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ORIGINAL PAPER

Genetic mapping of sulfur assimilation genes reveals a QTL for onion bulb pungency

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Abstract Onion exhibits wide genetic and environmental variation in bioactive organosulfur compounds that impart pungency and health benefits. A PCR-based molecular marker map that included candidate genes for sulfur assimilation was used to identify genomic regions affecting pungency in the cross 'W202A' × 'Texas Grano 438'. Linkage mapping revealed that genes encoding plastidic ferredoxin-sulfite reductase (SiR) and plastidic ATP sulfurylase (ATPS) are closely linked (1–2 cM) on chromosome 3. Inbred F_3 families derived from the F_2 population used to construct the genetic map were grown in replicated trials in two environments and bulb pungency was evaluated as pyruvic acid or lachrymatory factor. Broad-sense heritability of pungency was estimated to be 0.78–0.80. QTL analysis revealed significant associations of both pungency and bulb soluble solids content with marker intervals on chromosomes 3 and 5, which have previously been reported to condition pleiotropic effects on bulb carbohydrate composition. Highly significant associations (LOD 3.7–8.7) were observed between ATPS and SiR Loci and bulb pungency but not with bulb solids content. This association was confirmed in two larger, independently derived F_2 families from the same cross. Single-locus models suggested that the partially dominant locus associated with these candidate genes controls 30–50% of genetic variation in pungency in these pedigrees. These markers may provide a practical means to select for lower pungency without correlated selection for lowered solids.

Abbreviations

EPA	Enzymatically produced pyruvic acid
SSC	Soluble solids content
SSCP	Single-stranded conformation polymorphism
HDX	Heteroduplex
LF	Onion lachrymatory factor
CAPS	Cleaved amplified polymorphic sequence
ACSO	Alk(en)yl cysteine sulfoxide

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Introduction

Onion and other Allium vegetables have been valued since antiquity for their pungent flavour and aroma. Modern science has confirmed traditional beliefs that the organosulfur compounds that impart flavour also confer significant human health benefits (Griffiths et al. 2002; Rose et al. 2005) such as reduced blood clotting (Rendu et al. 2001) and antimicrobial properties (Kyung and Lee 2001). There is, however, evidence that some of these benefits are lost during cooking (Ali et al. 1999). It is desirable to identify breeding strategies to provide mild-flavoured, premium-value

cultivars with a combination of good health benefits and high agronomic quality.

The flavour precursors of onion are 1-propenyl-, propyl- and methyl cysteine sulfoxides. The pungent flavours of onion arise from the reactive sulfenic acid products formed when these alk(en)yl cysteine sulfoxide (ACSO) precursors are hydrolysed by the specific CS-lyase alliinase (Randle and Lancaster 2002). The tear-inducing action of onion is due to the lachrymatory factor (Z,E) thiopropanal-S-oxide (LF), which is produced by the action of lachrymatory factor synthase on 1-propenyl sulfenic acid (Imai et al. 2002). Sulfenic acids undergo a variety of spontaneous reactions, as well as back reactions with LF, producing a complex spectrum of volatiles with sensory and health attributes (Block 1992).

Since 1 mol of pyruvic acid is produced for each mole of ACSO hydrolysed by alliinase, enzymatically produced pyruvic acid (EPA) has long been used as a proxy for measuring pungency, since taste testing multiple onion samples is not feasible (Crowther et al. 2005; Schwimmer and Guadagni 1962; Schwimmer and Weston 1961). More recently, GC measurement of LF has been reported as a direct measure of this key flavourant (McCallum et al. 2005; Schmidt et al. 1996).

The onion flavour experience varies widely, and is a function of sweetness, overall pungency and the proportions of the different ACSO precursors (Randle and Lancaster 2002). Early studies revealed that pungency is strongly influenced by sulfur supply (Freeman and Mossadeghi 1970). Physiological studies have since detailed the influence of genotype, S, N, and other environmental factors on precursor levels and pungency (Randle 2000). Although the molecular basis for the genetic variation is not yet clear, physiological studies have revealed significant differences in the uptake, assimilation and partitioning of sulfate and organic sulfur compounds between mild and pungent varieties (McCallum et al. 2002; Randle 1992; Randle et al. 1999).

