Plant Physiology and Biochemistry 121 (2017) 176–186

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Selenium treatment differentially affects sulfur metabolism in high and low glucosinolate producing cultivars of broccoli (*Brassica oleracea* L.)

Marian J. McKenzie[a,*], Ronan K.Y. Chen[a], Susanna Leung[b], Srishti Joshi[b,1], Paula E. Rippon[c], Nigel I. Joyce[c], Michael T. McManus[b,2]

[a] New Zealand Institute for Plant & Food Research Ltd., Palmerston North, Private Bag 11600, Manawatu Mail Centre, Palmerston North, 4442, New Zealand
[b] Institute of Fundamental Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand
[c] New Zealand Institute for Plant & Food Research Ltd., Lincoln, Private Bag 4704, Christchurch Mail Centre, Christchurch, 8140, New Zealand

ARTICLE INFO

Keywords:
Broccoli
Selenium application
Sulfur metabolism
Glucosinolate content
O-acetylserine(thiol) lyase
APS kinase
ATP sulfurylase

ABSTRACT

The effect of selenium (Se) application on the sulfur (S)-rich glucosinolate (GSL)-containing plant, broccoli (*Brassica oleracea* L. var. *italica*) was examined with a view to producing germplasm with increased Se and GSL content for human health, and to understanding the influence of Se on the regulation of GSL production. Two cultivars differing in GSL content were compared. Increased Se application resulted in an increase in Se uptake *in planta,* but no significant change in total S or total GSL content in either cultivar. Also no significant change was observed in the activity of ATP sulfurylase (ATPS, EC 2.7.7.4) or *O*-acetylserine(thiol) lyase (OASTL, EC 2.5.1.47) with increased Se application. However, in the first investigation of APS kinase (APSK, EC 2.7.1.25) expression in response to Se fertilisation, an increase in transcript abundance of one variant of *APS kinase 1* (*BoAPSK1A*) was observed in both cultivars, and an increase in *BoAPSK2* transcript abundance was observed in the low GSL producing cultivar. A mechanism by which increased *APSK* transcription may provide a means of controlling the content of S-containing compounds, including GSLs, following Se uptake is proposed.

1. Introduction

The Brassicaceae are known for their capacity to produce and accumulate glucosinolates (GSLs), a group of S-containing glucosides that are proposed to contribute to the sharp/bitter taste reported for members of this family (Fenwick et al., 1983). GSLs are generated from amino acids in a multi-step process involving chain elongation, biosynthesis of the core structure, and then side chain modification (Sonderby et al., 2010). Together, these reactions produce the more than 120 glucosinolates identified today (Fahey et al., 2001). GSLs are thought to be involved in protection from general herbivory (Rask et al., 2000; Giamoustaris and Mithen, 1995), and bacterial and fungal disease (Brader et al., 2006). There is also strong evidence that their breakdown products have powerful anticarcinogenic effects in mammals (Stoner and Morse, 1997; Gupta et al., 2014) and therefore play an important role in human health (Verhoeven et al., 1997; Hecht, 1999). GSLs themselves are stable, inactive compounds that are hydrolysed by the action of the β-thioglucosidase myrosinase, which catalyses the first step in the formation of the bioactive isothiocyanates, indoles and nitriles (Rask et al., 2000). At the sub-cellular level, myrosinase remains physically separated from the glucosinolates until cellular disruption of the plant tissue by wounding, chopping or chewing (Bones and Rossiter, 1996).

The Brassicaceae are also notable for accumulating high endogenous concentrations of Se (up to 1000 mg kg^{-1} DW in some tissues) with little or no apparent detriment to the plant (Zayed et al., 1998), making this family members of the Se-accumulators. In non Se-accumulating plants, Se is tolerated at much lower levels (no more than 100 mg kg^{-1} DW) and is incorporated non-specifically into amino acids such as cysteine (Cys) or methionine (Met) in place of S to produce selenocysteine (SeCys) and selenomethionine (SeMet). These selenoamino acids are used during protein synthesis, resulting in toxicity symptoms such as stunting, necrosis of the leaves and reduced root growth when these plants are exposed to high levels of Se (Brown and

Abbreviations: APSK, Adenosine 5′-phosphosulfate kinase; ATPS, ATP sulfurylase; Cys, cysteine; GB, glucobrassicin; GI, glucoiberin; GSL, glucosinolate; Met, methionine; MeSeCys, methylselenocysteine; MGB, 1- and 4-methoxyglucobrassicin; OASTL, *O*-acetylserine(thiol) lyase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SeCys, selenocysteine; SeMet, selenomethionine; SMT, selenocysteine methyltransferase

* Corresponding author.
E-mail address: Marian.McKenzie@plantandfood.co.nz (M.J. McKenzie).

