Original Research

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

JEB™ p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

DOI : http://doi.org/10.22438/jeb/42/1/MRN-1408

Elucidation of induced host plant resistance to white backed plant hopper (WBPH) in response to zinc application in rice

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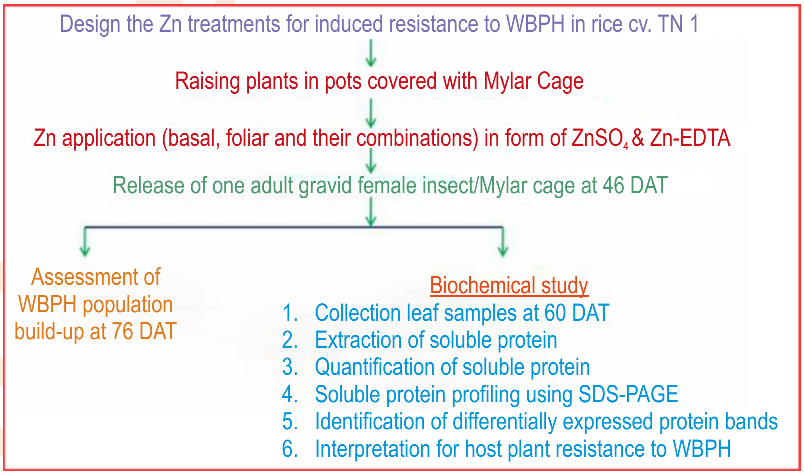
Received: 08.02.2020 *Revised*: 29.06.2020 *Accepted*: 07.08.2020

Abstract

Aim: The experiment was aimed to study the effects of various Zn formulations towards induced host plant resistance to WBPH in rice.

Methodology: Eight zinc treatments comprising basal and foliar applications(at 30 and 45 DAT) of Zn SO$_4$ and Zn-EDTA and their combinations were tested for induced host plant resistance to WBPH (a dreadful sucking insect) in a most sensitive rice variety TN 1. In addition, a treatment without any Zn application served as control. The pot grown plants were kept in the Mylar cages with top end covered by fine mesh muslin cloth and as such maintained till maturity. One adult gravid female insect was released into each cage (at 46DAT) and the insect population build-up was recorded after one month. Soluble protein profiling of leaf samples (at 60DAT) of each treatment was done by SDS-PAGE and data were analyzed for clustering pattern.



Design the Zn treatments for induced resistance to WBPH in rice cv. TN 1

Raising plants in pots covered with Mylar Cage

Zn application (basal, foliar and their combinations) in form of ZnSO$_4$ & Zn-EDTA

Release of one adult gravid female insect/Mylar cage at 46 DAT

Assessment of WBPH population build-up at 76 DAT

Biochemical study

1. Collection leaf samples at 60 DAT
2. Extraction of soluble protein
3. Quantification of soluble protein
4. Soluble protein profiling using SDS-PAGE
5. Identification of differentially expressed protein bands
6. Interpretation for host plant resistance to WBPH

Results: Altogether, 19 polypeptide bands (14.3-97.4kDa) were revealed. The low molecular weight proteins (14.3-25.1 kDa) were clearly absent in the control. T$_6$(Zn EDTA at basal and foliar) recorded least WBPH build–up and elicited highest number (15) of polypeptide bands including five new bands at 66.0, 37.0, 23.6, 15.8 and 14.3 kDa. Further, 66.0kDa, 37.0 kDa and 14.3 kDa polypeptide bands were commonly shared by T$_6$, T$_7$(ZnSO$_4$ basal+ EDTA foliar) and T$_8$(Zn EDTA basal + ZnSO$_4$) that recorded lower WBPH population and grouped together as compared to rest of the treatments and control. However, 23.6 kDa polypeptide band induced only in T$_6$ and T$_7$ seems to have greater role in manifestation of induced defence mechanism against WBPH in rice.

Interpretation: The differentially expressed proteins (as compared to control) revealed in response to Zn application may be considered as biochemical basis of induced resistance for the pest in rice.

Key words: Induced resistance, Soluble protein profiling, White backed plant hopper, Zinc

How to cite : Tripathy, S., L.K. Rath and S.K. Tripathy: Elucidation of induced host plant resistance to white backed plant hopper (WBPH) in response to zinc application in rice. *J. Environ. Biol.*, **42**, 154-161 (2021).

Introduction

Rice (*Oryza sativa* L.) is grown extensively in different parts of the world and one of the most important staple food crops of India and Odisha as well. Pests pose threats to rice production. Now-a-days, the situation is aggravated by climate change (Cabot *et al.,* 2019) and indiscrimilate use of pesticides leading to pest resurgence and drastic toxicidal effect on beneficial bioagents (Satpathy *et al.,* 2020). Micronutrient roles in plant defense are predominantly documented for Mn, Cu, Fe, and Zn (Fones and Preston, 2012). Zinc serves as cofactor for more than 300 enzymes (Marreiro *et al.,* 2017) and it is known to trigger plant growth and immunity system against biotic and abiotic stresses (Gupta *et al.,* 2012) although a general model for Zn-related defense mechanism is not clear. Zn seems to have antagonistic role on insects by inhibiting α-amylase, which is an important digestive enzyme required for their survival and growth (Kaur *et al.,* 2014). The Zn proteome is an important group of metalloprotein and it represents about 9% of the entire proteome in eukaryotes and 5-6% in prokaryotes (Andreini *et al.,* 2009). Insect infestation is reported to be related to the up-regulation of two zinc finger transcription factors in potato (Lawrence *et al.,* 2014). Similarly, Chang *et al.* (2012) reported increased Zn-SOD (super oxide dismutase) activity due to insect infestation and it is negatively correlated with foliar damage (Khederi *et al.,* 2018).

