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Influence of temperature on symptom expression, detection of host factors in virus infected *Piper nigrum* L.

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Expression of symptoms in black pepper plants (*Piper nigrum*) infected with Piper yellow mottle virus (PYMoV) vary depending on the season, being high during summer months. Here, we explored the influence of temperature on symptom expression in PYMoV infected *P. nigrum*. Our controlled environment study revealed increase in virus titer, total proteins, IAA and reducing sugars when exposed to temperature stress. There was change in the 2-D separated protein before and after exposure. The 2-D proteomics LC-MS identified host and viral proteins suggesting virus-host interaction during symptom expression. The analysis as well as detection of host biochemical compounds may help in understanding the detailed mechanisms underlying the viral replication and damage to the crop, and thereby plan management strategies.

Keywords: Black pepper, Piper yellow mottle virus, Stress, Virus-host proteins

Black pepper (*Piper nigrum* L.) is an important spice crop grown in tropical and subtropical regions of the world, namely Brazil, India, Indonesia, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam. The demand for this crop is increasing owing to its diversified uses in domestic and industry including pharmaceuticals. There are several production constraints in achieving the sustainable yield of this crop, among them diseases occupy predominant position, particularly the stunted disease. This disease, caused by Piper yellow mottle virus (PYMoV) (genus: *Badnavirus*), is characteristic with mottled and smaller leaves, shorter internodes, stunted vines and has been reported from all major black pepper-growing countries worldwide[1-4]. PYMoV is a dsDNA virus with a narrow host range limited to *Piper* spp. and is transmitted vegetatively through seeds and mealy bugs[5]. Earlier survey observations suggest that PYMoV symptom expression in black pepper is severe under summer months in all black pepper growing regions of India and Sri Lanka[2,6]. During winter months the plants are symptomless. Temperature dependent expression of Banana streak virus (BSV), the important member of *Badnavirus* genus is also known[7]. The serological relationship of PYMoV with BSV and Sugarcane bacilliform virus (SCBV) was also reported[6]. In light of this background information, we investigated the influence of temperature on virus expression by analyzing important physiological parameters, virus titer and the host protein expression in black pepper infected with PYMoV.

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Materials ad Methods

Source of plants and detection of virus by qPCR

Symptomless rooted cuttings of the black pepper variety Sreekara were obtained from nursery of Indian Institute of Spices Research, Kozhikode, India. SYBR-Green based quantitative real-time PCR (qPCR) was used to screen for presence or absence of the virus and also to determine the virus titer in the plants before and after exposure to temperature. Total DNA isolated from the plants was subjected to real-time PCR analysis in Rotor Gene Q real-time PCR system (Qiagen, Hilden, Germany) using PYMoV specific primers as described earlier[8]. The copy number (titer) of the virus in the sample was determined from the standard curve developed for PYMoV in our lab. Fifteen plants that tested positive for PYMoV and 5 plants that tested negative for PYMoV were used in the experiment.

Exposure to temperature and symptom expression

The plants were kept under controlled condition in Plant Growth Chamber (YORKO) at 36°C and 61% relative humidity for 8 h daily with watering once daily. The plants were monitored daily for symptom expression. The leaf samples from plants before and after symptom expression were taken and analyzed for virus titer, physiological parameters and the total protein expression.

Physiological parameters

Total chlorophyll, total phenols and reducing sugars were estimated as per the methodology described by Sadasivam and Manickam[9]. The total proteins were estimated as per Lowry *et al.*[10]. For IAA (indole-3-acetic acid) estimation, fresh samples were extracted in 80% cold methanol, centrifuged at 10000 rpm for 20 min at 4°C and the supernatant was collected. The methanol in the supernatant was removed by rotary flash evaporation system at 35°C and further extraction was done with minor modifications and estimated[11] using Salkowski's reagent.

Protein extraction, IEF, 2DE and image analysis.

