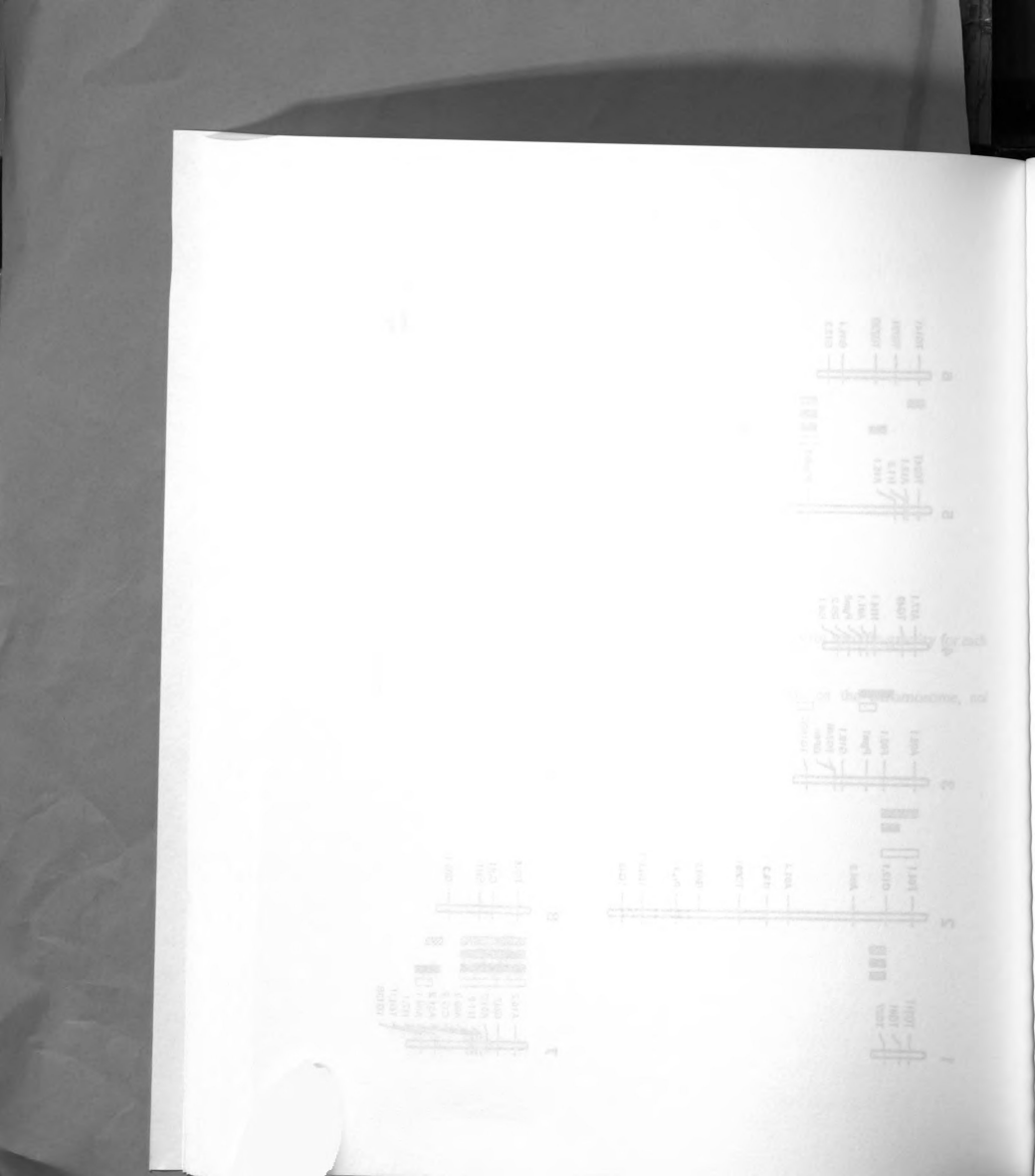

1
TG31T
TG81
TG27
2
F04.1
G12.1
A04.2
A01.4
I19.3
TG68T
G03.2
Prx3
TG131T
TG48
3
A08.1
F02.1
Pgm1
G19.1
TG74B
GP80
TG152C
4
A17.1
TG49
H14.1
A01.1
Pgm2
I20.2
I08.1
5
TG24T
A12.1
I11.3
A15.1
6Pgdh3
G17.1
H04.1
TG23
Dia1
TG60
TG69T
6
TG141
TG73T
TG73B
G05.4
G12.3
7
A15.2
Got2
A01.3
I11.2
A08.2
G17.2
A01.2
A04.1
I17.1
TG13T
TG13B
8
TG45
Got1
Est1
G05.1
9
10
I19.2
G10.1
G13.3
TG122C
TG122B
11
TG44
F13.2
TG30
H03.2
TG26
12
30cM
MES 90
CHES 90
MES 91
AVE

