Journal of Plant Physiology 167 (2010) 1525–1532

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Metabolic changes of *Brassica rapa* transformed with a bacterial isochorismate synthase gene

Sanimah Simoh[a,b], Huub J.M. Linthorst[c], Alfons W.M. Lefeber[d], Cornelis Erkelens[d], Hye Kyong Kim[a,*], Young Hae Choi[a], Robert Verpoorte[a]

[a] Division of Pharmacognosy, Section Metabolomics, Institute of Biology, Leiden University, Leiden, The Netherlands
[b] Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute, Kuala Lumpur, Malaysia
[c] Section Plant Physiology, Institute of Biology, Leiden University, Leiden, The Netherlands
[d] Division of NMR, Institute of Chemistry, Leiden University, Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 4 April 2010
Received in revised form 31 May 2010
Accepted 3 June 2010

Keywords:
Brassica rapa
Isochorismate synthase
Multivariate analysis
NMR spectroscopy
Salicylic acid

ABSTRACT

Metabolome analysis by 1-dimensional proton nuclear magnetic resonance (^{1}H NMR) coupled with multivariate data analysis was carried out in *Brassica rapa* plants transformed with a gene encoding bacterial isochorismate synthase (ICS). Partial least square-discrimination analysis (PLS-DA) on selected signals suggested that the resonances that were dominant in the transgenic plants corresponded to a glucosinolate (neoglucobrassicin), phenylpropanoids (sinapoyl malate, feruloyl malate, caffeoyl malate), organic acids (succinic acid and fumaric acid) and sugars (α- and β-glucose). In contrast, amino acids alanine threonine, valine, leucine were dominant in the untransformed controls. In addition, HPLC data showed that the transgenic plant accumulated salicylic acid (SA) at significantly higher levels than the control plants, whereas the phylloquinone levels were not affected. The results suggest that the expression of the bacterial isochorismate synthase gene in *B. rapa* does not affect fluxes into pathways to other groups of secondary metabolites through competition for the same precursor. On the contrary, the biosynthesis of isochorismate-derived products (SA) seems to induce the competitive pathways via phenylalanine (phenylpropanoids) and tryptophan (IAA and indole glucosinolates).

Introduction

The importance of salicylic acid (SA, 2-hydroxybenzoic acid) as a signal molecule in inducing the plant defense response both in local and systemic acquired resistance (SAR) is well known. Upon pathogen attack, SA mediates the oxidative burst that causes rapid cell death at the point of infection that leads to the hypersensitive response (HR). The HR prevents the systemic spread of the pathogen, and this is usually accompanied by the induction of SAR. Once SAR is established, the plant's resistance to subsequent infections by a broad spectrum of viral, bacterial and fungal pathogens is enhanced. The induction of SAR is strongly associated with the expression of a set of genes that leads to the accumulation of pathogenesis-related proteins (Linthorst, 1991; Yalpani et al., 1991). It has long been known that SA in higher plants is derived from the phenylalanine pathway (Sticher et al., 1997) via cinnamic acid, either by oxidative or non-oxidative pathways. In bacteria, it is synthesized via isochorismate, involving two enzymes: isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL). However in plants, the biosynthetic pathway leading to SA is not yet fully understood and is a matter of current debate. Recent studies have revealed that SA in plants also can be synthesized from chorismate through isochorismate, similar to bacteria (Van Tegelan et al., 1999; Wildermuth et al., 2001; Mustafa et al., 2009).

