IDENTIFICATION OF GENETIC FACTORS INFLUENCING CHIP COLOR IN DIPLOID POTATO (*Solanum spp.*)

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Abstract

A genetic map was constructed with a combination of isozymes, restriction fragment length polymorphisms (RFLPs) and randomly amplified polymorphic DNA (RAPDs) to apply quantitative trait loci (QTL) analysis to identify genetic factors that contribute to chip color in potato. The diploid population used was a cross between a *Solanum tuberosum* haploid and *S. chacoense* hybrid used as female parent and a *S. phureja* clone used as male. Chip color was determined visually on samples fried from tubers stored at 10C. On a scale of 1 (light color) to 10 (dark color), the population ranged from 2 to 8 while the parents average chip color was 3.5. Based upon one-way ANOVAs ($P < 0.05$), 13 genetic markers showed significant associations which represent a total of six QTLs. A multiple locus model based upon the markers that have the largest effect per QTL explained 43.5% of the phenotypic variation for chip color in the population and increased to 50.5% when one significant epistatic interaction was included in the model. All the significant marker associations were identifed in the *S. tuberosum-S. chacoense* hybrid. Through preliminary data, the results of this study suggest that additive effects contribute a significant portion of the genetic variation for chip color. The identification of these QTLs for chip color variation provides the means to apply marker-assisted selection to introgress these genes into the cultivated potato germplasm.

Compendio

Se construyó un mapa genético con una combinacion de isozimas, polimorfismos de restricción de la longitud de los fragmentos (RFLPs) y DNA polimórfico amplificado al azar (RAPDs), para aplicar el análisis de loci de caracteristicas cuantitativas (QTL) en la identificación de factores genéticos que contribuyen al color de la papa en la fritura a la inglesa. La población diploide utilizada fue un cruzamiento entre un haploide *Solanum tuberosum* y un híbrido *S. chacoense*, usado como progenitor femenino, y un clone *S. phureja* usado como macho. Se determinó visualmente el color de la papa

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frita a la inglesa sobre muestras fritas de tubérculos almacenados a 10 C. En una escala de 1 (color claro) a 10 (color oscuro), la población varió de 2 a 8, mientras que el color en los progenitores promedió 3.5. Basándose en ANOVAs de un solo sentido ($P < 0.05$), 13 marcadores genéticos mostraron asociaciones significativas que representan un total de seis QTLs. Un modelo de locus múltiples, basado en los marcadores que tuvieron el mayor efecto por QTL, explicó el 43.5% de la variación fenotípica de la población, para el color de la papa en fritura a la inglesa y se incrementó a 50.5% cuando se incluyó en el modelo una interacción epistática significativa. Se identificaron todas las asociaciones significativas de los marcadores en el híbrido *S. tuberosum-S. chacoense.* A través de la información preliminar, los resultados de este estudio sugieren que los efectos aditivos contribuyen en proporción significativa en la variación genética para el color de la papa frita a la inglesa. La identificación de estos QTLs, para variación del color en la fritura, provee los medios para aplicar la selección apoyada por marcadores en la introgresión de estos genes en el germoplasma de papa cultivada.

Introduction

The primary reducing sugars found in potato tubers are glucose and fructose (13) which are the products of sucrose hydrolysis (11). The reducing sugar levels in potato tubers is the primary factor affecting chip color upon frying (24, 28). Hughes and Fuller (12) found that 90% of the chip color variation could be related to the concentration of reducing sugars in tubers. The brown melanoidan pigments produced in dark chips is the result of the Maillard reaction, which is a non-enzymatic process where the carbonyl group of the monosaccharide reducing sugars reacts with the amino group of free amino acids during exposure to heat in processing (10, 22). High reducing sugar levels result in dark colored chips which carry an undesirable taste (27).

Various factors can affect the reducing sugar level in potato tubers. The genetic component has a strong influence upon initial reducing sugar levels in a mature tuber (23). As a result, cultivars have been bred specifically for chip processing (14, 18, 29). Numerous investigations into the genetics of chip color have been conducted with little concurrence between genetic models (1, 3, 16, 19).

