Journal of Experimental Botany Advance Access published March 18, 2015

Journal of Experimental Botany
doi:10.1093/jxb/erv096

RESEARCH PAPER

Global gene expression profiling of two switchgrass cultivars following inoculation with *Burkholderia phytofirmans* strain PsJN

Alejandra Lara-Chavez[1,*], Scott Lowman[1,2,*], Seonhwa Kim[1], Yuhong Tang[4,5], Jiyi Zhang[4,5], Michael Udvardi[4,5], Jerzy Nowak[2], Barry Flinn[1,2,3] and Chuansheng Mei[1,2,3,†]

[1] Institute for Sustainable and Renewable Resources, Institute for Advanced Learning and Research, Danville, VA 24540, USA
[2] Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA 24601, USA
[3] Department of Forest Resources and Environmental Conservation, Virginia Polytechnic Institute and State University, Blacksburg, VA 24601, USA
[4] Plant Biology Division, the Samuel Roberts Noble Foundation, Inc., Ardmore, OK 73401, USA
[5] BioEnergy Science Center, United States Department of Energy, Oak Ridge, TN 37831, USA

* These authors contributed equally to this work.
† To whom correspondence should be addressed. E-mail: chuansheng.mei@ialr.org

Received 1 December 2014; Revised 4 February 2015; Accepted 9 February 2015

Abstract

Improvement and year-to-year stabilization of biomass yields are primary objectives for the development of a low-input switchgrass feedstock production system using microbial endophytes. An earlier investigation of the effect of *Burkholderia phytofirmans* strain PsJN on switchgrass germplasm demonstrated differential responses between genotypes. PsJN inoculation of cv. Alamo (lowland ecotype) increased the plant root system, shoot length, and biomass yields, whereas it had no beneficial effect on cv. Cave-in-Rock (upland ecotype). To understand the gene networks governing plant growth promotion responses triggered by PsJN, the gene expression profiles were analysed in these two hosts, following seedling inoculation. The Affymetrix platform switchgrass expressed sequence tag (EST) microarray chip representing 122 972 probe sets, developed by the DOE BioEnergy Science Center, was employed to assess transcript abundance at 0.5, 2, 4, and 8 DAI (days after PsJN inoculation). Approximately 20 000 switchgrass probe sets showed significant responses in either cultivar. Switchgrass identifiers were used to map 19 421 genes in MapMan software. There were apparent differences in gene expression profiling between responsive and non-responsive cultivars after PsJN inoculation. Overall, there were 14 984 and 9691 genes affected by PsJN inoculation in Alamo and Cave-in-Rock, respectively. Of these, 394 are annotated as pathogenesis-related genes. In the responsive cv. Alamo, 68 pathogenesis-related genes were affected, compared with only 10 in the non-responsive cv. Cave-in-Rock. At the very early stage at 0.5 DAI, both cultivars exhibited similar recognition and defence responses, such as genes in signalling and proteolysis, after which the defence reaction in the responsive cv. Alamo became weaker while it was sustained in non-responsive cv. Cave-in-Rock.

Key words: Beneficial bacterial endophyte, *Burkholderia phytofirmans* strain PsJN, gene expression profiling, genotypic specificity, growth promotion, *Panicum virgatum* L.

Introduction

Reduction of our dependence on fossil fuels and greenhouse gas emissions has stimulated research efforts on bioenergy crops (Parrish and Fike, 2005). The US Department of Energy funded a series of studies at Oak Ridge National Laboratory (ORNL) which recommend switchgrass (*Panicum virgatum* L.) as one of the most promising bioenergy plant species for the

USA (McLaughlin and Kszos, 2005; Wright and Turhollow, 2010). Switchgrass is a C_4 warm-season perennial grass, native to the USA, with wide geographic distribution. It produces high biomass yields under low inputs and can be grown on marginal lands, minimizing erosion and enhancing carbon sequestration due to its deep root system (McLaughlin and Kszos, 2005; Parrish and Fike, 2005; Wright and Turhollow, 2010; Casler *et al.*, 2011). Recent field studies demonstrated that the lowland cultivar Alamo and upland cultivar Cave-in-Rock (CR) are currently the best commercial cultivars for the southern and northern USA, respectively (McLaughlin and Kszos, 2005). However, poor stand establishment and yearly location-dependent biomass yield variations are obstacles to their broad utilization and development of low-input and sustainable switchgrass production systems. To enhance and stabilize its field performance, the utilization of plant-beneficial bacterial endophytes as an intrinsic component of switchgrass feedstock production is proposed, especially on marginal lands (Kim *et al.*, 2012; Ker *et al.*, 2012; Lowman *et al.*, 2014).

It is well established that beneficial microbial endophytes promote host plant growth, enhance its nutrient acquisition, and improve stress tolerance (Ryan *et al.*, 2007; Mei and Flinn, 2010; Suárez-Moreno *et al.*, 2012). Recently, beneficial bacterial and fungal endophytes naturally inhabiting native switchgrass plants have been identified in Oklahoma (Ghimire *et al.*, 2011), Indiana and Illinois (Kleczewski *et al.*, 2012), and Quebec, Canada (Gagne-Bourgue *et al.*, 2013). In western Kentucky, 50% of the 76 endophytic bacteria isolated from switchgrass plants at two mining sites were capable of promoting leaf lamina expansion after inoculation of surface-sterilized switchgrass seeds (Xia *et al.*, 2012). Naturally occurring bacterial endophytes isolated from switchgrass grown in the field have also been shown to improve its stand establishment and seedling year biomass production (Ker *et al.*, 2012). Moreover, some isolates from switchgrass leaves were capable of solubilizing inorganic phosphorus (Gagne-Bourgue *et al.*, 2013).

The bacterial endophyte *Burkholderia phytofirmans* strain PsJN, originally isolated from onion roots (Frommel *et al.*, 1991; Nowak *et al.*, 1998), is one of the most studied plant growth-promoting microorganisms (Nowak and Shulaev, 2003; Sun *et al.*, 2009; Da *et al.*, 2012). It effectively colonizes a broad range of plant species, including potato (Frommel *et al.*, 1991), tomato (Pillay and Nowak, 1997; Sharma and Nowak, 1998; Nowak *et al.*, 2004), cucumber and sweet pepper (Nowak *et al.*, 2004), watermelon and cantaloupe (Liu *et al.*, 1995), grapevine (Compant *et al.*, 2005, 2008), and *Arabidopsis* (Poupin *et al.*, 2013). Its genome has been sequenced (Weilharter *et al.*, 2011), and genome analysis has recently been published by Mitter *et al.* (2013*a*, *b*). To date, the various mechanisms responsible for the beneficial effects of PsJN include: biocontrol through the secretion of siderophores, plant resistance to biotic stress through induced systemic resistance (ISR), synthesis of pathogenesis-related (PR) proteins, phenolics, and other compounds, plant tolerance to abiotic stress through production of trehalose, and plant growth promotion through the 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, phytohormone production, and quinolinate phosphoribosyl transferase activity (Lazarovits and Nowak, 1997; Barka *et al.*, 2002; Sessitsch *et al.*, 2005; Sun *et al.*, 2009; Fernandez *et al.*, 2012; Mitter *et al.*, 2013*a*, *b*).

In switchgrass, PsJN was able to colonize and penetrate roots of the lowland cultivar Alamo, translocate into leaf and sheath tissues, and significantly increase fresh weight by 57, 46, and 37% under *in vitro*, growth chamber, and greenhouse conditions, respectively (Kim *et al.*, 2012). PsJN also enhanced plant growth in field soil under suboptimal growth conditions, indicating a potential for the utilization of beneficial endophytic microorganisms in the development of low-input switchgrass feedstock production systems (Kim *et al.*, 2012). Recently, two field trials on soils with contrasting fertility showed that PsJN inoculation promoted switchgrass cv. Alamo growth on both soils, and the benefits of inoculation were significantly higher on a low nutrient soil (Lowman *et al.*, 2014). However, it was found that PsJN effects are genotype specific. In contrast to cv. Alamo, PsJN inoculation of the upland cv. CR had no beneficial effect. The genotype specificity of the plant hosts' responses to PsJN was also observed in potato (Frommel *et al.*, 1993; Conn *et al.*, 1997) and tomato (Pillay and Nowak, 1997). To understand the genotypic determinants of the PsJN effects on switchgrass germplasm, comparative global gene expression profile analyses have been conducted in cvs. Alamo (responsive) and CR (non-responsive) using switchgrass expressed sequence tag (EST) microarrays.

Materials and methods

Plant material for microarray

Seeds of switchgrass (*Panicum virgatum* L.) cultivars Alamo and CR were purchased from Warner Brothers Seed Co. (Lawton, OK, USA). Seed surface sterilization, germination, and growth conditions followed previous protocols (Kim *et al.*, 2012). Ten-day old seedlings were inoculated with *B. phytofirmans* strain PsJN by dipping them into the liquid bacterial suspension for 1 min as described before (Kim *et al.*, 2012). Non-inoculated seedlings were treated with PBS buffer alone as controls. The treated seedlings were blotted dry with sterile paper towels, and five seedlings were placed in one GA-7 Magenta container with the Murashige and Skoog (MS) medium described in Kim *et al.* (2012) and grown in the same environmental conditions. Whole seedlings (~0.1 g) were taken at 0 day (control, treated with PBS buffer), 0.5, 2, 4, and 8 days after inoculation (DAI) with PsJN and rapidly frozen in liquid nitrogen. The samples were then stored at –80 °C until RNA extraction. The experiment was conducted in a randomized fashion with three biological replicates.

RNA isolation

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's protocol, and then quantified using a Nanodrop-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The integrity was verified by agarose gel electrophoresis.