Quantitative genetic studies (Lin et al. 1995; Simon 1995) estimated broad-sense heritability of pungency in the range 0.5–0.7 and reported predominantly additive effects on EPA. Formal studies (Wall et al. 1996) and empirical breeding have shown good response to selection for lower pungency. The only marker-based QTL analysis of pungency published to date was conducted by Galmarini et al. (2001), who reported that a locus on chromosome 5 conditioned correlated effects on dry matter and pungency in the cross 'BYG15-23' × `AC43'.

Genetic analysis in onion has previously been limited by a lack of genetic and genomic resources. Populations are highly heterozygous and harbour substantial phenotypic variation. Inbreeding depression and the biennial nature of onion make development of genetic stocks laborious. The very large genome size of *Allium* species (Ricroch et al. 2005) complicates genomic cloning and sequencing strategies. The recent development of an EST resource and a PCR-based map of onion (Kuhl et al. 2004; Martin et al. 2005) has provided a basis for genetic analysis of economic traits. In the present study we used a partial genetic map based solely on PCR markers to detect genome regions affecting pungency in the cross between 'W202A' and 'Texas Grano 438', representatives of storage and sweet onion types widely used by breeders. This revealed co-incidence of a major QTL with two closely linked genes in the sulfate assimilation pathway.

Materials and methods

Plant materials

Field trials were conducted at Pukekohe (lat 37°12′) and Lincoln (lat 43°39′). General plant propagation, harvest and pollination practices were described previously (McCallum et al. 2006). Transplanted plots were arranged in five rows of 40 seedlings at 10 cm spacing. Several 'W202A' × 'Texas Grano 438' F_2 families were developed by self-pollinating single F_1 bulbs from a bulk cross as described previously (McCallum et al. 2006).

'W202A' × 'Texas Grano 438' population (F_2 family '47') Eighty-two F_2 bulbs grown at Lincoln were analysed for pungency and soluble solids and the male-fertile F_2s were self-pollinated to generate 59 F_3 families. In 2002/2003 these families were transplanted in replicated trials at Pukekohe and Lincoln (McCallum et al. 2006). In addition to standard crop management practices, supplementary sulfur fertilisation was applied as 160 kg/ha gypsum in early December at both sites. Plots were lifted at 90% tops down, field-cured for 10 days, hand-clipped and stored under ambient conditions for 8–10 weeks before analysis.

'W202A' × 'Texas Grano 438' population 2004/2005 trial (F_2 families 720 and 722) Seed was directly sown into single plots at West Melton (Lat 43° 39') in August 2004 and hand-thinned to 50–60 mm spacing at the third true leaf stage. Standard crop management was applied without additional S fertilisation. Bulbs were lifted at 90% tops down, hand-clipped, and field-cured

for 1 week. A total of 96 bulbs from each family were subjected to biochemical analysis and DNA was isolated from bulb extracts used for pungency analyses.

'W429A' × 'Houston' 2003/2004 trial Seedlings of parent lines and F_2 individuals were transplanted as described previously (McCallum et al. 2006). Management and S fertilisation were as previously. A total of 107 F_2 plants were tagged and sampled for DNA after bulbing. Bulbs were lifted at 90% tops down, hand-clipped, individually bagged, cured for 3 days at 30°C and stored under ambient conditions for 8–10 weeks before analysis.

Biochemical analysis

Analysis was performed on upper halves of bisected single bulbs at F_2 generations or bulked samples of 10–12 bulbs per plot at F_3 generations (McCallum et al. 2006). Bulb pungency was measured in 1:1 w/v homogenates of bulb tissue in water as EPA and lachrymatory factor (LF) as described previously (McCallum et al. 2005). Soluble solids content (SSC) of homogenates was measured as %Brix by digital refractometry.

Marker analyses

DNA isolation and analysis of SSR, SSCP and CAPS markers were carried out as described previously (Martin et al. 2005; McCallum et al. 2006, 2001b). For heteroduplex analysis, PCR cycling was conducted with addition of a final 95°C denaturation for 5 min followed by cooling to 42°C at a ramp rate of 2°C per minute. PCR products were mixed with non-denaturing sucrose buffer (50% sucrose, 0.3% bromophenol blue, 0.3% xylene cyanol FF, 0.3% orange G), electrophoresed on 6–8% non denaturing polyacrylamide gels and stained with ethidium bromide. Primer sets were previously reported by Kuhl et al. (2004). Additional markers based on new or existing primer sets used in this study are shown in Table 1.