Figure 3.2



trait explained by individual loci, determined by their R^2 value, ranged from 4% to 15.8%. Most loci had R^2 values between 4% and 8%. The highest values were identified in CHES90. Mean R^2 values per location were 6.2 for MES90; 9.56 for CHES90; 6.6 for MES91; and 8.64 for AVE.

The locus with highest R^2 value per QTL in each location was chosen to develop multilocus models. All loci segregating from the male were also included since their position was unknown and it could not be determined whether they were identifying different QTLs. The models developed are shown in Table 3.4. Seven loci were selected in MES90 (7 QTLs), eight loci in CHES90 (7 QTLs, 1 unmapped), seven loci in MES91 (5 QTLs, 2 unmapped), and 9 loci for the AVE model (7 QTLs, 2 unmapped). The amount of the phenotypic variation for the trait explained by each model its own data and that from the other environments is shown (Table 3.5).

Table 3.4. Multilocus models developed at each one of the environments and with the average data

MES90	CHES90	MES91	AVE
Main Effects			
F04.1 *Pgm-1* TG152C *6-Pgdh-3* I17.1 TG13B TG26	TG27 *Pgm-1* *6-Pgdh-3* H04.1 A15.1 A08.2 TG13T I20.1	TG27 G12.1 *6-Pgdh-3* *Got-2* TG26 I20.1 TG152B	TG27 G12.1 *Pgm-1* *6-Pgdh-3* TG24T *Got-2* TG13T TG152B TG14
Significant Interactions			
F04.1 * TG30 G12.1 * I11.2 I17.1 * *6-Pgdh-3* F13.2 * TG152C	I17.1 * *6-Pgdh-3* TG27 * TG13T	I20.1 * I17.1 I20.1 * *6-Pgdh-3* I17.1 * TG27 A15.2 * TG152B	I20.1 * *Pgm-1* I11.2 * TG152B I17.1 * *6-Pgdh-3*

Table 3.5. R^2 values obtained from the multiple analyses of variance using the multilocus models with data from the different environments

Environment[a]	Model Developed			
	MES90	CHES90	MES91	AVE
MES90	39.0[b] 56.5	27.3 -	32.7 -	37.4 41.1
CHES90	44.0 -	44.9 53.9	36.8 -	54.9 61.2
MES91	26.2 -	23.9 -	42.6 64.2	41.2 49.2
AVE	- -	- -	- -	57.1 62.4

[a] indicates environment used to test the models
[b] upper value is R^2 from main effects model; lower value is with inclusion of the significant interactions.

A total of 15, 10, 9 and 10 epistatic interactions were significant at MES90, CHES90, MES91 and AVE (data not shown). These correspond to 8.8%, 6.5%, 7.5% and 5.8% of all possible interactions between significant markers at each one of the locations. The interaction with highest R^2 was utilized when there were several that showed significance between the same pairs of QTLs. The interactions used for each one of the locations and AVE are shown (Table 3.4). The resulting R^2 values when the interactions were included in each of the main effects models are shown (Table 3.5).

DISCUSSION

Specific gravity is influenced by a number of environmental factors such as temperature, rainfall, day length, etc. (Stevenson et al., 1954). Genotype x environment interactions were found to be significant for this trait in studies with 4x potatoes (Johansen et al., 1967); however, inherent differences among varieties are apparent over a wide range of environmental conditions (Lana et al., 1970). Large genotype x

environmental effects were also found in diploid populations of *S. phureja* and *S. stenotomum* (Ruttencutter et al., 1979), and this is also confirmed in our results using *S. tuberosum, S. chacoense* and *S. phureja* material. This fact raises interesting questions that have been adressed in this study: a) what is the effect of environment on the QTLs detected; and b) is it possible to develop a model that will best explain the phenotypic variation for the trait across different environments and thus have predictive value.