Expression analysis with Affymetrix array

Purified RNA (500 ng) was amplified and labelled using a VT Express Kit (Affymetrix, Santa Clara, CA, USA). Microarray chips were hybridized, washed, and stained following the manufacturer's

recommendations. Data normalization was conducted by using the robust multiarray average (RMA) (Irizarry *et al.*, 2003). The Affymetrix platform switchgrass EST microarray chip was developed by BESC (the DOE BioEnergy Science Center at the Oak Ridge National Laboratory, Oakridge, TN, USA; Zhang *et al.*, 2013). This chip contains 122 972 probe sets for putative genes, including 122 868 probe sets corresponding to 110 208 PviUT sequences. These genes represent >85% coverage of the genome (Zhang *et al.*, 2013). Differentially expressed genes among the sample groups were selected using Associative Analysis as described by Dozmorov and Centola (2003). An expression value cut-off of 2 and Bonferroni-corrected *P*-value of 4.0659E-07 were derived from 0.05/*n* (*n*=122 972; the number of probe sets on the chip). For each sample group, the treated samples were compared against the untreated control samples to select important genes. The ratio generated by comparing the PsJN-inoculated treatment with the control was subjected to log2 transformation and then plotted into MapMan software (Usadel *et al.*, 2005) for visualization of metabolic pathways. Selected differentially expressed switchgrass probes were annotated using the 'Panicum virgatum/Pvi_cDNA a520831.mapping: 1.0' map identifiers downloaded from the MapMan website (mapman.gabipd.org). Up-regulated and down-regulated genes were defined as those showing a 2-fold increase (log2=1.00) and a 2-fold decrease (log2= –1.00), respectively.

qRT-PCR verification

RNA was extracted as described above after PsJN bacterization at 0, 0.5, 2, 4, and 8 d and stored at –80 °C until use. The specific gene primers were designed using the sequence provided by the Noble Foundation in Ardmore, OK, USA (CCGN10868.b1 CCGN *Panicum virgatum* etiolated seedlings) with Primer 3 software (Untergasser *et al.*, 2012). DNase treatment was performed with a DNA-free kit (Ambion, Foster City, CA, USA). cDNA synthesis was performed using SuperScript III (Invitrogen, Carlsbad, CA, USA) from 1 µg of total RNA following the manufacturer's protocol. Quanatitative real-time PCR (qRT-PCR) was performed with gene-specific primers with equal amounts of cDNA using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Ubiquitin gene expression was used as a normalizer. qPCR was performed with a Bio-Rad iQ™5 Multicolor Real-Time PCR Detection System.

H_2O_2 assay

Both controls and PsJN-inoculated Alamo and CR plantlets were removed from media at the corresponding time points and blotted dry on paper towels to remove excess media. The samples were then weighed, and 1× buffer was added at a ratio of 5:1. The plantlets were then ground with a mortar and pestle and centrifuged at 10 000 rpm for 10 min, and the supernatant was transferred into 1.5 ml tubes on ice until the assay was performed. The H_2O_2 assay was performed in 96-well plates, in duplicate, according to the manufacturer's protocol in the OxiSelect™ Hydrogen Peroxide assay fluorometric kit (Cell Biolabs Inc., San Diego, CA, USA). To initiate the reaction, 50 µl of working solution was added to 50 µl of sample.

Protease activity assays

Protein was extracted from the PsJN-inoculated plant materials using extraction buffer (50 mM TRIS-HCl pH 7.5, 50 mM NaCl, 5 mM $CaCl_2$, 100 µM $ZnCl_2$, 0.025% Brij 35, 0.02% NaN_3, 15% glycerol) at a ratio of 10 µl mg^{-1} tissue, and stored at –20 °C until used. Protein quantity was estimated by the modified method of Ghosh *et al.* (1988). Degradation of myelin basic protein (MBP) was assayed using bovine MBP (Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 0.2 mg ml^{-1} in the above extraction buffer without glycerol. A total of 1 µg of each protein extract was incubated with MBP in a 15 µl reaction volume for 7 h at 37 °C, and the products were analysed by SDS–PAGE to visualize MBP degradation. The gels were stained with LabSafe GEL Blue™ (G-Biosciences, St. Louis, MO, USA) for 1 h and then destained overnight in distilled water.

Results and Discussion

Global gene expression profiling

Gene expression profiling in switchgrass seedlings of the responsive (Alamo) and non-responsive (CR) cultivars showed that 19 421 probe sets were differentially expressed among four time points during 8 DAI with *B. phytofirmans* strain PsJN. The selected differentially expressed probe sets were used for pathway analysis with MapMan software (Usadel *et al.*, 2005).

The affected probes are listed in Supplementary Table S1 available at *JXB* online. In both cultivars, the largest number of up-regulated probes occurred at 0.5 DAI, and then the number remained more or less stable between 2109 and 2794 with no obvious trend at 2, 4, and 8 DAI. However, the number of down-regulated genes had a very different pattern. In Alamo the number increased from 2234 at 2 DAI to 3529 at 4 DAI and to 4714 at 8 DAI, while it decreased with time in CR from 3179 at 2 DAI to 2965 at 4 DAI and to 2426 at 8 DAI. Closer inspection of the 19 421 differentially expressed genes that were affected by PsJN inoculation in either genotype indicated that the global gene expression between Alamo and CR became more obvious with time after inoculation. There were 1257 probe sets that had much higher expression levels in Alamo, while there were only 475 in CR. Also, at the same time point, when differentially expressed genes were compared with the Venn diagram obtained at the online tool (http://omictools.com/venny-s6319.html) it was shown that there were more genes in common between these two genotypes at the earlier time point and fewer genes in common at later time points as the plants diverged in their responses to the bacterial infection, gradually dropping from 2866 to 1146 (Supplementary Fig. S1).

Gene verification utilizing qRT-PCR

To confirm the microarray data, nine different genes: peroxidase precursor, CSLC3-cellulose synthase-like family C, zinc-finger protein, 1,4-α-glucan-branching enzyme, 4-α-glucanotransferase, histidine-containing phosphotransfer protein, kinase domain containing protein, CML5-calmodulin-related calcium sensor, and EF Hand family protein, were chosen at 0.5 and 8 DAI to be verified via qRT-PCR. The results are summarized in Fig. 1. The overall trends of gene expression were similar between the microarray and qRT-PCR methods, confirming the reliability of the microarray data.

Analysis of gene regulation in various functional and metabolic pathways

MapMan software was employed to integrate the gene expression data into their functions in metabolic pathways. This

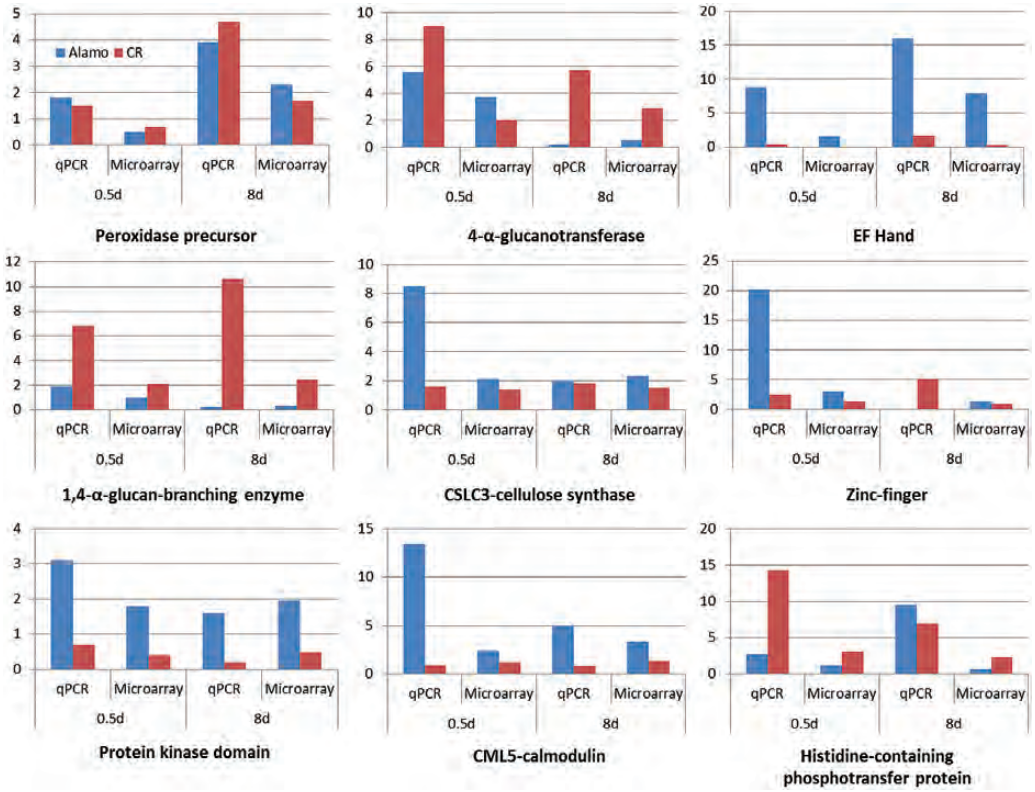

Fig. 1. Quantitative PCR verification of microarray gene expression levels at 0.5 d and 8 d. The *y*-axis represents relative expression levels.

enabled the identification of distinct differences between the responses of the two cultivars to PsJN inoculation, including redox, hormones, signalling, transcription factors, proteolysis, and abiotic stress metabolism.

Redox state

It has been reported that generation of reactive oxygen species (ROS) is one of the earliest plant response events to both pathogenic bacteria (van Loon *et al.*, 2008) and beneficial endophytes (Alqueres *et al.*, 2013). There was an increase in the number of up-regulated redox genes in Alamo at 0.5 DAI (27%), followed by a decline to 16% at 8 DAI (Fig. 2). In CR, 21–30% of genes were up-regulated during the entire period of the experiment. Moreover, 14% of genes were down-regulated in CR during 0.5–8 DAI, while in Alamo this increased from 14% to 27%. Interestingly, one gene for glutathione peroxidase, which protects hosts from oxidative damage, was greatly down-regulated in CR, with only 4–5% of 0 day control expression, whereas no changes were found in Alamo samples (Supplementary Table S2 at *JXB* online).

The redox state is also determined by many factors, such as respiratory burst oxidases, peroxidases, and glutathione *S*-transferases (GST). In the respiratory burst pathway, only one gene, respiratory burst oxidase homologue B, was up-regulated (130%) in Alamo at 0.5 DAI, with no up-regulated genes in CR. A significant down-regulation of respiratory burst oxidase homologue A and B genes (78% and 65% reduction, respectively) was found in Alamo at 8 DAI and only respiratory burst oxidase homologue A was reduced by 61% in CR at 8 DAI (Supplementary Table S2 at *JXB* online). GST catalyses the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification. A total of 25 gene probes were represented in MapMan, with 16, 28, 40, and 48% in Alamo and 28, 36, 44, and 44% of up-regulated genes in CR, respectively, at 0.5, 2, 4, and 8 DAI. However, the number of down-regulated genes in Alamo was higher than that in CR, with 12, 4, 12, and 4% in Alamo at 0.5, 2, 4, and 8 DAI and only 4% of genes in CR at 0.5 DAI, and then declining to 0% during the rest of the experimental period.