The development of saturated genetic maps provides means to dissect complex traits into discrete Mendelian components (25). In potato, genetic maps have been constructed with a combination of isozymes, restriction fragment length polymorphisms (RFLPs) and randomly amplified polymorphic DNA (RAPDs) markers (2, 5, 9) to apply Quantitative Trait Loci (QTL) analysis for various traits (6, 8, 26, 30). In this paper we report the use of QTL analysis to identify genetic factors that contribute to chip color in potato.

Materials and Methods

Plant Material

The population used (TRP133), is diploid and consists of 110 F_1 genotypes. The female parent used in the cross was clone 84SD22, which is a hybrid between haploid *S. tuberosum* (2x) and *S. chacoense,* while the male parent was *S. phureja* clone 84S10.

Chip Processing

On May 12, 1991, the two parents and 95 genotypes of population TRP133 were planted at the Montcalm Research Farm, Michigan. This was the second year of clonal propagation of the population, and genotypes used were ones which had enough tubers available for the trial. A randomized complete block design with two replications and eight plants per plot was used. Spacing was approximately 25 cm between plants within rows and 90 cm between rows. The trial was harvested 120 days after planting, and tubers with diameter greater than 3 cm were collected. These tubers were held for approximately three weeks at 15C, then placed into 10C storage. After 45 days, a sample of 10 tubers from each plot was sliced from apical to basal end and two 1/16 inch (0.4 cm) slices from each tuber were fried. Chip color determination was made visually on the 20-chip sample from each plot with use of the Potato Chip Color Reference Standards developed by Potato Chip Institute International, Cleveland, Ohio. For each genotype, the average chip data color from the two plots was then determined.

Genotyping with Molecular Markers

The individuals in population TRP133 were characterized with a total of 10 isozyme loci, 44 RFLP markers, and 63 RAPD markers that were segregating in a 1:1 ratio. Markers segregating from the female parent were used to construct a linkage map that covers 10 of the 12 potato chromosomes. The methodology used has been described elsewhere (5).

QTL Analysis

Single factor ANOVAs between the chip color data from the 95 individuals and each marker locus were conducted (PROC GLM, Statistical Analysis Systems, Cary, NC). F-tests were used to test the means of the genotypic marker classes for statistical difference ($P < 0.05$). A significant difference in chip color means was interpreted as linkage of a QTL to the marker locus. QTLs were localized based on the position of marker loci on the linkage map. Significant markers which are linked in the same chromosome were considered as one QTL if the distance between them did not exceed 50 cM (17). 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 chip color explained by the identified QTLs (15).

Epistatic interactions between significant loci were tested by two-way analyses of variance. Significant interactions were then included in the

