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INTRODUCTION

- Banana Bunchy Top caused by Banana Bunchy Top Virus (BBTV) is one of the most destructive viral diseases of banana. However, little is known about the molecular basis of symptom development, defense mechanism and interactions in banana against this virus.
- To gain a more global perspective on how viruses alter protein profile of the host, 2D gel electrophoresis and other proteomic methods are being applied.
- Despite advances in the identification of changes in the expression of plant genes in response to the infection of RNA viruses, similar studies have not been conducted, with ssDNA viruses.
- Thus, the characterization of virally induced changes in protein profile will provide valuable insight into the host's response towards viral activity and in the development of pathogen specific host biomarker.

MATERIALS AND METHODS

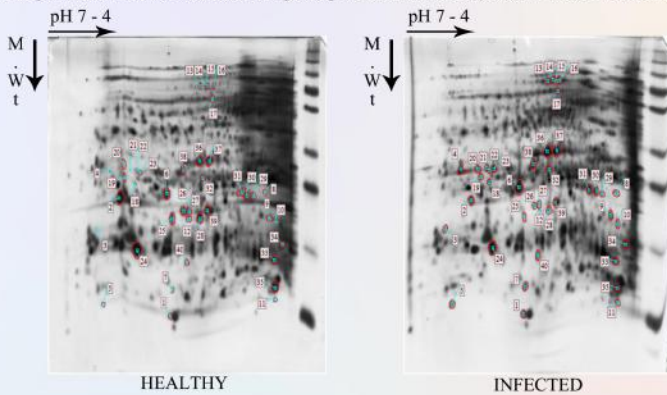
- Plant material - Cigar leaf of BBTV infected and healthy Virupakshi.
- Protein sample preparation - Phenol Ammonium Acetate Method (Carpentier et al, 2005).
- Protein quantification - Bradford method.
- 2DE analysis - 1st dimension - pH 4-7, 13 cm immobilized pH gradient (IPG) strips.
- Focusing - 30 min at 500 V, 30 min at 1000 V, 11 h at 3000 V.
- 2nd dimension - 12% Polyacrylamide SDS-PAGE gel.
- Gel scanning and image analysis - Transmission-light densitometer - Image Scanner.
- GE Healthcare. Image Master Platinum version 6.0 software (GE Healthcare).
- Database query - MASCOT (<http://www.matrixscience.com>).
- Annotation - Gene ontology (<http://www.amigo.geneontology.org>).

RESULTS AND DISCUSSION

- The differentially expressed proteins in leaves of BBTV infected cv. Virupakshi (hill banana) were identified by proteomic approach.
- A total of \approx 1100 reproducible spots were identified in each of the healthy and infected hill banana plants (Fig.1).
- Eighty protein spots that showed two-fold difference in intensity were selected for Peptide Mass Fingerprinting.
- Of those, 71 proteins identified by PMF, 41 were upregulated and 30 got downregulated in the BBTV-infected samples (Table 1).
- These proteins were found to be involved in defence, signal transduction, cell structure and function, photosynthesis and energy, plant growth, protein designation/storage and transcription/translation. (Fig. 3)
- Gene expression analysis was performed for 25 genes selected from different functional categories by Semi Quantitative PCR corroborated the proteomic results (Fig.2).
- Mitogen-activated protein kinase, Calmodulin motif containing protein, ABC Transporter, Cinnamyl alcohol dehydrogenase, Cinnamoyl-CoA reductase 1 and Feruloyl CoA ortho-hydroxylase 2 were upregulated upon infection and can be exploited as disease biomarkers.