Peroxidases play an important role in the plant's life cycle, including responses to abiotic and biotic stresses. Their level also increases during the early infection and colonization by symbiotic organisms (Passardi *et al.*, 2005). A total of 51 gene probes were identified in MapMan. Overall peroxidase gene expression levels after PsJN inoculation were higher in Alamo (31–55%) than in CR (20–41%). Interestingly, in Alamo 14% of peroxidase genes were up-regulated above an 8-fold level only at 0.5 DAI. This strong response indicates that the molecular mechanism of early recognition and modification of defence responses predisposes Alamo to symbiosis with endophytes (Passardi *et al.*, 2005). A few down-regulated genes were also found in both cultivars, 4–6% in Alamo and 2–4% in CR.

H_2O_2 acts as a secondary messenger in the induction of defence mechanisms in plants, namely via regulation of transcription factors (Marinho *et al.*, 2014), and is involved in hypersensitive responses to pathogen attack (Torres and Dangl, 2005). After PsJN inoculation, the number of up-regulated genes in the redox pathway was initially increased in both cultivars (0.5 DAI), with CR maintaining a high level of expression throughout the rest of the experiment, and expression declining in Alamo. The high level of ROS formation in CR implies

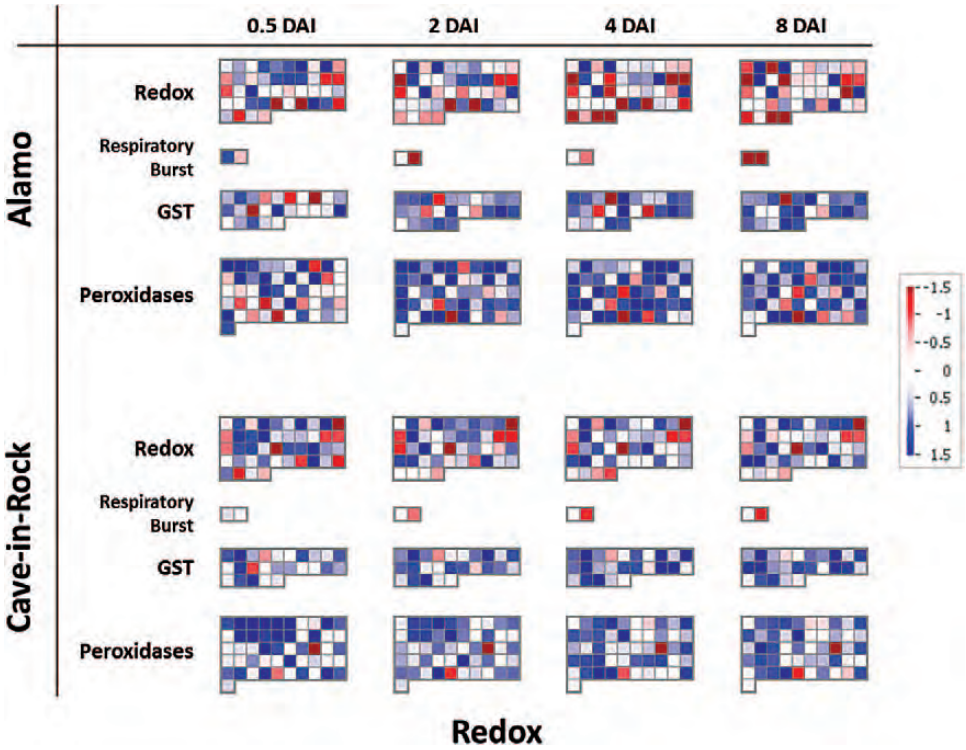

Fig. 2. MapMan heat maps of the redox bin representing differential gene expression between cultivars Alamo and CR at 0.5, 2, 4, and 8 DAI with *Burkholderia phytofirmans* strain PsJN.

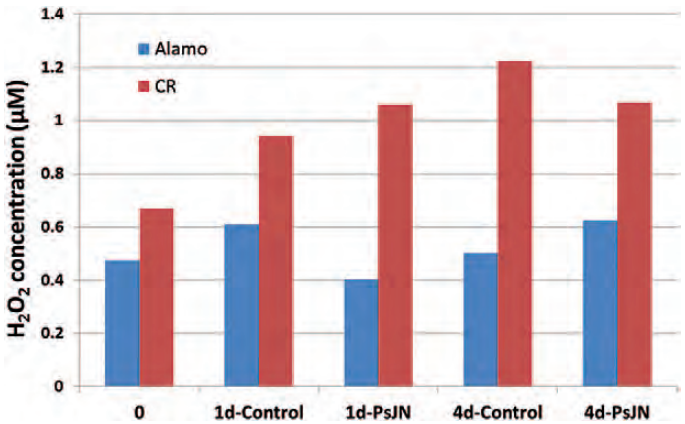

Fig. 3. H_2O_2 assay representing differential hydrogen peroxide changes between cultivars Alamo and CR at 0, 1, and 4 DAI with *Burkholderia phytofirmans* strain PsJN.

PsJN recognition by this cultivar as a pathogen. The H_2O_2 level in Alamo was low prior to inoculation and remained low after PsJN infection, while CR had a higher level of H_2O_2 that increased after PsJN infection (Fig. 3). This indicated a weaker defence response with Alamo after recognition at 0.5 DAI, while CR maintains a strong defence response to PsJN.

Hormones

Plant hormones are signal molecules that regulate metabolic pathways related to plant growth, development, and response to stress (Santner and Estelle, 2009). Microarray data analyses showed cultivar-specific expression patterns of hormonally regulated genes after PsJN inoculation; in salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA), and brassinosteroid (BR) pathways in particular (Fig. 4).

SA: SA is a phenolic compound produced in plants through the phenylpropanoid pathway, which plays a critical role in plant–pathogen interactions (Raskin, 1992). After PsJN inoculation, CR had a much higher number of up-regulated genes, with 40% at 0.5 DAI and 60% at 2–8 DAI, than Alamo which had 20% up-regulated genes during the entire experimental period. Furthermore, 20, 20, and 40% of genes in Alamo were down-regulated at 0.5, 2, and 4 DAI, respectively, while none were down-regulated in CR. *S*-Adenosylmethionine-dependent methyltransferase and benzoate carboxyl methyltransferase genes were significantly up-regulated in CR (Supplementary Table S3 at *JXB* online), indicating that CR may induce local defences as well as systemic acquired resistance (Ross *et al.*, 2007; Vlot *et al.*, 2009). Both enzymes produce volatiles as plant defence responses which can also act as intracellular regulators, a diffusible intercellular signal transducer, or an airborne signal mediating intra- and interplant communications (Seo *et al.*, 2001).

JA: JA and its derivatives are lipid-based hormone signals that regulate a wide range of processes in plants and also play an important role in plant defence, especially after mechanical wounding or insect and pathogen attack (Cheong *et al.*, 2002). Similarly to the SA pathway, the JA pathway was up-regulated significantly higher in CR than in Alamo, with 71, 57, 71, and 71% of genes up-regulated in CR at 0.5, 2, 4, and 8 DAI, respectively, versus 43% at 0.5 DAI, followed by a decline to 14% in Alamo (Fig. 4). The most up-regulated gene was lipoxygenase 2.1 (a chloroplast precursor) with a 1600% increase in CR at 0.5 DAI and only a 49% increase in Alamo. Lipoxygenase 2.1 was reported to catalyse the reduction of natural (+)-*cis*-OPDA for JA biosynthesis in rice (Tani *et al.*, 2008). Lipoxygenase 8, which

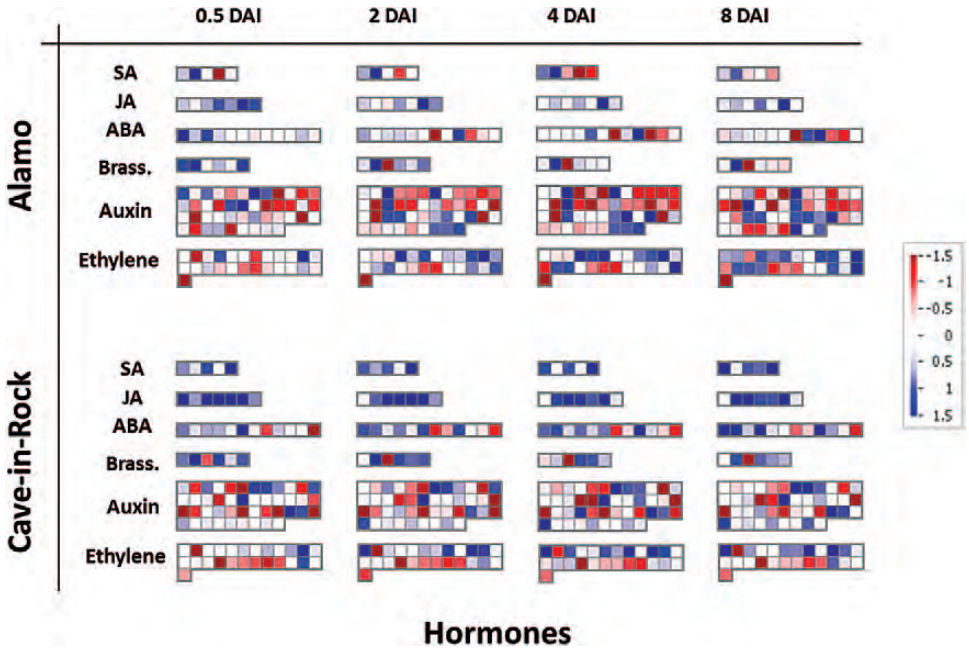

Fig. 4. MapMan heat maps of the hormone bin representing differential gene expression between cultivars Alamo and CR at 0.5, 2, 4, and 8 DAI with *Burkholderia phytofirmans* strain PsJN.

generates precursors (fatty acid hydroperoxides) of JA, was also up-regulated in CR samples throughout the entire experimental period (up to 200% increase); no lipoxygenase gene expression changes were observed in Alamo. The genes encoding two JA biosynthesis enzymes, 12-oxophytodienoate reductase 2, were also greatly up-regulated in CR (107–408% increase) with no changes in Alamo (Supplementary Table S3 at *JXB* online), all of these findings implying that more JA might be produced in CR.

