A proteomic approach to analysing rice allelopathy on barnyard grass (*Echinochloa crus-galli* L.)

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Abstract

Proteomic analysis was used to investigate the changing pattern of protein synthesis in allelopathic rice exposed to the stress from barnyard grass. Four differentially expressed proteins, 3-hydroxy-3-methylglutaryl-coA reductase 3 (HMGR3), phenylalanine ammonia-lase (PAL), thioredoxin-m and peroxidase precursor, which are related to the pathways of isoterpenoid and phenylpropanoid biosynthesis in the plant defence response, were detected by MALDI-TOF/MS analysis. The findings support the emerging concept that terpenoid and phenolic compounds are the allelochemicals against weeds.The proteomic approach in the discovery of genes and pathways conferring allelopathy is discussed.

Media summary

A proteomic approach was employed to analyse molecular mechanisms of allelopathy in rice induced by barnyardgrass at different densities.

Keywords

Allelopathy, barnyardgrass, proteomic analysis, rice (*Oryza sativa* L)

Introduction

Allelopathy has been defined as any direct or indirect beneficial or detrimental effect by one plant on another through production of chemical compounds released into their environment (Rice 1984). Many positive studies of allelopathy as a means of ecological weed control by selecting rice cultivars with higher allelopathic potential have been conducted. Although achievements have been made, the mechanism of allelopathic effect on weeds, especially at the molecular level, remains unknown. In this paper, a proteomic approach was used to analyse the molecular mechanism for allelopathic effects in rice exposed to the biotic stress of barnyard grass (BYD). This provides a view of the response to proteins in the rice plant..

Materials and methods

An experiment was done in Fujian Agriculture and Forestry University, Fuzhou, China, in 2003 with allelopathic rice PI 312777, introduced from USA. It was employed as the donor plant, and barnyardgrass (*E. crus-galli* L.) was used as the receiver plant to determine the differential expression of proteins in the allelopathic rice cultivar induced by the BYD. The trial with 3 replications was done in a mixed culture system (without soil) of the rice and the weed at ratios of 32 : 0 (control) and 2 : 30(rice : BYD). The roots of rice grown in the mixed system were sampled from each pot 15 d after being transplanted, which is an important competition phase between the rice and the weed (Lin, 2003). The sampled roots were put into liquid nitrogen in time for quick freezing, then stored at –80?C until the proteomic analysis was done.

The protein extraction and 2-D electrophoresis (2-DE) were carried out by using the method described by Shen et al. (2003). The stained gels were scanned at 600 dots per inch resolution with a UMAX Astra-2400S scanner. The spot detection and gel comparisons were made using MELANIE 3 software. The M_r

for the proteins was determined by co-migration of the protein standards.. Selected protein spots, which were differentially displayed in response to the stress of BYD at different densities, were manually excised from the colloidal stained preparative gels with silver for analysis using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry(MALDI-TOF/MS) to generate peptide mass finger-printing(PMF) profiles. Mass spectrometry(MS) analysis was done in School of Life Sciences , Xiamen University, China. The PMF data was used to search against the SWISS-PROT (Swiss Institute of Boinformatics, www.ebi.ac.uk/swissprot/) and NCBI(The National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/) non-redundant public protein database.

Results and discussion

An example of 2-DE maps from the roots of stressed and unstressed rice plants (control) is shown in Figure 1. There are four different highly repeatable root proteins in this trial. Individual protein spots were excised from the 2-DE gel and digested with the site-specific protease trypsin, resulting in a set of tryptic peptides. The peptide fragments were extracted and their masses measured by MALDI-TOF/MS as shown in Figure 2. All the proteins analysed by MS, were matched to those from rice (Table 1). The predicted proteins, 3-hydroxy-3-methylglutaryl-coA reductase 3 (HMGR3), phenylalanine ammonia-lase (PAL), thioredoxin-m and peroxidase precursor, were mainly involved in the pathway of isoprenoid and phenylpropanoid biosynthesis. The plant 3-hydroxy-3-methylglutaryl-coA reductase (HMGR, EC 1.1.1.34) catalysing the conversion of 3-hydroxy- methylglutaryl-coA (HMG-coA) to mevalonate, the specific precursor of all isoprenoid

