Development of a Model for Marker-Assisted Selection of Specific Gravity in Diploid Potato across Environments

R. Freyre* and D. S. Douches

ABSTRACT

Dry matter content in potato (Solanum tuberosum L.) is an important factor in processing and is estimated by specific gravity. We performed quantitative trait loci (QTL) analysis for this trait in 2x potato in three environments. A population of 110 individuals was derived from the cross of a hybrid of haploid S. tuberosum (2x) and S. chacoense Bitter, with a S. phureja Juz. & Buk. clone. This population was characterized for 10 isozyme loci, 44 restriction fragment length polymorphisms (RFLPs), and 63 random amplified polymorphic DNA (RAPDs). Field trials were conducted in two locations in Michigan in 1990 with three replications, and in one location in 1991 with 90 individuals and two replications. Specific gravity was determined through air weight/(air weight - water weight). QTLs were mapped separately for each location and for the average across environments by one-way analyses of variance for each marker locus. A total of 10 putative QTLs was identified over environments and they were localized on Chromosomes 1, 2, 3, 5, 7, and 11. Numbers and effects of QTLs detected varied across environments. The locus with highest Rs value per QTL in each environment was chosen to develop multilocus models, which explained a consistent proportion of the phenotypic variation for specific gravity. Each model was also tested with data from the other environments, and in general, the predictive value across environments was weaker. A model based on average specific gravity across environments explained a consistent proportion of the phenotypic variation for specific gravity when tested across environments. Using multiple environment data to develop models may be more valuable for marker-assisted selection in a potato breeding program than models based on single environments.

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 destructive of sampling material. A more common practice is the estimation of dry matter from specific gravity. These two characters, which are highly correlated, have a simple linear relationship; 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 product quality. 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, S. tuberosum spp. tuberosum, is tetraploid (2n = 4x = 48); however, over 70% of the tuber-bearing Solanums are diploid and include wild and cultivated species (Hawkes, 1990). These diploid species represent a valuable germplasm source 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

Abbreviations. CHES90, Clarksville Horticulture Exp. Sin. 1990 field trial; CM, centin,organ. Dia-1, diaphorase, locus 1; Est-1, esterase, locus 1; Got-1, gluconate oxaloacetate transaminase, locus 1; Got-2, gluconate oxaloacetate transaminase, locus 2; 1dh-1, NADH dehydrogenase, locus 1; Mdh-1, malate dehydrogenase, locus 1; MES90, Montcalm Exp. Sin. 1990 field trial; MES91, Montcalm Exp. Sin. 1991 field trial; 6-Pgdh-3, 6-phosphogluconate dehydrogenase, locus 3; Pgi-1, phosphogluconate isomerase, locus 1; Pgm-1, phosphoglucomutase, locus 1; Pgm-2, phosphoglucomutase, locus 2; Pre-3, peroxidase, locus 3; RAPDs, random amplified polymorphic DNA, RFLPs, restriction fragment length polymorphisms.

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species, and selection for high specific gravity among 2x populations has been successful (Rutten cutter et al., 1979). These diploid species can be crossed with haploids of cultivated species, and then the improved 2x germplasm can be transferred to the cultivated 4x level through 2n gametes (Chase, 1968; Iwanaga, 1985; Pelouquin 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 (Zea mays L.; Edwards et al., 1987; Stuber et al., 1987), tomato (Lycopersicon esculentum Mill.; Tanksley et al., 1982; Tanksley and Hewitt, 1988; Paterson et al., 1988), soybean [Glycine max (L.) Merr.]; Keim et al., 1990; Diers et al., 1992], wheat [Triticum aestivum (L.) em Thell; Miyura et al., 1992], and barley (Hordeum vulgare L.; Hayes et al., 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 chromosomal location can be estimated (Paterson et al., 1988; Lander and Botstein, 1989). Recently, the effect of environment on QTLs was studied in F2 and F3 populations of tomato (Paterson et al., 1991) and in F1 lines backcrossed to the parents in maize (Stuber et al., 1992).

Specific gravity is influenced by a number of environmental factors such as temperature, rainfall, and day length (Stevenson et al., 1954). Genotype × environment interactions were found to be significant for this trait in 4x potatoes (Johansen et al., 1967); however, inherent differences among cultivars are apparent over a wide range of environmental conditions (Lana et al., 1970). Large genotype × environment effects were also found in diploid populations of S. phureja and S. stenotomum (Rutten cutter et al., 1979). This fact raises the following interesting questions which we have attempted to answer in this study: (i) what is the effect of environment on the QTLs detected, and (ii) is it possible to develop a marker-based model that will best explain the phenotypic variation for the trait across different environments and thus have predictive value? The potato is a clonally propagated crop and offers a particular advantage for this type of analysis. Genotyping with molecular markers and evaluation of traits can be performed on cloned individuals from the first segregating generation, allowing the study of effects of QTLs across different environments.

