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Metabolic Response of Downy Mildew Resistant and Susceptible Maize Genotypes to *Peronosclerospora sorghi*

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The metabolic response of downy mildew resistant (MAI 756) and susceptible (CM 500) maize (*Zea mays* L.) genotypes to *Peronosclerospora sorghi* (Weston & Uppal) Shaw, the causal fungus of maize downy mildew was assessed 0, 24, 48 and 72 h after inoculation by using gas chromatography coupled to electron impact ionization-time of flight-mass spectrometry (GC/EI-TOF-MS). A total of 95 metabolites were identified and annotated using the Golm Metabolome Database and Tag Finder software. Among them, 19 metabolites were up-regulated and unique to MAI 756, 4 up-regulated metabolites were unique to CM 500 and 72 metabolites were common to both the genotypes. Infection by *P. sorghi* substantially alters the plant metabolic profile, including significant changes in sugars, amino acids and phenylpropanoids. Up-regulation of DL-ornithine, *cis*-aconitic acid, citric acid, phthalic acid, cellobiose and xylopyranose were detected in the resistant maize genotype 24 h after inoculation, whereas N-acetyl-L-lysine, L-threonic acid, 1-cyclohexene-1-carboxylic acid and cinnamic acid have been detected 48 h after inoculation. The metabolites *viz*., erythro-pentonic acid and octadecenoic acid were up-regulated in the susceptible genotype 48 h after inoculation. This investigation provides new insight into the mechanisms of resistance in maize to *P. sorghi* at the metabolite level.

Key words: Maize, *Zea mays*, downy mildew, *Peronosclerospora sorghi*, metabolomics.

Downy mildew (DM), caused by *Peronosclerospora sorghi* (Weston & Uppal) Shaw, is an economically important disease of maize (*Zea mays* L.) worldwide. This disease can occur at any stage of maize development from seedling to harvest, though it primarily infects soon after seedling emergence, until one month after planting. *P. sorghi* can cause significant yield losses under favourable environmental conditions and yield losses can reach 50-100% in susceptible cultivars (Lukman *et al*., 2013). Two pathotypes of *P. sorghi*, one capable of infecting both maize and sorghum and the other specific to maize have been reported (Anaso *et al*., 1987; Olanya and Fajemisin, 1993). Management of this disease primarily depends on seed treatment with systemic fungicide, metalaxyl (Anahosur and Patil, 1980). However, development of fungicide resistance within populations of *P. sorghi* has been reported (Isakeit and Jaster, 2005). Few resistant genotypes have been identified against this disease (Yen *et al*., 2004; Rashid *et al*., 2013). It has been well documented that during early phases of infection by plant pathogens a series of physiological and biochemical changes, together with the molecular response occur in plants. Among them metabolic alteration is a common response in both compatible and incompatible plant-pathogen interactions (Fiehn *et al*., 2000). The disease resistance or susceptibility depends on physiology of plants differing in their metabolic activities (Hamzehzarghani *et al*., 2005; Paranidharan *et al*., 2008). Hence comparing metabolic response of

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resistant and susceptible genotypes upon infection by pathogens possibly represents a powerful tool to unravel the biochemical pathways involved in plant defense. It is possible that the cultivar of maize differing in their resistance to *P. sorghi* also would produce certain metabolites to resist pathogen invasion. Such metabolites can be used as biomarkers or linked to genomic locations on chromosomes and used in the breeding programmes. In this study, we examined the differences in metabolites between downy mildew resistant (MAI 756) and susceptible (CM 500) maize plants infected with *P. sorghi* to understand the metabolic response of maize against the downy mildew pathogen.

MATERIALS AND METHODS

Greenhouse studies

Seeds of downy mildew susceptible (CM 500) and resistant (MAI 756) maize genotypes obtained from the Indian Institute of Maize Research, New Delhi, were sown in 30 cm diameter pots filled with sterilized maize field soil (clay loam with a pH of 7.5) at the rate of 5 seeds per pot and the plants were grown in a growth chamber maintained at 18°C-20°C temperature and >90% relative humidity.

