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QTL mapping of internal heat necrosis in tetraploid potato

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Abstract Internal heat necrosis (IHN) is a physiological disorder of potato tubers. We developed a linkage map of tetraploid potato using AFLP and SSR markers, and mapped QTL for mean severity and percent incidence of IHN. Phenotypic data indicated that the distribution of IHN is skewed toward resistance. Late foliage maturity was slightly but significantly correlated with increased IHN symptoms. The linkage map for 'Atlantic', the IHN-susceptible parent, covered 1034.4 cM and included 13 linkage groups, and the map for B1829-5, the IHN-resistant parent, covered 940.2 cM and contained 14 linkage groups. QTL for increased resistance to IHN were located on chromosomes IV, V, and groups VII and X of 'Atlantic', and on group VII of B1829-5 in at least 2 of 3 years. The QTL explained between 4.5 and 29.4% of the variation for mean severity, and from 3.7 to 14.5% of the variation for percent incidence. Most QTL detected were dominant, and associated with decreased IHN symptoms. One SSR and 13 AFLP markers that were linked to IHN were tested in a second population. One AFLP marker was associated with decreased symptoms in both populations. The SSR marker was not associated with IHN in the second population, but was closely linked in repulsion to another marker that was associated with IHN, and had the same (negative) effect on the trait as the SSR marker did in the first population. The correlation between maturity and IHN may be partially explained by the presence of markers on chromosome V that are linked to both traits. This research represents the first molecular genetic research of IHN in potato.

Keywords QTL · Internal heat necrosis · Linkage mapping · Potato · Tetraploid · *Solanum tuberosum*

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Introduction

Internal heat necrosis, or IHN, is a significant physiological disorder of potato tubers. It is characterized by necrotic patches in the parenchyma of the tuber, internal to the vascular ring (Larson and Albert 1945; Henninger et al. 1979). The necrotic patches typically appear at the apical end of the tuber, but in severe cases the affected area can encompass the entire parenchyma (internal to the vascular ring). Similar or identical necroses have been given various names by different researchers, including physiological internal necrosis (Larson and Albert 1945), internal brown

spot (Tzeng et al. 1986; Davies 1998), internal browning, internal brown fleck, and chocolate spot (Sterrett and Henninger 1997 and references therein).

Economic losses from IHN are extensive, and the disorder is a significant production constraint in the mid-Atlantic and southeastern United States, including Florida, North Carolina, Virginia, and New Jersey. Sterrett and Henninger (1997) developed a 9-point rating system to express the severity of IHN symptoms, with 9 being completely free from IHN, and 1 being almost completely necrotic. Potatoes rated a 7 or lower are considered off-grade by USDA grading standards. If greater than 5% of a load of US Number 1 potatoes are off-grade, the entire shipment may be rejected by chip processors (Sterrett et al. 2003; USDA-AMS 2008). Sterrett and Wilson (1990) reported that as much as 11% of the potato acreage in North Carolina is left unharvested in severe years due to high levels of IHN. A major reason for these losses is because 'Atlantic', a chipping cultivar grown on a majority of commercial acreage in the aforementioned regions, is highly susceptible to IHN (Webb et al. 1978; Henninger et al. 1979).

A review of methods to control IHN (Sterrett and Henninger 1997) concluded that the most efficacious option would be to breed for IHN resistance. Although broad-sense heritability for IHN resistance is high (Henninger et al. 2000), breeding for resistance is difficult. This is because the intensity of symptoms can vary from year to year due to the strong influence of environment on IHN expression, requiring genotype evaluations over several years and locations (Yencho et al. 2008; P. McCord, personal observation). In addition, there are no above-ground or external symptoms of IHN, necessitating that tubers be harvested and cut in order to observe the phenotype. Finally, IHN resistance/susceptibility is quantitative (reviewed in Yencho et al. 2008), making it more difficult to predict the behavior of individual genotypes. All of these situations increase the time and expense involved in breeding for IHN resistance. The development of molecular markers linked to IHN would greatly facilitate breeding by reducing the amount of land and time required to screen for the trait. When these markers are identified in the course of developing a genetic linkage map based on a segregating population, important information is also gained regarding the genetics of the trait, i.e., the number, effect and chromosomal location of genes and/or quantitative trait loci (QTL) involved.

Our objectives for this research were to generate a genetic linkage map and identify QTL for IHN resistance/susceptibility in a segregating population of tetraploid potato. Although linkage and QTL mapping is more easily performed at the diploid level, most of the breeding for commercial cultivars utilizes tetraploid materials. Disomic models of gene action may not be adequate to explain the behavior of loci in a tetrasomic context, because they do not account for the tri- and tetra-allelic interactions that can occur in tetraploids. Furthermore, polyploidy itself can induce changes in the organization of the genome and changes in gene expression, making it difficult to extrapolate map information from diploids (Song et al. 1995; Luo et al. 2004). The difficulty of mapping in autotetraploids is lessened through the use of the software package TetraploidMap for Windows (Hackett et al. 2007), hereafter referred to as TetraploidMap. In particular, TetraploidMap can handle the segregation patterns of simple sequence repeats (SSRs) and other multi-locus markers, and includes algorithms for interval mapping of QTL. We used amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995) for the bulk of our marker coverage, and included a small number of previously mapped SSR markers to anchor our linkage groups to known *Solanum* chromosomes. We also tested the utility of markers associated with QTL in a second tetraploid population.

Materials and methods

Populations

Population B2721, the primary mapping population, consisted of 160 individuals from the cross 'Atlantic' × B1829-5. 'Atlantic', a processing cultivar released in 1978 (Webb et al. 1978), is highly susceptible to IHN. B1829-5, a breeding line from the USDA-ARS Beltsville potato breeding program, is highly resistant. Progeny (20 genotypes) from this cross were first observed in 2004 and 2005 as part of a larger study of general combining ability for heat necrosis (Haynes, unpublished), and were found to be segregating for the trait. During 2005, minitubers generated from remnant seed from this cross were grown in the field as single plants. The first 160 plants yielding enough tubers for at least ten plants per genotype for the following year were collected to form the B2721 mapping population. Population NC206, the population used for testing B2721-derived markers, consisted of 163 individuals from the cross B2464-1 (IHN-susceptible) X B2471-5 (IHN-resistant). Both parents were identified in the general combining ability study mentioned earlier. In addition, both parents were derived from crosses between a 4*x*–2*x* hybrid between *S. tuberosum* and *S. phureja-stenotomum*, and 4*x Solanum tuberosum*. The diploid *phureja-stenotomum* is a source of high dry matter and reduced susceptibility to IHN (Sterrett et al. 2003). In 2006, 282 individuals from this cross producing more than one tuber as seedlings were grown as single plants at the USDA-ARS Chapman Farm, Presque Isle, Maine for seed increase (250 of this group were also planted in the greenhouse in North Carolina for data

collection). Of the individuals planted in Maine, 163 produced enough tubers for at least 9 plants, and these constituted population NC206.