Statistical methods

Linkage mapping, QTL mapping and calculation of heritability were performed as described previously (McCallum et al. 2006). Interval mapping was conducted using a genome-wide $P < 0.05$ significance threshold of LOD 2.3 determined by permutation testing. Mixed models were fitted with residual maximum likelihood (REML) in Genstat (Payne et al. 2005). Site, check line and marker genotype were included as fixed effects and test line and block were included as random effects. Significance of fixed effect terms was evaluated using Wald tests. Single marker models were fitted by regression using Genstat procedure AUNBALANCED and statistical significance was evaluated at a threshold of $P < 0.05$. Parameters and their standard error (SE) are presented in the form estimator (SE).

Results

Linkage mapping of ferredoxin sulfite reductase and plastidic ATP sulfurylase

A partial linkage map has been developed in an F_2 family (family '47') from the cross 'W202A' × 'Texas

Table 1 PCR-based markers used in this study and Genbank accession numbers of expressed sequences from which these were designed

PCR marker name	Homologous SNP or RFLP marker locus	Primer set name	Genbank accession number	L primer sequence R primer sequence	Analysis method
SiR1	SR-ScaI-10_5/; ACAAJ79	SiR1	CF434863	TGCAGCTCTTTCTCAAGTTGG CAGAGCAGGACATGCCATAG	SSCP/HDX
ATPS2	–	ATPS2	AF212154 AF403295	CCAAAACCGCCTAATCCTCA GGCACCGACATATTTACAAAGG	9 bp indel
API43_Mse1	API43G-E5-2-5_4 / API43G-E5-7_9	API43	AA451576	GGAGGTGAAAAGGATGTGGAG AGCAGCAGCAATAGGGTAGC	Mse1 CAPS
API89	API89B-E1-5_4-3	ACE6067CDS	AJ006067	CGTGAAGAACTGGATGAACG CCGTTGATGTCGTACCATTG	SSCP
ACM157B	–	ACM157	CF445533	GCTAGTTGTACCTGCGCCTC TTGTTGTTGGTGTTTCCAGG	SSR (multilocus)
AJK084E	AJK84-E1-15/ AJK84-E1-4-5	AJK084E	BE205623	CTCCCACTTTACCAGCGAAC TTTGAACGCAATCATAGACCA	SSCP

RFLP/SNP markers are anchor loci from map by Martin et al. (2005)

Grano 438' using co-dominant PCR markers, including EST-SSR and candidate gene markers. We previously reported mapping of the *Frc* locus (McCallum et al. 2006) and a serine acetyltransferase gene (McManus et al. 2005) in this population. Linkage groups to date cover significant portions of chromosomes 1–7. Markers for plastidic ATP sulfurylase (ATPS) and ferredoxin-sulfite reductase (SiR) were mapped in close proximity to one end of a chromosome 3 linkage group in this population. These genes respectively catalyse the initial activation of sulfate and the second reduction step leading to incorporation of inorganic sulfate into cysteine (Leustek et al. 2000). This chromosome 3 linkage group shares seven homologous markers with the 'BYG15-23' × 'AC43' map (Martin et al. 2005) and has similar marker order. The relatively small population sizes (82 and 59, respectively) in these studies limit the resolution of current linkage maps.

SiR was initially mapped by SSCP and heteroduplex polymorphisms in the amplicons from the primer set SiR1, which amplifies the region between exons 4 and 5, a region sharing homology with the pseudogene (DQ273270). Although the SSCP analysis revealed additional weaker segregating bands consistent with amplification of the pseudogene, these could not be reliably scored for mapping and the genomic location of the pseudogene has not yet been determined. The genomic location of the SiR gene was confirmed in the 'BYG15-23' × 'AC43' populations by mapping a Sca1 RFLP revealed by the partial C-terminal SiR cDNA (AF403293) and also a SNP in an upstream region of the gene that does not share homology with the pseudogene (EST ACAAJ79, CF434863; Martin et al. 2005).