Fig.1 Representative Silver-stained 2-DE gel of proteins from healthy and infected hill banana shoot

Table 1 Proteins identified from hill banana leaf by MALDI-TOF



Spot no	HHB1	HBI	Ig/Down	Score	Peptide match	Seq. Cnt.	Theoretical MW, pI	Expt. MW, pI	Protein
1	1.182	2.927	2.476	37	5/13	34	19.4/5.78	18.5.5	Oxidative stress 3 (OSX3) [Arabidopsis thaliana]
2	10.488	3.478	6.332	48	8/24	40	34.9/5.59	34.6.1	Putative LRR receptor-like serine/threonine-protein kinase [Argemone mexicana]
3	0.8841	1.501	17.844	46	9/53	40	23.38/5.2	29.6.5	Ras-related protein RAB11b OS=Arabidopsis thaliana GN=RAB11b PE=2 SV=1
4	0.815	6.876	454.41	32	7/43	21	43.3/6.3	43.6.3	Mitogen-activated protein kinase homolog NTF3 OS=Nicotiana glauca GN=NTF3 PE=1 SV=1
5	0.134	1.822	13.588	34	5/52	32	20/0.7.6	20.6.5	Ribulose biphosphate carboxylase small chain SSU5A, chloroplastic OS=Lemna gibba GN=SSU5A PE=3 SV=1
6	5.765	7.654	1.328	46	22/48	37	67.6/5.9	36.5/5.5	PREDICTED: MORC family CW-type zinc finger protein 4-like isoform X3 [Setaria italica]
7	0.384	2.555	6.641	35	14/55	62	22.4/5.31	22.5.4	Putative B3 domain-containing protein At4g03160 OS=Arabidopsis thaliana GN=At4g03160 PE=3 SV=1
8	0.431	1.241	2.874	33	4/33	44	7.5/4.56	35.4.5	Metallothionein type 2 [Coffea arabica]
9	0.900	2.701	2.000	66	15/34	30	57.7/7.4	34/4.4	Cytochrome P450
10	0.0663	1.453	230.02	53	18/30	49	35.8/9.7	31/4.2	Oxylase family protein 7 [Arabidopsis thaliana]
11	0.3218	0.958	2.978	36	10/27	86	8.4/10.0	18/4.2	NBS-39.2 [Cucumis melo]
12	1.042	0.029	0.028	30	20/24	49	35.2/5.8	32.5.4	Miscotic anagynic mutant 1, putative (Oryza sativa Japonica Group)
13	0.928	1.316	1.417	72	8/20	14	95.6/7.94	110/5.3	Pentatricopeptide repeat-containing protein Atg2020b-like [Fragaria vesca subsp. vesca]
14	0.013	1.226	89.866	52	11/21	13	139/6.9	110/5.2	ABC protein [Cephus japonicus]
15	1.254	1.097	0.875	52	17/17	26	83.3/7.2	110/5.1	Aukrin-3-like protein [Solomon lycopersicum]
16	1.017	1.593	1.566	56	10/19	31	41.5/5.12	110/5.1	ESP70-binding protein 1-like isoform X2 [Setaria italica]
17	1.159	0.857	0.633	97	9/21	45	35.5/5.18	80/5.2	ATP synthase beta subunit [Physalis sp. P139]
18	0.231	0.946	4.093	56	8/20	34	35.26/9.2	38/6.0	Serine/threonine-protein kinase Auerka-1-like [Solomon lycopersicum]
19	1.108	1.342	1.149	52	5/11	42	23/7.6	38/6.2	Ribulose biphosphate carboxylase small chain PWS4, chloroplastic [Trichum arvense]
20	1.266	2.576	2.035	61	10/20	37	47.5/5.7	39/6.1	26S proteasome regulatory complex component
21	0.002	1.378	528.74	52	5/20	28	39.1/5.66	39/6.5	Probable cinnamyl alcohol dehydrogenase OS=Eucalyptus botryoides GN=CADI PE=3 SV=1
22	0.248	0.869	3.492	53	8/28	86	9.6/6.45	40/6.0	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, partial [Asplenium normale]
24	17.19	9.458	0.550	35	4/12	30	21/5.78	25/5.8	Auxin-responsive protein IAA4 OS=Arabidopsis thaliana GN=IAA4 PE=1 SV=2
25	5.444	1.475	0.271	70	12/39	43	29.7/5.0	31/5.5	Breast carcinoma amplified sequence 2 (Coccomyxa subellipsoidea C-16)
26	4.490	1.373	6.293	45	10/26	42	26.7/5.99	34/5.45	Glutathione S-transferase F11-like [Solomon lycopersicum]
27	2.719	1.390	6.597	37	7/27	32	37.4/5.49	34/5.4	Phosphoenolpyruvate carboxylase family protein [Theobroma cacao]
28	6.840	1.388	6.233	63	8/18	46	31.7/4.74	32/5.2	Ribonuclease CAF1 [Medicago truncatula]
29	1.865	0.936	6.592	66	7/11	36	35.6/6.12	35/4.4	Feruloyl hydroxylase alpha subunit homologues
30	1.722	3.134	1.819	47	8/21	34	37.0/5.2	35/4.6	Glucosyl transferase (Oryza sativa Japonica Group)
31	1.626	4.416	2.716	74	10/26	33	41.0/6.4	36/4.8	PREDICTED: F-box protein At2g1290-like [Setaria italica]
32	0.815	1.771	2.172	76	7/25	36	33.9/5.51	45/5.3	Regulator of chromosome condensation (RCC1) repeat hypothetical protein [Arabidopsis thaliana]
33	1.766	2.190	1.240	70	9/31	48	23.2/8.3	25/4.2	Mal-like protein [Argemone mexicana]
34	0.463	1.930	4.161	52	10/20	53	36.8/10.3	28/4.1	Ribosomal protein S4, bacterial/vorganelle type [Populus trichocarpa]
35	5.119	0.375	6.073	49	4/10	65	14.9/10.0	22/4.2	TPA: putative RING zinc finger domain superfamily protein [Zea mays]
36	6.555	6.499	0.991	105	12/44	47	35.5/5.84	50/5.3	Oxygen-evolving enhancer protein 1, chloroplastic-like [Fragaria vesca subsp. vesca]
37	5.522	6.377	1.155	38	7/30	72	10.6/5.3	50/5.1	Full-Oxygen-evolving enhancer protein 1, chloroplastic; Short-OEE1
38	3.168	4.385	1.384	31	8/31	38	41.5/9.1	46/5.4	AP2/ERF and B3 domain-containing protein Ouf5g0549800 PE=2 SV=1
39	4.725	2.243	6.475	63	6/34	37	35.8/7.56	35/5.1	Flavonol synthase (flavonone 3-hydroxylase [Zea mays])
40	0.819	2.169	2.647	35	3/8	17	24.7/5.9	26/5.3	RING-H2 finger protein ATL40 OS=Arabidopsis thaliana GN=ATL40 PE=2 SV=1