Field experimental design and data collection

B2721 was grown in 2006 using an unreplicated design with 6 plants per plot. In 2007 and 2008, the population was planted in a randomized complete block design with 2 replications and 10 plants per plot. NC206 was grown in 2007 as an unreplicated design with 4 plants per plot, and in 2008 was grown as for B2721. All field trials were conducted at the North Carolina Department of Agriculture and Consumer Services, Tidewater Research Station (NCDA&CS TRS) in Plymouth, North Carolina, in a Portsmouth fine sandy loam. Fertilizer was applied at planting (18-18-18, 772.9 kg/ha and 30-0-0, 64.9 L/ha) and at hilling (30-0-0, 205 L/ha). Seed pieces were not treated prior to planting. Insect control was provided (depending on year) by carbamate, imidacloprid, imidacloprid/cyfluthrin (Leverage®), and spinosad using recommended rates. Early season weed control was provided by pre-emergent treatment with *s*-metolachlor and metribuzin; middle and late season weeding was done by hand.

Four and six-plant plots were dug with a single-row chain digger and harvested by hand. Ten-plant plots were harvested with a two-row potato harvester, modified to dig one row. Tubers were then washed and culled for rot only. For evaluation of IHN, tubers from each plot were quartered longitudinally, and the most affected quarter was retained for scoring. In 2006, a minimum of 40 tubers were cut per plot. If any of the first 40 tubers had IHN symptoms, all remaining tubers of marketable size were cut as well. In 2007 and 2008, 20 tubers were cut per plot. Due to lower rainfall and subsequent lower yields, 20 tubers were not always available in 2007 and 2008. In these cases, we cut all marketable tubers. Two measures of IHN were employed: severity and incidence. Severity refers to the mean severity rating of all tubers examined in a plot, based on a 9-point rating system developed by Sterrett and Henninger (1997), with 9 representing no symptoms and 1 representing completely necrotic tubers, respectively. Incidence was determined by calculating the percentage of sampled tubers displaying any level of IHN. Both populations were also rated for yield (on a per-plant basis), foliage maturity, skin texture, specific gravity, dry matter, and flower color (McCord et al. 2010). In addition, NC206 was rated for yellow flesh color. Variation of IHN symptoms due to year and population, and correlation between IHN and other measured traits was explored using the MIXED and CORR procedures, respectively, of SAS (SAS, Cary, NC). Years were treated as random when testing for population effects, and fixed when testing for year and year-by-population effects.

DNA extraction and marker generation

DNA for AFLP and SSR analysis was extracted primarily from greenhouse-grown leaf tissue (in three cases, DNA extractions were done using sprouts and surrounding tissue from tubers). Leaf tissue was placed in a microcentrifuge tube, which was then briefly dipped (not immersed) in liquid nitrogen. Tissue samples were then ground manually using miniature plastic pestles. After addition of CTAB extraction buffer (http://www.cipotato.org/csd/materials/Molecular/Molecular1.pdf), samples were incubated in a water bath at 65°C for 30–60 min. Following extraction with 24:1 chloroform:isoamyl alcohol, nucleic acids were precipitated with isopropranol and concentrated by centrifugation. Pellets were washed with 70% ethanol, dried, and resuspended in buffer (10 mM Tris, 0.1 mM EDTA pH 8) supplemented with RNase A.

Templates for AFLP fingerprinting with *Eco*RI and *Mse*I were prepared essentially as described in Myburg and Remington (2000), which is a version of the original protocol of Vos et al. (1995) modified to use fluorescence-based fragment detection. For fingerprinting with *Pst*I and *Mse*I, the second-digest AFLP (SD-AFLP) method was used, essentially following the method developed by Knox and Ellis (2001). The SD-AFLP method employs initial restriction, ligation, and preamplification reactions using only *Mse*I enzyme, adapters, and primers. This results in template DNA that is free of methylated cytosine residues (5-methylcytosine) to which *Pst*I is sensitive (McClelland et al. 1994 and references therein). Following restriction and ligation with *Pst*I enzyme and adapters, the SD-AFLP procedure is identical to the AFLP method. *Eco*RI and *Pst*I primers were labeled at the 5′ end with either IRD-800 or IRD-700 fluorescent dyes. Reaction products were separated and visualized using Li-Cor 4000, 4000L, and 4300 DNA analyzers (Li-Cor, Lincoln, NE).

SSR markers were selected from marker lists published by Frary et al. (2005) for tomato, and Feingold et al. (2005) for potato. Markers were screened for polymorphism in B2721 via electrophoresis in agarose or Spreadex® EL 800 (Elchrom Scientific, New Hyde Park, NY) gels, utilizing the parents and ten individuals. Two SSRs (SSR46 and SSR73) were analyzed across the entire population using agarose gel electrophoresis. The remaining SSR markers were amplified using a 3-primer M13 'tail' protocol (Schuelke 2000; Rampling et al. 2001), and analyzed using the Li-Cor systems as for AFLP. For all markers, gel images were adjusted for ease of scoring and converted to JPEG format using Adobe Photoshop (Adobe Systems Inc., San Jose, CA), FastStone Image Viewer (http://www.faststone.org), or GIMP (http://www.gimp.org). Markers were then scored using the program Cross Checker (Buntjer 2000). Some SSR primers resulted in banding patterns that were difficult

to score in a codominant fashion. In these cases, individual bands were scored as dominant markers. Information was lost regarding the locus per se, but this strategy still allowed us to anchor linkage groups to *Solanum* chromosomes.

Map construction and QTL analysis

For primary map construction and QTL analysis, we used the program TetraploidMap for Windows (Hackett et al. 2007). This software package is designed to infer parental genotype from the marker phenotypes of the parents and segregating progeny of a cross in an autotetraploid species. TetraploidMap for Windows and its predecessor TetraploidMap have been used to construct linkage maps in alfalfa (Julier et al. 2003; Robins et al. 2008) and potato (Bradshaw et al. 2004, 2008; Sagredo et al. 2006; Khu et al. 2008; Kelley et al. 2009). Once parental genotypes are determined at each locus (based on the marker phenotype or observed bands, and segregation ratios in the progeny), TetraploidMap attempts to combine the markers into linkage groups via cluster analysis, and determine the proper marker order. The software will accommodate dominant (AFLP) markers in simplex, duplex, or double-simplex configuration, and codominant (SSR, CAPS, and SCAR) markers, including any null alleles of codominant markers.

Based on experience using TetraploidMap, we selected the following types of markers for map construction: (1) simplex dominant markers (segregating 1:1) with a p value greater than 0.001 from a Chi-square test for goodness of fit; (2) duplex dominant markers (segregating 5:1) with a p value greater than 0.01; (3) double-simplex dominant markers (segregating 3:1) with a p value generally greater than 0.01 and which were known to be linked to at least one simplex marker; (4) all SSR (codominant) markers. Markers with greater than 10% of individuals missing were omitted. Following initial clustering of markers, the final linkage group composition and marker order were determined.