For the preparation of inoculum of *P. sorghi*, maize leaves (CM 500) showing symptoms of downy mildew were collected from the experimental farms of Tamil Nadu Agricultural University, Coimbatore, India and cut into 4-5 cm lengths and placed with their abaxial side facing up in 9 cm diameter Petri dishes lined with wet filter paper on both the sides. The plates were incubated in the dark for 6-7 h at 20°C for sporulation (Narayana *et al*., 1995). Conidia were harvested from the surface of leaves by gently washing them into cold distilled water using a camel hair brush. The concentration of conidia was adjusted to $1x10^8$conidia mL^{-1}. The wetting agent, Tween 20 (0.02%) was mixed with the conidial suspension before inoculation. The seedlings at two-leaf stage were spray inoculated with conidial suspension using a hand-held sprayer until run-off. The inoculated plants were incubated in a growth chamber at 20°C and >90% RH. Mock treated plants (sprayed with 0.02% Tween 20) and uninoculated plants (control plants) were maintained separately under the same growing conditions.

Sampling, metabolite extraction and derivatization

Leaf tissue samples from inoculated and control plants of each maize genotype were collected at 0, 24, 48 and 72 h after inoculation, frozen in liquid nitrogen and stored at -70°C until metabolite extraction. Five replicates were maintained for each time point. Polar primary metabolites were extracted from the leaf tissues and then subjected to derivatization by following the procedure described by Erban *et al*. (2007). Maize leaf samples were ground to a fine powder using a pre-chilled mortar and pestle. The leaf powder (120 mg) was transferred to 2 ml round bottom microcentrifuge tubes and 1.4 ml of 100% methanol and 60 µl of internal standard ribitol were added and mixed well by vortexing. The mixtures were incubated at 70°C for 10 min on a shaker at 150 rpm and then centrifuged at 11,000 xg for 10 min. To the supernatant, 750 µl of chloroform and 1.4 ml of double distilled water were added and vortexed for uniform mixing. The mixture was centrifuged at 2,200 xg for 15 min and 1 ml of the supernatant was transferred to 1.5 ml of micro centrifuge tube and concentrated in a vacuum concentrator. The dried samples were then stored at -70°C until derivatization. For derivatization, 300 µl of methoxyamination reagent (5 mg of 4-dimethylaminopyridine and 40 mg of methoxyamine hydrochloride dissolved in 1 ml of pyridine) was added to the microcentrifuge tube and shaken at 37°C for 2 h. The sample was vortexed every 30 min during 2 h of shaking to dissolve the sample completely in the reagent. After methoxyamination, the samples were centrifuged at 10,000 xg for 1 min and 40 µl of the supernatant was transferred to 1.5 ml microcentrifuge tube and 70 µl of *N, O*-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) and 10 µl of retention time standards (*n*-alkanes) were added to the sample and shaken at 37°C for 30 min. The *n*-alkanes mixture was prepared by using pyridine at a final concentration of 0.22 mg/ml each. The samples were then centrifuged at 10,000 xg for 1 min and 95 µl of the supernatant was transferred to GC-MS glass vial for analysis using GC-MS.

Gas chromatography and mass spectrometry (GC-MS) analysis

The sample was injected into Thermo Scientific TRACE Gas Chromatograph with Thermo MS DSQ II Operator and TR5MS column (30 m x 0.25 mm, 0.25 µm film thickness). The temperature was programmed at 30°C (1 min), 230°C (20 min) at the rate of 4°C per minute. Detector was heated at 250°C, injector at 230°C. Helium was used as carrier gas at 5 Psi pressure. Mass spectra were obtained by electron ionization at 70 eV (Belal *et al*., 2013).

Metabolite Identification

The metabolites were tentatively identified with the top five spectral hits with the NIST-05 (National Institute of Standards and Technology, Palisade Corp., NY) and Golm Metablome Database (GMD) (Kopka *et al*., 2005) libraries, using retention times as reference. For each peak, the spectra of four replicates of a treatment were compared and matched with that of NIST/GMD spectra. Analytes with good match factor (>60%) were considered and tentatively identified.

