

RESEARCH

Genetic Map and QTL Analysis of Agronomic Traits in a Diploid Potato Population using Single Nucleotide Polymorphism Markers

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ABSTRACT

Genetic maps now can be constructed using thousands of genomewide single nucleotide polymorphisms (SNPs) for identification of markers closely associated with agronomic traits. A diploid mapping population for potato (*Solanum tuberosum* L.) was developed from a pseudo-testcross between a homozygous line *S. tuberosum* Group Phureja DM 1-3 516 R44 and a heterozygous outcrossing *S. tuberosum* Group Tuberosum clone, RH89-039-16. The population of 96 individuals was evaluated for seven traits in two consecutive years (2012 and 2013). Yield (total tuber yield [TTY], average tuber weight [ATW], and number of tubers per plant [TS]), food quality (specific gravity [SPGR]), and plant development traits (vigor, maturity [Mat], and tuber end rot [TER]) were studied. Sixteen different quantitative trait loci (QTL) were identified. A QTL with major effects at 11.9 cM corresponding to 3.7 Mb on chromosome V of potato genome assembly explained between 20.3 and 75.7% of variance for TS, ATW, vigor, Mat, and TER. For TTY, ATW and SPGR, the QTL was detected at 6.4 and 12.9 cM. The other 15 QTL were located on chromosomes I, II, III, IV, V, VI, IX, X, and XII. In general, the results confirmed QTL previously identified for yield, SPGR, and Mat in diploid and tetraploid populations. The Infinium 8303 Potato Array provides an efficient means of scoring genomewide markers for constructing high-resolution genetic maps and thereby facilitates identification of genomic regions closely associated with genes coding for agronomic traits of interest.

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Abbreviations: ATW, average tuber weight; DH, doubled haploid; LOD, logarithm of odds; Mat, maturity; MIM, multiple interval mapping; MQM, multiple QTL models; QTL, quantitative trait locus or loci; REML, restricted maximum likelihood method; SNP, single nucleotide polymorphism; SPGR, specific gravity; TER, tuber end rot; TS, tubers per plant; TTY, total tuber yield.

GENETIC MAPS and quantitative trait locus (QTL) analysis of diploid potato have been valuable for studying the genetic basis of important agronomic traits. Multiple QTL throughout the genome have been reported as genetic factors controlling yield as well as developmental and quality traits in potato. Common QTL for tuber yield and specific gravity or tuber starch content have been reported on chromosomes I, II, III, V, and VII using different diploid populations (Bonierbale et al., 1993; Freyre and Douches, 1994; Schäfer-Pregl et al., 1998). On the basis of co-localization with QTL in diploid populations, further research using candidate gene and association mapping approaches in tetraploid populations allowed identifying diagnostic markers for superior alleles associated with tuber starch, sugar content, and

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yield (Draffehn et al., 2010; Gebhardt et al., 2005; Li et al., 2008; Li et al., 2005; Li et al., 2013). Linkage mapping analysis of agronomic traits in tetraploid potatoes also identified co-localization of QTL in previous reports using diploid populations (Bradshaw et al., 2008; McCord et al., 2011a). Quantitative trait loci for yield were identified on chromosomes I, II, V, VI, VIII, and XII and for specific gravity and dry matter on chromosomes II, V, VIII, IX, and XI. Starch content and specific gravity are important traits for industrial and food processing of potato, and increasing yield is a main goal for improving production efficiency for growers.

Plant maturity and tuberization are related physiological traits controlled by similar genetic factors and day-length. In potato, maturity is the physiological time point where plants have finished tuber bulking, the skin has set (periderm is developed), dry matter reaches its maximum, and the vines turn yellow and senesce (Herrman et al., 1995). The maturity type (early, intermediate, late) is an estimation of the length of the growing season required to harvest potatoes under temperate field conditions (Haga et al., 2012). Methods vary for measuring maturity. Breeders often estimate maturity by monitoring physiological changes in vine growth (completion of apical canopy growth, time of leaf senescence, and collapse of vines) and tuber bulking (time of tuber formation or rapid increase in harvest index) over many years (Haga et al., 2012; Kloosterman et al., 2013; Struik et al., 2005). Multiple QTL have been reported for maturity on all 12 chromosomes (Danan et al., 2011). However, a major effect QTL on chromosome V has been identified by measuring either tuber induction (earliness) (Fernandez-Del-Carmen et al., 2007; Simko et al., 1999; Van den Berg et al., 1996), onset and end of senescence (Celis-Gamboa et al., 2003; Hurtado et al., 2012; Malosetti et al., 2006), or foliage maturity (Bradshaw et al., 2004; Collins et al., 1999; Oberhagemann et al., 1999; Visker et al., 2003). The major gene for maturity on chromosome V was identified by Kloosterman et al. (2013). This locus encodes a transcription factor that regulates initiation of tuber development and plant maturity by acting in a photoperiod-mediated pathway that controls signals for tuberization.

Among the recently developed genomic resources for potato, the Infinium 8303 Potato Array presents a powerful tool for high-throughput genotyping with single nucleotide polymorphisms (SNPs). Single nucleotide polymorphisms on the array have genome-wide coverage and can be readily scored using the Illumina genotyping platform (Felcher et al., 2012). All 8303 Potato Array SNPs have been physically mapped on version 4.03 of the potato genome sequence assembly (<http://potato.plantbiology.msu.edu/cgi-bin/gbrowse/potato/>, accessed 1 June 2015) and their concordance with genetic maps has been confirmed (Felcher et al., 2012; Sharma et al., 2013).

Several high-density genetic maps have been constructed in diploid populations of potato (Felcher et al., 2012; Sharma et al., 2013; Van Os et al., 2006). In addition to offering genome-wide coverage and close mapping of traits of interest, these could constitute reference maps with transferable markers. Danan et al. (2011) mapped common markers in several segregating populations to construct a consensus map for late blight and maturity traits. Consensus maps could be useful for decreasing the QTL confidence intervals to identify a set of markers for selection of candidate genes in the anchored genome sequence. Likewise, dense genetic maps using transferable markers anchored to the genome sequence could be used as the starting point of a combined strategy with candidate gene and association mapping approaches to identify diagnostic markers for complex traits as proposed by Gebhardt et al. (2011). In the present study, the Solanaceae Coordinated Agricultural Project (SolCAP) Infinium 8303 Potato Array was used to build a genetic map of a diploid segregating population. The doubled monoploid *S. tuberosum* Group Phureja 1-3 516 R44-DM 1-3 (Paz and Veilleux, 1999) and the breeding line that is mostly *S. tuberosum* Group Tuberosum RH89-039-16-RH (Roupe van der Voort et al., 1997; Van Os et al., 2006) were selected as parental lines to discover and validate QTL and candidate genes associated with agronomic traits in potato on the basis of the available genomic resources (Hirsch et al., 2014) as well as their contrasting phenotypes. The population from the cross between DM 1-3 $\times$ RH was designated DRH (Felcher et al., 2012). This is the first instance of a diploid homozygous line of potato being used for both mapping and QTL analysis. Because of the 1:1 segregation of heterozygous alleles of RH, this represents a “one-way pseudo-testcross” in comparison with a two-way pseudo-testcross configuration for mapping intra- or interspecific crosses of highly heterozygous species proposed by (Gratapaglia and Sederoff, 1994). For QTL detection, up to three alleles could segregate at any locus in this population (Q1Q1 $\times$ Q2Q3). This allows the study of additive effects of RH allelic configurations in two genotypic classes (Q1Q2, Q1Q3) with a common female allele. The population was evaluated for seven agronomic traits over 2 yr, and QTL analysis was employed to identify associated markers. Most of the identified QTL co-localized with previously reported QTL and potential candidate genes that were successfully anchored to the potato genome. These results are part of a project to attempt fine mapping of genomic regions associated with fitness traits.