It was previously reported by us that it is possible to introduce microbial SA biosynthesis in plants via the isochorismate pathway (Verberne et al., 2000). Metabolic engineering of SA biosynthesis with the two bacterial genes *entC* (encoding for ICS) from *Escherichia coli* and *pmsB* (encoding for IPL) from *Pseudomonas fluorescens* led to the constitutive SA production in tobacco plants

Abbreviations: ABA, abscisic acid; AS, anthranilate synthase; BAP, benzyl amino purine; CaMV, cauliflower mosaic virus; CM, chorismate mutase; CSA, constitutive SA production; GBC, glucobrassicin; GBN, glucobrassicanapin; GFP, green fluorescence protein; GLS, glucosinolate; GNA, gluconapin; GNL, gluconapoleiferin; 4-OH, 4-hydroxyglucobrassicin; HR, hypersensitive response; IAA, indole 3-acetic acid; ICS, isochorismate synthase; IPL, isochorismate pyruvate lyase; 4-MeO, 4-methoxyglucobrassicin; NAA, 1-naphthaleneacetic acid; NEO, neoglucobrassicin; NMR, nuclear magnetic resonance spectroscopy; PCA, principal component analysis; PLS-DA, partial least square-discrimination analysis; PR, pathogenesis-related; PRO, progoitrin; RT-PCR, reversed transcription-polymerase chain reaction; SA, salicylic acid; SAG, salicylic acid glucoside; SAR, systemic acquired resistance; TMV, tobacco mosaic virus.

* Corresponding author at: Division of Pharmacognosy, Section Metabolomics, Institute of Biology, Leiden University, Einsteinweg 55, P.O. Box 9502, 2300 RA, Leiden, The Netherlands. Tel.: +31 71 527 4510; fax: +31 71 527 4511.
E-mail address: h.k.kim@chem.leidenuniv.nl (H.K. Kim).

0176-1617/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2010.06.022

(CSA plant) when the genes were targeted at the chloroplast. There have been several trials to uncover the effect of the insertion of ICS and IPL on the metabolic level. For example, the metabolic analysis of these CSA plants by high performance liquid chromatography (HPLC) showed the suppression of some flavonoids, such as quercetin, kaempferol, rutin, and also chlorogenic acid, all products of the phenylpropanoid pathways (Nugroho et al., 2002). However, most previous studies have used targeted analysis for a small range of metabolites. Recently, as a holistic approach, the metabolite profiles of tobacco mosaic virus-infected CSA plants (*Nicotiana tabacum*) by ^{1}H nuclear magnetic resonance spectroscopy (NMR) showed a clear discrimination between the transgenic and wild type plants. The major compounds contributing to the discrimination were chlorogenic acid, malic acid, glucose and sucrose (Choi et al., 2004). However, prior to drawing a general conclusion about the effect of the genetic modification of ICS and IPL on metabolome of plant, metabolomics of diverse CSA plants should be assessed.

The metabolome is defined as the quantitative complement of the low molecular weight compounds present in an organism under any specific condition (Fiehn et al., 2001). Metabolomics is a new 'omic' to complete the previously developed transcriptomics and proteomics. Any change in the physiological state of a cell as a result of gene deletion or overexpression can be reflected in its transciptome or proteome and should be measurable through the metabolome as the characterization of the phenotype (Kell et al., 2005). As a key tool to monitor the ultimate gene expression products (Oliver et al., 1998), profiling the metabolome offers a 'snapshot' of the physiological state of a cell in any given condition. By integrating all the 'omics' technologies, we may uncover a comprehensive and holistic understanding of the cellular response to a specific biological condition (Schauer and Fernie, 2006). Thus, metabolomics is a crucial part of systems biology. In genetic engineering, as another application, metabolome analysis allows us to monitor the 'substantial equivalence' between the genetically modified products and their wild type counterparts, or determine whether there is significant alteration of unexpected metabolites due to transgenic expression which might be detrimental to functional genomics.