TetraploidMap offers several strategies for marker ordering. In most cases the 'ripple' option was used due to its combination of speed and accuracy. Simulated annealing (Hackett et al. 2003) can give more accurate ordering results, but takes a long time to compute; it was generally used only for groups with fewer than 20 markers. Homologous chromosomes/linkage groups were identified via simplex markers linked in repulsion (detected automatically in TetraploidMap), duplex markers, and SSRs. Double-simplex markers, which are present in both parents but segregate in their progeny, were used along with SSR markers to align the parental maps.

TetraploidMap also includes an interval mapping (IM) routine for QTL analysis. This IM routine utilizes a multistep procedure. First, a graphical reconstruction is made of the chromosome/linkage group for each individual in the population, based on the parental map (the software considers data generated from only one parent at a time). In this first step, a branch and bound algorithm is used to create an individual's linkage group based on the fewest number of recombinations required to produce the observed segregation patterns (Hackett et al. 2001). Second, conditional probabilities of each possible QTL genotype (at 2 cM intervals) are calculated based on the chromosome reconstructions. Finally, phenotypic values for each trait are regressed on putative QTL genotypes, each genotype being weighted by its conditional probability (Hackett et al. 2001). The 'full' model includes six QTL genotypes, reflecting the six combinations in which any two parental alleles can be transmitted to offspring. Ten simpler models, which reflect a dominant effect of the QTL, are also analyzed and compared to the full model by a likelihood ratio test. These include the six duplex genotypes tested individually, and the four simplex genotypes.

We utilized the IM routine of TetraploidMap for almost all linkage groups identified. We performed permutation tests (Churchill and Doerge 1994) of 100 iterations generally for all LOD scores greater than or equal to 2.5. LOD scores were also tested which displayed a clear peak, but were slightly less than 2.5. In cases where the LOD peak was between the 90 and 95th percentile of the test distribution, the permutation test was rerun at least once more before accepting or rejecting the presence of a QTL at that position. LOD scores below the 90% distribution point were rejected as evidence for a QTL, except in cases where the LOD score was at least 1.8, and was also significant in a different year according to the more stringent criteria (and within 10 cM of the LOD score being considered). Traits were analyzed separately for each year. When a simpler model was not significantly different, and passed the permutation tests, we reported the simpler model. Empirically, we determined that p values for goodness of fit above 0.05 but below ~0.2 often did not pass the permutation test, while the full model did. In these cases, we reported the full model.

We found it was difficult to successfully perform the IM routine when analyzing a group with only one homolog. In these cases, we analyzed the marker class means using single-point ANOVA. Three linkage groups of B1829-5 (chromosomes I and XI and group XII) contained more than one homologous group, but could not be reliably analyzed via the IM routine of TetraploidMap. These groups were also tested for QTL using single-marker ANOVA. Only markers with associated p values <0.01 were reported, except in cases (as with interval mapping) where the marker was significant for the trait of interest in at least one other year. In these cases, markers with p values <0.05 were reported as well.

Results

Phenotypic data

Across all years, and both populations, the distributions of IHN incidence and severity were similar (Fig. 1a, b). They were all highly skewed, with most individuals displaying little to no IHN symptoms. Some transgressive segregation was observed, i.e., a handful of individuals were more susceptible to IHN than their susceptible parent. There were significant year effects on population mean IHN susceptibility (p values <0.0001 for incidence and severity), but population means were not significantly different from each other (p value = 0.33), nor was there a population by year interaction (p value = 0.58). However, a number of *individuals* in both populations showed widely varying IHN symptoms from year to year (data not shown). For example, 'Atlantic' had an average IHN incidence of 59, 15, and 20% for 2006, 2007 and 2008, respectively. This is in agreement with Henninger et al. (2000), who determined that 'Atlantic' is unstable in its expression of IHN symptoms, even when environmental variation is taken into account.

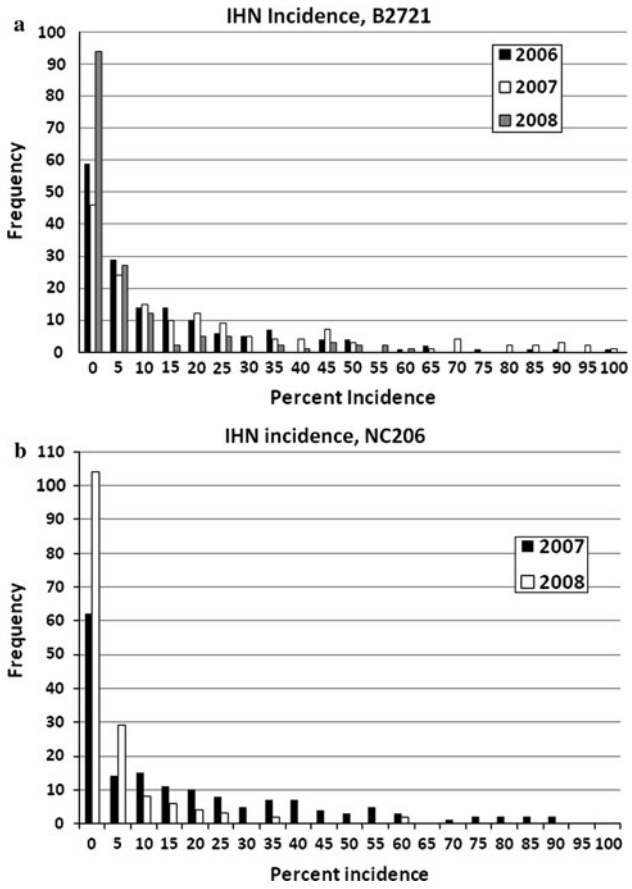

Fig. 1 **a** Frequency distribution of IHN incidence in B2721, 2006–2008. **b** Frequency distribution for IHN incidence in NC206, 2007–2008

In population B2721, IHN incidence and severity were highly negatively correlated ($r = -0.95$, Table 1), indicating that clones with a high percentage of IHN-affected tubers also have a low (more severe) severity rating. Correlations between IHN incidence or severity and other agronomic traits were either statistically insignificant, or had significant correlations of low magnitude ($r < 0.3$). The single exception was foliage maturity. Maturity was calculated as the area under the curve of a series of subjective visual assessments of foliage and vine senescence (McCord et al. 2010). Therefore, lower maturity ratings indicate later maturity, and vice versa. Earlier maturity was negatively correlated with IHN incidence ($r = -0.44$), and positively correlated with IHN severity ($r = 0.34$).

IHN severity and incidence were also highly negatively correlated in NC206 ($r = -0.94$, Table 2). As with B2721, earlier foliage maturity was also correlated negatively with incidence and positively with severity, though the coefficients were slightly lower ($r = -0.357$ and 0.284, respectively). In contrast to B2721, yield in NC206 was significantly correlated with IHN ($r = 0.455$ and -0.381 for incidence and severity, respectively).

