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# Genotypic variation in the sulfur assimilation and metabolism of onion (*Allium cepa* L.) I. Plant composition and transcript accumulation

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ABSTRACT

Organosulfur compounds are major sinks for assimilated sulfate in onion (*Allium cepa* L.) and accumulation varies widely due to plant genotype and sulfur nutrition. In order to better characterise sulfur metabolism phenotypes and identify potential control points we compared plant composition and transcript accumulation of the primary sulfur assimilation pathway in the high pungency genotype 'W202A' and the low pungency genotype 'Texas Grano 438' grown hydroponically under S deficient (S−) and S-sufficient (S+) conditions. Accumulation of total S and alk(en)yl cysteine sulfoxide flavour precursors was significantly higher under S+ conditions and in 'W202A' in agreement with previous studies. Leaf sulfate and cysteine levels were significantly higher in 'W202A' and under S+. Glutathione levels were reduced by S− treatment but were not affected by genotype, suggesting that thiol pool sizes are regulated differently in mild and pungent onions. The only significant treatment effect observed on transcript accumulation in leaves was an elevated accumulation of O-acetyl serine thiol-lyase under S−. By contrast, transcript accumulation of all genes in roots was influenced by one or more treatments. APS reductase transcript level was not affected by genotype but was strongly increased by S−. Significant genotype × S treatment effects were observed in a root high affinity-sulfur transporter and ferredoxin-sulfite reductase. ATP sulfurylase transcript levels were significantly higher under S+ and in 'W202A'.



## 1. Introduction

Members of the genus *Allium*, such as Chinese chive (*Allium tuberosum*), garlic (*Allium sativum*) and bulb onion (*A. cepa* L.), contain high levels of the reduced organosulfur compounds alk(en)yl cysteine sulfoxides (ACSOs), which confer characteristic flavours (Randle and Lancaster, 2002) and human health benefits (Griffiths et al., 2002). The major ACSOs of bulb onion are (+)-S-methyl-L-cysteine sulfoxide (MCSO) and *trans*-(+)-S-(1-propenyl)-L-cysteine sulfoxide (1-PrCSO; Kubec and Dadáková, 2009). ACSOs are biosynthetically derived from glutathione (Lancaster and Shaw, 1989) but the enzymatic steps have not been fully characterised. The pungent aromas of onion arise following cell disruption, when ACSOs are hydrolysed by alliinase (EC 4.4.1.4; Randle and Lancaster, 2002). The most characteristic feature of onion flavour is the tear-producing propanethial S-oxide (lachrymatory factor; LF), which is formed by the action of lachrymatory factor synthase (LFS) on 1-propenyl sulfenic acid produced by the action of alliinase on 1-PrCSO (Imai et al., 2002).

The levels of ACSOs and resulting pungency of onion bulbs vary widely due to genetic and environmental factors, most notably sulfur nutrition (Freeman and Mossadeghi, 1970; Randle and Lancaster, 2002). In a series of comparative hydroponic studies under differing S nutrition, Randle and colleagues demonstrated substantial genotypic and genotype × S treatment variability in partitioning of S into sulfate, ACSOs and other organosulfur compounds (Randle, 1992; Randle et al., 1995, 1999). We previously reported similar hydroponic studies in roots of young plants, prior to bulbing, of a mild and a pungent onion genotype with and without S deprivation (McCallum et al., 2002). These revealed significant genotypic differences in levels of sulfur- and nitrogen-containing metabolites and transcript levels of S assimilatory genes, suggesting that genetic variation in pungency may in part be conditioned by differences in regulation of the S assimilation pathway in onion. However these studies did not characterise metabolism of plants following initiation of bulbs, which constitute a strong sink for organosulfur compounds.

Although there are numerous studies reporting the effects on S phenotypes of manipulating single genes through genetic modification, principally in *Arabidopsis*, there are few concerning natural

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allelic variation associated with heritable genetic variation in such phenotypes. A mutation in adenosine 5′-phosphosulfate reductase (APR; EC 1.8.99.2) in *Arabidopsis* has been shown to condition natural variation in sulfate levels (Loudet et al., 2007). R2R3 MYB transcription factors have been shown to regulate gene expression associated with primary S metabolism (Yatusevich et al., 2010) and allelic variation in these modulates glucosinolate levels in crucifers (Sonderby et al., 2010). Comparative studies of responses by *Brassica oleracea* and onion to $H_2S$ treatment showed significant differences between these species in S accumulation (Durenkamp and De Kok, 2004) and regulation of APR (Durenkamp et al., 2007).

We previously obtained genetic evidence linking allelic variation in onion S assimilation pathway genes and quantitative variation in organosulfur phenotypes by quantitative trait locus (QTL) mapping of bulb pungency and LF levels in progeny of a cross between the mild onion 'Texas Grano 438' and the pungent onion variety 'W202A' (McCallum et al., 2006b). This revealed a QTL associated with closely linked genes on chromosome 3 encoding ATP sulfurylase (EC 2.7.7.4) and ferredoxin-sulfite sulfite reductase (EC 1.8.7.1; SiR). In order to understand the functional basis for these associations we compared metabolite and transcript accumulation profiles of S assimilation genes in the 'Texas Grano 438' and 'W202A' parental genotypes before and after bulb formation.

## 2. Results and discussion

### 2.1. Biomass and elemental composition

Mean leaf to root ratio was significantly lower in S-treatments (S+ ratio = 6.59; S− ratio = 5.08; $P = 0.001$) and leaf, but not root biomass accumulation (as FW) was significantly reduced by S− treatment after bulbing (Table 2; Fig. 1). Stunted growth and leaf yellowing typical of S deprivation were visible in some plants in S− treatments after bulbing. This suggests that sufficient S was available for immature plants prior to bulbing in the 18L tubs, such that uptake and growth was not restricted.

