

**UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.**

1. Project report No. 1st/2nd/3rd/Final : Final Report
2. UGC Reference No. : F.No. 41-559/2012(SR)
3. Period of report : from 1.07.2012 to 31.12.2015.
4. Title of research project : **“Identification and characterization of *Xa21* and *xa13* alleles conferring disease resistance to *Xanthomonas oryzae* pv *oryzae* from wild rice species *Oryza nivara*”**
5. (a) Name of the Principal Investigator : **Dr. Irfan Ahmad Ghazi**
 (b) Deptt. : Department of Plant Sciences,
 (c) University where work has progressed : University of Hyderabad, Hyderabad
6. Effective date of starting of the project : 1.07.2012
7. Grant approved and expenditure incurred during the period of the report:
 - a. Total amount approved Rs. : 7,22,500/-
 - b. Total expenditure Rs. : **6,57,147/-**
- c. Report of the work done : Separate sheet is attached
- i. Brief objective of the project:
 1. Expression analysis of *Xa21* and *xa13* alleles from *Oryza nivara* by semi-quantitative PCR.
 2. Cloning and sequencing of coding region of *Xa21* and *xa13* alleles.
 3. Study of host plant's response after infection with *Xanthomonas oryzae* through biochemical analysis, enzymatic assays and confocal studies.
- ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication : **One manuscript is published in Australian Journal of Crop Science**
 Kumar A, Gul MZ, Zeeshan A, Bimolata W, Qureshi IA, **Ghazi IA**. *Differential antioxidant responses of three different rice genotypes during bacterial blight infection. Australian Journal of Crop Science* 2013, 7(12): 1893-1900.
- ii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons

There are slight modifications in the project as we didn't get the preliminary expected result. We have included a new gene and also one more rice wild variety for further analysis and got significant results.

iv. Please indicate the difficulties, if any, experienced in implementing the project: In a preliminary screening analysis with marker, presence of resistance *Xa21* allele was not detected in *O. nivara* accessions used in our study. Hence, *Oryza longistaminata* is also included in our study.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet : Project is Completed and Separate sheet is attached.

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to the Commission : Project is completed and final report is attached.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

- a) One PhD and Two MSc students has been trained during this project
- b) PhD Awarded: One of the student has been awarded with PhD degree in which partial data from this project was used.
- c) One manuscript is Published with UGC acknowledgement.

**SIGNATURE OF THE
PRINCIPAL INVESTIGATOR**

**SIGNATURE OF THE
COINVESTIGATOR**

REGISTRAR

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

1. Title of the Project : **Identification and Characterization of *Xa21* and *Xa13* alleles conferring disease resistance to *Xanthomonas oryzae pv oryzae* from wild species *Oryza nivara*.**
2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR: Dr. Irfan Ahmad Ghazi, Dept. of Plant Sciences, University of Hyderabad, Hyderabad-500046.
3. NAME AND ADDRESS OF THE INSTITUTION: University of Hyderabad, Gachibowli, Prof CR Rao Road, Hyderabad-500046.
4. UGC APPROVAL LETTER NO. AND DATE: F.No. 41-559/2012(SR) and 18th July 2012.
5. DATE OF IMPLEMENTATION : 1st July 2012
6. TENURE OF THE PROJECT : Three Years

7. TOTAL GRANT ALLOCATED : Rs. 7,22,500/-

8. TOTAL GRANT RECEIVED : Rs. 6,55,000/-

9. FINAL EXPENDITURE : Rs. 6,57,147/-

6. 10. TITLE OF THE PROJECT : **Identification and Characterization of *Xa21* and *Xa13* alleles conferring disease resistance to *Xanthomonas oryzae pv oryzae* from wild species *Oryza nivara*.**

11. OBJECTIVES OF THE PROJECT :

- i. Expression analysis of *Xa21* and *xa13* alleles from *Oryza nivara* by semi-quantitative PCR.
- ii. Cloning and sequencing of coding region of *Xa21* and *xa13* alleles.
- iii. Study of host plant's response after infection with *Xanthomonas oryzae* through biochemical analysis, enzymatic assays and confocal studies.

12. WHETHER OBJECTIVES WERE ACHIEVED : Yes (Detail Report attached)

13. ACHIEVEMENTS FROM THE PROJECT :

- A. It was found that there were 121 polymorphic sites present in the promoter region of *xa13* gene.
- B. With reference to Os11N3, *Oryza nivara* possesses 95% and *Oryza longistaminata* possesses 89% sequence identity.
- C. One PhD and Two MSc students have been trained during this project

14. SUMMARY OF THE FINDINGS (IN 500 WORDS) :

We perform the characterization of Xa21 and xa13 alleles from wild species *Oryza nivara* and *Oryza longistaminata*. The genes of Xa13 were aligned from 10 different genotypes including IRBB13 and IR24. There were 121 polymorphic sites in the promoter region however, we didn't find any type of nucleotide changes in the UPT region among different accessions other than IRBB3. During Xoo infection in wild rice *O. nivara* 81832 and *O. longistaminata* OL1, these genes were analyzed. We checked the expression pattern of these genes in both varieties. These two accessions were selected for expression analysis based on the sequence analysis and genotypic screening analysis with pTA248 marker. IRBB21 and IR24 were used as positive and negative control. We also checked the chlorophyll content in three varieties (IRBB21, OLD and PB1) and found that bacterial blight (BB) disease led to decrease in Chl (a + b) content in all three genotypes due to lesion formation. The change in total chlorophyll content of PB-1 was minimal at first two stages of infection, however, this genotype showed drastic reduction on 15th DAI (1.53 ± 0.05 mg/g fw). Similar trend was observed with that of carotenoid content in all the three genotypes. In context with total polyphenol and flavonoid content the infected OL1 substantially produced double the amount of phenolics and flavonoids in comparison to control at 5 day after infection (DAI). The phenolic content reduced dramatically at the later stage of infection in all the three genotypes. The total antioxidative capacity of three rice genotypes sharply increased during initial stage of stress period. The effect of BB stress on the activities of total antioxidants participating in the scavenging of ROS which showed gradual decrease in all the three genotypes. Similar patterns of ferric reducing power (increased activity during first stage followed by decreased activity at the later stages) were also observed for all the three genotypes. The free amino acid pool did not change very much in control samples during the entire period of investigation, while in stress induced plants, total free amino acid content increased with increasing time period of stress in all three genotypes particularly at 15th day of infection. CAT activity increased significantly upon exposure to BB infection in all three genotypes. The highest activity was observed in IRBB21 at all time points followed by OL and PB-1 respectively. The peroxidase activity showed significant increase with regard to biotic stress in comparison to control plants.