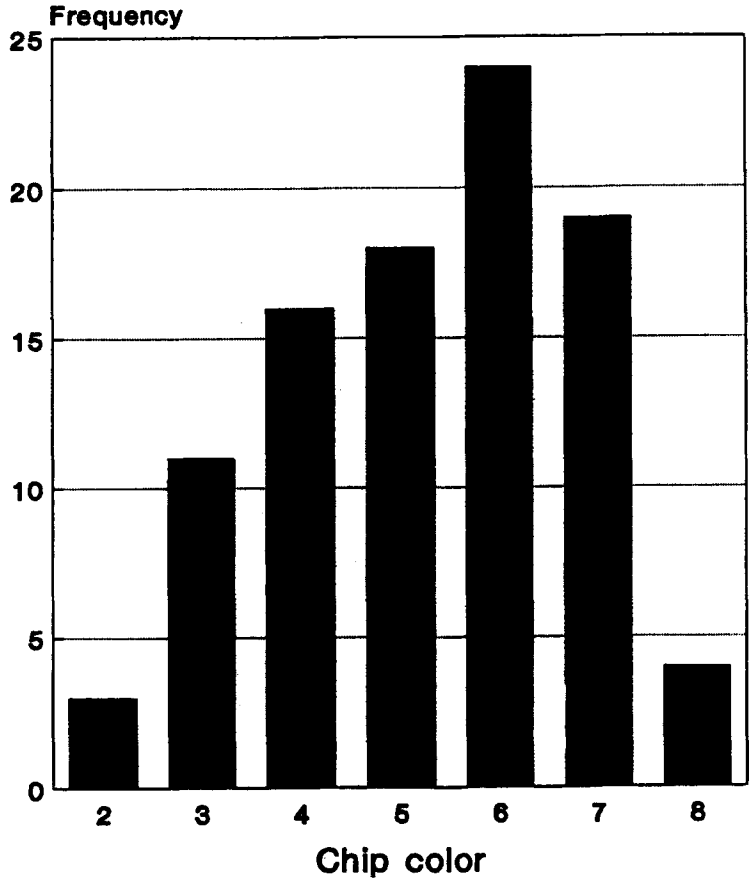

FIG. 1. Frequency distributions of chip color values in population TRP133.

TABLE 1.—*Significant association between markers and chip color.*

Marker[a]	Size (Kb)	Chrom.	R^2 (%)
A04.2	1.10	2	5.8 *
G05.5	1.30	2	14.9 ***
I10.1	0.56	2	5.1 *
Pgm-2		4	4.9 *
H14.1	0.75	4	10.2 **
A12.1'	1.15	5	6.0 *
A15.1	0.60	5	11.3 **
F06.2	0.80	5	7.0 *
H04.1	0.98	5	6.2 *
I11.1	0.83	5	9.9 **
I11.3	1.20	5	5.2 *
G13.3	0.90	10	11.6 ***
I19.2	0.83	10	5.1 *

[a]Isozyme locus is italicized; others are RAPD markers obtained with Operon 10-mer primers. Nomenclature for RAPDs is following Quiros *et al.* (20).