ABA: ABA is a hormonal regulator of defense gene expression in plants (Adie *et al.*, 2007). Its levels in plant tissues are determined by the rates of biosynthesis and catabolism (Nambara and Marion-Poll, 2005). Overall, CR had more genes up-regulated in the ABA pathway, with 8, 25, 17, and 33% of genes increased at least 2-fold at 0.5, 2, 4, and 8 DAI, respectively, as compared with 17, 8, 17, and 17% of genes up-regulated in the corresponding Alamo samples. Genotypic differences were also found in carotenoid cleavage dioxygenase 8 and abscisic-aldehyde oxidase, with a significant increase in CR but with slight down-regulation in Alamo (Supplementary Table S3 at *JXB* online). Interestingly, in the ABA induced-regulated-responsive-activated category, expression of FIP1 protein was significantly reduced across all time points in CR, but was slightly reduced in Alamo.

BRs: BRs are a class of polyhydroxysteroids biosynthesized from campesterol (Fujioka and Sakurai, 1997), which counteract both abiotic and biotic stresses in plants (Sharma *et al.*, 2008). More genes (67, 67, 33, and 17% at 0.5, 2, 4, and 8 DAI, respectively) were up-regulated in CR compared with Alamo (50, 17, 17, and 17%) after PsJN inoculation, particularly a delta14-sterol reductase, one enzyme in the BR biosynthetic pathway (Schrick *et al.*, 2000). Its expression was increased by 94–153% after inoculation in CR (at all sampling points), while no obvious increase was noted in Alamo.

Auxin: auxin functions in the promotion and regulation of growth and development in plants. Overall, Alamo exhibited slightly more up-regulated probes (9% at 0.5 DAI to 16% at 2 DAI and 20% at 4 and 8 DAI) than CR (13–16% throughout the sampling period) (Fig. 4). Protein OsSAUR5 (small auxin-up RNAs, member of an auxin-responsive SAUR gene family) and OsSAUR38 were rapidly increased in Alamo at 4 and 8 DAI compared with CR. However, CR exhibited significant reduction in gene expression levels of OsSAUR55, OsSAUR11, and OsSAUR17. Expression of OsSAUR45, OsSAUR20, OsSAUR44, OsSAUR48, and the axi1 protein coding genes was significantly up-regulated in CR (Supplementary Table S3 at *JXB* online). It was reported that SAUR genes induced minutes after auxin treatment with highly unstable mRNAs were bound to calmodulin *in vitro* (Jain *et al.*, 2006), which may provide a link between the calcium secondary messenger and auxin signalling.

Ethylene: ethylene is a plant growth inhibitor as well as a signalling molecule. Overall, in response to PsJN inoculation, Alamo had more up-regulated genes (8, 20, 32, and 36% at 0.5, 2, 4, and 8 DAI, respectively) than CR (8, 16, 24, and 12% in the corresponding samples). In the ethylene synthesis/degradation bin, Alamo had slightly more genes up-regulated after PsJN inoculation compared with CR (Fig. 4). Specifically, genes such as ACC synthase, ACC oxidase, and two oxidoreductases were up-regulated at 4 and 8 DAI (Supplementary Table S3 at *JXB* online). Expression of ACC synthase, coding for the enzyme responsible for converting *S*-adenosyl-L-methionine to ACC, was up-regulated in Alamo, and not in CR. Additionally, expression of one of the ACC synthase genes was greatly reduced in CR, whereas one of the ACC oxidases that catalyse oxidation of ACC to ethylene was up-regulated in Alamo samples. One of the oxidoreductase genes, which were reported to be involved in

defence responses in plants (van Damme *et al.*, 2008), was up-regulated up to 14-fold in CR at 8 DAI. In the ethylene signal transduction pathway more genes were down-regulated in CR at 0.5 and 2 DAI, including expression of an unknown gene that was only at the 2–4% level of control. In the ethylene induced-regulated-responsive-activated bin, both cultivars showed significant down-regulation of the universal stress protein (USP) family across all sampling times; to a greater extent in Alamo than in CR (15–31% versus 48–61% of control) (Supplementary Table S3).

The balance and interactions between signal molecules and/or plant hormones are important in plant growth and development as well as responses to abiotic and biotic stresses. Higher gene expression levels in SA, JA, ABA, and BR pathways would induce stronger defence signals against endophyte PsJN in the non-responsive cv. CR than in the responsive cv. Alamo. These interactions would probably be more complex *in vivo*; for example, SA-mediated disease resistance could repress the auxin-responsive genes (Wang *et al.*, 2007). Actual levels of different hormones in both cultivars still need to be determined, and their balance may be more important during PsJN colonization and then in plant growth and development.

Signalling

In response to PsJN inoculation, the two switchgrass cultivars showed distinct differences in the expression of genes classified as signalling associated. In Alamo, 24% of the genes were up-regulated at 0.5 DAI, after which the percentage of genes up-regulated declined to 13% over 2–8 DAI (Fig. 5). In contrast, the percentage of genes down-regulated increased steadily from 9% at 0.5 DAI to 30% at 8 DAI. In CR, 11% of the genes were up-regulated at 0.5 DAI and that level remained relatively stable (13%) up to 8 DAI. There was an initial increase in the percentage of genes down-regulated at 0.5 DAI (21%) which then declined (12%) at 8 DAI. Hence, the overall trend for the responsive cv. Alamo was an initial increase by 0.5 DAI in the number of up- and down-regulated genes, followed by a decline in the number of up-regulated genes and an increase in the number of down-regulated genes following PsJN inoculation, while the non-responsive cv. CR exhibited minimal changes in the number of up-regulated

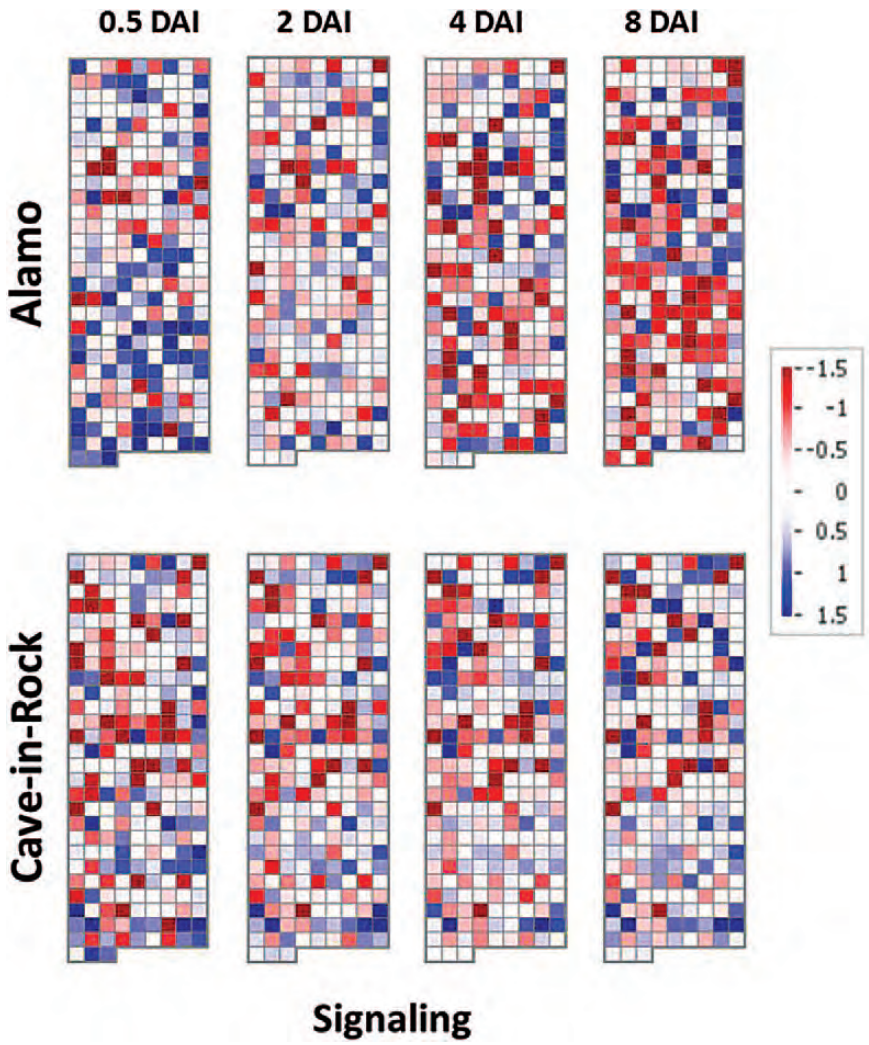

Fig. 5. MapMan heat maps of the signalling bin representing differential gene expression between cultivars Alamo and CR at 0.5, 2, 4, and 8 DAI with *Burkholderia phytofirmans* strain PsJN.

and a slight decline in the number of down-regulated genes after 0.5 DAI following PsJN inoculation.

Functional classification of the various up- and down-regulated genes was carried out using MapMan software (Fig. 5). Gene expression for several signalling-related pathways was modified, including genes representing light, calcium, G-protein, and receptor kinase signalling. Many of the genes classified as associated with light signalling and G-proteins were down-regulated over the 0.5–8 DAI period for Alamo, but did not change or were up-regulated for CR (Supplementary Table S4 at *JXB* online). The leucine-rich repeat (LRR) and domain of unknown function 26 (DUF26) receptor kinase classes also displayed changes in expression profiles in both cultivars following PsJN inoculation. In Alamo, the overall trend was a decline in the number of LRR receptor kinase genes up-regulated and an increase in the number of genes down-regulated between 0.5 and 8 DAI (e.g. Strubbelig Receptor Family SRF8, Transmembrane Kinase TMK1). In CR, the number of LRR receptor kinase genes up- and down-regulated remained relatively stable during 0.5–4 DAI, after which there was an increase in the number of genes up-regulated (e.g. ATP binding protein, Inflorescence Meristem Receptor Kinase IMK2). The DUF26 receptor kinase family was initially down-regulated in Alamo at 0.5 DAI, after which there was an increase in the number of genes up-regulated (e.g. receptor-like kinase CRK10, S-locus lectin protein kinase family protein). In CR, the number of DUF26 receptor kinase family members was initially decreased, and the number of up-regulated genes slowly increased between 2 and 8 DAI (e.g. receptor-like Ser/Thr protein kinase, receptor protein kinase ZmPK1). Various receptor kinases can serve as perception sites for pathogen-derived and beneficial microbe-derived signals/factors (Oldroyd, 2013), and their abundance may have a subsequent impact on cultivar responsiveness/non-responsiveness to PsJN. The general decline in LRR receptor kinases in Alamo and the temporal expression variation in DUF26 members further indicates differential stress response levels between Alamo and CR following PsJN inoculation.