15. CONTRIBUTION TO THE SOCIETY : (GIVE DETAILS)

- a. We found that the presence favourable form of R gene is limited to geographical region, related species and cultivars.
- b. Manpower trained from this project

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT: Yes

17. NO. OF PUBLICATIONS OUT OF THE PROJECT : One (attached)

FINAL REPORT

Title of the Project: Identification and characterization of *Xa21* and *xa13* alleles conferring disease resistance to *Xanthomonas oryzae* pv *oryzae* from wild rice species *Oryza nivara*

UGC Reference No. : F.No. 41-559/2012(SR)

Period of report : 1.07.2012 to 31.12.2015.

Summary of Work done in the Project:

1. Polymorphism analysis of UPT box of *Xa13* and *Os11N3* locus
2. Polymorphism and expression analysis of *Xa21* alleles in different accessions of *Oryza species*
3. Differential antioxidants responses of 3 different rice genotypes during bacterial blight infection
4. Enzymatic scavenging activity in *Oryza longistaminata*

Xa21 is the first BB *R* gene cloned with the help of map based positional cloning strategy by Song et al. (1995) which is 3.9 Kb in size. It was mapped on the long arm of chromosome 11 of rice. It is a broad spectrum disease resistance gene which was introgressed from wild species *O. longistaminata* into *O. sativa* (IR24) background. This gene codes for receptor like kinases with hydrophobic N terminal and four major domains, LRR, transmembrane, juxtamembrane (JM) and intracellular kinase domains. We have performed its polymorphism and expression analysis in accessions of *Oryza nivara* and *Oryza longistaminata*.

Xa13 (Os8N3) is susceptible in dominant form and susceptible in recessive form (*xa13*). The difference between *Xa13* and *xa13* lies in the UPT box of PthXo1 (recognition/binding site of *Xoo* effector PthXo1) present in the promoter of this gene. Due to the insertion of around 300 bp in *xa13* UPT_{PthXo1}, *xa13* doesn't recognize *Xoo* with effector PthXo1, its expression is not induced and hence, confer resistance against the pathogen. Similarly, *Os11N3* is also a susceptible gene whose expression is induced by the binding of effector AvrXa7/PthXo3 to the UPT box present in the promoter of this gene. Hence, we were interested in looking for presence of any natural mutations in their respective UPT boxes among different cultivated and wild

species of rice. If there are any mutations, that may help the plant in resistance against the disease.

Methodology

Plant material, Genomic DNA isolation and PCR:

Seventeen different rice genotypes MP, ML, PB1, R23, R47, TN1, GSL34 (*Oryza sativa*), WR14, WR84, WR86, WR102, ON4 (*Oryza nivara*), OL, (*Oryza longistaminata*), OL2 (*Oryza latifolia*), OA1, OA2, (*Oryza alta*), OE1 (*Oryza eichengeri*) and few other accessions of rice were used for polymorphism analysis, and genomic DNA was isolated by Cetyl trimethyl ammonium bromide (CTAB) method for the PCR amplification. Primers were designed covering the UPT box of PthXo1, PthXo3 and AvrXa7 in *Xa13* (*Os8N3*) and *Os11N3*, respectively. List of primer is given in table 1. The promoter region covering the UPT boxes were amplified by PCR from the mentioned genotypes and sequenced.

Table 1: Primer's name and their sequence in 5' -----3'

Primer name	Forward primer	Reverse primer
<i>Xa13</i>	5' TGGCATGTTGGTGTAGTGG 3'	5' CAACTGCATGTGTGGTTTGG 3'
<i>Os11N3</i>	5' CATGGCTGTGATTGATCAGG 3'	5' ATGGCCCCCTCTAATGTAAACC 3'

Sequence analysis:

Xa13 (*Os8N3*) promoter of IR24 and the promoter region of *xa13* in IRBB13 were used as reference for comparative analysis and also *Os11N3* sequence from Nipponbare was used as a reference for *Os11N3* gene. Sequences were subjected to multiple alignments using ClustalW.

RNA isolation and RT-PCR:

Total RNA was isolated following standard protocol from the young infected leaves of IRBB21, IR24, OL1 and OLD. cDNA was prepared using Invitrogen cDNA synthesis kit master mix. The primer used for the expression analysis was forward primer 5' GCTCGATGGGATTTATAGGG 3' and reverse primer 5' GCTTCCCGGTTACTATTTC 3'. Actin was used as an internal control.

Chlorophyll estimation:

On three different days of experiment, leaf discs were taken from five fully expanded leaves of comparable physiological age. Leaf sections were ground in 80% acetone and the total chlorophyll concentration was determined (Arnon et al., 1949).

