Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 9–17
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

cis-Jasmone induces accumulation of defence compounds in wheat, *Triticum aestivum*

Maria C. Blassioli Moraes [a,b], Michael A. Birkett [b,*], Ruth Gordon-Weeks [b], Lesley E. Smart [b], Janet L. Martin [b], Barry J. Pye [b], Richard Bromilow [b], John A. Pickett [b]

[a] *Embrapa Genetic Resources and Biotechnology, Cx. Postal: 02372, Cep. 70849-970 Brasília-DF, Brazil*
[b] *Biological Chemistry Department, Rothamsted Research, Harpenden, Herts. AL5 2JQ, United Kingdom*

Received 20 April 2007; received in revised form 14 June 2007
Available online 2 August 2007

Abstract

Liquid phase extraction (LPE) and vapor phase extraction (VPE) methodologies were used to evaluate the impact of the plant activator, *cis*-jasmone, on the secondary metabolism of wheat, *Triticum aestivum*, var. Solstice. LPE allowed the measurement of benzoxazinoids, i.e. 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA), and phenolic acids such as *trans*-*p*-coumaric acid, syringic acid, *p*-hydroxybenzoic acid, vanillic acid and *cis*- and *trans*-ferulic acid. Using LPE, a significantly higher level of DIMBOA was found in aerial parts and roots of *T. aestivum* following treatment with *cis*-jasmone, when compared with untreated plants. Similar results were obtained for phenolic acids, such as *trans*-ferulic acid and vanillic acid in roots. Using VPE, it was possible to measure levels of 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HBOA), benzoxazolin-2(3H)-one (BOA), ferulic acid, syringic acid and coumaric acid. The levels of HBOA in aerial parts and roots were significantly greater in *cis*-jasmone treated plants compared to untreated plants. *cis*-Jasmone is known to be a plant activator in terms of production of defence-related volatile semiochemicals that repel aphids and increase the foraging activity of aphid parasitoids. These results show, for the first time, that *cis*-jasmone also induces selective production of secondary metabolites that are capable of directly reducing development of pests, diseases and weeds.

Keywords: *Triticum aestivum*; Gramineae; Wheat; Liquid phase extraction; Vapor phase extraction; *cis*-Jasmone; Allelochemicals; Benzoxazinoids; Phenolic acids

1. Introduction

A substantial number of plant secondary metabolites are involved in direct and indirect plant defence against insect herbivores and pathogens. Wheat, *Triticum aestivum* L. (Gramineae) has been found to possess allelopathic potential, and this allelopathy has been shown to be associated with the presence of benzoxazinoids, such as 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA), and phenolic acids (Perez and Ormenonunez, 1991; Understrup et al., 2005).

It has been well documented that jasmonic acid (JA) and methyl jasmonate are activators of plant defence (Koch et al., 1999; Kessler and Baldwin, 2002). However, by comparison, few studies have been conducted to evaluate the impact of *cis*-jasmone, which is considered to be the final product in the jasmonic acid biosynthetic pathway from α-linolenic acid (Koch et al., 1997). *cis*-Jasmone is highly volatile, when compared with the other compounds of the JA pathway, and is an activator of chemical defence

Abbreviations: *BOA, benzoxazolin-2(3H)-one; HBOA, 2-hydroxy-7-methoxy-(2H)*-1,4-benzoxazin-3(4*H*)-one; DIBOA, 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one; DIMBOA, 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one; MBOA, 6-methoxy-benzoxazolin-2(3H)-one; HMBOA, 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one; PHB, *p*-hydroxybenzoic acid; VAN, vanillic acid; SYR, syringic acid; *cis*-FER, *cis*-ferulic acid; *trans*-*p*-COU, *trans*-*p*-coumaric acid; *trans*-FER, *trans*-ferulic acid.

* Corresponding author. Tel.: +44 1582 763133; fax: +44 1582 762595.
E-mail address: mike.birkett@bbsrc.ac.uk (M.A. Birkett).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.06.020

in plants, causing the release of volatile semiochemicals (Birkett et al., 2000). Bean plants, *Vicia faba*, treated with *cis*-jasmone showed a significant increase in the production of (*E*)-ocimene over a period of at least eight days, whereas when plants were exposed in the same way to methyl jasmonate, although there was an increase in the level of (*E*)-ocimene, the effect was short-lived (Birkett et al., 2000). In addition, changes in gene expression levels from *cis*-jasmone treated *V. faba* were recorded (Birkett et al., 2000). Bruce et al. (2003a, 2003b) showed in field studies that when *T. aestivum* is treated with *cis*-jasmone, there is a significant reduction in the development of grain aphid, *Sitobion avenae*, populations. This negative impact on aphids could be related to the induction of benzoxazinoid and phenolic acid production as a direct defence mechanism, as these compounds are known to confer plant resistance to insects and allelopathic effects (Guenzi and McCalla, 1966; Niemeyer, 1988).