Profiles of elemental and organosulfur content in the mild and pungent genotypes profiled in this study were broadly similar to those we previously reported with 'Houston Grano' and 'Canterbury Longkeeper' (McCallum et al., 2002). Leaf tissue sulfate concentrations were significantly higher in 'W202A' in S+ treatments ($P < 0.0001$ for genotype × S treatment interaction; Fig. 2). Total S accumulation in root, leaf and bulb showed similar significant reduction under S− at bulbing and accumulation was significantly higher in 'W202A' ($P < 0.0001$ for S treatment × genotype; data not shown). Leaf nitrate levels were significantly higher in 'Texas Grano' ($P < 0.01$).

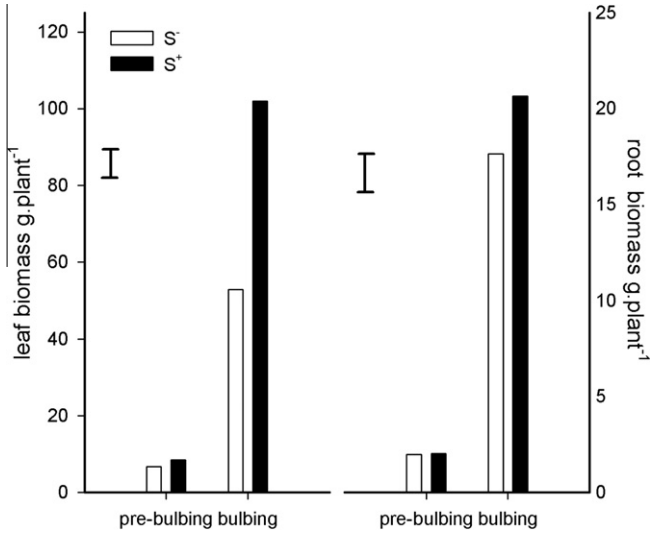


**Fig. 1.** Effects of S deprivation and plant maturity on mean leaf and root biomass (as FW). Error bars denote pooled LSD (df = 21) for comparing different levels of S treatment × plant maturity (pre-bulbing and bulbing) treatment combinations, as shown in Table 2.

### 2.2. Amino acid and organosulfur content

Significant differences were observed in the accumulation of ACSOs and amino acids similar to patterns we previously observed among mild and pungent cultivars (McCallum et al., 2002). Most notably, 'W202A' accumulated higher levels of 1-PrCSO in all tissues and exhibited more marked accumulation in response to S+ treatment ($P < 0.0001$ for genotype × S treatment interaction; Table 2). Leaf and bulb asparagine and glutamine levels were significantly higher under S deprivation and in 'W202A' (Table 1; McCallum et al., 2005). Levels of leaf glutathione responded strongly to S availability but were not affected by genotype (Fig. 2), and methionine levels were not affected by S treatments, as reported in *Arabidopsis* by Nikiforova et al. (2005). By contrast, levels of leaf cysteine were significantly higher in 'W202A' than in 'Texas Grano 438' under S+ (Fig. 2). Cysteine levels are tightly controlled in plants (Yi et al., 2010) and studies have previously suggested that the relatively high cysteine content of *Allium* tissues is a result of lower feedback inhibition by cysteine to serine *O*-acetyl transferase (SAT; E.C. 2.3.1.30; Urano et al., 2000), although one isoform of SAT characterised from onion is feed-back regulated by cysteine (McManus et al., 2005). While significant changes in cysteine pools have been reported in transgenic plants expressing modified SAT genes (Wirtz and Hell, 2003; Tabe et al., 2009), this appears to be the first report of significant natural genotypic variation in cysteine pools within a species.

### 2.3. Transcript accumulation

Transcript accumulation in leaves was not affected by S or other treatments, with the exception of OASTL I (*O*-acetyl-L-serine (thiol)-lyase; E.C. 4.2.99.8) which exhibited a statistically significantly increase in accumulation under S− (S+ 1.65; S− 2.31; *F*-test $P = 0.036$). A role for OASTL in biosynthesis of ACSOs has been suggested by the studies of Jones et al. (2004). By contrast, accumulation of all transcripts in roots was influenced by one or more treatments (Table 3). The largest variation in accumulation was observed in APR, which exhibited strong up-regulation by S−, consistent with reports in *Arabidopsis* and other species that APR is a key control point for S flux (Vauclare et al., 2002). However no significant genotypic differences were observed in APR transcript accumulation, suggesting that it is unlikely that variation in onion APR regulation conditions genotypic variation in sulfate pools, as reported in *Arabidopsis* (Loudet et al., 2007). Significant genotype × S treatment effects were observed in a group I high affinity sulphate transporter homolog (HAST) and SiR, but most strongly by ATP sulfurylase (Fig. 3), similar to trends we reported previously in a different pair of low and high-pungency lines (McCallum et al., 2002). Genetic marker studies using these three genes have shown that the ATP sulfurylase and SiR sequences used to design qPCR assays map as single loci (McCallum et al., 2006b) using PCR-based methods but that the HAST sequence reveals multiple loci (unpublished observations). The HAST gene family is complex, and exhibits complex patterns of expression among organs in response to developmental and environmental cues (Parmar et al., 2007). The HAST and ATP sulfurylase sequences were cloned from a differential cDNA library prepared from S-deprived versus S-sufficient roots and shown to exhibit significant responses to S-deprivation