MATERIAL AND METHODS

Plant Material

The DRH diploid mapping population comprised of 98 individuals was generated from a cross between the doubled monoploid *S. tuberosum* Group Phureja 1-3 516 R44 (female) and

the breeding hybrid *S. tuberosum* Group Tuberosum and Group Phureja RH89-039-16 (male). The RH clone, kindly provided by Wageningen University (Dr. Herman J. van Eck), was previously used to create an ultra-high density map of diploid potato (Van Os et al., 2006) and is further described by the Potato Genome Sequencing Consortium (2011). DM 1-3 is an anther-derived homozygous line, with short height, small leaves, and low production of elongated fingerling tubers with deep eyes, yellow flesh, and reddish skin. DM 1-3 also exhibits tuber stem end rot after harvest, which limits long-term storage of tubers. RH is more vigorous and adapted to potato production under temperate long-day conditions, with round white tubers and greater canopy size and height compared with DM 1-3.

Phenotyping

In 2012 and 2013, the DRH population was grown in the field at the Michigan State University Montcalm Research Center, Entrican, MI. Planting material consisted of greenhouse-grown tubers obtained from plants that were initiated from tissue culture and harvested in the Virginia Tech greenhouses after a 16-wk growing season. The planting and harvesting dates for 2012 were 23 May and 7–10 Sept., while for 2013 they were 14 May and 23 Sept. The experimental design was a randomized complete block with three replications of eight tuber plots, 2.4 m per plot. Seven traits associated with agronomic performance were evaluated. Yield was evaluated as three traits (total tuber yield [TTY] in kg/plant, number of tubers or tuber set [TS] per plant, and average tuber weight [ATW] in g), one for food quality (specific gravity [SPGR]) and three for physiologic plant development (vigor, maturity [Mat], and tuber end rot [TER]). Specific gravity was estimated using the formula $[\text{air weight}/(\text{air weight} - \text{water weight})]$ for a minimal sample size of 1 kg/plot. Plant vigor was scored approximately 3 mo after planting using a 1 to 5 scale (1: low vigor; 5: high vigor). The vigor rating increased for plants with greater leaf area, number of stems, height, and overall plant size. Maturity was evaluated on a 1 to 5 scale (1: early, vines completely dead; 5: late, full green vines and flowering) at about 120 d after planting before vine desiccation. DM 1-3 tubers exhibit a progressive rot from the basal end of the tuber at the point of stolon attachment affecting all periderm, vascular ring, and parenchymal tissue. This post-harvest deterioration of tubers has been constantly and indiscriminately observed regardless of growing conditions (field, greenhouse, or growth chamber) or location (Virginia Tech and Michigan State University). This trait had a wide segregation in the DRH progeny that was assessed. Presence and severity of TER was evaluated on a scale 0 to 5 (0: no TER; 5: severe TER). The mean values for each clone were used for QTL analysis of the seven traits.

Single Nucleotide Polymorphism Genotyping

Total genomic DNA was isolated from leaf samples using the DNeasy plant mini kit (QIAGEN). A final concentration of 50 ng/ μL was determined using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). Four μL of DNA per sample were SNP-genotyped with the Infinium 8303 Potato Array as described by Felcher et al. (2012). Single nucleotide polymorphism genotypes were called using the SolCAP custom three cluster-calling file (http://solcap.msu.edu/potato_infinium.shtml; accessed 15 June 2015) and the Illumina GenomeStudio 2011.1 software.

Low-quality and monomorphic SNPs, and those that physically mapped to more than one site on the potato genome sequence, were eliminated from the data set. Visual inspection of the cluster in the GenomeStudio graphs was done to eliminate SNPs with subclusters potentially located in paralogous regions in the genome and to manually correct genotype calls using the genotype calling function implemented in GenomeStudio as reported by Troggio et al. (2013).

Linkage Map

Unlike previous mapping populations in diploid potato, this is a homozygous by heterozygous cross, which allowed a study of recombination during gamete formation of a single meiosis from RH alleles. Segregating SNPs with the expected 1:1 segregation ratio, based on an α threshold of 0.001% of the Chi-square test, were initially used for mapping. JoinMap 4.1 software (Van Ooijen, 2006) was used to generate the 12 linkage groups using the cross-pollinated population type (coded as <nnxnp>) and the multipoint maximum likelihood method optimized with a Gibbs sampling procedure (Van Ooijen, 2011). Linkage groups were defined using an independence test with a logarithm of odds (LOD) score minimum of 5. Several rounds of mapping with increasing levels of distorted segregation were necessary to generate a high-quality genetic map with the greatest number of SNPs and distorted segregation. Graphical genotyping of mapped SNPs in JoinMap 4.1 was used to visualize recombination bins and discard individuals with an excessive number of recombination events. This process was also used to identify singletons, genotyping errors due to unlikely events of double recombination (Van Os et al., 2005) as described by Ward et al. (2013).

QTL Analysis

MapQTL 6 was used for QTL detection using the genotype coding (a, b) for doubled haploid (DH) population type (Van Ooijen, 2009). Since only the RH parental alleles can be observed to segregate in this population, the DH model with two genotypes ($a \times b$, $Q1 \times Q2$) takes advantage of using the linkage phase information generated in the cross-pollinated mapping type, and pseudo testcross approach for QTL identification. An LOD threshold for QTL detection was calculated for each trait by permutation tests with 1000 permutations to control for a genomewide error rate of 5%. Interval mapping was executed to identify main QTL. Then, either markers on QTL peaks or those selected with the automatic cofactor selection function were chosen as cofactors to fit the multiple QTL mapping (MQM) model. When new peaks arose from each MQM session, the linked markers were also added as cofactors, and the analysis was then repeated. One or several MQM sessions with different cofactors were executed until detection of QTL with greater values of LOD and explained variance. The final set of QTL detected per trait was reported. The 2-LOD confidence interval was calculated for each QTL peak.

