Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 1321–1333
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae)

Aaron J. Windsor, Michael Reichelt, Antje Figuth, Aleš Svatoš, Juergen Kroymann, Daniel J. Kliebenstein [1], Jonathan Gershenzon, Thomas Mitchell-Olds *

Max-Planck-Institute for Chemical Ecology, Genetics and Evolution, Hans-Knoell-Strasse 8, D-07745 Jena, Germany

Received 13 December 2004; received in revised form 21 January 2005
Available online 23 May 2005

Abstract

Glucosinolates are biologically active secondary metabolites that display both intra- and interspecific variation in the order Brassicales. Glucosinolate profiles have not been interpreted within a phylogenic framework and little is known regarding the processes that influence the evolution of glucosinolate diversity at a macroevolutionary scale. We have analyzed leaf glucosinolate profiles from members of the Brassicaceae that have diverged from *Arabidopsis thaliana* within the last 15 million years and interpreted our findings relative to the phylogeny of this group. We identified several interspecific polymorphisms in glucosinolate composition. A majority of these polymorphisms are lineage-specific secondary losses of glucosinolate characters, but a gain-of-character polymorphism was also detected. The genetic basis of most observed polymorphisms appears to be regulatory. In the case of *A. lyrata*, geographic distribution is also shown to contribute to glucosinolate metabolic diversity. Further, we observed evidence of gene-flow between sympatric species, parallel evolution, and the existence of genetic constraints on the evolution of glucosinolates within the Brassicaceae.

Keywords: Brassicaceae; *Arabidopsis lyrata*; Glucosinolate; Secondary metabolism; Genetic constraint; Evolution

1. Introduction

1.1. Phylogenetics and glucosinolate evolution

Glucosinolates, or mustard oil glycosides, are biologically active secondary metabolites found in the Brassicaceae and related families (Kjaer and Schuster, 1972; Kjaer, 1976; Doughty et al., 1991; Rodman et al., 1996, 1998; Raybould and Moyes, 2001; Fahey et al., 2001; Tokuhisa et al., 2004). These compounds are genetically variable within plant species, and strongly influence the feeding choices of insect herbivores (Lambrix et al., 2001; Kroymann et al., 2003). In *Arabidopsis thaliana*, genetic polymorphisms and loss-of-function mutations have allowed identification of genes encoding glucosinolate biosynthetic enzymes (Kliebenstein et al., 2001a,b; Hemm et al., 2003; Reintanz et al., 2001; Kroymann et al., 2001), and these results have been extended to *Brassica* relatives of *A. thaliana* (Gao et al., 2004; Li and Quiros, 2003; Quiros et al., 2001).

On a macroevolutionary time-scale, comparative phylogenetic analyses can elucidate biological patterns and processes influencing the evolution of secondary metabolism over millions of years. Although several compendia have cataloged glucosinolate profiles of tens or hundreds of species (Fahey et al., 2001; Daxenbichler et al., 1991), previous studies have not included a

* Corresponding author. Tel.: +49 3641 571401; fax: +49 3641 571402.
E-mail address: tmo@ice.mpg.de (T. Mitchell-Olds).
[1] Current address: Department of Vegetable Crops, University of California, Davis, CA 95616, USA.

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.04.016

detailed phylogenetic framework to interpret similarities and differences in glucosinolate patterns. Here we examine evolutionary changes in glucosinolate metabolism among close relatives of *A. thaliana*, which have diverged within the last 15 million years (Koch et al., 2001), to address evolutionary hypotheses and processes among species. In the following study, we have generated glucosinolate profiles for relatives of *A. thaliana* and used these data in a comparative framework to ask the following questions: (1) Are glucosinolate polymorphisms within *A. thaliana* also found in related species? (2) Do differences in glucosinolate profiles between species and within species involve changes to the structural portion of enzyme-encoding loci, or regulatory functions which impact gene expression? (3) Is there evidence for genetic constraints which limit the potential for future adaptation of glucosinolate profiles in entire lineages?