ATPS was mapped within 1 cM of SiR by an SSCP (McCallum et al. 2001b) and subsequently by a more specific primer set (ATPS2) targeting a 9 bp/Lys-Tyr-Pro insertion-deletion polymorphism in the N-terminal region of the mature protein between accessions AF403295 and AF212154 (McCallum et al. 2002). RFLP analysis in the 'BYG15-23' × 'AC43' families revealed that the ATPS locus was monomorphic and direct sequencing of ATPS2 PCR products from the parental lines revealed heterozygosity at the indel (data not shown). The close linkage between the SiR and ATPS loci was confirmed in a larger F_2 population of 93 individuals from 'W429A' × 'Houston Grano', which showed linkage of 2.2 cM (LOD 33).

Quantitative analyses

Development of the 'W202A' × 'Texas Grano 438' mapping population was initiated by phenotyping individual F_2 bulbs from a single F_1 (family '47') for SSC and EPA (Table 2). This revealed that the distribution of pungency in F_1 bulbs was skewed toward the more pungent parent. EPA and SSC were not well correlated ($r = 0.06$). Replicated trials in contrasting environments of inbred F_3 families derived from self-pollinations of male-fertile F_2 revealed a wide range of pungency (Table 2). EPA and LF values were correlated ($r > 0.7$) and appeared to follow a curvilinear relationship (Fig. 1). The observation that EPA and LF do not reveal identical trends, as observed in our earlier agronomic study (McCallum et al. 2005), confirms that these provide complementary measures of phenotypic variation in pungency.

Table 2 Generation means of soluble solids and pungency in 'W202A' × Texas Grano 438' family '47' used for QTL mapping

	Phenotype	SSC Brix% (SE)			EPA μmol pyruvic acid/g FW (SE)			LF nmol/g FW (SE)	
	Generation	F_2	F_3	F_3	F_2	F_3	F_3	F_3	F_3
	Environment	Lincoln	Lincoln	Pukekohe	Lincoln	Lincoln	Pukekohe	Lincoln	Pukekohe
Line	'Texas Grano 438'	9.80 (0.18) $N = 15$	9.88 (0.54) $N = 9$	7.86 (0.12) $N = 9$	5.07 (0.25) $N = 15$	5.27 (0.80) $N = 9$	4.40 (0.80) $N = 9$	405 (156) $N = 9$	211 (160) $N = 9$
	47 family	10.78 (0.12) $N = 82$	11.4 (0.07) $N = 45$	9.8 (0.07) $N = 58$	5.93 (0.16) $N = 82$	5.92 (0.12) $N = 45$	5.19 (0.11) $N = 58$	383 (23) $N = 45$	339 (23) $N = 58$
	F_1 bulbs	5.46 (0.11) $N = 15$	ND	ND	6.39 (0.27) $N = 15$	ND	ND	ND	ND
	'W202A'	10.00 (0.15) $N = 15$	12.14 (0.28) $N = 9$	11.09 (0.11) $N = 9$	7.08 (0.42) $N = 15$	6.23 (0.80) $N = 9$	6.31 (0.80) $N = 9$	562 (156) $N = 9$	489 (160) $N = 9$

Sample sizes denote number of individual bulbs analysed at F_2 generation and number of F_3 lines or plots of parent lines analysed at F_3
ND not determined