Determination of non-enzymatic antioxidants

The total phenolic content of the rice leaf extracts were estimated by the Folin- Ciocalteu method (Gul et al., 2011). A slightly modified version of the spectrophotometric method (Barreira et al., 2008) was used to determine the flavonoid content of samples. The antioxidant activity of leaf extracts were evaluated as per the protocol based on the reduction of Mo (VI) – Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto et al., 1999). The ability to reduce ferric ions was measured using the method given by Oyaizu et al., 1986. The DPPH free radical scavenging activity was carried out by employing the standard protocol given by Braca et al., 2002. The total amino acids were quantified by the ninhydrin method (Moore et al., 1968).

Evaluation of enzymatic antioxidants

CAT activity was determined by monitoring the disappearance of H_2O_2 at 240 nm ($\epsilon = 40\text{mM}^{-1}\text{cm}^{-1}$) (Aebi et al., 1984). Glutathione peroxidase (GPX) activity was determined as per the standard protocol (Hemeda et al., 1990). The percentage inhibitions of free radicals were calculated using the formula: $\text{Percentage Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$.

Results

The promoter region of *Xa13* gene was amplified from eight rice accessions using overlapping gene specific primers (Table 1). The size of sequenced alleles ranged from 692 to 800 bp in length.

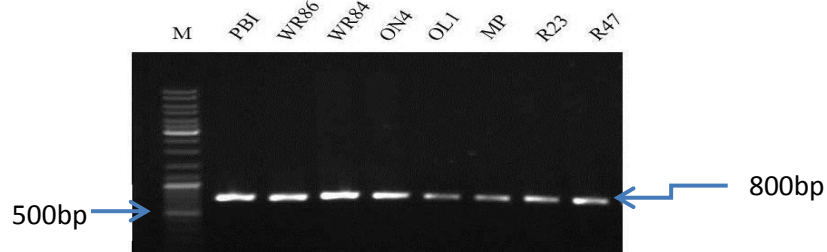


Fig.1 PCR amplification of *Xa13* (*Os8N3*)

Table 2: Sequence identity of seven rice genotypes with reference to IRBB13 and IR24

Species	Accession	Total Sequence(bp)	Identity with reference to IRBB13 (%)	Identity with reference to IR24 (%)
<i>O. sativa</i>	IRBB13	800	-	99
	IR24	702	99	-
	PB1	695	99	98
	MP	748	99	99
	R23	766	92	92
	R47	773	99	98
<i>O. nivara</i>	WR86	695	98	98
	WR84	688	98	98
	ON4	694	99	99
<i>O. longistaminata</i>	OL1	692	87	87

Os11N3 gene was also amplified from fourteen different accession using overlapping gene specific primers (fig. 2). The size of sequenced alleles *Os11N3* ranged from 547 to 673 bp in length. The sequence similarity of the *Os11N3* gene showed more than 90% sequence identity with reference to (Nipponbare susceptible). The sequence similarity of overall alleles is range from 88% to 99%, and it is mention in the table 3.

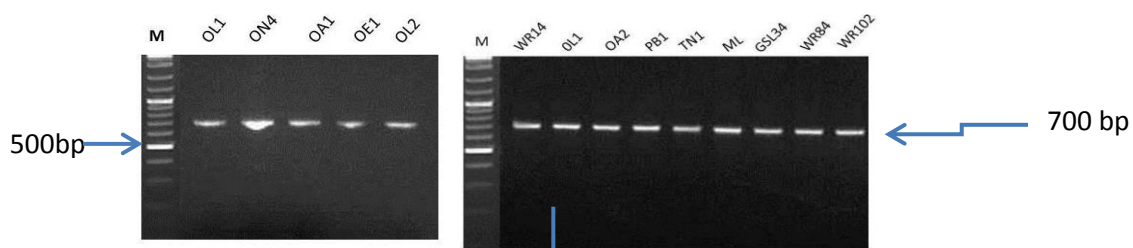


Fig 2: PCR amplification of *Xa13* (*Os8N3*) and *Os11N3* gene from different rice genotypes.

All PCR amplicons were resolved on 1% agarose gel in 1X TAE buffer. (M= Ladder mix)

Polymorphism analysis

The genes of *Xa13* were aligned from 10 different genotypes including IRBB13 and IR24. There were 121 polymorphic sites in the promoter region however, we didn't find any type of nucleotide changes in the UPT region among different accessions other than IRBB3 (Fig.3). Since, there were no changes in this region; all the isolated alleles will be similar to susceptible allele. Hence, expression analysis, cloning and sequencing of this gene from cDNA was not performed.

Os11N3 alleles were also aligned 14 different genotypes and length of total alignment ranged from 547 to 673 bp, including alignment gaps with promoter. It showed 151 polymorphic sites (in total 509 available sites). In the UPT_{AvrXa7} region isolated from different accessions we found nucleotide changes 'T' → 'A' in WR14 (*O. nivara*), 'G' → 'A' in TN1 and MP (both *O. sativa*) as shown in Fig. 4.

Table 3: Sequence identity of seven rice genotypes with reference to *Os11N3*

Species	Accession	Total Sequence(bp)	Identity with reference to <i>Os11N3</i> (%)
<i>O. sativa</i>	MP	547	99
	TN1	642	98
	GSL34	645	99
	ML	635	99
	PB1	644	99
<i>O. nivara</i>	WR14	643	95
	WR102	611	90
	WR84	673	88
	ON4	633	99
<i>O. alta</i>	OA1	640	99
	OA2	641	98
<i>O. eichenegri</i>	OE1	655	99
<i>O. longistaminata</i>	OL1	667	89
<i>O. latifolia</i>	OL2	634	99