In a previous study (Freyre and Douches, 1994) we described the use of isozymes for QTL analysis of specific gravity. Here we complement that research through the addition of RFLP and RAPD markers to the genetic linkage map. Multilocus models with markers representing the QTLs were developed for each of three environments to determine the contribution of the QTLs to phenotypic variation of specific gravity. Additionally, the data from the average of the three environments were used to develop a more stable model than those developed with data from separate environments.

**MATERIALS AND METHODS**

A diploid F1 population (TRP133) consisting of 110 genotypes was utilized in this study. This population was derived from the cross of clones 84SD22 (a hybrid between haploid S. tuberosum and S. chacoense) and 84S10 (S. phureja) as female and male parents, respectively. This population was initially planted for seed increase in 1989. The clonal material was planted at the Montcalm Experiment Station, Edmore, MI in 1990 and 1991 (MES90 and MES91), and at the Clarksville Horticultural Experiment Station. Clarksville, MI, in 1990 (CHES90). Both locations had McBride sandy loam soils (coarse, loamy, mixed, frigid, Alfic Fragiorthod) and had adequate insect, disease and weed control. The CHES location is not commonly used for potato production, while MES is located in a potato production region and optimal production practices were followed. In both locations a randomized complete block design was utilized, with eight plants per plot, and spacing of approximately 0.3 m within and 0.9 m between rows. In 1990, the 110 genotypes were planted with three replications, while in 1991 only two replications and 90 of the genotypes were used due to nonavailability of plant material. Dates of harvest were 119, 131, and 120 d from planting for MES90, CHES90, and MES91, respectively. After harvest at each environment, specific gravity was determined for all genotypes as air weight (air weight = water weight). A minimum sample size of 1 kg/plot was used. The value of specific gravity for each genotype in each environment was obtained by averaging across replications.

The genotypes in the population were characterized for the morphological marker yellow flesh (Y), 10 isozyme loci, 44 RFLPs, and 63 RAPDs. Methodology utilized has been described (Freyre et al., 1994). RFLP probes that were distributed throughout the potato genome were chosen based on previously published molecular maps (Gebhardt et al., 1991; Tanksley et al., 1992). These probes were provided by S. Tanksley at Cornell University (designated with TG or CD followed by a numeral), and C. Gebhardt at Max Planck Institut, Germany (designated with GP or CP followed by a numeral). RAPDs were resolved by commercial 10-mer primers (Operon Technologies, Alameda, CA). All markers used for analysis were heterozygous in one of the parents and homozygous in the other, thus segregating (1:1) in the progeny. Most 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 (Freyre et al., 1994).

The methodology utilized for QTL analyses has been previously described for another trait, tuber dormancy (Freyre et al., 1994). Briefly, linkage of a QTL to a marker locus was determined with a single factor analysis of variance for each marker locus (SAS Institute, 1988). A significant difference in genotypic class means (P < 0.05) was interpreted as linkage of the QTL to the marker locus. 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 environments and then judge the consistency of significant markers across environments. 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 specific gravity across environments based on the 90 individuals tested in all environ-
RESULTS

Frequency distributions (Fig. 1), and range of values and mean for genotypes and parents for the three environments are shown (Table 1). Values of specific gravity were generally higher at CHES90, and MES90 had higher values than MES91. For both MES90 and CHES90 the difference between the high and low specific gravity values was equivalent to 10.3% dry matter content, and at MES91 it was equivalent to 8.4% dry matter. For the average across environments, the mean was between that of CHES90 and MES90, and the difference between the highest and lowest specific gravity values was equivalent to 7.2% dry matter. The phenotypic
correlation coefficient for specific gravity between MES90 and CHES90 was 0.81; between MES90 and MES91 it was 0.72; and between CHES90 and MES91 it was 0.71. Results from the combined analysis of variance across environments indicated that environment, genotype, and genotype x environment effects were significant (data not shown). A total of 26 marker loci were significant in at least one environment (Table 2). Additionally, two loci, which were not significant in any single environment (TG24T and TG14), were significant in the across-environment analysis. Twenty-five of the significant loci were segregating from the female parent. These identified 10 QTLs, and their positions were localized on Chromosomes 1, 2, 3, 5, 7, and 11 (Fig. 2). Since the loci segregating from the male parent were not mapped, the positions of the three loci segregating from this parent were not identified. None of the RFLP probes selected for their putative positions on Chromosomes 9 and 12 (Bonierbale et al., 1988; Tanksley et al., 1992) could be scored successfully because of lack of polymorphism in this population or technical problems with some RFLP probes, so no loci could be assigned to these chromosomes. Among the 28 marker loci showing significant associations with specific gravity, six (21%) had significant interaction with the environment. These six loci represent four QTLs identified on Chromosomes 1, 3, 7, and 11 (Table 2).