Epistasis Analysis

Multiple interval mapping (MIM) method (Kao et al., 1999) implemented in WinQTLCart version 2.5 (Wang et al., 2012) was used to identify epistatic interactions between QTL found

for the same trait. Since WinQTLCart 2.5 does not support analysis for cross-pollinated populations, marker genotype data were translated to a backcross type using their phase and genotype configuration to account only for recombination bins across the population. QTL models were created per trait using the default MIM forward search procedure, 0.5 to 1 cM search walk speed, and 0.05 level of significance. For each QTL detected, the QTL position and MIM model were optimized using the refine model function. The criteria of MIM model selection were 0.05 significance level, 1 cM walk speed, and at 3 cM window size.

Heritability and Correlation Analysis

The restricted maximum likelihood method (REML) was used to calculate broad-sense heritability (H^2) with clone as random effect and year as fixed effect. The heritability was estimated on a genotype mean basis as the ratio of:

$$H^2 = \frac{\sigma_g^2}{\left(\sigma_g^2 + \frac{\sigma_{g \times y}^2}{m} + \frac{\sigma_e^2}{rm} \right)}$$

where (σ_g^2), ($\frac{\sigma_{g \times y}^2}{m}$), and ($\frac{\sigma_e^2}{rm}$) are the genetic, genotype $\times$ year interaction, and residual variance components, m is the number of years, and r is the number of replications.

Additionally, clone means of samples harvested in 2012 and 2013 were used to estimate Pearson correlations between generations as proposed by Frey and Horner (1957). Pearson correlation was also used to estimate correlations between traits using the REML method when samples were missing. Means, variances, correlation, and distribution analyses were calculated using JMP 10 (SAS Institute, Inc.).

RESULTS

Phenotypic Data

The data for both years from the evaluated traits exhibited continuous, unimodal, close to normal distribution for yield traits (TTY, TS, and ATW) and SPGR, while bimodal normal distribution for vigor, Mat, and TER were observed (Fig. 1). The analysis of variance for 89 samples with data for 2012 and 2013 showed significant differences among clones for all traits ($P < 0.0001$), with vigor only measured in 2012. There was a significantly greater population mean in 2012 than in 2013 for all traits ($P < 0.0001$) except for TER ($P = 0.74$). However, there was a significant genotype $\times$ year interaction for all traits ($P < 0.01$). Field data were collected only for the RH parental line because DM 1–3 plants were too weak for field conditions. RH means were close to population means, except for SPGR in 2012 and for TER (Table 1). In general, the population showed a lower SPGR than RH. Extreme values of progeny means showed wide distribution, thus good segregation for the evaluated traits. The significant correlations between progeny average in two consecutive years were low for TS (29.2%, $P = 0.005$) and for SPGR

(29.5%, $P = 0.006$), while high for Mat and TER (68.1 and 63.9%, $P < 0.0001$). Similar patterns were obtained for estimations of broad-sense heritability. There was no heritability estimation for TTY. The broad-sense heritability was 54.7% for TS, 28% for ATW, and 46.4% for SPGR, while 86% and 80.6% for Mat and TER, respectively. These results for Mat and TER were associated with the greater phenotypic variance explained by clones than by genotype $\times$ year interaction or residual variances.

Correlations Between Traits

The coefficients of correlation between traits are listed in Tables 2 and 3. For the yield traits, TTY was highly significantly correlated with TS and ATW for both years (0.60 and 0.41 for 2012 and 0.85 and 0.55 in 2013, respectively), while the correlation between TS and ATW was significantly negative in 2012 and not significant in 2013. On the basis of the coefficient of variation (mean/standard deviation), there was greater variation for TS in 2013 than in 2012 (0.76 vs. 0.42). Likewise, the coefficient of variation for TTY increased from 0.41 in 2012 to 0.95 in 2013. Total tuber yield did not correlate with any other trait in 2012 but with Mat and TER in 2013 (0.59 and -0.28 , respectively). Tuber set was positively correlated with Mat and slightly negatively correlated with TER in both years (0.51 and -0.36 for 2012 and 0.47 and -0.24 for 2013, respectively). Average tuber weight was slightly negative (-0.25) and positively (0.26) correlated with SPGR in 2012 and 2013, respectively. Maturity and vigor were highly correlated (0.81), with similar patterns of correlation with the other traits. Tuber end rot correlated negatively with SPGR and Mat both years (-0.4 and -0.62 , and -0.37 and -0.47 , respectively).

Linkage Map

A high-density linkage map with 1948 segregating SNPs and 813.2 cM length was constructed (Supplemental Fig. S1). The marker order and interval distance were consistent even when including SNPs with distorted segregation greater than an α threshold of 0.001% of the Chi-square test. Linkage groups were defined for all chromosomes at LOD score of 10 except for chromosome 12, which grouped at 5. Graphical genotyping analysis of the recombination events allowed identification of two individuals with high rates of recombination that were eliminated from the analysis. Likewise, a singleton SNP was identified; this corresponded to a 0.05% genotyping error rate in the SNP mapping dataset. This specific locus datum was recoded as missing data. The rate of missing data was of 1.04% (1 out of 96 individuals) in six loci. From the total mapped SNPs, 1366 SNPs cosegregated with the other 582 segregating SNPs. The SNPs with identical genotypes were detected on the basis of analysis of similarity of 1. With bin mapping positions as reference, the interval distance between