More than 100 species of insects have been reported to attack the rice crop (Krishanaiah *et al.,* 2008). Among these white backed plant hopper (WBPH, *Sogatella furcifera*) is a major sucking pest of rice that causes nearly 35-95% yield loss under favourable condition (Sidhu, 1979; Sogawa *et al.,* 2009). Hopper burn due to intensive sucking by WBPH together with its high fecundity and long-distance migration ability pose serious threat to rice cultivation (Zhai *et al.,* 2013). Unfortunately, no truly resistant variety has been so far developed against this insect. However, application of various micronutrients can be an alternate non-conventional and eco-friendly sustainable plant protection approach to manage this pest through induction of host plant resistance. Zinc is one such micronutrient which can induce defence mechanism in rice against the sucking pest (Rath, 2004).

Out of four micronutrients (iron, zinc, copper and manganese), zinc is reported to be the most effective to induce antibiosis effect against BPH, WBPH and GLH (Rath, 2006) leading to reduced nymphal survival, growth index and population build up. Besides, the BPH incidence is reported to be inversely related with zinc ($r = -0.2690$) and sulphur ($r = -0.2689$) content of rice foliage (Dash *et al.,* 2007) following application of $ZnSO_4$ along with NPK fertilizers. Increase in expression of defense related protein after infestation (Edwards and Wratten, 1983) and induction of a specific protein (53 kDa) due to leaf folder infestation in resistant and moderately resistant rice varieties have been reported by Das *et al.* (1999). Besides, Punithavalli *et al.* (2013) reported increased expression of a 38 kDa protein due to leaf folder infestation, which served as a key for identification of leaf folder tolerant or resistant genotypes. Besides, elicitation of defense related protein in response to basal or foliar application of zinc in rice could be a biochemical basis of induced resistance to insects. Therefore, an experiment was designed to study the extent of induced resistance to WBPH in rice by application of zinc and to explore the role of Zn-induced defense related protein for induced host plant resistance mechanism.

Materials and Methods

Zinc treatments: Twenty days old seedlings of a WBPH susceptible rice variety TN 1 were transplanted on 10 kg capacity earthen pots filled with puddled soil. Various Zn treatments taken were, T_1: $ZnSO_4$ basal (25 kg ha^{-1}), T_2: Zn EDTA basal (40 kg ha^{-1}), T_3: $ZnSO_4$ Foliar spray (0.5 %) (30 & 45 days after transplanting (DAT)), T_4: Zn EDTA foliar spray (0.8%) (30 & 45 DAT), T_5: $T_1 + T_3$, T_6: $T_2 + T_4$, T_7: $T_1 + T_4$, T_8: $T_2 + T_3$ and T_9 along with a treatment without any Zn application to serve as control. After application of recommended fertilizer dose and basal Zn treatment in respective pots, they were covered by the Mylar cages of 45 cm height with top end covered by fine mesh muslin cloth. The plants were maintained as such under cage till maturity. At 30 and 45 DAT; foliar applications of Zn were imposed on the plants. All the experiments were done in green house of the Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar.

Assessment of WBPH population: At 46 DAT, 3-4 days old, one adult gravid female insect was released into each cage. After one month of release, the population of WBPH was counted, replication-wise as per Heinrichs *et al.* (1985).

Soluble protein extraction and quantification: As the WBPH population build-up attained highest at 60DAT in the control (without Zn application in cv. TN 1); leaf samples from different treated caged plants were collected at this stage for quantitative and qualitative analysis of protein and also to explore the presence of any new defence protein responsible for inducing host plant resistance. For the purpose, soluble protein was extracted from leaves using Tris extraction buffer (20mM Tris-HCl, pH 7.5; 50mM $MgCl_2$, 2% PVP, 1mM PMSF) followed by precipitation with 50% TCA + 1% β-ME; and solubilisation in sample buffer (9M Urea, 4% CHAPS, 1% DTT and 1mM PMSF, 2% Bio-Lyte) @ 1mg dried protein powder/0.1ml buffer at 4oC overnight. The total soluble protein of each sample was quantified by the method suggested by Lowry *et al.* (1951).

Soluble protein profiling and gel documentation: Total soluble protein profiling was carried out by Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) according to Lagrimini and Rothstein (1987). Reproducibility was confirmed by minimum of two repeats of each run of SDS-PAGE under similar electrophoretic conditions. After electrophoresis, gels were stained with 0.125% w/v coomassie brilliant Blue R 250, 50%v/v methanol, and 10% v/v glacial acetic acid for four hours with intermittent shaking followed by destaining overnight in 50% methanol and 10% glacial acetic acid; and finally, several washings with 5% methanol and 7% glacial acetic acid. Thereafter, the gels were placed on Gel Documentation System (Fire Reader-Uvtec, Cambridge, UK) for assessment of banding pattern and photographed. The presence and absence of polypeptide bands were scored as 1 and 0 respectively to determine variation among various zinc treatments. The relative mobility of each polypeptide band was calculated using software of the Gel doc system. The molecular weights of the dissociated polypeptides were determined by using molecular weight marker of protein standards which consisted six standard proteins of known molecular weight i.e. lysozyme (14.3kDa), soybean trypsin inhibitor(20.1 kDa), carbonic anhydrase (29kDa), ovalbumin (43kDa), bovine serum albumin (66kDa) and phosphorylase-b (97.4kDa). Differentially expressed unique polypeptide bands were noted to find relationship with WBPH tolerance.