[1] Present address; Bioseperation and Bioprocess Laboratory, Department of Chemical Engineering, Indian Institute of Technology, Delhi, India.

[2] Dedicated to the memory of Michael McManus, September 1957–July 2015.

http://dx.doi.org/10.1016/j.plaphy.2017.10.027
Received 28 August 2017; Received in revised form 19 October 2017; Accepted 27 October 2017
Available online 31 October 2017

Shrift, 1982). In contrast, Se-accumulators have an additional enzyme, selenocysteine methyltransferase (SMT, EC 2.1.280), which catalyses the methylation of Se-Cys to form methylselenocysteine (MeSeCys) (Lyi et al., 2005; Cai et al., 1995). This diverts the Se-amino acids away from protein synthesis, thereby protecting the Se-accumulators from Se toxicity. In addition, from a human health point of view, Se ingested as MeSeCys has been shown to have greater cancer-prevention properties than other Se-containing compounds (Ip et al., 1991; Whanger, 2002) due to its efficient conversion to methylselenol in the body (Ip et al., 1999). Methylselenol is a non-toxic compound with demonstrated anti-cancer effects including the induction of apoptosis and the reduction of angiogenesis in cancerous tissues (Medina et al., 2001).

With this connection to human health, members of the Brassicaceae have now been investigated to determine their ability to simultaneously produce the GSLs and the Se-based compounds, such as MeSeCys, to high levels. However, Se and S are known to compete for uptake and metabolism in plants because of their chemical similarity, with most S-assimilatory enzymes unable to distinguish between the two elements (Sors et al., 2005). Indeed, several reports have shown that Se fertilisation resulted in reduced S uptake and/or GSL production in the Brassicas (Robbins et al., 2005; Charron et al., 2001; Toler et al., 2007). Conversely, others have reported increased S uptake and GSL formation in response to Se fertilisation or no change in content (Barickman et al., 2013; Hsu et al., 2011; Ramos et al., 2011). At the molecular level, Se fertilisation of broccoli has been shown to alter the transcript accumulation of genes involved in S uptake and assimilation, such as sulfate transporters (*BoSULTR1;1, 1;2, 2;1, 2;2*) and ATP sulfurylase 1 (*BoAPS1*), but only in certain cultivars (Hsu et al., 2011; Ramos et al., 2011). In contrast, *SMT* expression appears to be consistently up-regulated by Se fertilisation regardless of cultivar (Lyi et al., 2005; Ramos et al., 2011).

We were interested in examining the influence of adding Se, at rates suitable for commercial production, on the production of GSLs in broccoli. We focused on two cultivars that differ in GSL content; one having relatively high content, the other having relatively low content, for comparison. We determined the total Se, S and GSL contents in floret tissue following harvest at commercial maturity. To further investigate the effects of Se fertilisation on S metabolism, we determined the activity of key enzymes associated with the reductive (primary) S-assimilation pathway, ATP-sulfurylase and *O*-acetylserine(thiol) lyase, and isolated the broccoli orthologs of *Arabidopsis thaliana* APS kinase (*APSK*) *1* and *2* for the first time.APS kinase is a key branch point enzyme involved in channelling S into GSL production via 3′-phosphoadenosine 5′-phosphosulfate (PAPS). We investigated APSK transcription for both cultivars in different tissues, at different developmental stages, and following Se fertilisation, to determine if the transcription of this gene acts as a point of regulatory control for GSL production in broccoli on treatment with Se.

2. Materials and methods

2.1. Growth and treatment of plant material in the field

Ten broccoli cultivars (*Brassica oleracea* L. var. *italica* cv. Booster, Diplomat, Heritage, Irons, Legacy, Legato, Marathon, Monterey, Shadow and Triathlon) where grown in three replicates according to a standard block design in the field at The New Zealand Institute for Plant & Food Research Limited in Palmerston North. Standard agronomic conditions were applied and floret material harvested from each plant at commercial maturity. Each replicate comprised the combined floret material of three plants grown in the same block and was snap frozen in liquid nitrogen and stored at −80 °C until analysis.