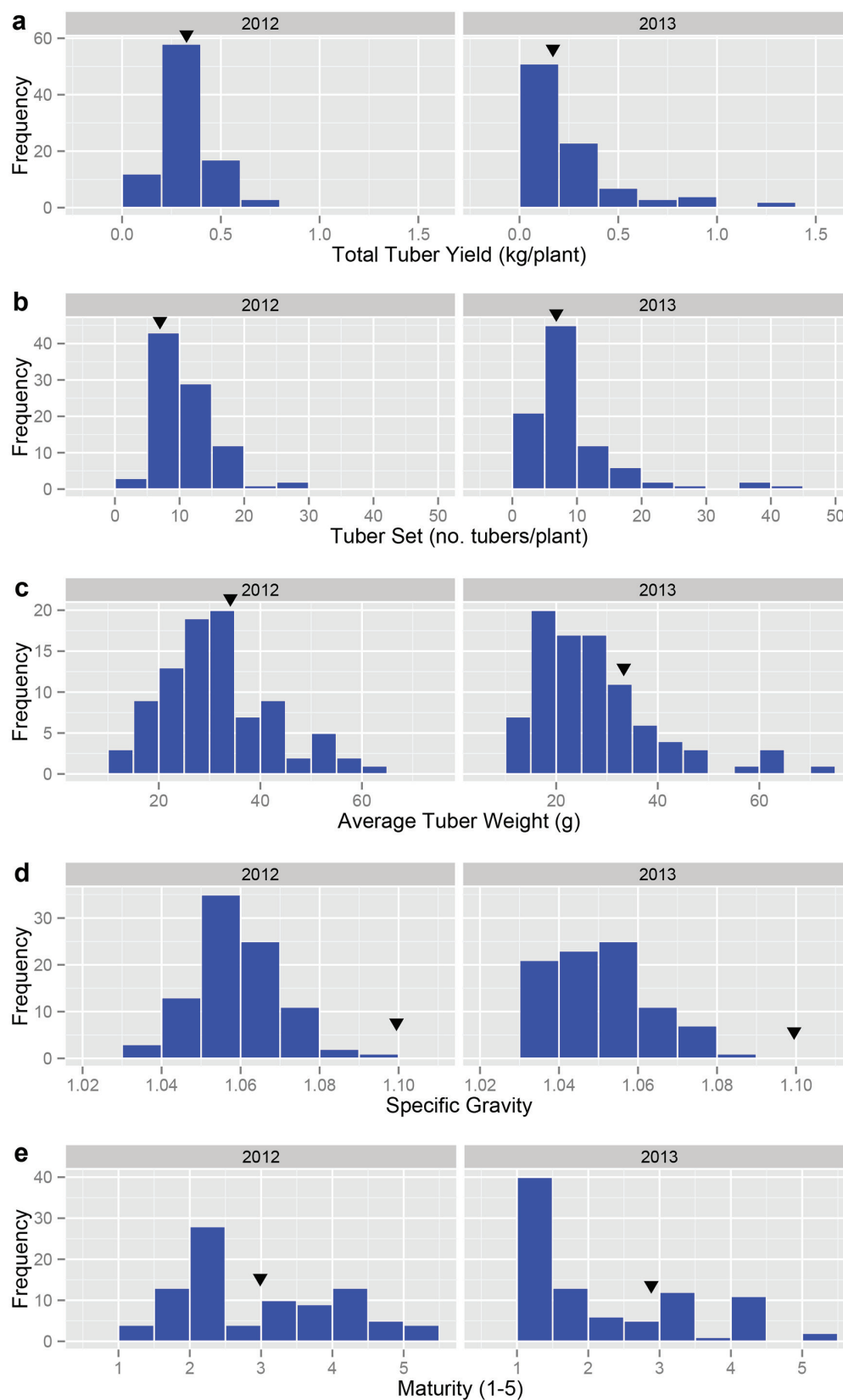


Figure 1. (continued on next page) Frequency distribution of 2012 and 2013 clone means for seven agronomic traits. (a) total tuber yield (kg/plant); (b) tuber set (no. tubers/plant); (c) average tuber weight; (d) specific gravity; (e) maturity (1 = early and 5 = late); (f) tuber end rot (0 = no rot and 5 = high-severity tuber end rot); (g) vigor (1 = weak and 5 = vigorous). RH parent mean denoted by a black triangle.



Figure 1. Continued.

Table 1. Mean performance of RH parent and DRH progeny, population extremes, and standard deviation (SD) for 2012 and 2013.

Trait [†]	Year	RH mean	DRH	SD	Min.	Max.
TTY	2012	0.3	0.3	0.1	0.1	0.8
	2013	0.2	0.3	0.3	0.03	1.4
TS	2012	7	11	4.6	3.1	26.7
	2013	6.7	9	6.8	1.9	41
ATW	2012	34.1	31.4	10.9	10.5	64.6
	2013	34.4	27.9	12.5	12.3	70
SPGR	2012	1.12	1.06	0.01	1.04	1.09
	2013	1.06	1.05	0.01	1.03	1.08
Mat	2012	3	2.9	1.1	1.3	5
	2013	2.5	2.1	1.1	1	5
TER	2012	0	1.3	1	0	4.3
	2013	0	1.3	0.8	0	4.5
Vigor	2012	2	2.7	0.8	1	5

[†] TTY, total tuber yield in kg/plant; TS, tuber set; ATW, average tuber weight in g; SPGR, specific gravity; Mat, maturity; and TER, tuber end rot.

markers varied from 1.1 to 11.7 cM, with an average density of 2 cM between SNPs. The 12 linkage groups were well defined and identified on the basis of SNP positions on the pseudomolecule assembly version 4.03 of the potato genome sequence (Sharma et al., 2013). The genomewide coverage of SNPs from the Illumina 8303 Potato Array is 720.6 Mb, while that for SNPs mapped in the DRH population was 703.7 Mb with 99% correspondence. Between 88 (chromosome III) and 259 (chromosome I) SNPs were mapped per chromosome (Table 4).

Table 2. Coefficient of correlation among traits in 2012.

Trait [†]	TTY	TS	ATW	SPGR	Mat	Vigor	TER
TTY	–						
TS	0.60***	–					
ATW	0.41***	–0.43***	–				
SPGR	–0.11	0.08	–0.25*	–			
Mat	0.06	0.51***	–0.50***	0.21*	–		
Vigor	0.05	0.43***	–0.45***	0.26*	0.81***	–	
TER	0.08	–0.36***	0.51***	–0.40***	–0.62***	–0.61***	–

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

[†] TTY, total tuber yield in kg/plant; TS, tuber set; ATW, average tuber weight in g; SPGR, specific gravity; Mat, maturity; and TER, tuber end rot.

Table 3. Coefficient of correlation among traits in 2013.

Trait [†]	TTY	TS	ATW	SPGR	Mat	TER
TTY	–					
TS	0.85***	–				
ATW	0.55***	0.13	–			
SPGR	0.19	0.06	0.26*	–		
Mat	0.59***	0.47***	0.48***	0.46***	–	
TER	–0.28**	–0.24*	–0.14	–0.37***	–0.47***	–

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

[†] TTY, total tuber yield in kg/plant; TS, tuber set; ATW, average tuber weight in g; SPGR, specific gravity; Mat, maturity; and TER, tuber end rot.

Table 4. Summary of single nucleotide polymorphism (SNP) marker information of DRH population linkage map. Chr, chromosome; cM, centimorgan; Mb, megabase pair.

Chr	Mapped SNPs	cM	Mb	No. bins
I	259	96.2	87.7	55
II	209	66.3	40.7	37
III	88	67.5	57.6	24
IV	222	75.8	72	47
V	133	71.1	51.9	29
VI	205	52.2	56.5	33
VII	150	52.3	55.4	28
VIII	144	58.9	56.3	32
IX	166	86.2	61.4	44
X	113	62.2	59.3	29
XI	128	64.7	44.1	31
XII	131	59.7	60.8	25
Total	1948	813.2	703.7	414

QTL Analysis

The 582 segregating SNPs mapped to 414 unique bin positions that were used for the QTL analysis. A total of 16 different QTL positions on nine chromosomes was identified using MQM in MapQTL 6 (Table 5). A common QTL on chromosome V at 11.9 cM position was identified for TS, Mat, and TER for 2012 and 2013. This QTL also was detected for ATW and vigor in 2012. The proportion of trait phenotypic variance explained (R^2) by this QTL varied from 20.3% for TS in 2012 to 75.7% for Mat in 2012. A close QTL at 6.4 cM was identified for TTY and ATW in 2013 with R^2 of 27.4 and 21.6%, respectively, and at 12.9 cM for SPGR in 2013 with 49.4% of the phenotypic variance explained. In fact, when means of samples with homozygous vs. heterozygous genotypes for the SNP located at 11.9 cM (solcap_snp_c1_3793) were compared, the heterozygous genotype was highly significantly ($P < 0.0001$ for single marker ANOVA analysis) associated with greater TTY (nn = 0.18, np = 0.38 in 2013), TS (nn = 9.3, np = 13.5 and nn = 7, np = 12 for 2012 and 2013, respectively), SPGR (nn = 1.05, np = 1.06 in 2013), more vigorous plants (nn = 2.2, np = 3.4 in 2012), later Mat (nn = 2.1, np = 4 and nn = 1.5, np = 3 for 2012 and 2013, respectively), and lower TER (nn = 1.8, np = 0.6 and nn = 1.6, np = 0.8 for 2012 and 2013, respectively).