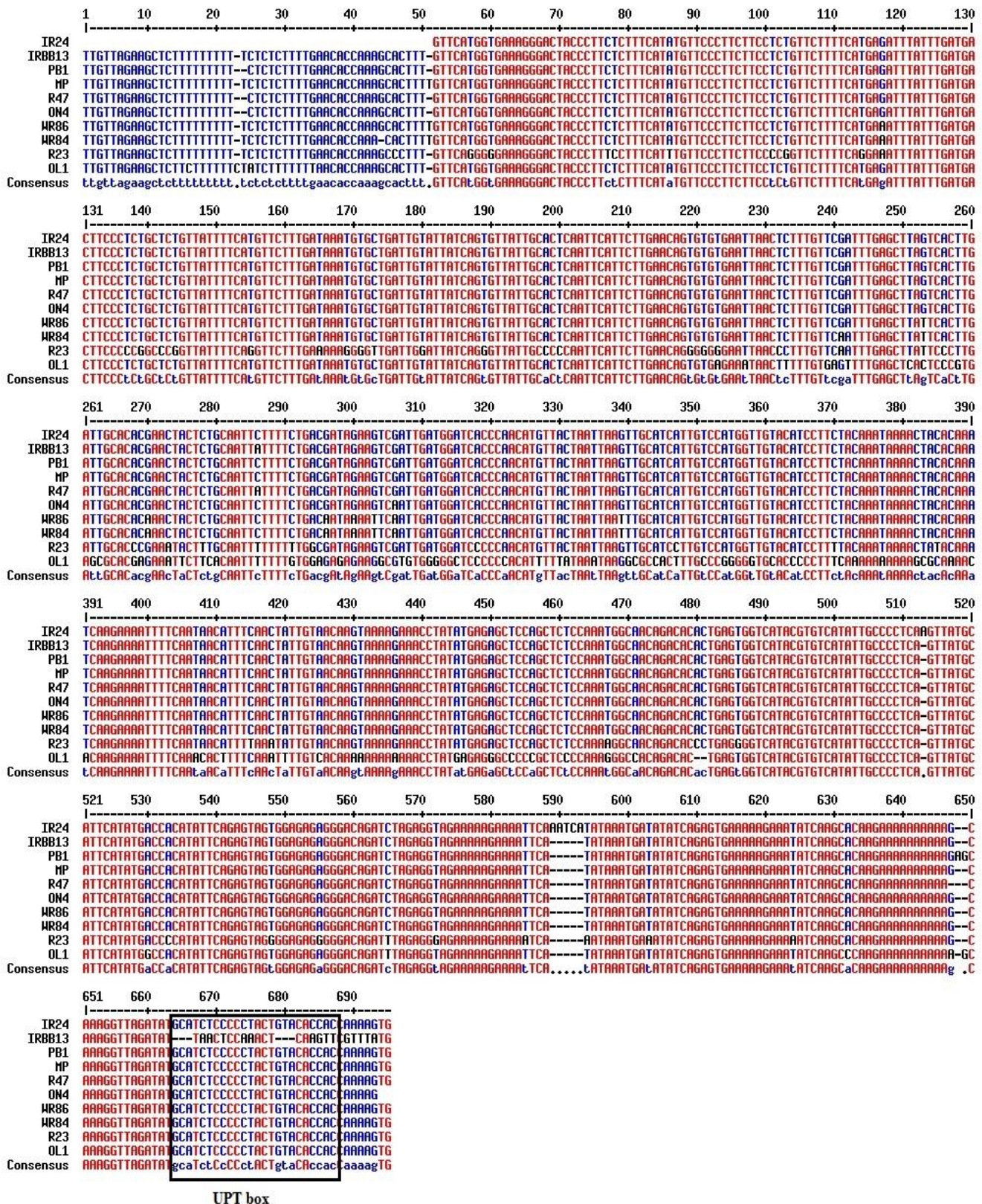


Fig 3: Multiple alignment of promoter region of *Xa13* covering the predicted UPT box

