Journal of Tropical Agriculture 62(1): 72-80, 2024

Efficient screening of brinjal genotypes for bacterial wilt resistance at seedling stage: Unravelling the mechanisms through biochemical parameters

Niharika Muslepally[1] and Raji Vasudevan Namboodiri*[2]

[1]*Department of Genetics and Plant Breeding, College of Agriculture, Kerala Agricultural University, Padannakkad 671 314, Kerala, India*
[2]*Centre for Intellectual Property Rights Technology Management & Trade, College of Agriculture, Kerala Agricultural University, Vellanikkara 680 656, Kerala, India*

Received on 21 October 2023; received in revised form 03 April 2024, accepted 10 May 2024.

Abstract:
Brinjal genotypes were screened for resistance to bacterial wilt (BW) caused by *Ralstonia solanacearum* at the seedling stage and biochemical parameters were analysed to uncover the underlying resistance mechanisms. Three-week-old seedlings of 31 brinjal genotypes including four resistant checks(RC: Ponny, Haritha, Surya and Neelima) and two susceptible checks [SC:SM-26,(*S.incanum*),SM-27(*S.insanum*)] were subjected to a screening procedure that combined soil drenching and root dip inoculation.Based on percentage disease incidence (PDI), two accessions SM-15 and SM-25 were rated as highly resistant(HR: 0 PDI) and accession SM-1 was rated as resistant(R: 1-10PDI). Accessions SM-1, SM-2, SM-5, SM-11, SM-17,SM-24 and KAU hybrid Neelima were rated as moderately resistant (MR: 11-20 PDI). Accessions SM-4, SM-8, SM-14 and SM-19 were rated as susceptible (S: 21-30 PDI). Moderately susceptible (MS: 31-40 PDI) accessions were SM-3, SM-32, SM-33, SM-13 and SM-16. Accessions SM-9, SM-10, SM-12, SM-18, SM-20, SM-21, SM-22, SM-23, SM-26 and SM-27 were rated as highly susceptible (HS:>40PDI). Biochemical analysis showed higher phenol content, Polyphenol Oxidase (PPO) activity, and total sugars in resistant and moderately resistant groups at 12 and 24 hours post inoculation (hpi). PPO activity, phenolic and total sugars had a strong negative correlation with disease incidence at 12 and 24 hpi.

Keywords: Biochemical analysis, Brinjal genotypes, Disease resistance, *Ralstonia solanacearum*

Introduction

In Asia, brinjal is the third most significant vegetable, but its productivity faces constraints from pests, diseases, and environmental stresses, despite a global increase in production in recent years (Alam and Salimullah, 2021). One of the prominent challenges it confronts is susceptibility to various diseases, with bacterial wilt (BW) being a major concern. In India, BW is responsible for substantial yield losses, which is estimated as 11.67-96.67 per cent (Bainsla et al., 2016). As the name suggests, the primary symptom of this disease is plant wilting, and it is caused by the soil-borne bacterium *Ralstonia solanacearum*.

Conventional disease management techniques, including crop rotation, timing of planting, cultural practices, and soil treatments, are ineffective against this soil-borne pathogen, particularly given its wide range of host plants (Cao et al., 2009). Many commercial brinjal varieties and hybrids are highly susceptible to this pathogen. Given the pathogen's ability to persist at significant soil depths and its

*Author for Correspondences: Phone:9495326220; Email:raji.namboodiri@kau.in

presence within the plant's xylem, chemical control methods are not cost-effective, especially under field settings. Therefore, the most economically and environmentally viable approach for managing this pathogen remains the pursuit of resistant sources and the development of resistant brinjal cultivars.

Creating a resistant brinjal variety requires a screening process to identify and choose resistant plants as part of a crop improvement program. Implementing a scaled-down screening process using root inoculation with soil drenching (Singh et al.,2018) ,within a semi-open net house at the pro-tray stage offers a viable alternative. This approach allows for quicker execution, cost efficiency, reduced space requirements, thorough evaluations under controlled environmental conditions, and the creation of gnotobiotic conditions(without interference from external or unwanted microorganisms) unavailable in the field. Additionally, this screening process can be combined with biochemical analysis to investigate the fundamental mechanisms of resistance against BW.