While both cultivars displayed an up-regulation of signalling-associated genes early after inoculation (0.5 DAI), distinct cultivar differences were evident during the 2–8 DAI, with a pronounced decline in overall signalling-associated gene expression for the responsive cultivar Alamo. In contrast, for the non-responsive cultivar CR, signalling-associated gene expression remained similar. The early up-regulation of signalling-associated gene expression for both cultivars was not surprising, as both would be expected to perceive the microbial inoculation and initiate a response (Fujita *et al.*, 2006). Various mechanisms are associated with plant–microbe interactions, including calcium (Vadassery and Oelmüller, 2012), light (Kangasjärvi *et al.*, 2012), GTP (Bernoux *et al.*, 2011), receptor (Segonzac and Zipfel, 2011), and kinase (Tena *et al.*, 2011) signalling processes. The present analysis of the top up- and down-regulated genes over the 8 DAI indicated that many of the above processes were represented, although it was noted that in some cases different genes were regulated for the different cultivars, indicating that the signalling-associated processes were different between the responsive and non-responsive cultivars.

Functional analysis of gene expression changes through MapMan indicated that genes associated with light- and G-protein-associated processes were down-regulated in Alamo, but not in CR. As examples, COP1 and DET1, while are commonly associated with repression of light-induced gene expression via the ubiquitin-mediated degradation pathway, also mediate general stress response genes (Mayer *et al.*, 1996). Furthermore, another down-regulated gene in Alamo, VAR3, encodes a protein which interacts with and is believed to inhibit the carotenoid cleavage dioxygenase CCD4 (Huang *et al.*, 2009), an enzyme which is up-regulated in response to stress and ABA (Wang *et al.*, 2013). Hence, the down-regulation of these genes in Alamo but not in CR suggests differential stress response recognition of PsJN between the responsive cv. Alamo and the non-responsive cv. CR.

Transcription factors

Transcription factors are involved in every aspect of plant growth and development. Distinct transcription factor gene expression differences were noted in response to PsJN inoculation between Alamo and CR (Fig. 6). The primary families with significant response to the bacterial inoculation were MAPK, AP2/EREBP, WRKY, MYB, DOF, and bZIP.

MAPK: mitogen-activated protein kinase (MAPK) plays important roles in signal transduction leading to transcription factor gene expression. In Alamo only a MAP3K-like protein kinase gene was up-regulated at 4 DAI, while in CR there were two genes (MAP3K-like protein kinase and MAPK8) up-regulated at 0.5 and 8 DAI. Many differences were found in down-regulated genes between both cultivars. Alamo had a much higher number of down-regulated genes than did CR, with 38, 13, 38, and 50% in Alamo at 0.5, 2, 4, and 8 DAI, respectively, versus 13% and 38% in CR at 0.5 and 2 DAI and no down-regulated genes at 4 and 8 DAI.

AP2/EREBP: this gene family represented the most noticeable changes compared with any other transcription factor class. It includes the ethylene-responsive element-binding protein, one of the most critical elements of metabolic regulation in plants (Singh *et al.*, 2002). Overall, the number of down-regulated genes in this family was 86, 64, 57, and 50% in CR at 0.5, 2, 4, and 8 DAI, respectively, compared with 43, 21, 14, and 14% in the corresponding Alamo samples. The most common genes that were down-regulated in CR were the transcription factor TINY followed by the AP2 domain-containing protein and BABY BOOM 1. The down-regulated genes in Alamo were primarily BABY BOOM 1. In contrast, only the AP2/EREBP transcriptional factor WRI1 was up-regulated at 4 DAI in Alamo, and no genes were up-regulated in CR (Fig. 6).

WRKY: the WRKY gene family plays an important role in the regulation of gene transcriptional reprogramming involved in plant stress responses (Chen *et al.*, 2012). Alamo had more up-regulated genes than CR, with Alamo having 33% at 2 and 4 DAI, which increased to 50%, while CR had only 17% of up-regulated genes at 8 DAI. More WRKY

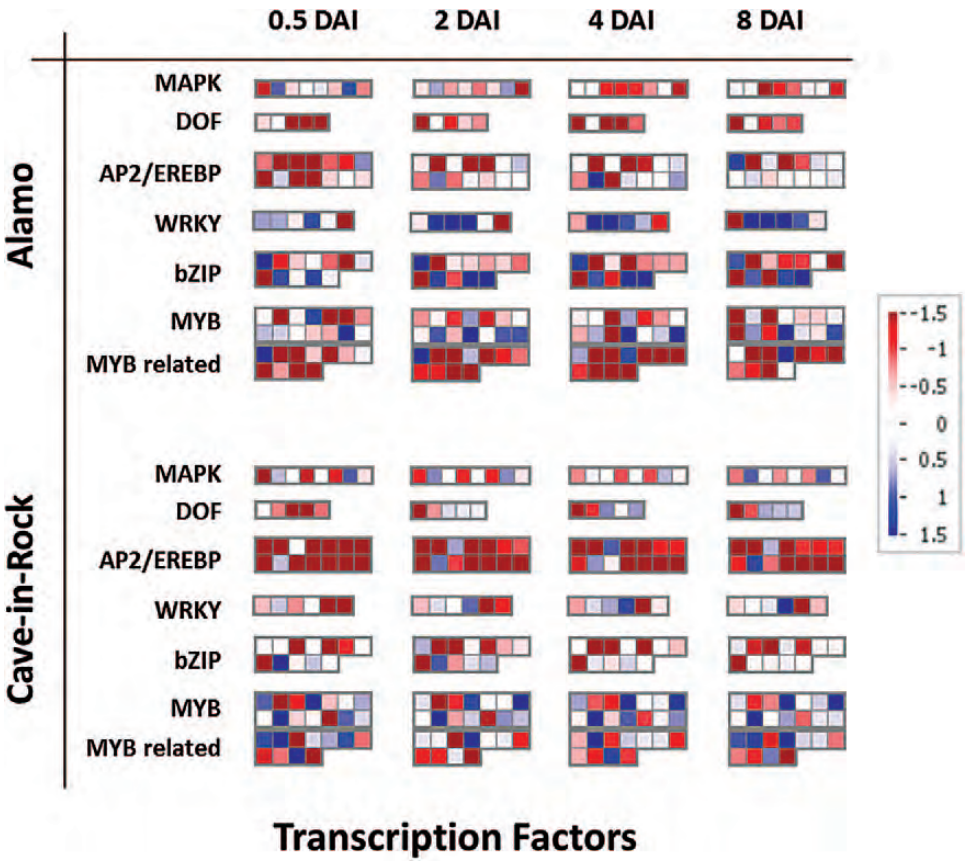

Fig. 6. MapMan heat maps of the transcription factor bin representing differential gene expression between cultivars Alamo and CR at 0.5, 2, 4, and 8 DAI with *Burkholderia phytofirmans* strain PsJN.

genes were down-regulated in response to PsJN in CR than in Alamo. In CR, 33% of genes were down-regulated at 0.5 DAI, which declined to 17%, while in Alamo 17% of genes were down-regulated at 0.5 and 2 DAI, which declined to 0. WRKY36 and the WRKY DAN binding domain were up-regulated in Alamo; WRKY21/calmodulin binding/transcription factor was down-regulated across all samples in CR.

DOF: DOFs are plant-specific transcription factors with a highly conserved DNA-binding domain and function as transcriptional activators or repressors involved in diverse biological processes. Both cultivars showed a significant number of down-regulated genes following PsJN inoculation, with Alamo having more down-regulated genes. No genes were found to be up-regulated (Fig. 6).

bZIP: in plants, bZIPs (basic leucine zippers) are key regulators of growth and development, and are functionally involved in oxidative stress (Corrêa *et al.*, 2008). In general, in Alamo a higher number of these genes were up-regulated after PsJN inoculation (8–25%) than in CR, which had an initial increase (8%) at 0.5 DAI, followed by a decline to 0% from 2 to 8 DAI. Both cultivars exhibited a similar number of down-regulated genes. Transcription factor HBP-1a and light-inducible protein CPRF-2 were the most down-regulated genes in both cultivars, but to a greater extent in Alamo (Supplementary Table S5 at *JXB* online).

MYB and MYB-related genes: the MYB transcription factor family is large and controls many physiological processes including biotic and abiotic stress responses (Ambawat *et al.*, 2013). Overall and across all time points, CR had a similar average of up- and down-regulated genes (24% and 21%, respectively). Alamo exhibited a differential expression pattern, with only 4% of genes up-regulated compared with 42% down-regulated (Fig. 6). Significant differences were found in MYB-related genes between the responsive and non-responsive cultivars, with a much greater number of genes down-regulated in Alamo (55, 45, 73, and 45% of genes down-regulated at 0.5, 2, 4, and 8 DAI, respectively) than in CR (18, 18, 0, and 9%, respectively).

Proteolysis

The two switchgrass cultivars displayed distinct differences in the expression of proteolysis-associated genes in response to PsJN inoculation (Fig. 7). In Alamo, 19% of the genes were up-regulated at 0.5 DAI, after which the percentage of genes up-regulated declined to 7% over 2–4 DAI, with a further decline to 4% by 8 DAI (Supplementary Table S6 at *JXB* online). In contrast, the percentage of genes down-regulated increased at each sampling point, from 18% at 0.5 DAI to 42% at 8 DAI.