The total leaf protein was extracted[12] and the concentration was estimated using 2 D Quant kit (GE health care). The protein pellets were dissolved in solublization buffer as described above and was added with rehydration buffer (8 M urea, 2% w/v CHAPS, 18 mM DTT, 0.5% w/v IPG buffer pH 3-11 and 0.002% bromo phenol blue). The IPG strips (immobilized pH gradient strips) were rehydrated passively for 16 h with 60 µg protein. The first dimension IEF (isoelectric focusing) was carried out using non linear IPG strips (pH 3-11) using Ettan IPGphor system from GE health care (step1-500Vh, step2 Gradient-800Vh, step3 Gradient-16500Vh, step4-22200Vh). Prior to second dimension electrophoresis the strips were equilibrated by placing the strips in equilibration tube. The first equilibration was done for reduction of proteins in the strip with 2.5 mL of equilibration buffer (50 mM Tris Cl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS (sodium dodecyl sulfate), traces of bromophenol blue) containing 0.5% (w/v) DTT for 20 min and the second equilibration was done to alkylate the proteins, with 2.5 mL equilibration buffer containing 2.5% (w/v) iodoacetamide solution. After equilibration, the strips were dipped for 2 min in fresh 1x gel running buffer and then placed on a 12% (40% T, 3% C Acrylamide:Bisacrylamide) continuous SDS PAGE (1 mm thick) avoiding any air bubbles in between the strip and the gel. The strip was sealed with agar overlay solution (0.25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS, traces of bromophenol blue, 0.5% agarose). The lower tank buffer was 1x and the upper tank buffer was 2x running buffer and the electrophoresis was done at 80 V for 15 min, and then 100 V till the bromo phenol blue reached the bottom of the gel. Protein patterns were observed by staining with Coomassie 250. Stained gels were scanned and calibrated with Labscan 6.0 software (GE health care) including IQTL calibration converter and the Image analysis was carried out with image master platinum 6.0 (GE health care). The Protein bands were excised with sterile surgical blade and samples were subjected to in-gel digestion[13] and with additional alkylation and reduction.

MS/MS analysis and database search

Digested peptides were reconstituted in 15 µL of the 0.1% formic acid and 1 µL of samples were injected in to the column and digested peptides were subjected to 70 min RPLC (high pH reversed-phase liquid chromatography) gradient, followed by acquisition of the data on LTQ-Orbitrap-MS (mass spectrometry) (Thermo) with collision induced dissociation as the fragmentation method. The precursor mass tolerance was at 10 ppm and fragment mass tolerance at 0.8 Da with 3, maximum cleavage sites. The dynamic modifications set were carbamidomethyl (C), Oxidation (M), Carboxymethyl (C), GLN->Pyro-Glu (N-termQ) and Glu->Pyro-Glu (N-term E) with the protein relevance Threshold 20 and protein relevance factor 1. Minimum of two high confident peptides was used as a prerequisite to identify the proteins.12.5 fmoles standard BSA digest was analyzed at the beginning and end of sequence to check the performance of the instrument.12.5 fmoles of standard BSA was successfully found back with the sequence coverage of 49.92%, 47.61% before and after ending sequence, respectively. The generated data was searched following standard approach for the identity using MASCOT 2.4 as search engine on Proteome discoverer 1.4. The data was searched against Uniprot TrEMBL and Swiss Prot database from NCBI.

Statistical analysis

For biochemical/protein studies, three biological replicates were taken and pooled for each analysis. For 2-D electrophoresis two replicate gels were run under each category and the replicate gels were merged and analyzed with image master platinum 6.0 software with saliency value of 700 for spot detection. The spot intensity was compared between the gels by scatter plot and also by histogram pattern. For the matched proteins, the relative value was obtained along with the histogram.

Results and Discussion

The qPCR analysis of 15 PCR positive plants showed an virus copy number ranging from 282 to 3786 (with an average of 1073) in different plants before exposure to temperature stress while PCR negative plants did not show the copy number. Out of 15 PCR positive plants, 7 started showing typical viral symptom on 10th day but PCR negative plants did not show any symptom. On 20th day, all PCR positive plants and two PCR negative plants showed symptom. The copy number ranged from 46011 to 475746 (with an average of 244130) in PCR positive plants, thus indicating an increase of 229 fold upon subjecting to stress. Plants that tested negative in qPCR before subjecting to stress also showed presence of virus with copy number ranging from 842 to 3741 (with an average of 2477) after subjecting to stress. This result indicates that these plants had very low virus copy before subjecting to stress thus probably went undetected and indicates that even if the virus is present in low copy number the temperature would have the influence in its symptom expression. Viruses are known to adopt different strategy to replicate when the host is under stress. It is also reported that symptom expression does not necessarily correlate with the virus titer, indicating that disease can be the result of specific interactions between virus and host components[14].