The progress of metabolome research is limited by the analytical methods. As the ultimate goal of metabolome analysis is the unbiased and non-targeted identification and quantification of all the metabolites present in the organism (Verpoorte et al., 2007), it requires that all the metabolites are analyzed simultaneously in a small number of steps. At present, there is no single analytical method capable of this. The analytical methods used for metabolomics are normally based on chromatography hyphenated diverse spectroscopy such as ultraviolet or mass spectrometry, or spectroscopy as itself, e.g. NMR and Fourier transform – infra red spectroscopy (FT-IR). The application of NMR as a promising analytical tool in metabolomics has been highlighted (Verpoorte et al., 2007) due in part to its potential to quantify and identify diverse groups of metabolites, including amino acids, carbohydrates, lipids, phenolics, and terpenoids (Moing et al., 2004; Choi et al., 2006; Kim et al., 2006). Although NMR is considered less sensitive than any other method, such as GC/LC–MS, it provides a direct profile of the system in a single spectrum in which the metabolite information obtained can be further extracted by multivariate data analyses (Manetti et al., 2006). In the field of plant biotechnology, NMR-based metabolomics has been increasingly utilized, e.g., to identify any unintended metabolic effects and to monitor the substantial equivalent in the transgenic tomatoes (Gall et al., 2003). Also, metabolic profiles of transgenic potatoes (Defernez et al., 2004) and tobacco (Choi et al., 2004) have been reported. The advancement of such analytical technologies for measurement of the metabolome generates massive data sets in which the identification of compounds remains a major challenge. The application of multivariate-handling methods such as principal component analysis (PCA), partial least square regression (PLS) and partial least square-discrimination analysis (PLS-DA) is of great importance for data handling; it can reduce the complex data sets, allowing them to be analyzed and interpreted.

To assess the metabolic effect of a gene involved in plant SA biosynthesis, a single ICS gene was transformed into *Brassica rapa* ssp. *oleifera* in which this gene was targeted at the chloroplast (Simoh et al., unpublished data). We used only ICS, as the IPL overexpression might result in channeling isochorismate away from essential metabolic pathways, such as for phylloquinone. SA as a signal molecule might have a wide range of influence on biosynthesis of diverse metabolites. In *B. rapa*, metabolic alteration can be expected (Fig. 1). This study aimed at the identification of metabolic pathways that are affected by the introduction of the ICS gene in *B. rapa*. NMR-based metabolomics coupled with supervised or unsupervised multivariate data analysis was applied to distinguish between the control and greenhouse-grown transgenic (T_0) plants.

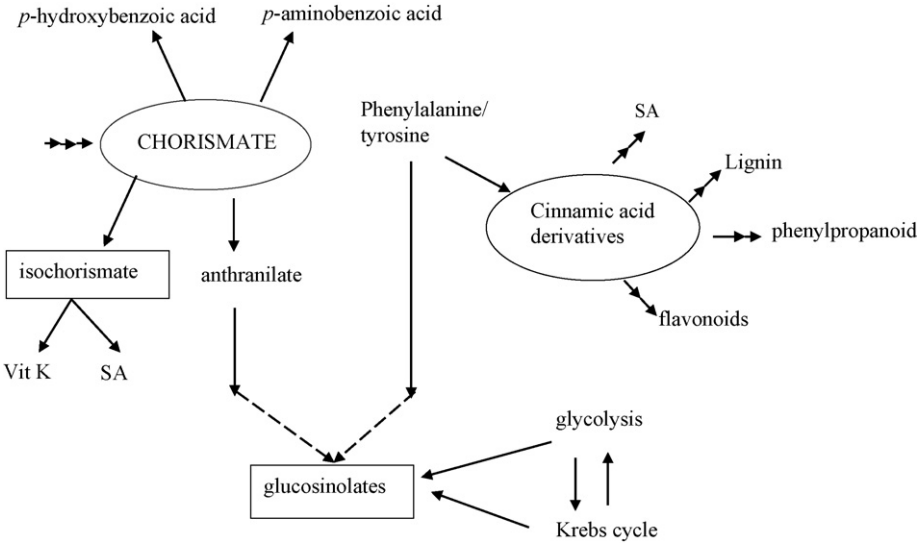

Fig. 1. Schematic representation of metabolic pathway originated from shikimate.