Statistical analysis: The data sets for total soluble protein(mg/g) and WBPH population build-up per plant were subjected to Duncan's multiple range test (Duncan,1955). Means superscripted by same letter were considered not significantly different at $P \leq 0.05$. The binary data set (1/0 score) of soluble protein profile was subjected to estimation of Jaccard's similarity co- efficient values and construction of dendrogram (clustering pattern) using the procedures of Jaccard (1908), and Sokal and Michener (1958) respectively.

Results and Discussion

Preference/non-preference of a variety by the insect is greatly assessed by the population load of the insect on the variety within a stipulated time. Higher degree of suitability of the plant variety by insect(s) results typical susceptibility (more insect population) reaction while, in-build resistance mechanism(s) in the host plant leads to unsuitability by same insect. A perusal of data depicted in Table 1 revealed that the population build-up of WBPH on rice plants subjected to various application of Zn treatments varied significantly among the treatments after a constant time interval. In the present study, population build up of WBPH was found to be highest in the control treatment (79.2 per hill) which was significantly very high than all other zinc treatments. The lowest population of WBPH (31.40) was visualized in T_6 which was statistically different from all other treatments. Even the treatment T_6 revealed nearly 60 % less population when compared with control. Closely following T6, the treatment T_7 and T_8 supported 38.20 and 41.60 insects respectively as compared to the control. There was variation between T_1 and T_2 but T_2 did not differ from T_3 and T_4 treatments. Earlier workers (Rath, 2004; Dash and Mishra, 2009) have also witnessed low population build-up of WBPH in rice being subjected to zinc application. In this context, increased silica and zinc content in plants is reported to offer induced resistance to stem borer in paddy (Chandramani *et al.,* 2010; Dash *et al.,* 2011, Madhuri *et al.,* 2017).

Total soluble protein content of leaves of rice cv. TN 1 was estimated to be 8.76 mg g^{-1} in the untreated control, but it was increased in all zinc treatments except T_1 which was at par with control. Further, it is worth to note that protein content (Table 1) was highest in T_6 (10.51 mg g^{-1}) and it was at par with T_8 (10.43 mg g^{-1}) and T_7 (9.93 mg g^{-1}). Interestingly, these treatments revealed less pest population build-up of WBPH. Similarly, Hori and Atalay (1980) observed increase in soluble protein content up to the 3rd day after infestation by Lygus disponsi bug in cabbage. The initial increase in protein content may be due to over expression of defense related proteins. However, exact role of Zn application in variation of soluble protein content is not clear in this study. In contrast, Raghumoorthy and Gunathilagaraj (1988) reported decrease in total seed proteins in the resistant rice varieties CO1, CO24 and CO32 to angoumois grain moth.

Similar results have also been reported on corn leaves of resistant and susceptible varieties by Beck *et al.* (1983). A slight decrease in the total protein content was invariably revealed in

Table 1: Impact of zinc application on total soluble protein content and population build-up of white backed plant hopper (WBPH) in rice

Treatments	Total soluble protein (mg g^{-1})	Population Build-up/plant*
T_1: $ZnSO_4$ basal (25 kg ha^{-1})	8.89^{c}	53.60^{b}
T_2: Zn EDTA basal (40 kg ha^{-1})	9.15^{bc}	51.20^{bc}
T_3: ZnSO4 foliar spray @ 0.5% twice at 30 and 45 DAT	9.31^{bc}	49.80^{bc}
T_4: Zn EDTA Foliar spray @ 0.8% twice at 30 and 45 DAT	9.62^{abc}	49.00^{cd}
T_5: ZnSO4 basal (25 kg ha^{-1}) + $ZnSO_4$ foliar spray @ 0.5% twice at 30 and 45 DAT	9.67^{abc}	45.20^{de}
T_6: Zn EDTA basal (40 kg ha^{-1})+ Zn EDTA Foliar spray@ 0.8% twice at 30 and 45 DAT	10.51^{a}	31.40^{g}
T_7: ZnSO4 basal(25 kg ha^{-1})+ Zn EDTA Foliar spray @ 0.8% twice at 30 and 45 DAT	9.93^{ab}	38.20^{f}
T_8: Zn EDTA basal (40 kg ha^{-1}) + $ZnSO_4$ foliar spray @ 0.5% twice at 30 and 45 DAT	10.43^{a}	41.60^{ef}
T_9: Control	8.76^{c}	79.20^{a}
$SE_{(m)}\pm$	0.327	1.406
C.D.(0.05)	0.97	4.03

*Mean of five replications. Means followed by same letter are not significantly different from each other

Table 2: SDS-PAGE polypeptide banding pattern of total soluble protein samples extracted from rice leaves after application of zinc