Total chlorophyll and the total phenols in PCR negative plants was more than that of PCR positive plants initially while total proteins and total sugars were found to be higher after symptom expression (Fig. 1). IAA content in PCR negative plants remained same before (1.62 µg/g) and after (1.91 µg/g) stress while the PCR positive plants showed higher content after the stress (5.11 µg/g). The increase in total sugar, IAA, phenolics and the reduction in chlorophyll indicates that the important plant biochemical factors may have a role in PYMoV expression in black pepper. Studies on mesta plants infected with yellow vein mosaic virus also showed similar findings for chlorophyll and phenolics[15]. Accumulation of sugar and starch in virus infected plants was correlated with symptom development such as chlorosis, mosaic and stunted phenotype[16]. Many of the symptoms in virus infected plants can be linked to plant hormones, interaction as observed with Tobacco mosaic virus replicase and auxin/IAA proteins that affect auxin mediated pathway, and thereby produces disease symptom[17].

There was change in the 2-D separated protein pattern (Fig. 2 a-d). The analysis by image master platinum 6.0 at 700 saliency value for protein spots showed that the temperature stress had increased the number of protein spots both in the PCR positive (22 spots before and 65 spots after stress) and PCR negative (13 spots before and 20 spots after stress) plants showing the possible effect of temperature in general. Comparison between PCR positive and negative plants after temperature stress gave significantly different protein spots in the case of PCR positive plants showing additional proteins.

Protein spots that are present in all before and after temperature stress, present only in PCR negative after temperature stress, spots present only in PCR positive before stress and after stress were selected randomly for MS analysis. The 16 kDa calmodulin protein, 17.6 kDa class I heat shock proteins, Rubisco, super oxide dismutase, chaperonin HSp 70 family were identified from PCR negative samples after stress. Earlier report on Soybean mosaic virus (SMV) infected plants showed increased transcript level at 14 days after infection and reduced considerably after some period for calmodulin, HSp 70 and super oxide dismutase and the alteration in biosynthetic