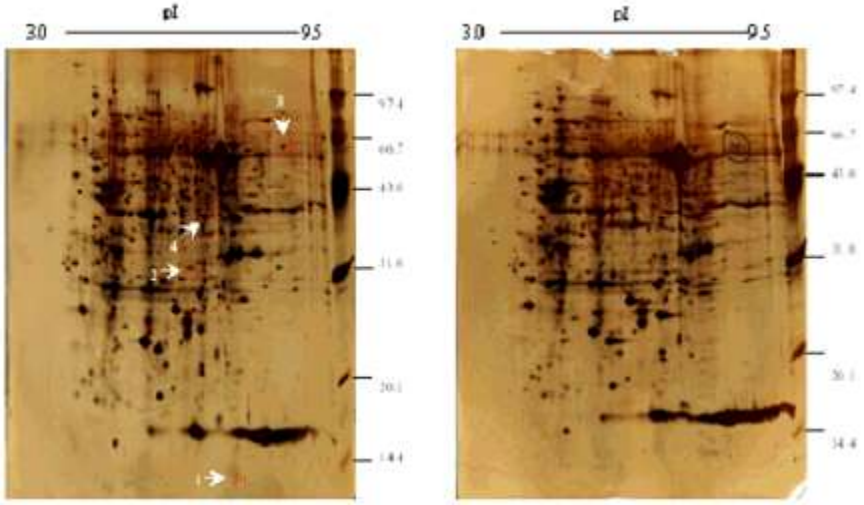

Figure 1. Analytical silver-stained two-dimensional electrophoresis root protein profiles of allelopathic rice in response to the stress of barnyard grass (a) control without the weed and (b) with the weed.

compounds present in plants, has been considered as a potential rate-limiting enzyme in biosynthesis of phytosterols which originates from cytosolic acetate/mevalonate pathway. The HMGR members that are encoded by multigenes responded differently to various external stimuli including weed and pathogen infection. The induction pattern of HMGR was correlated with terpenoid synthesis. The precise physiological roles of terpenoid have not been defined but it is generally agreed that they mediate plant-environment interactions by playing roles in defence and plant-plant communication. There also was evidence that terpenoids have potential use as allelochemicals. Because of their relative low toxicity to vertebrates, they offer significant advantage in weed and pest control application compared with conventional methods (Ha, 2003)

Phenylalamine ammonia-lase (PAL) is a key enzyme of plant metabolism catalyzing the first reaction in the phenylpropanoid biosynthesis from L-phenylalanine of a wide variety of natural product based on the

phenylpropane skeleton. Phenylpropanoid metabolism comprises a complex series of branching biochemical reactions which provide the plant with a host of important phenolic compounds (Razal, 1996 and Dixon, 2002). It has generally been assumed that the appearance of phenylpropanoid metabolites during a plant's response to weed and pest infection is a result of the transcriptional activation of the various biosynthetic pathway genes. It has been documented that thioredoxin m and f(Trx-m and Trx-f) link the light-triggered generations of reducing power in thylakoid membranes with the regulation of metabolism in the soluble stroma in higher plant chloroplasts (Duck, 2001) photochemically reduced ferredoxin and an iron sulfer protein cleave the unique disulfide bond of Trx which in turn reduces disulfide bonds of chloroplast enzyme. Trx-f is particularly efficient in the stimulation of enzyme involved in the photosynthetic fixation of CO_2, whereas Trx-m functions in the inhibition of one enzyme related to the catabolism of carbon compounds, glucose-6-p- dehydrogenase, which favors hexose monophosphate pathway, HMP, to increase the carbon source for phenylpropanoid synthesis (Razal, 1996, Dixon, 2002 and Lin, 2003).