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

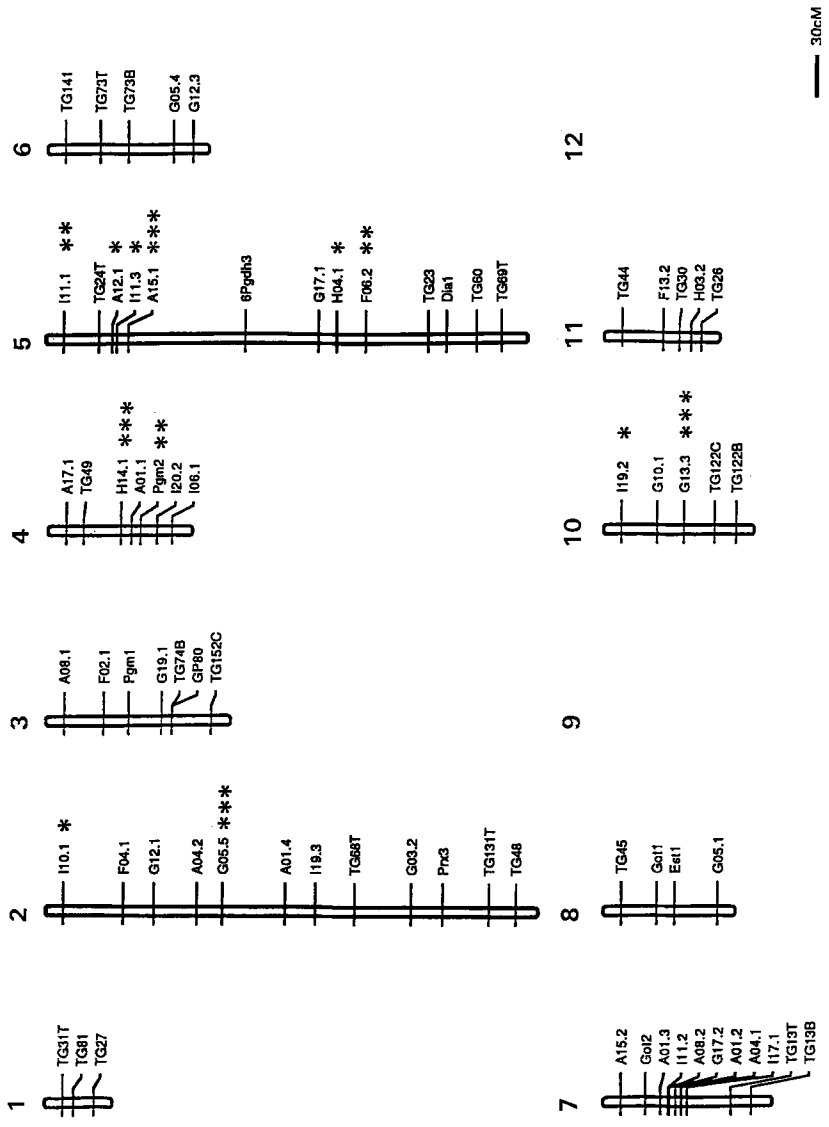

FIG. 2. Molecular linkage map and localization of QTLs for chip color variation.

multiple analysis of variance to determine their contribution in the phenotypic variation for chip color. When there were several interactions between the same pairs of QTLs, the one with the highest R^2 value was utilized.

Results

Chip color values, on a scale of 1 to 10, ranged from 2 to 8 in the population and had a mean of 5.5. (Fig. 1). Even though the mode of the population was class 6 (with 24 individuals), the frequency distribution of the population did not differ significantly from a normal distribution. Both parents, 84S10 and 84SD22, had average chip color values of 3.5. Results from the one-way analyses of variance indicated that one isozyme locus and twelve RAPD markers showed significant association with chip color (Table 1). Of these, RAPD marker A04.2 showed a distorted segregation. All the significant markers were segregating from the female parent. These markers represent a total of six QTLs: two on chromosome 2, one on 4, two on 5, and one on 10 (Fig. 2). The proportion of phenotypic variation for chip color explained by individual markers, as determined by the R^2 values in the one-way analyses of variance, ranged from 4.9% to 14.9% (Table 1).

Epistatic interactions between significant markers were tested through two-way analyses of variance. Only the interaction between the RAPD markers I10.1 and I11.1 showed significance. These markers were mapped on chromosomes 2 and 5, respectively.

The markers which had the largest effect per QTL were chosen to develop a multilocus model. This model consisted of markers A15.1, G05.5, G13.3, H14.1, I10.1, H04.1 and Pgm-2. This model was used in a multilocus analysis of variance, and the resulting R^2 of 43.5% indicates the proportion of the phenotypic variation for chip color explained by these QTLs. This value increased to 50.5% when the significant epistatic interaction was included in the model.

Discussion

In this study six QTLs were identified that contributed to chip color variation from potatoes stored at 10C (50F). These QTLs are distributed over four of the 10 chromosomes that were mapped (Fig. 2). RAPD marker G05.5 is highly significant for chip color variation ($P < .001$) along with G13.3 and H14.1, while F06.2 and A15.1 are significant at $P < .01$. A15.1 and F06.2 are two loci which have two and one flanking markers, respectively, showing linkage to a QTL (Fig. 2). These additional markers proximal to the QTLs strengthen our confidence that we tagged chromosomal segments which contribute to chip color variation. RAPD markers I10.1 (chromosome 2) and I19.2 (chromosome 10) mark two independent QTLs, but the low level of significance along with the lack of the flanking markers may suggest that they may be false positive statistical tests or QTLs with a

small effect. G05.5, on chromosome 2, is highly significant, and A04.2, which is 17.7cM apart from G05.5 is also significant ($P > 0.0305$), but also has a highly distorted segregation which may be affecting this result.