The majority of additional QTL identified were for ATW and TER. For ATW, one QTL was identified on chromosome I (at 41.7 cM in 2012 and 2013), two QTL on chromosome IV (at 15 cM in 2012 and 22.4 cM in 2013), and another one on chromosome VI (at 5.3 cM in 2012). Two of the QTL identified on chromosome IV as well as V were located in different positions each year. In general, the QTL detected for ATW showed the greatest values for additive effects and switched from positive to negative between 2012 and 2013 on chromosome V. The additive effects calculated by the QTL mapping algorithm for DH population

type, correspond to the mean difference between phased a and b genotypic classes divided by two. However, for our population type, where a combination of two alleles segregates per genotypic class (nn, np recoded as a and b), the additive effects correspond only to one allele substitution, thus to the difference between a and b. Tuber end rot had one QTL on three chromosomes: I (at 44.9 cM in 2012), III (at 19.2 cM for both years), and VI (at 35.3 cM for both years). Total tuber yield had one QTL on chromosomes II and XII (at 36.1 cM and 27.2 cM in 2012, respectively). Tubers per plant and SPGR had a QTL on chromosome V and IX at 26.7 cM in 2013 and 23.3 cM in 2012, respectively. For vigor we detected two QTL on chromosomes III and X at 0 and 44.9 cM in 2012. The variation explained by this set of QTL varied between 10.3 and 28.3%.

Maturity effects were removed by regressing each trait using Mat as predictor. The residuals from the regression analysis were used to repeat the entire QTL analysis. Similar results were obtained using either residual or raw data. Only two QTL from a total of 16 were identified using the residuals. These were on chromosomes I and VI, both for ATW. Using the residuals for QTL analysis also increased LOD and R^2 values of QTL on chromosome IV for ATW. Removing the variance due to Mat effect allowed the identification of some minor-effect QTL and increased the resolution of some major-effect QTL.

Epistasis Analysis

Multiple QTL were detected for most of the traits. Multiple interval mapping in WinQTLCart 2.5 was executed to identify epistatic interactions between QTL for each trait. Three epistatic interactions were detected (Table 6). For TTY a novel QTL on chromosome II (7.4 cM, LOD = 3.9 and R^2 = 10.6) was interacting with the QTL on chromosome V in 2013. The allelic genotype of SNPs on chromosome II and V (solcap_snp_c1_11344 and solcap_snp_c1_3793) had means of nn = 0.19, np = 0.32 and nn = 0.18, np = 0.39, respectively. For TER, the QTL on chromosomes I and V showed interaction for 2012 (solcap_snp_c1_9573 and solcap_snp_c1_3793 with means nn = 1.0, np = 1.7 and nn = 1.8, np = 0.6), and the QTL on chromosomes III and VI in 2013 (solcap_snp_c2_20347 and solca_snp_c1_10130 with means nn = 1.03, np = 1.7 and nn = 1.6, np = 1.1). Specific allelic combinations at both loci were associated with either yield or TER. The QTL positions detected by MIM were similar to the QTL detected by the MQM method and corresponded to the same SNP or a nearby locus. In general the SNP genotypes from chromosome V with greater TTY and lower TER inherited the same haplotype from RH.

DISCUSSION

DRH is the first segregating diploid population of potato using one homozygous parental line crossed to a

Table 5. Quantitative trait loci (QTL) identified in the DRH population. Chr, chromosome; LOD, logarithm of odds; % R^2 , percentage of explained variance.

Trait [†]	QTL locus [‡]	Chr	cM position	cM Interval	LOD [§]	% R^2	Additive effect	Year
TTY	chr02_35.2_c2_40169	II	36.1	33.9–39.3	4.7	12.6	0.1	2012
	chr05_3.1_c1_3840	V	6.4	4.2–6.4	6.2	27.4	0.2	2013
	chr12_6.5_c2_1542	XII	27.2	27.2–32.2	4.3	13.4	0.3	2012
TS	chr05_3.7_c1_3793	V	11.9	6.4–12.9	4.6–7.3	20.3–31.4	2.1–6.2	2012–2013
	chr05_12.2_c2_38456	V	26.7	23.5–27.7	3.1	11.9	–3.5	2013
ATW	chr01_71.5_c2_12126	I	41.7	39.6–44.9	4.3–3.3	18.4–15.1	–4.4––4.2	2012–2013
	chr04_5.4_c1_16079	IV	15	118–16.1	5.8	23.8	–4.6	2012
	chr04_46.8_c1_3319	IV	22.4	22.4–25.6	7	28.3	–17.9	2013
	chr05_3.7_c1_3793	V	11.9	6.4–12.9	7.7	31.6	–6.2	2012
	chr05_3.1_c1_3840	V	6.4	6.4–12.9	4.7	21.6	6.5	2013
	chr06_4.2_c2_3104	VI	5.3	3.2–6.3	3.1	10.3	3	2012
SPGR	chr05_5.5_c2_51464	V	12.9	11.8–14	12.9	49.4	0.01	2013
	chr09_6.4_c2_13242	IX	23.3	17.8–23.3	5.1	20.8	0.01	2012
Vigor	chr03_4.4_c1_13052	III	0	0–2.1	3.6	13.8	–0.3	2012
	chr05_3.7_c1_3793	V	11.9	6.4–12.9	15.7	53.9	0.6	2012
	chr10_56_c2_48126	X	44.9	43.9–49.3	4.7	18.7	–0.4	2012
Mat	chr05_3.7_c1_3793	V	11.9	6.4–12.9	28.6–14.7	75.7–53.2	1–1.2	2012–2013
TER	chr01_72.5_c1_9573	I	44.9	41.7–45.9	4.3	12.4	–0.4	2012
	chr03_44.3_c1_20437	III	19.2	17.1–21.3	3.3–4.8	13.6–20.3	0.4–0.4	2012–2013
	chr05_3.7_c1_3793	V	11.9	6.4–12.9	10–6.4	34.1–27.1	–0.6––0.5	2012–2013
	chr06_49.9_c1_10130	VI	35.3	33.2–36.4	3.1–2.8	12.8–11.3	0.4–0.3	2012–2013

[†] TTY, total tuber yield in kg/plant; TS, tuber set; ATW, average tuber weight in g; SPGR, specific gravity; Mat, maturity; and TER, tuber end rot.