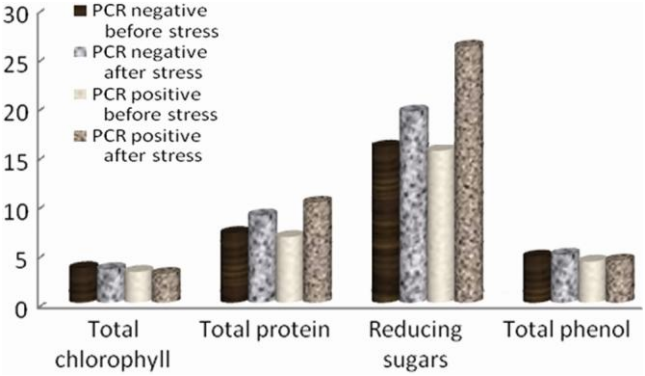

Fig. 1—Dynamics of biochemical parameters in PYMoV infected black pepper plants

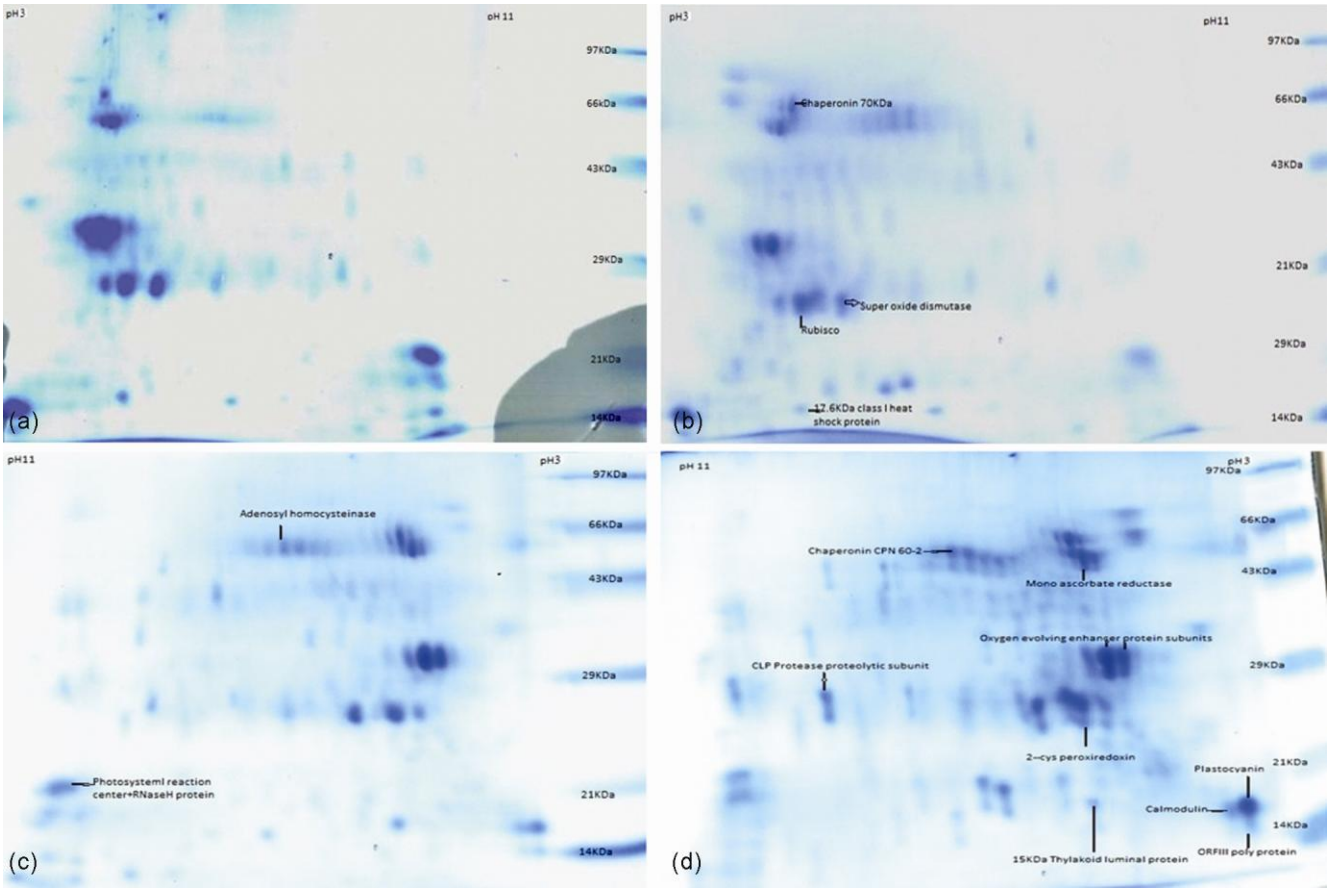

Fig. 2—Protein pattern from PCR Negative (a and b); and Positive (c and d) plants before and after temperature stress, respectively.

gene including rubisco[18]. The proteins identified in PCR positive plants were of photosystem I reaction center subunit II and the adenosyl homocysteinase. Many studies on virus symptom expression have identified the role of photosystem proteins. Adenosyl homocysteinase is an important protein which plays a major role in sulphur uptake and assimilation. Yang *et al.*[19] reported that Turnip mosaic virus infection suppressed the expression of genes involved in sulphur uptake pathway, affecting plant growth and development which included adenosyl homocysteinase. Taken together, our analysis also suggest possible role of these two proteins in virus expression.

The PCR positive plants after stress had subunits of oxygen evolving enhancer protein I, 16 kda plastocyanin, mono dehydro ascorbate reductase, 2cys peridoxin, chaperonin CPN 60-like 1 protein, thylakoid luminal 15 kDa family protein and the CLP protease. Tobamovirus and Cauliflower mosaic virus were reported to differentially affect the components of oxygen evolving complex[20]. Mono dehydro ascorbate reductase, 2cys peridoxin, adenosyl homocysteinase transcripts were found to be upregulated in SMV infection studies using microarray[17]. Chaperonin CPN 60-like 1 protein was found to be associated with Rubisco activase for protecting photosynthesis during heat stress[21]. Thylakoid luminal 15 KDa family proteins which is a heat shock family protein was found to have antioxidant/free radical scavenging activity and the CLP protease proteolytic subunit (serine type endopeptidase) is a host defense protein. Three proteins were found to be of matched proteins (calmodulin, plastocyanin and Rubisco) from PCR negative and PCR positive (before and after stress) and showed difference in expression during gel analysis when analyzed by image analysis software. Hence, the relative values were obtained with the histogram pattern (Fig. 3). Calmodulin had high relative value (expression) in PCR negative after stress compared to before stress, was down regulated in PCR positive before and after stress. Plastocyanin showed high relative value in PCR positive after

