Multiple Alleles for Tuber Shape in Diploid Potato Detected by Qualitative and Quantitative Genetic Analysis Using RFLPs

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ABSTRACT

Tuber shape in potato is commonly regarded as displaying continuous variation, yet at the diploid level phenotypes can be discerned visually, having round or long tubers. Inheritance of qualitative tuber shape can be explained by a single locus Ro, round being dominant to long. With restriction fragment length polymorphisms (RFLPs) the Ro locus was mapped on chromosome 10. Tuber shape was also studied as a qualitative trait, using the length/width ratio as trait value. The estimated broad sense heritability was $h^2 = 0.80$. The morphologically mapped Ro locus explained 75% of the genetic variation, indicating the presence of a major quantitative trait locus (QTL) at the Ro locus and minor genetic factors. RFLP alleles linked with Ro alleles were used to divide the progeny into four genotypic classes: Ro$^r$Ro$^b$:Ro$^a$ro:roRo$^b$:roro = 1:1:1:1. The recessive ro allele is identical by descent in both parents. The significantly different effects ($P = 0.0157$) of the non-identical alleles Ro$^a$ and Ro$^b$ provided evidence for multiallelism at the Ro locus. Linkage mapping of the Ro locus was compared with QTL mapping. Only those markers which are polymorphic in both parents allow accurate QTL mapping when genetic factors segregate from both parents. This finding applies to QTL mapping in all outbreeders without homozygous inbred strains.

Since the introduction of restriction fragment length polymorphism (RFLP) markers many applications of this technique have proven to be very useful for the identification of genetic variability in practical breeding and scientific research. Initially attention has focused on the construction of genetic linkage maps. The convenient availability of saturated RFLP maps has greatly stimulated quantitative genetic investigations. Individual loci of quantitative traits (QTLs) have been recognized and mapped on the genome (PATTERSON et al. 1988). Recently, RFLP markers have also been used to investigate inter- and intralocus interactions of loci involved in quantitative traits. FATOKUN et al. (1992) reported epistasis between QTLs: an interlocus interaction. STUBER et al. (1992) detected QTLs playing a significant role in heterosis: an intralocus interaction. Another example of an intralocus interaction is the interaction between multiple alleles. Multiallelism is a common and frequently reported phenomenon for qualitative traits. The existence of multiple alleles for quantitative trait loci has only been considered in a theoretical model (FOREMANN and SEYFFERT 1977); experimental evidence is not available.

The experimental model used in this study is tuber shape in diploid potato. Cultivated potato is a highly heterozygous tetraploid outbreeder. The shape of the tuber is one of the most eye-catching traits of the potato crop. The tuber shape selected by breeders is determined by the preference of consumers and the processing industry. The consumer preference may vary with country. To minimize waste, varieties with long tubers are preferred for French fries, but varieties with round tubers are used for crisps. Although tuber shape is an easily selectable trait for breeders, the continuous variation complicates classification into Mendelian ratios. The range from round to oval or long suggests a polygenic inheritance. Studies on tuber shape carried out at the tetraploid level did not give any clear genetic model (DEJONG and BURNS 1993; DEMAIN and FLEMING 1991). Genetic studies are complicated by the heterozygosity, the high genetic load and the tetrasomic inheritance. However, at the diploid level, DEJONG and ROWE (1972), TAYLOR (1978), OKWUAGWU (1981) and MASSON (1985) concluded that the inheritance of tuber shape is monogenic. A single dominant gene Ro was postulated by MASSON (1985); round being dominant to long.

Our objectives in this research were: (1) to describe inheritance of tuber shape both in a qualitative and in a quantitative way, (2) to map the Ro locus on the potato genome, (3) to compare the results of linkage analysis using qualitative data with QTL mapping and (4) to elucidate the phenotypic effect of the different alleles at the Ro locus.
MATERIALS AND METHODS

Plant material: The experimental material was developed by crossing two diploid \(2n = 2x = 24\) potato clones. The female parent, clone US-W5337.3 (HANNEMAN and PELQUIN 1967) was a hybrid between \textit{Solanium phureja} Pl225669.1 and the dihaploid US-W42 extracted from cv. Chippewa. This clone is widely used in many types of research. The male parent, clone 77.2102.37 (JACOBSEN 1980), was a hybrid between VH'4211 (a \textit{Solanium vernei-Solanum tuberosum} backcross) and the US-W5337.3 clone. Both parental clones have round tubers. Descendants derived from this cross are maintained as a population for mapping studies. Morphological observations on tuber shape were done when the population size was 102 clones. RFLP analysis was performed in a smaller randomly chosen subset. From 50 clones (virus-free, with good tuber formation), three plants were grown in a greenhouse during the summer in 10-liter pots. The tubers were harvested when mature and bulked per clone.

