Molecular Approaches to Characterizing and Improving Bulb Composition in Onion

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Abstract

Onion bulb composition varies widely, both within and between varieties. Development of Allium gene sequence and mapping resources has enabled dissection of bulb composition traits through genetic analysis and physiological studies. The collaborative use of complementary genetic stocks has proved critical to this progress. Mutation scanning of the 5' regions of genes has proved the most robust source of portable markers to date. Both marker and QTL studies to date confirmed high levels of heterozygosity within bulb onion. The most surprising finding to date is that a single genetic locus, Frc, conditions the majority of the variation in reducing sugar and fructan contents between sweet and storage onion types. Heterozygosity at this dominant locus could underlie substantial within-population variation in bulb composition. Another well-supported locus conditions variation in dry matter and pleiotropic effects on pungency. Biochemical and genetic studies have supported earlier suggestions that differences in S assimilation underlie some genetic variation in pungency.

INTRODUCTION

Onion bulbs are a unique functional food, delivering prebiotic fructans, antioxidant flavonoids and organosulfur compounds with anticancer and cardiovascular health benefits (Griffiths et al., 2002). These same compounds impart the visual and taste attributes sought by customers and it is therefore of interest to breeders to better understand the wide variation in these compounds within and between varieties.

Despite the global culinary and economic significance, genetic research of onion has greatly lagged that of other major vegetable crops. This has been due in part to the difficulty of developing and propagating genetic stocks and a lack of sequence resources. Another barrier to more rapid progress has been the small community engaged in Allium genetic research.

Biochemical and physiological studies of onion bulb composition and development have documented the wide variation in dry matter, carbohydrate composition, pungency and organosulfur compounds and pigments in cultivated onion. Many of the key enzymes involved in biosynthesis and metabolism of these compounds have been characterised and cloned, though details of some key steps in flavour precursor biosynthesis remain unclear (Randle and Lancaster, 2002). Studies of expression and sequence variation in flavonoids biosynthetic enzymes have shown putative associations between alleles and bulb colour (Kim et al., 2004, 2005). However, these loci have not been mapped and colour has yet to be subjected to formal QTL or multi-locus genetic analyses. Studies using alien addition lines have however revealed chromosomal locations of key regulatory and structural genes for flavonoid metabolism (Masuzaki et al., 2006;
Extensive physiological studies have detailed the impact of sulfur nutrition and genotype on pungency (Randle and Bussard, 1993; Randle et al., 1995) and have suggested that mild land pungent varieties differ in assimilation and partitioning of sulfate (Randle et al., 1999). Although marker-free quantitative genetic studies have been conducted on inheritance of pungency and solids contents (Lin et al., 1995; Simon, 1995), the results did not reveal any major clues about the genetic control of bulb composition.

This paper will outline recent progress in dissection of bulb carbohydrate composition and pungency made possible using a framework molecular marker map based on onion EST resources and collaborative use of complementary genetic stocks.

GENETIC MAP DEVELOPMENT IN ONION

The first public genetic map of onion published in 1998 (King et al., 1998) was based primarily on RFLP markers evaluated on F3 families from the cross between the pungent storage inbred ‘Brigham Yellow Globe’ (BYG15-23) and mild sweet show onion ‘Alisa Craig’ (AC43). Since evaluation of RFLP markers is technically demanding due to the large genome size of onion, we sought to convert these to PCR-based assays and showed that single-stranded conformation polymorphism (SSCP) or CAPS assays could be developed from many of these (McCallum et al., 2001). Subsequent efforts to develop markers from the larger EST resource developed by (Kuhl et al., 2004) revealed that the 5' UTR and N-terminal coding regions of genes were good targets for developing EST-SSR and/or EST-SNP markers. Preliminary surveys of EST-SSR allelic variation in a broad sample of cultivated onion germplasm have revealed high heterozygosity but limited allele size variation (McCallum and Havey, unpublished). These markers have proved highly portable between laboratories, crosses and Allium species and greatly improved the usability of the improved BYG15-23 × AC43 map (Martin et al., 2005).

Another major improvement to this map was the chromosomal assignment of linkage groups based on assignment of single-locus PCR markers in a panel of alien monosomic addition lines of Allium fistulosum with extra chromosomes from shallot (Shigyo et al., 1996). These lines were similarly used previously to assign linkage groups of the AFLP-based linkage map of an A. cepa × A. roylei interspecific cross (van Heusden et al., 2000). We are currently adding codominant EST-SSR and EST-SNP markers to this highly polymorphic cross (McCallum et al., unpublished).