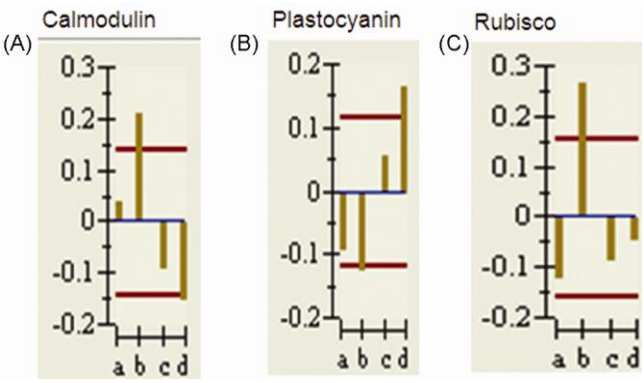

Fig. 3—Histogram of relative values of proteins. Blue horizontal line is the chosen central tendency and the red line delimits the range.

stress compared to before stress, the PCR negative before and after stress had less expression. Rubisco protein showed high relative value only in PCR negative after stress.

MS data revealed photosystem I protein binding to virus counterpart. Two highest hits from MS data protein similarity showed this protein homology with RNaseH (ribonuclease H) protein of BSV (Accession Id: ACL37070.1) (a member of the genus *Badnavirus* to which PYMoV also belong) with 70% identity in Blastp. The report on Sugarcane mosaic virus[22] revealed the association of Hc-pro protein of virus with the host photosystem I reaction center subunit II (encodes ferridoxin docking protein).When analyzed, the 16 KDa plastocyanin molecule was found to bind with viral protein. The similarity search for 2 highest hits with 116.97 score from MS analysis showed this protein homology with RNaseH protein of BSV (Accession ID: ACL37070.1) with 70% identity in Blastp. Earlier report[23] had also revealed the association of this host protein with the coat protein of Potato virus *X* during symptom severity in *Nicotiana*. Chaperonin CPN 60-2 protein also had binding protein. The highest hit search with 98.99 score for the homology of this protein yielded the protein similarity towards ORF III polyprotein (Accession ID: AAE86310.1) of BSV with 50% identity in Blastp.

The data on protein profile (Table 1 and 2) from PYMoV infected black pepper upon temperature stress shows that the temperature is an important factor for the virus to produce symptom. In our present study also, we observed the involvement of host biochemical factors/proteins in symptom expression of PYMoV upon temperature stress. The results suggest that alteration in hormone synthesis, chloroplast, photosynthesis and heat shock proteins may contribute to symptom expression. This preliminary report on the protein expression before and after exposure to temperature stress in PCR positive and negative plants has given indication of host proteins with virus derived proteins such as photosystem I protein-RNaseH protein of BSV, plastocyanin-RNaseH protein of BSV, Chaperonin CPN 60-2 protein -ORF III polyprotein. Though these initial results throw light on the involvement of host factors in symptom expression, the analysis on the entire protein profile to pinpoint the specific up-/down- regulation of host factors, interaction of host factors with virus factors for the altered physiology and disease development is needed for the development of defined and more potent management strategies to reduce symptom expression.

Table 1—Nano LC-MS identified proteins with accession, molecular weight and the protein score

Protein name/MW	Accession	Score
Calmodulin /16KDa[3,4]	P93171	72.83
Photosystem I reaction center/25KDa[1]	A5AEB4	437.33
Adenosyl homocysteinase/70KDa[1]	O23255	350.22
17.6 K Da classI heat shock proteins/17.65KDa[5]	P30693	77.15
Rubisco large subunit/28KDa[5]	A2YVR7	109.64
Superoxide dismutase/25KDa[5]	P33DZ8	193.92
Chaperonin HSP 70 family/70KDa[5]	MORGDO	193.92
Plastocyanin/16 KDa[2]	POO297	67.78
Oxygen evolving enhancer protein/35KDa[2]	F2XX49	363.144
Oxygen evolving enhancer protein/35KDa[2]	F2XX49	363.144
Monohydro ascorbate reductase/45KDa[2]	A5JPK7	151.26
Chaperonin CPN 60-2/60KDa[2]	Q43298	98.66
2-Cys peroxiredoxin/25 K Da[2]	B6TDA9	142.69
CLP protease proteolytic subunit(Serine type endopeptidase)[2]	ATIG12410.1	164
Thylakoid luminal 15 KDa family protein/18KDa[2]	222851000	183.163