[‡] The QTL locus name represents, separated by underline, the pseudomolecule and Mb position in the latest potato genome assembly (version 4.03), and the Solanaceae Coordinated Agricultural Project (SolCAP) single nucleotide polymorphism (SNP) identification.

[§] LOD threshold > 3 for QTL identified in a single year and > 3.7 for those detected simultaneously in 2012 and 2013 on the basis of the 95th percentile in a permutation test or at least 1000 iterations.

^{||} Additive effects calculated as the mean difference between phased a and b genotype divided by two (half the value for the cross-pollinated population).

Table 6. Significant epistatic interactions detected by multiple interval mapping. Chr, chromosome; LR, likelihood ratio; LOD, logarithm of odds; % R^2 , percentage of explained variance.

Trait [†]	QTL locus [‡]	Chr	cM	LR	LOD	% R^2	Additive effect	Year
TTY	chr02_21.7_c1_11344	II	7.4	17.8	3.9	10.6	0.2	2013
	chr05_3.7_c1_3793	V	9.4	37.1	8.1	28.5	–0.3	2013
	Interaction	II*V		17.5	3.8	9.8	0.4	2013
TER	chr01_72.5_c1_9573	I	44.9	15.2	3.3	9.6	0.5	2012
	chr05_3.7_c1_3793	V	9.4	65.2	14.2	38	1.3	2012
	Interaction	I*V		20.9	4.5	10.1	–1.2	2012
	chr03_44.3_c1_20347	III	19.2	19.2	4.2	14.5	–0.6	2013
	chr06_49.9_c1_10130	VI	35.2	14.2	3.1	5.5	0.5	2013
	Interaction	III*VI		16.1	3.5	8.7	–1.1	2013

[†] TTY, total tuber yield in kg/plant; TER, tuber end rot.

[‡] The QTL locus name represents, separated by underline, the pseudomolecule and Mb position in the latest potato genome assembly (version 4.03), and the Solanaceae Coordinated Agricultural Project (SolCAP) single nucleotide polymorphism (SNP) identification.

heterozygous parent used for QTL analysis. The resulting population would be expected to segregate in a 1:1 ratio for loci of the heterozygous parent; therefore, this is a “one-way pseudo-testcross” genotyped with biallelic SNP markers where only the segregation of RH alleles was monitored during the mapping and QTL analysis process. The simpler genetic and allelic interaction of this population allowed the identification of regions associated with the traits by specifically studying the segregation of

two genotypic classes. In a full-sib segregating population from a cross of two heterozygous parents, up to four alleles could segregate per locus ($ab \times cd$). Therefore, both markers and QTL could have different segregation patterns and linkage phases between them. Three types of segregation patterns could be identified from locus to locus: testcross (1:1 segregation), F_2 cross (1:2:1 segregation), and full cross (1:1:1:1 segregation). The models and statistical methods for linkage analysis in outcrossing species have

been widely developed. In general, the estimation of the recombination frequencies has been based on two-point (Maliepaard et al., 1997; Ritter et al., 1990; Ritter and Salamini, 1996), three-point (Ridout et al., 1998; Wu et al., 2002), and different multipoint maximum likelihood methods (Tong et al., 2010; Van Ooijen, 2011). Similarly, QTL models for complex pedigree and biparental outbred populations have been developed and described (Gazaffi et al., 2014; Tong et al., 2012). The QTL detection and estimation of allelic effects are based on conditional QTL probabilities segregating for up to four genotypic classes in 1:1:1:1 and their linkage phases. In our pseudo-testcross ($Q1Q1 \times Q1Q2$ or $Q1Q1 \times Q2Q3$), two or three alleles could segregate in two genotypic classes with dominance ($Q1Q1$ vs. $Q1Q2$) or allelic interaction ($Q1Q2$ vs. $Q1Q3$). Comparison between genotypic classes from either segregation, two or three alleles, allowed the estimation of RH allele configuration effects for greater yield, SPGR, vigor, intermediate Mat, and low TER. DM 1-3 is a *S. tuberosum* Group Phureja line whereas RH is a hybrid with *S. tuberosum* Group Tuberosum and Group Phureja pedigree. Therefore, a simple two-genotype QTL model was appropriated to detect QTL and its estimated effects. The DH population type algorithm uses the phase information to associate QTL with either genotype a (representing the haplotype with “a” genotype with phase type [0] or “b” genotype with phase type [1]) or genotype b (that corresponded to “b” genotype with phase type [0] or “a” genotype with phase type [1]).

The trait correlations ranged from 0.21 to 0.85. There were some patterns of correlation in both years for certain traits. For example, later Mat correlated with greater levels of SPGR and TS and lower TER. Similarly, for yield traits, greater TTY correlated with greater TS and ATW. The greater variation for TTY and TS in 2013 could have affected the correlation of ATW with other traits in 2013 and the heritability for yield traits. As expected, there was strong positive correlation between TTY and TS, but the size and the number of tubers define the ATW and may justify why this trait could invert its correlation with other traits and be more complex to understand. Low percentages of heritability were found for TTY and ATW (<30%), intermediate for TS and SPGR (30–55%), and high for Mat and TER (>65%). The growing conditions in 2013 were affected by herbicide sensitivity reaction that was scored and used to correct the data. However, similar results were obtained using either corrected or raw data in the entire set of analyses (data not reported). Besides this effect, there was strong interaction between years and genotypes for all traits. The results of this study were dissimilar from previous analyses with regard to three points: correlations among all yield traits were positive and greater than 60%, the heritabilities were greater than 54% (Bradshaw et al., 2008), and early Mat was correlated with greater SPGR

(Bradshaw et al., 2008; McCord et al., 2011a). However, our results were similar for Mat with high heritability (Bradshaw et al., 2008; D’hoop et al., 2014), and later Mat correlated with greater yield (D’hoop et al., 2014; McCord et al., 2011a). Later maturity will lead to better canopy development, providing photosynthetic capacity to increase yield and starch accumulation. Lower heritabilities were found when comparing observations from multi-year-multi-location, while high heritabilities were observed from data from a single growing season (D’hoop et al., 2014). The environmental effect was the main reason for low reproducibility for the yield traits measured.