In CR, 12% of the genes were up-regulated at 0.5 DAI, declining further by 2 DAI (8%) and 4 DAI (5%), with 9% of genes up-regulated by 8 DAI. In terms of down-regulated genes, 21% of genes were down-regulated at 0.5 DAI, with a decline to 14–15% by 2–4 DAI, and a further decline to 8% by 8 DAI. Hence, the overall trend for responsive cv. Alamo was an initial increase by 0.5 DAI in the number of

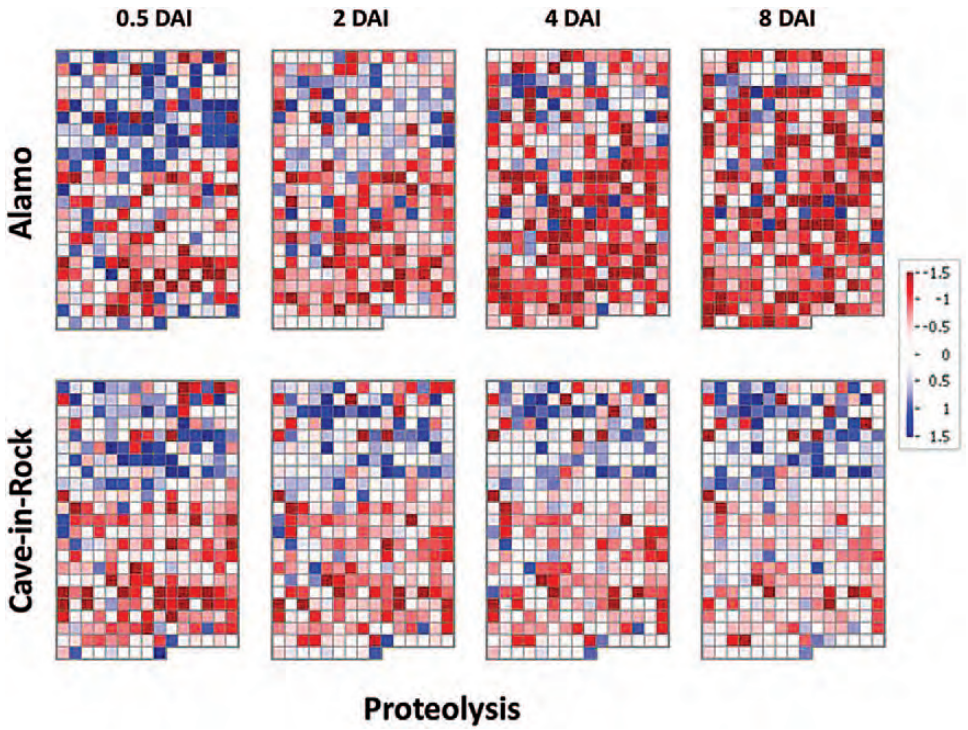

Fig. 7. MapMan heat maps of the proteolysis bin representing differential gene expression between cultivars Alamo and CR at 0.5, 2, 4, and 8 DAI with *Burkholderia phytofirmans* strain PsJN.

up- and down-regulated genes, followed by a decline in the number of up-regulated genes and an increase in the number of down-regulated genes following PsJN inoculation. In contrast, the non-responsive cv. CR displayed an initial increase by 0.5 DAI in the number of up- and down-regulated genes, after which the number of up- and down-regulated genes decreased.

Both cultivars also displayed differences in protease functional classes over the period of study (Supplementary Table S6 at *JXB* online). Overall the numbers of cysteine proteases declined somewhat over the 8 d period for both Alamo and CR. However, there was a down-regulation in the number of serine proteases and metalloproteases in Alamo, while the numbers of these functional classes increased in CR.

As mentioned above, the microarray data suggested a more pronounced decline in proteolysis-associated gene expression for Alamo. To determine whether this was associated with any detectable changes in protease activity, an MBP degradation protease assay was carried out using protein extracts from day 0 and day 8 post-PsJN inoculation plantlets (Fig. 8). At day 0 (prior to PsJN inoculation), there was more proteolytic activity present in plant extracts of Alamo, and less in CR, as evidenced by the higher abundance of intact MBP in the CR sample lane. Comparison of the MBP degradation pattern with day 8 protein extracts revealed little change in the level of intact MBP with the Alamo samples, suggesting little overall change in proteolytic activity between day 0 and day 8. In contrast, there was a decline in intact MBP between day 0 and day 8 with the CR samples, suggesting an increase in overall proteolytic activity by day 8 for CR. However, there did not appear to be any difference in overall proteolytic activity between Alamo and CR at day 8.

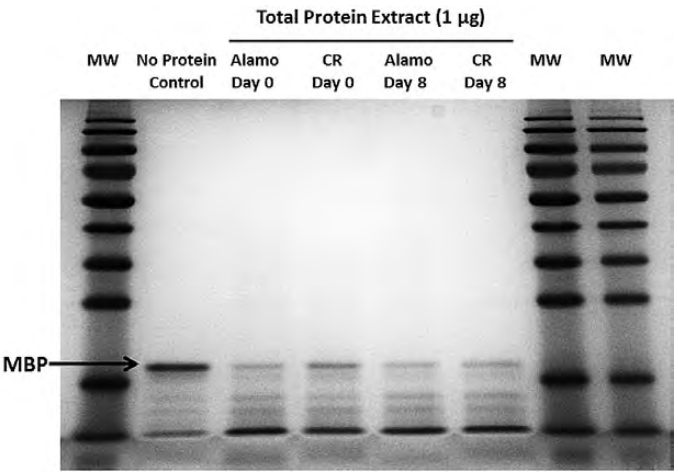

Fig. 8. Proteolysis activity assay with myelin basic protein (MBP). A total of 1 µg of each protein extract was incubated with MBP at 0.2 mg ml^{-1} in a 15 µl reaction volume for 7 h at 37 °C, and products were analysed by SDS–PAGE.

Various proteases are up-regulated following pathogen infection, and, as an example, following corn exposure to corn rust, various genes associated with proteolysis were primarily up-regulated (Wang *et al.*, 2012), including those associated with various protease classes (subtilisin-like, serine carboxypeptidase, prolyl endopeptidase, metalloprotease, cystatin cysteine-protease inhibitor), and ubiquitin–proteasome system- (UPS) associated proteins (RING finger, F-Box, kelch-containing protein, proteasome subunit, ubiquitin). In the system used here, the initial up-regulation of proteolysis-associated genes in both cultivars suggests that the first response of both to PsJN is a defence or wound response.

However, the subsequent reduction of proteolysis-associated gene expression in Alamo may provide a more welcoming environment for bacterial survival and colonization.

The combined observations on the types of genes up- and down-regulated following PsJN inoculation for both cultivars indicated both similarities and differences. In both cultivars, the top up- and down-regulated genes represented various protease classes, although the overall pattern was down-regulation of protease gene expression for Alamo. However, these top regulated genes represented different gene family members, and the overall proteolytic functional gene classes were modulated differently between the two cultivars. Many protease gene classes have been reported as induced by biotic and/or abiotic stress (van der Hoorn and Jones, 2004; Yee and Goring, 2009; Wang *et al.*, 2012), and may also be expressed during plant growth, impacting development and cell fate (van der Hoorn, 2008). Furthermore, the proteolytic machinery plays a role in plant microbial and pest defence responses (van der Hoorn and Jones, 2004). Hence, the prominent down-regulation of specific protease genes in cv. Alamo over the 8 d period may reflect a perceived 'less stressed' environment for this responsive cultivar, rather than a defence type of response associated with the non-responsive cv. CR.

Abiotic stress

There were different trends in the genes involved in abiotic stress between the responsive and non-responsive cultivars following PsJN inoculation. Overall, more genes were up- and down-regulated in Alamo than in CR (Fig. 9). Alamo had an initial increase in the number of up-regulated genes at 0.5 DAI (28%), which declined to 13%, whereas CR had an initial increase at 0.5 DAI (15%), which declined to 8, 9, and 12% at 2, 4, and 8 DAI, respectively. Furthermore, the number of genes down-regulated was also higher in Alamo than in CR (i.e. 23, 19, 27 and 39% at 0.5, 2, 4, and 8 DAI in Alamo, respectively), with an increasing trend, while these numbers were 17, 15, 12, and 16% in corresponding CR samples. For example, Protein fb2 gene, protein wound inducive gene, HORVU low temperature-induced protein, and protein 3Fe–4S ferredoxin were more highly expressed in Alamo compared with CR (Supplementary Table S7 at *JXB* online). These higher expression levels in Alamo may indicate a better ability to deal with stress resulting from the presence of endophyte PsJN.

Summary

Burkholderia phytofirmans strain PsJN is one of the most studied beneficial bacterial endophytes which possesses the *acdS* gene (encoding ACC deaminase) and exhibits high activity of the enzyme, a general feature in the genus *Burkholderia* (Onofre-Lemus *et al.*, 2009). ACC deaminase degrades ACC, a precursor of ethylene, resulting in lower levels of the plant growth-inhibiting hormone ethylene, so promoting plant growth (Sessitsch *et al.*, 2005; Sun *et al.*, 2009). However, PsJN-mediated growth promotion is genotype specific, as observed in other plant species. Identifying the underlying genetic causes of this phenomenon will increase understanding of plant-beneficial microbe interactions and aid in the development of more responsive plant cultivars. With the responsive cv. Alamo and non-responsive cv. CR, a switchgrass EST microarray chip was used to measure gene expression levels of both cultivars following PsJN inoculation at early stages (0–8 DAI) with subsequent data analysis using MapMan software to establish the transcriptome network. Using a MapMan Biotic Stress diagram, modifications were made based on the data (Fig. 10). The first step in plant and microbe interaction is recognition, which could be seen at 0.5 DAI; both the responsive and the non-responsive cultivars showed similar reactions, such as redox, signalling, and proteolysis. After recognition at 0.5 DAI, responsive and non-responsive cultivars reacted in totally different ways. In general, the responsive cultivars showed a weak defence response, and the relationship became mutual or beneficial while the non-responsive cultivars maintained a relatively strong defence response throughout the experimental period, which includes redox, signalling, proteolysis, and abiotic stress. Signal molecules and hormones also play critical roles in host plant–endophyte interaction, determining their relationships, whether mutual or antagonistic. The most dramatic differences between cultivars were the up-regulation of SA, JA, and ABA pathways in CR, which may induce or enhance resistance to PsJN. This finding supports previous studies where SA plays a role in activating defence responses against both biotrophic and hemi-biotrophic pathogens (Zhao and Qi, 2008; Bari and Jones, 2009; Ding *et al.*, 2011), and is mediated by several resistance genes involved in the induction of local defences as well as the establishment of systemic acquired resistance (Ross *et al.*, 2007; Vlot *et al.*, 2009).

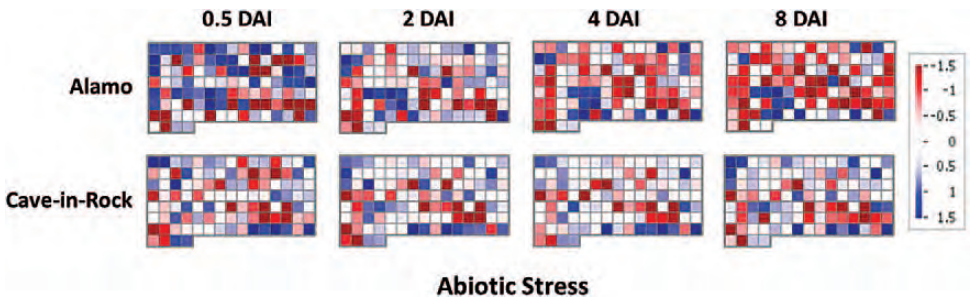

Fig. 9. MapMan heat maps of the abiotic stress bin representing differential gene expression between cultivars Alamo and CR at 0.5, 2, 4, and 8 DAI with *Burkholderia phytofirmans* strain PsJN.