Poly-peptide band	**Mol. Wt. (kDa)**	**T_1 $ZnSO_4$ basal (25 kg ha^{-1})**	**T_2 Zn EDTA basal (40 kg ha^{-1})**	**T_3 $ZnSO_4$ foliar spray (0.5%) (30 and 45 DAT)**	**T_4 Zn EDTA foliar spray (0.8%) (30 and 45 DAT)**	**T_5 (T_1+T_3)**	**T_6 (T_2+T_4)**	**T_7 (T_1+T_4)**	**T_8 (T_2+T_3)**	**T_9 (Control)**	**Total bands**
B1	97.4	1	1	1	1	1	1	1	1	1	9
B2	90.0	1	1	1	1	1	1	1	1	1	9
B3	85.5	0	1	1	1	1	1	1	1	1	8
B4	78.0	1	1	1	1	1	1	1	1	1	9
B5	72.2	1	1	1	1	1	1	0	1	1	8
B6	66.0	1	0	0	0	0	1	1	1	0	4
B7	56.8	0	1	1	1	1	1	1	1	1	8
B8	40.2	1	1	1	1	1	1	1	1	1	9
B9	37.0	1	0	0	0	0	1	1	1	0	4
B10	35.0	0	1	1	1	1	1	1	1	1	8
B11	33.0	1	0	0	0	0	0	0	0	0	1
B12	29.0	0	1	1	1	1	1	1	1	1	8
B13	27.3	1	1	1	1	1	1	1	1	1	9
B14	25.1	1	0	0	0	0	0	0	0	0	1
B15	23.6	0	0	0	0	0	1	1	0	0	2
B16	22.0	0	1	1	1	1	0	1	0	0	5
B17	20.1	0	1	1	0	0	0	0	0	0	2
B18	15.8	0	0	0	1	1	1	0	1	0	4
B19	14.3	0	1	1	1	1	1	1	1	0	7
Total bands		10	13	13	13	13	15	14	14	10	115
WBPH population build- up		**53.60**	**51.20**	**49.80**	**49.00**	**45.20**	**31.40**	**38.20**	**41.60**	**79.20**	

N.B.: Presence or absence of polypeptide bands marked by 1 and 0 respectively

Table 3: Similarity coefficient between zinc treatments for induced resistance to white backed plant hopper (WBPH) in rice

Treatments	**T_1 $ZnSO_4$ basal (25 kg ha^{-1})**	**T_2 Zn EDTA basal (40 kg ha^{-1})**	**T_3 $ZnSO_4$ foliar spray (0.5%) (30 and 45 DAT)**	**T_4 Zn EDTA foliar spray (0.8%) (30 and 45 DAT)**	**T_5 T_1 + T_3**	**T_6 T_2 + T_4**	**T_7 $T_{1+}T_4$**	**T_8 $T_{2+}T_3$**	**T_9 Control**
T_2 : Zn EDTA basal (40 kg ha^{-1})	0.35								
T_3 : $ZnSO_4$ foliar spray (0.5%) (30 and 45 DAT)	0.35	1.00							
T_4 : Zn EDTA foliar spray (0.8%) (30 and 45 DAT)	0.35	0.86	0.86						
T_5 : T_1 + T_3	0.35	0.86	0.86	1.00					
T_6 : T_2 + T_4	0.47	0.65	0.65	0.75	0.75				
T_7 : T_1 + T_4	0.41	0.69	0.69	0.69	0.69	0.81			
T_8 : T_2 + T_3	0.50	0.69	0.69	0.80	0.80	0.93	0.75		
T_9 : Control	0.43	0.77	0.77	0.77	0.77	0.67	0.60	0.71	

the leaf folder infested resistant and moderately resistant genotypes (Ptb 33, TKM6, LFR831311, ASD16, *O. minuta* and *O. rhizomatis*) of rice as compared to control healthy plants (Punithavalli *et al.,* 2013). Thus, the present finding indicated that a sub-set of protein content indirectly influence insect resistance in rice plants. Plants respond to insect attack through a variety of defense mechanisms which may be either morphological adaptation (trichomes, pubesence, waxy cuticle), or elicitation of biochemical and molecular mode of defense systems (Belete, 2018). Induction of defense enzymes, bio-chemicals and resistant proteins by insect feeding has been reported in many insect-plant interactions (Radja Commare *et al.,* 2002). Host plant

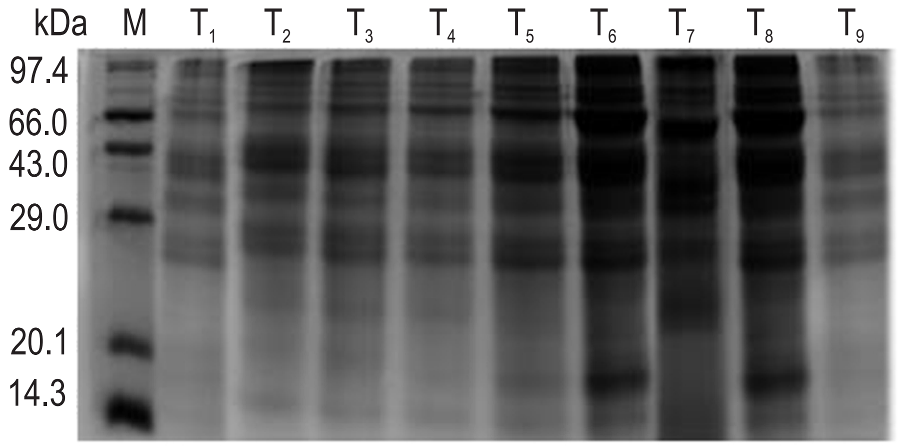


Fig. 1: SDS-PAGE soluble protein profile of various Zn treatments in rice cv. TN 1 for elucidation of induced host plant resistance to WBPH. T1-T9: various Zn treatments (basal and/or foliar applications), M- Molecular weight marker.