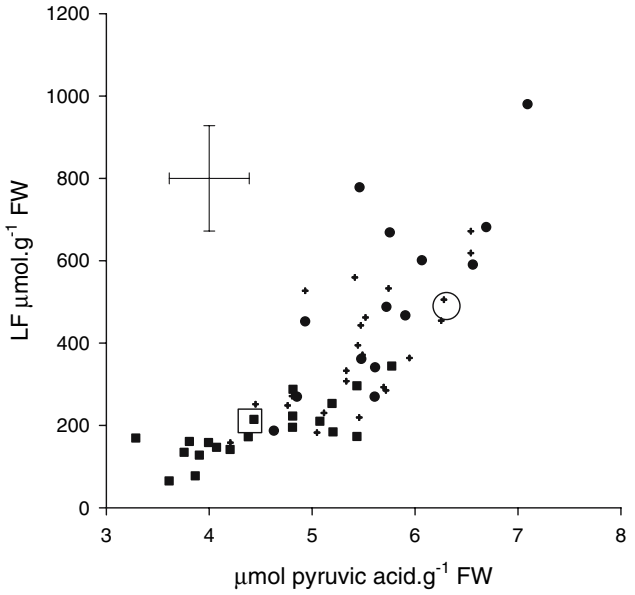

Fig. 1 Mean bulb pungency of parental lines (*open square* Texas Grano 438; *open circle* 'W202A') and F_3 families, plotted by ATPS2 marker genotype (*filled square* homozygous for 'Texas Grano 438' allele; *filled circle* homozygous for 'W202'A allele; cross-heterozygous). *Bars* denote approximate mean 95% confidence interval for line means

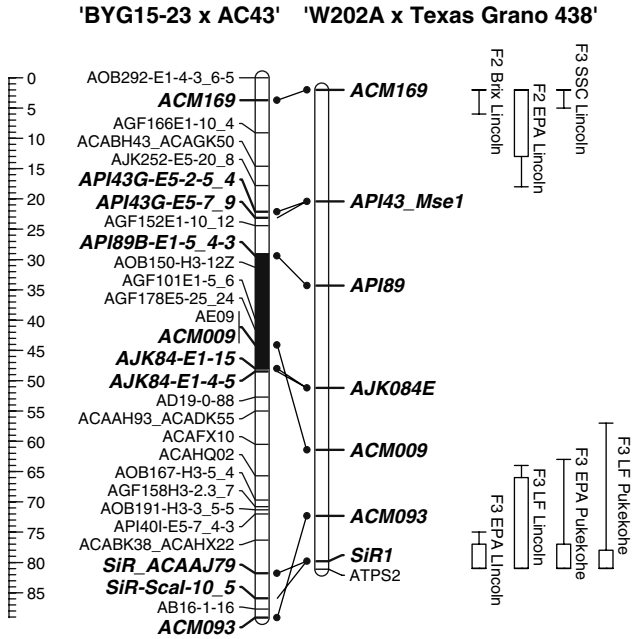

Fig. 2 QTL mapping for bulb pungency on onion chromosome 3 in 'W202A' × 'Texas Grano 438' and alignment with 'BYG15-23' × 'AC43' linkage map. The scale denotes Kosambi distance. The interval reported by Galmarini et al. (2001) to affect dry matter is shown in *bold*. Common markers are denoted in *bold italics*. Locations of QTL detected by standard interval mapping are denoted as regions with LOD > 2.3 (outer bounds) and 1-LOD interval surrounding peak (*inner bar*)

REML models showed significant environmental variation within and between environments. The Lincoln and Pukekohe sites differed in terms of mean pungency as EPA ($\chi^2 = 70.6$; 1 *df*; $P < 0.001$) and LF ($\chi^2 = 6.7$; 1 df; $P = 0.01$) (Table 2). Residual variance for both traits was significantly higher at Pukekohe [EPA: Pukekohe 0.24 (0.04), Lincoln 0.38 (0.05); LF: Pukekohe 20820 (2856), Lincoln 9326 (1379)], suggesting higher within-site variability. Broad-sense heritability estimates obtained from these models were correspondingly higher at Lincoln [LF 0.85 (0.08); EPA 0.88 (0.06)] than at Pukekohe [LF 0.78 (0.10) ;EPA 0.81 (0.08)]. These estimates are higher than estimates of 0.5–0.7 reported in earlier studies (Lin et al. 1995; Simon 1995). Bulb pungency measured as EPA was more strongly correlated with SSC at Lincoln ($r = 0.35$) than at Pukekohe ($r = 0.25$).