Fig.2 Validation of the differentially expressed proteins from shoot by semi quantitative PCR

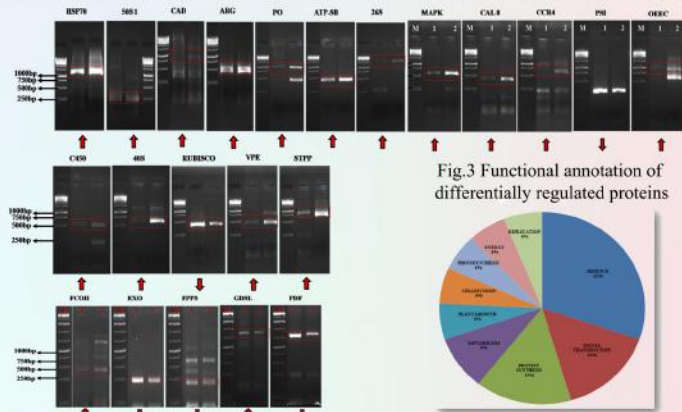
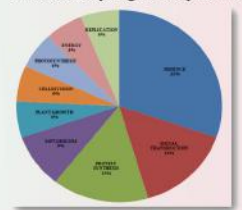


Fig.3 Functional annotation of differentially regulated proteins



CONCLUSION

- This is the first report on the application of proteomics to unravel BBTV-Banana interaction and these results will contribute for a better understanding of the molecular basis of host responses to BBTV.
- Two proteins (Mitogen-activated protein kinase and Cinnamoyl-CoA reductase) were confirmed to be induced at higher levels during BBTV infection, and they qualify as the first disease marker candidates that may prove to be useful for BBTV detection.
- This information would be useful in serological based kit development for effective diagnosis and management of this disease.