The utilization of a significance level of 0.01 or 0.001 has been recommended in QTL analysis to reduce the risk of accepting false positives (Lander and Botstein, 1989). However in this study we chose to use the less stringent level of 0.05 as indicated by Soller and Brody (1976) for the individual locations and then judge the consistency of significant markers across locations. The total number of significant loci detected was 28, 25 of which were segregating from the female parent and were mapped. This hybrid parent had more heterozygous loci and higher specific gravity than the male consistently across all locations, therefore its larger contribution of loci associated with the trait is not surprising. Nevertheless, three loci segregating from the male parent were also identified.

There were differences in the significant loci identified at each environment, even though the total number of loci in each one of them was very similar. These loci identified a total of 10 QTLs on 6 chromosomes. One QTL was identified on each of chromosomes 1, 2 and 11; 2 distinct QTLs were identified on chromosomes 3 and 7; and 3 QTLs on chromosome 5. The marker loci were not evenly spaced across the genome and in some cases there was a cluster of significant loci identifying the same QTL. For example, on chromosome 7, nine significant loci were mapped to a chromosome region spanning 49 cM. The locus with the highest R^2 value at a given QTL was not always consistent across environments, for example on chromosome 7, A08.2 was highest in MES90, I17.1 in CHES90, and *Got-2* in MES91 and AVE. Also, on all cases except for MES91, there are multiple peaks on this QTL as indicated by the individual R^2 values. Nevertheless, due to the closeness between the loci, this would not necessary indicate multiple QTLs (Paterson et al., 1991).

Two of the ten QTLs on chromosomes 5 and 7 were identified in all environments, therefore showing a strong and stable association with specific gravity.

Interestingly, these two QTLs also showed association with another quantitative trait in potato, tuber dormancy (Chapter 2), even though there is no correlation between the two tuber traits in this material. The similarity of results for the two traits suggest either pleiotropic effects of single QTLs, or clustering of different QTLs into closely linked groups as explained by Paterson et al. (1991) in tomato. Five other QTLs (50%) were identified in two of the environments and 2 of them were significant also with AVE. The other 3 QTLs (30%) were specific for only one of the environments. One of these was also significant with AVE, while the other two, TG152C in MES90 and H04.1 in CHES90 have low significance levels (4.6% and 4%, respectively) and therefore could possibly be false positives. There is more consistency among the tagged QTLs across environments in this study than in the similar study in tomato (Paterson et al., 1991), where 14%, 34% and 52% of the QTLs detected were identified in 3, 2 and 1 environment, respectively. This could be due to the fact that the environments they used (California and Israel) were more different than the ones used in this study, and as noted in their article, their comparison across environments was confounded by the use of different generations and methods of trait evaluation.

The results from the comparison of QTLs across environments, seem to indicate that the two MES trials were more similar to each other than to CHES, even though they were performed in different years. This seems contradictory to the fact that the correlation coefficient was highest between MES90 and CHES90. Nevertheless, this value indicates a similar response of genotypes in different environments and not the overall response of the population in these environments. Also, lower correlation values with MES91 data could be confounded by the use of the smaller population size compared to 1990 (90 versus 110 individuals).

The values of phenotypic variation for the trait estimated by the R^2 values of individual loci ranged from 4% to 15.8%. These values correspond to a difference of 0.7% and 1.3% dry matter between the means of the marker classes, respectively, as estimated by different methods (Schippers, 1976; Simmonds, 1977; Wannamaker et al., 1992). These are important differences when considering that a difference between 1.075 and 1.080 specific gravity, that can determine acceptance of processing potato varieties,

represents a difference of only 1% dry matter. Most loci have only small effects on the trait, as indicated by the fact that most R^2 values are between 4% and 8%. On average, R^2 values were highest at CHES90, which was the only environment with R^2 values higher than 14%. This can be due to the longer growing season for this trial (11 more days to harvest) as compared with both trials at MES.