Linkage maps

Forty *Eco*RI/*Mse*I primer combinations, seven *Pst*I/*Mse*I primer combinations, two SCARs, and 14 SSRs were analyzed in B2721, resulting in 674 scored markers. The AFLP

Table 1 Correlations between IHN incidence and severity, and other agronomic traits measured in population B2721

	Incidence	Severity	Yield	SG[a]	DM[b]	Texture[c]	Maturity[d]
Incidence		−0.95 (<0.0001)	0.13 (0.007)	−0.09 (0.05)	0.01 (0.81)	0.17 (0.0003)	−0.44 (<0.0001)
Severity	−0.95 (<0.0001)		−0.08 (0.09)	0.07 (0.13)	−0.03 (0.56)	−0.15 (0.002)	0.34 (<0.0001)

p values of correlations are listed in parentheses

[a] Specific gravity

[b] Tuber dry matter

[c] Skin texture

[d] Foliage maturity

Table 2 Correlations between IHN incidence and severity, and other agronomic traits measured in population NC206

	Incidence	Severity	Yield	SG	DM	Texture	Maturity	Flesh color
Incidence		−0.94 (<0.0001)	0.45 (<0.0001)	0.08 (0.15)	0.17 (0.002)	0.26 (<0.0001)	−0.36 (<0.0001)	−0.03 (0.56)
Severity	−0.94 (<0.0001)		−0.38 (<0.0001)	−0.08 (0.13)	−0.17 (0.003)	−0.21 (0.0002)	0.28 (<0.0001)	0.02 (0.72)

p values of correlations are listed in parentheses

primer combinations, and the number of markers generated per combination, are listed in Supplement 1. The parental genotypes of 24 markers could not be reliably determined from their segregation ratios, and were not used. Following the marker selection criteria described in the "Materials and methods" section, subsets of the marker pool were used to create a linkage map for each parent.

The map for 'Atlantic' included 274 AFLP markers, of which 174 were simplex, 24 were duplex, 69 were double-simplex, and seven SSRs, four of which were scored as dominant markers. The markers were resolved into 18 linkage groups. In several cases two or more groups were combined based on common linkage (via double-simplex markers) to a single group from B1829-5, resulting in a total of 13 linkage groups for 'Atlantic'. Nine of these groups contained all four homologs, one group (chromosome IV) contained three homologs, and the remaining three (chromosome VIII, group XIII, and group XIV) contained one homolog each. Chromosomes I, II, III, IV, V, VI, VIII, and IX were anchored through the use of SSR markers, either directly in the 'Atlantic' map, or through the linkage of double-simplex markers to SSRs mapped in B1829-5. Thirty-two markers remained unlinked (this includes unanchored linkage groups with only two markers).

A total of 244 markers were used to generate the map for B1829-5. These included 135 simplex markers, 25 duplex markers, 74 double-simplex markers, and 10 SSRs (five scored as dominant). This dataset was organized initially into 16 linkage groups, later condensed to 14 groups when some were combined via common association to linkage groups from 'Atlantic'. Five of these groups (chromosomes II, III, VI, IX, and group VII) contained all four homologs. Three homologs were identified in each of chromosomes I, IV, and V, two homologs each in chromosomes VIII, XI, and group XII. The remaining three groups (X, XIII, and XV) each consisted of a single homolog. Chromosomes I, II, III, IV, V, VI, VIII, IX, and XI were anchored via the use of previously mapped SSR markers. Twenty-five markers remained unlinked. Using double-simplex and microsatellite markers, alignment of linkage groups from parental maps was achieved for all but one group from each parent (group XIV from 'Atlantic', and group XV from B1829-5). Overall map lengths were 1,034.4 cM for 'Atlantic', and 940.2 cM for B1829-5.

Chromosomes and linkage groups with interval-mapped QTL or significant single markers are shown graphically in Fig. 2. As maturity was the only other trait correlated with IHN, only maturity, IHN severity, and IHN incidence QTL and markers are shown on the map. The full map (containing all chromosomes and groups) can be seen in Supplement 2. The data in Fig. 2 and Supplement 2 are presented as overall maps, with data from all homologs collapsed into one group as done by Bradshaw et al. (2008).

IHN severity

In 'Atlantic', nine QTL for IHN severity were detected via interval mapping (Table 3). Three QTL were identified in roughly the same position on chromosome IV, one for each year; the variation accounted by these three QTL ranged from 4.5 to 16.2% depending on the year. Four QTL (two in 2006, one in both 2007 and 2008) were also identified on chromosome V, though their position was more variable than those detected on chromosome IV. These loci explained from 9.3 to 16.9% of the observed variation, except for the second QTL found in 2006. Due to the constraints of interval mapping, which assumes there is only one QTL per linkage group corresponding to the maximum LOD score, the effects of the second QTL were not estimable. On linkage group VII, two QTL (one estimable) were identified in 2007 explaining 29.4% of the variation. Of the seven QTL that could be modeled for dosage and effect, four were modeled as duplex alleles with dominant effect, while the remaining three QTL were best explained using the original 6-mean additive/complex model. In each of these first four cases, the QTL was associated with decreased IHN severity (i.e., a higher score).

Five QTL for severity were identified in B1829-5 (Table 3). Three of these were found in a similar location (~34 cM) on linkage group VII (one in each year). They were all modeled as duplex dominant alleles, with reducing effects on severity. A second, non-estimable QTL was also found on group VII in 2006 only. The dominant QTL explained from 6.6 to 10.2% of variation. One additive/interactive QTL was detected for severity in 2007 on chromosome V that explained 6.7% of observed variation.

A number of single markers in B1829-5 were associated with IHN severity, as revealed by single-point ANOVA (Table 4). On chromosome I, five linked markers were