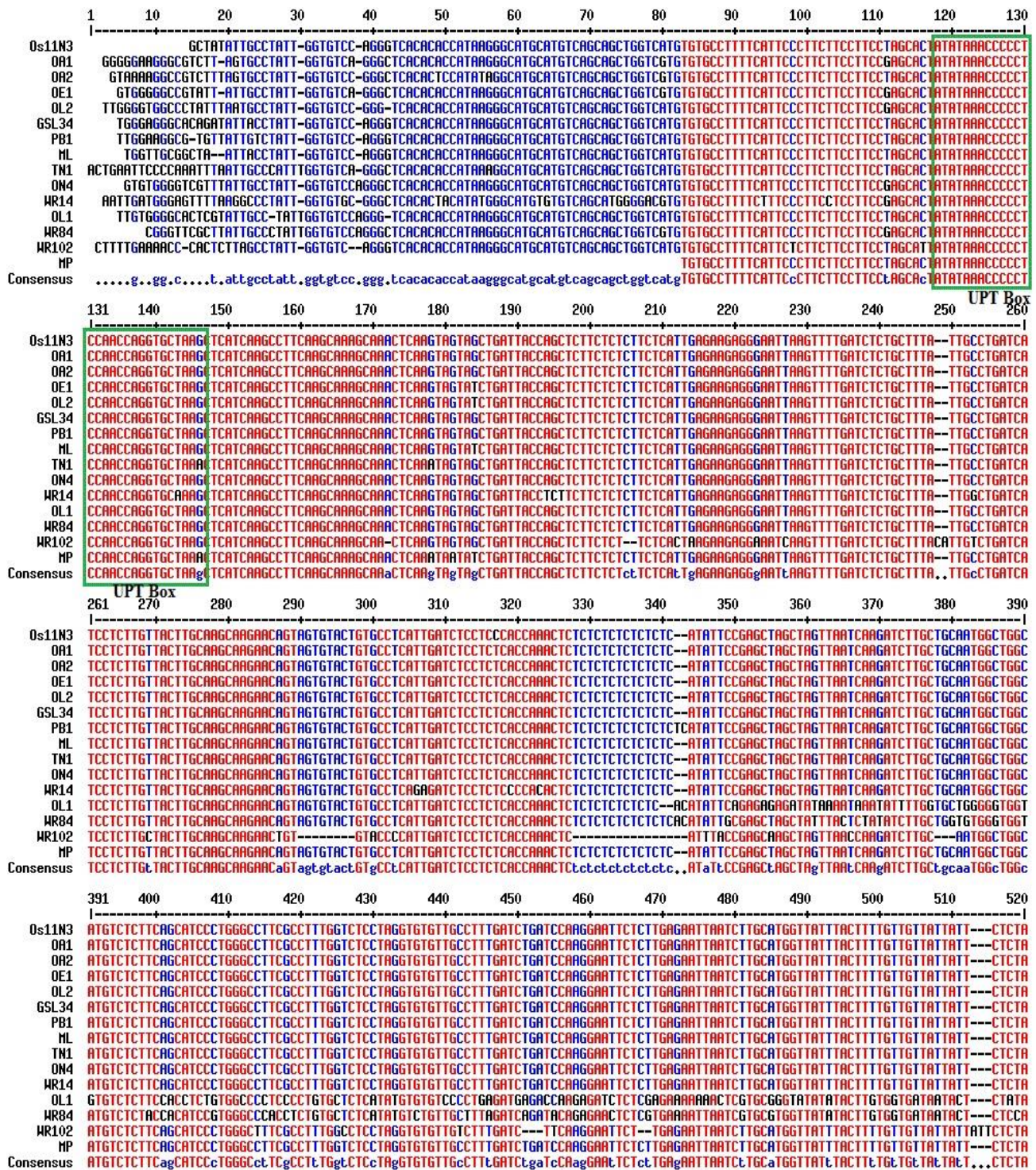


Fig 4: Multiple alignment of promoter region of Os11N3 covering the predicted UPT box

Polymorphism and Expression analysis of *Xa21* alleles (OL)

We screened the different accessions of rice genotypes with PTA248 marker for *Xa21* gene (Fig 5).

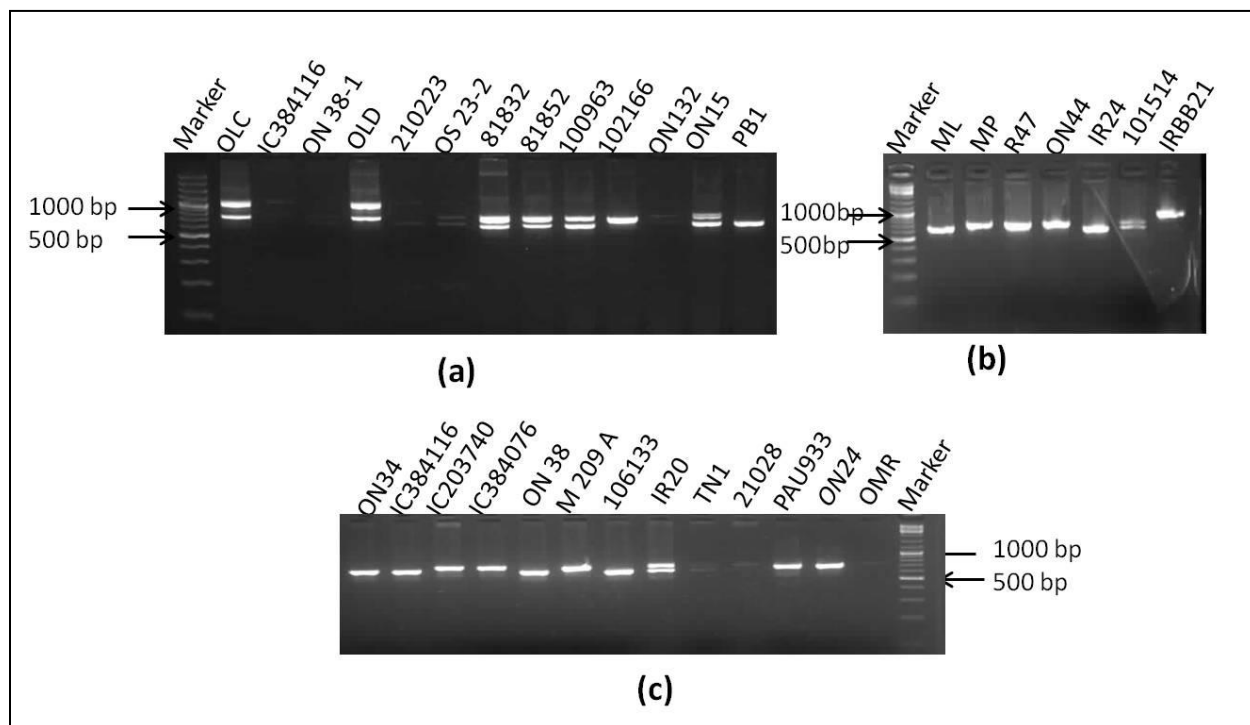


Fig. 5: Schematic presentation of genotypic screening using PTA248 marker (Ronald et al. 1996). (a), (b) & (c) Gel picture showing PCR product after resolving on 2% agarose gel. The primer was designed at the polymorphic site between dominant (*Xa21*) and recessive (*xa21*). Dominant alleles showed a band size around 1 Kb. Accession numbers of the genotypes and the cultivars are mentioned above the respective wells. The DNA marker used is 100 bp plus (100 bp – 3000 bp).