Even though there were altered correlations of TTY and ATW with other traits, the QTL results were consistent between years. Major effects driven by a QTL affecting most of the traits were detected on chromosome V. This QTL shifted the position toward 6.4 cM for TTY, 11.9 cM for TS, Mat, vigor, and TER, and 12.9 cM for SPGR, while ranging from 6.4 cM to 11.9 cM for ATW between years. This QTL with major effects was expected on the basis of the strong correlation of Mat with other traits. The overarching effect of Mat is explained by the physiological development role of the DNA-binding transcription factor *CDF1* gene (PGSC0003DMG400018408) associated with early maturity and initiation of tuberization (Kloosterman et al., 2013). The *solcap_snp_c1_3793* SNP in the QTL location of 11.9 cM is physically located at 3.9 Mb in the potato genome assembly 4.03. This is close to the *CDF1* gene location at 4.5 Mb. Eight loci cosegregate with this SNP marker; they are physically located at 3.7, 4, 4.3, 4.9, and 5.1 Mb. *CDF1* downregulates the two *CONSTANS* genes of potato (*StCO1* and *StCO2*), which enables expression of the tuberigen (*StSP6A*) signal to induce tuberization. Tuberization is a critical developmental stage shift that alters the entire plant metabolism toward storage of carbohydrates to the tubers. For this reason, the agronomic traits studied in this paper have strong correlation with Mat, and this QTL with major effect was simultaneously detected for all of them.

Several authors have reported similar QTL on chromosome V for yield and tuber size (Bradshaw et al., 2008; Li et al., 2008; McCord et al., 2011a; Schäfer-Pregl et al., 1998). Likewise for SPGR, and colocalizing with candidate genes associated with starch and sugar content (Bradshaw et al., 2008; Freyre and Douches, 1994; Li et al., 2008; McCord et al., 2011a; Schäfer-Pregl et al., 1998; Werij et al., 2012). Some of the QTL markers reported were ST1032 and STM3179 that are located at 4.8 Mb and 6 Mb on the potato genome pseudomolecule assembly version 4.03 for chromosome V. Besides the previously associated candidate genes, some regulatory and carbohydrate metabolism genes are located in the consolidated QTL interval that spans the QTL detected for several traits in this study (Fig. 2). On the basis of the physical map

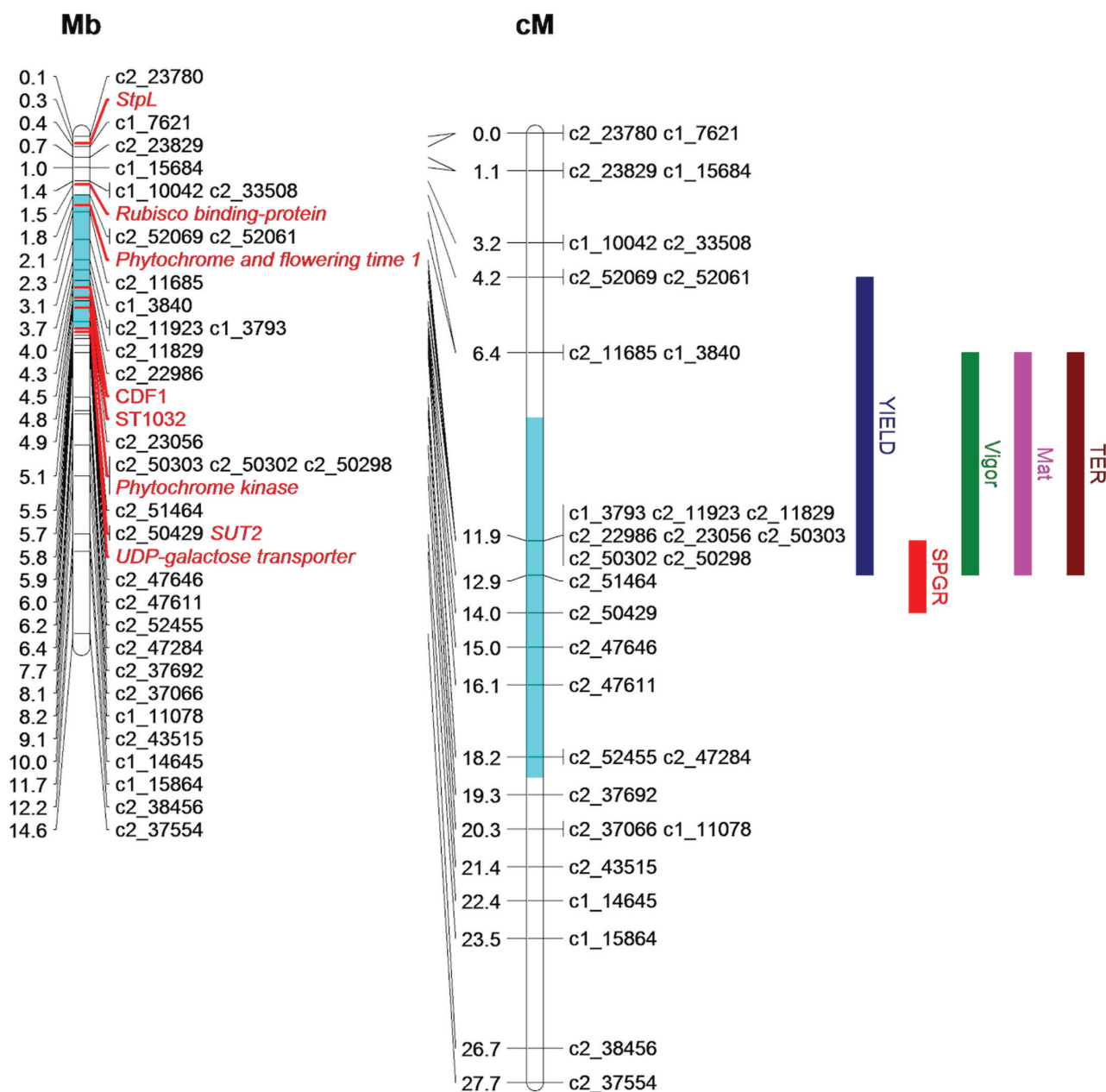


Figure 2. Physical (Mb) and genetic (cM) map of chromosome V arm with a quantitative trait locus (QTL) for multiple agronomic traits. Regulatory and carbohydrate metabolism genes (*L*-type starch phosphorylase *StpL*, *Rubisco binding-protein*, *Phytochrome*, and *flowering time 1*, DNA-binding transcription factor *CDF1*, *Phytochrome kinase*, *Sucrose transporter SUT2*, and *UDP-galactose transporter*) and markers *ST1032* and *STM3179* (red), located in the QTL interval 1.8 to 5.7 Mb and 4.2 to 13.9 cM (light blue inside chromosome bar), for yield traits. SPGR, specific gravity; Mat, maturity; and TER, tuber end rot.

position of mapped SNPs for the DRH population, this area corresponds to 1.8 to 5.7 Mb of pseudomolecule chromosome V. Probably both environmental effects and additional QTL interactions could have triggered the shifting position of this QTL for the different traits, and between years for ATW. The additive effects for ATW also shifted from positive to negative, suggesting some additional gene interactions that could influence the final phenotype. We also identified an additional QTL for TS at 26.7 cM in

2013, which was simultaneously detected with the QTL with major effects at 11.9 cM.