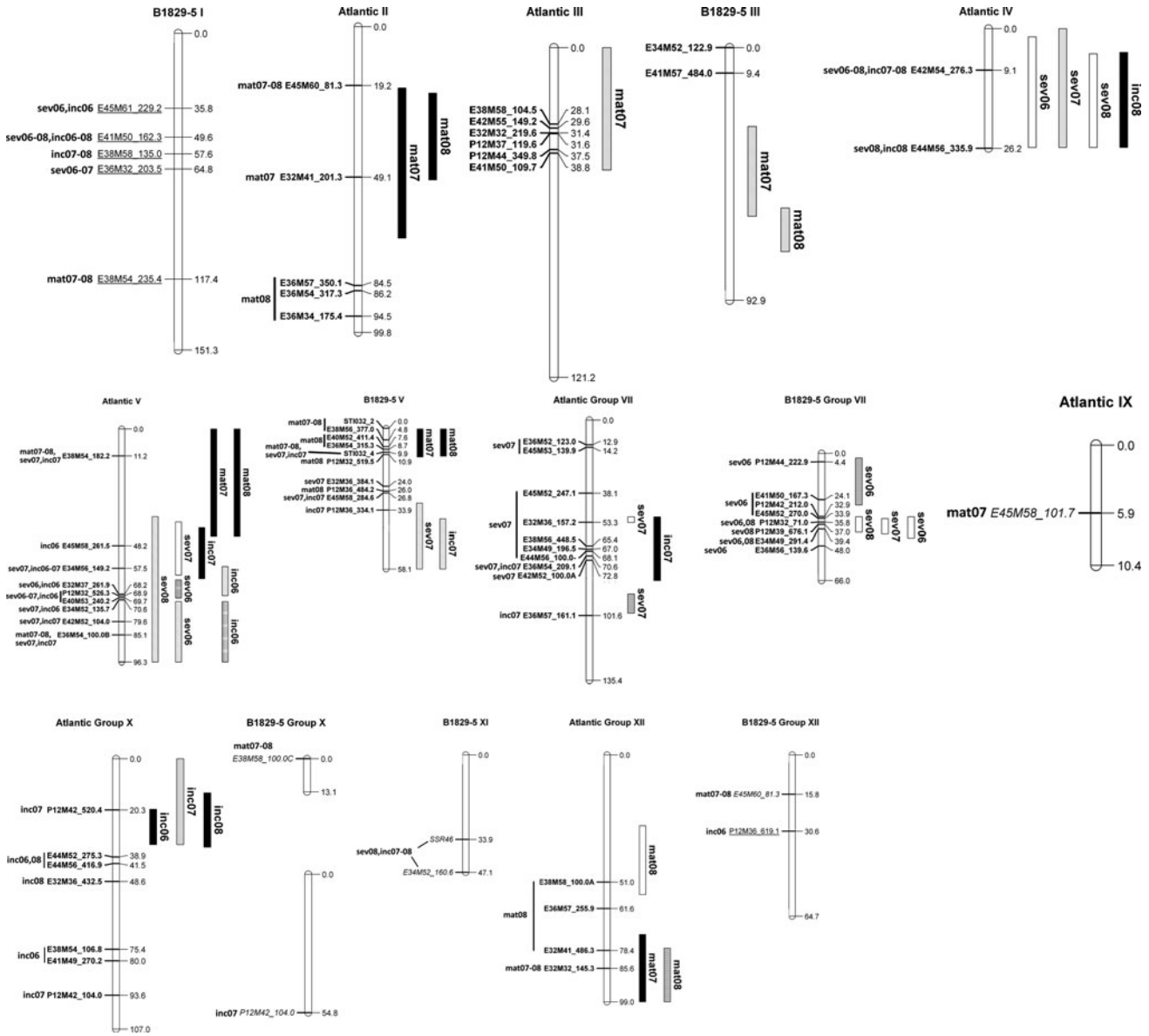

Fig. 2 Linkage maps of 'Atlantic' and B1829-5, that contain interval-mapped QTL or single markers linked to IHN incidence, severity, and foliage maturity. Unless specified by the term 'group', *Roman numerals* refer to actual chromosomes. AFLP markers are denoted by the Keygene system of primer combinations (http://www.keygene.com/keygene/pdf/KF%20Primer%20enzyme%20combinations.pdf). The *number after the underscore symbol* is the approximate size in base pairs. Size estimates followed by *letters* (or a *minus sign*) refer to markers that were shorter than the shortest size marker, and are ranked alphabetically by size (*A* = largest, then *B*, and so forth). Tomato SSR markers are denoted by the prefix *SSR*, and potato SSR markers by the prefix *STI*. Significant single markers are annotated by the trait(s) they are associated with, and are *underlined* if they affect the trait in a positive direction, or italicized if they have a negative effect. QTL are identified by *shaded bars* which represent a 1-LOD interval. *Bar shading* codes are the following: *black* dominant negative effect, *white* dominant positive effect; *light gray* complex additive effect; *dark gray* non-estimable effect. *Markers in bold* are significantly ($p < 0.05$) associated with the mapped trait. Maps were drawn using MapChart (Voorrips 2002), with adjustments made using GIMP (http://www.gimp.org)

strongly associated with reduced severity in at least 1 year. One marker, E41M50_162.3, was detected in all 3 years. This simplex marker was highly skewed (*p* value from Chi-square test = 0.0007), but was retained because of its significance. Two markers on chromosome XI were associated with increased IHN severity in 2007 and 2008.

IHN incidence

As expected from the high correlation between IHN incidence and severity, the QTL detected for incidence both through IM and single-point ANOVA were generally collinear with those for IHN severity. Eight QTL for IHN

Table 3 List of QTL detected for IHN incidence (inc), severity (sev) and foliage maturity (mat) in 2006–2008

Trait	Parent	LG[a]	Position (cM)	LOD score	*R* squared	Dosage/effect	QTL Present Mean (SE[c])	QTL Absent Mean (SE[c])
inc06	Atlantic	V	64	4.58	13.1	Additive		
inc06	Atlantic	V	78	4.13	NE[b]	NE[b]		
inc06	Atlantic	X	30	3.02[d]	10.7	Duplex, dominant	10.2 (1.7)	30.2 (4.5)
inc07	Atlantic	V	50	2.44[d]	13.0	Duplex, dominant	14.9 (2.1)	43.0 (5.7)
inc07	Atlantic	VII	51	2.68[d]	14.5	Duplex, dominant	15.1 (2.2)	42.8 (5.3)
inc07	Atlantic	X	30	1.89[e]	3.7	Additive		
inc07	B1829-5	V	50	3.49	8.6	Additive		
inc08	Atlantic	IV	24	4.81	14.4	Duplex, dominant	3.6 (1.0)	16.3 (2.2)
inc08	Atlantic	X	32	3.26[d]	10.8	Duplex, dominant	4.5 (1.1)	18.7 (4.5)
sev06	Atlantic	IV	24	2.58	7.1	Duplex, dominant	8.78 (0.05)	8.30 (0.12)
sev06	Atlantic	V	78	4.06	9.3	Additive		
sev06	Atlantic	V	64	3.80[d]	NE	NE		
sev06	B1829-5	VII	38	3.61	10.2	Duplex, dominant	8.78 (0.06)	8.17 (0.13)
sev06	B1829-5	VII	12	3.32	NE	NE		
sev07	Atlantic	IV	12	2.41[d]	4.5	Additive		
sev07	Atlantic	V	50	3.02[d]	16.9	Duplex, dominant	8.62 (0.08)	7.35 (0.22)
sev07	Atlantic	VII	51	5.76	29.4	Duplex, dominant	8.65 (0.08)	7.07 (0.20)
sev07	Atlantic	VII	96	3.64	NE	NE		
sev07	B1829-5	V	58	3.22[d]	6.7	Additive		
sev07	B1829-5	VII	38	2.12[e]	7.5	Duplex, dominant	8.58 (0.08)	7.83 (0.19)
sev08	Atlantic	IV	24	5.47	16.2	Duplex, dominant	8.93 (0.03)	8.59 (0.06)
sev08	Atlantic	V	76	2.70[e]	5.3	Additive		
sev08	B1829-5	VII	36	2.22[e]	6.6	Duplex, dominant	8.90 (0.03)	8.66 (0.07)
mat07	II	ATL	38	1.91[e]	7.9	Simplex, dominant	66.3 (2.9)	78.7 (2.1)
mat07	III	ATL	38	3.56[e]	8.8	Additive		
mat07	V	ATL	20	4.20	14.3	Duplex, dominant	71.6 (1.7)	90.7 (3.5)
mat07	XII	ATL	86	1.84[e]	5.3	Duplex, dominant	72.9 (1.8)	85.8 (3.9)
mat07	III	1829-5	40	4.18	18.7	Additive		
mat07	V	1829-5	2	3.23	9.6	Duplex, dominant	61.5 (3.5)	77.6 (1.7)
mat08	V	ATL	22	3.83	13.1	Duplex, dominant	76.0 (2.6)	103.8 (5.3)
mat08	XII	ATL	48	2.70	7.9	Duplex, dominant	98.6 (5.2)	76.8 (2.7)
mat08	XII	ATL	86	2.48[d]	NE	NE		
mat08	III	1829-5	66	3.87	10.4	Additive		
mat08	V	1829-5	2	5.23	15.6	Duplex, dominant	57.0 (5.0)	87.1 (5.5)