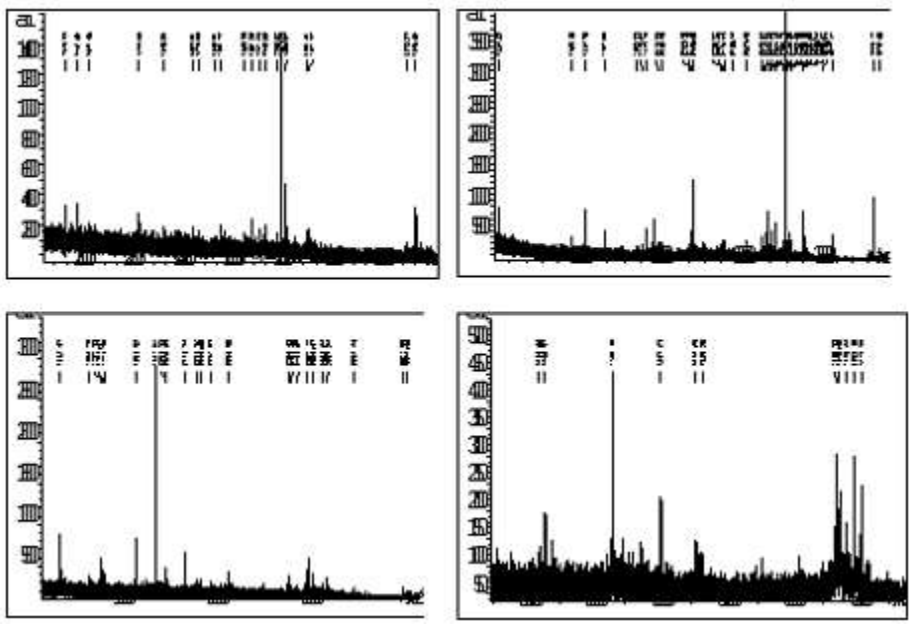

Figure 2. Identification of protein spots from 2-DE gels by MADI-TOF/MS.

Kim *et al* (2000) found that the allelopathic effect in rice was increased as the density of barnyard grass co-cultured with the rice was increased, suggesting that the rice cultivars resulted in higher allelopathic effect grown under more competitive conditions with barnyard grass, which was consistent with our previous studies (Lin, 2000 and Lin, 2003). We also found that environmental stress including biotic and abiotic factors, such as low nutrient condition and target weed density, stimulated the production of active oxygen species (AOS) and allelochemicals, which in turn induced antioxidant enzyme activities of superoxide dismutase (SOD) and peroxidase (POD) to prevent the accumulation of AOS and increased allelopathic effect in rice exposed to the stress conditions (He, 2004 and Shen, 2004). This physio-biochemical process coupled with the cascades that amplified and transmitted initial signal transduction to activate the relative enzyme gene expression is involved in the pathway of isoterpenoid and phenylpropanoid synthesis. It therefore could be inferred that the barnyard grass induced differential expression of the proteins in allelopathic rice in the mixed system, and this triggered the pathway related to plant defence (He, 2002).

Table 1. Results of protein identification by peptide mass finger-printing analysis in rice.

Spot	Accession	Protein match to	M_r	pI	Peptide Coverage
1	Q9ZP20	Thioredoxin M-type, chloroplast precursor (TRX-M)	18517	8.16	18
2	P37835	Peroxidase 2 precursor (POD)	32586	5.51	26
3	Q9XHL5	3-hydroxy-3-methylglutaryl-coenzyme A reductase 3(HMGR)	59415	7.91	7
4	P14717	Phenylalanine ammonia-lyase (PAL)	75761	8.52	7

Conclusions

In this experiment, we successfully employed the proteome analysis technique as a tool to investigate differential expression of proteins in allelopathic rice exposed to barnyard grass stress. Our results have provided useful information about the induced proteins, which are closely related to the pathways of isoprenoid and phenylpropanoid biosynthesis to increase metabolite of allelochemicals in the plant's defence response. These findings support the emerging concept that terpenoid and phenolic compounds are the allelochemicals against weeds.

