GENETIC ANALYSIS OF POTATO TUBER FLESH PIGMENTATION AND SHAPE

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

By

Yongfei Zhang

May 2009

GENETIC ANALYSIS OF POTATO TUBER FLESH PIGMENTATION AND SHAPE

Yongfei Zhang, Ph.D.

Cornell University 2009

Diploid potato clone 10618-01, which has partially pigmented flesh, was crossed with diploid 320-02, which has white flesh. QTLs influencing extent of flesh pigmentation were detected on chromosomes 5, 8 and 9. The potato homolog of *Petunia an1*, a bHLH transcriptional regulator of anthocyanin biosynthesis, was found to co-localize with the QTL on chromosome 9. A CAPS marker based on this gene was used to evaluate a collection of 21 tetraploid potato clones with highly or fully pigmented flesh, as well as 53 cultivars with white or yellow flesh. All pigmented-flesh clones shared a marker allele that was present in only 21 of the 53 white and yellow clones, suggesting that a common bHLH allele contributes towards, although not sufficient for, pigmented tuber flesh in cultivated potato.

It was previously reported that *dfr* co-segregates with *R*. To test directly whether *R* corresponds to *dfr*, the allele of *dfr* was put into the potato cultivar Prince Hairy (genotype *dddd rrrr P*----), which has white tubers and pale blue flowers. Transgenic Prince Hairy tubers remained white, but flower color changed to purple. Three independent transgenic lines, as well as a vector-transformed line, were then crossed with the red-skinned variety Chieftain (genotype *D*--- *R*--- *pppp*), to establish populations that segregated for *D*, *R*, *P*, and the *dfr* transgene or empty vector. Progeny carrying the empty vector in the genetic background *D*--- *rrrr* produced white or purple tubers, while progeny with the same genotype and the *dfr* transgene produced red or purple tubers. HPLC and LC-MS/MS analyses revealed no

qualitative differences. Thus, *dfr* can fully complement *R*, both in terms of tuber color and anthocyanin composition.

The last chapter adds to knowledge about potato tuber shape. In addition to confirming the known *Ro* locus on chromosome 10, which explained 55% or more of the variation for ratio of tuber length to width, we identified a novel QTL, *ts*5.1, which explained 8.9% of the variation. CAPS marker CT217 was found to be tightly linked with *Ro* in the diploid population. Evaluation of a panel of 84 tetraploid potato varieties with CT217 revealed a banding pattern highly correlated with long tuber shape. An anatomical comparison of round versus long tubers revealed that long tuber shape results from an increase in cell number, rather than an increase in cell length, along the longitudinal axis.

BIOGRAPHICAL SKETCH

Yongfei Zhang was born in the city of Shaoyang in Hunan province, China. He enrolled in Hunan Agriculture University in 1984 and graduated with a Bachelors degree in 1988; his major was in tea breeding and processing. His undergraduate thesis explored the regeneration of tea plantlets from *in vitro* callus culture, and was ranked first among his peers. He then went to graduate school and received an M.S. degree from Yunnan University in 1991, with a major in crop physiology. His M.S. thesis focused on detecting changes in phytohormone levels related to the release of potato tuber dormancy. He then spent one year teaching English in a rural middle school in a small county of Yunnan province, as a member of a volunteer poverty-relief team. He helped over 10 students achieve their dream of attending college that year. In the Fall of 1992, he joined the faculty of Yunnan Normal University (YNU) as a potato research scientist and taught undergraduate plant physiology. During his eleven years at YNU he released three potato varieties, including “Cooperation-88”, which has been widely adopted by farmers in south-west China. In 2002 and 2003 he was a key member of the organizing committee for the Fifth World Potato Congress that was held in Kunming in 2004. The congress was a great success, but he did not get a chance to attend as he came to Cornell as a visiting scientist in early 2003, where he joined Dr. De Jong’s research team. In Spring 2004, he became a graduate student in the Department of Plant Breeding and Genetics at Cornell, where he conducted his PhD studies.

This dissertation is dedicated to those who have educated me and those who believe in education can make a change.

ACKNOWLEDGMENTS

I am very grateful to my mentor Dr. Walter De Jong for his patience and guidance. My background in molecular genetics was almost zero when I joined his lab. He coached me in such a way that not only built my lab skills but also provided strategic and practical insight for pursuing independent research on my own in the future. My interests were broad and to some extent I spread myself thin, but he persuaded me to just “take one candy out of the jar at a time”. My progress was initially slow, but he still gave me time to try what I wanted to test, including some experiments that he already knew would not succeed. He has always been ready to help me out when I got lost. In this way I picked up skills, ideas and understanding. His character of combining a sharp view of potato molecular genetic research, broad expertise in different facets of classical potato genetics, his critical but constructive thoughts on filling the gap between conventional and molecular breeding, and his humbleness and kindness, though hard to match, have set up a model for my future career.

I am also very grateful to Dr. Jian Hua, who has been quite encouraging and always responded to my questions with generous advice. She quenched my panic over fast evolving molecular methods by guiding me to focus on fundamental skills.

My sincere thanks also go to Dr. Martha Mutschler. She kindly agreed to replace Dr. Molly Jahn on my committee after Molly moved to the University of Wisconsin-Madison. I have learned a lot from Martha about genetic analysis, as well as about comparing tomato and potato to extract useful information.

I also appreciate Dr. Molly Jahn very much, she was on my committee for the first half of my study at Cornell. She was so compelled to educate me that she even gave me extra hours each week to discuss and answer all the questions I had when I

took her graduate genetics course.

I am grateful to Dr. Chun Suk Jung, Shuping Cheng, Darlene De Jong and Dr. Helen Griffiths, the potato crew and other part time workers in the potato lab for their help in lab work. Without them my study would have been even tougher. Dr. Jung was very generous and kind in sharing with me his experience and understanding of quantitative genetic analysis.

Dr. Peter Davies, Dr. Hubert Zandstra and Mr. Allan Parker initiated my road to Cornell. Dr. Peter Davies also helped me in setting up at Cornell, showed me around Ithaca, and remained a good friend during my stay at Cornell. To them I owe heartfelt thanks.

Dr. Robert Turgeon, Dr. Lailiang Chen, Dr. Pengmin Li, Dr. Rayko Halitschke helped me with data collection. I greatly appreciate their help.

Dr. Mark Sorrells, Dr. Ronnie Coffman, Dr. Henry De Jong, Dr. Robert Plaisted, Dr. Lisa Earle, Dr. Peter Gregory and Dr. Michael Mazourek inspired me in many ways. The staff and faculty in the Plant Breeding and Genetics Department and SYNAPSIS club members treated me as family. I deeply appreciate this.

Finally, I want to thank my wife Hui Li, my son Dingquan Zhang and my parents and siblings, especially my wife who sacrificed her own career just to enable me to fulfill my dream. They have been giving me unlimited support and love. No words can express my gratitude to them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

GENETIC ANALYSIS OF PIGMENTED TUBER FLESH IN POTATO

Abstract

Interest in anthocyanin-pigmented potato tuber flesh is increasing. To genetically map and characterize loci that influence this trait, diploid potato clone 10618-01, which has partially pigmented flesh, was crossed with diploid 320-02, which has white flesh. Almost all progeny exhibited purple coloration in the flesh, with some clones having only a small percentage of tissue pigmented, other clones having most tissue pigmented, and the majority of clones showing intermediate color phenotypes. The two parents and 228 progeny were genotyped with 493 AFLP, 8 CAPS and 13 SSR markers. QTLs influencing extent of flesh pigmentation were detected on chromosomes 5, 8 and 9. The potato homolog of *Petunia an1*, a basic helix-loop-helix (bHLH) transcriptional regulator of anthocyanin biosynthesis, was found to co-localize with the QTL on chromosome 9. A CAPS marker based on this gene was used to evaluate a collection of 21 tetraploid potato clones with highly or fully pigmented red or purple flesh, as well as 53 cultivars with white or yellow flesh. All 21 pigmented-flesh clones shared a marker allele that was present in only 21 of the 53 white and yellow clones, suggesting that a common bHLH allele contributes towards, although it is clearly not sufficient for, highly or fully pigmented tuber flesh in cultivated potato.

Introduction

Consumer interest in potatoes with red or purple flesh has been increasing over the past decade, in part because of novel appearance, and in part because of the perceived benefits of higher antioxidant content (Tsuda et al 2000; Ross and Kasum 2002; Brown et al 2003; Scalbert et al 2005; Brown et al 2005; Brown et al 2007). Red and purple tuber flesh color results from the accumulation of anthocyanin pigments (Lewis et al 1998; Rodriguez-Saona et al 1998; Naito et al 1998; Eichhorn and Winterhalte 2005).

Pigmented tuber flesh is conferred by the *Pf* locus (De Jong 1987). *Pf* is tightly linked with *I*, which is required for pigmentation of tuber skin and maps to chromosome 10 (De Jong 1987; Dodds and Long 1955; van Eck et al 1994). The *I* locus is also known as *D* in tetraploid potatoes (Salaman 1910). *Pf* alone is not sufficient for tuber flesh to be completely pigmented; potatoes with this gene may exhibit a small, intermediate or large degree of flesh coloration. In many plants, tissue-specific accumulation of anthocyanins is mediated by R2R3MYB genes and/or bHLH regulators (Cone et al 1986; Ludwig et al 1989; Ludwig and Wessler 1990; Quattrocchio et al 1998; Quattrocchio et al 1999; Selinger et al 1998). The potato ortholog of *Petunia an*2, an R2R3MYB regulator of anthocyanin production, maps to the same region of the genome as *Pf* and *I* (De Jong et al 2004).

Several other potato genes are also known to influence potato color. The *R* locus, which co-segregates with dihydroflavonol 4-reductase (De Jong et al 2003), is required for the production of red anthocyanins, while the *P* locus, which codes for flavonoid 3',5'-hydroxylase (Jung et al 2005), is required for production of purple pigments.

Several recent studies have reported on potential health benefits of consuming potatoes with anthocyanin-pigmented flesh. E.g., rats fed with purple potato flakes

have significantly higher serum antioxidant potential and hepatic Cu/Zn-superoxide dismutase in the liver (Han et al 2006). Potato anthocyanin may also help combat both prostate cancer (Reddivari et al 2007) and breast cancer (Thompson et al 2008). To more efficiently manipulate tuber flesh color in our applied breeding program, we would like to better understand the genetic basis for flesh pigmentation. Toward this end, we constructed a diploid population that segregates for degree of tuber flesh coloration, and report here on a QTL analysis of the pigmented flesh trait, as well as a follow-up marker analysis of tetraploid potato cultivars with and without pigmented tuber flesh.

Materials and Methods

Plant materials

Diploid potato clone 10618-01, which has purple, partially-pigmented tuber flesh, was crossed as a female with white-fleshed diploid 320-02 to form an F_1 mapping population consisting of 228 clones. Both diploid parents were kindly provided by H. De Jong (AAFC, Fredericton, NB). WIS clones with pigmented tuber flesh were kindly provided by S. Janksy (USDA-ARS, Madison, WI). POR04PG01-2 was kindly provided by C. Brown (USDA-ARS, Prosser, WA). NY clones were provided by the Cornell University potato breeding program.

Phenotyping

The extent of tuber flesh coloration was evaluated with tubers produced in the greenhouse. Each clone was grown in two pots, and the largest tuber from each pot was scored. The average score of the two tubers was used for QTL analysis. All 228 progeny, as well as both parents, were genotyped. After genotyping, two daughter

clones appeared to be identical; one of the two was excluded from subsequent analysis. Thirteen of the remaining 227 clones did not tuberize well or died in 2006, and were not included in any QTL analyses. A further 15 clones did not tuberize well or died in 2007, and were not used for QTL analyses that year. Flesh coloration was scored on a 1-10 scale (1 = no flesh pigmentation, 10 = almost completely pigmented), where the distribution of pigment was assessed in equatorial cross-sections of mature tubers. Flesh coloration was scored in both 2006 and 2007.

Marker analysis

Genomic DNA was extracted from plants grown in the greenhouse using a Qiagen DNA kit, following the manufacturer's instructions. AFLP markers were generated with 14 Pst+2/Mse+3 and 10 Eco+2/Mse+3 AFLP primer combinations according to Vos et al (1995), using ^{33}P-labeled *Pst* I or *Eco* R1 primers. AFLP amplification products were separated on a 5% denaturing polyacrylamide gel. Sizes of amplification products were estimated by comparison to a Sequamark 10 base ladder (Research Genetics, Huntsville, Alabama). Images were visualized by exposing film against dried acrylamide sequencing gels. Eight CAPS markers (Konieczny and Ausubel 1993) (SbeII - Chen et al 2001; F35H-4F/4R – Jung et al 2005; 21BA, *bch*6, CT203, GP24, *UGPase*, and *zep* are described in Table 1) and 13 polymorphic SSR markers of known chromosomal location (STM0003, STM1104, STM1106, STM1053, STM2020, STM2022, STM3009, STM3010, STM3016, StI011, StI014, StI041, and StI049) (Feingold et al 2005, Milbourne et al 1998) were used to identify chromosomes.

Table 1. CAPS markers developed in this study

Marker	Approx product size (bp)	Restriction enzyme	Chromosome	Primer sequences
21BA	470/400	none	10	F: GTGATTATGTCATCCAAAAGTTTATAG
				R: GAATTTCTGAGGTTGAGGTCTTA
ans	700	*Hae*III	8	F: TATTGCTTGTACTTCTATTTTTCGAGATAG
				R: CTTGGCATATTCACTTGTTGCT
bch6	400	*Bcr*I	6	F: AACAACCTCACATGTTTCTCCAA
				R: CAAATGTACCCAACATTTCGGTTA
chi	800	*Mse*I	5	F: ATAGAGGTTTGGAGATTGAAGG
				R: ACTACACTTTGCTGCAGGGGA
chs	1600	*Alu*I	5	F: GCGACTCCTTCGAACTGTG
				R: TGAAGTTTTTCGGGCTTTAGGC
CT203	760	*Alu*I	10	F: AGTGACGATGATGACAGAGGAGAA
				R: AAATGGACTAAAGCATATAGCCGG
GP24	900	*Alu*I	6	F: CTGCAGTCAAGGGATACATTT
				R: GCGTCTCTGCAATCTATTTCT
jaf13	1480	*Rsa*I	8	F: GAAGATCCTAACCTCATTCAGCAAATAAAA
				R: GTTGCTTAAAATTATGGAGGCACTGA
Stan1	1600	*Taq*I	9	F: CGGCCCTAGTTATGATGAATTATCACA
				R: ACCTCCACTTTAAGTTCCCTTAGC
UGPase	600	*Rsa*I	11	F: CACCTTGACTGATGAGGGCTAT
				R: TGGCACCAGCAGCTACTCTA
zep	1000	*Bfu*CI	2	F: AGAGGGATTTAAGTGCTATCAGAG
				R: CCAGTATAACAAGTGTAGCCAGAG

Mapping

Marker data were analyzed with JoinMap 3.0 (Van Ooijen and Voorrips 2001). Linkage groups were assembled using the Kosambi function (Kosambi 1943). Twenty-two linkage groups were assembled at LOD thresholds of 8 or greater. Chromosomes 2 and 7 of female parent 10618-01 were assembled at LOD 6 and LOD 5, respectively. Each linkage group was labeled with at least one anchor marker of known location.

QTL analysis

QTL analysis was performed with the program MapQTL 5 (Van Ooijen 2004). Two analysis models (Kruskal-Wallis and Interval Mapping) were used. A LOD threshold of 2.85 for declaring significance ($P<0.05$) for interval mapping was established by empirically permuting the data 1000 times. Linkage map and QTL locations were visualized using MapChart 2.1 (Voorrips 2002). QTL analysis was repeated with phenotypic data from 2006 and 2007.

Association of QTLs with anthocyanin pathway genes

CAPS markers were designed against five potato anthocyanin pathway genes (*ans*, *Stan1*, *chi*, *chs*, *jaf13*; Table 1). For each CAPS marker, genomic DNA was amplified using the following thermal profile: 94C for 2 min, then 35 cycles of [94C, 20 sec; 72C, 60 sec; 56C, 30 seconds]. PCR products were digested with the corresponding restriction enzyme for 3 hours, then visualized on a 2% agarose gel.

Results

The progeny of a cross between diploid 10618-01, which has purple skin and partially-colored (purple and white) tuber flesh, and diploid 320-02, which has red

skin and white flesh, segregated extensively for extent of purple color in tuber flesh. After harvest in 2006, the flesh of 11 progeny did not appear to be pigmented at all, the flesh of 10 progeny were heavily pigmented, while the remaining 193 progeny displayed intermediate degrees of purple flesh coloration (Figure 1). All progeny had purple tuber skin. The extent of tuber flesh coloration was scored on a 1-10 scale (Figure 1).

To identify loci influencing extent of flesh coloration, the progeny and both parents were evaluated with 514 molecular markers including 493 AFLP, 13 SSR and 8 CAPS markers. Analysis with JoinMap 3.0 readily separated markers into 12 maternal and 12 paternal linkage groups. 496 of the markers could be placed in linkage groups with a LOD score of 5 or higher. The map of 10618-01 totaled 753 cM in length and comprised 212 markers, while the map of 320-02 totaled 907 cM in length and was made up of 284 markers. All 24 linkage groups included at least one anchor marker of known chromosomal location.

Marker and year 2006 trait data were then analyzed using both non-parametric (Kruskal-Wallis) and parametric (interval mapping) approaches. Kruskal-Wallis analysis revealed significant ($P<0.001$) loci on chromosome 5 of both parents: for 10618-01, at AFLP marker E32M49-442, and for 320-02, at marker E53M54-162 (Table 2). In addition, highly significant loci were detected on chromosome 8 of 320-02 at marker P14M37-134 ($P<0.0005$) and on chromosome 9 of 10618-01 at marker E32M48-233 ($P<0.0001$) (Table 2). QTLs at comparable locations were identified by interval mapping (Figure 2 and Table 2). The same loci were detected when phenotypic data for year 2007 was analyzed separately. Tuber pigmentation scores were not identical in 2006 and 2007, but were highly correlated ($r^2 = 0.61$). Two

Figure 1. Distribution of flesh color phenotypes observed in 2006 in the F_1 progeny of a cross between diploid clones 10618-01 and 320-02.

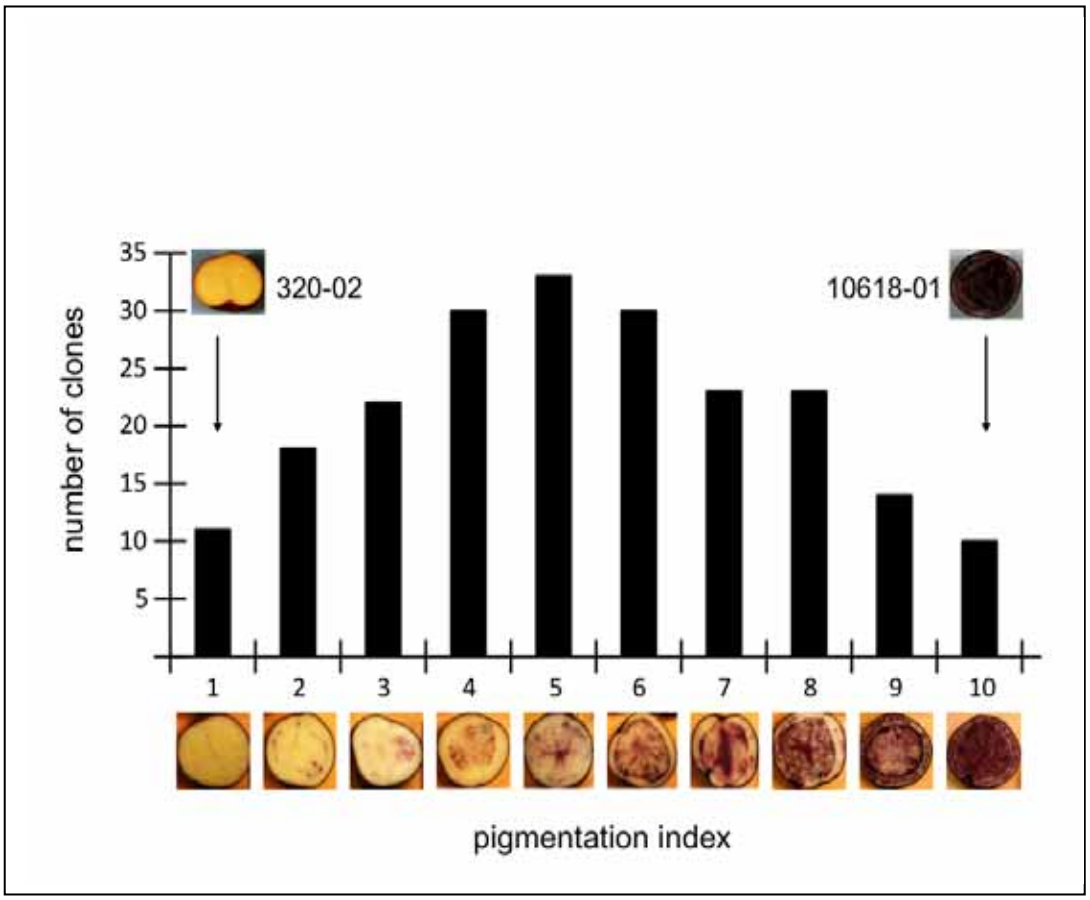

number of clones
35
30
25
20
15
10
5
320-02
10618-01
1
2
3
4
5
6
7
8
9
10
pigmentation index

Table 2. QTLs detected by Kruskal-Wallis (KW) and Interval Mapping (IM) using phenotype data from 2006.

		QTL parameters			
QTL Model	Parent	Chromosome	Marker [a]	Significance value	Percent variation (r^2)
KW	10618-01	9	E32M48-233	0.0001	N/A
		5	E32M49-442	0.001	N/A
	320-02	8	P14M37-134	0.0005	N/A
		5	E35M54-162	0.001	N/A
IM	10618-01	9	E32M48-233	LOD 3.6	8.1
		5	E32M49-442	LOD 3.6	8.1
	320-02	8	P14M37-134	LOD 2.9	6.5
		5	E35M54-162	LOD 3.7	8.1

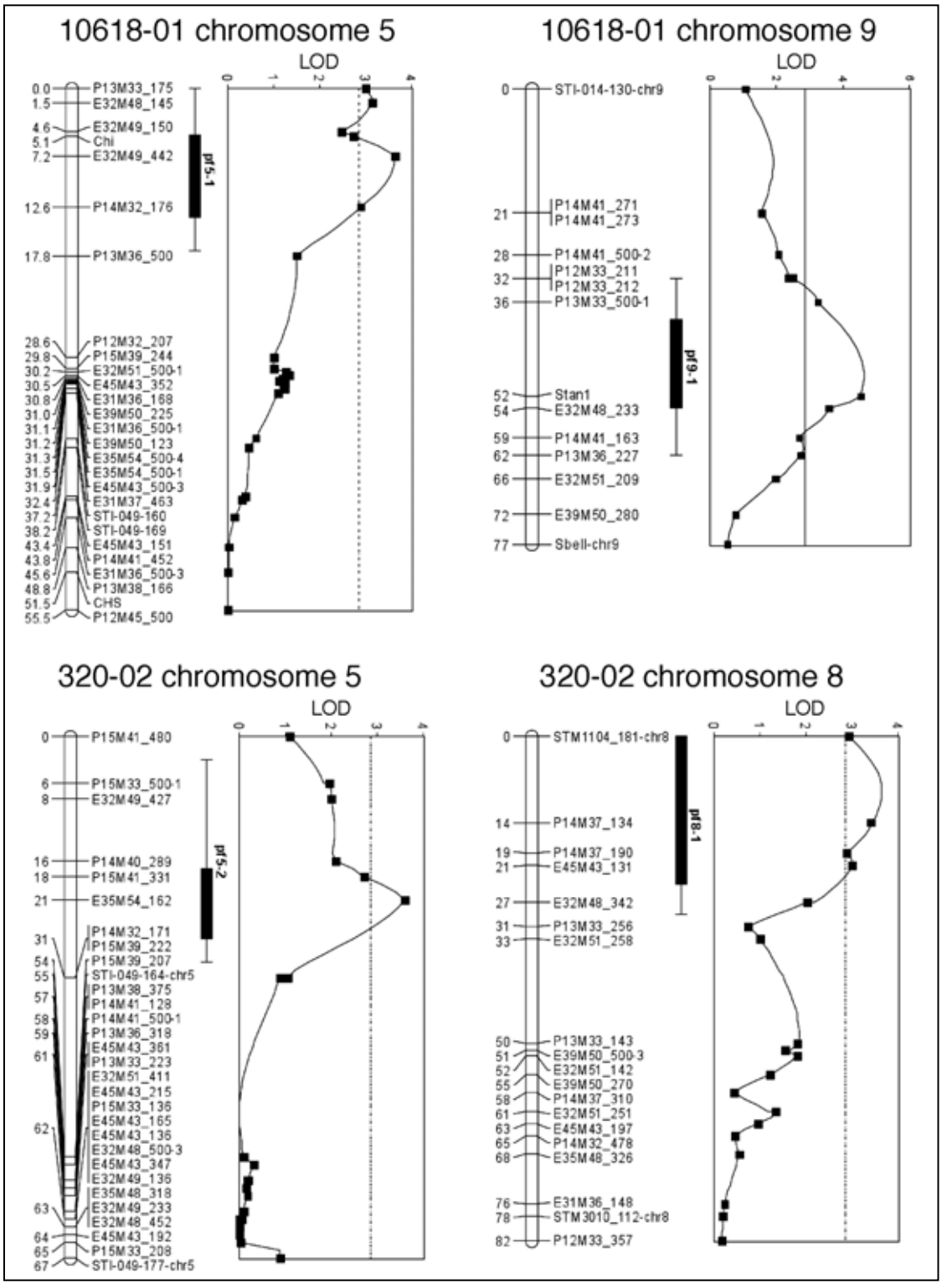
[a] Marker with highest significance score

additional loci were detected only in 2007, on chromosome 8 of 10618-01 at marker E32M48-318 (LOD 3.1) and chromosome 3 of 320-02 at marker E39M50-249 (also with LOD 3.1) (data not shown).

We subsequently tested whether any known anthocyanin biosynthetic or regulatory genes co-localize with these QTLs. Potato chromosome 5 is known to code for at least two anthocyanin biosynthesis genes, chalcone isomerase (*chi*) and chalcone synthase (*chs*); chromosome 8 is known to harbor a basic helix-loop-helix (bHLH) anthocyanin regulatory gene (homolog of *Petunia jaf13*), as well as anthocyanidin synthase (*ans*); and chromosome 9 is known to code for a bHLH gene homologous to petunia *anthocyanin 1* (*an1*) (Spelt et al 2000; De Jong et al 2004). CAPS markers were developed for all these genes (Table 1). Two of the five genes mapped under QTLs detected in 10618-01: *chi* on chromosome 5 and *Stan1*, the potato homolog of *an1*, on chromosome 9 (Figure 2). *Stan*1 explained more phenotypic variation – 11% – than AFLP marker E32M48-233, which explained 8.1% (Table 2). The relationship between the potato homologs of *jaf13* and *ans* with the QTL on chromosome 8 could not be evaluated, as neither CAPS marker was polymorphic in 320-02. The fifth gene, *chs*, mapped far from the QTL on chromosome 5 of 10618-01 (Figure 2).

CAPS markers based on *ans*, *chi*, *Stan1*, and *jaf13* were tested for possible relationship with pigmented flesh in a panel of diverse potato germplasm consisting of 21 tetraploid potato clones with red or purple flesh and 53 clones with white or yellow tuber flesh. The *Stan1* CAPS marker revealed a common digestion product, about

Figure 2. Location of QTLs that influenced extent of tuber flesh coloration in 2006. Map locations for anthocyanin-related genes *chi*, *chs* and *Stan1* are also shown.