The standard errors of trait means are in parentheses. For QTL with additive/complex effects, there are six means, one for each QTL genotype (see Fig. 3); they are not listed in this table

[a] Linkage group/chromosome

[b] Not estimable (occurs when a secondary LOD peak is detected)

[c] Standard error

[d] LOD scores between the 90 and 95th percentile in a permutation test of at least 100 iterations

[e] LOD score of at least 1.8, were reported if a QTL was found in a similar location at least one other year, and met the more stringent LOD score and permutation test criteria

incidence were detected in 'Atlantic', of which seven were estimable with respect to dosage and effect (Table 3). Five of these eight QTL for IHN incidence were collinear with those for IHN severity; the three exceptions were on linkage group X, where QTL for incidence were found in all 3 years, but not for severity (LOD peaks were evident for severity on this linkage group, but did not pass the permutation tests). Again, most had dominant effects, and all dominant QTL were associated with decreased incidence of IHN. Variation explained by these QTL ranged from 3.7 to

Table 4 List of single markers declared significant for IHN severity/incidence, and foliage maturity

Trait	Marker	Parent	LG	*p* value	Mean (0)[a]	Mean (1)[b]	SED[c]
inc06	E45M61_229.2	B1829-5	I	0.009	17.7	8.6	3.0
inc06	E41M50_162.3	B1829-5	I	0.017	16.9	8.5	3.0
inc06	P12M36_619.1	B1829-5	XII	0.008	17.1	8.9	3.0
inc07	E41M50_162.3	B1829-5	I	0.002	25.9	13.6	4.0
inc07	E38M58_135	B1829-5	I	0.031	24.7	15.5	4.2
inc07	E36M32_203.5	B1829-5	I	0.015	24.5	14.6	4.0
inc07	E34M52_160.6	B1829-5	XI	0.025	14.0	23.8	4.3
inc07	SSR46	B1829-5	XI	0.01	12.1	22.6	4.0
inc08	E38M58_135.0	B1829-5	I	0.027	8.9	4.2	2.1
inc08	E41M50_162.3	B1829-5	I	0.007	9.2	3.7	2.0
inc08	E34M52_160.6	B1829-5	XI	0.011	3.1	8.8	2.2
inc08	SSR46	B1829-5	XI	0.004	2.2	8.1	2.0
sev06	E41M50_162.3	B1829-5	I	0.017	8.56	8.81	0.10
sev06	E45M61_229.2	B1829-5	I	0.009	8.53	8.81	0.11
sev06	E36M32_203.5	B1829-5	I	0.049	8.59	8.77	0.11
sev07	E41M50_162.3	B1829-5	I	0.002	8.20	8.67	0.15
sev07	E36M32_203.5	B1829-5	I	0.004	8.22	8.66	0.15
sev07	P12M42_104.0	B1829-5	X	0.004	8.69	8.40	0.17
sev08	E41M50_162.3	B1829-5	I	0.006	8.78	8.93	0.05
sev08	E38M58_135.0	B1829-5	I	0.027	8.79	8.91	0.05
sev08	E34M52_160.6	B1829-5	XI	0.034	8.93	8.81	0.06
sev08	SSR46	B1829-5	XI	0.009	8.95	8.82	0.05
mat07	E45M58_101.7	ATL	IX	0.008	81.8	72.0	3.6
mat07	E38M54_235.4	1829-5	I	0.03	81.8	72.8	4.2
mat07	E38M58_100.0C	1829-5	X	0.01	82.4	72.5	4.0
mat07	E45M60_81.3	1829-5	XII	0.02	81.6	72.6	3.9
mat08	E38M54_235.4	1829-5	I	0.004	96.2	78.1	6.1
mat08	E38M58_100.0C	1829-5	X	0.02	92.6	78.5	6.0
mat08	E45M60_81.3	1829-5	XII	0.04	89.9	77.9	5.8

Markers were tested using single-point ANOVA, and were declared significant if they had a *p* value <0.01 in a single year, or <0.05 in multiple years

[a] Mean (0) = mean of individuals with marker genotype 0 (absent)

[b] Mean (1) = mean of individuals with marker genotype 1 (present)

[c] SED = standard error of the difference between marker class means

14.5%. One QTL with additive/complex effect was detected in 2007 on chromosome V of B1829-5, which was collinear with a QTL for IHN severity. This QTL explained 8.6% of the observed variation. Five markers from chromosome I were associated with reduced incidence in at least 1 year, as was a single marker from group XII. Two markers from chromosome XI (including an SSR, SSR46 from tomato) were associated with increased incidence in 2007 and 2008. All of these markers were present in B1829-5.

In summary, based on observation over 3 years, we have reliably detected QTL for IHN on chromosomes IV and V and linkage groups VII and X, the majority of which exhibit dominant action. Based on single-marker ANOVA, QTL also appear to be on chromosomes I and XI, and possibly on linkage group XII.

Maturity

QTL for foliage maturity were detected on 'Atlantic' chromosomes II and V, and linkage group XII in both 2007 and 2008, and on chromosome III in 2008 only (Fig. 2; Table 3). Of the estimable QTL, all but the one on chromosome III had dominant effects. The QTL from chromosomes II, V, and group XII (in 2007) were associated with later maturity (lower AUSC scores), while the QTL on group XII in 2008 was associated with earlier maturity (higher AUSC scores). The 'late' QTL on group XII was also detected in 2008, but had a lower LOD score than the 'early' QTL, and was not estimable. The variation explained by these QTL ranged from 5.3 to 14.3%. A single marker on chromosome IX, E45M58_101.7 was associated with later maturity in 2007. In B1829-5, QTL were found in 2007 and 2008 on chromosomes III and V. The QTL on chromosome III displayed additive/interactive effects, while those on chromosome V were dominant, and were associated with later maturity. The variation explained by these QTL ranged from 9.6 to 18.7%. One marker each was found on chromosome I, group X, and group XII. These markers were significant in 2007 and 2008, and all were associated with later foliage maturity.