[1], PCR positive before stress; [2], PCR positive after stress; [3], PCR positive (before/after) stress; [4], PCR negative (before/after); and [5], PCR negative after stress

Table 2—Identified peptide sequences and the peptide mass from LTQ-Orbitrap

Protein Name	Peptide Sequeces from LTQ-Orbitrap (Peptide Mass)
Calmodulin	EAFSLFDKDGDGcITTK (1903.87406) VFDKDQNGFISAAELR (1809.91166)
Plastocyanin	GTYSFYcSPHQGAGmVGKVTVN (2376.07523) GTYSFYcSPHQGAGmVGK (1962.85076)
16 KDa heat shock protein	AGLENGVLTVTVPK (1397.79875) FRLPENAK (974.541429)
Ribulose Large subunit	DTDILAAFR (1021.53063) EITLGFVDLLR (1275.73113)
SOD	LVVETTANQDPLVTK (1627.89092) KLVVETTANQDPLVTK (1755.98699)
Oxygen evolving enhancer protein	DGIDYAAVTVQLPGGER (1760.88274) GGSTGYDNAVALPAGGR (1562.75823) GGSTGYDNAVALPAGGRGDEEELLKENIK (2960.46292) GDEEELLKENIK (1416.72234) RLTYDEIQSK (1252.65332) FEEKDGIDYAAVTVQLPGGER (2294.12894 GGSTGYDNAVALPAGGRGDEEELLK (2476.19731) LTYDEIQSK (1096.55278)
Chaperonin CPN 60	AAVEEGIVVGGGcTLLR (1700.89971) LSGGVAVIQVGAQTETELK (1900.0396) GGYPILIIAEDIEQEALATLVVNK (2569.41043) EVELEDPVENIGAK (1541.77043) GYISPYFVTDSEK (1505.70976) LADLVGVTLGPK (1182.7083) SQYLDDIAILTGATVIR (1849.00577)
2-cys peroxiredoxin	APDFEAEAVFDQEFINVK (2068.98882) LNTEVLGVSIDSVFSHLAWVQTDR (2686.38608) EGVIQHSTINNLAIGR (1721.93132) GLFIIDKEGVIQHSTINNLAIGR (2508.39402)
Chaperonin HSP 70	QVANATNDVAGDGTTcATILTR (2249.08452) MISTSEEIAQVGTISANGER (2093.01885) QRPLLIVAEDVESDALATLILNK (2521.4249) SVAAGmNAmDLR (1267.57683) NVVIEQSFGAPK (1288.69072) LLEQENPDLGYDAAK (1675.81963) IGVQIIQNALK (1196.73613)
Photosystem reaction center II subunit	EQIFEmPTGGAAImR (1682.78984) QGVGVNFR (876.468492) EQIFEmPTGGAAIMR (1666.79265) EQcLALGTR (1047.52556) VFPNGEVQYLHPK (1527.79595)
CLP Protease	EYGLIDGVImNPLK (1577.82463) ANLNGYLAYHTGQSLEK (1878.93535) INQDTDRDFFmSAK(1703.7719)
Thylakoid luminal protein	AGNVQPYQFILPPTWK1858.98833 VANILSGNYcQPK1463.73161
Mono dehydro ascorbate reductase	TSVPDVYAVGDVATFPLK1878.98601 FGTYWIK1914.476976

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References

1 Mishra RK & Singh SK, Antispermatogenic and antifertility effects of fruits of *Piper nigrum* L. in mice. *Indian J Exp Biol*, 47 (2009) 706.

2 Lockhart BEL, Kirtisak KA, Jones P, Padmini DS, Olsziewski NE, Lockhart N, Nuarchan D, Sangalang J, Identification of *Piper yellow mottle virus*, a mealybug–transmitted badnavirus infecting *Piper* spp. in southeast-Asia. *Eur J Plant Pathol*, 103 (1997) 303.

3 *de* Silva DPP, Jones P & Shaw MW, Identification and transmission of *Piper yellow mottle virus* and *Cucumber mosaic virus* infecting black pepper (*Piper nigrum* L.) in Sri Lanka. *Plant Pathol*, 51 (2002) 537.