RESULTS AND DISCUSSION

In the present study, the metabolic response of downy mildew resistant (MAI 756) and susceptible (CM 500) maize genotypes to *P. sorghi* was assessed 0, 24, 48 and 72 h after inoculation by using gas chromatography coupled to electron impact ionization-time of flight-mass spectrometry (GC/EI-TOF-MS) (Fig. 1). A total of 95 metabolites were identified and annotated using the Golm Metabolome Database and Tag Finder software (Data not shown). Among them, 19 metabolites were up-regulated and unique to MAI 756 (Table 1), 4 up-regulated metabolites were unique to CM 500 (Table 2), and 72 metabolites were common to both cultivars. Infection by *P. sorghi* substantially altered the plant metabolic profile, including significant changes in sugars, amino acids and phenylpropanoids. In the resistant genotype, DL-ornithine, *cis*-aconitic acid, citric acid, phthalic acid, cellobiose and xylopyranose were detected 24 h after inoculation, whereas N-acetyl-L-lysine, L-threonic acid, 1-cyclohexene-1-carboxylic acid and cinnamic acid were detected 48 h after inoculation (Table 3).

Table 1. Metabolites up-regulated in downy mildew resistant maize genotype (MAI 756) after inoculation with *P. sorghi*

Metabolites	Retention Time(RT) (min)	Molecular weight	Molecular Formula	Peak area	Chemical Group
n-Butylamine	5.18	217	$C_{10}H_{27}NSi_2$	25641938.87	Amines
Geranyl isovalerate	7.47	238	$C_{15}H_{26}O_2$	17962184.97	Unknown
N-acetyl-L-Lysine	7.98	404	$C_{17}H_{40}N_2O_3Si_3$	214049679.23	Amino acids
DL-Ornithine	8.35	348	$C_{14}H_{36}N_2O_2Si_3$	90182123.20	N -Compounds
Benzoic acid	9.43	278	$C_{17}H_{26}O_3$	11967721.24	Organic acids
L-Threonic acid	9.95	424	$C_{16}H_{40}O_5Si_4$	654315968.46	Polyhydroxy acids
Arabitol	11.69	512	$C_{20}H_{52}O_5Si_5$	9800504115.07	Polyols
cis-aconitic acid	12.18	390	$C_{15}H_{30}O_6Si_3$	4817711504.37	Organic acids
Citric acid	12.48	408	$C_{15}H_{32}O_7Si_3$	283880299.91	Organic acids
6-Methoxy-2-benzoxazolinone	12.61	165	$C_8H_7NO_3$	204361391.02	Unknown
1-Cyclohexene-1-carboxylic acid	12.91	462	$C_{19}H_{42}O_5Si_4$	223019247.26	Organic acids
Phthalic acid	13.20	376	$C_{23}H_{36}O_4$	11444674.33	Organic acids
Cinnamic acid	15.64	338	$C_{16}H_{26}O_4Si_2$	79261569.56	Organic acids
Galacturonic acid	18.68	554	$C_{21}H_{50}O_7Si_5$	76592669.08	Polyhydroxy acids
Glucuronolactone	20.26	392	$C_{15}H_{32}O_6Si_3$	141400348.70	Sugars
Cellobiose	22.48	947	$C_{37}H_{89}NO_{11}Si_8$	35415978.72	Sugars
Xylofuranose	23.30	438	$C_{17}H_{42}O_5Si_4$	34936643.36	Sugars
Xylopyranose	25.33	438	$C_{17}H_{42}O_5Si_4$	1990760.53	Sugars
Hexopyranose	26.68	540	$C_{21}H_{52}O_6Si_5$	14665348.69	Sugars

Plants produce thousands of metabolites and they can be broadly grouped into primary and secondary metabolites (Yazdani *et al*., 2011). Several of these metabolites are involved in the defense of plants against abiotic and biotic stresses. These metabolites can occur constitutively in plants or induced following infection by pathogens. Polyamines are small, positively charged aliphatic amines at cellular pH values and therefore bind to negatively charged molecules, including nucleic acids, acidic phospholipids and proteins (Cohen, 1998). Consequently, they modulate DNA-protein and protein-protein interactions (Thomas and Thomas, 2001). Common natural polyamines include putrescine, spermidine and spermine. The amino acid ornithine is converted into putrescine by the enzyme ornithine decarboxylase (ODC) and putrescine is then converted to spermidine and spermine by spermidine synthase and spermine

Table 2. Metabolites up-regulated in downy mildew susceptible maize genotype (CM 500) after inoculation with *P. sorghi*

Metabolites	Retention Time(RT) (min)	Molecular weight	Molecular Formula	Peak area	Chemical Group
2-Azetidinone	9.20	341	$C_{17}H_{35}NO_2Si_2$	25834007.56	lactams
Erythro-Pentonic acid	11.67	438	$C_{17}H_{42}O_5Si_4$	45509890.97	Fatty acids
Octa decenoic acid	21.28	444	$C_{28}H_{44}O_4$	15212426.69	Organic acids
Disiloxane	26.80	162	$C_6H_{18}OSi_2$	4038921.57	Alkanes