In order to confirm the functional significance of alleles identical to resistant *Xa21* (IRBB21), we checked the expression of *Xa21* alleles in *O. longistaminata* (OL). These two accessions were selected for expression analysis based on the sequence analysis and genotypic screening analysis with pTA248 marker. IRBB21 and IR24 were used as positive and negative control. Unfortunately, these two tested alleles didn't show any expression after the infection or before infection. We could see the up-regulation of this gene only in IRBB21 infected (Fig.6). Though,

Xa21 allele in OL failed to express, it was highly resistant to the disease hence, we went further with the antioxidant activity assays.

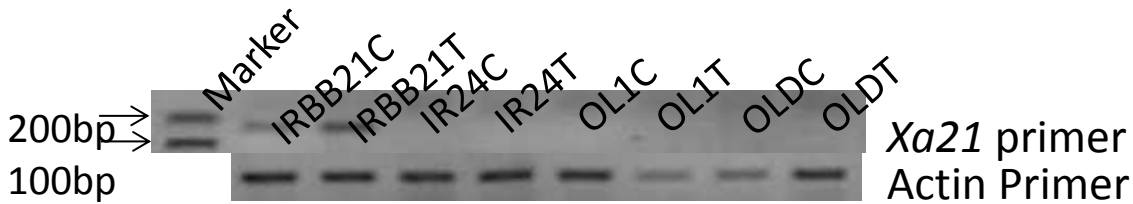


Fig. 6 RT- PCR of *Xa21* alleles in control (C) and *Xoo* infected (T) samples. First lane shows amplification with *Xa21* primer and 2nd lane shows amplification with internal control actin primer.

Chlorophyll estimation

Bacterial blight disease led to decrease in Chl (a + b) content in all three genotypes (IRBB21, OLD and PB1) due to lesion formation (Table 4). The change in total chlorophyll content of PB-1 was minimal at first two stages of infection, however, this genotype showed drastic reduction on 15th DAI (1.53 ± 0.05 mg/g fw). Almost similar trend was observed in IRRB21 wherein chlorophyll content depleted progressively, whereas in OL, we found that the chlorophyll content was maintained at the same level even at 15 DAI. Similar trend was observed with that of carotenoid content in all the three genotypes.

Table 4. Bacterial blight stress induced changes in chlorophyll and carotenoid contents (mg/g fw) of three rice genotypes.

Sample	Chl (a+b)		Carotenoid	
	Untreated	Treated	Untreated	Treated
5th Day after Infection (5th DAI)				
OL	6.06±0.02	5.24±0.07*	3.09±0.07	3.03±0.03
PB-1	6.47±0.03	4.99±0.07*	3.08±0.05	2.99±0.04*
IRBB21	6.43±0.02	4.93±0.10*	3.08±0.02	3.00±0.04
10th Day after Infection (10th DAI)				
OL	5.43±0.08	5.00±0.09*	3.06±0.06	2.98±0.05
PB-1	5.66±0.08	4.81±0.08*	3.07±0.06	2.98±0.04
IRBB21	4.99±0.05	4.02±0.07*	3.03±0.08	2.65±0.03*
15th Day after Infection (15th DAI)				
OL	5.37±0.08	3.07±0.13*	3.04±0.04	2.22±0.06*
PB-1	5.62±0.08	1.53±0.10*	3.05±0.04	1.07±0.06*
IRBB21	5.83±0.08	2.21±0.07*	3.01±0.05	1.38±0.07*

Values are presented as mean of triplicate determinations ± standard deviation.

(* = $p \leq 0.001$; ANOVA one way variance, Student's t test).

Total polyphenol and flavanoid content in infected rice

Infected OL1 substantially produced double the amount of phenolics and flavonoids in comparison to control at 5 DAI. The phenolic content reduced dramatically at the later stage of infection in all the three genotypes. The highest rate of phenolic content reduced after infection being observed in OL (from 10.82±0.01 to 4.81±0.06 mg/g fw), followed by PB-1 (from 12.23±0.06 to 4.96±0.05 mg/g fw) and IRBB21 (from 11.28±0.05 to 4.31±0.04 mg/g fw)

Like phenolics, flavonoid content in OL showed highest production at the 5th DAI (18.6±0.03 mg QE/g fw) in comparison to control plants (8.7±0.05 mg QE/g fw) and started to decrease and reached to (7.0±0.06 mg QE/g fw) at 15th DAI. The other two genotypes, PB-1 and IRBB21 showed similar pattern (Table 5). The change in flavonoid content in stressed PB-1 was

found 22.2 ± 0.02 to 8.73 ± 0.04 mg QE/g fw and in IRBB21; the reduction was from 19.1 ± 0.05 to 8.98 ± 0.05 mg QE/g fw.

Total antioxidant activity

The total antioxidative capacity of three rice genotypes sharply increased during initial stage of stress period. The effect of BB stress on the activities of total antioxidants participating in the scavenging of ROS which showed gradual decrease in all the three genotypes is shown in Table 5. The level of total antioxidant and ferric reducing power increased in the infected plants in comparison to the control plants on the onset of infection. The maximum antioxidant level was observed in treated OL (16.19 ± 0.02 mg AAE/g fw) compared to control (9.48 ± 0.02 mg AAE/g fw).

Similar patterns of ferric reducing power (increased activity during first stage followed by decreased activity at the later stages) were also observed for all the three genotypes (Table 2). OL was found to be more active as compared to other genotypes with maximum level of ferric reducing power value on 5th DAI (10.09 ± 0.02 mg AAE/g fw), while there was less significant change in the FRAP activity in other two genotypes, PB-1 and IRBB21, 9.23 ± 0.02 and 10.48 ± 0.01 mg AAE/g fw, respectively on the same day in comparison to control plants (8.53 ± 0.06 and 9.16 ± 0.01 mg AAE/g fw for PB-1 and IRBB21, respectively).