Table 5 List of single markers tested in NC206, and found to be significantly associated with IHN incidence and severity

Trait	Marker	Parent	*p* value	Mean (0)	Mean (1)	SED
inc08	E38M58_135	B2464-1	0.004	5.8	1.7	1.4
sev07	E38M58_135	B2464-1	0.017	8.39	8.68	0.12
sev08	E38M58_135	B2464-1	0.006	8.88	8.97	0.03

Marker testing in NC206

Four AFLP primer combinations (all *Eco*RI/*Mse*I) and one SSR marker, STI032_4 (the fourth largest allele of the SSR, scored as a dominant marker) were used to screen population NC206. One primer combination, E38M58, was chosen because it produced a marker shown to be associated with IHN incidence and severity. STI032_4 was chosen because it was associated with increased IHN symptoms (both severity and incidence) in 2007 and 2008, and was linked to QTL for IHN incidence and severity on chromosome V. Primer combination E36M57 was chosen because it produced markers associated with yield, texture, and flower color (McCord et al. 2010). The remaining primer combinations (E32M40 and E36M32) had already been used to develop markers in NC206 in an initial attempt to generate a second set of linkage maps. In B2721, the primer combinations produced 13 IHN-associated markers, on chromosomes I, III, VI, and group XII; two markers were unlinked. In NC206, the AFLP primer combinations produced 63 markers, which were tested for their association with IHN, and their relationship (i.e., molecular weight) to the original B2721 markers. Eight of the original B2721 markers were polymorphic in NC206. One marker, E38M58_135, was strongly associated with reduced IHN severity and incidence in 2008 (Table 5). This marker is located on chromosome I of B1829-5, and was significantly associated with reduced incidence and severity over several seasons in this population as well (see Fig. 2; Table 5). In contrast to B2721, where the marker was contributed by the resistant parent, in NC206 the marker was contributed by B2464-1, the susceptible parent. STI032_4 was not associated with IHN per se in NC206, but it was in repulsion-phase linkage (i.e., on a different homolog) at a distance of 2.9 cM to an AFLP marker, E32M40_180.8, that was itself associated with increased IHN symptoms (severity and incidence) in both 2007 and 2008 (data not shown). STI032_4 was associated with increased IHN symptoms in B2721 as well, so we believe we have identified the same QTL in both populations. In the case of E32M40_180.8, the marker was contributed by the susceptible parent.

Discussion

QTL for IHN

This paper presents the first attempt to map and characterize QTL responsible for internal heat necrosis (IHN). The population distributions of IHN severity and incidence lead us to hypothesize that these two traits were controlled by relatively few genes of major effect. Skewed phenotypic distributions caused by the segregation of a major gene or genes have been demonstrated in other species, including *Mimulus* (flower carotenoids, Bradshaw et al. 1995), chickpea (ascochyta blight resistance, Cho et al. 2004), and citrus [citrus tristeza virus (CTV) resistance, Asins et al. (2004)]. The QTL for IHN detected in our research explained up to 29.4 and 14.5% of the variation for severity and incidence, respectively. These figures are not as large as for other major QTL [for example, Bradshaw et al. (1995) detected a QTL which explained 88.3% of the variation for flower carotenoids], but they still account for a sizable portion of the phenotypic variation, at least in the case of IHN severity. When the variation explained in a given year was summed over all QTL for each measure of IHN, they explained an average of 39.9% of the variation for severity, and 29.6% of the variation for incidence. These numbers would almost certainly be higher if the identification of additional homologs allowed interval mapping to be performed on additional chromosomes with significant single markers, particularly chromosomes I and XI. Of the 23 QTL detected, 12 had dominant effects, and several of the additive/complex QTL could be modeled as dominant in years that they had stronger effects (incidence QTL on chromosome IV and group X are two examples). In addition, the QTL with largest effects were generally dominant ones. Given these observations, we have revised our hypothesis slightly. Expression of IHN symptoms is not a monogenic or oligogenic trait, but rather a moderate number of QTL with mostly dominant gene action are likely producing the skewed phenotypic distribution we have observed.

Given that most of the QTL were found in the susceptible parent, ‘Atlantic’, and were associated with decreasing IHN symptoms, the question remains; why is ‘Atlantic’ susceptible to IHN? There are at least two possibilities. First, though most ‘Atlantic’ QTL were associated with resistance, we did find markers associated with susceptibility, particularly those linked to the QTL on chromosome V and linkage group X; these regions may have enough influence on IHN to result in ‘Atlantic’ being susceptible. Second, it is also possible that these alleles associated with susceptibility interact with alleles at other loci which are not segregating in the B2721 population, thereby preventing their detection. In that case, it would be useful to

develop a map in another population (such as NC206) to search for these additional loci. We also point out that 'Atlantic' has variable susceptibility to IHN; as mentioned earlier, Henninger et al. (2000) determined that 'Atlantic' is an unstable clone with respect to this trait. This phenomenon could have limited our ability to detect more QTL and/or QTL alleles associated with susceptibility. Future research on the genetics of IHN should perhaps utilize material that is more highly and stably susceptible. Certain members of the B2721 population fit these criteria, as does B2464-1, the susceptible parent of NC206.

Portability of QTL and markers

In order to test the utility of the markers in B2721 we screened several of them in a secondary population that also segregated for IHN. The QTL for IHN resistance on chromosome I was detected in the NC206 population, via the AFLP marker E38M58_135. A QTL allele for IHN susceptibility on chromosome V of B1829-5, represented by the SSR marker STI032_4, was also located in NC206. In this case, the SSR itself was not associated with IHN, but was closely linked in repulsion to an AFLP marker that was associated with susceptibility. In NC206, both of these markers were present in the susceptible parent. The fact that the parent was highly susceptible to IHN (more so than 'Atlantic') despite the presence of E38M58_135 suggests that the QTL on chromosome I does not have a major effect on IHN, or does so only in certain genetic backgrounds. Clearly, more markers from B2721 need to be tested in NC206 or other populations segregating for IHN, to determine how useful any markers derived from that population would be in selecting for IHN resistance in other genetic backgrounds. It is too early to tell whether or not IHN resistance/susceptibility is governed by a common or diverse set of loci, but these initial results indicate that at least some of the loci involved in IHN are common across these two populations. If a common set of loci determine IHN resistance/susceptibility, a set of markers for breeding IHN-resistant cultivars would have universal application in this regard, and would eliminate a great deal of the costly and time-consuming fieldwork that is currently required.

Map characteristics

To date, the most comprehensive linkage maps for tetraploid potato are those developed by Bradshaw et al. (2008). They obtained map lengths of 1,202 cM for 12601ab1, the female parent, and 1,234 cM for 'Stirling', the male parent of the mapping population. Our estimates of 1,034.4 cM for 'Atlantic' and 940.2 cM for B1829-5 compare favorably with the estimates of Bradshaw et al. (2008). Khu et al. (2008) obtained longer distances for their tetraploid maps, 2,940 and 1,929 cM for the female and male parents, respectively. However, it is unclear whether these distances are based on the length of overall linkage groups, or the length of each homolog within a linkage group. The ultra high-density (UHD) linkage maps of diploid potato assembled by van Os et al. (2006) were 751 and 773 cM for the female and male parents, respectively. Our map distances are slightly longer, in basic agreement with the observation by Bradshaw et al. (2008) that marker order between the UHD and their tetraploid maps was generally the same, but with longer map distances between markers.