resistance can be developed by appropriate breeding strategies. Besides, adequate level of resistance can be engineered genetically to build up endogenous defense biomolecules within the host plants to confer resistance to insects (Gatehouse, 2013). Many often, in absence of above inherent insect resistance mechanisms; induced resistance can be elicited in the host plants by use of chemical elicitors of secondary metabolites, potassic fertilizers or even chemical compounds containing silicon, zinc and ferrous. Zinc is needed for plant growth and resistance to biotic and abiotic stresses. Besides, zinc seems to be a major player in defense related response in plants (Gupta *et al.,* 2012). Sufficient accumulation of zinc (either through uptake from soil or by foliar spray) followed by its cellular sequestration make the plants climatically more smart (abiotic stress tolerant), but the underlying mechanism is not explicitly delineated. It activates/stabilizes the activity of a number of metalloenzymes (Fones and Preston, 2012). Zn-SOD (super oxide dismutase) activity is commonly increased in herbivore/pathogen-challenged plants (Deepak *et al.,* 2006). Besides, insect infestation in vogue elicits ZFN-transcriptional factor(s) synthesis (Lawrence *et al.,* 2014) to impart host plant resistance.

Recent advances in microarray and proteomic approaches have revealed that a wide spectrum of plant resistance protiens is involved in plant defense against insects (Belete, 2018). Direct defenses, such as trypsin protein inhibitors in rice has anti-digestive or toxic effects on insect herbivores (Qi *et al.,* 2018). In the present context, protein profile of various treatments exposed to different zinc fertilizer in pot culture experiment were analysed by SDS-PAGE method to ascertain whether zinc is responsible for production of any new defensive protein against WBPH or not. Altogether, nineteen polypeptide bands were revealed in response to extraneous application of zinc in form of basal and foliar application of $ZnSO_4$ and Zn EDTA. Polypeptide bands i.e. B1(97.4kDa), B2 (90kDa), B4 (78 kDa), B8 (40.2kDa) and B13 (27.3kDa) in the zymogram are monomorphic over all the treatments and control indicating their expression independent of the zinc application (Table 2 and Fig. 1). It was observed that polypeptide bands of 29.0 kDa, 35.0 kDa, 56.8 kDa and 85.5 kDa were absent in basal application of $ZnSO_4$, but induced in all treatments and even in control. In contrast, 33.0 kDa and 25.1 kDa polypeptides were induced by basal application of $ZnSO_4$, but down regulated in all treatments including control. Zinc uptake in case of basal application is determined by presence of Zn-transporter genes in root cells followed by transport to stem and foliage, while foliar application can bypass such genetic system and avail zinc directly to the enzymes required for plant growth and metabolism (Zhao *et al.,* 2014). Basal application of Zn EDTA and also foliar application of $ZnSO_4$ alone produced a polypeptide band of 20.1 kDa.

Two polypeptide bands *viz.,* 37.0 kDa and 66 kDa were induced in $ZnSO_4$ basal, but not expressed in control as well as in response to its foliar spray alone or its additional application as foliar spray. Even these two polypeptide bands were also not expressed in Zn-EDTA basal or foliar spray alone, but induced by combination of basal + foliar spray of Zn-EDTA as well as combination of basal $ZnSO_4$ + foliar Zn EDTA or vice versa. The low molecular weight proteins ranging from 14.3-25.1 kDa were clearly absent in the control, but zinc application in form of above sources as basal or foliar treatment elicited biosynthesis of new polypeptide bands. For instance, a polypeptide band (14.3 kDa) was noticed in all the zinc treatments except $ZnSO_4$ basal. Besides, foliar spray of Zn-EDTA in combination with basal application of either $ZnSO_4$ or Zn-EDTA induced new polypeptide band at 23.6 kDa. In the present investigation, elucidation of the induced polypeptide bands revealed in response to various treatments of zinc-application may throw light for better understanding about the biochemical basis of induced host plant