Data collection: Qualitative data on tuber shape were obtained by dividing the clones of progeny US-W5337.3 \(\times\) 77.2102.37 visually into two phenotypic classes: round and long. This classification was based not only on the visual perception of tuber shape, but also on tapering. Long tubers often taper at the apical and stolon ends (rose and heel), while round tubers may have a longitudinal axis which is shorter than the transversal axis.

Quantitative data were obtained by measuring the length/width ratio of the tubers. The length of a tuber is defined as the distance between the apex (rose) and the place of stolon attachment (heel). The width of the tuber is the length of the transversal axis perpendicular to the longitudinal axis. This method of measuring the transversal axis is very sensitive for irregular tuber shape and deviant traits like "kidney" and "pebble." Therefore, the width was measured twice, in such a way that the two directions were representative for the irregularity of the tuber. In most cases we took the largest transversal axis (if it could be found unambiguously), and the width perpendicular to the previous direction. The length/width ratio is a numerical measure describing the phenotypic value of tuber shape, the width being the mean of the two measurements in both directions perpendicular to the longitudinal axis.

RFLP markers and linkage analysis: The progeny used in this experiment was also used to construct a genetic map of potato in our laboratories. This map is comprised of morphological, isozyme and RFLP markers (VAN ECK et al. 1993). Additional RFLP markers were obtained from S. D. TANKSLEY (Cornell University, Ithaca, New York) to align the linkage groups with the published genetic map of potato (TANKSLEY et al. 1992). Linkage analysis was performed using JoinMap (STAM 1993) and Linkage-1 (SUTTER et al. 1983). The significance of pairwise linkage between loci is expressed in LOD scores, the logarithm of the odds ratio of the likelihood of the data assuming that two loci are linked with a given recombination value over that assuming the two are not linked. A LOD value of three means that the chances are greater than thousand to one that the loci are linked for a given recombination estimate.

Statistical analysis and QTL mapping: The quantitative data on tuber shape were analyzed with the statistical computer program SAS (SAS Institute Inc. 1990). The variation in L/W ratio between "long" (roro) clones and between tubers within a "long" clone is much larger than the variation between and within "round" clones (Ro). Therefore, log transformation was performed on the L/W ratio to produce a normally distributed error term.

With the procedure of generalized linear models (GLM) the log transformed data on the L/W ratio were analyzed using standard analysis of variance (ANOVA) methods. Variance was partitioned according to the experimental factors: (i) phenotypic classes for the morphological types round = Ro or long = roro, (j) genotypic classes within morphological classes \(\{\text{Ro}^\prime\text{Ro}: \text{roro} : \text{Ro}'\text{ro} : \text{roro}\}\), (k) clones within genotypic classes and (l) tubers within clones. This unbalanced nested design is represented by the mixed model:

\[
\log(L/W)_{ij} = \mu + Mo_{i} + Ge_{j} + Cl_{ip} + Tu_{ij}.
\]

The terms \(Cl\) and \(Tu\) for clones and tubers are considered random. The other terms are considered fixed. The total variance explained by the model was partitioned into components of variance belonging to the terms of the model. Since the terms for morphological and genotypic classes (Mo and Ge) represent fixed terms, the variation explained by these terms is not expressed as a variance component \(\sigma^2\) but is indicated by \(Q\) (SAS Institute Inc. 1990). The \(F\) value was calculated using the type I mean square (SAS Institute Inc. 1990) and the appropriate error term. (Type I sums of squares are the incremental improvement in error sum of squares as each effect is added to the nested model. They can be computed by fitting the model in steps and recording the difference in error sum of squares at each step.)