The number of significant marker loci identified was 19 in MES90, 18 in CHES90, 16 in MES91, and 19 across environments, representing seven, seven, five, and seven QTLs, respectively. Two of 10 QTLs were identified in every analysis. Five were identified in two environments, and three of these were identified across environments. Four were identified in only one environment and two of these across environments. The number of QTLs in common between environments was three between MES90 and CHES90, four QTLs between MES90 and MES91, and three QTLs between CHES90 and MES91. From the QTLs identified in two environments, two were at both MES90 and MES91, one at MES90 and CHES90, and one at CHES90 and MES91. The amount of phenotypic variation for the trait explained by individual loci, determined by their $R^2$ value, ranged from 4.0 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 environment were 6.2 for MES90, 9.6 for CHES90, 6.6 for MES91, and 8.6 across environments.

The locus with highest $R^2$ value per QTL in each environment was selected to develop multilocus models. All loci segregating from the male were also included since their positions were unknown and it could not be determined whether they were identifying different QTLs. The loci chosen for each environment are shown in Table 3. Seven loci were selected for MES90 (seven QTLs), eight loci for CHES90 (seven QTLs, one unmapped), seven loci for MES91 (five QTLs, two unmapped), and nine loci for the across-environment data across environments.

Table 2. Significant loci for specific gravity identified in each of three testing environments, chromosome locations, $R^2$ values in single-factor ANOVAs, and $F$ values for marker x environment interactions.

<table>
<thead>
<tr>
<th>Loci</th>
<th>Chromosome location</th>
<th>MES90</th>
<th>CHES90</th>
<th>MES91</th>
<th>Across environments</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG24T</td>
<td>4</td>
<td>NS</td>
<td>7.5**</td>
<td>5.7*</td>
<td>6.7*</td>
<td>3.8</td>
</tr>
<tr>
<td>TG34.1</td>
<td>5</td>
<td>5.9*</td>
<td>NS</td>
<td>NS</td>
<td>5.7*</td>
<td>NS</td>
</tr>
<tr>
<td>TG11.2</td>
<td>6</td>
<td>5.8*</td>
<td>4.8*</td>
<td>NS</td>
<td>6.6*</td>
<td>NS</td>
</tr>
<tr>
<td>TG24T</td>
<td>7</td>
<td>7.0*</td>
<td>8.2**</td>
<td>NS</td>
<td>5.8*</td>
<td>6.0</td>
</tr>
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<td>TG152C</td>
<td>8</td>
<td>6.4*</td>
<td>NS</td>
<td>NS</td>
<td>6.0</td>
<td>NS</td>
</tr>
<tr>
<td>TG24T</td>
<td>9</td>
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<td>4.5*</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>A15.2</td>
<td>10</td>
<td>4.5*</td>
<td>10.1**</td>
<td>10.1*</td>
<td>13.0***</td>
<td></td>
</tr>
<tr>
<td>A01.3</td>
<td>11</td>
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<td>NS</td>
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<td></td>
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<tr>
<td>A08.2</td>
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<td>13.4***</td>
<td>5.4*</td>
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</tr>
<tr>
<td>A08.2</td>
<td>12</td>
<td>7.7*</td>
<td>15.0***</td>
<td>6.8*</td>
<td>13.1***</td>
<td></td>
</tr>
<tr>
<td>A01.3</td>
<td>13</td>
<td>6.0*</td>
<td>14.9**</td>
<td>4.9*</td>
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<td></td>
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<tr>
<td>A01.3</td>
<td>13</td>
<td>5.9*</td>
<td>11.3*</td>
<td>5.4*</td>
<td>9.1**</td>
<td></td>
</tr>
<tr>
<td>A04.1</td>
<td>14</td>
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<td></td>
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<tr>
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<td>15</td>
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<td>5.1*</td>
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<td>F13.2</td>
<td>18</td>
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<td></td>
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<td>NS</td>
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<td></td>
</tr>
<tr>
<td>H03.2</td>
<td>20</td>
<td>5.7*</td>
<td>NS</td>
<td>5.3*</td>
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<td>NS</td>
<td>9.6*</td>
<td>NS</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* ** * * Significant at the 0.05, 0.01 and 0.001 probability levels, respectively; NS not significant.