10618-01 chromosome 5
LOD
0 1 2 3 4
0.0 P13M33_175
1.5 E32M48_145
4.6 E32M49_150
5.1 Chi
7.2 E32M49_442
12.6 P14M32_176
17.8 P13M36_500
28.6 P12M32_207
29.8 P15M39_244
30.2 E32M51_500-1
30.5 E45M43_352
30.8 E31M36_168
31.0 E39M50_225
31.1 E31M36_500-1
31.2 E39M50_123
31.3 E35M54_500-4
31.5 E35M54_500-1
31.9 E45M43_500-3
32.4 E31M37_463
37.2 STI-049-160
38.2 STI-049-169
43.4 E45M43_151
43.8 P14M41_452
45.6 E31M36_500-3
48.8 P13M38_166
51.5 CHS
55.5 P12M45_500
pf5-1
10618-01 chromosome 9
LOD
0 2 4 6
0 STI-014-130-chr9
21 P14M41_271
P14M41_273
28 P14M41_500-2
32 P12M33_211
P12M33_212
36 P13M33_500-1
52 Stan1
54 E32M48_233
59 P14M41_163
62 P13M36_227
66 E32M51_209
72 E39M50_280
77 Sbell-chr9
pf9-1
320-02 chromosome 5
LOD
0 1 2 3 4
0 P15M41_480
6 P15M33_500-1
8 E32M49_427
16 P14M40_289
18 P15M41_331
21 E35M54_162
31 P14M32_171
P15M39_222
54 P15M39_207
55 STI-049-164-chr5
57 P13M38_375
P14M41_128
58 P14M41_500-1
59 P13M36_318
61 E45M43_361
P13M33_223
E32M51_411
E45M43_215
P15M33_136
62 E45M43_165
E45M43_136
E32M48_500-3
E45M43_347
E32M49_136
E35M48_318
63 E32M49_233
E32M48_452
64 E45M43_192
65 P15M33_208
67 STI-049-177-chr5
pf5-2
320-02 chromosome 8
LOD
0 1 2 3 4
0 STM1104_181-chr8
14 P14M37_134
19 P14M37_190
21 E45M43_131
27 E32M48_342
31 P13M33_256
33 E32M51_258
50 P13M33_143
51 E39M50_500-3
52 E32M51_142
55 E39M50_270
58 P14M37_310
61 E32M51_251
63 E45M43_197
65 P14M32_478
68 E35M48_326
76 E31M36_148
78 STM3010_112-chr8
82 P12M33_357
pf8-1

980bp in size, in all 21 of the clones with pigmented flesh (Figure 3 and Table 3). This same digestion product was present in only 21 of 53 white- and yellow-fleshed clones (Figure 3 and Table 3), suggesting that a common bHLH allele contributes towards, but is not sufficient, for the ability to accumulate anthocyanin in potato tuber flesh. No association with flesh color was observed with CAPS markers based on *jaf13*, *ans* or *chi* in the same panel.

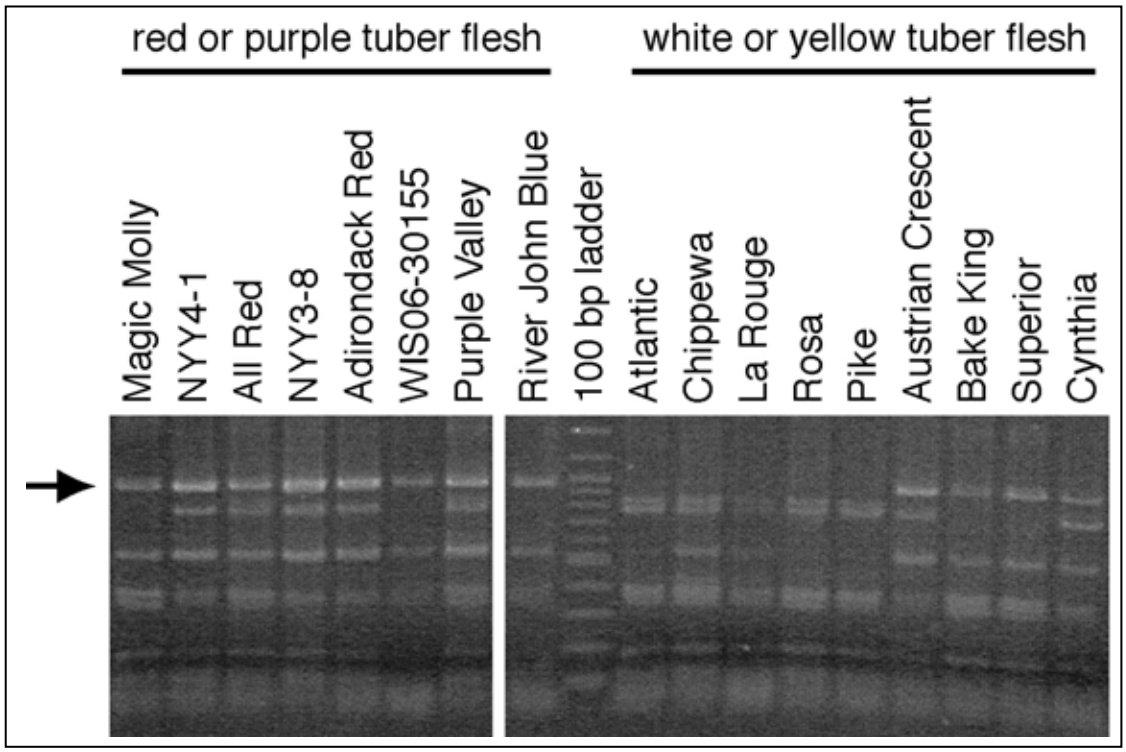


Figure 3. Association between colored tuber flesh and a CAPS marker allele based on the potato homolog of Petunia an1. Genomic DNA was amplified with Stan1 primers (Table 1), restricted with Taq I, and electrophoresed through a 2% agarose gel. An arrow denotes the approximately 980 bp band present in all clones tested with red or purple tuber flesh.

Table 3. Presence/absence of ≈980 bp *Stan1* marker allele in a panel of potato clones with and without anthocyanin-pigmented tuber flesh.

Potato clone	Flesh color	*Stan1* 980 bp fragment present (1= yes, 0 = no)
Adirondack Blue	purple	1
Adirondack Red	red	1
All Red	red	1
Huckleberry	red	1
Magic Molly	purple	1
NYH52-1	purple	1
NYS48-6	purple	1
NYY3-8	red	1
NYY4-1	red	1
POR04PG01-2	purple	1
Purple Peruvian	purple	1
Purple Valley	purple	1
River John Blue	purple	1
WIS00-4252-1	purple	1
WIS01-1131-1	purple	1
WIS01-1131-5	red	1
WIS06-3124	purple	1
WIS06-30155	purple	1
WIS06-30244	purple	1
WIS06-30340	purple	1
WIS99-2743	purple	1
Allegany	white	0
Amandine	yellow	1
Andover	white	0
Atlantic	white	0
Austrian Crescent	yellow	1

Figure 3 (Continued)

Bake King	white 1
Bintje	yellow 1
Carola	yellow 0
Chieftain	white 0
Chippewa	white 0
Cynthia	yellow 0
Desiree	yellow 1
Eva	white 0
German Butterball	yellow 1
Idarose	white 1
Katahdin	white 0
Kennebec	white 0
Keuka Gold	yellow 1
La Rouge	white 0
Lehigh	yellow 0
Lenape	white 0
Monona	white 0
Nordonna	white 1
Norland	white 1
NY97	white 1
NY99	white 0
NY115	white 0
NY118	white 1
NY120	white 1
NY121	white 0
NY123	white 0
NY127	white 0
NY128	white 0
NY129	white 0
NY130	white 0
NY132	white 0

Figure 3 (Continue)

NYT15-1	white 0
Pike	white 0
Prince Hairy	white 1
Reba	white 0
Red La Soda	white 0
Redsen	white 1
Rideau	white 1
Rosa	white 0
Salem	white 0
Sandy	yellow 0
Serrana Inta	yellow 1
Snowden	white 0
Stirling	white 1
Superior	white 1
Sylvia	yellow 0
Yagana	yellow 1
Yukon Gold	yellow 1

Discussion

This study detected loci on three chromosomes – 5, 8, and 9 – that mediate degree of tuber flesh pigmentation. Alleles influencing this trait descended from both the white and purple-fleshed parents, with the white-fleshed parent contributing alleles from chromosome 5 and 8, and the purple-fleshed parent contributing alleles from chromosomes 5 and 9.

The only locus that has previously been implicated in pigmentation of tuber flesh, *Pf*, is presumably located on chromosome 10, as *Pf* is tightly linked to *I* (De Jong 1987), and *I* has been mapped to chromosome 10 (Van Eck et al 1994). If *Pf* segregated in this cross, we would have expected half the progeny to exhibit white

flesh. Instead, only 11 of 214 progeny had unpigmented flesh, suggesting that 10618-01 is homozygous for *Pf*, and that *Pf* is necessary, but not sufficient, for anthocyanin-pigmented tuber flesh. No polymorphic markers from chromosome 10 segregated aberrantly, so *Pf* must have been transmitted to either half or all progeny. As tuber skin color did not segregate in this cross – all progeny had purple-skinned tubers – the genes required for anthocyanin production *per se* were present in all progeny. Thus, the relatively few white-fleshed progeny must have been white for a reason other than lacking a necessary biosynthetic gene.

Although the genes underpinning flesh coloration QTLs were not conclusively established in this study, two promising candidates – *chi* (for a QTL on chromosome 5) and a bHLH transcription factor similar to *Petunia an1* (for a QTL on chromosome 9) – were identified. Both of these genes mapped close to, or under, the peak of the respective QTLs. It is not obvious how *chi*, an anthocyanin biosynthetic gene, might influence degree of flesh pigmentation; perhaps this gene exhibits functional variation in its promoter region, leading to differences in the range of tissues in which it can be expressed. That the potato homolog of *Petunia an1* may play a role in tissue-specific expression was not surprising, as bHLH regulators of anthocyanin biosynthesis, such as *delila* in *Antirrhinum majus* (Goodrich et al 1992), *B* in *Zea mays* (Selinger et al 1998), *ivs* in *Ipomoea tricolor* (Park et al 2004), *tt8* of *Arabidopsis thaliana* (Nesi et al 2000; Baudry et al 2006), the rice *Purple leaf* (*Pl*) locus (Sakamoto et al 2001) and the rice red grain locus *Rc* (Sweeney et al 2006) are all known to mediate tissue-specific expression of anthocyanins.

Further evidence that the potato homolog of *Petunia an1* (or a gene tightly linked to it) is associated with pigmented tuber flesh came from a comparison of varieties with and without pigmented flesh. All 21 pigmented flesh clones tested to date share an approximately 980 bp CAPS marker allele. Eight of the pigmented flesh clones

evaluated were developed in Wisconsin (WIS clones), six were developed in New York (NY and Adirondack clones), one was developed in Alaska (Magic Molly), one was developed in Washington (POR clone), one was developed in Korea (Purple Valley), and the remaining four are of unknown origin. Though potato clones that accumulate anthocyanin in tuber flesh are not uncommon in Andean landraces, this trait has generally been selected against in modern potato breeding, just as pigmented kernels were selected against in maize (Johannessen et al 1970) and pigmented grains were selected against in rice (Sweeney et al 2007). Nevertheless, as understanding of the potential health benefits conferred by anthocyanins has increased over the past decade, interest in consuming anthocyanin-rich plant tissues has also increased dramatically. Markers based on *Stan1* may thus prove useful for those seeking to more efficiently manipulate the nutritionally important trait of pigmented tuber flesh in applied potato breeding programs.

ACKNOWLEDGEMENTS

We thank Shuping Cheng for helping with SSR data collection, and the field crew of the Cornell potato breeding program for maintaining the diploid population.

REFERENCES

Baudry A, Caboche M, Lepiniec L (2006) TT8 controls its own expression in a feedback regulation involving TTG1 and homologous MYB and bHLH factors, allowing a strong and cell-specific accumulation of flavonoids in *Arabidopsis thaliana*. Plant J 46:768-779

Brown CR, Wrolstad R, Durst R, Yang CP, Clevidence B (2003) Breeding studies in potatoes containing high concentrations of anthocyanins. Amer J of Potato Res 80:241-250

Brown CR, Culley D, Yang CP, Durst R, Wrolstad R (2005) Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines. J Amer Soc Hort Sci 130:174-180

Brown CR, Culley D, Bonierbale M, Amoros W (2007) Anthocyanin, carotenoid content, and antioxidant values in Native South American Potato Cultivars. Hortscience 42:1733-1736

Chen X, Salamini F, Gebhardt C (2001) A potato molecular-function map for carbohydrate metabolism and transport. Theor Appl Genet 102:284-295

Cone KC, Burr FA, Burr B (1986) Molecular analysis of the maize anthocyanin regulatory locus C1. Proc Natl Acad Sci USA 83:9631-9635

De Jong H (1987) Inheritance of pigmented tuber flesh in cultivated diploid potatoes. Am Potato J 64:337-343

De Jong WS, De Jong DM, De Jong H, Kalazich J, Bodis M (2003) An allele of dihydroflavonol 4-reductase associated with the ability to produce red anthocyanin pigments in potato (Solanum tuberosum L.). Theor Appl Genet 107:1375-1383

De Jong WS, Eannetta NT, De Jong DM, Bodis M (2004) Candidate gene analysis of anthocyanin pigmentation loci in the Solanaceae. Theor Appl Genet. 108:423-32.

Dodds KS, and DH Long (1955) The inheritance of colour in diploid potatoes: types of anthocyanidins and their genetic loci. J Genetics 53:136-149

Eichhorn S, Winterhalter P (2005) Anthocyanins from pigmented potato (Solanum tuberosum L.) varieties. Food Research International 38: 943-948

Feingold S, Lloyd J, Norero N, Bonierbale M, Lorenzen J (2005) Mapping and characterization of new EST-derived microsatellites for potato (Solanum tuberosum L.). Theor Appl Genet. 111:456-466

Goodrich J, Carpenter R, Coen ES (1992) A common gene regulates pigmentation pattern in diverse plant species. Cell 68:955-964

Han KH, Sekikawa M, Shimada K, Hashimoto M, Hashimoto N, Noda T, Tanaka H, Fukushima M (2006) Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats. Br J Nutr 96:1125-33

Johannessen CL, Wilson MR, Davenport WA (1970) The domestication of maize: process or event? Geographical Review 60:393-413

Jung CS, Griffiths HM, De Jong DM, Cheng S, Bodis M, De Jong WS (2005) The potato P locus codes for flavonoid 3',5'-hydroxylase. Theor Appl Genet 110:269-275

Konieczny A, Ausubel FM (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403-410

Kosambi DD (1943) The estimation of map distances from recombination values. Annals of Eugenics 12:172-175

Lewis CE, Walker JRL, Lancaster, JE, Sutton KH (1998) Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of Solanum tuberosum L. Journal of the Science of Food and Agriculture 77:45–57

Ludwig SR, Wessler SR (1990) Maize R gene family: tissue-specific helix-loop-helix proteins. Cell 62:849-851

Ludwig SR, Habera LF, Dellaporta SL, Wessler SR (1989) Lc, a member of the maize R gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the myc-homology region. Proc Natl Acad Sci USA 86:7092-7096

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterisation and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233-245

Naito K, Umemura Y, Mori M, Sumida T, Okada T, Takamatsu N, Okawa Y, Hayashi K, Saito N, Honda T (1998) Acylated pelargonidin glycosides from a red potato. Phytochemistry 47:109–112

Nesi N, Debeaujon I, Jond C, Pelletier G, Caboche M, Lepiniec L (2000) The TT8 gene encodes a basic helix-loop-helix domain protein required for expression of DFR and BAN genes in *Arabidopsis* siliques. Plant Cell 12:1863-1878

Park KI, Choi JD, Hoshino A, Morita Y, Iida S (2004) An intragenic tandem duplication in a transcriptional regulatory gene for anthocyanin biosynthesis confers pale-colored flowers and seeds with fine spots in Ipomoea tricolor. Plant J 38:840-849

Quattrocchio F, Wing JF, van der Woude K, Mol JN, Koes R (1998) Analysis of bHLH and MYB domain proteins: species-specific regulatory differences are caused by divergent evolution of target anthocyanin genes. Plant J 13:475-488

Quattrocchio F, Wing J, van der Woude K, Souer E, de Vetten N, Mol J, Koes R (1999) Molecular analysis of the anthocyanin2 gene of petunia and its role in the evolution of flower color. Plant Cell. 11:1433-1144

Reddivari L, Vanamala J, Chintharlapalli S, Safe SH, Miller JC (2007) Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells through activation of caspase-dependent and caspase-independent pathways. Carcinogenesis 28:2227-2235

Rodriguez-Saona LE, Giusti MM, Wrolstad RE (1998) Anthocyanin pigment composition of red-fleshed potatoes. Journal of Food Science 63:458–465

Ross JA, Kasum CM (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 22:19-34

Sakamoto W, Ohmori T, Kageyama K, Miyazaki C, Saito A, Murata M, Noda K, Maekawa M (2001) The Purple leaf (Pl) locus of rice: the Pl(w) allele has a complex organization and includes two genes encoding basic helix-loop-helix proteins involved in anthocyanin biosynthesis. Plant Cell Physiol 42:982-991

Salaman RN (1910) The inheritance of colour and other characters in the potato. J Genet 1:7-46.

Scalbert A, Johnson IT, Saltmarsh M (2005) Polyphenols: antioxidants and beyond. Am J Clin Nutr 81(1 Suppl):215S-217S

Selinger DA, Lisch D, Chandler VL (1998) The maize regulatory gene B-Peru contains a DNA rearrangement that specifies tissue-specific expression through both positive and negative promoter elements. Genetics 149:1125-1138

Spelt C, Quattrocchio F, Mol JN, Koes R (2000) anthocyanin1 of petunia encodes a basic helix-loop-helix protein that directly activates transcription of structural anthocyanin genes. Plant Cell 12:1619-1632

Sweeney MT, Thomson MJ, Pfeil BE, McCouch S (2006) Caught red-handed: Rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. Plant Cell 18:283-94

Sweeney MT, Thomson MJ, Cho YG, Park YJ, Williamson SH, Bustamante CD, McCouch SR (2007) Global dissemination of a single mutation conferring white pericarp in rice. PLoS Genet 3(8):e133

Thompson MD, Thompson HJ, McGinley JN, Neil ES, Rush DK, Holm DG, Stushnoff C (2008) Functional food characteristics of potato cultivars (Solanum tuberosum L.): phytochemical composition and inhibition of 1-methyl-1-nitrosourea induced breast cancer in rats. Journal of Food Composition and Analysis (in press).

Tsuda T, Horio F, Osawa T (2000) The role of anthocyanins as an antioxidant under oxidative stress in rats. Biofactors 13:133–139

Van Eck HJ, Jacobs JME, Van Den Berg PMMM, Stiekema WJ, Jacobsen E (1994)

The inheritance of anthocyanin pigmentation in potato (*Solanum tuberosum* L.) and

mapping of tuber skin colour loci using RFLPs. Heredity 73:410-421.

Van Ooijen JW and Voorrips RE (2001) JoinMap® 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands

Van Ooijen, JW (2004) MapQTL ® 5, software for the zapping of quantitative trait loci in experimental populations. Kyazma B.V., Wageningen, Netherlands

Voorrips, RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. The Journal of heredity 93:77-78

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 21: 4407-4414

CHAPTER 2

THE POTATO R LOCUS CODES FOR DIHYROFLAVONOL-4-REDUCDASE

Abstract

The potato *R* locus is required for the production of red pelargonidin-based anthocyanin pigments in potato (*Solanum tuberosum* L.). Red color also requires tissue-specific regulatory genes, such as *D* (for expression in tuber skin) and *F* (expression in flowers). A related locus, *P*, is required for production of blue/purple anthocyanins; *P* is epistatic to *R*. We have previously reported that the dihydroflavonol 4-reductase gene (*dfr*) co-segregates with *R*. To test directly whether *R* corresponds to *dfr*, we placed the allele of *dfr* associated with red color under the control of the CaMV 35S promoter and introduced it into the potato cultivar Prince Hairy (genotype *dddd rrrr P*---), which has white tubers and pale blue flowers. Transgenic Prince Hairy tubers remained white, but flower color changed to purple. Three independent transgenic lines, as well as a vector-transformed line, were then crossed with the red-skinned variety Chieftain (genotype *D*--- *R*--- *pppp*), to establish populations that segregated for *D*, *R*, *P*, and the *dfr* transgene or empty vector. Markers were used to genotype progeny at *D* and *R*. Progeny carrying the empty vector in the genetic background *D*--- *rrrr* produced white or purple tubers, while progeny with the same genotype and the *dfr* transgene produced red or purple tubers. HPLC and LC-MS/MS analyses of anthocyanins present in Chieftain and in a red-skinned progeny clone with the *dfr* transgene in a *D*--- *rrrr* background revealed no qualitative differences. Thus, *dfr* can fully complement *R*, both in terms of tuber color and anthocyanin composition.

Introduction

The potato *R* locus, first described by Salaman (1910), is required for the production of red anthocyanin pigments in any tissue of a potato plant. The related *P* locus (Salaman 1910) is required for production of purple anthocyanins. *P* has been shown to code for the anthocyanin biosynthetic enzyme flavonoid 3',5'-hydroxylase (Jung et al. 2005). *R* and *P* are, nevertheless, not sufficient for red and purple color, respectively; both require the activity of additional tissue-specific regulatory genes such as Developer (*D*) (Salaman 1910) for anthocyanin production in tuber periderm or *F* for anthocyanin synthesis in flowers (Dodds and Long 1956). *D* is also known as the *I* locus in diploid potato (Dodds and Long 1955, Dodds and Long 1956). *P* is known to be epistatic to *R* (Dodds and Long 1955).

We have previously reported that a specific allele of dihydroflavonol 4-reductase (*dfr*), another anthocyanin biosynthetic gene, was present in every red-skinned or red-flowered potato clone examined (De Jong et al. 2003a), and absent in many, but not all, white potato clones. This result provided strong circumstantial evidence, but did not prove, that *R* codes for *dfr*.

DFR catalyzes an essential reaction in each of the three primary branches of anthocyanin synthesis, reducing dihydrokaempferol (DHK), dihydroquercitin (DHQ), and dihydromyricetin (DHM) en route to the production of red pelargonidin, pink cyanidin, and purple delphinidin-based anthocyanins, respectively (Holton and Cornish 1995). In some genera, like *Gerbera*, DFR is able to efficiently reduce all three substrates (Johnson et al. 2001), while in other genera, like *Petunia* (Forkmann and Ruhnau 1987) and *Cymbidium* (Johnson et al. 1999), DFR can efficiently reduce DHQ and DHM, but not DHK. The precedent of differences in substrate specificity between species provides a possible explanation for how variation between alleles of potato *dfr* might lead to differences in potato color; it suggests that the 'red allele' of

potato DFR is capable of reducing DHK, while other potato DFR alleles cannot (De Jong et al 2003a). Simple RFLP banding patterns suggest that *dfr* is a single copy gene in both potato and tomato (De Jong et al 2003a; Bongue-Bartelsman et al 1994).

To test the relationship between *R* and *dfr* more thoroughly, we report here on the construction and evaluation of transgenic potato lines that express the 'red allele' of *dfr*, and show that this allele is able, in a genetic background that includes *D*, to direct synthesis of red pelargonidin-based anthocyanin pigments in tuber skin.

Materials and Methods

Plant materials

Diploid potato clone 320-02 (kindly provided by H. De Jong, AAFC, Fredericton, NB) and tetraploid potato cultivars Prince Hairy (also known as NYL235-4) (Plaisted et al. 1992) and Chieftain (Weigle et al. 1968) were propagated as sterile plantlets *in vitro*, as well as via tubers in both the greenhouse and field. Transformed plants were maintained in the greenhouse under 14 hr photoperiod and at temperatures of 24 to 28°C during the day and 16 to 18°C at night. Tubers were harvested after the vines had naturally senesced.

Transformation construct

The open reading frame of the potato *dfr* allele that co-segregates with red color (De Jong et al. 2003a) was amplified from diploid W5281.2, which is homozygous *RR*, with primers DFRadaptorF (5′ GCG CCA TGG CAA GTG AAG TTC AT) and DFRadaptorR (5′ CGC GGT ACC CTA GAT TTC ACC ATT GGT). The fourth through ninth nucleotides of primer DFRadaptorF create an *Nco* I restriction site, while the last 18 nucleotides correspond to the first 18 nucleotides of the *dfr* open reading frame. The fourth through ninth nucleotides of primer DFRadaptorR create a

Kpn I site, while the last 18 nucleotides anneal to the final 18 nucleotides of the *dfr* open reading frame. The resulting PCR product was digested with *Nco* I and *Kpn* I, and then cloned into the *Nco* I and *Kpn* I sites of intermediate vector pIBT210.1 (Haq et al. 1995). The *dfr* open reading frame was then transferred, as an *Xho* I – *Kpn* I fragment, into the *Xho* I and *Kpn* I sites of binary vector pPS1 (Huang and Mason 2004). This vector drives expression with a doubled CaMV 35S promoter and carries a tobacco etch virus translational enhancer to further increase transgene expression. Sequencing the transgene construct revealed a single, translationally silent nucleotide change in the open reading frame: the 256th codon of the *dfr* construct is CAT, while the same codon of native *dfr* is CAC; both code for the amino acid histidine. The construct was introduced into *Agrobacterium tumefaciens* strain LBA4404, and then transformed into potato cultivar Prince Hairy as described (Jung et al. 2005).

Crossing

Four transgenic Prince Hairy lines – three transformed with the *dfr* construct, and one transformed with an empty pPS1 vector – were crossed, as females, to Chieftain. Seeds from the four crosses were harvested and planted in the greenhouse the following year. Approximately 200 hundred seeds from each of the three plants transformed with *dfr* and the line transformed with an empty vector were sown.

Genotyping

Genomic DNA was isolated from 353 F_1 progeny of the three crosses to *dfr*-transformed lines and 87 F_1 progeny from the cross to the empty vector-transformed line using a quick extraction method (Edwards et al. 1991). PCR assays were used to genotype progeny at the *D* and *R* loci, and for the presence/absence of the transgene or empty vector. To test for the presence of the native allele of *dfr* associated with red

color, DNA was amplified with primers potDFR1 and potDFR2 and digested with *Bam* HI (De Jong et al. 2003a). The same assay was used to test for the presence of the *dfr* transgene; because the transgene lacks introns, amplification with the same primers yields smaller products. To test for the presence of *D*, DNA was amplified with primers 21BAClaI-F2 (5′ GTG ATT ATG TCA TCC AAA AGT TTA TAG) and 21BAClaI-R1 (5′ GAA TTT CTG AGG TTG AGG TCT TA) and digested with *Cla* I; these primers amplify part of an R2R3MYB gene tightly linked to *D* (Jung and De Jong, unpublished). To test for the presence of the empty vector, DNA was amplified with primers pPS13162F (5′ CGA ATC TCA AGC AAT CAA GCA) and pPS13443R (5′ CGT AGG TAC GTG GAG TGT CTT); these flank the cloning sites of pPS1 and amplify a 282 bp product from an empty vector. PCR products were visualized on a 2% agarose gel. A total of 293 plants from all four crosses were selected based on genotype data generated by these markers. These were grown to maturity, when tuber color was recorded.

Anthocyanin extraction

One tenth of a gram of tuber periderm tissue was frozen in liquid nitrogen and ground to a fine powder. Anthocyanins were extracted using one ml of 1% (v/v) HCl in methanol for five minutes. The homogenate was centrifuged for 10 min at 12,000 g. The supernatant was filtered through a 0.45 µm regenerated cellulose syringe filter (Grace, Deerfield, IL) prior to HPLC analysis.

HPLC analysis

Anthocyanin compounds were analyzed using a HP1100 Liquid Chromatograph equipped with a diode array detector (DAD) (Agilent Technology, Palo Alto, CA, USA). An Inertsil ODS-3 column (5.0 µm particle size, 4.6 mm × 250 mm, GL

Sciences Inc., Tokyo, Japan) was used in the separation, preceded by an Inertsil ODS-3 Guard Column (5.0 μm, 4.0 mm × 10 mm). Five μl of filtered supernatant was injected for HPLC. A binary gradient of a mixture of water-acetonitrile-formic acid was used. Solvent A was 10% (v/v) formic acid in water and solvent B was 10% (v/v) formic acid in acetonitrile. The gradient for HPLC analysis was linearly changed as follows (total 75 min): 95% A (0 min), 85% A (25 min), 78% A (42 min), 64% A (60 min), 95% A (65 min). Post run-time was 10 min. Flow rate was 1.0 ml/min at 30°C. Anthocyanins of each sample were compared by their UV-Vis spectra and retention times. A DAD chromatogram was generated using 520 nm as the detection wavelength.