Table 3. Time course of induction of metabolites in downy mildew resistant (MAI 756) and susceptible (CM 500) maize genotypes after inoculation with *P. sorghi*

Metabolites	Retention	Susceptible genotype (CM 500)				Resistant genotype (MAI 756)			
	Time (RT)	C	24h	48h	72h	C	24h	48h	72h
n-Butylamine	5.18	-	-	-	-	-	-	-	+
Geranyl isovalerate	7.47	-	-	-	-	-	-	-	+
N-Acetyl-L-Lysine	7.98	-	-	-	-	-	-	+	+
DL-Ornithine	8.35	-	-	-	-	-	+	+	+
2-Azetidinone	9.20	-	-	-	+	-	-	-	-
Benzoic acid	9.43	-	-	-	-	-	-	-	+
L-Threonic acid	9.95	-	-	-	-	-	-	+	+
Erythro-Pentonic acid	11.67	-	-	+	+	-	-	-	-
Arabitol	11.69	-	-	-	-	-	-	-	+
cis-aconitic acid	12.18	-	-	-	-	-	+	+	+
Citric acid	12.48	-	-	-	-	-	+	+	+
6-Methoxy-2-benzoxazolinone	12.61	-	-	-	-	-	-	-	+
1-Cyclohexene-1-carboxylic acid	12.91	-	-	-	-	-	-	+	+
Phthalic acid	13.20	-	-	-	-	-	+	+	+
Cinnamic acid	15.64	-	-	-	-	-	-	+	+
Galacturonic acid	18.68	-	-	-	-	-	-	-	+
Glucuronolactone	20.26	-	-	-	-	-	-	-	+
Octa decenoic acid	21.28	-	-	+	+	-	-	-	-
Cellobiose	22.48	-	-	-	-	-	+	+	+
Xylofuranose	23.30	-	-	-	-	-	-	-	+
Xylopyranose	25.33	-	-	-	-	-	+	+	+
Hexopyranose	26.68	-	-	-	-	-	-	-	+
Disiloxane	26.80	-	-	-	+	-	-	-	-

'+' present; '-' absent

synthase respectively (Kumar *et al*., 1997). Alterations in the level of polyamines have been reported during plant microbe interactions (Walters, 2000). Increase in the activities of ODC and arginine decarboxylase and elevated concentrations of their products and conjugates during hypersensitive reaction in tobacco plants infected with Tobacco mosaic virus (TMV) have been reported (Negrel *et al*., 1984; Yoda *et al.,* 2003). Marini *et al*. (2001) observed accumulation of polyamines in tobacco cultivars resistant to TMV, but not in TMV-susceptible counterparts. It has been reported that spermine accumulates in intercellular spaces and induces pathogenesis-related proteins in tobacco during hypersensitive response (Yamakawa *et al*., 1998). Yoda *et al.* (2003) reported that polyamines are critical components in induction of hypersensitive cell death during pathogen attack in tobacco plants, and that this is mainly based on production of hydrogen peroxide through their degradation by polyamine oxidase (Yoda *et al*., 2003). The up-regulation of ornithine in the resistant maize genotype upon inoculation with *P. sorghi* suggests their possible involvement in plant defense.

The activation of plant defense mechanisms and growth promotion requires an increased energy supply that eventually must come from photosynthesis, and probably accompanied by greater respiratory rates (Bolton *et al*., 2008; Shoresh *et al*., 2010). The tricarboxylic acid cycle (TCA cycle) is a central hub in plant metabolism, giving rise to many primary and secondary metabolites including several intermediates involved in amino acid biosynthesis and nitrogen assimilation (Foyer *et al*., 2011). Shoresh and Harman (2008) reported that the most commonly altered differential proteins in