RESULTS

Inheritance and mapping of tuber shape as a qualitative trait: The differences in tuber shape between descendents of the cross US-W5337.3 \(\times\) 77.2102.37 allowed a preliminary visual separation into two morphological classes. Nevertheless, considerable and typical differences could be observed between clones ordered within the same morphological group. Based on this preliminary classification, a segregation in tuber shape of 68:29 = "round": "long" was observed. As both parents have "round" tubers this segregation fits a 3:1 monogenic ratio \(X^2_{[\text{3:1}]} = 1.24; P = 0.26\); round being dominant to long. The genetic model proposed for this cross \((\text{Roro} > \text{Raro} \rightarrow \text{Ro} + \text{raro})\) was confirmed by intercrossing several "round" and "long" descendents and by backcrossing with the parents. (Data not shown). The gene symbol \(Ro\) is used according to MASSON (1985), who also reported a single dominant gene for tuber shape, as did TAYLOR (1978), OKWUAGWU (1981) and DEJONG and BURNS (1993).

Co-segregation between RFLP markers and tuber shape (visually classified) was observed for several markers located on chromosome 10 (Figure 1; see Table 3). The RFLP markers Ac38-46, TG303, ST06, TG63, Ac15-7 and ST15a detected polymorphism between alleles segregating from the female parent as well as between alleles segregating from the male parent. Markers Tac20 and TG43 were polymorphic in the male parent, but were homozygous in the female parent. Markers Tac15b and ST05 showed polymorphism only for the female alleles. For the construction of the map a strategy was chosen not to evaluate the data on the genotypes of the descendents of this cross, but to evaluate the data on the genotypes of the male and female gametes which produced the descendents. The resulting linkage maps represent the independent maps of the male and female
Quantitative genetic analysis of tuber shape: From 50 clones the tubers were harvested and measured. The number of tubers per clone ranged from 3 to 42, with an average of 19.8 ± 9.2 tubers per clone. To calculate the broad sense heritability ($h^2$) of tuber shape, the genetic variance between clones ($\sigma_{gw}^2$) and the environmental variance between clones ($\sigma_{me}^2$) were estimated according to the model $\log(L/W)_{hi} = \mu + \text{Cl}_i + \text{Tub}_i$, assuming the clone effect was random. The calculated heritability $h^2$ (see Table 1) of 0.80 indicated that tuber shape is hardly affected by environmental factors.

Nevertheless, tuber shape is not completely determined by the Ro locus and environmental factors, because differences can be observed between clones belonging to the same morphological class (e.g., “round” and “long”). These differences have to be associated with minor genetic factors. To calculate the amount of the genetic variance explained by minor genetic factors ($\sigma_{gen-min}^2$) and the Ro locus ($\sigma_{gen-max}^2$), the following model was used: $\log(L/W)_{hi} = \mu + \text{Ro}_i + \text{Cl}_i + \text{Tub}_i$. Table 1 shows the estimates of the variance components. From the total genetic variation estimated by this model 75% was due to the $\text{Ro}_i$ term representing the qualitative classification and 25% was due to genetic variation between clones within the same morphological class.

Establishing the underlying genotypes of the phenotype “round”: The pedigree of the material shows that the male parent 77.2102.37 of this cross is a descendant of the female parent US-W5337.3. As a consequence of the relatedness of the parents, this cross can be regarded as a backcross. The segregation of RFLPs which are polymorphic in both parents can be described by the general genetic model $ab \times bc \rightarrow \frac{1}{4} ab + \frac{1}{4} ac + \frac{1}{4} bb + \frac{1}{4} bc$. At every locus both parents have at least one allele in common (allele b). This common allele is the allele which was transmitted from clone US-W5337.3 to clone 77.2102.37. Figure 1 shows the distribution of alleles at the RFLP loci flanking the Ro locus on the chromatids. Coupling and repulsion phases of alleles within a parent were deduced from the cosegregation of alleles in the offspring. The recessive allele ro in clone 77.2102.37 is identical by descent to the recessive allele ro in clone US-W5337.3, while the dominant alleles are indicated with the symbols Ro* and Ro‡ to distinguish between their origin. Figure 1 also shows that two crossing over events occurred in clone US-W5337.3 when the gamete was formed which contributed to clone 77.2102.37. The configuration of the alleles of markers TG303 and Ac15-7 in the male parent is unexpected, because the alleles from these loci that are transmitted from the parent US-W5337.3 are in repulsion phase with alleles at other loci. For the clones descending from the cross US-W5337.3 × 77.2102.37 it is possible to deduce the genotype at the Ro locus using the linkage with unique alleles at RFLP loci closest flanking the Ro locus. The initial 3:1 segregation into two morphological classes (“round”: “long” = Ro:ro = 3:1) can be separated into a 1:1:1:1 segregation of the four genotypic classes Ro‡Ro‡:Ro*ro:roRo*:roro. The prediction of the phenotypes based on this method was in agreement with the initially used visual classification.