LC/MS analysis

HPLC-MS/MS analyses on a Quantum Access triple quadrupole system (Thermo Finnigan LLC, San Jose, CA) was used to confirm anthocyanin identity. Anthocyanin extracts were separated on a ThermoFisher Accela HPLC equipped with a Gemini C18 reversed phase column (3μm, 150 x 4.6 mm, Phenomenex, Torrance, CA) using a solvent gradient (solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in acetonitrile): 0-4 min 5% B, 24 min 60% B; 34 min 95% B, 40 min 95% B at a flow rate of 0.7 mL min^{-1}. The MS detector was equipped with an electrospray ionization (ESI) probe operated under the following conditions: spray voltage 4.5 kV, capillary temperature 300°C, sheath gas (N_2) pressure 30 arbitrary units, auxiliary gas (N_2) pressure 55 arbitrary units. Mass spectra were recorded in positive mode between *m/z* 100 and *m/z* 1200 to determine molecular ions $[M+H]^+$. Aglycone and glycosylation pattern were analyzed by collision-induced dissociation (CID energy 30 V, CID gas (Ar) pressure 1.5 mTorr) of selected molecular ions and compared with reported anthocyanin fragmentation data (Eichhorn and Winterhalter 2005).

Results

To test if the allele of *dfr* associated with red color corresponds to *R*, we introduced it, under the control of a doubled CaMV 35S promoter and tobacco etch virus translational enhancer, into the potato cultivar Prince Hairy. Wild type Prince Hairy produces white tubers and pale blue flowers (Figure 1). The blue flower color indicates that Prince Hairy must have a functional allele at the *P* locus (genotype *P*---). In addition, because Prince Hairy tubers are white, this cultivar must lack a functional allele at *D* (genotype *dddd*). It has previously been shown that Prince Hairy lacks the allele of *dfr* associated with red color (De Jong et al. 2003a). We would have preferred to introduce the red allele of *dfr* directly into a white potato with genotype *D--- rrrr pppp*, to test if the transformation would change skin color from white to red, but did not have such a genotype available. Thus an indirect approach, one that required subsequent crossing, was used in this study instead.

If *dfr* corresponds to *R*, we anticipated that flower color would change in transgenic Prince Hairy, from light blue to purple, as the flowers would produce a mixture of blue and red pigments. Indeed, of the 12 independent transgenic Prince Hairy lines generated, 10 exhibited purple flowers (Figure 1). Flower color in the remaining two transgenic lines was unchanged. None of the seven independent Prince Hairy lines transformed with an empty vector developed purple flowers; their flowers all remained pale blue. Tubers of all transgenic Prince Hairy lines, whether transformed with *dfr* or an empty vector, were invariably white (Figure 1).

To determine how the red allele of *dfr* would interact with *D*, three independent transgenic Prince Hairy lines, all with purple flowers, were crossed (as females) with the red skinned cultivar Chieftain, which is known to be simplex at *R* (genotype *D--- Rrrr pppp*) (De Jong et al. 2003b). We anticipated that the progeny of these crosses would segregate for native *R* as well as the *dfr* transgene, and would likely segregate

Figure 1. Tubers and flowers of the cultivar Prince Hairy, before and after transformation with the ‘red allele’ of potato dihydroflavonol 4-reductase.


Prince Hairy
Prince Hairy + 35S::dfr

for *D* and *P* as well. The progeny of most interest would be those that carried the transgene in a genetic background of *D--- rrrr pppp*, as their phenotype would allow us to determine if *dfr* could substitute for *R*. As a control, a Prince Hairy line transformed with an empty vector was also crossed with Chieftain.

Progeny of these three crosses were genotyped with PCR assays to assess presence of *R*, presence of the transgene or empty vector, and presence of an R2R3 MYB gene tightly linked to *D* (see Methods). All eight possible combinations of these loci were observed. The phenotype(s) observed for each genotypic class are summarized in Figure 2. Because the three crosses with lines carrying the *dfr* transgene yielded similar results (not shown), data from these three crosses were pooled in Figure 2C. Note that the *P* locus was not genotyped in this study.

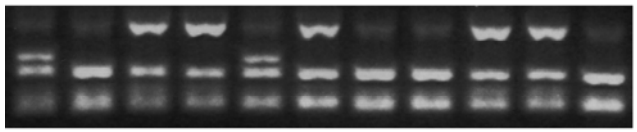
As expected, all progeny lacking the marker tightly linked to *D* produced white tubers, reflecting the requirement of *D* for red or purple tuber skin. Progeny with this marker were red, purple, or white, presumably reflecting offspring that contained *R* but not *P*, *P* with or without *R*, and neither *R* nor *P*, respectively (Figure 2). The genotype of most interest was that which contained *D* and the transgene, but lacked *R*. If *dfr* corresponds to *R*, segregants with this genotype should be red or purple, but never white. Of 62 clones with this genotype, 28 red- and 34 purple-skinned clones were observed, and no clone produced white tubers (Figure 2C). Of the six progeny that carried *D* and the empty vector, but lacked native *R*, four produced purple tubers, and two produced white tubers; none produced red tubers (Figure 2D). Thus, the allele of *dfr* that co-segregates with *R*, but not an empty vector, can complement *R*.

Since *D* appeared to segregate in a 5:1 ratio when the data from all four crosses were considered together (239 colored:54 white, χ^2= 0.66, P= 0.42), Chieftain appears to be duplex for *D* (i.e., genotype *DDdd*). Similarly, since the skin color of all progeny

Figure 2. Predicted and observed tuber color, sorted by genotype, in F_1 progeny of crosses between transgenic Prince Hairy and Chieftain. **A** and **B** illustrate the PCR assays used to genotype at *R*/*dfr* and *D*, respectively. **C** and **D** show predicted and observed tuber color for each genotype, where the transgene is the 'red allele' of *dfr* in **C**, and an empty vector in **D**. Representative tubers for each genotype are shown on the right side of panels **C** and **D**.

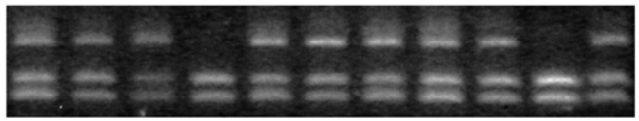
A

genotyping at *R/dfr*



B

genotyping at *D*



C

predicted / observed tuber color; transgene (*T*) = *dfr*

genotype (+/- *P*)	number harvested	predicted color(s)	observed color(s)
D--- R--- T---	56 (28 red, 28 purple)	red or purple, no white	
D--- rrrr T---	62 (28 red, 34 purple)	red or purple, no white	
D--- R--- tttt	39 (19 red, 20 purple)	red or purple, no white	
D--- rrrr tttt	41 (17 white, 24 purple)	white or purple, no red	
dddd R--- T---	4 (4 white)	all white	
dddd rrrr T---	22 (22 white)	all white	
dddd R--- tttt	5 (5 white)	all white	
dddd rrrr tttt	10 (10 white)	all white	

D

predicted / observed tuber color; transgene = empty vector

genotype (+/- *P*)	number harvested	predicted color(s)	observed color(s)
D--- R--- T---	16 (5 red, 11 purple)	red or purple, no white	
D--- rrrr T---	6 (2 white, 4 purple)	white or purple, no red	
D--- R--- tttt	12 (2 red, 10 purple)	red or purple, no white	
D--- rrrr tttt	7 (1 white, 6 purple)	white or purple, no red	
dddd R--- T---	1 (1 white)	all white	
dddd rrrr T---	5 (5 white)	all white	
dddd R--- tttt	4 (4 white)	all white	
dddd rrrr tttt	3 (3 white)	all white	

combined with *D* and native *R* segregated in an apparent 1:1 ratio (54 red: 69 purple, χ^2= 1.83, P= 0.18), Prince Hairy appears to be simplex at the *P* locus.

To determine if the anthocyanins produced in red untransformed tubers with genotype *D*--- *R*--- were comparable in composition to those produced in red tubers expressing the *dfr* transgene, pigment profiles were compared by HPLC. As shown, the red-skinned variety Chieftain and a transgenic progeny clone with genetic background *D*--- *rrrr* (i.e., lacking native *R*) produced indistinguishable pigment profiles (Figure 3). Both were clearly distinct from the white-skinned variety Prince Hairy and a white-skinned progeny clone transformed with an empty vector, neither of which produced detectable anthocyanin (Figure 3).

Additional characterization of tuber skin pigments by LC-MS/MS also revealed no substantial differences between Chieftain and *dfr*-transgenic (*D*--- *rrrr*) tubers (Figure 4 and Table 1). In both cases, based on the pattern of molecular mass and fragment ions, and comparison to previous potato analyses (Eichhorn and Winterhalter 2005, Naito et al. 1998, Rodriguez-Saona et al. 1998), the primary pigments were pelargonidin-3-coumaryl-rutinoside-5-glucoside, pelargonidin-3-feruloyl-rutinoside-5-glucoside, and pelargonidin-3-rutinoside-5-glucoside (Table 1). Lesser quantities of three additional anthocyanins, where peonidin took the place of pelargonidin, were also observed (Table 1). The identity of the individual anthocyanins in the two extracts was verified by comparing the $[M+H]^+$ and fragmentation patterns. Prince Hairy and empty vector-transformed Prince Hairy produced no anthocyanins detectable by our LC-MS/MS analysis (Table 1).

Figure 3. HPLC analysis of pigments in the varieties Chieftain, Prince Hairy, and progeny of a cross between Chieftain and transgenic Prince Hairy, where the transgene is either the 'red allele' of *dfr* or an empty vector control.

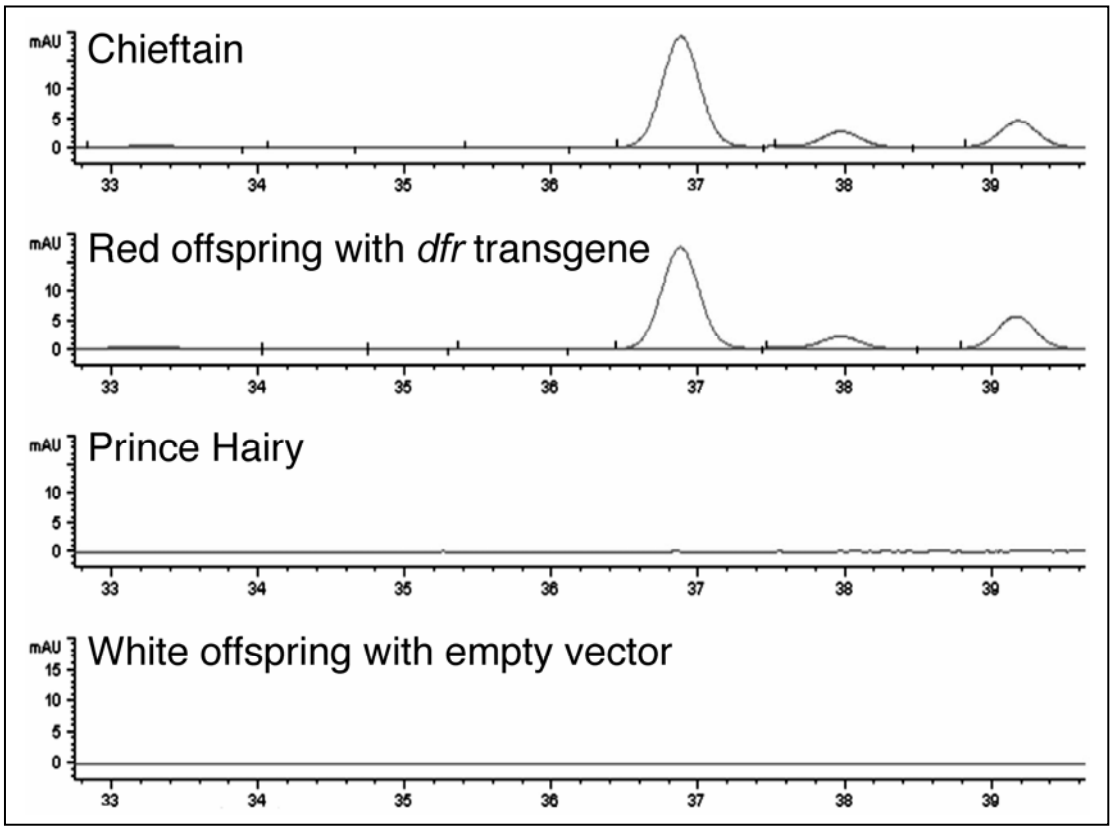

mAU
Chieftain
10
5
0
33
34
35
36
37
38
39
mAU
Red offspring with *dfr* transgene
10
5
0
33
34
35
36
37
38
39
mAU
Prince Hairy
10
5
0
33
34
35
36
37
38
39
mAU
White offspring with empty vector
15
10
5
0
33
34
35
36
37
38
39

Figure 4. LC-MS analysis of anthocyanins produced by transgenic and natural *dfr* alleles. **A** illustrates the mass spectra of anthocyanins from a plant carrying the *dfr* transgene in a genetic background of D---rrrr at the transgene at retention time 32.6 min, and **B** illustrates mass spectra from a naturally red potato (genotype D---R---), also at retention time 32.6 min.

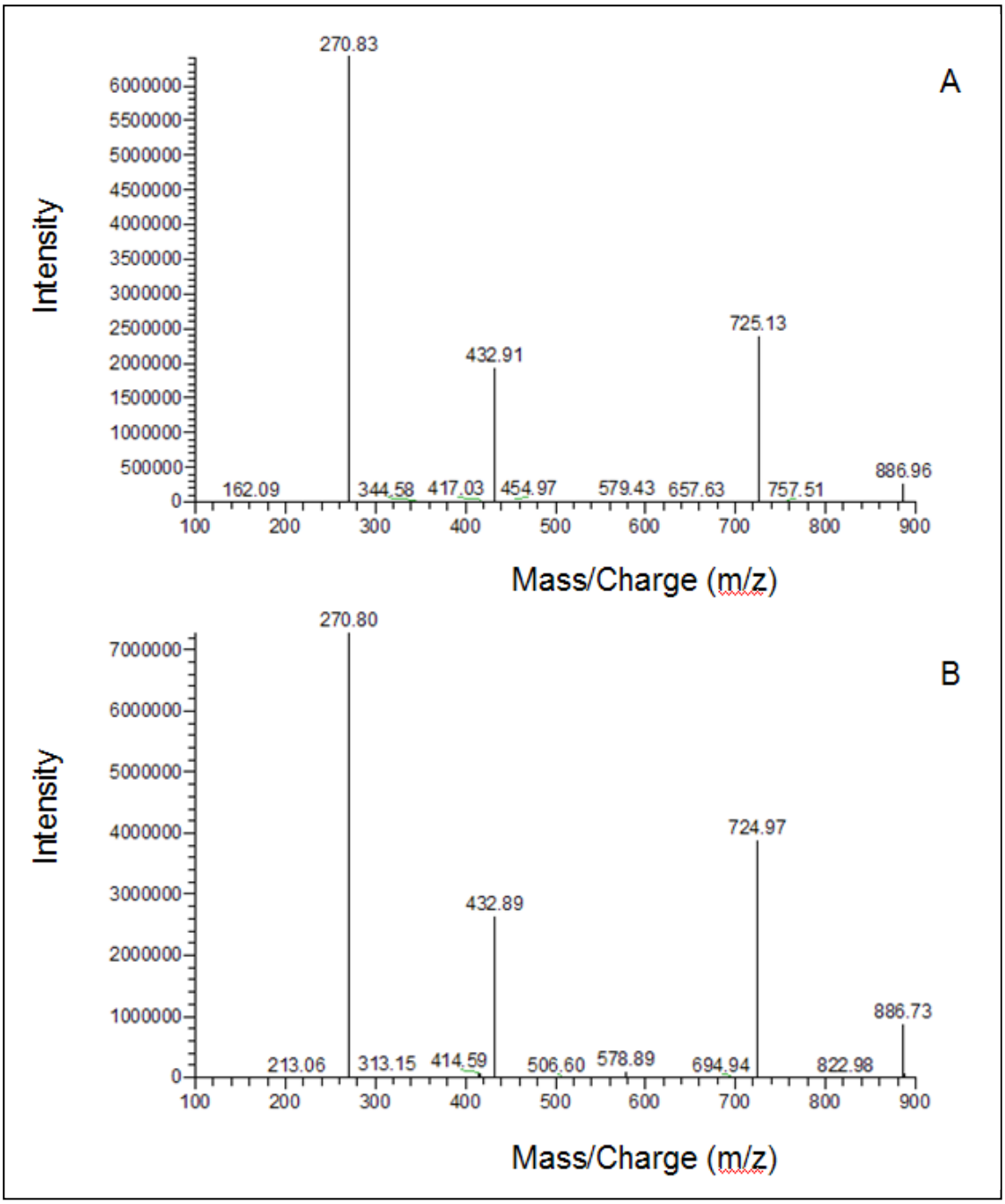

A
Intensity
270.83
725.13
432.91
886.96
162.09
344.58
417.03
454.97
579.43
657.63
757.51
Mass/Charge (m/z)
B
Intensity
270.80
724.97
432.89
886.73
213.06
313.15
414.59
506.60
578.89
694.94
822.98
Mass/Charge (m/z)

Table 1. Anthocyanins detected in tubers by LC-MS/MS

#	R_t [min]	inferred compound	$[M+H]^+$ [*m/z*]	fragment ions [*m/z*]	relative amount [% peak area]			
					Chieftain	Prince Hairy	dfr-transgenic clone	empty vector
1	9.8	pel-3-rut-5-glc	741	579, 433, 271	11	nd[a]	16	nd
2	10	peo-3-rut-5-glc	771	609, 463, 301	2	nd	2	nd
3	14	pel-3-coum-rut-5-glc	887	725, 433, 271	59	nd	53	nd
4	14.1	peo-3-coum-rut-5-glc	917	755, 463, 301	6	nd	4	nd
5	14.2	pel-3-ferul-rut-5-glc	917	755, 433, 271	17	nd	19	nd
6	14.4	peo-3-ferul-rut-5-glc	947	785, 463, 301	5	nd	6	nd

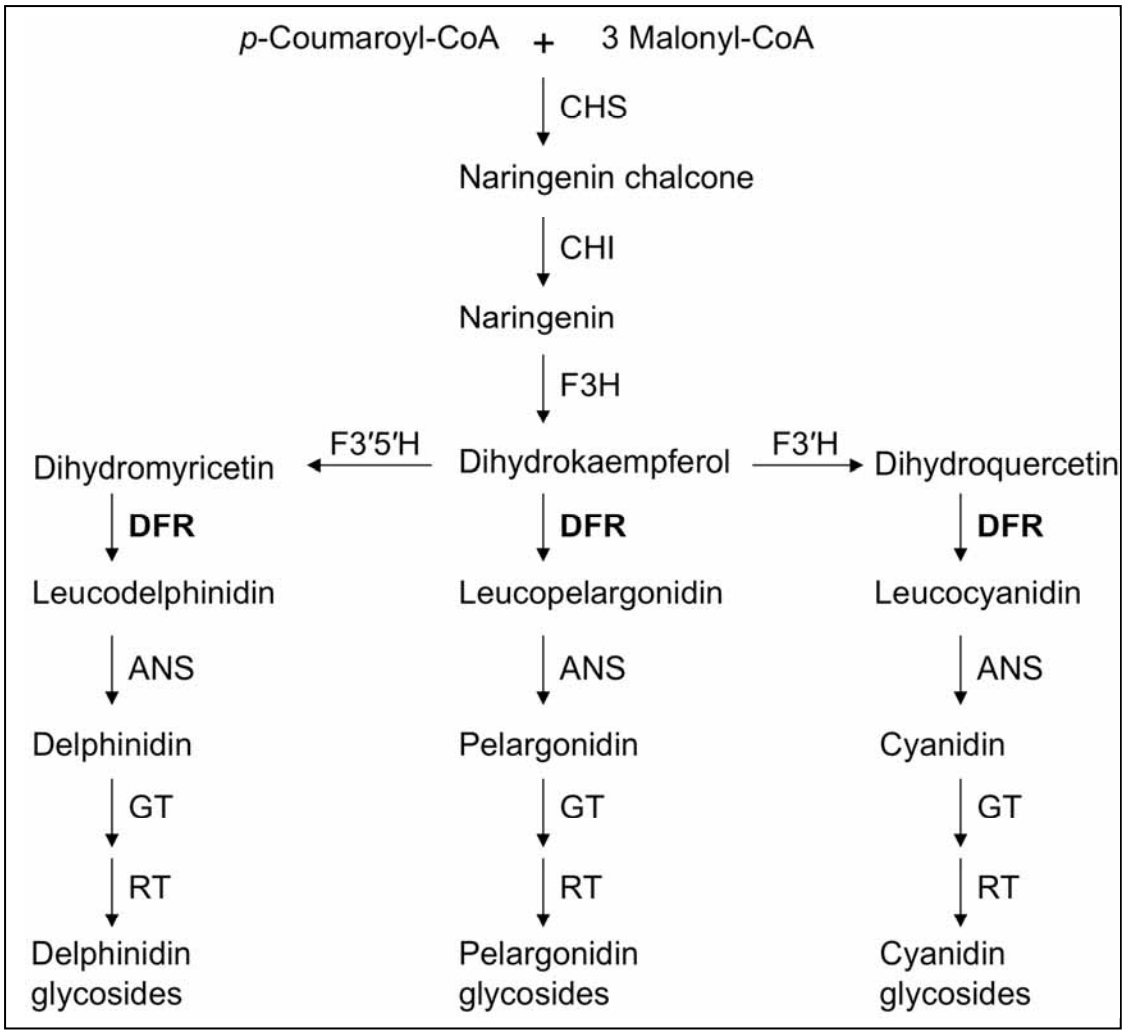
Discussion

We have provided direct evidence that the potato *R* locus codes for dihydroflavonol 4-reductase, as the allele of *dfr* previously shown to co-segregate with the dominant allele at the *R* locus (De Jong et al. 2003a) can transgenically complement *R* with respect to both tuber color and anthocyanin composition.

More than one anthocyanin was observed in both Chieftain and *dfr*-transformed potato skin (Figure 3, Table 1), consistent with previous studies that have also reported more than one anthocyanin present in red-skinned or red-fleshed tubers (Lewis et al. 1998; Naito et al. 1998; Eichhorn and Winterhalter 2005). DFR is required for the production of red, pink and purple anthocyanins in plants (Holton and Cornish 1995; Figure 5). It is worth noting that while only one allele of potato *dfr* appears able to direct synthesis of red anthocyanins, both it and other allele(s) of potato *dfr* can support production of purple anthocyanins. As shown in Figure 2, many potato genotypes without *R* or the *dfr* transgene produced purple-skinned tubers. Similarly, potatoes that are homozygous at *R*, like purple-skinned diploid W5281.2 (De Jong and Burns 1993), can produce purple anthocyanins in the presence of *P*. Thus the key difference between 'red' and 'not-red' alleles of *dfr* does not appear to be an issue of enzyme functionality *per se*, as both types of alleles code for catalytically-active enzymes.

In order for a plant to produce red pelargonidin-based anthocyanins, DFR must be able to reduce DHK, while reduction of DHM is required to produce purple delphinidin or petunidin-derived compounds (Figure 5). Thus, the enzyme encoded by the red allele of potato *dfr* must be capable of reducing both DHK and DHM, while

Figure 5. Generalized anthocyanin biosynthetic pathway (adapted from Holton and Cornish 1995), illustrating the various chemical reactions that DFR can, in principle, catalyze en route to producing red (pelargonidin-based), pink (cyanidin-based) and purple (delphinidin-based) anthocyanin pigments. Enzyme abbreviations: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3´H, flavonoid 3'-hydroxylase; F3´5´H, flavonoid 3´,5´-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; GT, UDP-glucose anthocyanidin 3-O-glucosyltransferase; RT, UDP-rhamnose anthocyanidin-3-glucoside rhamnosyltransferase.


p-Coumaroyl-CoA + 3 Malonyl-CoA
CHS
Naringenin chalcone
CHI
Naringenin
F3H
F3′5′H
F3′H
Dihydromyricetin
Dihydrokaempferol
Dihydroquercetin
DFR
DFR
DFR
Leucodelphinidin
Leucopelargonidin
Leucocyanidin
ANS
ANS
ANS
Delphinidin
Pelargonidin
Cyanidin
GT
GT
GT
RT
RT
RT
Delphinidin glycosides
Pelargonidin glycosides
Cyanidin glycosides

enzymes coded by other potato allele(s) can reduce DHM, but not DHK. The peonidin derivatives observed in Chieftain and red-skinned transgenic progeny clones (Table 1) suggest that the enzyme coded by the 'red allele' of *dfr* can also utilize DHQ as a substrate, since peonidin is simply methylated cyanidin.

Transgenic expression of *dfr* to alter color of economically important plant tissue was first reported in *Petunia* (Meyer et al. 1987), and has since been reported in other floral crops such as *Torenia fournieri* (Aida et al. 2000), rose (Katsumoto et al. 2007) and *Osteospermum hybrida* (Seitz et al. 2007). In a suitable genetic background, ***i.e.***, one that contains a functional allele at *D* and lacks a functional allele at *P*, it should be possible to convert a white-skinned potato into a red-skinned one. Of relevance to applied breeding, the red allele of *dfr* did not appear to be silenced after sexual hybridization, as all progeny carrying *D* and the transgene were red or purple, and never white (Figure 2).

It should be possible, through reciprocal exchange of fragments between 'red' and 'not red' alleles of potato *dfr*, to precisely delineate the amino acids that permit the 'red allele' to utilize DHK as a substrate. Comparable experiments, exchanging portions of *dfr* between *Gerbera* and *Petunia*, and subsequent site-directed mutagenesis (Johnson et al. 2001), have implicated a single amino acid, corresponding to potato amino acid position 145, in the ability to utilize DHK. Potato amino acid 145 does not differ between 'red' and 'not red' alleles, although potato positions 143 and 154 are polymorphic (De Jong et al. 2003a). The crystal structure of grape DFR has recently been described (Petit et al. 2007). Thus, if the potato amino acid changes that influence substrate specificity can be identified, their role in influencing DFR activity could be interpreted at high resolution.

ACKNOWLEDGEMENTS

We thank Drs. Lailiang Cheng and Pengmin Li for help with HPLC.