2.2. Growth and selenium treatment of plant material in the greenhouse

Plants of two cultivars of broccoli (*Brassica oleracea* L. var. *italica* cv. Triathlon and cv. Booster) were grown in potting mix in 8 L-capacity bags under standard greenhouse conditions. The soil was irrigated once daily for 1 min by watering spikes so the soil was fully moist, but no liquid was dripping from the bags. For the assessment of APSK gene expression in different plant tissues and developmental stages triplicate samples of meristem/head, leaf, stem and tap root tissues were taken at 1) six weeks after germination, termed 'young' tissues and 2) when the head tissue was present in the commercially harvested form, termed 'mature' tissues. For Se fertilisation, Se application commenced following the emergence of immature floret material at the apical meristem. Plants were fed via the soil with 20 mL of sodium selenate at varying concentrations (0–0.5 mM) twice weekly for four weeks following watering (Matich et al., 2012). At maturity, triplicate samples of head material were harvested. For both experiments each triplicate comprised the combined material of two plants and was snap frozen in liquid nitrogen and stored at −80 °C until analysis.

2.3. Measurement of total sulfur, selenium, methyl selenocysteine and glucosinolates

Frozen broccoli samples were freeze dried, ground to a fine powder in a coffee grinder and total S and Se content determined by ICP-MS at RJ Hill Laboratories Limited (Hamilton, New Zealand). The GSL profile and content of each sample was determined by HPLC analysis, based on the method of West and co-workers (West et al., 2002) using a Waters solvent delivery system with variable wavelength UV detector (models W2965 and W2996 PDA; Waters, Milford, MA). Freeze dried samples (200 mg) were extracted into 25 mL of ultrapure boiling water. Aliquots of filtered supernatant (20 μL) were injected onto a RP-18 column (Prevail C18, 5 μm, 250 × 4.6 mm; Grace Davison Discovery Sciences, Deerfield, IL) at 30 °C protected with an appropriate guard column. Glucosinolates were eluted at a flow rate of 1.0 mL/min. Solvents were 65 mM ammonium acetate in water (A), methanol (B) and water (C). The initial solvent composition was 80% A and 20% C, held for 6 min and then a linear 34 min solvent gradient from 80 to 75% A, 0–25% B and 20–0% C simultaneously was used, which was then held for 9 min. The column was returned to initial solvent composition over 1 min and re-equilibrated for 10 min before the next analysis. Eluted components were monitored at 230 nm and quantified based on a sinigrin (Sigma-Aldrich, USA) external calibration curve. Methylselenocysteine content was determined by LCMS as described in McKenzie et al. (2009).

2.4. Enzyme activity analysis

For the determination of ATPS, tissue extraction and enzyme assay was performed as described by Thomas and co-workers (Thomas et al., 2011), while for OASTL, tissue extraction and assay was as described by Rolland and co-workers (Rolland et al., 1992).

2.5. Isolation and characterisation of APS kinase homologues

Total RNA was extracted using the hot borate method (Hunter and Reid, 2001). A 0.2 g aliquot of frozen broccoli florets was ground to fine powder in liquid nitrogen and homogenised in 1 mL of hot (90 °C) extraction buffer (200 mM di-sodium tetraborate, 30 mM EGTA, 1% (w/v) sodium deoxycholate, 1% (w/v) SDS, 2% (w/v) PVP, 1% (v/v) IGEPAL and 10 mM DTT, pH 9.6). Proteins in the extract were digested with 150 μg of Proteinase K (Roche Diagnostics GmbH, Germany), and the RNA precipitated with 2 M LiCl at 4 °C for 16 h. The RNA pellet was washed with 80% (v/v) ethanol, resuspended in 20 μL DEPC-treated water and then quantified by NanoDrop® ND-1000 spectrophotometer V3.6 (Thermo Fisher Scientific Inc., USA).

To clone the *APSK* genes, degenerate primers were used (Table S2) and the PCR products cloned into pGEM-T *Easy* (Roche), sequenced and gene-specific primers developed for use in 3′-RACE. Subsequent sequencing revealed two distinct 3′-UTRs for *BoAPSK1* (designated