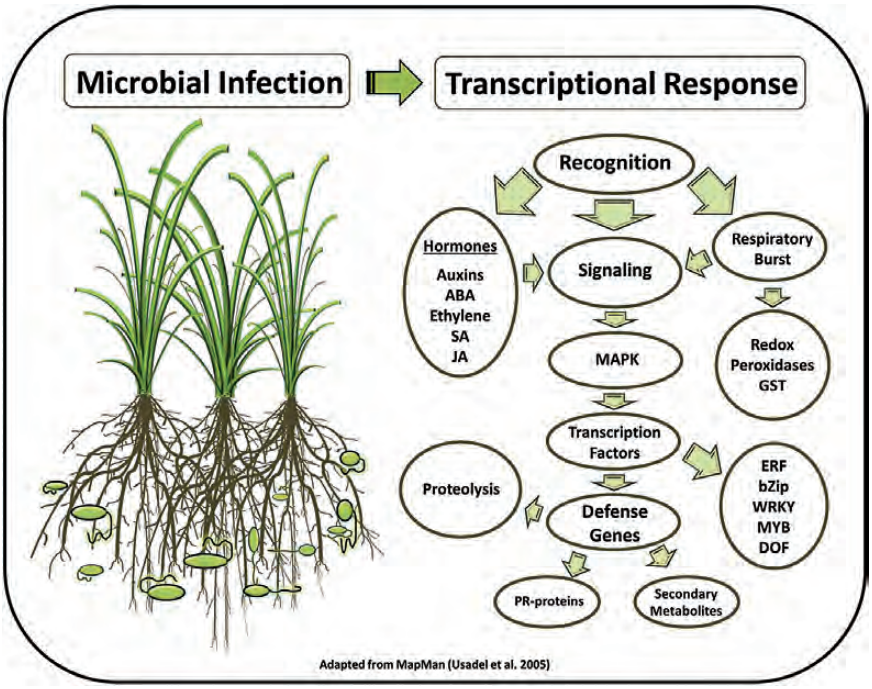

Fig. 10. A flow chart representing the cascade of genes modified after inoculation with *Burkholderia phytofirmans* strain PsJN.

Most defence responses are associated with the hypersensitive response (HR) and cell death; the non-responsive cv. CR did not exhibit any apparent HR or cell death following exposure to PsJN, suggesting that some level of stress signalling is different between the response to a beneficial microbe and the response to a pathogenic microbe. Comparative plant differential defence responses to the beneficial endophyte PsJN and to the pathogenic bacterium *Pseudomonas syringae* were investigated in grape cell suspension cultures (Bordiec *et al.*, 2011). The non-responsive cv. CR had a sustained up-regulation of genes involved in the redox reaction, and SA and JA pathways, which were more similar to the response of grape cell cultures to pathogenic *P. syringae*, but probably the expression levels were different. Endophytic bacteria must be compatible with their plant host to colonize its tissues successfully. The defence network activated and utilized by the host plant depends on the nature of the pathogen and its mode of pathogenicity (Bari and Jones, 2009). In summary, the present results demonstrated significant differences in transcript responses between the responsive and non-responsive cultivars to PsJN inoculation. Microarray data analysis revealed differences in metabolic pathways of redox, hormones, and signalling molecules, and transcription factors. The PsJN-responsive cultivar had the ability to reduce stress caused by endophytic bacterial infection and colonization and slightly enhanced plant defence response. However, the non-responsive cv. CR had a strong defence response including strong SA, JA, and ABA signalling which may help resist endophytic bacterial infection and colonization.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. The Venn diagram shows common and differently affected genes between Alamo and CR after PsJN inoculation.

Table S1. Number of differentially expressed probe sets at each time point in comparison with the non-inoculated control.

Table S2. Redox and related genes.

Table S3. Hormone genes.

Table S4. Signalling genes.

Table S5. Transcription factor genes.

Table S6. Proteolysis genes.

Table S7. Abiotic stress genes.

Acknowledgements

This work was funded by the Office of Science (BER), US Department of Energy for Plant Feedstock Genomics for Bioenergy Program (DE-SC0004951).

References

Adie BA, Pérez-Pérez J, Pérez-Pérez MM, Godoy M, Sánchez-Serrano JJ, Schmelz EA, Solano R. 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. *The Plant Cell* **19,** 1665–1681.

Ambawat S, Sharma P, Yadav NR, Yadav RC. 2013. MYB transcription factor genes as regulators for plant responses: an overview. *Physiology and Molecular Biology of Plants* **19,** 307–321.

Alquéres S, Meneses C, Rouws L, Rothballer M, Baldani I, Schmid M, Hartmann A. 2013. The bacterial superoxide dismutase and glutathione reductase are crucial for endophytic colonization of rice roots by *Gluconacetobacter diazotrophicus* PAL5. *Molecular Plant-Microbe Interactions* **26,** 937–945.

Bari R, Jones JD. 2009. Role of plant hormones in plant defence responses. *Plant Molecular Biology* **69,** 473–488.

Barka EA, Gognies S, Nowak J, Audran JC, Belarbi A. 2002. Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. *Biological Control* **24,** 135–142.

Bernoux M, Ellis JG, Dodds PN. 2011. New insights in plant immunity signaling activation. *Current Opinion in Plant Biology* **14,** 512–518.

Bordiec S, Paquis S, Lacroix H, Dhondt S, Ait Barka E, Kauffmann S, Jeandet P, Clément C, Baillieul F, Dorey S. 2011. Comparative analysis of defence responses induced by the endophytic plant growth-promoting rhizobacterium *Burkholderia phytofirmans* strain PsJN and the non-host bacterium *Pseudomonas syringae* pv. Pisi in grapevine cell suspensions. *Journal of Experimental Botany* **62,** 595–603.

Casler MD, Tobias CM, Kaeppler SM, Buell CR, Wang ZY, Cao P, Schmutz J, Ronald P. 2011. The switchgrass genome: tools and strategies. *The Plant Genome* **4,** 273–282.

Chen L, Song Y, Li S, Zhang L, Zou C, Yu D. 2012. The role of WRKY transcription factors in plant abiotic stresses. *Biochimica et Biophysica Acta* **1819,** 120–128.

Cheong YH, Chang HS, Gupta R, Wang X, Zhu T, Luan S. 2002. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. *Plant Physiology* **129,** 661–677.

Compant S, Kaplan H, Sessitsch A, Nowak J, Barka EA, Clément C. 2008. Endophytic colonization of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN: from the rhizosphere to inflorescence tissues. *FEMS Microbiology Ecology* **63,** 84–93.

Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Barka EA. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology* **71,** 1685–1693.

Conn KL, Lazarovits G, Nowak J. 1997. A gnotobiotic bioassay for studying interactions between potatoes and plant growth-promoting rhizobacteria. *Canadian Journal of Microbiology* **43,** 801–808.

Corrêa LGG, Riaño-Pachón DM, Schrago CG, Dos Santos RV, Mueller-Roeber B, Vincentz M. 2008. The role of bZIP transcription factors in green plant evolution: adaptive features emerging from four founder genes. *PLoS One* **3,** e2944.

Da K, Nowak J, Flinn B. 2012. Potato cytosine methylation and gene expression changes induced by a beneficial bacterial endophyte, *Burkholderia phytofirmans* strain PsJN. *Plant Physiology and Biochemistry* **50,** 24–34.

Ding L, Xu H, Yi H, *et al.* 2011. Resistance to hemi-biotrophic F. graminearum infection is associated with coordinated and ordered expression of diverse defense signaling pathways. *PLoS One* **6,** e19008.

Dozmorov I, Centola M. 2003. An associative analysis of gene expression array data. *Bioinformatics* **19,** 204–211.

Fernandez O, Vandesteene L, Feil R, Baillieul F, Lunn JE, Clément C. 2012. Trehalose metabolism is activated upon chilling in grapevine and might participate in *Burkholderia phytofirmans* induced chilling tolerance. *Planta* **236,** 355–369.

Frommel M, Nowak J, Lazarovits G. 1993. Treatment of potato tubers with a growth promoting Pseudomonas sp.: plant growth responses and bacterium distribution in the rhizosphere. *Plant and Soil* **150,** 51–60.

Frommel MI, Nowak J, Lazarovits G. 1991. Growth enhancement and developmental modifications of *in vitro* grown potato (*Solanum tuberosum* spp. tuberosum) as affected by a nonfluorescent Pseudomonas sp. *Plant Physiology* **96,** 928–936.

Fujioka S, Li J, Choi YH, *et al*. 1997. The Arabidopsis deetiolated2 mutant is blocked early in brassinosteroid biosynthesis. *The Plant Cell* **9,** 1951–1962.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K. 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology* **9,** 436–442.

Gagne-Bourgue F, Aliferis KA, Seguin P, Rani M, Samson R, Jabaji S. 2013. Isolation and characterization of indigenous endophytic bacteria associated with leaves of switchgrass (*Panicum virgatum* L.) cultivars. *Journal of Applied Microbiology* **114,** 836–853.

Ghimire SR, Charlton ND, Bell JD, Krishnamurthy YL, Craven KD. 2011. Biodiversity of fungal endophyte communities inhabiting switchgrass (*Panicum virgatum* L.) growing in the native tallgrass prairie of northern Oklahoma. *Fungal Diversity* **47,** 19–27.

Ghosh S, Gepstein S, Heikkila J, Dumbrov EB. 1988. Use of a scanning densitometer or an ELISA plate reader for measurement of nanogram amounts of protein in crude extracts from biological tissues. *Analytical Biochemistry* **169,** 227–233.

Huang FC, Molnár P, Schwab W. 2009. Cloning and functional characterization of carotenoid cleavage dioxygenase 4 genes. *Journal of Experimental Botany* **60,** 3011–3022.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4,** 249–264.

Jain M, Tyagi AK, Khurana JP. 2006. Genome-wide analysis, evolutionary expansion, and expression of early auxin-responsive *SAUR* gene family in rice (*Oryza sativa*). *Genomics* **88,** 360–371.

Kangasjärvi S, Neukermans J, Li S, Aro EM, Noctor G. 2012. Photosynthesis, photorespiration, and light signalling in defence responses. *Journal of Experimental Botany* **63,** 1619–1636.

Ker K, Seguin P, Driscoll BT, Fyles JW, Smith DL. 2012. Switchgrass establishment and seeding year production can be improved by inoculation with rhizosphere endophytes. *Biomass and Bioenergy* **47,** 295–301.