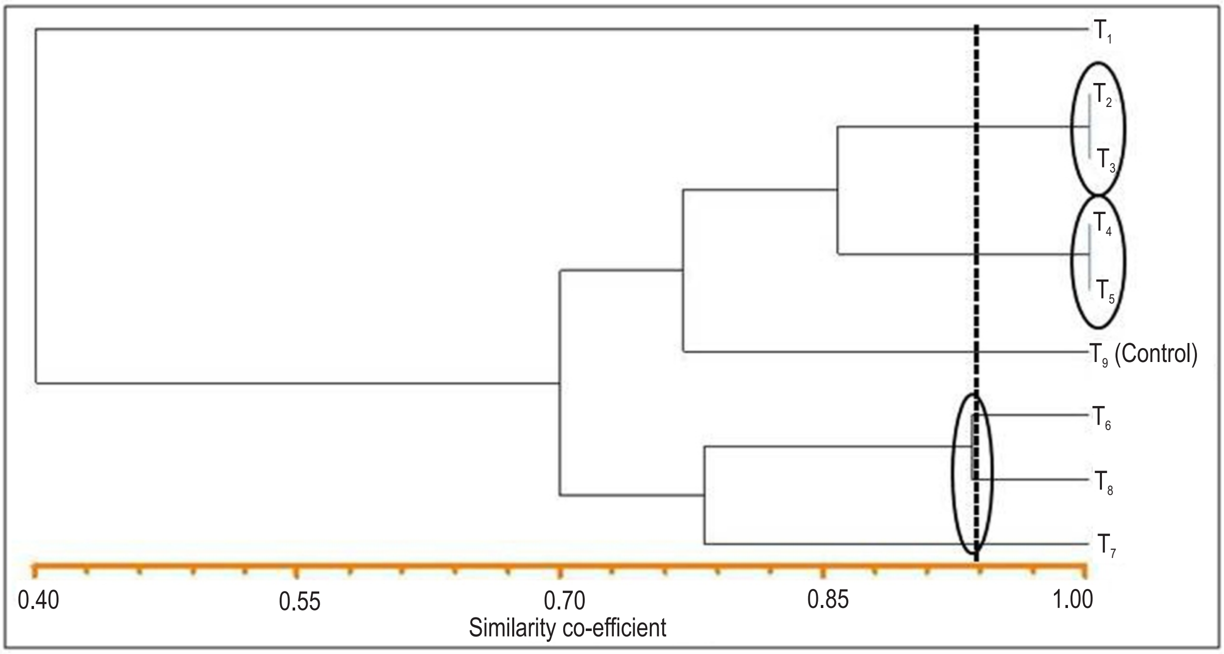


Fig. 2: Dendrogram depicting inter-relationship among various Zn treatments for induced resistance to WBPH in rice.

resistance to WBPH. Sinha *et al.* (2005) revealed over-expression of high molecular weight protein (>97kD) and a specific 38 kD polypeptide band in leaf folder infested resistant parent as well as resistant RILs derived from a cross IR 36 (susceptible) x TNAULFR 831311 (Resistant) in rice. Thus, the induced polypeptide bands identified in the present investigation may serve as biochemical marker for WBPH resistance in rice.

WBPH is a dreadful sucking insect of rice and it usually attacks at culm base of rice plant at tillering and flowering stage causing drastic reduction in crop growth and seed yield (CRIDA,2019). Zinc application either as basal and/or foliar application reduced the population build up of WBPH ranging from 31.40 to 53.60 insects/hill as against control (79.20 insects/hill). Combination treatment of basal and foliar application of zinc formulation in the form of Zn EDTA (T_6 : $T_2 + T_4$) resulted maximum dividend followed by T_7 ($T_1 + T_4$) and T_8 ($T_2 + T_3$) in term of decrease in WBPH population. In this context, it is worth to note that the most responsive zinc combination treatment (T_6) elicited highest number (15) of polypeptide bands against ten normal protein bands in the control. Expression of five new polypeptide bands at 66.0, 37.0, 23.6, 15.8 and 14.3 kDa induced by T_6 can be considered as biochemical basis of induced resistance in rice against WBPH. Among these, 66.0, 37.0, 23.6 and 14.3 kDa protein bands were induced by T_7 and 66.0, 37.0, 15.8 and 14.3 kDa protein bands induced by T_8, were common to that of T_6, which were new types and not expressed in control treatment. Further, the soluble protein profiling study revealed that 66.0kDa, 37.0 kDa and 14.3 kDa polypeptide bands were significantly induced and commonly shared in T_6, T_7 and T_8 that recorded lower WBPH population. However, 23.6 kDa polypeptide band induced only in T_6 and T_7 seems to have greater role in manifestation of induced resistance to WBPH in rice. Induction of a defense related protein (53 kDa), due to leaf folder infestation in resistant and moderately resistant rice varieties have been reported by Das *et al.* (1999). Similarly, Sinha *et al.* (2005) and Punithavalli *et al.* (2013) have also noticed enhanced expression of a high molecular weight (> 97 kDa) protein in all the genotypes but there was an increased induction of a 38 kDa protein in leaf folder infested resistant rice genotypes, which was absent in uninfested plants. They reported these as a defense related proteins. Defence related proteins encoded by R genes play critical roles in plant resistance to insects and pathogens (Dodds and Rathjen, 2010). More than 25 R genes related to rice defense against BPH have been found (Cheng *et al.,* 2013, Zhao *et al.,* 2016).

A novel protein encoded by Bph 32 gene that offers resistance to BPH in rice has been identified (Ren *et al.,* 2016). Therefore, the present finding lies in conformity with the finding of above authors. Further, clustering pattern (dendrogram) (Fig. 2) based on pair-wise similarity coefficient values (Table 3) revealed that T_1, T_9 (Control), T_6, T_7 and T_8 formed single treatment cluster while, both T_2 and T_3 together and also T_4 and T_5 combinedly formed separate clusters at 100% phenon level. Had there been fine tuning of the protein profiling, T_2 and T_3 might be separated and this difference could have been interpreted in terms of per cent damage difference between T_2 and T_3. Similarly, T_4 and T_5 could have been differentiated as difference between these was revealed in field and pot culture experiment. However, the difference between T_4 and T_5 was not so much vigilant as compared to their effect with either of T_6, T_7 or T_8, which revealed higher induction of host plant resistance to WBPH. Thus, plants respond to physical and chemical changes associated with insect feeding which in turn influences on the degree of damage by WBPH infestation. In the present study, low pest population build-up consequent with repression of few specific poypeptides and/or elicitation of new polypeptides induced in protein profile of WBPH-challenged rice plants following Zn application clearly indicates Zn-induced host plant resistance to the pest.

Acknowledgements

The financial assistance provided by the Department of Science and Technology (DST), Govt. of India, New Delhi for the research work in terms of INSPIRE Fellowship to the first author is highly acknowledged. Further, Laboratory facilities provided by the Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar is gratefully acknowledged.