For the 50 clones used in this experiment their underlying Ro genotypes were determined. This resulted in eight homozygous dominant clones Ro‡Ro‡, nine heterozygous clones Ro*ro, 10 heterozygous clones roRo* and eleven homozygous recessive clones roro. For the twelve remaining clones, the genotypes could not be determined unambiguously. Eight clones could not be identified because of crossing over between flanking markers, the crossover events occurring in five cases in
the female meiosis and in three cases on the male side. Missing RFLP data prevented the determination of the Ro genotype of four clones.

To estimate again the amount of the genetic variation explained by the major gene at the Ro locus, but now using the 1:1:1:1 classification instead of the 3:1 classification, the following model was used: \( \log(L/W) = \mu + \text{Cl}_{jk} + \text{Tu}_{jk} \). From the total genetic variance estimated by this model 80% was due to the \( \text{Cl}_{j} \) term representing the 1:1:1:1 classification (Table 1). The higher value of the variation explained by this classification compared with the value of 75% for the 3:1 segregation indicates the presence of genetic variation within the "round" class and a smaller influence of minor loci.

**Detection of multi allelism:** Genetic variance due to the major QTL for tuber shape at the Ro locus between the three genotypic classes Ro\(^5\)Ro\(^6\), Ro\(^5\)ro and roRo\(^6\) was expected. Typical differences were already observed between clones belonging to the same morphological class. The mean length/width ratios of the four genotypic classes, listed in Table 2, show that heterozygous clones which are "round" due to the presence of the Ro\(^6\) allele have a much lower length/width ratio than "round" clones having the Ro\(^5\) allele. The difference in tuber shape between the four genotypic classes was investigated using mixed model analysis of variance and was significant \( (F = 44.72, P < 0.0001) \). The high \( F \) value was mainly due to the difference between round and long clones. With the subset including only data from the two heterozygous genotypic classes, Ro\(^5\)ro and roRo\(^6\), the \( F \) value for the mixed model analysis of variance was still 12.24 \( (P = 0.0028) \). The significance of the difference between the heterozygous genotypes analyzed by a protected least significant difference test between the least significant means of the classes Ro\(^5\)ro and roRo\(^6\) was \( t = -2.5437 \) \( (P = 0.0157) \). With the subset including only data from the two heterozygous genotypic classes Ro\(^5\)ro and roRo\(^6\) the significance improved slightly: \( t = -3.0803 \) \( (P = 0.0068) \). The homozygous dominant genotype Ro\(^5\)Ro\(^6\) gave an intermediate round tuber shape and did not differ significantly from both the heterozygous genotypes. The finding of a significant difference in the mean tuber shape varying with the parental origin of the dominant Ro allele provides evidence for the presence of three alleles at the Ro locus.

**QTL mapping of the Ro locus:** The position already given of the Ro locus on chromosome 10 of the potato genome was determined by a qualitative approach. A QTL mapping procedure is expected to give a similar result. QTL mapping was performed by analysis of variance using \( \log(L/W) \) value as the dependent variable and the genotypic classes of the RFLP loci as the treatment source of variation. The results of this analysis presented in Table 3 reveal that proximal RFLP markers ST06, TG63 and Ac15-7 are associated with tuber shape, whereas the distal markers Ac38-46, TG303 and ST15