The impact of plant activators upon induced plant secondary metabolism has been studied up to now using a variety of separate and complex analytical techniques on different parts of the plant. Volatile organic compounds (VOCs) emitted by plants are typically studied by the collection of the headspace of parts of, or the whole plant, and are analysed by high-resolution gas chromatography (GC) (e.g. Agelopoulos et al., 1999). However, the impact of external signals on internal plant physiology typically is studied by extraction of plant tissue (roots and/or leaves), with different solvents sometimes followed by chemical derivatization and analysis by GC or by high-pressure liquid chromatography (HPLC). This has been applied to hydroxamic acid analysis in cereals (Wu et al., 1999). Qualitative and quantitative changes in plant metabolism are assessed by mass spectrometry, coupled to either GC or LC, using conventional electron impact mass spectrometry (EI-MS), or by more sophisticated techniques such as electrospray-ionization-mass spectrometry (ESI-MS). In either case, identification can be enhanced by the use of tandem mass spectrometry techniques (MS–MS). However, few attempts have been made to assess the impact of external signals on the secondary metabolites of the whole plant. Schmelz et al. (2003, 2004) proposed the simultaneous quantification of phytohormone, phytotoxin and VOC production in plants using vapor phase extraction (VPE) in various model systems: thale cress, *Arabidopsis thaliana*, following infection with the pathogen, *Pseudomonas syringe*; maize, *Zea mays*, during herbivory by the corn earworm, *Helicoverpa zea*; tobacco, *Nicotiana tabacum*, after mechanical damage, and tomato, *Lycopersicon esculentum*, during drought stress. VPE offers advantages and potential applicability in the preparation and simultaneous GC–MS analysis of phytotoxins (coronatine), phytohormones (salicylic acid, jasmonic acid, indole-3-acetic acid and abcisic acid) and VOCs from low levels (milligrams) of plant tissue. This approach enabled the exploration of interactions between physiologically and ecologically relevant chemical signals at the level of production, and to rationalise previous findings on induced plant defence.

The aim of this study was to investigate the impact of *cis*-jasmone upon secondary metabolism in wheat, *T. aestivum* (var. Solstice). For comparison, liquid phase extraction (LPE), which uses different solvents (polar and non-polar) to obtain the partition of the compounds with different polarities from biological matrices, was used alongside VPE. For both techniques, derivatization was carried out prior to analysis, with the reagents *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and trim-

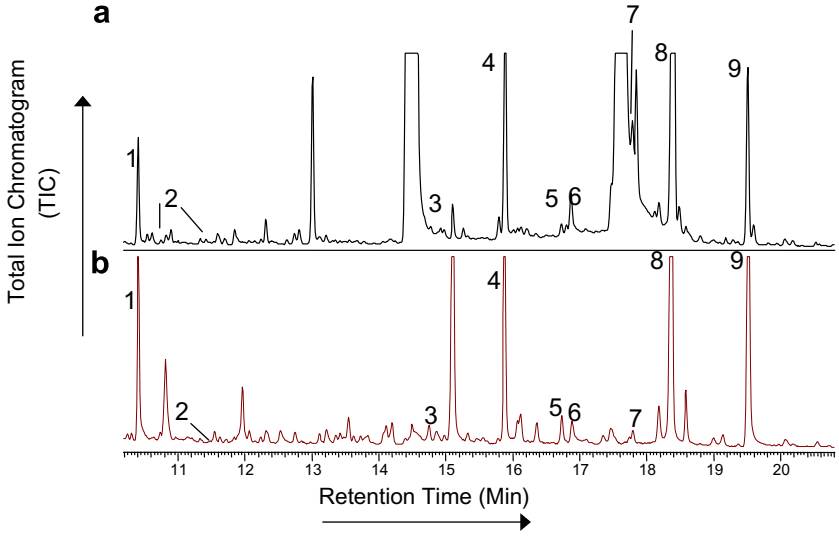

Fig. 1. Typical GC–MS profiles of extracts of aerial parts (a) and roots (b) obtained from wheat, *T. aestivum,* obtained by liquid-phase extraction (LPE). The compounds were identified as TMSi derivatives. MBOA (1), *p*-hydroxybenzoic acid (2), vanillic acid (3), HMBOA (4), syringic acid, (5), *cis*-ferulic acid, (6), *trans*-*p*-coumaric acid (7), DIMBOA (8), *trans*-ferulic acid (9).