Add-on Information

Authors' contribution: S. Tripathy: Contribution: Carried out the experiment and recorded data; **L. K. Rath:** Conceptualized and designed the experiment; **S. K. Tripathy:** Carried out data analysis and wrote the paper.

Research content: The research contents is original and has not been published elsewhere

Ethical approval: Not Applicable.

Conflict of interest: The author declares that there is no conflict of interest.

Data from other sources: Not Applicable

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

References

Andreini, C., I. Bertini and A. Rosato: Metalloproteomes: A bioinformatic approach. *Acc. Chem. Res.,* **42**, 1471-1479 (2009).

Beck, D. L., G.M. Dunn, D.O. Routley and J.S. Bowman: Biochemical basis of resistance in corn to the corn leaf aphid. *Crop Sci.,* **23**, 995-998 (1983).

Belete, T.: Defense mechanisms of plants to insect pests: From morphological to biochemical approach. *Tren. Techni. Sci. Res.,* **2**, 30-37(2018).

Cabot, C., S. Martos, M. Lugany, B. Gallego, R. Tolrà and C. Poschenrieder: A role for zinc in plant defense against pathogens and herbivores. *Fron. Plant Sci.,* **10**, 1171(2019).

Chandramani, P., R. Rajendran, C. Muthiah and C. Chinniah: Organic source induced silica on leaf folder, stem borer and gall midged population and rice yield. *J. Biopesti.,* **3**, 423-427(2010).

Chang, R.L., R.J. Arnold and X.D. Zhou: Association between enzyme activity levels in Eucalyptus clones and their susceptibility to the gall wasp, *Leptocybe invasa,* in South China. *J. Tropi. Fore. Sci.,* **24**, 256-264 (2012).

Cheng, X.Y., L.L. Zhu and G.C. He: Towards understanding of molecular interactions between rice and the brown planthopper. *Molecular Plant,* **6**, 621-634 (2013).

Das, A., S. Balasaraswathi, K. Sadasivam and S. Palaniswamy: Biochemical variations between leaf folder (*Cnaphalocrosis medinalis* Gunee) resistant and susceptible rice (*Oryza sativa* L.) varieties in healthy and infested plants. *Indian J. Agricul. Biochem.,* **12**, 1-4 (1999).

Dash, D. and P.R. Mishra: Influence of rice varieties and graded levels of plant nutrients on the effectiveness of insecticide against white backed plant hoppers (WBPH), *Sogatella furcifera* (Horvath). *J. Entomol. Res.,* **33**, 105-108 (2009).

Dash, D., B. Senapati and H.P. Patnaik: Influence of rice varieties and plant nutrients on the incidence of yellow stem borer, *Scirpophaga incertulas* (Walker) under protected condition. *Indian J. Entomol.,* **73**, 196-200 (2011).

Dash, D., L.K. Rath and B.K. Mishra: Studied on nutrient status in rice foliage and its relationship with leaf folder and brown plant hopper incidence. *Indian J. Plant Prote.,* **35**, 243-247(2007).

Deepak, S. A., H. Ishii and P. Park: Acibenzolar-S-methyl primes cell wall strengthening genes and reactive oxygen species forming/scavenging enzymes in cucumber after fungal pathogen attack. *Physiol. Molec. Plant Pathol.,* **69**, 52–61 (2006).

Dodds, P.N. and J.P. Rathjen: Plant immunity: Towards an integrated view of plant-pathogen interactions. *Natu. Revi. Gene.,* **11**, 539-548 (2010).

Duncan, D.B.: Multiple range and multiple F-tests. *Biometrics,* **11**, 1- 42 (1955).

Edwards, P.J. and S.D. Wratten: Wound induced defences in plant and their consequences for patterns of insect grazing. *Oecologia,* **59**, 88-93 (1983).

Fones, H.N. and G.M. Preston: Reactive oxygen and oxidative stress tolerance in plant pathogenic *Pseudomonas. FEMS Microbiol. Lett.,* **327**, 1-8 (2012).

Gatehouse, J.A.: Genetic engineering of crops for insect resistance. In: Sustainable Food Production. (Eds.: P. Christou, R. Savin, B.A. Costa-Pierce, I. Misztal and C.B.A. Whitelaw). Springer, New York, (2013). doi: https://doi.org/10.1007/978-1-4614-5797-8_239

Gupta, S.K., A.K. Rai, S.S. Kanwar and T.R. Sharma: Comparative analysis of zinc finger proteins involved in plant disease resistance. *PLoS ONE*, **7**, e42578 (2012). doi: 10.1371/journal. pone. 0042578

Heinrichs, E.A., F.G. Medrana and H.R. Rapusas: Genetic evaluation for insect resistance in rice. Int. Rice Res. Institute, Los Banos, Philippines, pp. 356 (1985).

Hori, K. and R. Atalay: Biochemical changes in the tissue of chinese cabbage injured by the bug *lygus disponsi*. *Appl. Entomol. Zool.,* **15**, 234-241(1980).

Jaccard, P.: Nouvelles recherches Sur la distribution florale. *Bull. Soc. Vaud Sci. Natio.,* **44**, 223-270(1908).

Kaur, R., A.K. Gupta and G.K. Taggar: Zinc as an important factor determining resistance against *Helicoverpa armigera* herbivory in pigeon pea (*Cajanus cajan* L.). *Curr. Sci.,* **107**, 1871(2014).