REFERENCES

Aida R, Kishimoto S, Tanaka Y, Shibata M (2000) Modification of flower color in torenia (*Torenia fournieri* Lind.) by genetic transformation. Plant Sci 153: 33–42

Bongue-Bartelsman M, O'Neill SD, Tong Y, Yoder JI (1994) Characterization of the gene encoding dihydroflavonol 4-reductase in tomato. Gene 138: 153-157

De Jong H, Burns VJ (1993) Inheritance of tuber shape in cultivated diploid potatoes. Amer Pot J 70: 267–283

De Jong WS, De Jong DM, De Jong H, Kalazich J, Bodis M (2003a) An allele of dihydroflavonol 4-reductase associated with the ability to produce red anthocyanin pigments in potato (*Solanum tuberosum* L.). Theor Appl Genet 107: 1375–1383

De Jong WS, De Jong DM, Bodis M (2003b) A fluorogenic 5' nuclease (TaqMan) assay to assess dosage of a marker tightly linked to red skin color in autotetraploid potato. Theor Appl Genet 107: 1384–90

Dodds KS, Long DH (1955) The inheritance of colour in diploid potatoes: types of anthocyanidins and their genetic loci. J Genetics 53: 136–149

Dodds KS, Long DH (1956) The inheritance of colour in diploid potatoes: II. A three-factor linkage group. J Genetics 54: 27–41

Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19: 1349

Eichhorn S, Winterhalter P (2005) Anthocyanins from pigmented potato (*Solanum tuberosum* L.) varieties. Food Res Int 38: 943–948

Forkmann G, Ruhnau B (1987) Distinct substrate specificity of dihydroflavonol 4-reductase from flowers of *Petunia hybrida*. Z Naturforsch 42c: 1146–1148

Haq TA, Mason HS, Clements JD, Arntzen CJ (1995) Oral immunization with a recombinant bacterial antigen produced in transgenic plants. Science 268: 714–716

Holton TA, Cornish EC (1995) Genetics and biochemistry of anthocyanin biosynthesis. Plant Cell 7: 1071–1083

Huang Z, Mason HS (2004) Conformational analysis of hepatitis B surface antigen fusions in an Agrobacterium-mediated transient expression system. Plant Biotechnol J 2: 241–249

Johnson ET, Yi H, Shin B, Oh B-J, Cheong H, Choi G (1999) *Cymbidium hybrida* dihydroflavonol 4-reductase does not efficiently reduce dihydrokaempferol to produce orange pelargonidin-type anthocyanins. Plant J 19: 81–85

Johnson ET, Ryu S, Yi H, Shin B, Cheong H, Choi G (2001) Alteration of a single amino-acid changes the substrate specificity of dihydroflavonol 4-reductase. Plant J 25: 325–333

Jung CS, Griffiths HM, De Jong DM, Cheng S, Bodis M, De Jong WS (2005) The potato P locus codes for flavonoid 3',5'-hydroxylase. Theor Appl Genet 110: 269–275

Katsumoto Y, Fukuchi-Mizutani M, Fukui Y, Brugliera F, Holton TA, Karan M, Nakamura N, Yonekura-Sakakibara K, Togami J, Pigeaire A, Tao G-Q, Nehra NS, Lu C-Y, Dyson BK, Tsuda S, Ashikari T, Kusumi T, Mason JG, Tanaka Y (2007) Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. Plant Cell Physiol 48: 1589-1600

Lewis CE, Walker JRL, Lancaster, JE, Sutton KH (1998) Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L. J Sci Food Agr 77: 45–57

Meyer P, Heidmann I, Forkmann G, Saedler H (1987) A new petunia flower colour generated by transformation of a mutant with a maize gene. Nature 30: 677–678

Naito K, Umemura Y, Mori M, Sumida T, Okada T, Takamatsu N, Okawa Y, Hayashi K, Saito N, Honda T (1998) Acylated pelargonidin glycosides from a red potato. Phytochemistry 47: 109–112

Petit P, Granier T, d'Estaintot BL, Manigand C, Bathany K, Schmitter JM, Lauvergeat V, Hamdi S, Gallois B (2007) Crystal structure of grape dihydroflavonol 4-reductase, a key enzyme in flavonoid biosynthesis. J Mol Biol 368: 1345–1357

Plaisted RL, Tingey WM, Steffens JC (1992). The germplasm release of NYL 235-4, a clone with resistance to the Colorado potato beetle. Amer Pot J 69: 843–846

Rodriguez-Saona LE, Giusti MM, Wrolstad RE (1998) Anthocyanin pigment composition of red-fleshed potatoes. J Food Sci 63: 458–465

Salaman RN (1910) The inheritance of colour and other characters in the potato. Journal of Genetics 1: 7–46

Seitz C, Vitten M, Steinbach P, Hartl S, Hirsche J, Rathje W, Treutter D, Forkmann G (2007) Redirection of anthocyanin synthesis in *Osteospermum hybrida* by a two-enzyme manipulation strategy. Phytochemistry 68: 824–833

Weigle JL, Kehr AE, Akeley RV, Horton JC (1968) Chieftain: a red-skinned potato with attractive appearance and broad adaptability. Amer Pot J 45: 293–296

CHAPTER 3

GENETIC AND HISTOLOGICAL ANALYSIS OF POTATO TUBER SHAPE

Abstract

Potato shape is an important consideration in both marketing and breeding. Prior genetic studies have shown that a single gene, *Ro*, on chromosome 10, controls most variation for round versus long tuber shape. To characterize the genetics of tuber shape more thoroughly, an F_1 population of 228 individuals from a cross between long-shaped diploid 10618-01 and round-shaped diploid 320-02 was evaluated with AFLP, SSR, and CAPS markers. In addition to confirming the known *Ro* locus on chromosome 10, which explained 55% of the variation for ratio of tuber length to width, we identified a novel QTL, *ts*5.1, which explained 8.9% of the variation. CAPS marker CT217 was found to be tightly linked with *Ro* in the diploid population. Evaluation of a panel of 84 tetraploid potato varieties with CT217 revealed a banding pattern highly correlated with long tuber shape. This marker may thus prove useful in applied potato breeding. An anatomical comparison of round versus long tubers revealed that cell shape is similar in both, indicating that long tuber shape results from an increase in cell number, rather than an increase in cell length, along the longitudinal axis.

Introduction

Potato tuber shape is an important trait for marketing and breeding, as different end products require different shapes of tubers. The chipping industry, for example, prefers round tubers, while the ideal tuber for French fries is long. Modern cultivars are generally round, oblong or long, while the shape of primitive varieties exhibits much more diversity, including round, ovate, tapered, rectangular, kidney, sickle, coiled and stick-like shapes (Glendinning 1983). The genetics of potato tuber shape are nevertheless not well understood. Almost a century ago Salaman (1910) proposed that tuber shape is controlled by a Mendelian locus and that long tubers are dominant to round. A subsequent study also reported that long tuber shape is dominant to short, but not completely, with modifier genes involved (Black 1930). Bartosch et al (1930) and Huber et al (1930) suggested that tuber shape was controlled by three or four genes. De Jong (1972) and Taylor (1978) reported that tuber shape is controlled by one major gene in diploid and tetraploid germplasm, respectively, with round dominant over long. Masson (1985) proposed the symbol *Ro* for this gene, which was subsequently mapped, in a small diploid F_1 population of 50 clones, to chromosome 10 (Van Eck et al. 1994). A recent association study implicated 3 loci, on chromosomes 2, 4 and 11, that influence round versus long tuber shape (D'hoop et al 2008).

In contrast to potato tubers, the genetics of solanaceous fruit shape have been relatively well characterized (reviewed in Tanksley 2004, Paran and Knaap 2007). Three major tomato fruit shape QTLs (*fs*7.1, *fs*8.1, and *fs*8.2) have been identified (Ku et al 1999, Glandino et al 1999, van der Knaap et al 2001). A weak fruit shape QTL has also been reported on tomato chromosome 10 (Ku et al 1999). The tomato *sun* locus, which underlies *fs*7.1 (Van der Knaap et al 2001), was recently cloned (Han et

al 2008), and encodes a putative calcium-binding protein. The tomato *ovate* gene, which maps to chromosome 2 (Ku et al 1999), controls ovate versus round fruit shape and codes for a VWFC domain, suggesting that it might impact shape by modulating cell division (Liu et al 2002). In pepper, QTLs that control fruit shape have also been identified, including *fs*3.1 (Ben Chaim et al 2001; Ben Chaim et al 2003a; Rao et al 2003), *fs*2.1 and *fs*4.1 (Zygier et al 2005), and *fs*10.1 (Ben Chaim et al 2003b). Based on map location, the pepper *fs*10.1 locus may represent an ortholog of potato *Ro* (Ben Chaim et al 2003b).

To provide additional understanding of the genetics and cellular basis of tuber shape, we report here on the characterization of a large (n = 228) diploid population that segregates for long versus round tuber shape. Several loci of small effect, in addition to the large-effect *Ro* locus, were detected. An anatomical evaluation of long and round potato tubers revealed that long potatoes have more cells than round potatoes along the longitudinal axis. A chromosome 10 marker correlated with long tuber shape in cultivated germplasm is also described.

Materials and methods

Plant materials: Round-shaped diploid potato clone 320-02 was crossed as the pollen donor to long-shaped diploid potato clone 10618-01. The male parent 320-02 was previously shown to be heterozygous at *Ro* (De Jong and Burns 1993), while 10618-01 is homozygous recessive. Two hundred twenty-eight seedlings from this cross were grown in the greenhouse in 2006 under a 14 hr photoperiod and temperatures of 24-28 C during the day and 16 C at night. Tubers were harvested after plants senesced naturally. Two hundred and fourteen of these clones were grown in 2007.

Phenotypic data: The maximum longitudinal diameter (length) and maximum equatorial diameter (width) of the four largest tubers for each genotype was measured each year. After genotyping, two clones appeared to be identical; one of them was excluded from subsequent analysis. Thirteen clones died or did not tuberize in 2006, thus tuber shape data was based on the measurement of 214 clones that year. Length to width ratios were calculated for each tuber; a tuber shape index was then calculated by averaging the four ratios obtained for each genotype. Length and width measurements were repeated in 2007. Fifteen more clones died or did not tuberize well in 2007, thus one hundred and ninety nine clones were measured that year.

Anatomical analysis: Portions of mature tubers of 320-02 and 10618-01, as well as tubers of the long cultivar 'Russet Burbank' and round cultivar 'Atlantic', were sectioned into slices 0.1 mm in thickness and 4 x 8 mm in area. Two slices were taken from each of three locations in each tuber: 0.2 cm from the apical end, 0.2 cm from the stolon end, and at the equatorial center. Two tubers were examined for each genotype. Slices were stained with 1% Safranin and then photographed under 200X magnification. Cell width and length of 60 cells of each slice, aligned linearly from the apical end to the stolon end, were measured, and ratio of cell length/width was used to calculate a cell shape index. Cell shape indices were compared using Student's t-test.

AFLP analysis: DNA was extracted using a Qiagen kit following the manufacturers' instructions. AFLP markers (Vos et al 1995) were generated with 14 Pst/Mse and 10 Eco/Mse AFLP primer combinations, using ^{33}P-labeled *Pst* I or *Eco*RI primers. After PCR, samples were electrophoresed through a denaturing 5% acrylamide gel. A DNA size standard was run along with samples. AFLP banding patterns were visualized by

exposing film against a dried gel. Polymorphic AFLP markers were scored for mapping purposes.

Anchor markers: Thirteen polymorphic SSR (Milbourne et al 1998; Feingold et al 2005), as well as eight CAPS markers based on known genes (Solanaceae Genomics Network; De Jong et al 2004; Han et al 2008, Zhang et al submitted) were also used to screen the diploid population, to create anchor points for orienting linkage maps. Six other CAPS markers developed in the course of this project are listed in Table 1.

Mapping: JoinMap 3.0 (Van Ooijen and Voorrips 2001) was used to construct separate male and female linkage maps, using only markers that segregated in the progeny and were present in one parent but not the other (*i.e.*, 1:1 markers). The Kosambi function was used to determine genetic distance between markers (Kosambi 1943).

QTL analysis: MapQTL 5 (Van Ooijen 2004) was used for QTL analyses. Data was analyzed by Interval Mapping (IM). Empirical LOD thresholds for $P < 0.05$ were determined by permuting the data 1000 times. QTLs were visualized with MapChart 5 (Voorrips 2002). QTL analysis was performed separately with tuber shape data from years 2006 and 2007.

Interactive QTL marker analysis: Interactions (epistases) among the marker loci were tested using Qgene 3.0 software (Nelson 1997). Three cases of interactions were investigated: interactions among the three major QTLs detected in this study through tests for main effects; interactions between the three major QTLs and seven other loci that were shown to be significant at a low significance threshold level ($\alpha = 0.01$ for

Table 1: Primers and chromosome location of PCR markers developed in this study

Marker	Approx product sizes (bp) after digestion	Enzyme	Chromosome	Primer sequence
CT217	500/450	*Alu* I	10	F: CTG CCC ACC CAT CAT TTT CTT
				R: TGA AGG AAC AAT AAC AGT ACG CAT C
CAPS 01990	1400/650/600	*Rsa* I	10	F: CAA ACC TTC CTA TCA AGG TTC CTA ACA
				R: GGC CTC ATC TTT TGT CTT TAC CAA ATG
CT203	610/350	*Alu* I	10	F: AGT GAC GAT GAT GAC AGA GGA GAA
				R: AAA TGG ACT AAA GCA TAT AGC CGG
poSUN	900/650	*Mse* I	10	F: CTA GCA AGA CTG GTT GTT TAA TGT TAC C
				R: AGC ACT TTG GAT TCT AAT AGC AGC
21BA	470/440[a]	none	10	F: GTG ATT ATG TCA TCC AAA AGT TTA TAG
				R: GAA TTT CTG AGG TTG AGG TCT TA
chs	800/700	*Alu* I	5	F: GCG ACT CCT TCG AAC TGT G
				R: TGA AGT TTT TCG GGC TTT AGG C

[a] 21BA PCR products were polymorphic in size and did not require digestion

Kruskal-Wallis or LOD = 1.95 for interval mapping) or only significant in one of two years; and interactions among these seven weak loci. These ten loci were then checked for interaction, in an experiment-wise manner, against markers in the rest of the genome. Statistical significance for detection of QTLs by interaction is determined by the frequency (α) of false positive associations that we are willing to accept, and α = 0.05 was chosen as an acceptable overall (genome-wise) error rate. Only those cases of epistasis where the interaction was significant at $P \leq 0.01$ are reported.

Verification of major shape QTL marker with commercial varieties: a group of 84 varieties varying in tuber shape were used to test whether marker CT217 can predict tuber shape. Forty-six long-shaped cultivars and breeding clones with shape index equal to or greater than 1.5 were evaluated: A02089-3, A0008-1TF, A00324-1LB, A00646-4, A00727-1, A0082-6, A01010-1, A01025-4, A01124-3, A01235-33LB, A02060-3TE, A02062-1TE, A02507-2LB, A03005-2, A03077-1, A03103-3, A03293-2, A03988-2, A88338-1, A95109-1, A95409-1, A96104-2, A96814-65LB, A97066-42LB, A98134-2, A98289-1, A98345-1, A98374-1, AOA95154-1, AOA95155-7, Alturas, Blazer Russet, Bannock Russet, Carola, Defender, German Butterball, Highland Russet, La Ratte, Magic Molly, NY99, PAO3NM5-1, Premier Russet, Ranger Russet, Russet Burbank, Russet Norkotah, and Stampede Russet. Thirty-eight cultivars or breeding lines with a tuber shape index of less than 1.5 were also evaluated: A02463-101, Adirondack Blue, Adirondack Red, Andover, Atlantic, Bake-King, Bintje, Chieftain, Chippewa, Elba, Eva, IdaRose, Katahdin, Kennebec, Keuka Gold, Marcy, Nordonna, NY115, NY120, NY121, NY125, NY126, NY127, NY128, NY131, NY138, NY139, Pike, Prince Hairy, Purple 5, Reba, Redsen, Rideau, Rosa, Salem, Shepody, Snowden, and Superior. Genomic DNA of these varieties were

extracted from tuber sprouts. Three tubers were measured for shape index and mean shape index was used to test for correlation with CT217 marker data.

Results

Tuber shape. In 2006, 214 progeny of a cross between round diploid 320-02 and long diploid 10618-01 were scored for their length and width; the distribution of length to width ratios is shown in Figure 1. Tuber shape in this population does not segregate in a perfect bimodal fashion, as two major peaks overlap (Figure 1). Tuber shape in this population is also skewed towards long (Figure 1). That two major peaks are nevertheless visible suggests that a single gene controls much of the variation for tuber shape, while the overlap and skewness suggest that additional minor gene(s) are also segregating.

Anatomical analysis. An anatomical evaluation of tuber cell shape from three locations (apical end, equatorial center, stolon end) of round- and long-shaped tubers showed no statistically significant differences between round diploid 320-02 and long diploid 10618-01 (Table 2 and Figure 2), or between tetraploid cultivars Atlantic (round) and Russet Burbank (long) (data not shown). This indicates that differences in cell shape are not the primary basis for difference in shape between round and long shaped tubers. It appears, instead, that long tubers have more cells along the longitudinal axis compared to round tubers of comparable width. No obvious differences in arrangement of cells were observed between round and long tubers; in both cases cells appeared to be arranged randomly, as if cell-division had occurred in multiple planes.

Figure 1. Frequency distribution of tuber shape index in 2006 for progeny of a cross between diploids 10618-01 (long) and 320-02 (round). Shape index is the average length to width ratio for four tubers of each clone.

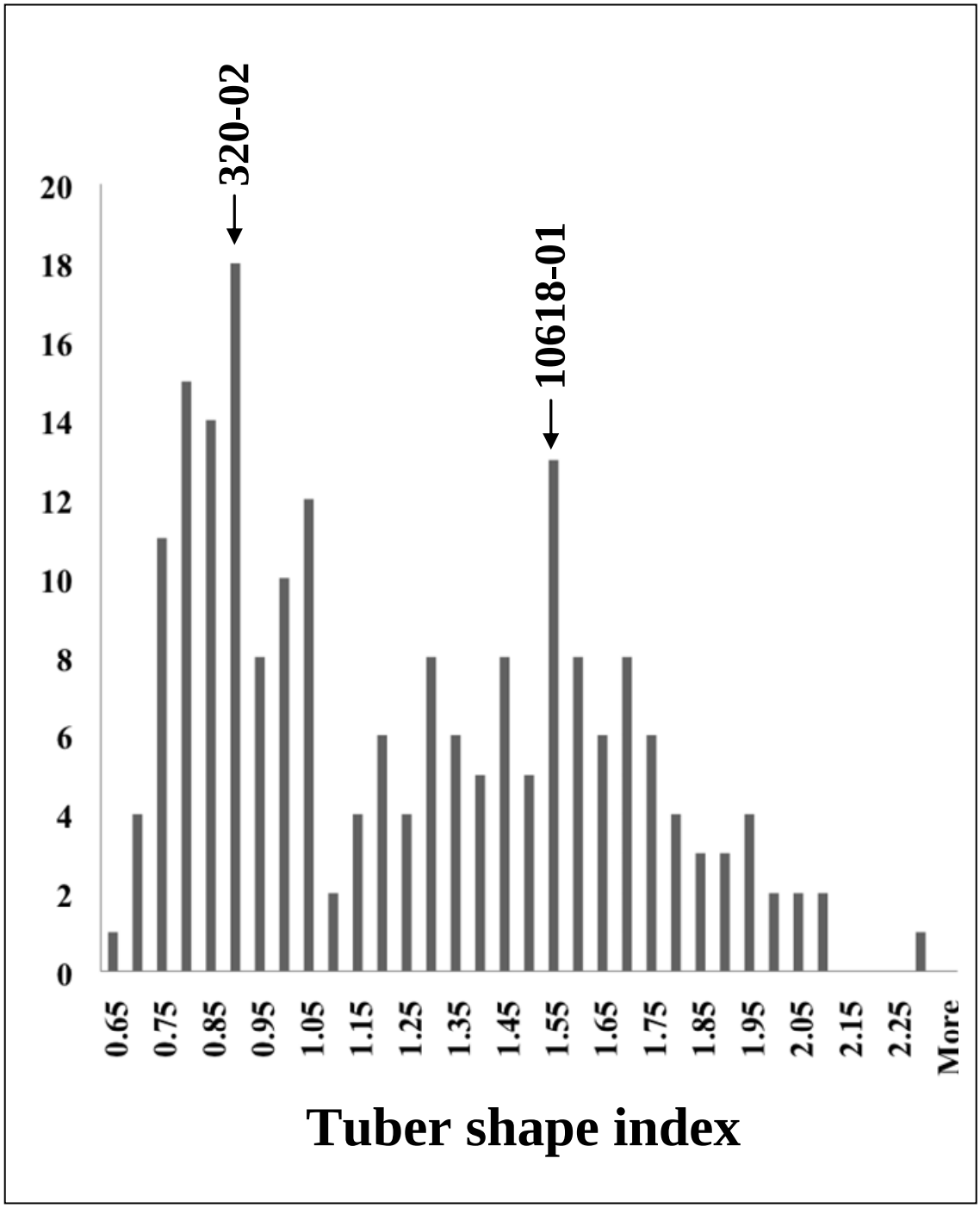
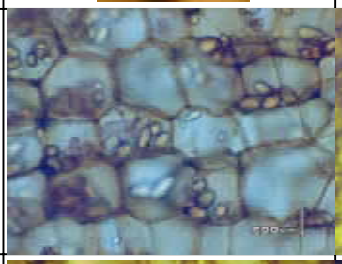
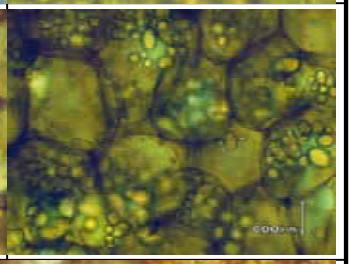
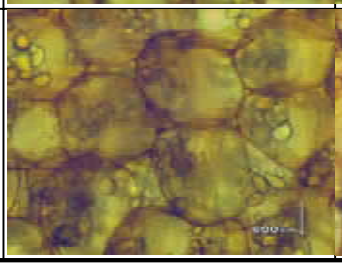

320-02
10618-01
20
18
16
14
12
10
8
6
4
2
0
0.65
0.75
0.85
0.95
1.05
1.15
1.25
1.35
1.45
1.55
1.65
1.75
1.85
1.95
2.05
2.15
2.25
More
Tuber shape index

Table 2. Comparison of cell length to width ratios from apical, equatorial and stolon regions of tubers[a]

Sample locality within tuber	Round-shaped parent 320-02	Long-shaped parent 10618-01
Cells from apical end tissue	0.6859	0.7028
Cells from equatorial tissue	0.9987	0.9987
Cells from stolon end tissue	0.9439	0.9581

[a] Sixty cells were measured under 200X magnification for each location, in two tubers for each genotype. Length to width ratios for each location were not statistically different between 320-02 and 10618-01 when compared using Student's t-test.

Figure 2. Longitudinal cross sections of round-shaped parent 320-02 and long-shaped parent 10618-01, as observed 2 mm from the tuber apex, at the tuber equator, and 2 mm from the stolon end. Sections were stained with 1% Safranin and magnified 200X. The black scale bar is 1000 μm in length.

Sample location	320-02 	10618-01 
Apical end cells		
Equatorial center cells		
Stolon end cells		

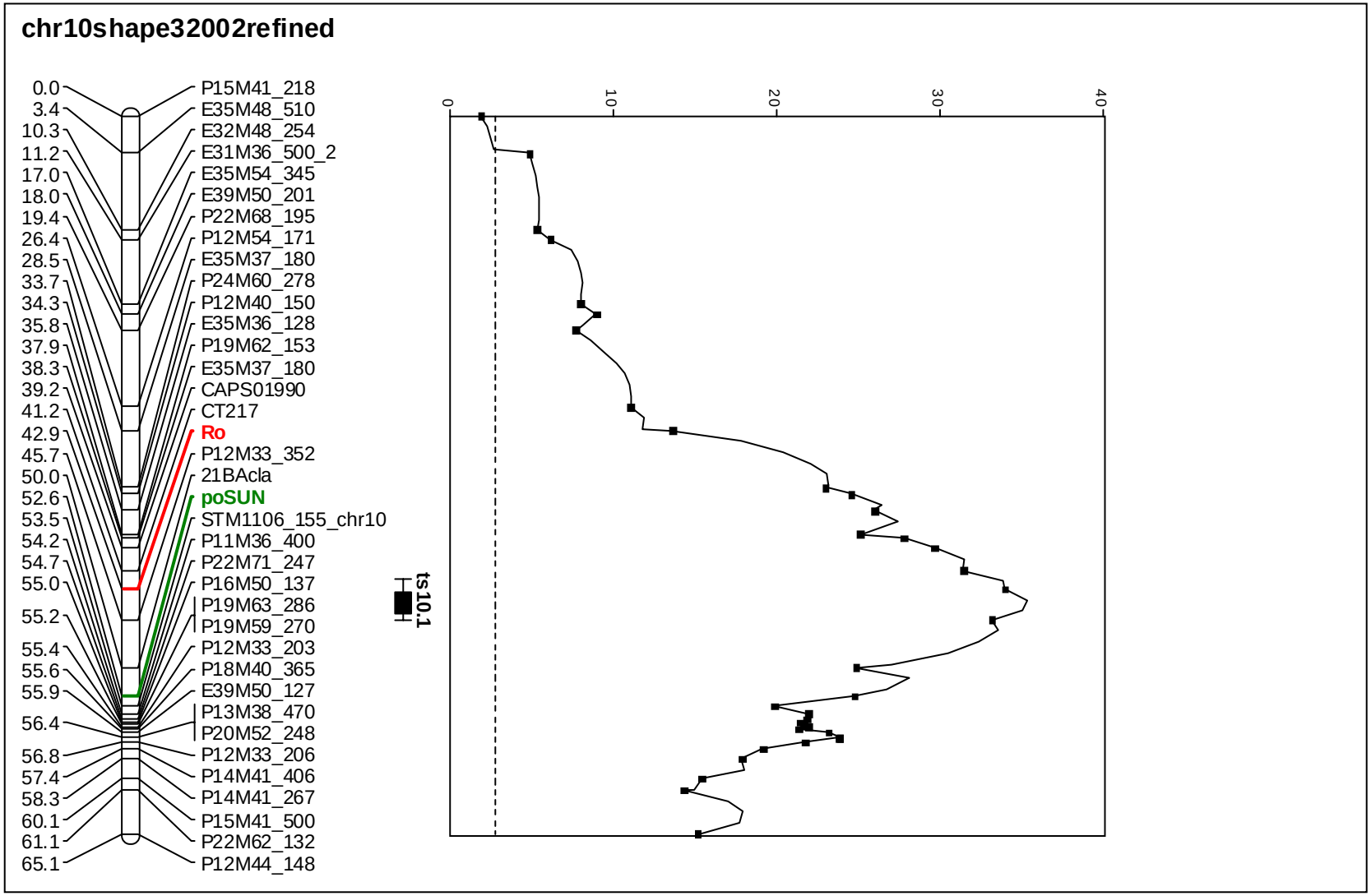
1000 μm

Linkage Maps. A total of 514 markers including 493 AFLP markers, 13 SSR, and 8 CAPS were used for mapping. Of these, 497 markers could be mapped, while 22 markers could not. Two hundred and eighty-five markers, segregating from round parent 320-02, formed twelve distinct linkage groups, while two hundred and twelve other markers, segregating from long parent 10618-01, were assembled into 12 additional linkage groups. Each linkage group was labeled with at least one SSR or CAPS anchor marker of known chromosomal location, so that chromosome identity could be assigned.

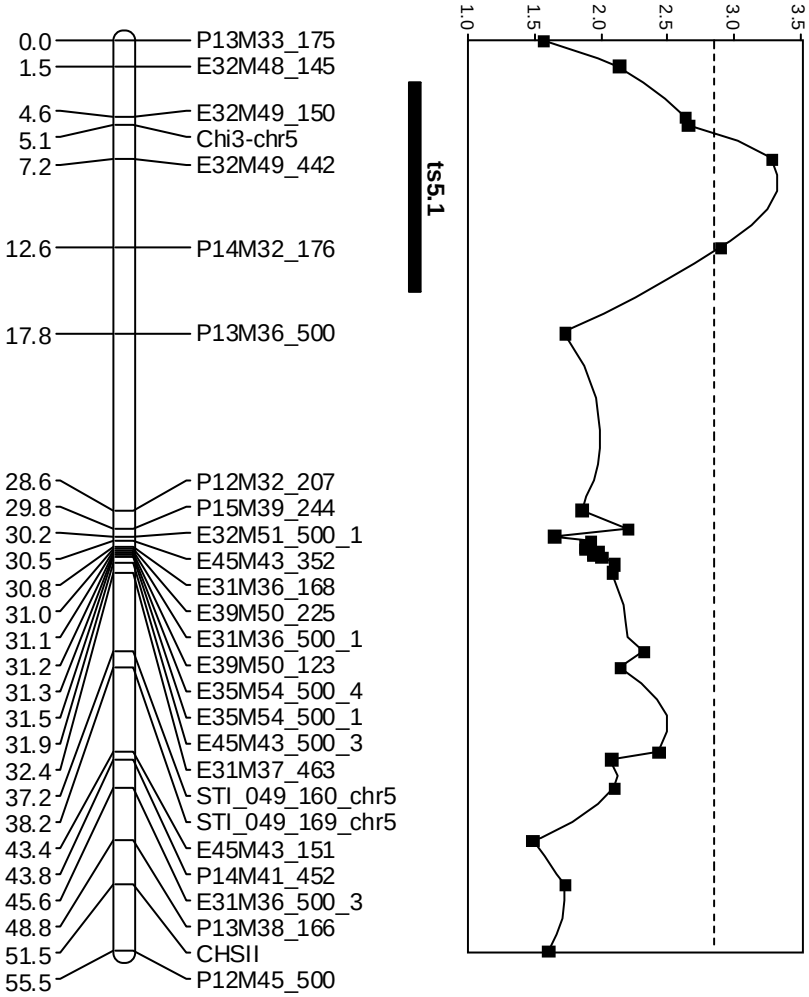
To facilitate future map-based cloning, as well as marker-assisted selection of *Ro* in potato breeding programs, primer pairs were designed against three RFLP (Tanksley et al. 1992), six COS (Fulton et al. 2002), and four COSII (Wu et al. 2006) tomato markers that appeared to map close to *Ro* based on comparison of potato and tomato genetic maps (Tanksley et al 1992). Four of these (CT217, CAPS01990, 21BA and poSUN) proved polymorphic and could be mapped in the 10618-01 x 320-02 population (Figure 3).