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**TABLE 1**

<table>
<thead>
<tr>
<th>Term</th>
<th>Estimated component</th>
<th>( \log(L/W) )</th>
<th>( L/W )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model: ( \log(L/W) = \mu + \text{Tu}_{jk} )</td>
<td>( \sigma_{\text{total}} )</td>
<td>0.12536</td>
<td>0.10404</td>
</tr>
<tr>
<td>Model: ( \log(L/W) = \mu + \text{Cl}<em>{jk} + \text{Tu}</em>{jk} )</td>
<td>( \sigma_{\text{Cl}} )</td>
<td>0.09864</td>
<td>0.08337</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{\text{Tu}} )</td>
<td>0.02672</td>
<td>0.02068</td>
</tr>
<tr>
<td>Model: ( \log(L/W) = \mu + \text{Mo}<em>{jk} + \text{Cl}</em>{jk} + \text{Tu}_{jk} )</td>
<td>( \sigma_{\text{mo}} )</td>
<td>0.07883</td>
<td>0.06225</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{\text{Cl}} )</td>
<td>0.01981</td>
<td>0.02112</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{\text{Tu}} )</td>
<td>0.02672</td>
<td>0.02068</td>
</tr>
<tr>
<td>Model: ( \log(L/W) = \mu + \text{Ge}<em>{j} + \text{Cl}</em>{jk} + \text{Tu}_{jk} )</td>
<td>( \sigma_{\text{pen}} )</td>
<td>0.01910</td>
<td>0.01694</td>
</tr>
<tr>
<td></td>
<td>( \sigma_{\text{ge}} )</td>
<td>0.02987</td>
<td>0.02121</td>
</tr>
</tbody>
</table>

* Untransformed data.
* Log transformed data.
* Not a variance component.

**TABLE 2**

<table>
<thead>
<tr>
<th>Class</th>
<th>Mean ( L/W ) ratios ± se</th>
<th>LSD ( (\alpha = 0.05) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tubers</td>
<td>0.9782 ± 0.0112</td>
<td>0.0517</td>
</tr>
<tr>
<td>Morphological class &quot;round&quot;</td>
<td>0.9133 ± 0.0270</td>
<td>0.0517</td>
</tr>
<tr>
<td>Morphological class &quot;long&quot;</td>
<td>1.5112 ± 0.0517</td>
<td>0.0517</td>
</tr>
<tr>
<td>Genotype Ro(^5)Ro(^6)</td>
<td>0.8057 ± 0.0665</td>
<td>0.0517</td>
</tr>
<tr>
<td>Genotype Ro(^5)ro</td>
<td>0.7530 ± 0.0472</td>
<td>0.0517</td>
</tr>
<tr>
<td>Genotype roRo(^6)</td>
<td>0.8898 ± 0.0499</td>
<td>0.0517</td>
</tr>
<tr>
<td>Genotype toro</td>
<td>1.5112 ± 0.0517</td>
<td>0.0517</td>
</tr>
</tbody>
</table>

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Multiple Alleles of a QTL in Potato

TABLE 3

<table>
<thead>
<tr>
<th>LOD scores of genetic linkage between RFLP markers along chromosome 10 and the qualitative Ro locus, and significance of association between RFLP markers and log(L/W) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFLP marker</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Ac38-46</td>
</tr>
<tr>
<td>TG303</td>
</tr>
<tr>
<td>Tac13b</td>
</tr>
<tr>
<td>Tac20</td>
</tr>
<tr>
<td>TG43</td>
</tr>
<tr>
<td>ST06</td>
</tr>
<tr>
<td>TG63</td>
</tr>
<tr>
<td>Ac15-7</td>
</tr>
<tr>
<td>ST05</td>
</tr>
<tr>
<td>ST15</td>
</tr>
</tbody>
</table>

lack significant association. Markers Tac20 and TG43 failed to show a highly significant association with tuber shape represented by the log(L/W) value. This lack of significance is caused by the type of polymorphism displayed by these RFLP markers. Five types of polymorphisms can be found at RFLP loci in the offspring of outbreeders: (1:1), (1:1), (3:1), (1:2:1) and (1:1:1:1). The first and second type of polymorphism is found at loci where either the female or male parent is segregating (heterozygous), while the other parent is homozygous at that locus. The third and fourth type of polymorphism is found at loci where both parents, with the same genotype, are heterozygous for a dominant (e.g., RAPD markers) and codominant marker, respectively. When both parents have different heterozygous genotypes the fifth type is found (multiple alleles, ab × be).