4 Hareesh PS & Bhat AI, Detection and partial nucleotide sequence analysis of *Piper yellow mottle virus* infecting black pepper (*Piper nigrum* L.) in India. *Indian J Virol*, 19 (2008) 160.

5 Hareesh PS & Bhat AI, Seed transmission of Piper yellow mottle virus in black pepper (*Piper nigrum* L.). *J Plant Crops*, 38 (2010) 62.

6 Bhat AI, Devasahayam S, Venugopal MN & Suseela Bhai R, Distribution and incidence of viral diseases of black pepper in Karnataka and Kerala, India. *J Plant Crops*, 33 (2005) 59.

7 Dahal G, Hughes DA, Thottappilly G & Lockhart BEL, Effect of temperature on symptom expression and expression and reliability of banana streak badnavirus detection in naturally infected plantain and banana (*Musa* spp.). *Plant Dis*, 21 (1998) 8216.

8 Bhat AI & Siljo A, Detection of viruses infecting black pepper by SYBR Green-based real-time PCR assay. *J Plant Pathol*, 96 (2014) 105.

9 Sadashivam S & Manickam A, *Biochemical Methods for Agricultural Sciences* (Wiley Eastern Limited, New Delhi) (1992) 5.

10 Lowry OH, Rosebrough NJ, Farr AL & Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193 (1951) 265.

11 Glickmann E & Dessaux YA, Critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl Environ Microbiol*, 61 (1995) 793.

12 Umadevi P & Anandaraj M, An efficient protein extraction method for proteomic analysis of black pepper (*Piper nigrum* L.) and generation of protein map using nano LC-LTQ Orbitrap mass spectrometry. *Plant Omics*, 8 (2015) 500.

13 Shevchenko A, Thomas H, Havlis J, Olsen JV & Mann M, In gel digestion for mass spectrometric characterization of proteins and proteomes. *Nature Prot*, 1 (2007) 2856.

14 Pallas V & Garcia A, How do plant viruses induce disease? Interactions and interference with host components. *J Gen Virol*, 92 (2011) 2691.

15 Chatterjee A & Ghosh SK, Alteration in biochemical components in mesta plants infected with yellow vein mosaic disease. *Braz J Plant Physiol*, 4 (2008) 11.

16 Herbers K, Tacke E, Hazirezaei M, Krause KP, Melzer M, Rohde W & Sonnewald U, Expression of a luteoviral movement protein in transgenic plants leads to carbohydrate accumulation and reduced photosynthetic capacity in source leaves. *Plant J*, 10 (1997) 1045.

17 Padmanabhan MS, Shiferaw H & Culver JN, The Tobacco mosaic virus replicase protein disturbs the localisation and function of interacting Aux/IAA proteins. *Mol Plant Microbe Interact*, 19 (2006) 864.

18 Babu M, Gargarnova AG, Brandle JE & Wang A, Association of the transcriptional response of soybean plants with soybean mosaic virus systemic infection. *J Gen Virol*, 89 (2008) 1069.

19 Yang C, Guo R, Jie F, Nettleton D, Peng J, Carr T, Yeakley JM & Fan JB, Whitham SA, Spatial analysis of *Arabidopsis thaliana* gene expression in response to turnip mosaic virus infection. *Mol Plant Microbe Interact*, 20 (2007) 358.

20 Sui C, Fan ZF, Wong SM & Li HF, Cloning of cDNAs encoding the three subunits of oxygen evolving complex in *Nicotiana benthamiana* and gene expression changes in tobacco leaves infected with Tobacco mosaic virus. *Physiol Mol Plant Pathol*, 68 (2006) 61.

21 Salvucci ME, Association of Rubisco activase with chaperonin -60: a possible mechanism for protecting photosynthesis during heat stress. *J Exp Bot*, 59 (2008) 1923.

22 Ma Y, Zhou T, Hong Y, Fan Z & Li H, Decreased level of ferredoxin I in Tobacco mosaic virus-infected tobacco is associated with development of the mosaic symptom. *Physiol Mol Plant Pathol*, 72 (2008) 39.

23 Qiao Y, Li HF, Wong SM & Fan ZF, Plastocyanin transit peptide interacts with potato virus X coat protein, while silencing of plastocyanin reduces coat protein accumulation in chloroplasts and symptom severity in host plants. *Mol Plant Microbe Interact*, 22 (2009) 152