QTL analysis. Interval mapping of tuber shape data collected in 2006 revealed two statistically significant QTLs for tuber shape. A QTL of large effect, *ts*10.1, explained 55% of the phenotypic variation and mapped to chromosome 10 of round parent 320-02 (Figure 3). We presume that *ts*10.1 corresponds to *Ro*. A QTL of smaller effect, *ts*5.1, explained 8.9% of the phenotypic variation and mapped to chromosome 5 of long parent 10618-01 (Figure 3). A locus possibly allelic to *ts*5.1 was observed in male parent 320-02, but its statistical significance was under threshold. We named this candidate QTL *ts*5.2 (Table 3). When QTL analyses were repeated for tubers produced in 2007, QTLs at comparable positions to *ts*10.1, *ts*5.1 and *ts*5.2 were detected again,

Figure 3. Potato tuber shape QTLs identified in 2006 in a cross between diploids 10618-01 (long) and 320-02 (round). A LOD threshold of 2.85 for significance ($P<0.05$) was empirically determined by permuting the data 1000 times. In female parent 10618-01 the shape QTL *ts5.1* was identified to be closely linked with marker E32M49_442. In the male parent 320-02, two QTLs, *ts10.1* and *ts5.2* were identified, *ts10.1* is close to marker CT217 and *ts5.2* is close to P13M38_375, respectively.


chr10shape32002refined
0.0 P15M41_218
3.4 E35M48_510
10.3 E32M48_254
11.2 E31M36_500_2
17.0 E35M54_345
18.0 E39M50_201
19.4 P22M68_195
26.4 P12M54_171
28.5 E35M37_180
33.7 P24M60_278
34.3 P12M40_150
35.8 E35M36_128
37.9 P19M62_153
38.3 E35M37_180
39.2 CAPS01990
41.2 CT217
42.9 Ro
45.7 P12M33_352
50.0 21BAcla
52.6 poSUN
53.5 STM1106_155_chr10
54.2 P11M36_400
54.7 P22M71_247
55.0 P16M50_137
55.2 P19M63_286
P19M59_270
55.4 P12M33_203
55.6 P18M40_365
55.9 E39M50_127
56.4 P13M38_470
P20M52_248
56.8 P12M33_206
57.4 P14M41_406
58.3 P14M41_267
60.1 P15M41_500
61.1 P22M62_132
65.1 P12M44_148
ts10.1
0
10
20
30
40

2ndQTLofshape



32002ch5shapeqtl

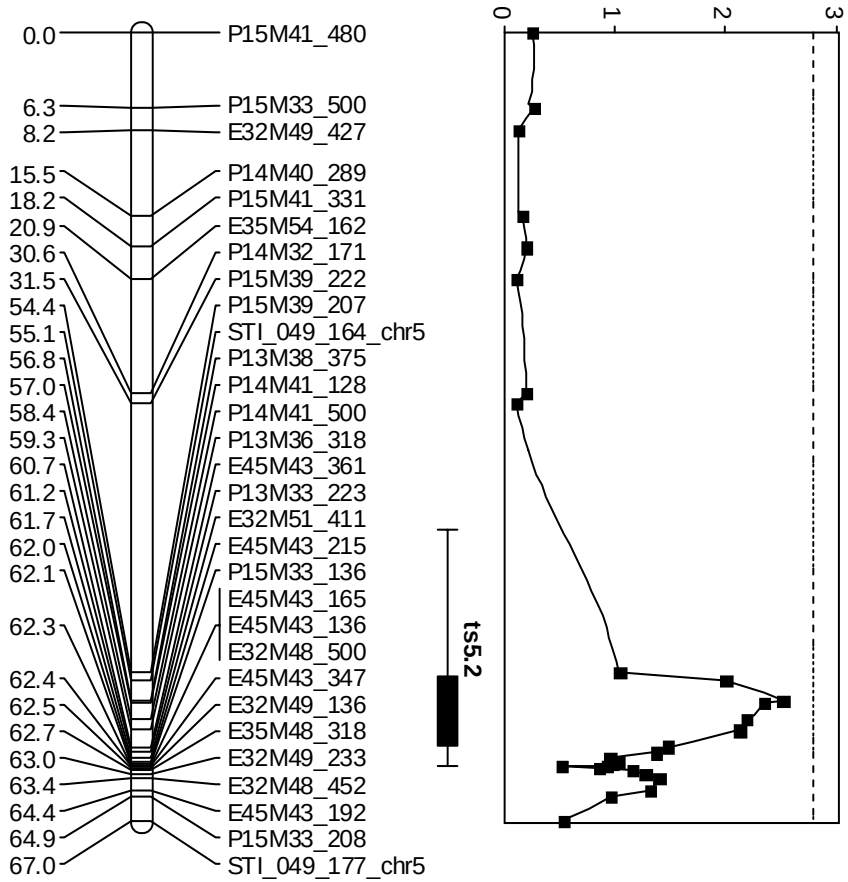


Table 3. Tuber shape QTLs detected by Interval Mapping in 2006 and 2007

				2006 results		2007 results	
Parent	locus	Chromosome	Marker	Sig value	% variation (r^2)	Sig value	% variation (r^2)
10618-01	ts5.1	5	E32M49_442	LOD 3.3	8.9	LOD 3.17	7.4
	ts5.3	5	P12M45_500	LOD 1.4	3.4	LOD 5.4	14
320-02	ts10.1	10	CT217	LOD 30.7	55.1	LOD 43.1	75.4
	ts5.2	5	P13M38_375	LOD 2.5	5.3	LOD 2.6	6.2
	ts2.2	2	P14M40_172	LOD 0.81	1.7	LOD 2.7	6.5

with a LOD score of 43 for *ts*10.1, explaining 75.4% of the variation, a LOD score of 3.2 for *ts*5.1, explaining 7.4% of the variation, and a LOD score of 2.6 for *ts*5.2, explaining 6.2% of the variation (Table 3).

In 2007 two possible QTls were detected that were not observed in 2006. The LOD score (2.7) for a candidate fourth QTL, *ts*2.1, was slightly under the empirically determined threshold of 2.85 for genome-wide significance at $P<0.05$. A fifth QTL, *ts*5.3, explained 14% of the variation and mapped to a location distinct from *ts*5.1.

A potato chromosome 10 gene (poSUN) with 93% sequence identity to tomato SUN (Han et al 2008) mapped 12 cM away from *ts*10.1 (Figure 3). Thus poSUN does not correspond to *ts*10.1.

Interactions between QTLs. The QTLs *ts*10.1, *ts*5.1 and *ts*5.2 were examined for interaction employing Qgene's two-way ANOVA interaction test (Nelson 1997). No significant interaction was detected at $P \leq 0.01$. These three QTLs were then tested for interactions with seven other loci that were detected through main effects with LOD scores above 2 by interval mapping or at $p < 0.05$ by Kruskal-Wallis analysis. These seven loci were P12M33_285 (chromosome 2 of the female parent, abbreviated as 2F and similarly hereafter), E32M49_420 (3F), CHSII (5F), E35M54_162 (5M), E31M37_181 (7M), E31M37_460 (8F), and E45M43_301 (11M). QTL *ts*10.1 was found to be involved in a significant interaction at $P \leq 0.003$ with P12M33_285 (2F) (Table 4). The interaction explained 4.3% of the phenotypic variation based on analysis of 2006 tuber shape data. This interaction was again detected with 2007 data at $P \leq 0.0223$.

Ability of CT217 to predict shape in tetraploid potato. Since CT217 maps close to *ts*10.1 we wondered if it might be able to predict tuber shape in tetraploid potato. To test this, 84 autotetraploid potato cultivars and breeding clones were evaluated with

Table 4. Interaction between loci detected by multiple regression in 2006.

*ts*10.1	*ts*2.1	tuber length to width ratio
+	+	1.02
+	-	0.99
-	+	1.73
-	-	1.49

Probability that interaction is not significant < 0.0029

CT217. Two amplification products were observed in the panel – one about 500 bp in length, the other about 450 bp. Potato clones which only exhibited the 500 bp band were almost invariably long (Figure 4 and data not shown). Of the 35 potatoes with this banding pattern, all 35 produced long tubers (Figure 4 and data not shown). Potatoes that exhibited both 450 and 500 bp bands tended to be round or oblong, but did include some clones (like Premier Russet and Defender) that are unambiguously long.

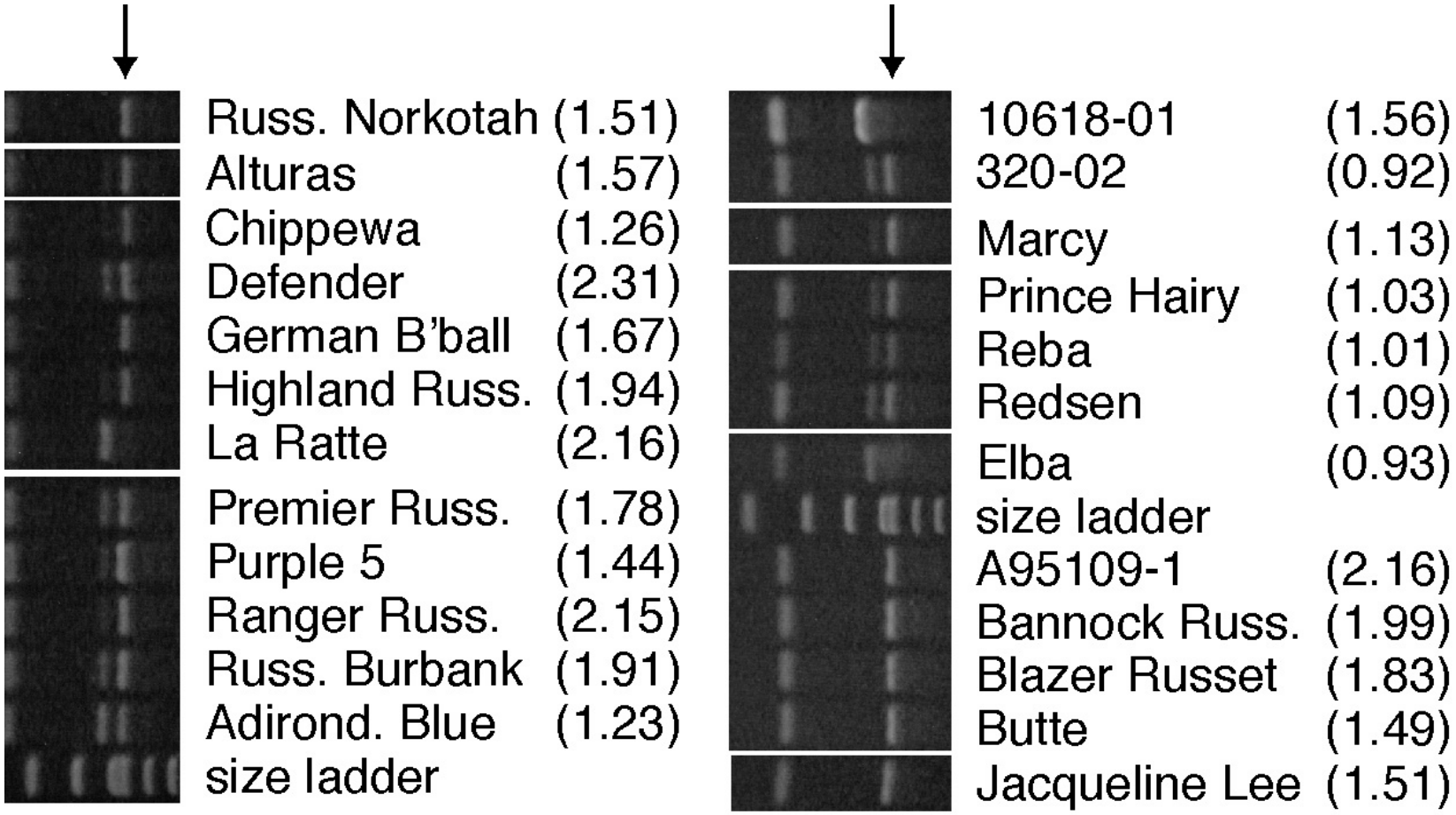
Discussion

This study has presented comparative anatomical data from round and long-shaped tubers which demonstrates that cell shape is similar in round and long tubers; thus long tubers appear to differ from round tubers primarily by having more cells along the longitudinal axis.

We identified two QTLs (*ts*10.1 and *ts*5.1) that influenced tuber shape in both 2006 and 2007. *ts*10.1 mapped to chromosome 10 and has the largest effect on tuber shape. It very likely corresponds to the classical tuber shape locus *Ro* described by De Jong and Burns (1993) and mapped by van Eck et al (1994). The remaining reproducible QTL, *ts*5.1, had a smaller effect on shape, mapped to chromosome 5, and has not previously been described. We also detected an interesting epistatic QTL in 2006, *ts*2.1, that interacts with *ts*10.1.

We had originally hoped that our diploid population could serve as a basis for map-based cloning of *Ro*. After examination of the distribution of tuber length to width ratios, however, it quickly became apparent that the population did not segregate in a tidy bi-modal fashion. Instead, the distribution was continuous, so that some clones could not be unambiguously characterized as round or long. The distribution was

Figure 4. The genotype of selected potato clones as assessed with marker CT217. Tuber shape indices (in parentheses following each clone name) were determined by measuring length and width for three tubers of each clone. An arrow denotes the 500 bp band referred to in the text.


Russ. Norkotah (1.51)
Alturas (1.57)
Chippewa (1.26)
Defender (2.31)
German B'ball (1.67)
Highland Russ. (1.94)
La Ratte (2.16)
Premier Russ. (1.78)
Purple 5 (1.44)
Ranger Russ. (2.15)
Russ. Burbank (1.91)
Adirond. Blue (1.23)
size ladder
10618-01 (1.56)
320-02 (0.92)
Marcy (1.13)
Prince Hairy (1.03)
Reba (1.01)
Redsen (1.09)
Elba (0.93)
size ladder
A95109-1 (2.16)
Bannock Russ. (1.99)
Blazer Russet (1.83)
Butte (1.49)
Jacqueline Lee (1.51)

also skewed toward long tuber shape. The skewed distribution could, in principle, be explained as the result of the combined effects of *ts*10.1, *ts*5.1, and *ts*2.1. Marker CT217 maps very close to *ts*10.1. Interestingly, the variance (0.017) of tuber shape in the presence of the allele of CT217 linked in coupling with round shape was significantly smaller than the variance (0.125) of tuber shape when the alternative allele from 320-02 was present ($P \leq 1.3E\text{-}20$). Again, the difference in variances between the two groups may be related to composite QTL effects from *ts*10.1, *ts*5.1, *ts*5.2 and an inter-genic interaction between *ts*10.1 and *ts*2.1 (Table 4).

Marker CT217 was not only tightly linked to tuber shape in our experimental diploid population, but one marker configuration (500 bp only pattern) proved to be correlated with long tuber shape in a panel of tetraploid cultivars and breeding clones. This marker could conceivably be used in applied breeding programs to drive germplasm toward long shape. Nevertheless, as almost all the long potatoes evaluated in this study were long russets developed in the northwestern USA, we cannot rule out the possibility that the apparent association is accidental, caused by differences in CT217 allele frequency in northwestern potato breeding programs compared to those located elsewhere. Since the banding pattern associated with long shape in the tetraploid potato panel was opposite to that observed with diploid parents 320-02 and 10618-01 (Figure 4), even if CT217 is useful for tetraploid breeding, it won't necessarily be predictive across diploid germplasm.

The tomato *sun* locus, which confers long shape to tomato fruit, is located on chromosome 7 (van der Knaap et al. 2001), while a gene closely related to SUN is located on tomato chromosome 10 (van der Knaap et al. 2004; Han et al. 2008). We identified a potato counterpart of *sun* (poSUN) that shares 93% sequence identity with *sun*, but it mapped 12 cM away from *ts*10.1. Therefore, *ts*10.1 does not appear to be related to *sun*, although we cannot rule out the possibility that both *sun* and *ts*10.1 are

members of the same gene family. In pepper, a major fruit shape QTL (*fs*10.1) maps to chromosome 10 (Chaim et al. 2001; Rao et al. 2003). In tomato, a weak QTL related to ovate fruit shape also maps to chromosome 10 (Ku et al. 1999). There may thus be a common gene on chromosome 10 that controls organ shape across solanaceous genera, where the potato otholog primarily influences tuber shape.

It is likely that there are more genes that impact tuber shape than those mapped in this study. In tomato sixteen QTLs have been identified so far that control fruit shape (Grandillo et al 1996, 1999, Ku et al 1999, 2000, Van der Knaap et al 2001, 2002). Different types of long-shaped tubers, such as cylindrical, spheroid and/or pear shaped almost certainly require additional loci. In a recent association mapping study, loci influencing tuber shape were located on chromosomes 2, 4 and 11 (D'hoop et al 2008). It is interesting that the locus of largest effect in our conventional QTL mapping study, *ts*10.1, was not detected by association mapping. The *ts*2.1 QTL may correspond to the chromosome 2 locus reported by D'hoop et al (2008).

ACKNOWLEDGEMENTS

We thank Dr. Robert Turgeon for assistance in measuring tuber cell shape, and the field crew of the Cornell potato breeding program for maintaining the diploid population.

REFERENCES

Ben Chaim A, Paran I, Grube R, Jahn M, van Wijk R, Peleman J (2001) QTL mapping of fruit related traits in pepper (Capsicum annuum). Theor Appl Genet 102: 1016-1028

Ben Chaim A, Borovsky E, Rao GU, Tanyolac B, Paran I (2003a) fs3.1: a major fruit shape QTL conserved in Capsicum. Genome 46: 1-9

Ben Chaim A, Borovsky E, De Jong W, Paran I (2003b) Linkage of the A locus for the presence of anthocyanin and fs10.1, a major fruit-shape QTL in pepper. Theor Appl Genet 106: 889-894

Black W (1930) Notes on progenies of various potato hybrids. Journal of Genet 22: 27-44

De Jong H, Rowe PR (1972) Genetic markers in inbred clones of cultivated diploid potatoes. Potato Research 15: 200-208

De Jong H, Burns V.J (1993) Inheritance of tuber shape in cultivated potato. American Potato Journal 70: 267-283

De Jong WS, Eannetta NT, De Jong DM, Bodis M (2004) Candidate gene analysis of anthocyanin pigmentation loci in the Solanaceae. Theor Appl Genet 108:423-432

D'hoop BB, Paulo MJ, Mank RA, van Eck HJ, van Eeuwijk FA (2008) Association mapping of quality traits in potato (Solanum tuberosum L.). Euphytica 161: 47-60

Feingold S, Lloyd J, Norero N, Bonierbale M, Lorenzen J (2005) Mapping and characterization of new EST-derived microsatellites for potato (Solanum tuberosum L.). Theor Appl Genet 111:456-66

Fulton TM, Van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14:1457-1467

Glendinning DR (1983) Potato introductions and breeding up to the early 20th century. New Phytologist 94: 479-505

Gonzalo MJ, van der Knaap E (2008) A comparative analysis into the genetic bases of morphology in tomato varieties exhibiting elongated fruit shape. Theor Appl Genet 116:647-656

Grandillo S, Ku HM, Tanksley SD (1996) Characterization of fs8.1, a major QTL influencing fruit shape in tomato. Mol Breed 2: 251–260

Grandillo S, Ku H-M, Tanksley SD (1999) Identifying loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99: 978–987

Han X, Jiang N, Schaffner E, Stockinger EJ, van der Knaap E (2008) A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. Science 319:1527-1530

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12: 172-175

Ku H-M, Doganlar S, Chen K-Y, Tanksley SD (1999) The genetic basis of pear-shaped tomato fruit. Theor Appl Genet 99: 844–850

Ku H-M, Grandillo G, Tanksley SD (2000) *fs*8.1, a major QTL, sets the pattern of tomato carpel shape well before anthesis. Theor Appl Genet 101: 873–878

Liu J, Van Eck J, Cong B, Tanksley SD (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA 99: 13302–13306

Liu J, Tanksley SD (2003) Generation and analysis of an artificial gene dosage series in tomato to study the mechanisms by which the cloned QTL fw2.2 controls fruit size. Plant Physiol 132: 292–299

Masson MF (1985) Mapping, combining abilities, heritabilities and heterosis with 4X x 2X crosses in potato. PhD thesis. Univ. Wisconsin

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterisation and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233-45

Nelson JC 1997 QGENE: Software for marker-based genomic analysis and breeding. Molecular Breeding 3: 239-245

Paran I, van der Knaap E (2007) Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. J Exp Bot 58:3841-3852

Rao GU, Ben Chaim A, Borovsky E, Paran I (2003) Mapping of yield related QTLs in pepper in an inter-specific cross of Capsicum annuum and C. frutescens. Theor Appl Genet 106: 1457-1466

Salaman RN (1910) The inheritance of colour and other characters in the potato. Journal of Genet 1:7-46

Solanaceae Genomics Network [Online] http://www.sgn.cornell.edu

Taylor LM (1978) Variation patterns of parthenogenetic plants derieved from "unreduced" embryo-sac of Solanum tuberosum subspecies andigena (Juz et Buk.). Theor Appl Genet 52:241-249

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, and Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141-1160

Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. Plant Cell 16 Suppl: S181-9

van der Knaap E, Tanksley SD (2001) Identification and characterization of a novel locus controlling early fruit development in tomato. Theor Appl Genet 103: 353–358

van der Knapp E, Lippman ZB, Tanksley SD (2002) Extremely elongated tomato fruit controlled by four quantitative trait loci with epistatic interactions. Theor Appl Genet 104: 241–247

van der Knaap E, Tanksley SD (2003) The making of a bell pepper-shaped tomato fruit: Identification of loci controlling fruit morphology in Yellow Stuffer tomato. Theor Appl Genet 107: 139–147

van der Knaap E, Sanyal A, Jackson SA, Tanksley SD (2004) High-resolution fine mapping and fluorescence in situ hybridization analysis of sun, a locus controlling tomato fruit shape, reveals a region of the tomato genome prone to DNA rearrangements. Genetics 168:2127-40

van Eck HJ, Jacobs JME, Stam P, Ton J, Stiekema WJ, and Jacobsen E (1994) Multiple alleles for tuber shape in diploid potato detected by qualitative and quantitative genetic analysis using RFLPs. Genetics 137:303-309

Van Ooijen JW,,Voorrips RE (2001) JoinMap® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wagengen, the Netherlands

Van Ooijen JW (2004) MapQTL ® 5, Software for the zapping of quantitative trait loci in experimental populations. Kyazma B.V., Wageningen, Netherlands

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 21: 4407-4414

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of heredity 93: 77-78

Wu F, Mueller LA, Crouzillat D, Pétiard V, Tanksley SD (2006) Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: a test case in the euasterid plant clade. Genetics 174:1407-20

Zhang YF, Jung CS, De Jong WS (2009) Genetic analysis of pigmented tuber flesh in potato. Submitted

Zygier S, Chaim AB, Efrati A, Kaluzky G, Borovsky Y, Paran I (2005) QTLs mapping for fruit size and shape in chromosomes 2 and 4 in pepper and a comparison of the pepper QTL map with that of tomato. Theor Appl Genet 111:437-45

APPENDIX I

Figures that are relevant to the thesis research but are not reported in papers.

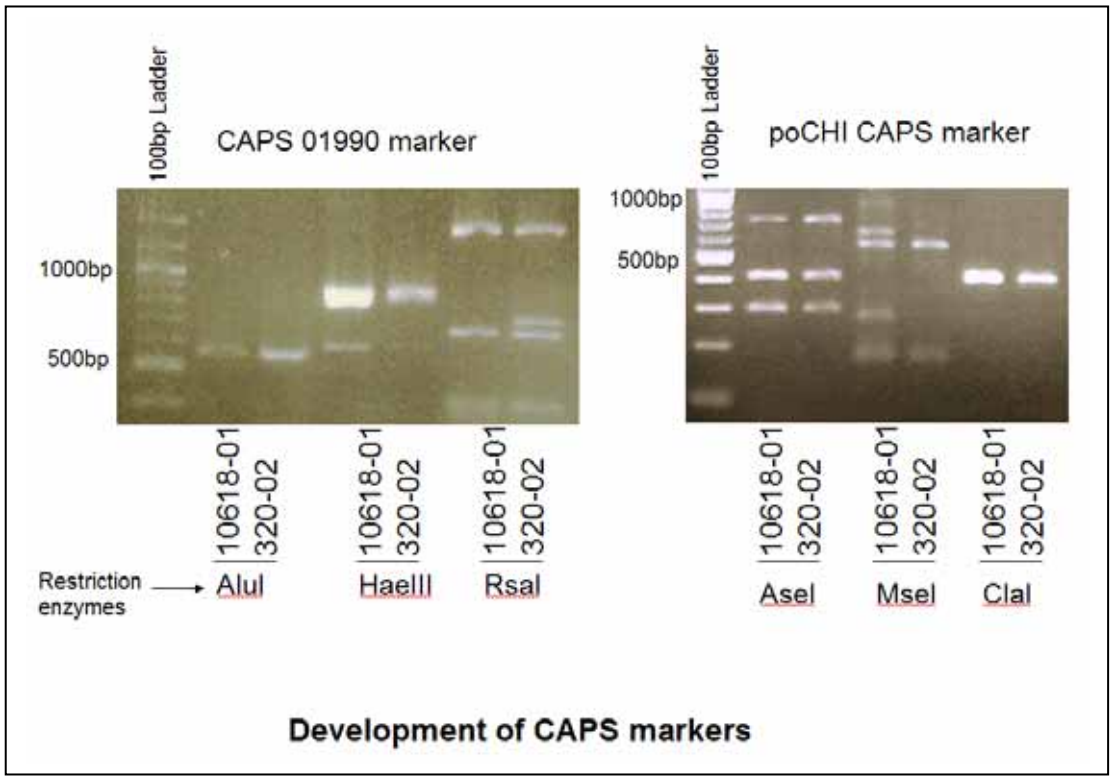


Figure 1. The development of CAPS 01990 and poCHI CAPS markers. PCR products were amplified with primers designed against potato EST sequences. Then PCR products were digested with restriction enzymes, and digestion products were visualized by electrophoresis through a 1.5% agarose gel.

Figure 2. AFLP markers generated with Pst16/Mse50. Product sizes can be estimated comparison with a Sequamark 10 base ladder (Research Genetics, Huntsville, Alabama). Markers specific to the round-shaped parent are indicated by a full arrow, whereas markers that are specific to the long-shaped parent are indicated with an empty arrow. A total of 24 AFLP combinations were used to evaluate 222 progeny clones.