1 The positions of markers segregating from the male parent have not been identified.
2 $F$ values for marker x environment interaction significant at the 0.05 level.
Fig. 2. Molecular linkage map and localization of QTLs for specific gravity for each environment and across environments. QTLs are indicated by bars which define the position on the chromosome, not necessarily the significant markers. MES90—Montcalm Experimental Station, 1990 field trial; CHES90—Clarksville Horticulture Experimental Station, 1990 field trial; MES91—Montcalm Experimental Station, 1991 field trial; AVE—across environments analysis.

(7 QTLs, two unmapped). Each multilocus model was used in a multiple analysis of variance where the $R^2$ indicated the total variation for specific gravity explained by the identified QTLs. For comparison, the multilocus models were also tested with data from the other environments (Table 4).

A total of 15, 10, 9, and 10 epistatic interactions were significant at MES90, CHES90, MES91, and across environments, respectively (data not shown). These correspond to 8.8, 6.5, 7.5, and 5.8% of all possible interactions between significant markers. The interaction with highest $R^2$ was utilized in the multiple analysis of variance when there was more than one interaction that showed significance between the same pairs of QTLs. The interactions used in the multiple analysis of variance for each environment and across environments are shown (Table 3). The resulting $R^2$ values when the interactions were included in each of the main effects models are shown (Table 4). The inclusion of these interactions resulted in an increase of 17.5, 9.0, and 21.6% of phenotypic variation explained over the main effects models for MES90, CHES90, and MES91, respectively, and 5.3% for the across-environments model.

**DISCUSSION**

The total number of significant marker loci detected was 28, 25 of which were segregating from the female
Table 4. $R^2$ values obtained from multiple analyses of variance using multilocus models representing QTLs for specific gravity in diploid potato.

<table>
<thead>
<tr>
<th>Environment used to test models</th>
<th>MES90</th>
<th>CHES90</th>
<th>MES91</th>
<th>Across environments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES90</td>
<td>39.01</td>
<td>27.3</td>
<td>32.7</td>
<td>37.4</td>
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<tr>
<td>CHES90</td>
<td>56.5</td>
<td>44.9</td>
<td>36.8</td>
<td>54.9</td>
</tr>
<tr>
<td>MES91</td>
<td>-</td>
<td>53.9</td>
<td>-</td>
<td>61.2</td>
</tr>
<tr>
<td>Across environments</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>57.1</td>
</tr>
</tbody>
</table>

1. MES90 - Montcalm Experimental Station, 1990 field trial; CHES90 - Clarksville Horticulture Experimental Station, 1990 field trial; MES91 - Montcalm Experimental Station, 1991 field trial.
2. Upper value is $R^2$ from male effects model; lower value includes significant epistatic interactions.

Light-seven marker loci were used to map QTLs for specific gravity in this study. With this dense genetic map, a large number of evaluations were conducted to detect QTLs. We chose a significance level of 0.05 to judge the consistency of significant markers across locations. At this level of significance, this process leads to spurious associations between the marker locus and QTL. Based on comparison-wise tests conducted at the 0.05 level, we expect, on average, four significant associations or a total of 12 associations over the three environments occurring by chance. Our study identified 28 significant associations, thus, about 40% of these may be spurious. If more stringent statistical criteria were followed in this study, a nominal significance level of about 0.002 would be necessary for a true experiment-wise confidence level of 95% (Lander and Botstein, 1989). This would eliminate the QTLs claimed on Chromosomes 1, 2, and 11. The QTL on Chromosome 5 would be identified in CHES90 and across environments, while evidence for a QTL on Chromosome 7 would be strong across all environments. However, using the 0.05 level comparison-wise tests, we have found a consistent association of Pgm-1 with specific gravity across two environments in two different potato populations (Freyre and Douches, 1994). These associations were not significant at the 0.002 level. We interpret this linkage as a potential with a small effect or loose linkage rather than a spurious association. Therefore, in this study we report the QTLs on Chromosomes 1, 2, and 11 as putative associations that may need further testing. This rationale should also be considered with the significant epistatic interactions.

Two of the 10 QTLs, on Chromosomes 5 and 7, were identified in all environments, showing a strong and stable association with specific gravity. Interestingly, these two QTLs also showed association with another quantitative trait in potato, tuber dormancy (Freyre et al., 1994), even though no correlation was found between the two tuber traits in this germplasm. The similarity of results for the two traits suggests 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 of the 10 QTLs were identified in two environments and three of them were also significant across environments. The other three QTLs were specific for only one of the environments. One of these was also significant across environments, while the other two, TQ152C in MES90 and H04.1 in CHES90, have low significance levels (4.6 and 4.0%, respectively) and could possibly be false positives or QTLs with small effects. More consistency was found among the tagged QTLs across environments in our study than in a similar study in tomato (Paterson et al., 1991), where 14, 34, and 52% of QTLs detected were identified in three, two and one environments, respectively. This could be related to the greater diversity among environments (California and Israel), and as they noted, comparison across environments was confounded by the use of different generations and methods of trait evaluation.