When clones are classified according to a poly- morphic marker of the second type (1:1), the clones are classified with respect to the paternal alleles, but lumped together with respect to the maternal alleles (Ro6 Ro6 + roRo6 vs. Ro6 ro + roro). The contrast in tuber shape (Length/Width) between these groups is expected to be small, because the genotypic class Ro6ro, with the smallest L/W value, and the genotypic class roro, with the highest L/W value (shown in Table 2), are in this situation lumped together into the same class. Figure 2 illustrates that allele a of marker TG43 indicates both genotypic classes Ro6ro and roro. The distribution of genotypic classes Ro6 Ro6 and roRo6 indicated by allele b coincides with the minimum in the bimodal distribution indicated by allele a. This explains why markers Tac20 and TG43 cannot detect significant association with tuber shape despite their close linkage. On the other hand, when the clones are lumped together in groups according to the maternal RFLP alleles and the paternal alleles are ignored, the contrast between the groups is expected to be much larger. The highly significant association between RFLP marker Tac13b and tuber shape can be explained by this lumping together of clones with different paternal alleles and classifying according to maternal alleles. This is also illustrated by the still significant F value for marker ST05 although it is located at 30 cM map distance from Ro.

To verify the point that different types of polymorphism have different powers to detect a QTL, the 1:1:1:1 segregating RFLP loci were converted into φ1:1 and ψ1:1 segregating loci. Table 3 shows the resulting pattern of F values. These F values which display the effect of lumping are in good accordance with the different effect of the Ro6 and Ro6 allele reported in Table 2. The higher recombination frequency in the female meiosis is disturbing the effect for the markers Ac38-46 and TG303 which are not linked to Ro in a map based on the female meiosis alone.

DISCUSSION

Inheritance and qualitative mapping of tuber shape:
The visual classification of tuber shape is not unambiguous, but it was demonstrated to be a suitable approach not only in this study, but also in previous studies using diploid material (TAYLOR 1978; OKEWAGWU 1981; MASSON 1985; DEJONG and BURNS 1993). In this study the visual classification appeared to be completely in agreement with the predictions based on the flanking RFLP markers. The localization of the Ro locus on potato chromosome 10 described in this paper should help in revising the classical genetics of the potato. Many contradictory genetic models were postulated to explain the inheritance of tuber shape (DEJONG and BURNS 1993). A genetic model with a major QTL with multiple alleles will explain the data of many of the earlier papers.

Localization of the Ro locus on chromosome 10 is in agreement with the earlier observation of linkage between tuber shape and skin color (DEJONG and ROWE 1972; MASSON 1985; DEJONG and BURNS 1993), for skin color is now also localized on chromosome 10 (GEHRARDT et al. 1989). Probably, the locus Ro is
the only major QTL for tuber shape in Group Tuberosum germplasm, since the linkage between tuber shape and skin color was reported several times. In several other crosses which were studied in our laboratory, tuber shape always mapped on chromosome 10. This justifies the use of the symbol Ro after MASSON (1985). Moreover, clone US-W5373.3 was also used by MASSON.

**Detection of multiple alleles:** Multiple alleles were detected at the Ro locus after making a distinction among the descendants between the four possible genotypes which can be obtained with three different alleles (Ro\(^6\)Ro\(^5\), Ro\(^5\)ro, roRo\(^5\) and roro). The correct genotype of each clone of the progeny was uncovered by using the information of the flanking RFLP alleles. The disadvantage of this method is the absence of the truly homozygous genotypes Ro\(^6\)Ro\(^6\) and Ro\(^5\)Ro\(^5\). However, it is not possible to design one single cross from which Ro\(^5\)Ro\(^5\), Ro\(^5\)Ro\(^5\) and roro can segregate, and therefore, these genotypes cannot be selected within (on average) the same genetic background. Without RFLP techniques there is an alternative method to compare the effect of multiple alleles in a common genetic background, i.e., the development of near isogenic lines by repeated backcrossing. Unfortunately, this technique is limited to species which can be selfed indefinitely. Furthermore, the diploid potato is self-incompatible, and only one generation per year can be obtained. A common genetic background is required to study the effects of a single locus. A minimum number of eight clones per (marker) genotypic class would be adequate to cancel the effects of other chromosomal regions on tuber shape, outside chromosome 10.