Khederi, S. J., M. Khanjani, M. Gholami, O. Panzarino and E. de Lillo: Influence of the erineum strain of *Colomerus vitis* (Acari: Eriophyidae) on grape (*Vitis vinifera*) defense mechanisms. *Exp. Appli. Acarol.,* **75**, 1-24 (2018).

Krishnaiah, N.V., V. Jhansilakshmi, I.C. Pasalu, G.R Katti and C. Padmavathi: Insecticides in Rice IPM: past, present and future. *Directorate of Rice Research, Hyderabad,* pp. 146 (2008).

Lagrimini, L.M. and S. Rothstein: Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. *Plant Physiol.*, **84**, 438-442 (1987).

Lawrence, S.D., N.G. Novak, R.W. Jones, R.R. Farar and M.B. Blackburn: Herbivory responsive C2H2 zinc finger transcription factor proteins StZFP2 from potato. *Plant Physiol. Biochem.,* **80**, 226-233 (2014).

Lowry, D.H., N.J. Rosebrough, A.L. Farr and J.L. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chemi.,* **193**, 265-275 (1951).

Madhuri, G., P.C. Dash and P. Pruthvi : Impact of different graded levels of fertilizers on the incidence of yellow stem borer *Scirpophaga incertulas* (Walker), leaf folder *Cnaphalocrocis medinalis* (Guenee) and whorl maggot, *Hydrellia philippina* (Ferino) in paddy. *Int. J. Curr. Microbiol. Appli. Sci.,* **6**, 793-803 (2017).

Marreiro, D.D., K.J. Cruz, J.B. Morais, J.B. Beserra, J.S. Severo and A.R. de Oliveira: Zinc and oxidative stress: Current mechanisms. *Antioxidants (Basel)*, **6**, 24 (2017).

Punithavalli, M., N.M. Muthukrishnan and M. Balaji Rajkumar: Defensive responses of rice genotypes for resistance against rice leaf folder, *Cnaphalocrocis medinalis*. *Rice Sci.,* **20**, 363-370 (2013).

Qi, J., S. Malook, G. Shen, L. Gao, C. Zhang, J. Li, J. Zhang, L. Wang and J. Wu: Current understanding of maize and rice defense against insect herbivores. *Plant Divers.,* **40**, 189-195 (2018).

Radja Commare, R., R. Nandakumar, A. Kandan, S. Suresh, M. Bharathi, T. Raguchander and R. Samiyappan: *Pseudomonas fluorescences* based bio-formulation for the management of sheath blight and leaf folder insect in rice. *Crop Prote.,* **21**, 671-677 (2002).

Raghumoorthy, K.N. and K. Gunathilagaraj: Field incidence of and host resistance to Angoumois grain moth (AGM). *Int. Rice Res. Newsletter,* **13**, 12 (1988).

Rath, L.K.: Micronutrient induced antibiosis in rice against brown plant hopper, *Nilaparvata lugens* (Stal). *J. Plant Prote. Environ.,* **3**, 29-33 (2006).

Rath, L.K.: Influence of certain micronutrients on the growth and development of white backed plant hopper, *Sogatella furcifera* (Horvath) infesting rice. *J. Plant Prote. Environ.,* **1**, 19-23 (2004).

Ren, J., F. Gao, X. Wu, X. Lu, L. Zeng, J. Lv, X. Su, H. Luo and G. Ren: Bph32, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. *Scientific Reports,* **6**, Article No- 37645 (2016).

Satpathy, S., B.S. Gotyal and V. Ramesh Babu: Role of novel insecticides in crop protection and their selectivity to natural enemies: A review. *J. Environ. Biol.,* **41**, 149-160 (2020).

Sidhu, G.S.: Need for varieties resistant to white backed plant hopper in Punjab. *Int. Rice Res. Newsletter,* **14**, 6-7(1979).

Sinha, S., R. Balasaraswathi, K. Selvaraju and P. Shanmugasundaram: Molecular and biochemical markers associated with leaf folder (*Cnaphalocrocis medinalis* G.) resistance in rice (*Oryza sativa* L.). *Indian J. Bioche. Biophy.,* **42**, 228–232 (2005).

Sogawa, K., G. Liu and Q. Qiang: Prevalence of white backed plant hoppers in Chinese hybrid rice and white backed plant hopper resistance in Chinese japonica rice. In: Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia. (Eds.: K.I. Heong and B. Hardy), Los Banos (Philippines), pp. 257-280 (2009).

Sokal, R.R. and C.D. Michener: A statistical method for evaluating systematic relationships. University Kansas Science Bulletin, **28**, 1409-1438 (1958).

Zhai, H.W., Y.F. Zhao, X.W. Gao and M.X. Wu: Efficacy of *Empedobacter brevis* in controlling rice stem borer. *Bio. Disas. Sci.,* **36**, 291-294 (2013).

Zhao, A.Q., X.H. Tian, Y.X. Cao, X.C. Lu and T. Liu: Comparison of soil and foliar zinc application for enhancing grain zinc content of wheat when grown on potentially zinc-deficient calcareous soils. *J. Sci. Food Agricul.,* **94**, 2016-2022 (2014).

Zhao, Y., J. Huang and Z.Z. Wang: Allelic diversity in an NLR gene BPH9 enables rice to combat planthopper variation. In: Proceedings of National Academy of Sciences, USA, **113**, 12850-12855 (2016).