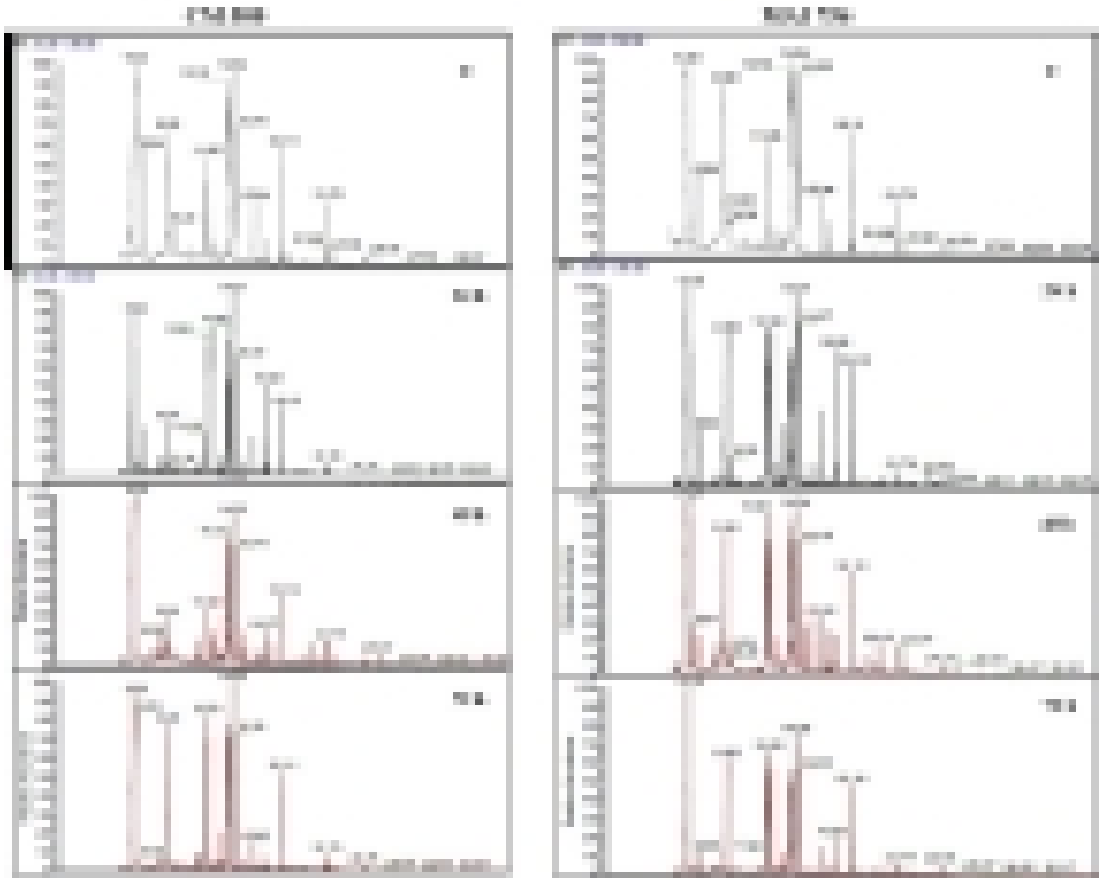

Fig. 1. Total ion chromatogram of downy mildew resistant (MAI 756) and susceptible (CM 500) maize genotypes sampled at different time intervals after inoculation with *P. sorghi*

Trichoderma harzianum inoculated maize plants were those involved in carbohydrate metabolism, especially those in the glycolytic, TCA or respiratory pathways. Transcript and metabolite analysis of the *Trichoderma* induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana* revealed significant changes in amino acids, polyamines, sugars and citric acid cycle intermediates (Brotman *et al*., 2012). The results of the present study indicate that the metabolites belonging to TCA cycle such as citric acid and *cis*-aconitic acid were up-regulated in the inoculated leaves of downy mildew resistant maize genotype (Table 3). The induced metabolites belonging to the TCA cycle might have contributed for an increased energy supply required for the activation of plant defences in the resistant maize genotype. However, Cagirgan *et al*. (2011) reported that the endogenous concentrations of citric and oxalic acid were lower in chickpea genotypes resistant to *Ascochyta* blight, caused by *Ascochyta rabiei* than the susceptible genotype and malic acid was higher in the resistant genotypes than the susceptible one. Phthalic acid is known to induce oxidative stress and alter the activity of some antioxidant enzymes in roots of apple (Bai *et al*., 2009). Ju and Han (2002) reported that phthalic acid at high-concentration exhibited allelopathic inhibitory effects on the growth of soybean root rot fungus. The up-regulation of phthalic acid may also account for resistance of MAI 756 to downy mildew.