Total free amino acid

The free amino acid pool did not change very much in control samples during the entire period of investigation, while in stress induced plants, total free amino acid content increased with increasing time period of stress in all three genotypes particularly at 15th day of infection (Table 5). Mean TFA (total free amino acid) levels ranged from 21.0 to 29.9 $\mu\text{g/g}$ fw at 15th DAI under stress conditions. The highest accumulation of the amino acid was recorded in PB-1 (29.9 $\mu\text{g/g}$ fw), followed by OL (26 $\mu\text{g/g}$ fw) and IRBB21 (21 $\mu\text{g/g}$ fw). During the early stages of stress, all the three genotypes evidenced the minimal accumulation of total free amino acids.

DPPH activity

The leaf tissues of OL under bacterial stress condition at the 5th DAI showed a significantly higher capacity to detoxify oxygen radicals ($51.2 \pm 0.01\%$) over the antioxidant potential of the leaves of non-treated ($18.06 \pm 0.01\%$). In other two genotypes, PB-1 and IRBB21, the activity was similar throughout the experimental period which shows that only OL had evident tendency to detoxify oxygen radicals to counter act the stress conditions (Fig 7).

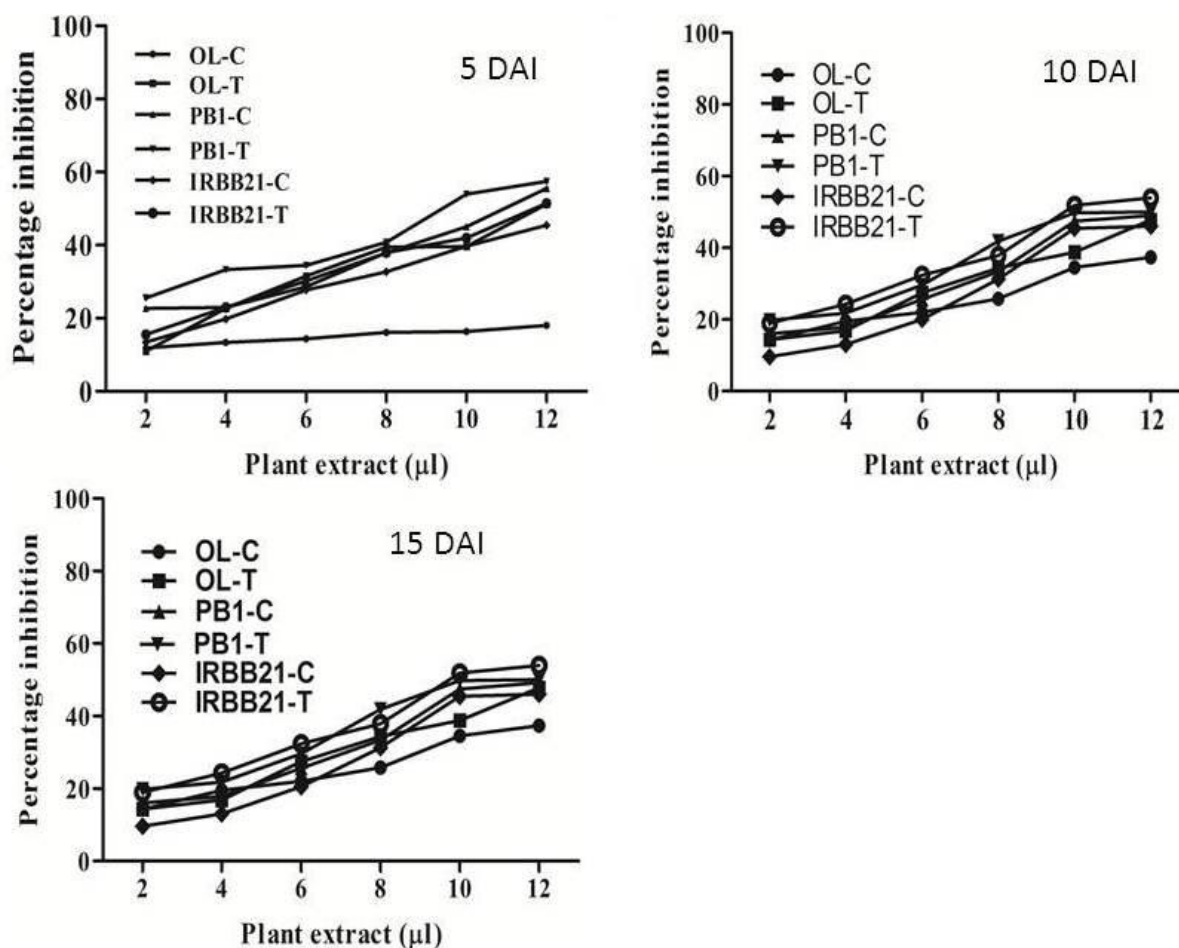


Fig 7. DPPH scavenging activity of extracts of different genotypes.

Scavenging activity of CAT and GPX

CAT activity increased significantly upon exposure to BB infection in all three genotypes. The highest activity was observed in IRBB21 at all time points followed by OL and PB-1 respectively.

The peroxidase activity showed significant increase with regard to biotic stress in comparison to control plants (Fig 8). Peroxidase activity significantly increased with increasing stress period in OL at 10th DAI, however, slight decrease in activity was observed upon increasing exposure to bacterial stress.