QTL mapping

Single marker analyses and interval mapping using the partial genetic map revealed highly significant associations of EPA and LF with markers on chromosome 3. (Fig. 2; Table 3). In both F_2 and F_3 generations in the Lincoln environment, statistically significant associations were observed both with the marker ACM169 and the region containing ATPS and SiR, at each end of the linkage group. At Pukekohe there was only significant association with ATPS/SiR (Figs. 1, 2; Table 3). The ACM169 region, but not the ATPS/SiR region, showed association with SSC in both generations in the Lincoln environment. This suggests that this locus exerts a pleiotropic effect on pungency in this environment through its effects on bulb solids. Galmarini et al. (2001) reported effects of the neighbouring genome region on solids in 'BYG15-23' × 'AC43'. Model estimates showed evidence of dominance at the ATP/SiR locus, with the F_2 generation exhibiting complete dominance of the W202A allele over the Texas Grano 438 allele.

Statistically significant associations were also observed in both generations and environments between pungency and the marker ACM171 on chromosome 5 (Table 3). We previously reported that this region has a significant effect on bulb dry matter in this population (McCallum et al. 2006). Galmarini et al. (2001) also reported that this region, which contains a phloem-unloading sucrose transporter locus (API66), affected bulb dry matter and pungency, confirming that this region also exerts a significant pleiotropic effect on pungency.

Estimates from single locus regression and interval mapping models suggested that the locus in the ATPS/SiR region controlled ~50% of the heritable variation

Table 3 QTL for pungency and soluble solids detected by interval mapping on chromosomes 3 and 5 of 'W202A' × 'Texas Grano 438' population (family '47')

Chromo-some	Marker interval	Generation	Environment	Trait	LOD	%var	Effects	
							A	D
3	ACM169	F_2	Lincoln	SSC	2.59	14	−0.27	0.16
3	ACM169	F_2	Lincoln	EPA	4.56	25	0.49	0.16
3	ATPS/SiR	F_2	Lincoln	EPA	2.46	13	0.29	0.29
3	ATPS/SiR	F_3	Lincoln	EPA	3.7	32	0.52	0.35
3	ATPS/SiR	F_3	Lincoln	LF	3.87	33	98	66
3	ATPS/SiR	F_3	Pukekohe	EPA	5.59	36	0.60	0.26
3	ATPS/SiR	F_3	Pukekohe	LF	8.65	50	161	25
5	ACM133-ACM171	F_3	Lincoln	EPA	2.51	27	0.63	0.18
5	ACM133-ACM171	F_3	Lincoln	SSC	4.4	38	0.58	0.38
5	ACM133-ACM171	F_3	Pukekohe	LF	2.50	19	106	−70

LOD score and percentage of explained phenotypic variance were determined from single locus models. Additive effects were associated with alleles from 'W202A'

in pungency. Since these genes are candidates for conditioning variation in S assimilation and partitioning of organosulfur precursors to flavour, we sought to verify this association in independent pedigrees. Additional larger F_2 families were grown at Lincoln in subsequent years in single plots and individual bulbs were phenotyped and genotyped at the ATPS2, ACM171 and ACM169 loci. Since DNA can be readily isolated from the same bulb juice samples used for phenotyping, this is a particularly economical approach to genetic analysis of onion composition. The population 'W429A' × `Houston Grano' exhibited wide variation in pungency but did not exhibit statistically significant associations of pungency with SiR or ATPS genotypes (data not shown). Two additional F_2 families derived independently from two other 'W202A' × 'Texas Grano 438' F_1 bulbs were also evaluated in this manner. Both families exhibited significant association between ATPS2 genotypes and LF (Table 4). Analysis of bulb fructan content suggested that the '720' family was segregating at the *Frc* locus (fructan content range 7.3–34.8% DW) but that the '722' family was fixed for low fructan (fructan content range 4.3–15.9% DW). This observation suggests that 'W202A' is not fixed at the *Frc* locus. The families differed in correlations between EPA and SSC and association between markers and these traits. The '720' family exhibited a moderate correlation between EPA and SSC (r = 0.45) but this was not so in family '722' ($r = 0.06$). The '720' family, but not the '722' family, exhibited significant associations ($P < 0.05$) between ACM169 and ACM171 marker genotypes and both EPA and SSC content.