Other QTL identified for yield were located on chromosomes I, II, IV, VI, and XII. The QTL found on chromosome I for ATW at 41.7 cM (physically mapped at 71.5 Mb) co-localized with QTL previously reported for yield, tuberization, and starch content (Bradshaw et al., 2008; Schäfer-Pregl et al., 1998; Van den Berg et al., 1996). A starch synthesis candidate gene (glucose-1-phosphate

adenylyltransferase *AGPaseS*) has also been reported on chromosome I, associated with tuber starch and starch yield (Chen et al., 2001). Some allele-specific sequences of *AGPaseS* on chromosome I have been characterized in tetraploid potato and used as diagnostic markers to select for starch content, starch yield, and chip quality (Li et al., 2013). Two QTL on chromosome II for TTY at 36.1 cM in 2012 and 7.4 cM in 2013 co-localized with QTL for yield identified by Schäfer-Pregl et al. (1998) and McCord et al. (2011a). The physical positions of our QTL on chromosome II are 35.2 and 21.7 Mb, respectively. The marker STM0038 located at 19.6 Mb (Li et al., 2008) is close to the RFLP (GP23) reported by Schäfer-Pregl et al. (1998); while the marker ST1052 located at 39 Mb was reported by McCord et al. (2011a). The chloroplastic ribulose biphosphate carboxylase small chain C is a photosynthesis-associated candidate gene for this trait suggested by Chen et al. (2001).

The QTL on chromosome IV for ATW are located at 15 and 22.4 cM. Two QTL were previously reported on chromosome IV for yield measured as tuber size and the other associated with tuberization (D'hoop et al., 2014; Van den Berg et al., 1996). The QTL on chromosome VI found for ATW at 5.3 cM was in a similar position on the distal arm to that reported for yield and tuberization traits (McCord et al., 2011a; Schäfer-Pregl et al., 1998; Van den Berg et al., 1996), while the QTL found by Bradshaw et al. (2008) was in a less distal position. The QTL on chromosome XII for TTY at 27.2 cM in 2012 co-localized with QTL for yield reported by McCord et al. (2011a).

The second QTL identified for SPGR on chromosome IX co-localized with QTL and candidate genes for specific gravity, starch content, sugar (fructose and sucrose) content, and chip quality (Li et al., 2005; McCord et al., 2011a; Menendez et al., 2002; Schäfer-Pregl et al., 1998; Werij et al., 2012). QTL on chromosome III and X at 0 and 44.9 cM were identified for vigor. To our knowledge, only the QTL on chromosome V has been reported for vigor (Collins et al., 1999; Oberhagemann et al., 1999).

For TER, we identified four QTL on chromosomes I, III, V, and VI. Tuber end rot could be a physiological disorder of potato tubers with similar symptoms as the “internal necrosis” syndrome well described by Yencho et al. (2008). In our case, a progressive rot from the stem end of the tuber affecting all periderm, vascular ring, and parenchymal tissue appears after harvest. This has been a common postharvest deterioration that has been observed in DM 1–3 tubers grown in growth-chamber, greenhouse, and field conditions. The TER broad-sense heritability was high (64.6%), implying a strong genetic control of this trait as for internal heat necrosis (IHN) (Henninger et al., 2000). A major-effect QTL on chromosome V for severity and incidence of IHN was reported for a tetraploid mapping population of potato (McCord et al., 2011b). This QTL was in a distal position from the Mat locus, whereas that for TER was

in the same position. Markers associated (ANOVA) with lower incidence and severity of IHN were found on chromosome I for two populations in the study previously cited and on chromosomes III, VI, and XII for one population. A microarray analysis of tuber tissue with different levels of IHN identified some differentially expressed candidate genes (McCord, 2009). Since temperature, water relations, and soil nutrients have an important role in the expression of IHN symptoms (Yencho et al., 2008), it was not surprising that some genes, up- or down-regulated in response to cold and heat stress, were found in the microarray analysis. Those genes were located on chromosomes I, II, and VIII. Different studies have also shown that low calcium (Ca) levels could be correlated with increased tuber IHN (Yencho et al., 2008). In our study, QTL in chromosomes I, III, and VI were detected for TER. Future studies could elucidate if similar genetic factors as for physiological disorder of IHN are associated with this trait. High accumulation of sugar in the stem end could produce appropriate conditions for postharvest deterioration.

The candidate genes previously reported and also mentioned in this study, as well as some regulatory and carbohydrate metabolism genes located in the QTL intervals, are listed in Supplemental Table 1.

Allelic interactions are critical for attaining better performance of traits. Alleles at loci encoding ribulose-biphosphate carboxylase/oxygenase activase (*Rca*) on chromosome X, sucrose phosphate synthase (*Sps*) on chromosome VII, and vacuolar invertase (*Pain1*) on chromosome III were most frequently involved in significant epistatic interactions with effect on tuber starch content and starch yield (Li et al., 2010). This analysis showed the clear correlation of gene network and functional interaction with the effect in the measured traits. These genes function in photosynthesis and starch metabolism, two interconnected pathways for synthesis and accumulation of starch. Even though the right allelic combination could improve performance of a trait, the incompatibilities between alleles and/or dosage effect in a breeding population could neutralize the desired effect in the progeny (Li et al., 2013). We identified one marker interaction for yield traits and two for TER that explained between 8.7 and 10.1% of phenotypic variance. In two of the interactions, the major effect of QTL on chromosome V was associated. Primary metabolism, physiological development, and specialized genes are on chromosome V, and different allelic interactions and networks could be simultaneously affecting different traits.

The high-quality SNP markers used in this study, with an error rate of 0.05%, allowed the construction of a high-density map, the resolution of which could be improved by increasing the population size. The SNPs provided genomewide coverage; however, because they were selected using a transcriptome-based approach (Hamilton

et al., 2011), a genome sequencing approach could help to identify new SNP markers to fill the gaps and increase mapping resolution close to centromere regions.

In the future, extreme phenotypes of the DRH population could be studied for better understanding of the genetic bases of these traits and to identify specific haplotype blocks associated with each trait.

As reported by Felcher et al. (2012), blocks of markers with distorted segregation were mainly identified on chromosomes IX and XII at significance levels of 0.1 and 0.001% (we have an ongoing study to characterize segregation distortion regions in diploid mapping populations of potato). Several rounds of mapping with increasing levels of distorted segregation were necessary to generate a high-quality genetic map with the greatest number of SNPs and distorted segregation. This process was supported by the use of JoinMap 4.1 software features: (i) use of an enhanced multipoint maximum likelihood algorithm for cross-pollinated populations, (ii) checking of the strongest cross-link parameter to identify markers with weak linkage, and (iii) the use of tests for independence of segregation, which is not affected by distorted segregation, at a threshold value of 10 to calculate linkage groups. We did not identify any QTL in the distorted segregation regions of chromosome IX and XII. Regardless of the effect of markers with distorted segregations on QTL detection, the significant QTL will depend on linkage distance to the QTL, the degree of dominance of QTL, and the population size (Zhang et al., 2010). The distorted segregation could even benefit the QTL detection by increasing genetic variance and power.

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