Plant cell wall modifications are known to induce plant defense reactions even prior to pathogen attack and reduce pathogen penetration and growth (Underwood, 2012). β- 1, 4-linked xylopyranose is the principal component of plant cell wall hemicellulose, which is the most abundant polysaccharide found in the nature (Subramaniyan and Prema, 2002). In cell wall of cereals, phenolic acids form complex with hemicelluloses and form FAXX, *O*-[5-*O*-(*trans*-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose. The FAXX is subjected to the formation of covalent cross-links by extracellular peroxidase of the plant cell wall (Eraso and Hartley, 1990). The diferulate cross-links are known to strengthen the cell wall and provide structural resistance to invading fungi (Assabgui *et al*., 1993). The up-regulation of xylopyranose indicates an important contribution of this compound in strengthening of cell wall and in resistance of MAI 756 to *P. sorghi*.

Plant secondary metabolites play important roles in plant defense and chemical signalling (Inderjit and Weston, 2003). Phenolic compounds are one of the most important groups of secondary metabolites produced by plants (Michalak, 2006). They are mainly synthesized from cinnamic acid, which is formed from phenylalanine by the action of the enzyme phenylalanine ammonia- lyase (Dixon and Paiva, 1995; Michalak, 2006). In this study, upregulation of cinnamic acid in the resistant genotype was observed 48 h after inoculation with *P. sorghi*. The induction of cinnamic acid in the resistant genotype suggests that this could be involved in resistance of maize plant against *P. sorghi*.

Octadecenoic acid was found only in downy mildew susceptible *plants* inoculated with *P. sorghi* (Table 2). Octadecanoic acid pathway produces signal molecules with vital roles in regulating secondary pathways (Blechert *et al*., 1995). Jasmonic acid plays a key role in inducing defense responses to microbial pathogens (Reinbothe *et al*., 1994; Yang *et al*., 1997) and exogenous addition of JA can increase the resistance of plants to pathogens (Jayaraj *et al*., 2004). JA is synthesized from linolenic acid via the octadecanoid (oxylipin) pathway (Hamberg and Gardner, 1992). Linolenic acid can also be converted to epoxy fatty acids by peroxygenase. One of these epoxy fatty acids, cis-12, 13-epoxy-9(*Z*)-octadecenoic acid, has been shown to be a strong inhibitor of allene oxide cyclase (Ziegler *et al*., 1997), an enzyme necessary for the conversion of linolenic acid to JA. Therefore, the octadecenoic acid might have rendered the host susceptible to infection by *P. sorghi* by suppression of JA synthesis. The present investigation provides new insight into the mechanisms of resistance to *P. sorghi* in maize at the metabolite level and indicates a large number of potential metabolic targets for further in depth investigations.

To our knowledge this study represents the first comprehensive analysis of metabolic changes in maize during compatible and incompatible interactions with *P. sorghi.*