An important part of this study was the development of multilocus models to estimate the phenotypic variation for the trait explained by the QTLs at each one of the locations, make comparisons across them, and develop a model with the best predictive value. However, it is important to note that the analyses are affected by different factors: a) the use of 110 individuals in tests using 1990 data versus only 90 individuals for 1991 and AVE; b) the models were tested only on individuals that had complete sets of data for all loci involved, and due to missing values, in some extreme cases this was limited to numbers as small as 64 individuals; c) the different number of loci utilized in each one of the models.

There were differences in the models developed for each of the locations due to variations in the locus with the highest R^2 value per QTL, and in the loci segregating from the male parent that were also included. Nevertheless, the portion of the phenotypic variation of specific gravity explained by the respective models is quite similar: 39% in MES90, 44.9% in CHES90, and 42.6% in MES91. The poor predictive values of each of these models is demonstrated by the lower R^2 values when they are tested with data from the other environments: 44% and 26.2% for the MES90 model tested on CHES90 and MES91, respectively; 27.3% and 23.9% for the CHES90 model tested on MES90 and MES91; and 32.7% and 36.8% for the MES91 model tested on MES90 and CHES90. On the other hand, the model developed with data from the average of the three environments (AVE) explains a distinctively higher portion of the variation of specific gravity when tested with its own data (57.1%). Moreover, when tested with data from each of the locations it gives consistent results, which are comparable to using the best model for each location: 37.4% in MES90, 54.9% in CHES90, and 41.2% in MES91. The AVE model consists of 7 loci (the same number as MES90 and CHES90 models) plus 2 unmapped loci (one more than the CHES90 model), so the results can not

be attributed to the use of a vastly larger number of loci. CHES90 has high values throughout the analysis which could be due to the stronger effect of the loci as indicated by their R^2 values, or by being influenced by the confounding factors already mentioned.

The numbers of epistatic interactions that were significant represent 8.8%, 6.5%, 7.5% and 5.8% of all possible interactions for MES90, CHES90, MES91 and AVE, respectively. These values, according to Paterson et al. (1991), would represent only minimal evidence of epistasis. Nevertheless, their effects can not be underestimated, since they give increments of 17.5%, 9% and 21.6% with respect to the R^2 values from the main effects models for each one of the locations. Here too, a difference between environments can be detected on the numbers of significant interactions identified, the QTLs involved, and their effects on the trait. However, when included in the AVE main effects model the effects are not as dramatic and they give increments of 5.3%, 3.7%, 6.3% and 8% when tested with AVE, MES90, CHES90 and MES91 data, respectively.

This study has unequivocally demonstrated the influence of environmental effects on QTLs associated with specific gravity in potato, by testing the same plant material across three different environments. For breeding purposes, it is important to note that the predictive value of multilocus models developed with data from individual locations is not always effective when used on other environments. On the other hand, the best multilocus model was developed when the data was averaged over the three environments. The value of this model is that the loci involved could be tested in marker-assisted selection in future generations using this material. Moreover, it is of interest to investigate the consistency of these markers in germplasm involving other wild relatives of potato, and the transfer of the QTLS from the 2x to the 4x level.

Specific gravity is relatively easily evaluated and traditional potato breeders may argue that there is no value in attempting an indirect selection method. Nevertheless, in potato breeding, both at 2x and at 4x levels, determination of specific gravity is usually not performed at earlier stages of selection but up to 4 years after the initial cross was made. Even though a selection method for high dry matter in seedling generation was reported (Lam and Grenard, 1976), the estimations of specific gravity based on only one plant, and the utilization of a small sample size can not be accurate. In addition,

greenhouse-grown 2x seedling tubers in particular are usually very small and have therefore low specific gravity values and high variability (Cole, 1975). Even in field-grown plants, the small sample size from one seedling plant generally resuls in inaccurate estimates of specific gravity. An indirect selection method based upon tagged QTLs associated to the trait is feasible at the seedling stage, and may prove adequate and time-saving to improve the dry matter content in potato.