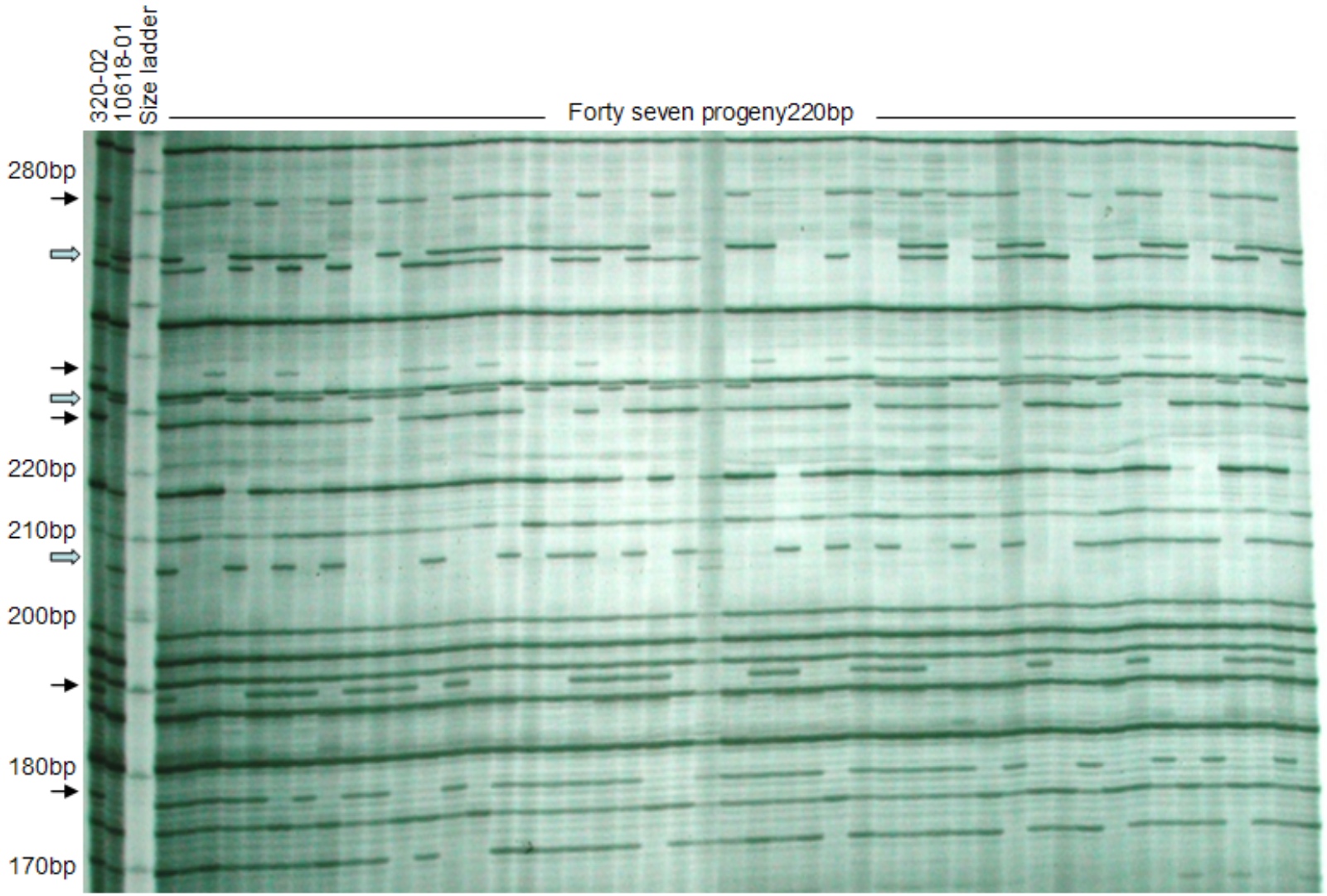

320-02
10618-01
Size ladder
Forty seven progeny220bp
280bp
220bp
210bp
200bp
180bp
170bp

Figure 3. Anthoocyanin biosynthetic pathway relevant to chapter one and chapter two.

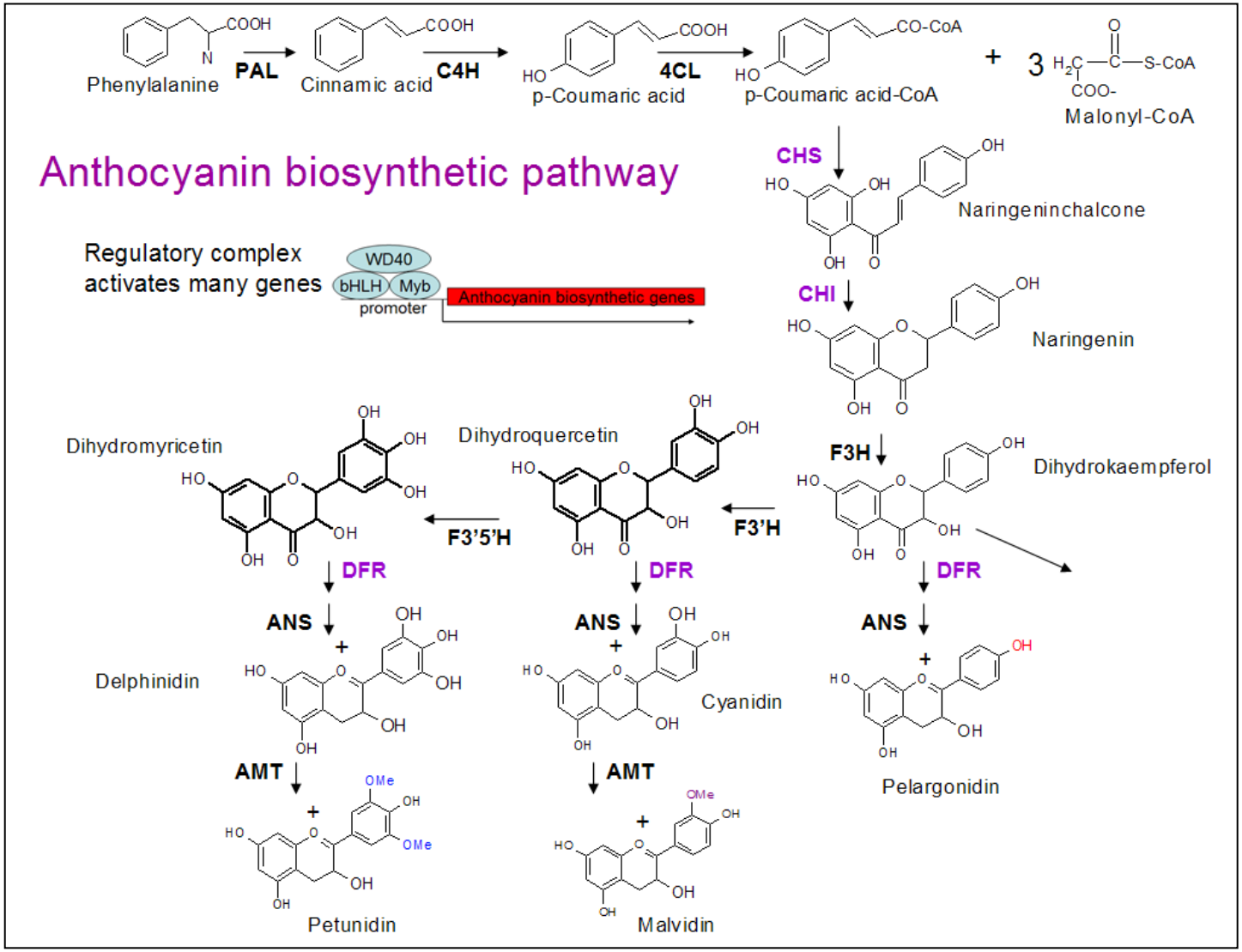
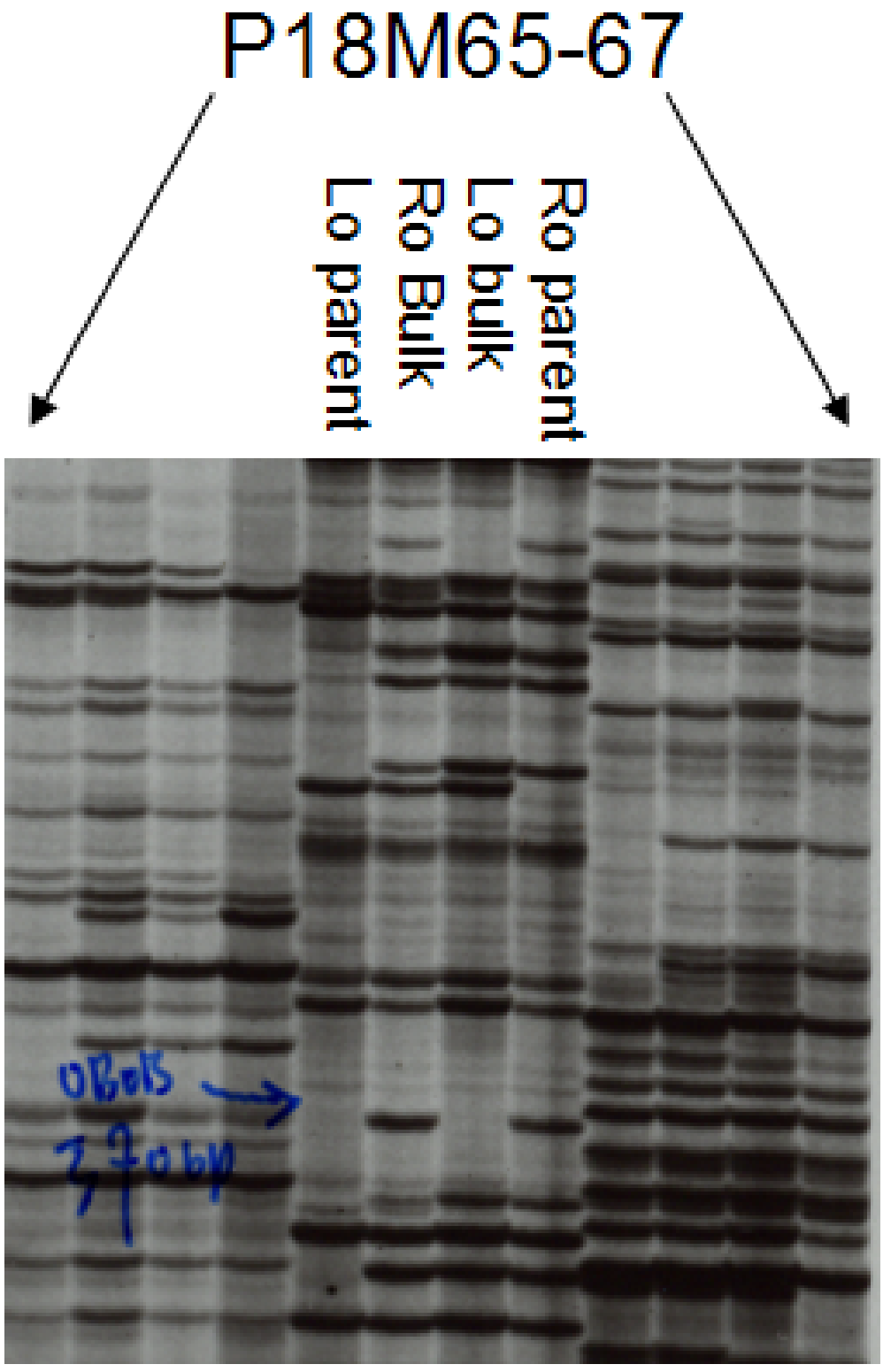
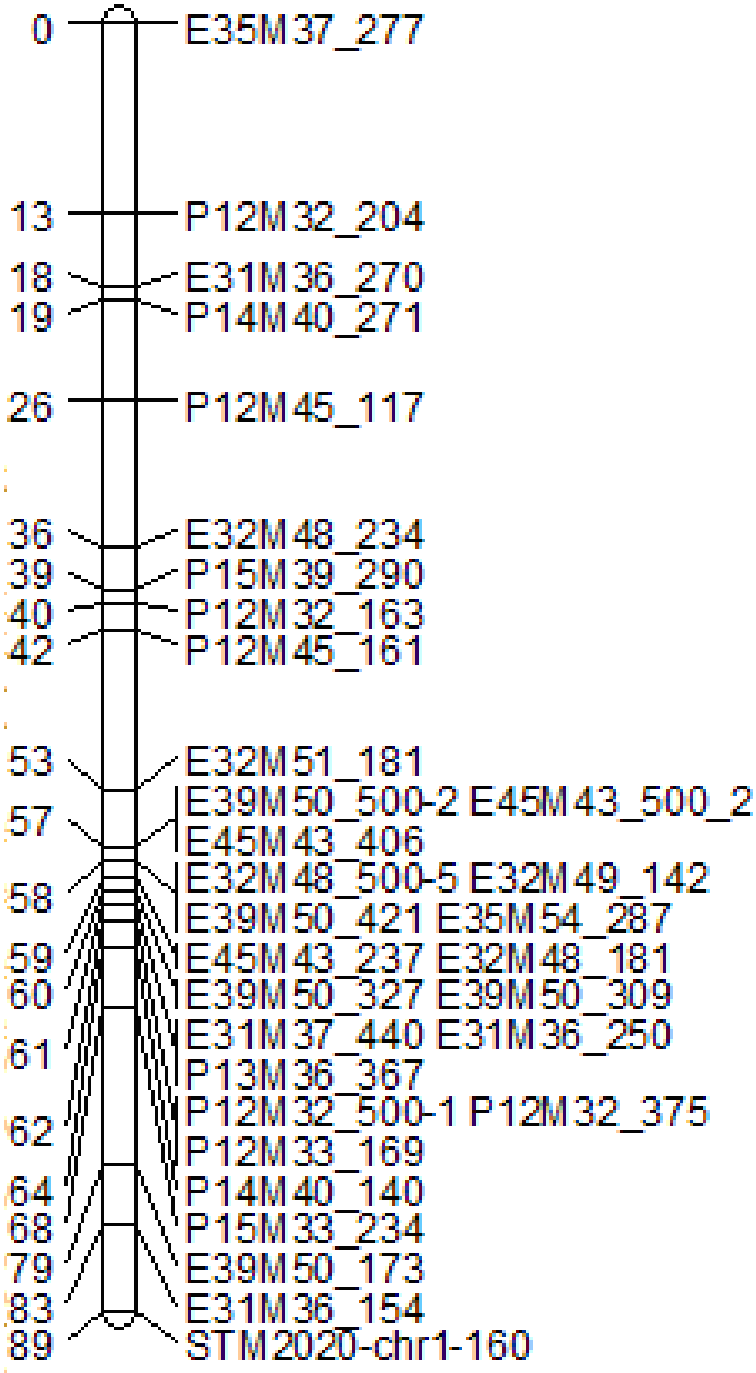

Phenylalanine
PAL
Cinnamic acid
C4H
p-Coumaric acid
4CL
p-Coumaric acid-CoA
+ 3
Malonyl-CoA
Anthocyanin biosynthetic pathway
CHS
Naringeninchalcone
Regulatory complex
activates many genes
WD40
bHLH
Myb
promoter
Anthocyanin biosynthetic genes
CHI
Naringenin
F3H
Dihydrokaempferol
Dihydromyricetin
Dihydroquercetin
F3'5'H
F3'H
DFR
DFR
DFR
ANS
ANS
ANS
Delphinidin
Cyanidin
Pelargonidin
AMT
AMT
Petunidin
Malvidin

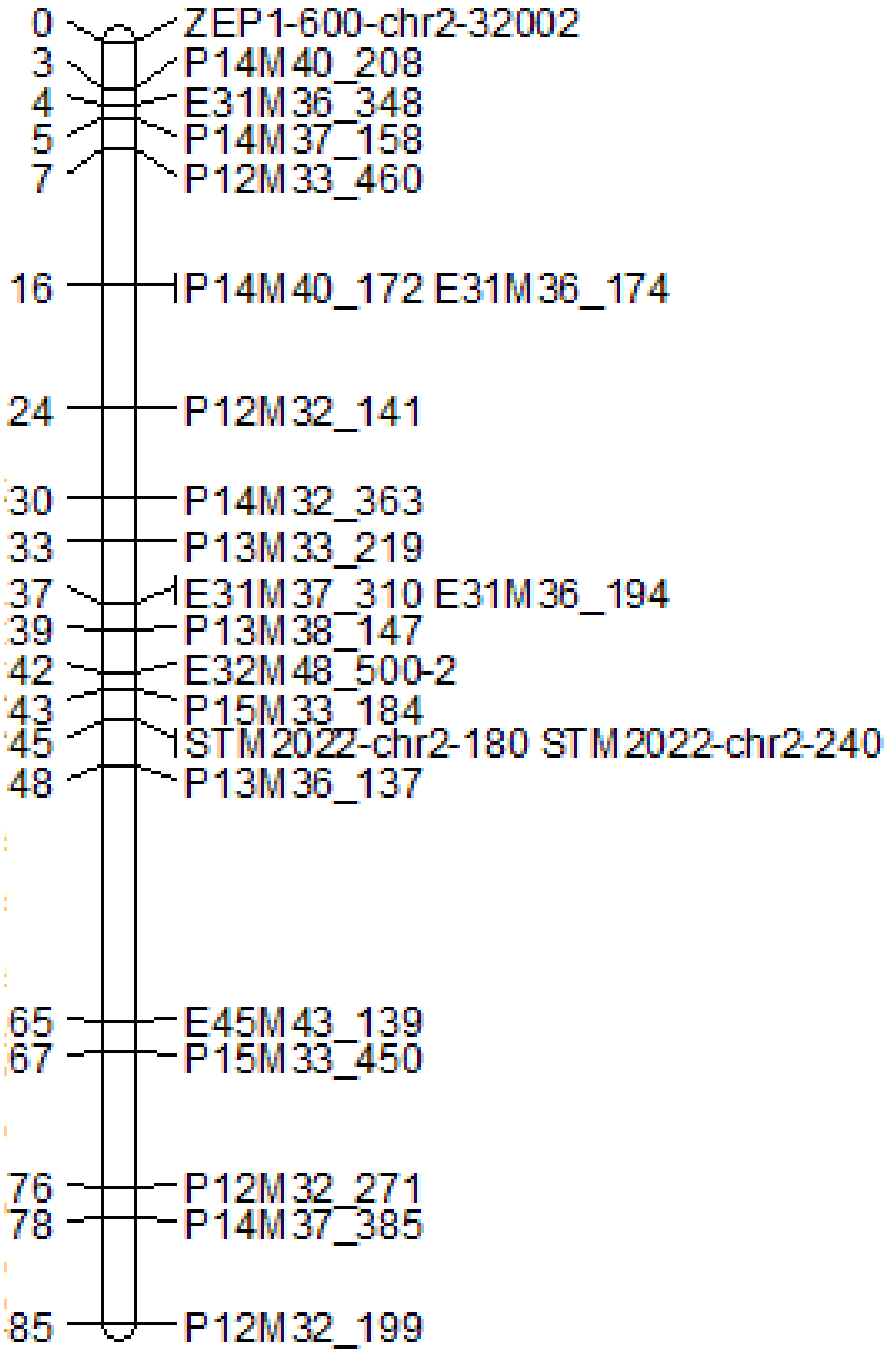
Figure 4. Generation of bulked segregant analysis (BSA) markers that were used to saturate the genetic map around *Ro*. Genomic DNA was extracted from both parents as well as fifteen round and fifteen long-shaped individuals. Equal amounts of DNA from each round individual were pooled, this became the round bulk; equal amounts of DNA from each of the 15 long-shaped individual were pooled to form the long bulk. Then different AFLP combinations were used to compare these two parents and two bulks. The AFLP markers that are seen only in the round parent and round bulk are potentially linked in coupling to *Ro*, whereas those seen only in the round-shaped parent and long bulk are potentially linked in repulsion to *Ro.* AFLP marker P18M66_370 is shown to be potentially linked in coupling with *Ro* in this figure.