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REFERENCES

1. Lukman, R., Afifuddin, A. and Lubberstedt, T. Unraveling the genetic diversity of maize downy mildew in Indonesia. *J. Plant Pathol. Microbiol.,* 2013; **4**: 162 doi:10.4172/2157-7471.1000162.
2. Anaso, A. B., Tyagi, P. D., Emechebe, A. M. and Manzo, S. K. Identity of a downy mildew in Nigeria Guinea savannah. *Samaru J. agric. Res.,* 1987; **5**:13-22.
3. Olanya, O. M. and Fajemisin, J. M. Host range and survival studies of *Peronosclerospora sorghi* at two locations in Nigeria. *Int. J. Pest Manage.,* 1993; **39**: 13-18.
4. Anahosur, K. H. and Patil, S. H. Chemical control of sorghum downy mildew in India. *Plant Dis.,* 1980; **64**: 1004-1006.
5. Isakeit, T. and Jaster, J. Texas has a new pathotype of *Peronosclerospora sorghi*, the cause of sorghum downy mildew. *Plant Dis.,* 2005; **89**: 529.
6. Yen, T. T. O., Prasanna, B. M., Setty, T. A. S. and Rathore, R. S. Genetic variability for resistance to sorghum downy mildew (*Peronosclerospora sorghi*) and Rajasthan downy mildew (*P. heteropogoni*) in the tropical/ sub-tropical Asian maize germplasm. *Euphytica,* 2004; **138**: 23-31.
7. Rashid, Z., Zaidi, P. H., Vinavan, M. T., Sharma, S. S. and Srirama Setty, T. A. Downy mildew resistance in maize (*Zea mays* L.) across *Peronosclerospora* species in lowland tropical Asia. *Crop Prot.,* 2013; **43**: 183-191.
8. Fiehn, O., Kopka, J., Dormann, P., Altmann, T., Trethewey, R. N. and Willmitzer, L. Metabolic profiling for plant functional genomics. *Nat. Biotechnol.,* 2000; **18**: 1157–1161.
9. Hamzehzarghani, H., Kushalappa, A. C., Dion, Y., Rioux, S., Comeau, A., Yaylayan, V., Marshall, W. D. and Mather, D. E. Metabolic profiling and factor analysis to discriminate quantitative resistance in wheat cultivars against *Fusarium* head blight. *Physiol. Mol. Plant P.,* 2005; **66**: 119–133.
10. Paranidharan, V., Abu-Nada, Y., Hamzehzarghani, H., Kushalappa, A. C., Mamer, O., Dion, Y., Rioux, S., Comeau, A. and Choiniere, L. Resistance-related metabolites in wheat against *Fusarium graminearum* and the virulence factor deoxynivalenol (DON). *Botany,* 2008; **86**: 1168-1179.
11. Narayana, Y. D., Mughogho, L. K. and Bandyopadhyay, R. Evaluation of greenhouse inoculation techniques to screen sorghum for resistance to downy mildew. *Euphytica,* 1995; **86**: 49-53.
12. Erban, A., Schauer, N., Fernie, A. R. and Kopka, J. Non supervised construction and application of mass spectral and retention time index libraries from time-of-flight gas chromatography-mass spectrometry metabolite profiles. *Methods Mol. Biol.,* 2007; **358**: 19-38.
13. Belal, E. B., Kamel, S. M. H. and Hassan, M. M. Production of antimicrobial metabolites by *Bacillus subtilis* and their applications. *Asian Network for Scientific Information,* 2013; **12**: 14-24.
14. Kopka, J., Schauer, N., Krueger, S., Birkemeyer, C., Usadel, B., Bergmuller, E., Dormann, P., Weckwerth, W., Gibon, Y., Stitt, M., Willmitzer, L., Fernie, A. R. and Steinhauser, D. GMD@CSBDB: The Golm Metabolome Database. *Bioinformatics,* 2005; **21**: 163-538.
15. Yazdani, D., Tan, Y. H., Zainal Abidin, M. A. and Jaganath, I. B. A review on bioactive compounds isolated from plants against plant pathogenic fungi. *J. Med. Plants Res.,* 2011; **5**: 6584-6589.
16. Cohen, S. S. A guide to the polyamines. Oxford University Press, Oxford, UK. 1998.
17. Thomas, T. and Thomas, T. J. Polyamine in cell growth and cell death: molecular mechanisms and therapeutic applications. *Cell. Mol. Life Sci.,* 2001; **58**: 244-258.
18. Kumar, A., Altabella, T., Taylor, M. A. and Tiburcio, A. F. Recent advances in polyamine research. *Trends Plant Sci.,* 1997; **2**: 124-130.
19. Walters, D. R. Polyamine in plant-microbe interactions. *Physiol. Mol. Plant P.,* 2000; **57**: 137-146.
20. Negrel, J., Vallee, J. C. and Martin, C. Ornithine decarboxylase activity and the hypersensitive reaction of tobacco to tobacco mosaic virus in *Nicotiana tabacum*. *Phytochemistry,* 1984; **23**: 2747–2751.
21. Yoda, H., Yamaguchi, Y. and Sano, H. Induction of hypersensitive cell death by hydrogen peroxide produced through polyamine degradation in tobacco plants. *Plant Physiol.,* 2003; **132**: 1973-1981.
22. Marini, F., Betti, L., Scaramagli, S., Biondi, S. and Torrigiani, P. Polyamine metabolism is upregulated in response to tobacco mosaic virus in hypersensitive but not in susceptible tobacco. *New Phytol.,* 2001; **149**: 301-309.