The proportion of phenotypic variation in specific gravity explained by individual loci ranged from 4.0 to 15.8%. These values correspond to a difference of 0.7 and 1.3% dry matter between the means of 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, which can determine acceptability of potato processing cultivars, represents a difference of only 1% dry matter. Most loci have only small effects on the trait, as indicated by most $R^2$ values 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%. The trial at CHES had a longer growing season (11 more days to harvest) as compared with both trials at MES, which can account for more mature tubers, with higher specific gravity values.

Based on marker by location analysis of variance, six of the marker loci that had significant associations with specific gravity also had significant marker by environ-
ment interactions (Table 3). These loci represented four QTLs on four chromosomes. Five loci represent QTLs on Chromosomes 1, 3, and 11. These QTLs did not show consistent significant associations across all three environments. The locus located on Chromosome 7 may have been identified by chance since it was the only locus on this QTL showing this interaction, and also, this QTL is consistent across all environments.

Since genotype × environment interactions are important for phenotypic expression of specific gravity, an important part of this study was the development of multilocus models to estimate the phenotypic variation for the trait explained by QTLs in each environment, make comparisons across them, and develop a model with the best predictive value for marker-assisted selection. However, the analyses are affected by the following different factors: (i) 110 individuals were used from 1990 data versus only 90 individuals for 1991 and across environment tests; (ii) models were tested only on individuals with 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; and (iii) a different number of loci was utilized in each one of the models.

Differences were found in models developed for each environment due to variations in the marker locus with highest $R^2$ value per QTL, and in the loci segregating from the male parent. Nevertheless, the proportion of phenotypic variation of specific gravity explained by the respective models is similar: 39.0% in MES90, 44.9% in CHES90, and 42.6% in MES91. The weakness of the single environment models is demonstrated by lower $R^2$ values when they are tested with data from other environments: 44.0 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 37.7 and 36.8% for the MES91 model tested on MES90 and CHES90. On the other hand, the across-environments model explains a distinctively higher proportion of variation in specific gravity when tested with its own data (57.1%). Moreover, when tested with data from each environment, it gives consistent results, which are comparable to those of the best model for each environment: 37.4% in MES90, 54.9% in CHES90, and 41.2% in MES91. The across-environments model consists of seven loci (the same number as MES90 and CHES90 models), plus two unmapped loci (one more than the CHES90 model), so the results cannot 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 across-environments, respectively. These values, according to Paterson et al. (1991), would represent only minimal evidence of epistasis. Nevertheless, their effects cannot 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 environment. 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 across environments main effects model, the effects are not as dramatic, giving increments of 5.3, 3.7, 6.3, and 8.0% when tested with the across-environments, MES90, CHES90, and MES91 data, respectively.

By testing the same plant material across three different environments we have demonstrated the influence of environmental effects on QTLs associated with specific gravity in potato. For breeding purposes, the predictive value of multilocus models developed with data from individual environments is not always effective with potatoes grown in other environments. On the other hand, the best multilocus model was developed when the data were averaged over three environments. The value of this model is that the loci involved could be tested in marker-assisted selection in future generations of this material. With this further testing, it would be possible to judge the adequacy of the significance level chosen for this experiment. The use of the more stringent significance level may prove necessary if selection of markers results in transfer of relatively few genes with large effects for specific gravity while markers identified with significance level of >0.002 have little or no contribution to higher specific gravity. Additionally, it is vital to investigate the consistency of markers associated with specific gravity in germplasm involving other wild relatives of potato, and the transfer of the QTLs from the 2x to the 4x level.

One may argue that there is no value in attempting indirect selection methods with markers, because specific gravity is easily evaluated. 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 yr after the initial cross is made. Even though a selection method for high dry matter in seedling generations was reported (Lam and Grenard, 1976), the estimation of specific gravity based on only one plant, and the utilization of a small sample size, are seldom indicative of future performance. In addition, greenhouse-grown 2x seedling tubers, in particular, are usually very small and have low specific gravity values and high variability (Cole, 1975). Even in field-grown plants, the small sample size from one seedling plant generally results in inaccurate estimates of specific gravity. An indirect selection method based on tagged QTLs associated with specific gravity 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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