In our study, the identification of six QTLs in this population supports a polygenic model to explain chip color variation. The results of Pereira, *et al.* (19), examining chip color and reducing sugar content in 4x crosses, also support a polygenic model. In their crosses, transgressive segregants for low and high reducing sugar were found in all except one set of progenies. Cunningham and Stevenson (3) examined crosses between good and poor chip processors and the progeny showed a continuous variation of chip colors. Loiselle, *et al.* (16) divided the genetics of chip color into two components: chip-processing stability and overall chip-processing ability. They determined that both general and specific combining ability contributed to genetic variation. In comparison, Accatino (1) investigated the inheritance of reversion resistance and reconditioning ability and estimated that chip color was determined by two loci. Due to the large amount of variation explained by the multiple ANOVA model in our study, we conclude that additive effects contributes a major portion of the genetic variation for chip color variation in this population.

The population analyzed provided a wide range of chip color variation. The parents used to produce the population are classified as having moderate chip-processing ability, therefore both parents should contribute both positive and negative QTLs for chip color. The multiple ANOVA, including the significant interaction, explained over 50% of the phenotypic variation for chip color. None of this variation was based upon marker segregation in the male parent. This observation is probably partly due to the greater marker density in the female parent 84SD22. In addition, corresponding markers to those significant QTLs in 84SD22 were not segregating in 84S10. The development of a more dense linkage map for 84S10 may identify further QTLs in this population. Eight of the marker loci were linked in coupling with positive chip color effects while five were linked in repulsion. The identification of these linkages suggests that the species, *S. chacoense*, along with *S. tuberosum* can contribute positive alleles for low reducing sugar accumulation. Further analyses may identify positive QTLs in 84S10, a *S. phureja* selection.

Three classes of genetic markers were utilized for mapping: isozymes, RFLPs, and RAPDs. Interestingly, 12 of the 13 significant markers were RAPDs with no RFLP marker tagging a significant QTL in this population for chip color variation. For other tuber traits in this population (4, 5), we also observed the RAPD markers to tag a majority of the QTLs. This observation was surprising since the RFLP markers were chosen according to their distribution in the potato genome. On the other hand, the RAPD loci used in this study were based upon random sampling of polymorphisms rather than genomic distribution. It may be that the RAPD markers are

more efficient in tagging traits. However, this observation may partially be explained by the greater number of RAPD markers that were used compared to RFLPs (63 versus 44, respectively).

The identification of QTLs which explain chip color variation indicates that genes controlling reducing sugar accumulation have been tagged. These genes are mapped to chromosomes 2, 4, 5 and 10. Two other traits, tuber dry matter content and dormancy, were examined in this population in previous studies (5, 6). The genetic markers linked to chip color variation are different than those for dry matter and dormancy, except for *Pgm-2*, which was also significant for specific gravity (5). Van Den Berg, *et al.* (26) identified major QTLs for tuber dormancy, tuberization and stem branching on chromosomes 2 and 4 and tuber dormancy on chromosome 5. Gebhardt (7) identified QTLs for resistance to *Phytophthora infestans* also on chromosomes 2, 4 and 5. In addition, the PVX resistance gene is localized to chromosome 5 (21). At this time we cannot determine if any linkages exist between chip color and these other agronomic trait QTLs because of different markers used in the mapping.

This study was a preliminary investigation for alternate methodologies to evaluate chip color variation. Six QTLs were identified which contribute to chip color variation. Over 50% of the chip color variation in the population could be explained by these QTLs and one epistatic interation. The identification of further markers in the male parent may identify additional QTLs. The identification of QTLs for chip processing can be of value to potato breeders. These QTLs can be introgressed via marker assisted selection into the cultivated potato germplasm to broaden the genetic base for chip-processing ability (or low reducing sugar levels). In this study, chip processing was studied directly out of 10C storage. Chip processing directly out of 4C storage was poor; however, chip color variation was also noted out of 7.8C storage in a sample of this population (data not shown). Other diploid populations that segregate for chip processing out of 4C storage could be identified for which to conduct QTL analysis. These additional studies would allow for a comparison of QTLs at both 10 and 4C storage regimes. In addition, this population with mapped QTLs for chip color variation may also be valuable to study the relationship of biochemistry of reducing sugar accumulation with the mapped QTLs.

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