These results suggest proteomics are an effective tool for physiological and genetic studies in rice allelopathy. It is documented that allelopathy in rice is quantitatively inherited (Lin, 2000 and Lin, 2003). Recently, quantitative trait loci (QTL) technique has been employed to intensively make gene mapping conferring allelopathic traits. However, the gene discovery process is one of elimination and consideration. When a QTL is mapped to an interval of 5-10 cm on a chromosome, there may be 100 genes or more in that interval in which genes become “positional candidate genes” for the QTL. It is therefore difficult to get the functional genes. Proteomics can contribute to the identification of positional, functional and expressional genes. Comparison of 2-DE protein pattern obtained for key tissue of stressed and control plants will identify a set of stress-responsive proteins encoded by expressional candidate genes. Sequencing of these stressed-responsive proteins will then reveal that some of them have functions clearly consistent with the stress tolerant trait. The encoding genes will thus be both the expressional and functional candidate genes. This result encouraged us to ascertain the function by transformation and/or reverse genetics by using the functional cloning strategy.

Acknowledgements

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Reference

Rice E.L. (1984) Allelopathy , 2nd ed. Academic Press Orlando pp 320-343

Saletedch G.H. (2002) Approach to analyzing drought and salt-responsiveness in rice Field Crops Research 76: 199-219

Brensegen F. (2001) The role of active oxygen species in plant signal transduction. Plant Science 161: 405-414

Duck P. (2001) Rapseed chloroplast thioredoxin-m modulation of the affinity for target proteins. Biochimica et Biophysica Acta. 1546,299-311

Razal R.A. (1996) Nitrogen recycling in phenylppanoid metabolism. Phytochemistry 41, 31-35

Dixon R.A. (2002) The phenylpropanoid pathway and plant defense-a genomics perpective. Molecular Plant Pathology 3, 371-390

Ha S.H. (2003) Molecular characterization of three 3-hydroxyl-3-methylglutaryl-coA-reductase genes including pathogen-induced Hmg2 from pepper. Biochemica et. Biophysica Acta 1625: 253-260

Shen S.H. (2003) Proteomics approach to identify wound-response relate proteins from rice leaf sheath. Proteomics 3,527-535

W.X. Lin (2003) The performance of allelopathic heterosis in rice (*Oryza sativa* L) Allelopathy J 12,179-188

W.X. Lin (2000) Allelopathic potential in rice (*Oryza sativa* L) and its possible modes of action on barnyardgrass (*Echinochloa crus-galli*). Allelopathy J. 7,215-224

W.X. Lin (2003) Analysis on dynamic heterosis for allelopathy in rice under different environment conditions. Agricultural Sciences in China 2,707-714

K.U.Kim ed. (2000).Rice Allelopathy,Proceedings of the workshop on in rice allelopathy, 17-19 August, Taegu,Korea pp49-56

H.Q.He (2004).Study on rice (*Oryza sativa* L) allelopathy and its molecular mechanism. Ph.D thesis, Fujian Agriculture and Forestry University, Fujian, PR. China pp61-84

L.H Shen (2004).Studies on the characteristics of rice allelopathy on barnyard grass (*Echinocloa crusgalli* L.) exposed to low phosphorus condition. MS thesis, Fujian Agric. and Forestry Uni., Fujian, China pp14-28

S.L.He (2002). Induction of sesquiterpene cyclase gene expression and antioxidant enzymes regulated by exogenous salicylic acid in leaves of *Capsicum annum* Chin.J.Appl.Ecol.13(5): 569-572.