Discussion

This is the first report of a QTL affecting onion pungency without pleiotropic effects on bulb solids content. The close association of two sulfur assimilation genes with pungency suggests that mutations in one of these genes or cis-elements may condition the observed variation. We are conducting functional studies of the

Table 4 ANOVA tests of association between marker genotype and bulb composition phenotypes for individual F_2 bulbs (family '47' $N = 82$; 720, 722 $N = 96$ individuals) from three families of the 'W202A' × `Texas Grano 438' cross

Family	Chromo some	Marker	EPA			LF			SSC		
			A	D	F prob	A	D	F prob	A	D	F prob
47	3	ACM169	0.48	−0.03	<0.001				−0.19	0.12	0.081
	3	ATPS2	0.37	0.32	0.003				0.02	0.09	0.797
	5	ACM171	0.24	0.03	0.293				0.22	0.26	0.006
720	3	ACM169	0.48	0.07	<0.001	154	15	<0.001	0.36	0.02	0.017
	3	ATPS2	0.21	0.07	0.128	106.5	8.5	0.002	−0.09	−0.32	0.171
	5	ACM171	0.25	−0.07	0.038	41.5	−9.5	0.35	0.44	−0.20	<0.001
722	3	ACM169	0.20	−0.07	0.198	74	24	0.044	0.12	−0.08	0.618
	3	ATPS2	0.23	0.35	0.005	142	98	<0.001	0.02	0.49	0.022
	5	ACM171	0.22	−0.03	0.109	36.5	−53.5	0.253	0.17	−0.08	0.365

Dominance (D) and additive (A) effects are calculated from genotypic means (standard errors are omitted for clarity)

sulfate assimilation pathway in the two parent lines to identify if there are varietal differences in expression of these enzymes.

The different patterns of phenotypic correlation observed between sites and families suggest that the loci conditioning pleiotropic effects on solids and pungency are heterozygous in the parents and affected by environment. Previous quantitative genetics studies revealed widely differing patterns of phenotypic correlation and dominance. Simon (1995) observed some dominance for low pungency. The inter-relationship of bulb solids, dry matter, water content and storability is still poorly understood (Sinclair et al. 1995). The observation that segregating families derived from the same cross between 'mild sweet' and 'pungent storage' onion parents can differ widely in terms of trait segregation and correlation implies that multiple families should be evaluated under contrasting environments to unravel the genetics of bulb composition. The major barrier to this is the cost of developing, replicating and propagating inbred F_3 families such as those used in this study and previous QTL mapping (Galmarini et al. 2001). The major disadvantages of inbred family development in onion are the potential for losses and population skewing due to poor adaptation or storage and lack of segregating markers due to parental heterozygosity.

The results obtained in this study and previous analyses of bulb fructan (McCallum et al. 2006) demonstrate that a more economical and informative approach is to analyse the genotypes and phenotypes of larger samples of single bulbs from multiple families from multiple pedigrees. With the availability of a medium-density framework map of PCR-based markers in *Allium* this is the most practical approach to expanding knowledge of onion genetics.

The observation of significant dominance at the ATPS/SiR locus has practical implications for onion breeding. A significant advantage of genetic analysis in F_2 onion populations is the ability to more easily identify dominant variation at major QTL. We recently reported that the *Frc* locus, which conditions large differences in bulb fructan and reducing sugar content, also exhibits complete dominance of high fructan over sweet, low fructan phenotypes (McCallum et al. 2006). Marker-aided selection at these loci would be an efficient means to fix sweetness and pungency phenotypes without self-pollinations.

The observation that at least two loci on chromosomes 3 and 5 condition pleiotropic effects on solids and pungency suggests that phenotypic selection for reduced pungency could lead to correlated selection for reduced solids, with possible negative impact on storage. Conversely, phenotypic selection for storage life may lead to selection at these loci for higher solids, with correlated increase in pungency. We speculate that such heavy storage selection led to the skewing of New Zealand onion germplasm toward higher pungency (McCallum et al. 2001a).

Simon (1995) noted the feasibility of independent selection for pungency and solids and these results suggest two practical strategies for achieving this. The first is to analyse the phenotypes of individual bulbs from multiple families to identify families exhibiting low phenotypic correlation between pungency and solids, from which low pungency lines can be selected. The second is to employ marker-aided selection at ATPS/SiR or other pungency-specific loci. The ATPS2 insertion/deletion marker is a user-friendly marker for selecting the lower pungency phenotype from 'Texas Grano 438.'

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