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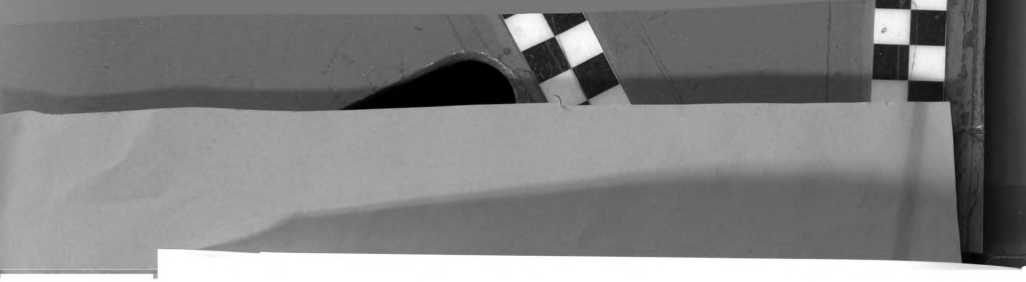

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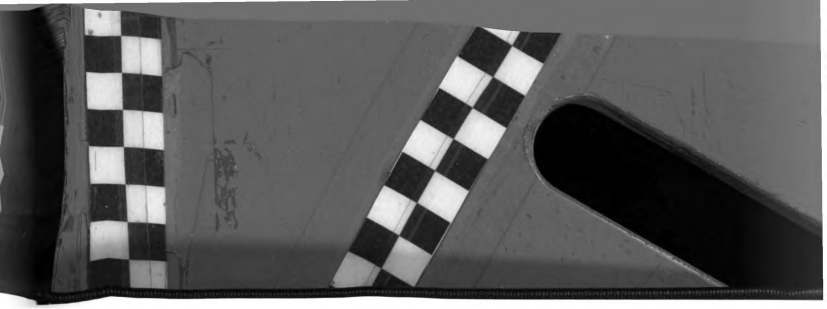

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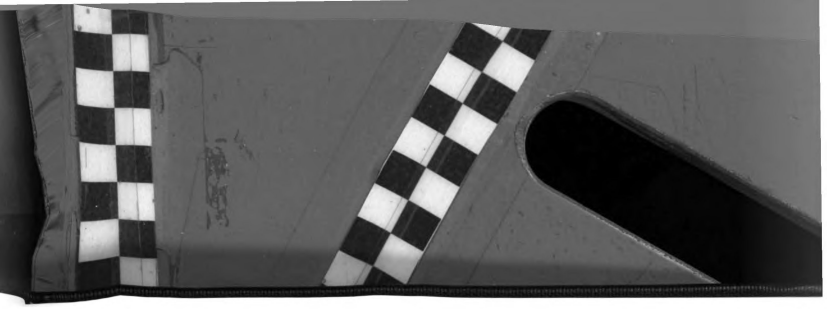
CONCLUSIONS

This research is one of the first quantitative trait loci studies using molecular markers in potato. Two diploid populations of *Solanum* spp. were characterized with isozyme loci, and QTL analysis was performed for two traits. The consistency of significant markers across environments and genetic backgrounds was evaluated. One of the populations was further characterized with RFLPs and subsequently RAPDs when this technology became available and was optimized for potato. This resulted in the utilization of 127 marker loci. Eighty-seven of these loci were used to construct a linkage map. Six QTLs with significant effect on tuber dormancy were identified. One of the QTLs clustered several loci and had the highest effect on the trait, and could possibly be of value for tagging this trait in future generations. For specific gravity, a total of 10 QTLs were identified over three environments. Utilizing the average data from the three environments a model with 9 loci was developed, which was consistent when tested separately with data from each one of the environments. This model has potential value for marker-assisted selection for this trait.

This study has thus provided the initial information on two tuber traits with polygenic inheritance. There are many directions in which future studies can be directed. The population could be further characterized with markers on chromosomes 9 and 11 to complete and saturate the linkage map and see whether additional QTLs affecting the traits can be identified. Furthermore, with tagged traits the feasibility of using marker-assisted selection in potato breeding can be tested. Also, the importance of these markers at the tetraploid level after 4x x 2x crosses could be studied. These could be used to monitor the introgression of the desirable traits from the diploid wild species, and therefore increase the efficiency of their use in potato breeding. For specific gravity, it would be possible to study the correlation between QTLs tagged for specific gravity and cloned genes involved in the starch production pathway. For tuber dormancy, the same type of QTL study could be performed in haploid *S. tuberosum* populations which do not have dominance effects from wild species and therefore not have skewed frequency



distributions. In this research, the basic methodology for QTL analysis was developed and incorporated into the Potato Breeding Program at MSU and is now available for future studies with other traits and *Solanum* germplasm.