Two distinct dominant alleles and one recessive allele could be distinguished in this cross; the presence of other alleles in potato germplasm is very likely, especially in cultivated species where other types of tuber shape can be observed (See illustrations in DEJONG and BURNS 1993.) Further research may reveal to what extent this variation is due to other minor loci or to other alleles of the Ro locus. Studies on tuber shape at the tetraploid level have never resulted in a clarification of the inheritance of this trait (DEMAINE and FLEMING 1991; DEJONG and BURNS 1993). An explanation as to why these investigations have been so difficult may be the large amount of intralocus interaction between the four possibly different alleles in the tetraploid genome.

Although experimental evidence for the presence of multiple alleles for a QTL is only now being reported, this study in potato can be compared with a case reported in maize. BEAVIS et al. (1991) showed associations between the chromosomal localization of QTLs for plant height and qualitative genetic loci like the GA dwarf locus, d3, on chromosome 9. Their results provide circumstantial evidence in support of ROBERTSON’s hypothesis on the relationship of qualitative mutants to quantitative traits. ROBERTSON (1985) proposed that major mutants are actually null or near-null alleles at a QTL. According to this approach the recessive ro allele for tuber shape can be regarded as the null or near-null allele which is recognized qualitatively. The variation among dominant Ro alleles is of a quantitative nature.

The view that the (most) recessive allele can be regarded as a null or near-null allele is in accordance with the model suggested by FORKMANN and SEYFFERT (1977) to describe the quantitative effects at a multiallelic locus. In the parameterization of FORKMANN and SEYFFERT the most recessive homozygote is the reference point and all contributions of the other alleles are unidirectional and
positive, whereas the interactions between alleles are unidirectional and negative. This parameterization may, to some extent, reflect the biological and/or biochemical basis of gene action. However, its usefulness in terms of operational and observable parameters on the population level, which are necessarily allele frequency dependent, is questionable. When more metric traits are resolved into Mendelian factors, in experimental designs which use heterozygous parents, statements can then be made concerning the importance of multiple alleles relative to multiple loci in explaining quantitative genetic variation. In this mapping population multiple alleles were detected at more than one third of the RFLP loci. This abundance of multiple alleles at the DNA sequence level may be indicative for a resembling profusion of multiallelism for quantitative trait loci.

**QTL mapping of the Ro locus:** By carrying out linkage analysis of tuber shape as a qualitative trait prior to the QTL analysis we were able to demonstrate the possible weakness of a QTL analysis in a heterozygous outbreeding crop. The qualitative analysis resulted in location of the Ro locus on the map and also revealed the presence of multiple alleles. Had we started with the QTL analysis by treating tuber shape as a true quantitative character we would not have been able to map the major QTL at the Ro locus properly. Most QTL mapping procedures are designed for autogamous species, in many cases assuming a typical F2 or BC type of segregation. Standard QTL mapping software cannot properly deal with multiallelism resulting in a 1:1:1:1 ratio for markers. One way to get around this is to carry out two analyses, one for the paternally segregating alleles (disregarding the maternal segregation) and one for the maternally segregating alleles (disregarding the paternal segregation). In doing so we demonstrated that this grouping of alleles may lead to false conclusions: in our case the effect of the paternally segregating alleles is almost completely masked by the joint effect of the maternal alleles. Had all of the markers been segregating in a 1:1 ratio, either from the paternal or the maternal side, only the maternal segregation would have indicated a QTL for tuber shape. Moreover, had we not known (from the pedigree and the qualitative analysis) the heterozygous state at the Ro locus in the male parent, we would not have detected it by a QTL analysis based on 1:1 ratio’s only.

Where dominant markers are used, such as RAPDs, to analyze the offspring in an outbreeding crop, these markers will segregate in a 1:1 ratio either from the male or female side (a dominant marker which is heterozygous in both parents is not likely to be used, because it detects no polymorphism between the parents in the first place). Our present analysis clearly demonstrates the limitations of a straightforward QTL analysis in such a situation; a QTL effect may go unnoticed due to heterozygosity of the other parent. Therefore we recommend performing QTL analysis with markers which detect different polymorphisms in both parents (1:1:1:1:1) when quantitative genetic factors are also segregating from both parents.

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**LITERATURE CITED**


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