P18M65-67
Ro parent
Lo bulk
Ro Bulk
Lo parent
OBo15
277 bp

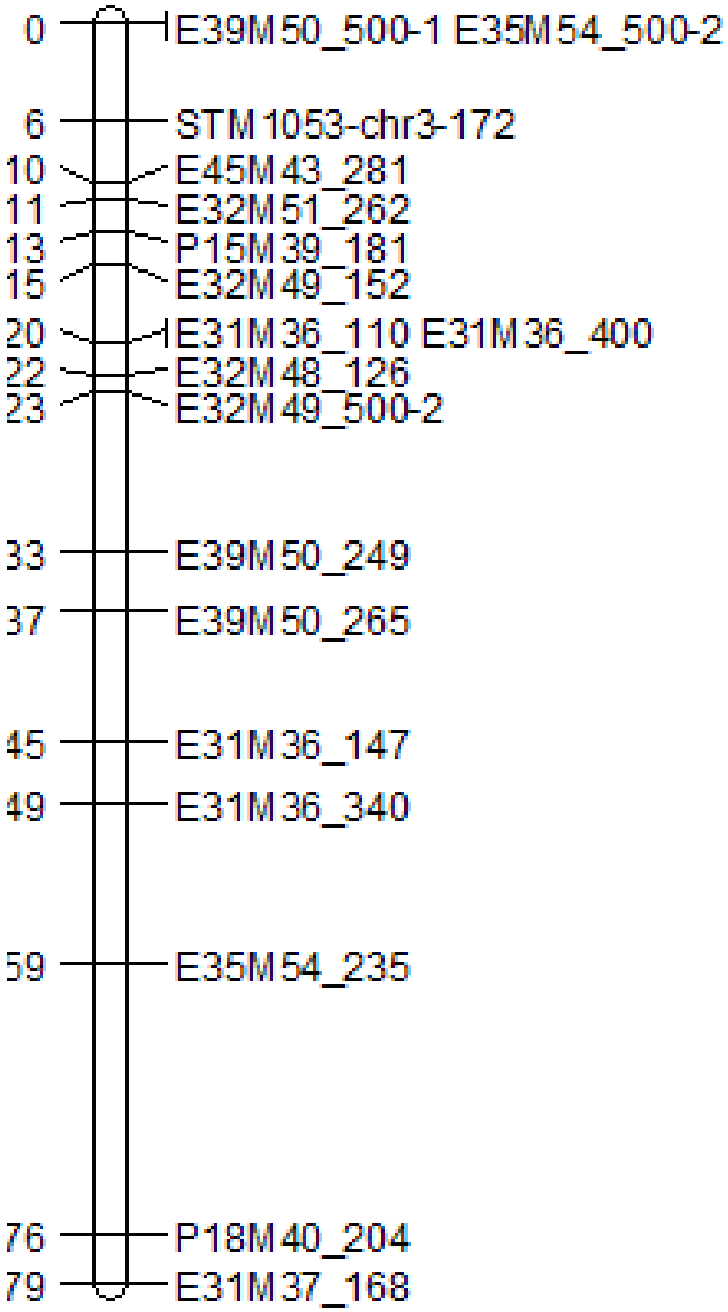
APPENDIX II



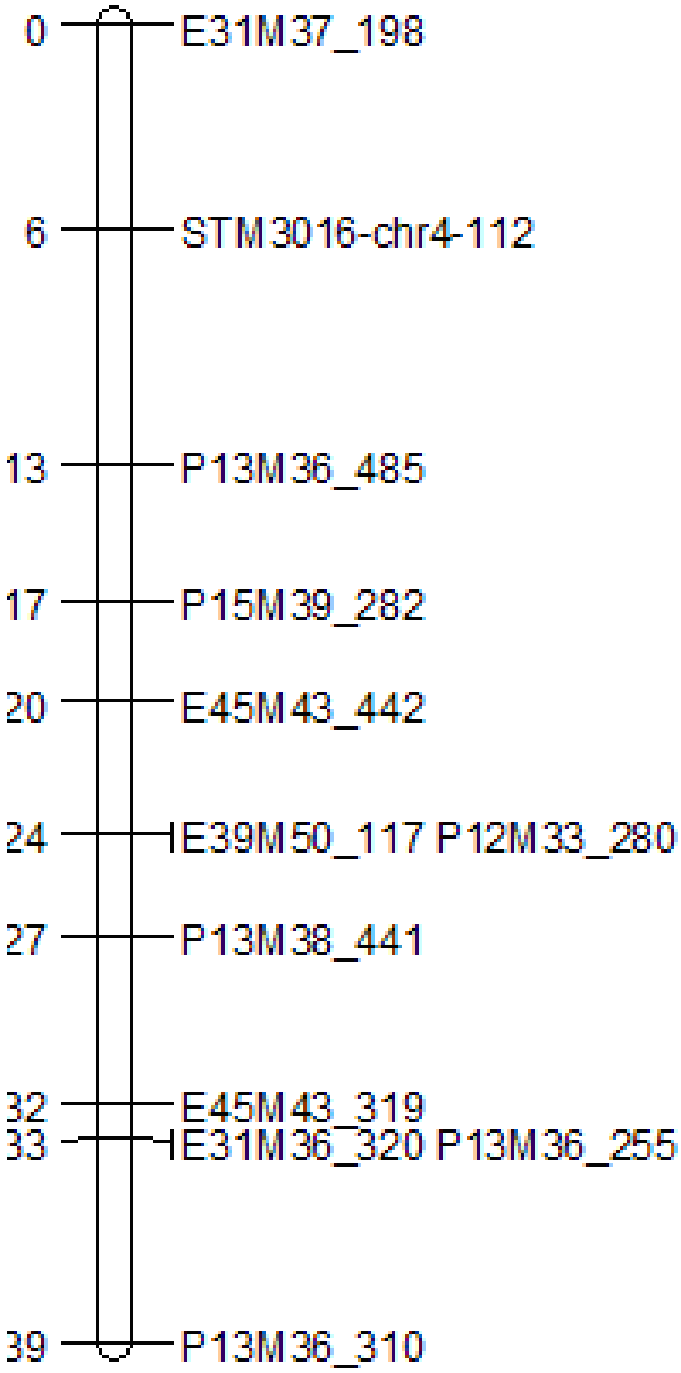
320-02 Chromosome 1



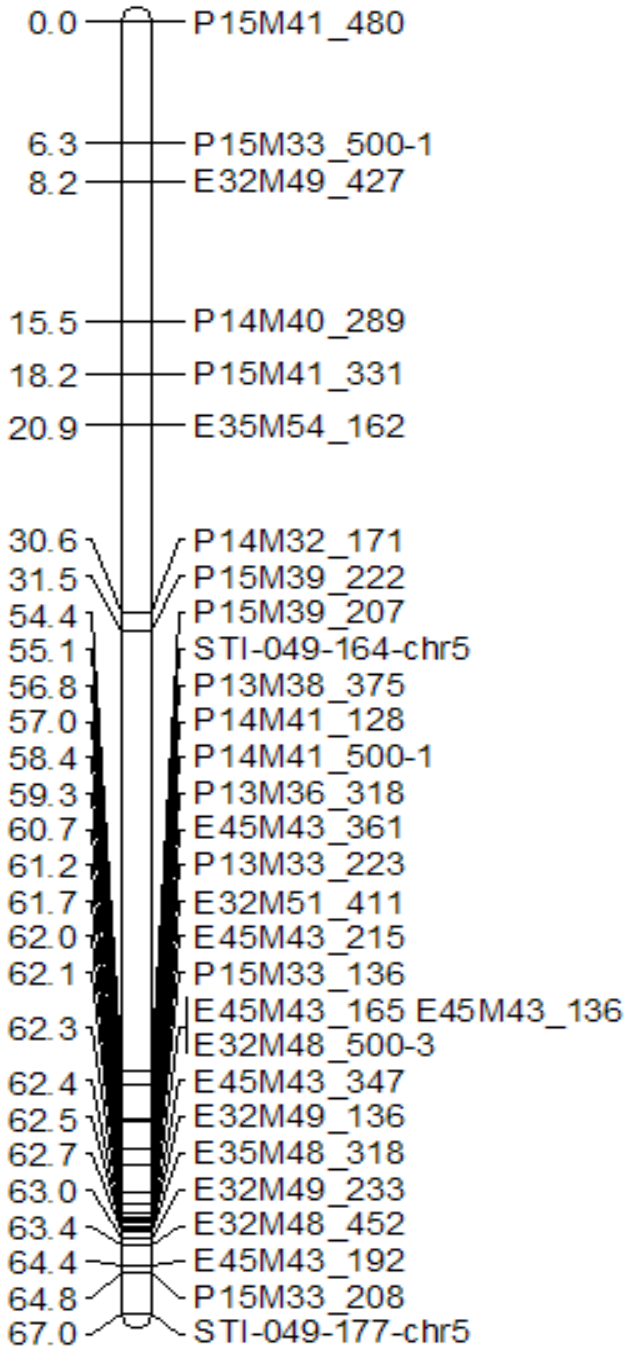
320-02 Chromosome 2



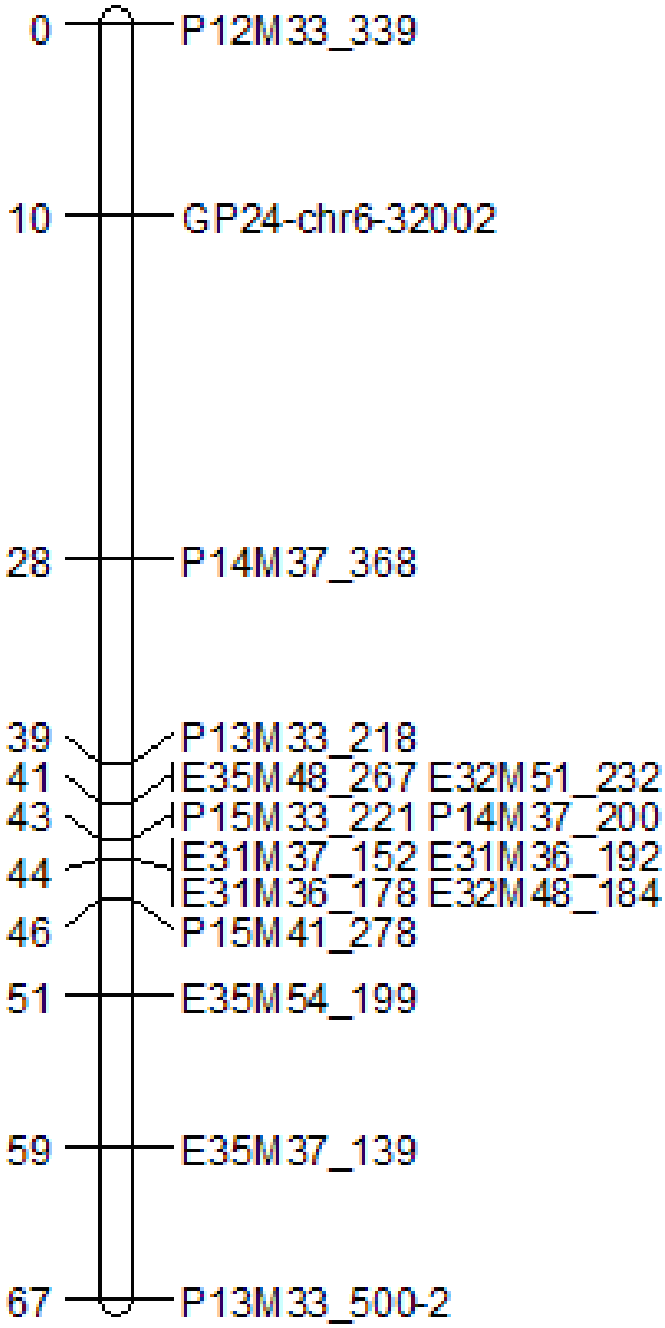
320-02 Chromosome 3



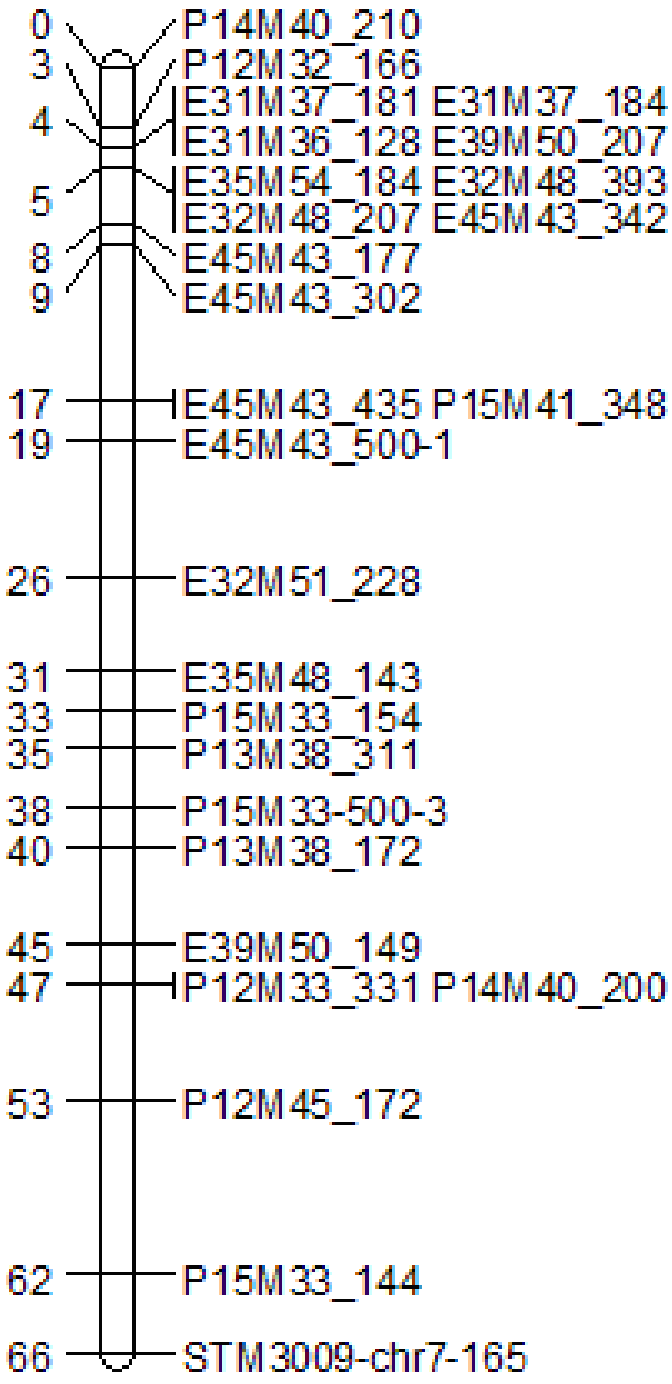
320-02 Chromosome 4



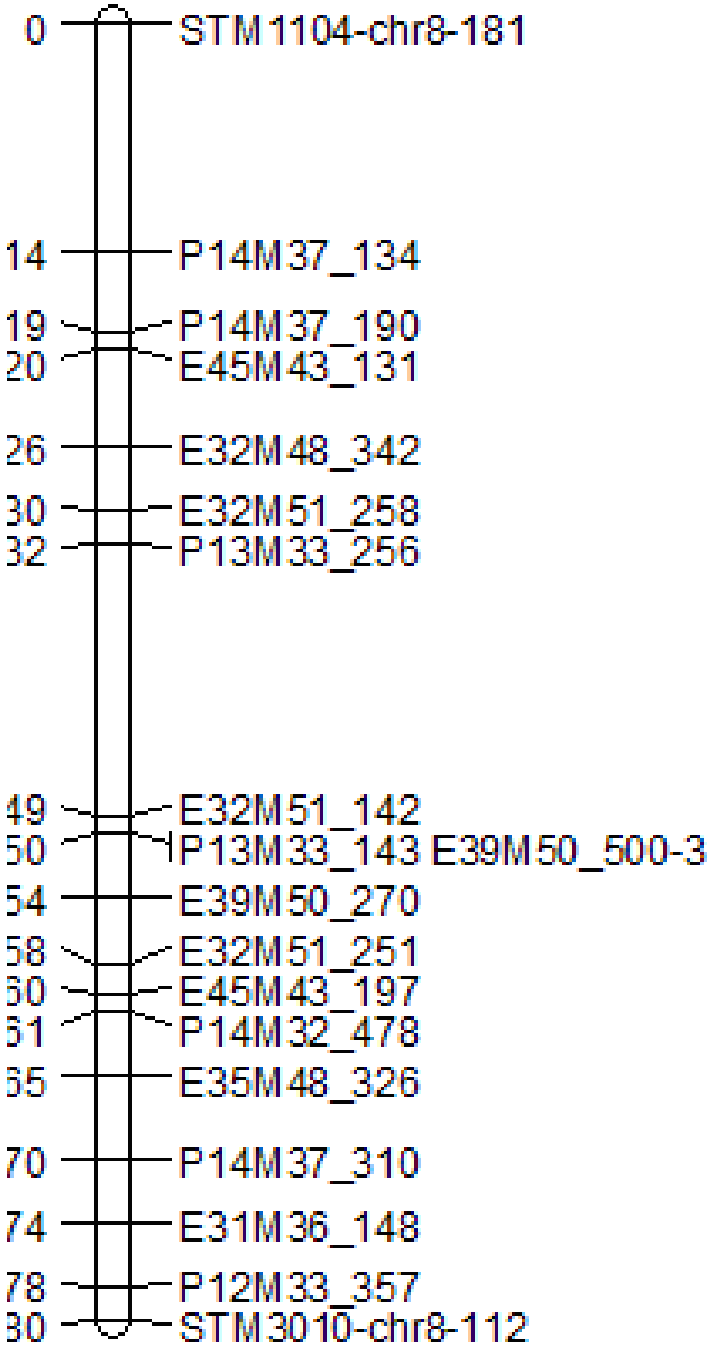
320-02 Chromosome 5



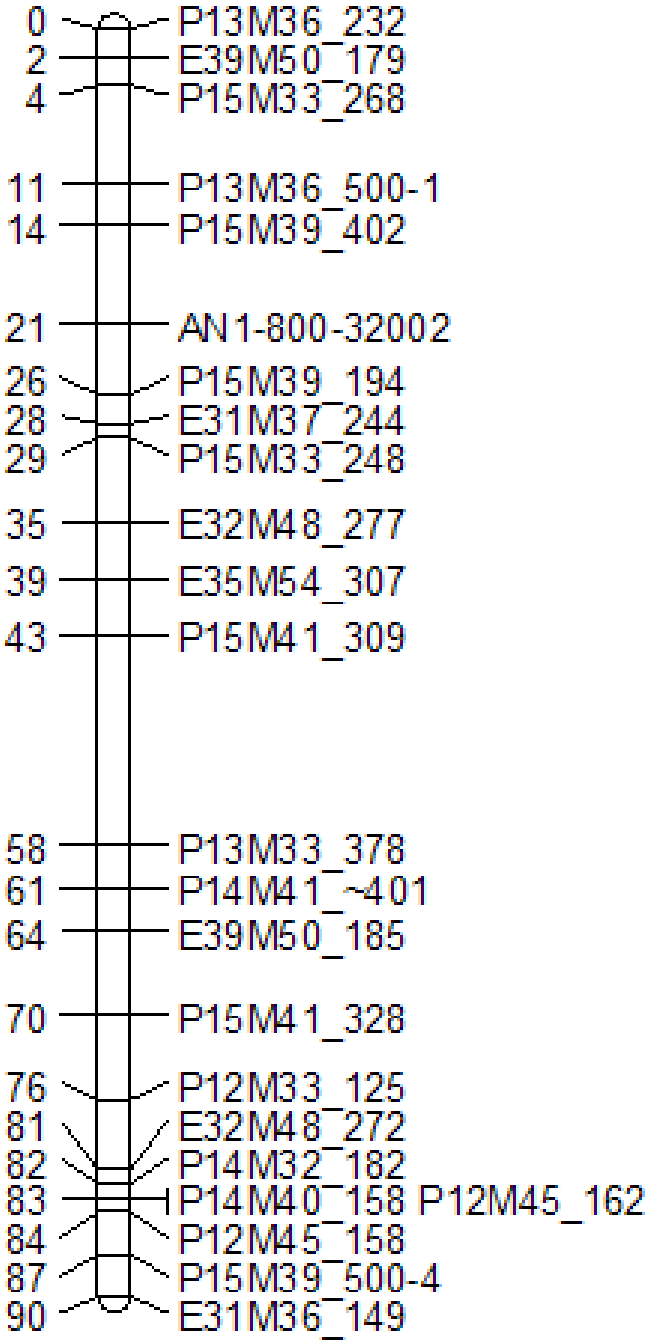
320-02 Chromosome 6



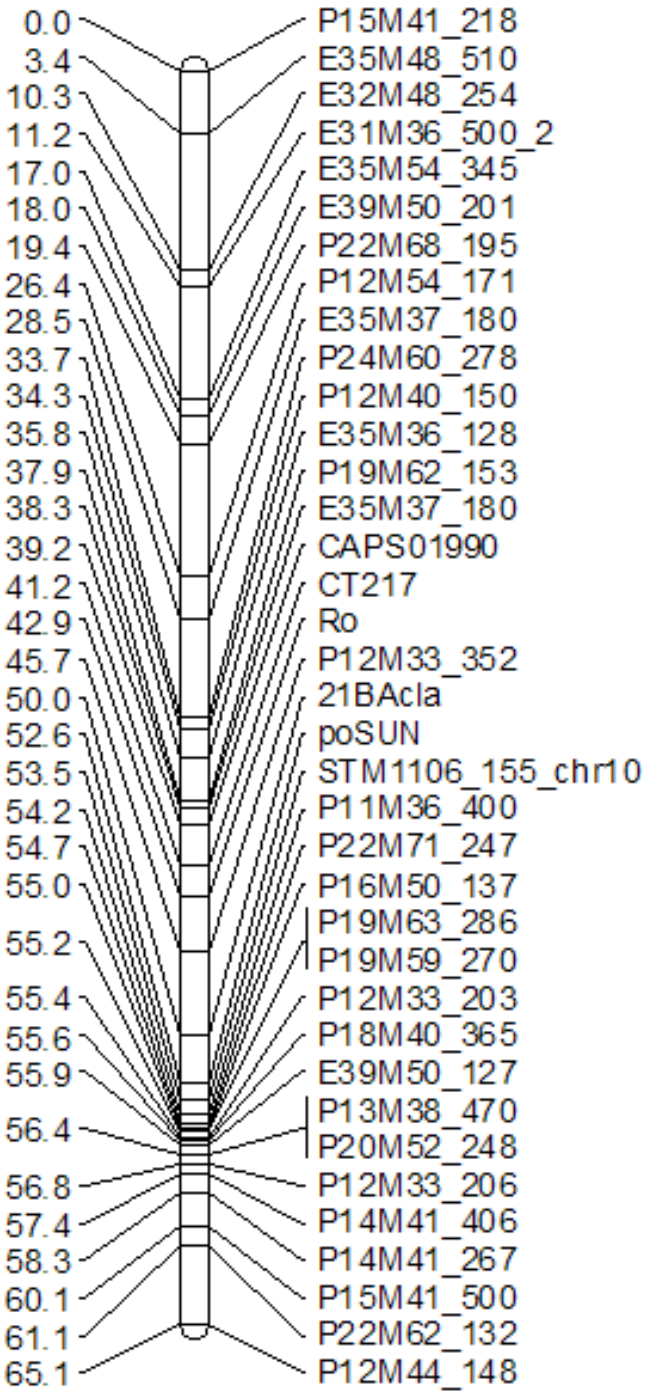
320-02 Chromosome 7



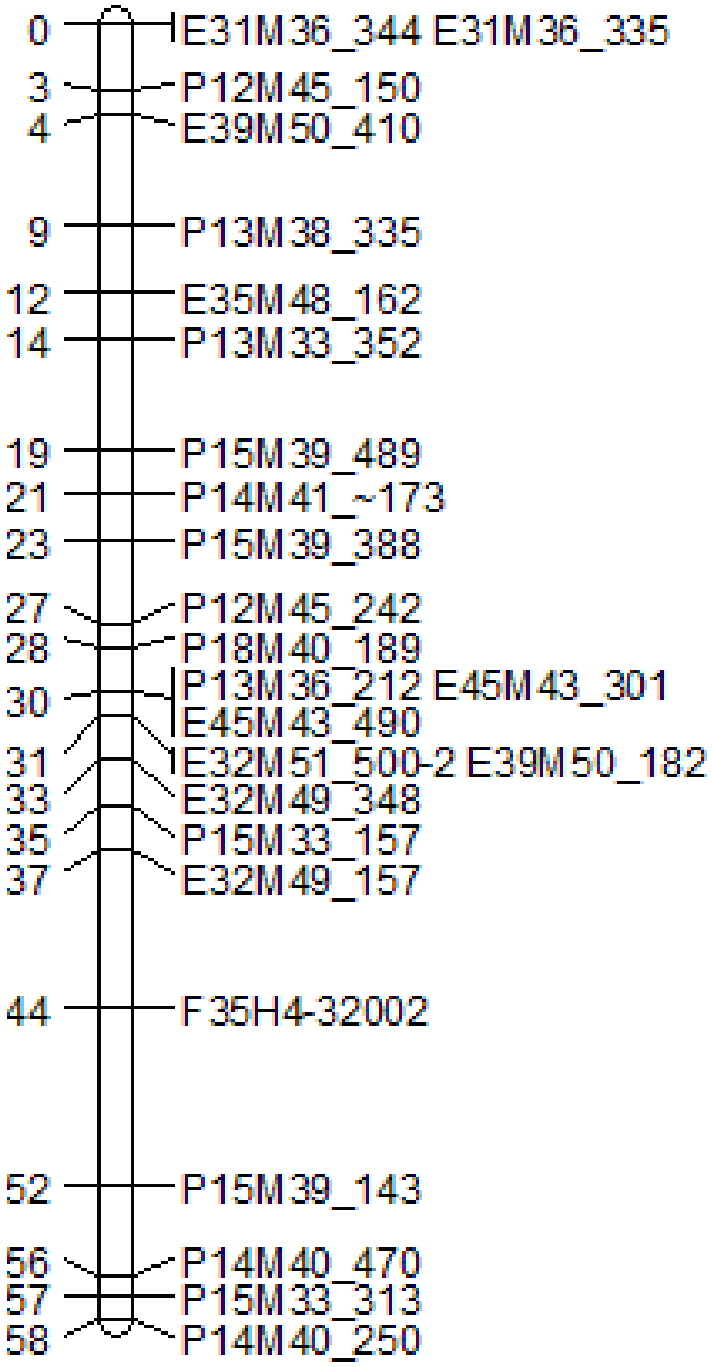
320-02 Chromosome 8



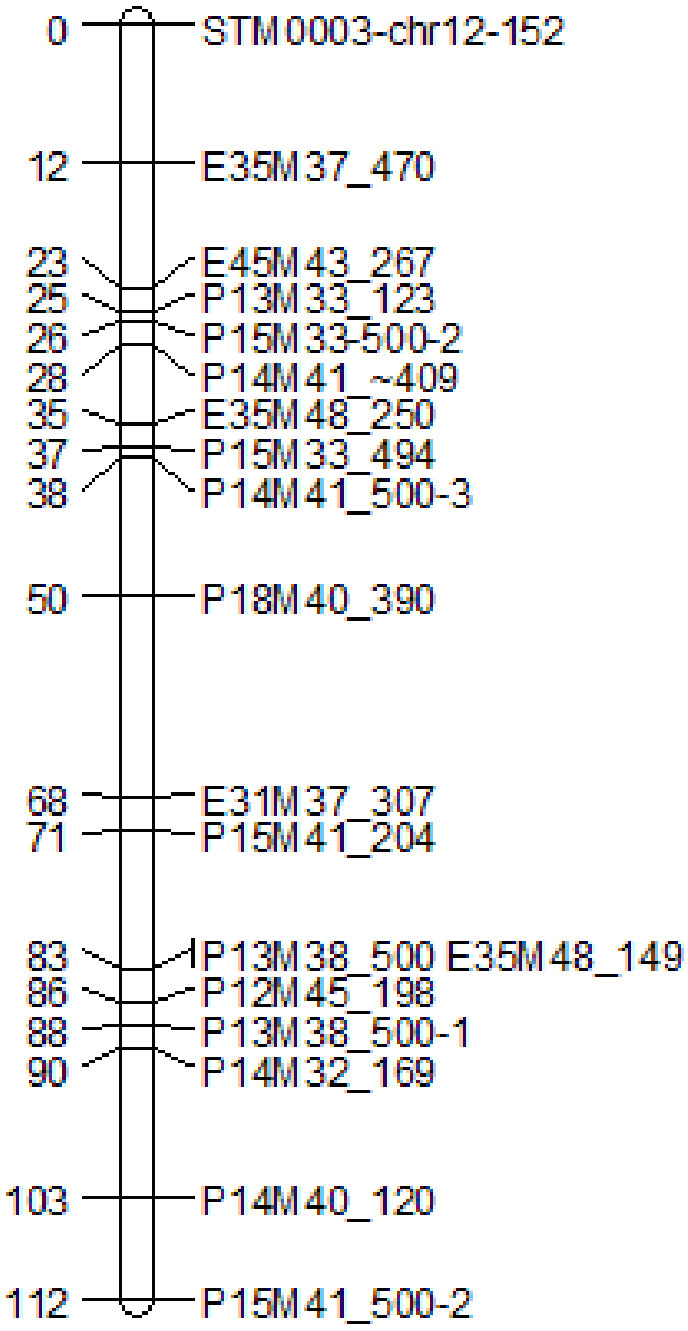
320-02 Chromosome 9



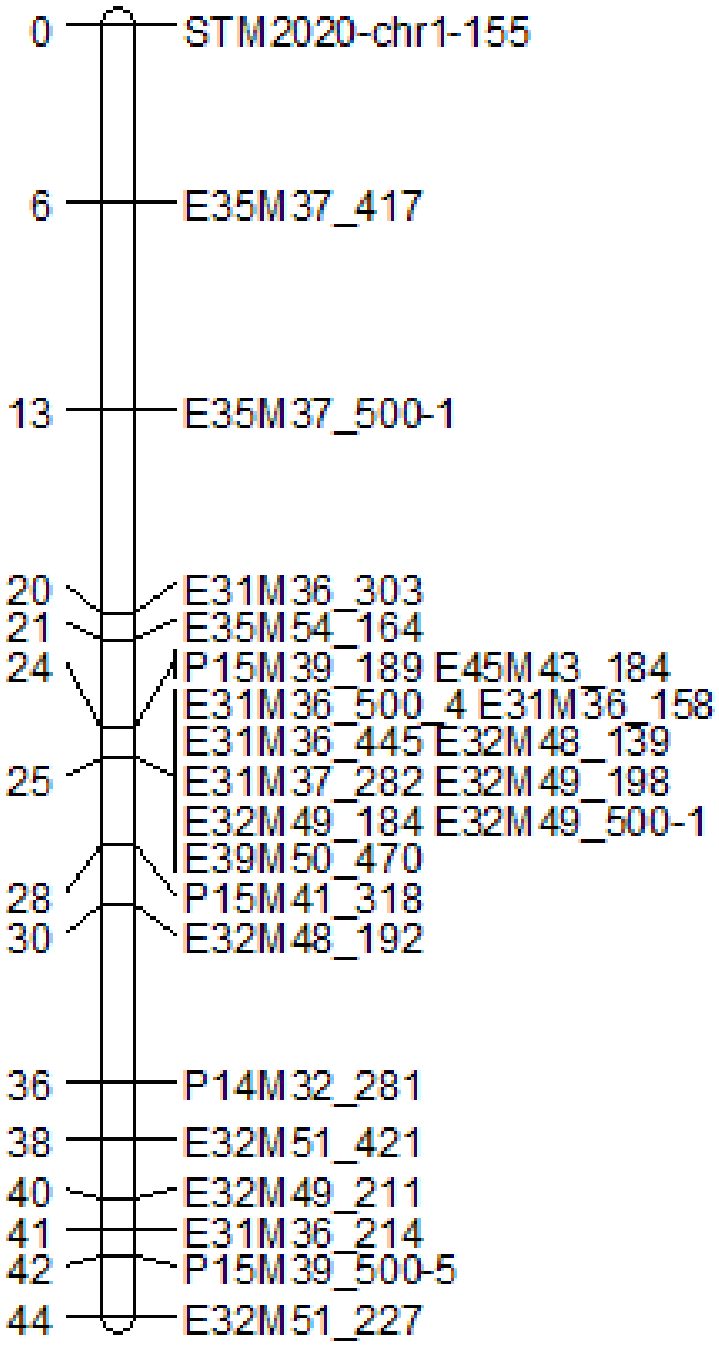
320-02 Chromosome 10



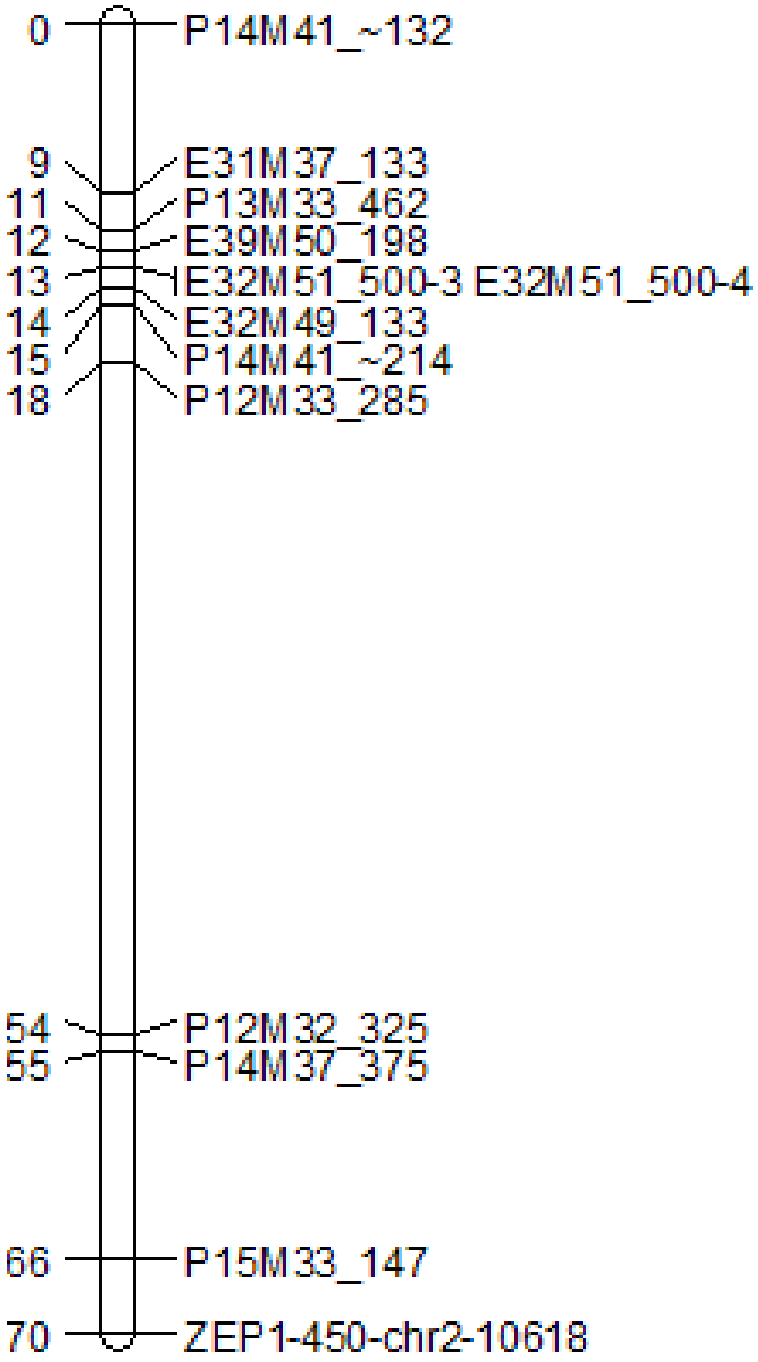
320-02 Chromosome 11