Kim S, Lowman S, Hou G, Nowak J, Flinn B, Mei C. 2012. Growth promotion and colonization of switchgrass (*Panicum virgatum*) cv. Alamo by bacterial endophyte *Burkholderia phytofirmans* strain PsJN. *Biotechnology for Biofuels* **5,** 37.

Kleczewski N, Bauer J, Bever J, Clay K, Reynolds H. 2012. A survey of endophytic fungi of switchgrass (*Panicum virgatum*) in the Midwest, and their putative roles in plant growth. *Fungal Ecology* **5,** 521–529.

Lazarovits G, Nowak J. 1997. Rhizobacteria for improvement of plant growth and establishment. *HortScience* **32,** 188–192.

Liu Z, Pillay V, Nowak J. 1995. *In vitro* culture of watermelon and cantaloupe with and without beneficial bacterium. *Acta Horticulturae (ISHS)* **402,** 58–60.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* **25,** 402–408.

Lowman JS, Lara-Chavez A, Kim-Dura S, Flinn B, Nowak J, Mei C. 2015. Switchgrass field performance on two soils as affected by bacterization of seedlings with *Burkholderia phytofirmans* strain PsJN. *BioEnergy Research* **8**, 440–449.

Marinho HS, Real C, Cyrne L, Soares H, Antunes F. 2014. Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biology* **2,** 535–562.

Mayer R, Raventos D, Chua NH. 1996. det1, cop1, and cop9 mutations cause inappropriate expression of several gene sets. *The Plant Cell* **8,** 1951–1959.

McLaughlin SB, Adams Kszos L. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass and Bioenergy* **28,** 515–535.

Mei C, Flinn BS. 2010. The use of beneficial microbial endophytes for plant biomass and stress tolerance improvement. *Recent Patent Biotechnology* **4,** 81–95.

Mitter B, Petric A, Chain P, Trognitz F, Nowak J, Compant S, Sessitsch A. 2013*a*. Genome analysis, ecology, and plant growth promotion of the endophyte *Burkholderia phytofirmans* strain PsJN. In: de Bruijn FJ, ed. *Molecular microbial ecology of the rhizosphere* , **Vols 1 and 2**. Hoboken, NJ: John Wiley & Sons, Inc., 865–874

Mitter B, Petric A, Shin MV, Chain PSG, Hauberg-Lotte L, Reinhold-Hurek B, Nowak J, Sessitsch A. 2013*b*. Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Frontiers in Plant Science* **4,** 1–15.

Nambara E, Marion-Poll A. 2005. Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56,** 165–185.

Nowak J, Asiedu S, Bensalim S, Richards J, Stewart A, Smith C, Stevens D, Sturz A. 1998. From laboratory to applications: challenges and progress with *in vitro* dual cultures of potato and beneficial bacteria. *Plant Cell, Tissue and Organ Culture* **52,** 97–103.

Nowak J, Sharma VK, A'Hearn E. 2004. Endophyte enhancement of transplant performance in tomato, cucumber and sweet pepper. *Acta Horticulturae (ISHS)* **631,** 253–263.

Nowak J, Shulaev V. 2003. Priming for transplant stress resistance in *in vitro* propagation. *In Vitro Cellular and Developmental Biology-Plant* **39,** 107–124.

Oldroyd GE. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* **11,** 252–263.

Onofre-Lemus J, Hernández-Lucas I, Girard L, Caballero-Mellado J. 2009. ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species, and its growth-promoting effect on tomato plants. *Applied and Environmental Microbiology* **75,** 6581–6590.

Parrish DJ, Fike JH. 2005. The biology and agronomy of switchgrass for biofuels. *Critical Reviews in Plant Sciences* **24,** 423–459.

Passardi F, Cosio C, Penel C, Dunand C. 2005. Peroxidases have more functions than a Swiss army knife. *Plant Cell Reports* **24,** 255–265.

Pillay V, Nowak J. 1997. Inoculum density, temperature, and genotype effects on *in vitro* growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a pseudomonad bacterium. *Canadian Journal of Microbiology* **43,** 354–361.

Poupin MJ, Timmermann T, Vega A, Zuñiga A, González B. 2013. Effects of the plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN throughout the life cycle of *Arabidopsis thaliana*. *PLoS One* **8,** e69435.

Raskin I. 1992. Role of salicylic acid in plants. *Annual Review of Plant Biology* **43,** 439–463.

Ross CA, Liu Y, Shen QJ. 2007. The WRKY gene family in rice (*Oryza sativa*). *Journal of Integrative Plant Biology* **49,** 827–842.

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. 2007. Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters* **278,** 1–9.

Santner A, Estelle M. 2009. Recent advances and emerging trends in plant hormone signalling. *Nature* **459,** 1071–1078.

Schrick K, Mayer U, Horrichs A, Kuhnt C, Bellini C, Dangl J, Jürgens G. 2000. FACKEL is a sterol C-14 reductase required for organized cell division and expansion in Arabidopsis embryogenesis. *Genes and Development* **14,** 1471–1484.

Segonzac C, Zipfel C. 2011. Activation of plant pattern-recognition receptors by bacteria. *Current Opinion in Microbiology* **14,** 54–61.

Seo HS, Song JT, Cheong JJ, *Lee Y-H, Lee Y-W, Hwang I, Lee JS Choi YD*. 2001. Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. *Proceedings of the National Academy of Sciences, USA* **98,** 4788–4793.

Sessitsch A, Coenye T, Sturz A, *et al.* 2005. *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant-beneficial properties. *International Journal of Systematic and Evolutionary Microbiology* **55,** 1187–1192.

Sharma V, Nowak J. 1998. Enhancement of verticillium wilt resistance in tomato transplants by *in vitro* co-culture of seedlings with a plant growth promoting rhizobacterium (*Pseudomonas sp.* strain PsJN). *Canadian Journal of Microbiology* **44,** 528–536.

Sharma P, Bhardwaj R, Arora N, Arora HK, Kumar A. 2008. Effects of 28-homobrassinolide on nickel uptake, protein content and antioxidative defence system in *Brassica juncea*. *Biologia Plantarum* **52,** 767–770.

Singh KB, Foley RC, Oñate-Sánchez L. 2002. Transcription factors in plant defense and stress responses. *Current Opinion in Plant Biology* **5,** 430–436.

Suárez-Moreno ZR, Caballero-Mellado J, Coutinho BG, Mendonça-Previato L, James EK, Venturi V. 2012. Common features of environmental and potentially beneficial plant-associated Burkholderia. *Microbial Ecology* **63,** 249–266.

Sun Y, Cheng Z, Glick BR. 2009. The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN. *FEMS Microbiology Letters* **296,** 131–136.

Tani T, Sobajima H, Okada K, Chujo T, Arimura SI, Tsutsumi, N., Yamane, H. 2008. Identification of the OsOPR7 gene encoding 12-oxophytodienoate reductase involved in the biosynthesis of jasmonic acid in rice. *Planta* **227,** 517–526.

Tena G, Boudsocq M, Sheen J. 2011. Protein kinase signaling networks in plant innate immunity. *Current Opinion in Plant Biology* **14,** 519–529.

Torres MA, Dangl JL. 2005. Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Current Opinion in Plant Biology* **8,** 397–403.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012. Primer3—new capabilities and interfaces. *Nucleic Acids Research* **40,** e115.

Usadel B, Nagel A, Thimm O, Redestig H, Blaesing OE, Palacios-Rojas N, Selbig J, Hannemann J, Piques MC, Steinhauser D. 2005. Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of coresponding genes, and comparison with known responses. *Plant Physiology* **138,** 1195–1204.

Vadassery J, Oelmüller R. 2009. Calcium signaling in pathogenic and beneficial plant microbe interactions: what can we learn from the interaction between *Piriformospora indica* and *Arabidopsis thaliana*. *Plant Signaling and Behavior* **4,** 1024.

Van Damme M, Huibers RP, Elberse J, Van den Ackerveken G. 2008. Arabidopsis DMR6 encodes a putative 2OG-Fe (II) oxygenase that is defense-associated but required for susceptibility to downy mildew. *The Plant Journal* **54,** 785–793.

van der Hoorn RA. 2008. Plant proteases: from phenotypes to molecular mechanisms. *Annual Review of Plant Biology* **59,** 191–223.

van der Hoorn RA, Jones JD. 2004. The plant proteolytic machinery and its role in defence. *Current Opinion in Plant Biology* **7,** 400–407.

van Loon LC, Bakker PA, van der Heijdt WH, Wendehenne D, Pugin A. 2008. Early responses of tobacco suspension cells to rhizobacterial elicitors of induced systemic resistance. *Molecular Plant-Microbe Interactions* **21,** 1609–1621.

Vlot AC, Dempsey DMA, Klessig DF. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology* **47,** 177–206.

Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X. 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology* **17,** 1784–1790.

Wang RK, Wang CE, Fei YY, Gai JY, Zhao TJ. 2013. Genome-wide identification and transcription analysis of soybean carotenoid oxygenase genes during abiotic stress treatments. *Molecular Biology Reports* **40,** 4737–4745.

Wang X, Liu T, Li C, Zhao Z. 2012. Gene expression profiles in maize (*Zea mays* L.) leaves inoculation with southern corn rust (Puccinia polysora Underw.). *Acta Physiologiae Plantarum* **34,** 997–1006.

Weilharter A, Mitter B, Shin MV, Chain PSG, Nowak J, Sessitsch A. 2011. Complete genome sequence of the plant growth-promoting endophyte *Burkholderia phytofirmans* strain PsJN. *Journal of Bacteriology* **193,** 3383–3384.

Wright L, Turhollow A. 2010. Switchgrass selection as a 'model' bioenergy crop: a history of the process. *Biomass and Bioenergy* **34,** 851–868.

Xia Y, Greissworth E, Mucci C, Williams MA, DeBolt S. 2012. Characterization of culturable bacterial endophytes of switchgrass (*Panicum virgatum* L.) and their capacity to effect plant growth. *GCB Bioenergy* **5,** 674–682.

Yee D, Goring DR. 2009. The diversity of plant U-box E3 ubiquitin ligases: from upstream activators to downstream target substrates. *Journal of Experimental Botany* **60,** 1109–1121.

Zhang JY, Lee YC, Torres-Jerez I, Wang M, Yin Y, Chou WC, Udvardi MK. 2013. Development of an integrated transcript sequence database and a gene expression atlas for gene discovery and analysis in switchgrass (*Panicum virgatum* L.). *The Plant Journal* **74,** 160–173.

Zhao S, Qi X. 2008. Signaling in plant disease resistance and symbiosis. *Journal of Integrative Plant Biology* **50,** 799–807.