Although they do not reveal size polymorphisms, some onion EST-SSR markers can amplify in A. fistulosum crosses and reveal SNPs (Hikaru Tsukazaki, pers. comm.). We have developed a partial linkage map in the onion cross ‘W202A × Texas Grano 438’ using EST-SSR and EST-SNP markers, many of which are shared with the BYG15-23 × AC43 and interspecific maps (McCallum et al., 2006; McManus et al., 2005). The sharing of common portable PCR-based markers and linkage information between these Allium crosses and the systematic use of alien addition lines is permitting the development of an increasingly large set of chromosomally assigned, portable, PCR-based markers suitable for QTL mapping and other genetic analyses in Allium.

GENETIC ANALYSIS OF BULB COMPOSITION

The genetic map of BYG15-23 × AC43 was first used by Galmarini et al. (2001) to conduct QTL analysis of pungency, dry matter and soluble solids content. The most notable finding of this study was that two QTL affecting solids and dry matter content were identified in regions flanked by RFLP revealed by candidate genes for carbohydrate metabolism - an acid invertase (API89) on group D (chromosome 3) and a phloem-unloading sucrose transporter (API66) on group E (chromosome 5). The locus on chromosome 5 also affected pungency, suggesting that this locus may exert pleiotropic effects on bulb composition. A further QTL study using this map in conjunction with more detailed analysis of bulb carbohydrates again revealed significant effects of the chromosome 3/acid invertase region on bulb sucrose content (Havey et al., 2004).

During early efforts to develop an F2 mapping population in the cross ‘Colossal Grano’ × ‘Early Longkeeper P12’ we observed strongly bimodal segregation for bulb
fructan content when samples were freeze-dried prior to analysis. Subsequent analyses of other storage × sweet onion crosses, including families from ‘W202A × Texas Grano 438’, also showed the same pattern of 3:1 high fructan/low reducing sugar: low fructan/ high reducing sugar phenotypes. Fortuitous identification of an unlinked EST-SSR marker in ‘W202A × Texas Grano 438’ in strong disequilibrium with fructan content permitted rapid assignment of the putative locus to chromosome 8 using the interspecific mapping population (McCallum et al., 2006). The improved map of Martin et al. (2005) then provided markers for more detailed QTL mapping in ‘Colossal Grano’ × ‘Early Longkeeper P12’ and ‘BYG15-23 × AC43’, confirming that in all these populations a single dominant locus on chromosome 8, dubbed Frc, conditioned most heritable variation in fructan content. This study also confirmed the importance of the chromosome 5 locus identified by Galmarini et al. (2001) in control of bulb dry matter content. The observation that a gene on chromosome 8 of *A. cepa* conditions major shifts in fructan accumulation was independently confirmed by Hang et al. (2004), who observed that chromosome 8 shallot additions in *A. fistulosum* lines caused an increase in accumulation of non-reducing sugars in winter leaf blades.

In parallel with these studies of carbohydrate composition we have also conducted genetic analysis of pungency in ‘W202A × Texas Grano 438’ and extensive comparative physiological studies of sulfur assimilation physiology in the parent lines. In general our physiological studies have supported earlier suggestions that mild and pungent onions differ in expression and regulation of the sulfur assimilation pathway (McCallum et al., 2002; Randle et al., 1999). A key observation is that foliar cysteine, but not glutathione levels are significantly higher in the more pungent line ‘W202A’. Use of EST resources and homology data has permitted us to clone major sulfur assimilation genes, providing genetic markers (McCallum et al., 2001, 2002; McManus et al., 2005) as well as permitting development of antibodies to recombinant proteins. Linkage mapping has revealed that two key sulfur assimilation genes, ATP sulfurylase (ATPS) and sulfite reductase (SiR) are closely linked on chromosome 3 (McCallum et al., submitted). QTL analysis in F2 and replicated F3 families of ‘W202A × Texas Grano 438’ in two environments revealed that this genome region contains a QTL affecting bulb pungency. We verified this association in two additional F2 families independently derived from the same cross. This QTL analysis also confirmed that the chromosome 5/API66 region exerted pleiotropic effects on pungency and solids, as well as another locus on chromosome 3, close to the API89 acid invertase region detected by (Galmarini et al., 2001). Genetic studies using much larger populations are desirable in order to study epistatic interactions between these genes and Frc in the control of bulb composition.

**CONCLUSION**

Genetic analysis aided by a framework molecular marker map has allowed rapid advances in the understanding of variation in onion bulb composition, revealing at least four unlinked genome regions with significant effects. The effectiveness of shallot - *A. fistulosum* alien addition lines for chromosomal assignment of genes associated with flavonoid and carbohydrate composition suggests that these will continue to be a key resource for functional and genetic studies of major genes. A key approach in our recent studies has been the use of single bulb genotypes and phenotypes from multiple large F2 populations. Greater automation of this approach, combined with a growing collection of PCR-based markers, including those from key structural and regulatory genes, will enable further refinement of genetic models and provide breeder-friendly markers for economic bulb composition traits.

**Literature Cited**


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