1.2. Biology of glucosinolates

Glucosinolates are synthesized from a limited repertoire of amino acids (Fig. 1) with a core structure consisting of an (*Z*)-*N*-hydroximinosulfate ester, a β-thio-linked glucose moiety, and a variable amino acid derived R-group (Fig. 1). In the chemical defense of plants against insect herbivores, glucosinolates function with a specific class of β-thioglucosidases, the myrosinases. Glucosinolates and myrosinase coexist temporally in intact plant tissues, but are separated spatially (Eriksson et al., 2001; Husebye et al., 2002; Geshi et al., 1998; Thangstad et al., 2004). These two elements come into contact when tissue damage occurs, leading to cleavage of the glucosinolate thioglucoside linkages, thereby liberating D-glucose and a thiohydroximate-*O*-sulfate. Thiohydroximate-*O*-sulfates are labile and undergo spontaneous rearrangements to form a variety of toxic products including nitriles, isothiocyanates, and thiocyanates. It is the quantity and spectrum (i.e. quality) of the end-products of the glucosinolate–myrosinase interaction that confer the biological activity associated with glucosinolates; the end-products defend against generalist insect herbivores, or can act as ovipositional cues and feeding stimulants for specialist insect herbivores (Raybould and Moyes, 2001; Pivnick et al., 1994; Marazzi and Staedler, 2004). Several intraspecific studies have documented genetic variation for glucosinolate type and concentration (Kjaer, 1976; Louda and Rodman, 1983; Kliebenstein et al., 2001a; Fahey et al., 2001; Kroymann et al., 2003; Castro et al., 2004; Font et al., 2004; Charron and Sams, 2004) and demonstrated their functional significance in resistance to generalist insect herbivores (Raybould and Moyes, 2001; Kroymann et al., 2003; Kroymann and Mitchell-Olds, 2004). These results, in plant species separated by tens of millions of years of independent evolution (Koch et al., 2001), underscore the ecological importance of these secondary metabolites.

While structural variation among glucosinolates is dependent on the precursor amino acids, modifications of the R-groups also contribute enormous diversity to this class of compounds. Carbon chain-elongation of methionine (Fig. 2; Kroymann et al., 2001; Field et al., 2004; Textor et al., 2004) prior to the core pathway of glucosinolate biosynthesis is a common and well characterized example of side chain elaboration. After core biosynthesis, chemical modification of R-groups can occur through oxidation (Fig. 2), hydroxylation, and

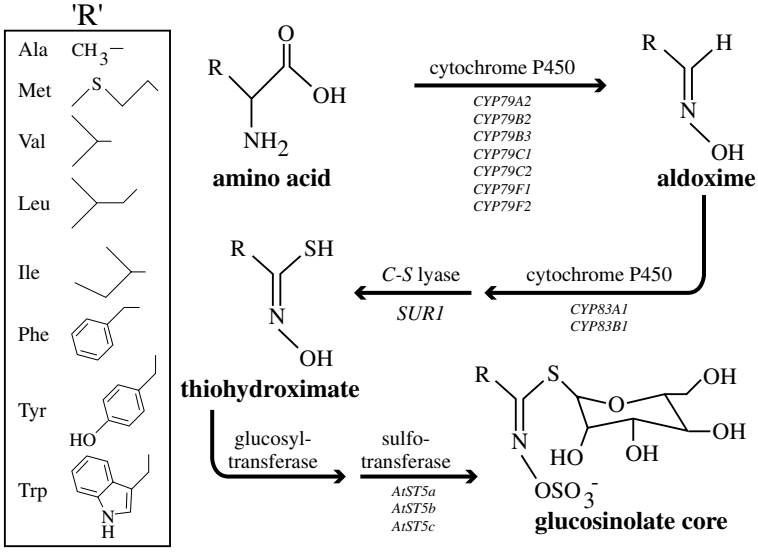

Fig. 1. The core pathway for biosynthesis of glucosinolates from amino acids. R-groups for each of the eight precursor amino acids are indicated. Loci from *A. thaliana* which encode the first three steps are indicated. Recently, the glucosinolate *C-S* lyase has been identified as the protein product of the *SUPERROOT1* (*SUR1*) locus in *A. thaliana* (Mikkelsen et al., 2004).