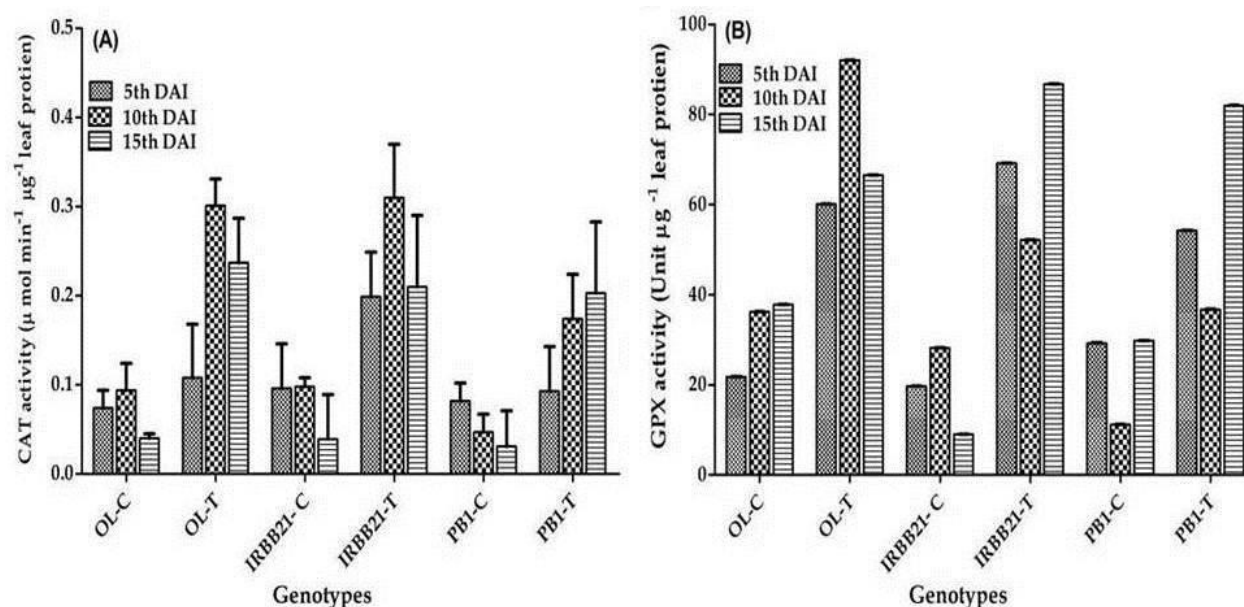


Fig 8. Activity of antioxidative enzymes, CAT (A) expressed as Units $\mu\text{mol min}^{-1} \mu\text{g}^{-1}$ protein and GPX (B) as Units μg^{-1} protein. Values are mean $\pm$ SD for three observations ($n=3$). The highest activity was observed in IRBB21 at all time points followed by OL and PB-1 respectively.

Table 5: Effect of bacterial blight on total polyphenol content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), ferric reducing power (FRAP) and free amino acid content (FAA) on three rice genotypes.

<i>Sample</i>	<i>TPC^a</i>		<i>TFC^b</i>		<i>TAC^c</i>		<i>FRAP^c</i>		<i>FAA^d</i>	
	<i>Control</i>	<i>Treated</i>	<i>Control</i>	<i>Treated</i>	<i>Control</i>	<i>Treated</i>	<i>Control</i>	<i>Treated</i>	<i>Control</i>	<i>Treated</i>
<i>5th Day after Infection (5th DAI)</i>										
OL	5.52±0.03	10.82±0.01 [†]	8.70±0.05	18.60±0.03 [†]	9.48±0.02	16.19±0.02 [†]	6.47±0.05	10.09±0.02 [†]	6.43±0.05	8.30±0.42 [†]
PB-1	10.14±0.01	12.23±0.06 [†]	19.32±0.07	22.20±0.02 [†]	13.13±0.02	17.05±0.03 [†]	8.53±0.06	9.22±0.02 [†]	2.82±0.04	4.14±0.52 [†]
IRBB21	9.75±0.03	11.28±0.05 [†]	16.40±0.01	19.10±0.05	15.59±0.03	15.25±0.05	9.16±0.01	10.48±0.01 [†]	7.92±0.02	9.54±0.34 [†]
<i>10th Day after Infection (5th DAI)</i>										
OL	7.68±0.03	10.47±0.03 [†]	11.90±0.09	18.03±0.03 [†]	11.90±0.02	17.15±0.01 [†]	8.32±0.05	9.75±0.06 [†]	2.31±0.02	6.54±0.11 [†]
PB-1	9.40±0.02	11.11±0.06 [†]	14.28±0.03	17.73±0.05 [†]	15.27±0.05	16.03±0.04	7.74±0.04	10.38±0.01 [†]	4.42±0.01	11.34±0.98 [†]
IRBB21	10.07±0.05	12.80±0.04 [†]	16.28±0.02	19.85±0.06	14.85±0.01	15.18±0.02	8.45±0.05	9.83±0.06 [†]	6.34±0.08	13.10±0.36
<i>15th Day after Infection (15th DAI)</i>										
OL	2.77±0.02	4.81±0.06 [†]	5.45±0.03	7.0±0.06 [†]	5.08±0.02	10.28±0.04 [†]	5.04±0.01	6.02±0.02 [†]	4.12±0.02	26.89±0.78 [†]
PB-1	4.30±0.07	4.96±0.05 [†]	7.40±0.08	8.73±0.04 [†]	6.53±0.05	10.35±0.02 [†]	5.64±0.03	5.93±0.05 [†]	4.35±0.02	29.21±0.25 [†]
IRBB21	4.94±0.04	4.31±0.02 [†]	7.78±0.09	8.98±0.05 [†]	8.91±0.04	9.03±0.02 [†]	5.83±0.06	6.10±0.04 [†]	8.65±0.06	21.82±0.48 [†]

a: gallic acid; b: quercetin; c & d: ascorbic acid equivalents mg/g fw plant material respectively; Values are presented as mean of triplicate determinations ± standard deviation. († = p≤0.01; ANOVA one way variance, Bartlett's test).