Using a total of 14 SSR markers from tomato and potato, we were able to tag (in one or both parents) chromosomes I, II, III, IV, V, VI, VIII, IX, and XI. We were most successful in the case of chromosome III, where three SSR markers (SSR111, SSR22, and SSR300) were successfully mapped. Chromosomes VII, X, and XII were not tagged. Our maps contain large linkage groups, such as groups X and XII of 'Atlantic' and group VII in both parents (Fig. 2), that likely correspond with the remaining three chromosomes. Using agarose gel electrophoresis, we putatively identified one SSR (two for chromosome VII) for each of these chromosomes that was polymorphic in B2721. Marker STI008, located on chromosome VII, was unlinked in our study, and the second chromosome VII marker, STI064, was in actuality monomorphic. SSR4 and SSR20, located on chromosomes 10 and 12, respectively, did not amplify when using the M13-tailed primer method, a phenomenon that is rare, but known to occur (R. Bravo and J. Lyerly, personal communication). Cost constraints prohibited us from screening more SSRs, but we are confident that any future attempts to tag the three remaining chromosomes using B2721 would be successful. Khu et al. (2008), using a smaller population (92 individuals) and more SSR markers (95), were able to tag all 12 potato chromosomes.

Allelic effects

When a simple simplex or duplex dominant model does not adequately describe QTL effects, the six-mean models developed by TetraploidMap (representing the six possible ways a parent can transmit any two alleles to its offspring) can be difficult to interpret. Figure 3 illustrates the effects of the six genotypes for the *inc06* QTL on 'Atlantic' chromosome V. Allele 3 appears to be epistatic to the other three alleles, while allele 4 seems to have a dominant negative effect over alleles 1 and 2. These estimates demonstrate the complex genetic effects present in polyploids, which are absent in diploids. QTL mapping software developed for diploid systems are designed to handle one 'homolog' at a time. This can also be done in polyploids but requires the presence of simplex markers on each homolog, in contrast to TetraploidMap, which incorporates all marker types and

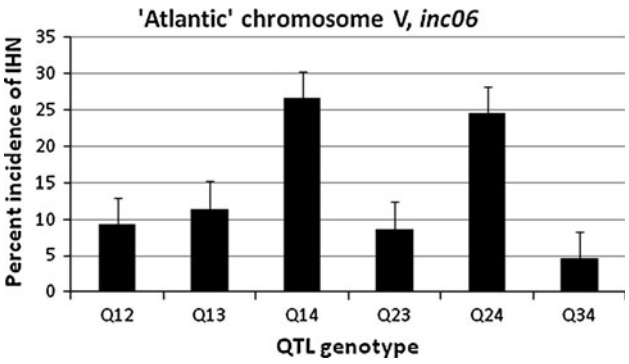

Fig. 3 The effects of six possible QTL genotypes on the incidence of IHN in 2006. This QTL is centered at 64 cM on chromosome V. *Error bars* represent an average of the standard errors of all genotypes. *Q* refers to the putative QTL; 1–4 refer to the four homologs of each chromosome. There are six possible ways to combine the 2 homologs contributed by one parent, hence the six means

is therefore more efficient. If sufficient simplex markers were available, we would likely gain more information about intralocus interactions. Although TetraploidMap is superior to other software in terms of its ability to incorporate dominant and codominant markers in an autotetraploid population, the IM model of TetraploidMap does not provide estimates of interactions between QTL. Again, the presence of more simplex markers covering all four homologs would allow for these more advanced analyses using software such as Qgene (Joehanes and Nelson 2008) or QTL Cartographer (Wang et al. 2007). Through the SOLCAP project (http://solcap.msu.edu), large numbers of sequence-specific, portable SNP markers are expected to be available soon, in the Illumina Infinium platform (Douches 2010). The B2721 population will be genotyped using this array (D. Douches, personal communication), resulting in many more markers that could be incorporated into the maps to improve map coverage and QTL analyses. A further advantage of the SNP array is the SNPs are derived from expressed sequences; these are a source of candidate genes for additional, more targeted experiments. In addition, the new SNP array would facilitate association mapping of IHN across a broad array of germplasm. This approach would likely be more efficient than more traditional linkage-based QTL mapping, especially for determining if SNPs relating to IHN are population dependent, or more universally involved in the trait.

Maturity

There is a small, but significant correlation between maturity and internal heat necrosis, with earlier-maturing clones showing lower levels of IHN, and vice versa. We have detected a maturity QTL on chromosome V, in both parents. This chromosome also carries QTL for IHN incidence and severity, detected in both parents. Upon closer inspection of the markers on these chromosomes, we noticed two AFLP markers from 'Atlantic' and an allele of STI032 from B1829-5 that were associated with later maturity, and higher levels of IHN. The inverse relationship between maturity and IHN is most likely due to these markers (or loci linked to them). The effects of the loci on chromosome V may be pleiotropic with respect to IHN. For example, it is known that IHN symptoms get worse as the season progresses (Sterrett et al. 1991; P. McCord, personal observation), perhaps a cumulative response to the stresses of the growing season. The converse may also be true; individuals that complete their life cycle relatively early in the growing season may be able to escape some of the environmental stresses responsible for the disorder. Sterrett and Henninger (1997) discussed harvesting earlier in the season to avoid IHN symptoms, but pointed out that this tended to reduce yields. Early-maturing varieties that size quickly might be able to avoid this yield penalty. In any case, the fact remains that the correlation between IHN and maturity, though real, is relatively low in absolute terms; early maturity is not a cure for IHN.

Conclusion

Because of its significant economic impact, the difficulty of screening in the field, and its quantitative distribution, IHN is a good candidate for QTL analysis. The QTL mapping reported in this paper is an important first step towards understanding the genetics of the disorder, and developing markers for selection. As a prerequisite for QTL mapping, we successfully generated linkage maps of tetraploid potato, and anchored most of the linkage groups to the known *Solanum* chromosomes. We have reliably identified QTL for IHN incidence and/or severity on chromosomes IV and V, and linkage groups VII and X, and have shown that in most cases, these QTL exhibit dominant action for resistance, which likely explains the skewed phenotypic distribution of IHN. Markers linked to these QTL, and markers from other linkage groups identified using single-point ANOVA, are now candidates for further testing for their utility in selection. We have initiated this process by testing several of these markers in a secondary mapping population; in two cases, we have demonstrated that certain QTL are common to different breeding populations. Much research remains to be done to detect additional QTL and to better quantify the effects and possible interactions of the QTL we have already identified, and to test the efficacy of markers linked to these QTL. The research we have presented provides a solid foundation for further pursuit of this important and intriguing disorder of potato.

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