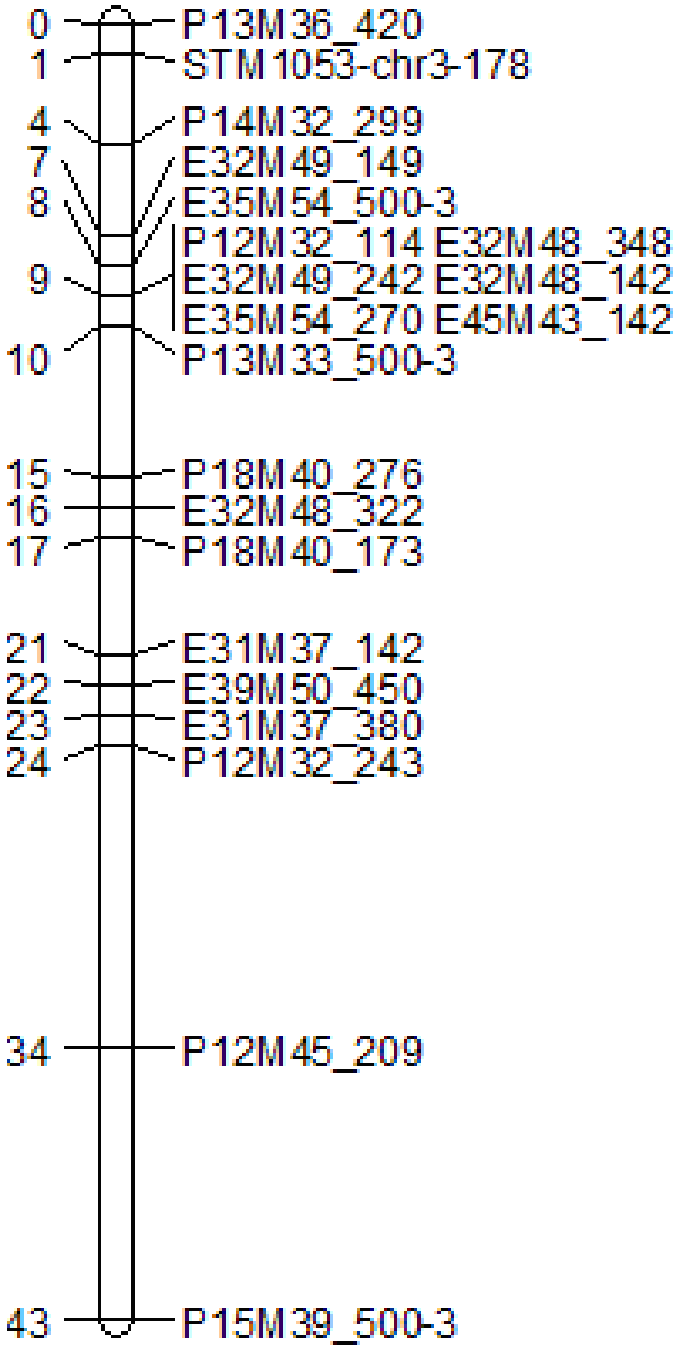
320-02 Chromosome 12



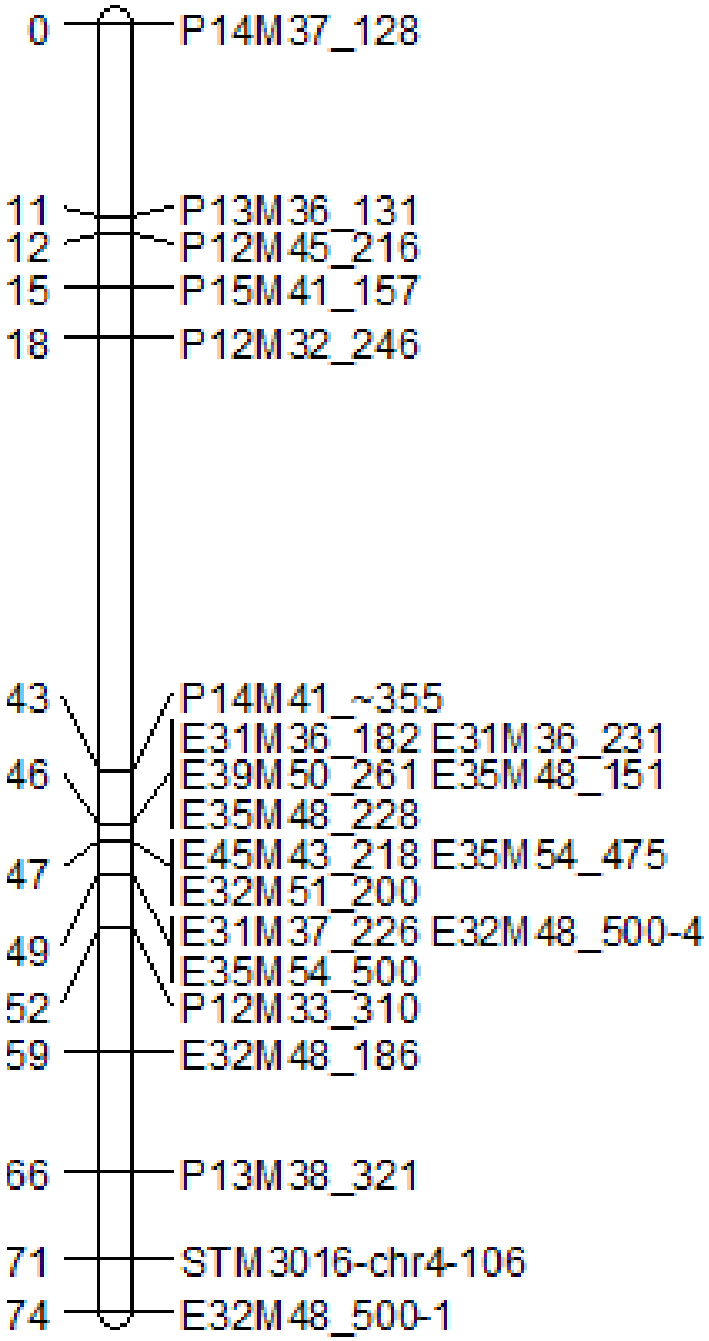
10618-01 Chromosome 1



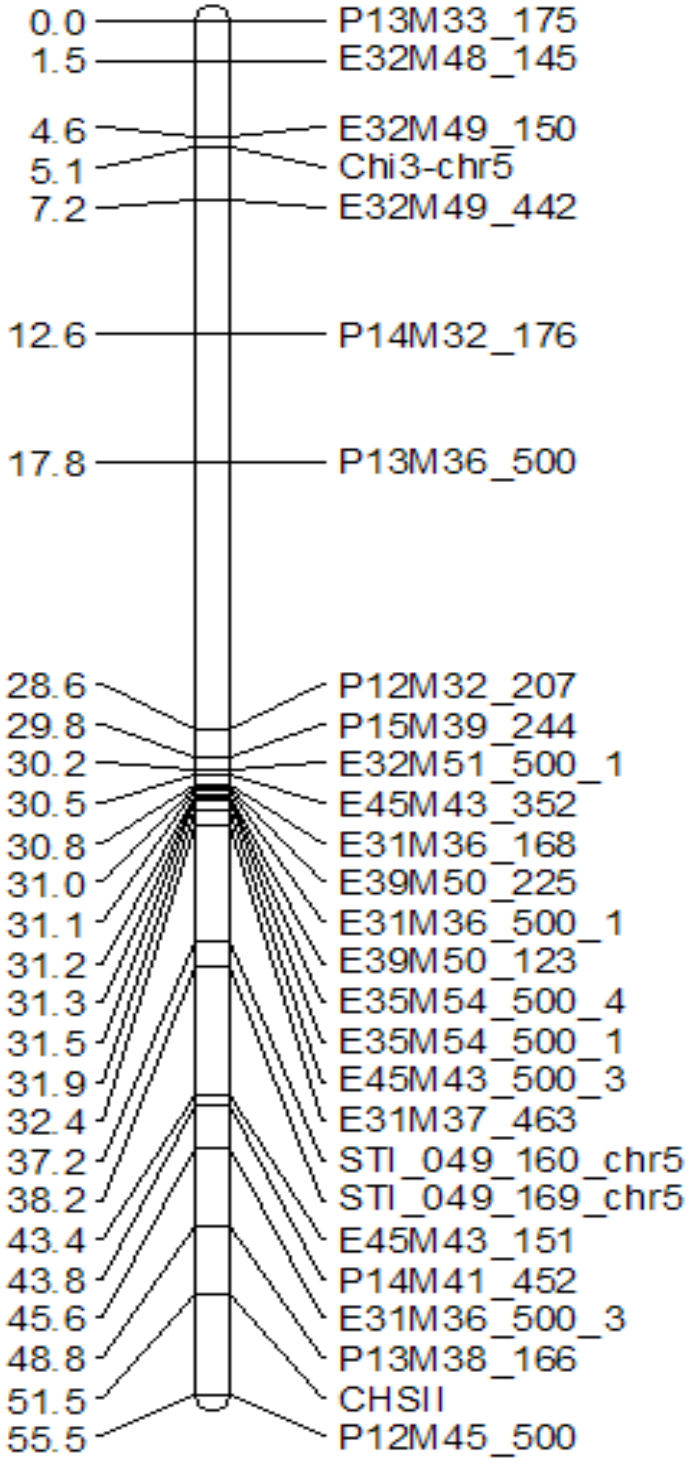
10618-01 Chromosome 2



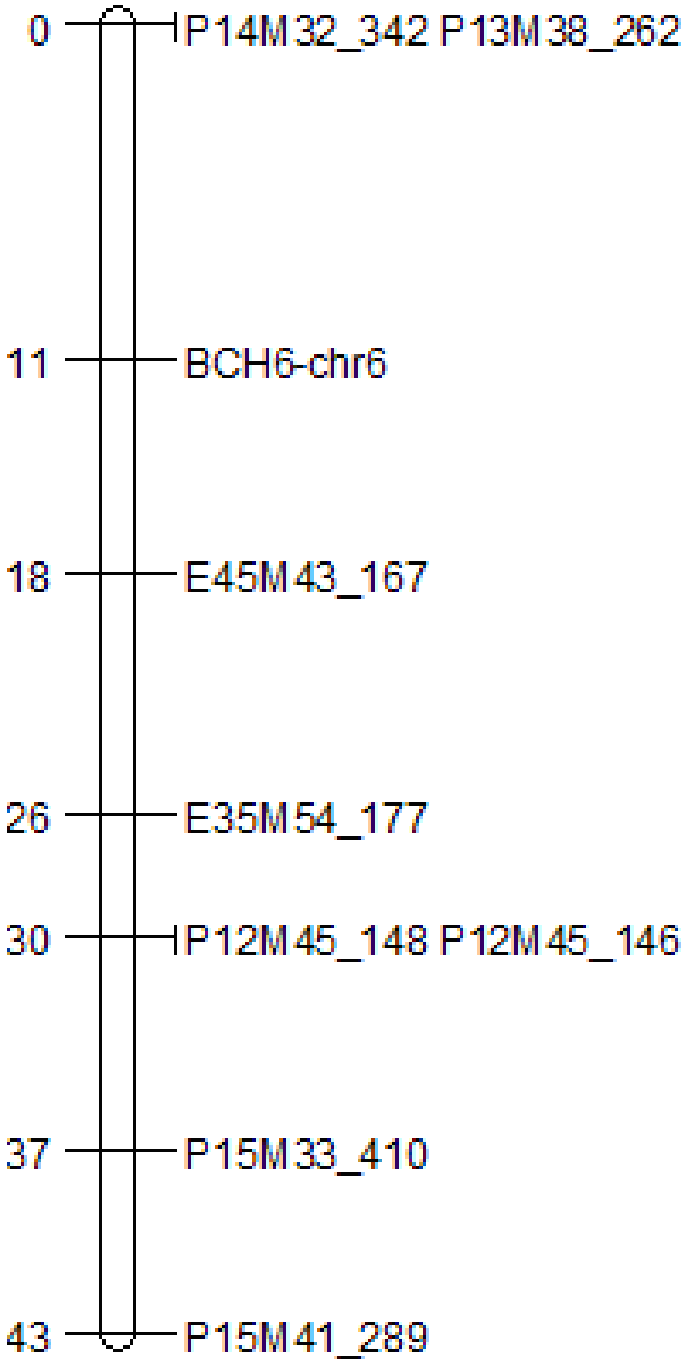
10618-01 Chromosome 3



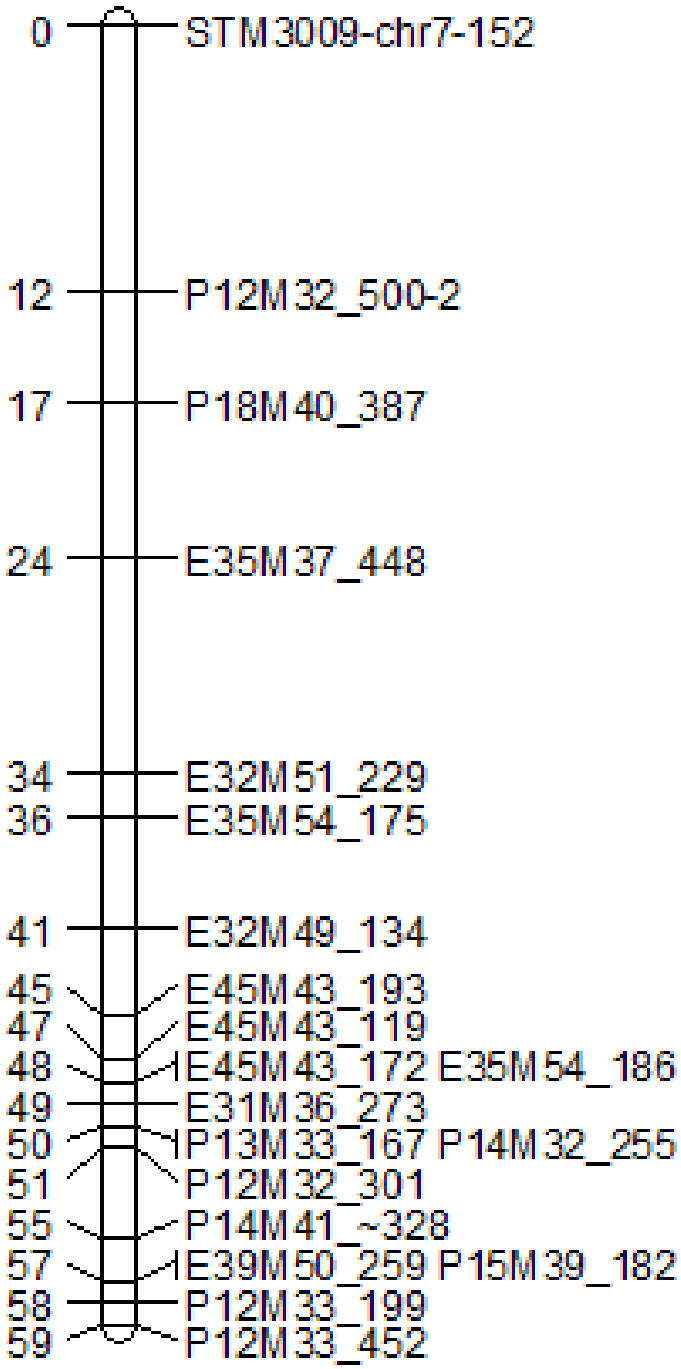
10618-01 Chromosome 4



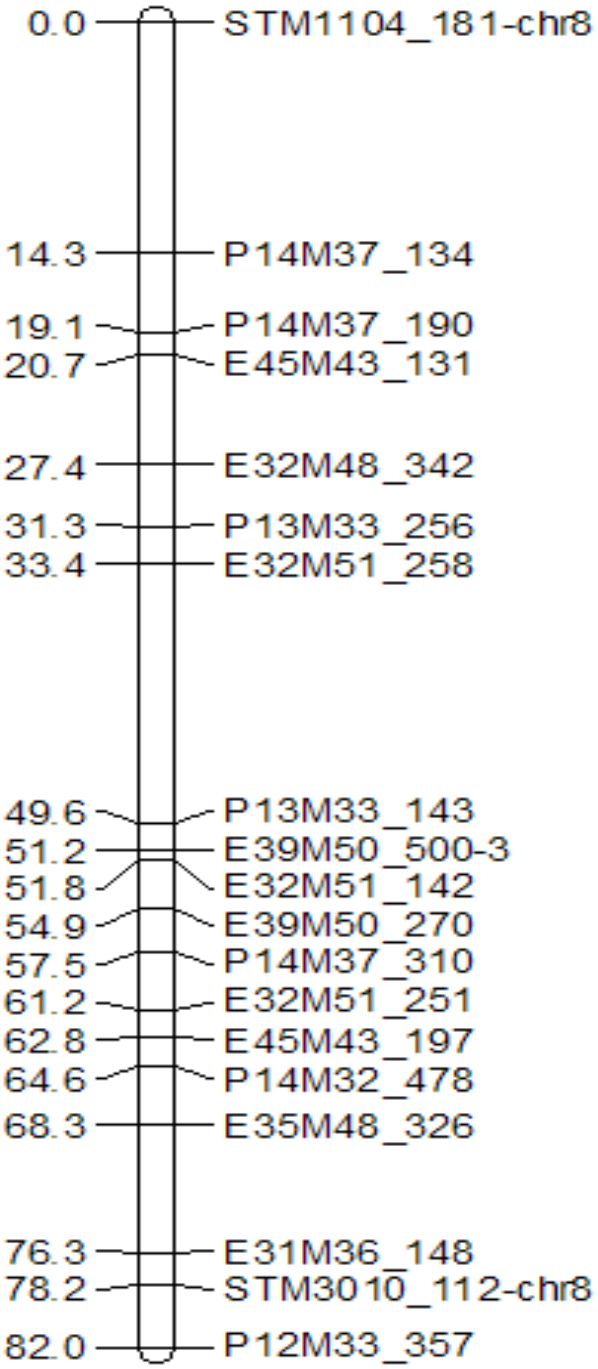
10618-01 Chromosome 5



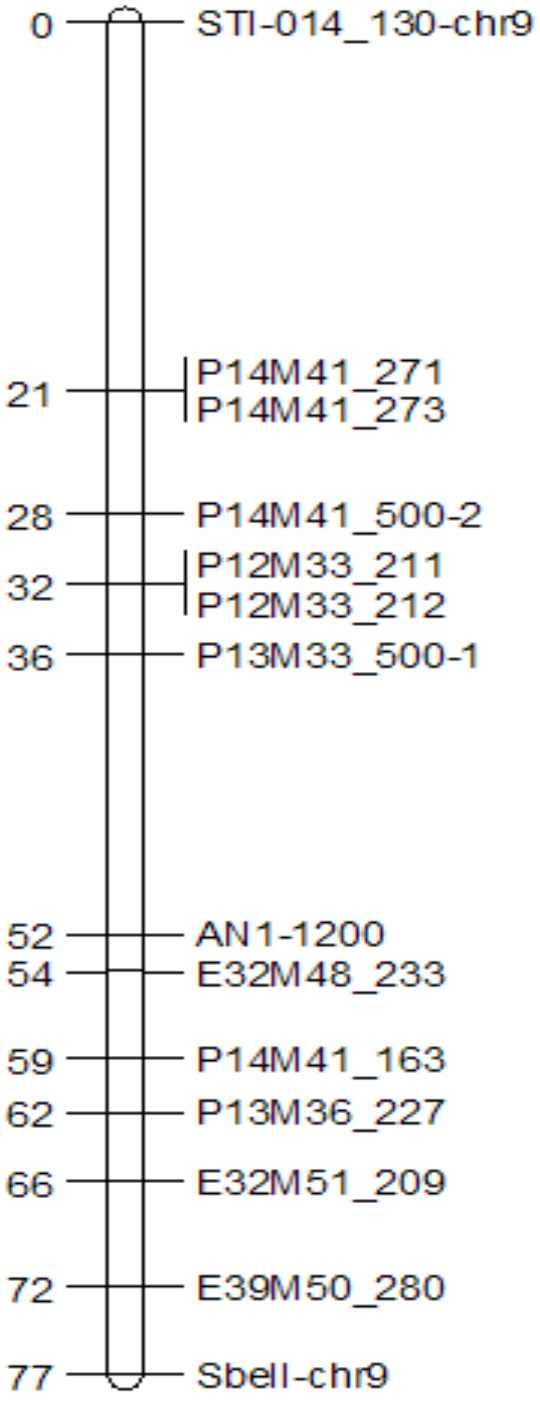
10618-01 Chromosome 6



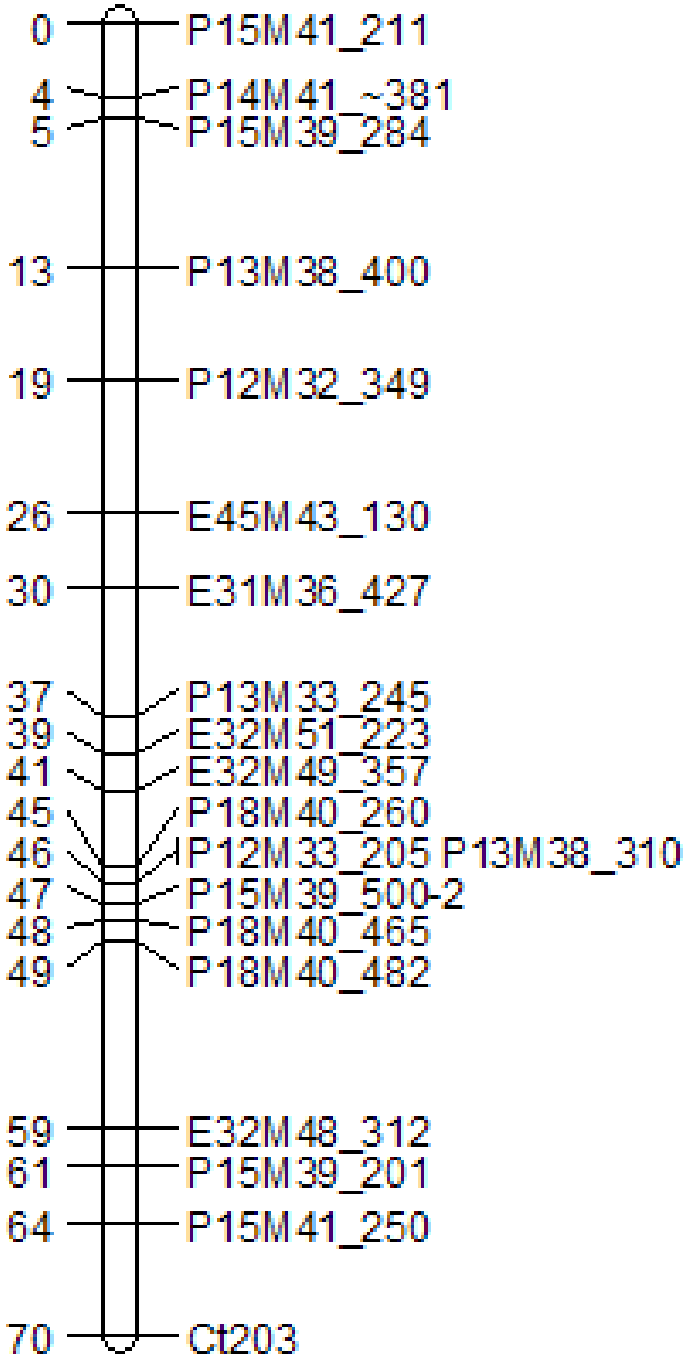
10618-01 Chromosome 7



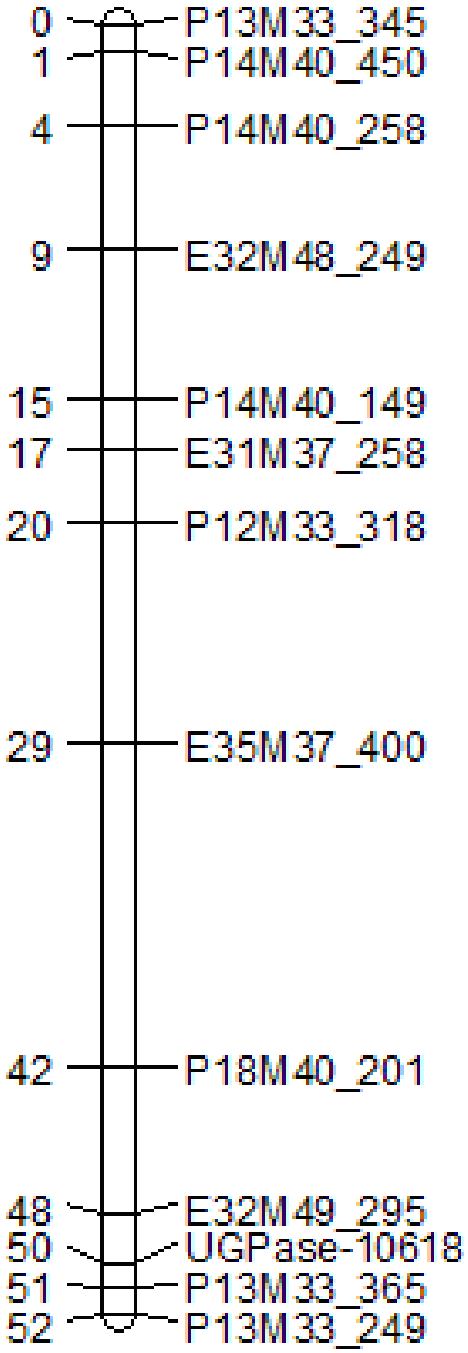
10618-01 Chromosome 8



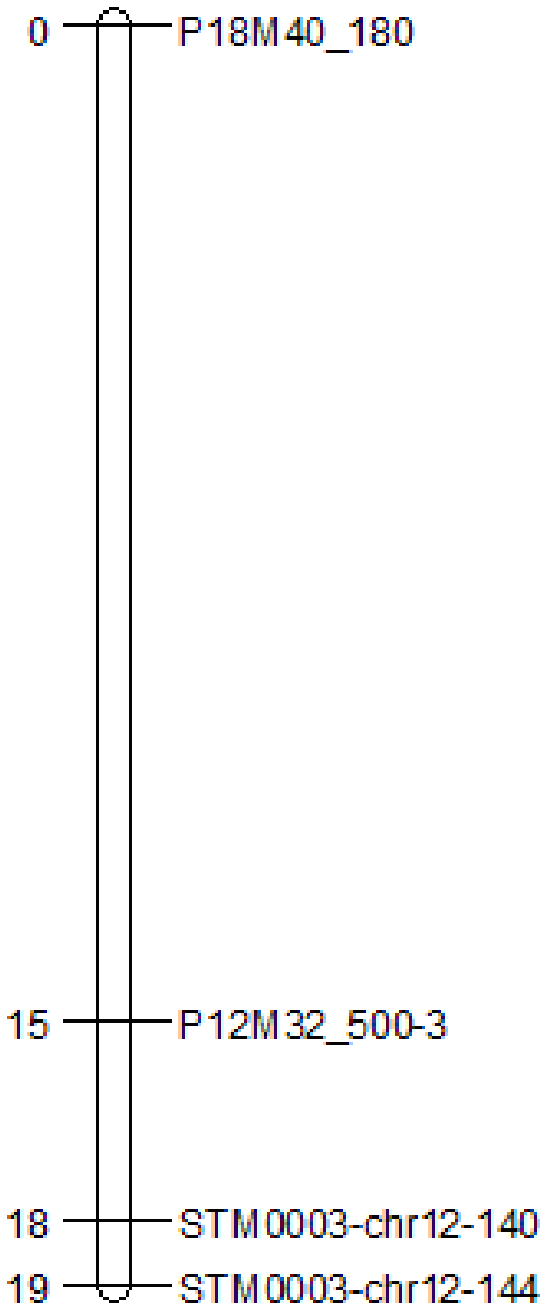
10618-01 Chromosome 9



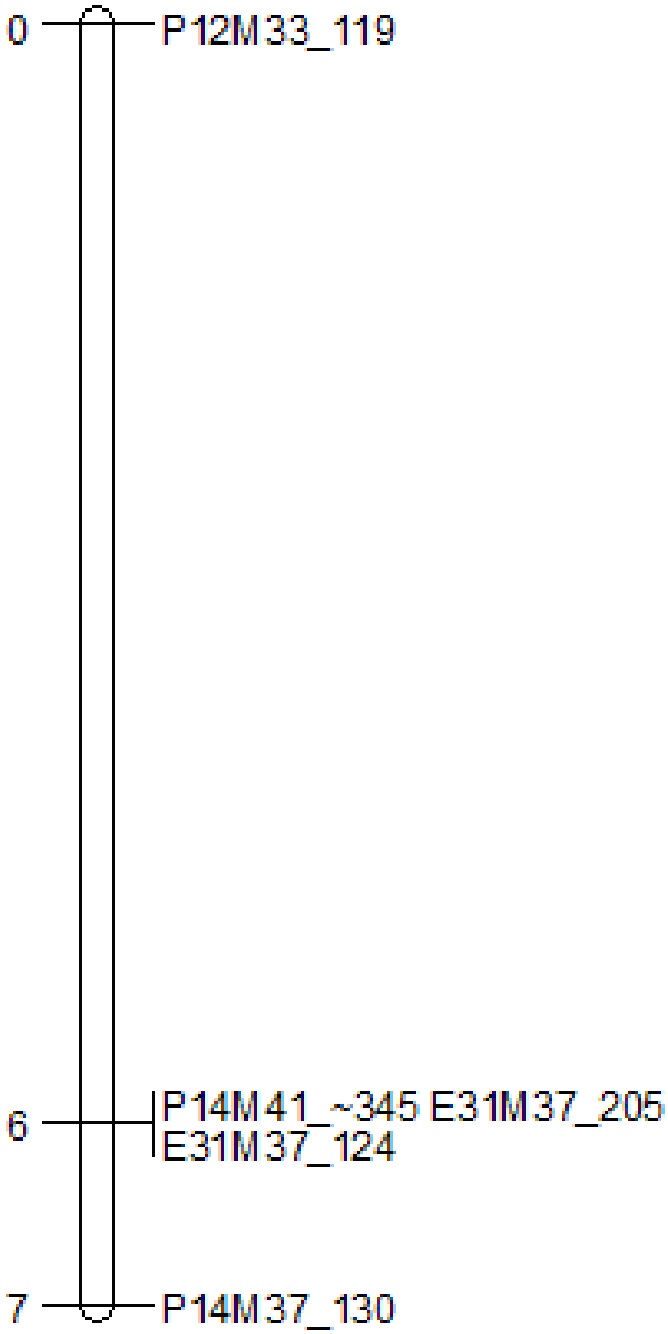
10618-01 Chromosome 10



10618-01 Chromosome 11



10618-01 Chromosome 12



10618-01 linkage group unlabeled