23. Yamakawa, H., Kamada, H., Satoh, M. and Ohashi, Y. Spermine is a salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. *Plant Physiol.*, 1998; **118**: 1213–1222.
24. Bolton, M. D., Kolmer, J. A., Xu, W. W. and Garvin, D. F. Lr34- mediated leaf rust resistance in wheat: transcript profiling reveals a high energetic demand supported by transient recruitment of multiple metabolic pathways. *Mol. Plant Microbe Interact.*, 2008; **21**: 1515–1527.
25. Shoresh, M., Harman, G. E. and Mastouri, F. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol.*, 2010; **48**: 21–43.
26. Foyer, C. H., Noctor G and Hodges M Targeting mitochondria-associated metabolism as a means to enhance nitrogen use efficiency. *J. Exp. Bot.*, 2011; **62**: 1467-1482.
27. Shoresh, M. and Harman, G. E. The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. *Plant Physiol.*, 2008; **147**: 2147–2163.
28. Brotman, Y., Lisec, J., Meret, M., Chet, I., Willmitzer, L. and Viterbo, A. Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. *Microbiology*, 2012; **158**: 139–146.
29. Cagirgan, M. I., Toker, C., Karhan, M., Aksu, M., Ulger, S. and Canci, H. Assessment of endogenous organic acid levels in *Ascochyta* blight [*Ascochyta Rabiei* (Pass.) Labr.] susceptible and resistant chickpeas (*Cicer arietinum* L.). *Turkish J. of Field Crops*, 2011; **16**: 121-124.
30. Bai, R., Ma, F., Liang, D. and Zhao, X. Phthalic acid induces oxidative stress and alters the activity of some antioxidant enzymes in roots of *Malus prunifolia*. *J. Chem. Ecol.*, 2009; **35**: 488-494.
31. Ju, H. Y. and Han, L. M. Influence of root exudations on pathogenic fungi produced by root rot and seed germination in continuous cropping soybean. *J. Jilin Agric. Uni.*, 2002; **24**: 45-49.
32. Underwood, W. The plant cell wall: a dynamic barrier against pathogen invasion. *Front. Plant. Sci.*, 2012; **3**: 85.
33. Subramaniyan, S. and Prema, P. Biotechnology of microbial xylanases: enzymology, molecular biology and application. *Crit. Rev. Biotechnol.*, 2002; **22**: 33-64.
34. Eraso, F. and Hartley, R. D. Monomeric and dimeric phenolic constituents of plant cell walls. *J. Sci. Food Agric.*, 1990; **51**: 163-170.
35. Assabgui, R. A., Reid, L. M., Hamilton, R. I. and Arnason, J. T. Correlation of kernel (E)-ferulic acid content of maize with resistance to *Fusarium graminearum*. *Phytopathology*, 1993; **83**: 949-953.
36. Inderjit and Weston, I. A. Root exudation: an overview, In: Root Ecology, Ed. De Kroon and Visser EJW), Springer Verlag, Heidelberg, Germany, 2003; 235–251pp.
37. Michalak, A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol. J. Environ. Stud.*, 2006; **15**: 523-530.
38. Dixon, R. A. and Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell*, 1995; **7**: 1085–1097.
39. Blechert, S., Brodschelm, H., Holder, S., Kammerer, L., Kutchan, T. M. and Mueller, M. J. The Octa decanoic pathway: signal molecules for the regulation of secondary pathways. *Proc. Natl. Acad. Sci.*, 1995; **92**: 4099–4105.
40. Reinbothe, S., Mollenhauer, B. *and* Reinbothe, C. JIPs and RIPs: the regulation of plant gene expression by jasmonates in response to environmental cues and pathogens. *Plant Cell*, 1994; **6**: 1197–1209.
41. Yang, Y., Shah, J. *and* Klessing, D. F. Signalling during plant–pathogen interactions. *Gene Dev.*, 1997; **11***:* 1621–1639.
42. Jayaraj, J., Muthukrishnan, S., Liang, G. H. and Velazhahan, R. Jasmonic acid and salicylic acid induce accumulation of β-1, 3-glucanase and thaumatin-like proteins in wheat and enhance resistance against *Stagonospora nodorum*. *Biol. Plantarum*, 2004; **48**: 425-430.
43. Hamberg, M. *and* Gardner, H. W. Oxylipin pathway to jasmonates: biochemistry and biological significance. *J. Cell Biol.*, 1992; **1165***:* 1–18.
44. Ziegler, J., Hamberg, M., Miersch, O. *and* Parthier, B.. Purification and characterization of allene oxide cyclase from dry corn seeds. *Plant Physiol*., 1997; **114***:* 565–573.