Given the background information, the current study made use of a collection of brinjal germplasm preserved at the Department of Plant Breeding and Genetics, College of Agriculture, Padannakkad. The primary aim of this study was to conduct a comprehensive assessment of these brinjal genotypes to determine their resistance to BW and to explore the mechanisms of this resistance by analyzing biochemical parameters.

Materials and Methods

The experimental material comprised of a collection of 31 brinjal genotypes consisting of ten local genotypes from North Kerala, seventeen accessions from NBPGR regional station, Thrissur and four released varieties of KAU. The details of the accessions are mentioned in Table 1. The initial screening experiment was conducted using a completely randomized design, involving 31 genotypes. The screening process was repeated three times, with ten seedlings per replication and a control group per each genotype was also maintained. The experiment was carried out in open conditions from March to May 2022, near the Department of Plant Breeding and Genetics, College of Agriculture, Padannakkad.

Bacterial source and identification

Ralstonia solanacearum was obtained from College of Agriculture, Padannakkad- Instructional Farm-1 ($12^{o}15'22"$ N & $75^{o}6'59"$ E).The bacteria was isolated and cultured on a semi-selective Kelman's tetrazolium chloride (TZC) medium.The pathogen's identity was confirmed through a series of biochemical tests viz., Gram staining, KOH solubility, casein test, urease test and carbohydrates fermentation tests. Molecular methods, were employed, where Polymerase Chain Reaction (PCR) amplification was utilized to targetand amplify the 16S rRNA gene for the identification of bacterial strain at Eurofins Genomics Lab, Bangalore. (Accession number-CP011998.1).

Artificial screening of brinjal seedlings through root dip inoculation method

Screening brinjal seedlings via root dip inoculation method (Singh et al., 2018) involved preparing a 10^{8} CFU/ml bacterial inoculum in a 150 ml sterile container. Three-week-old brinjal seedlings were uprooted, wounded, and immersed in the inoculum, ensuring root exposure to the pathogen (Fig.1). These seedlings were then planted in sterile paper cups filled with sterile soil media. Suspension culture A 1-1.5 ml) of bacterial inoculum was added

Table 1: List of accessions used for screening

Sl. No	Collection number	Collection Source
1	SM-1, SM-2, SM-3,4 SM-,5, SM-8,SM-9, SM-10, SM-32, SM-33	Local germpalsm collection of North Kerala
2	SM-11 to SM-27	NBPGR, Thrissur
3	Haritha, Surya, Ponny, Neelima	KAU released varieties and hybrid

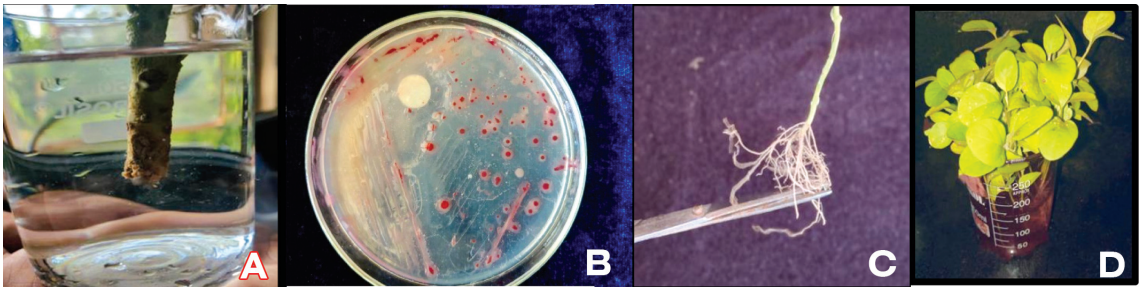

Figure 1: Screening forBW incidence in seedling stage(2-3weeksold) by root inoculation method. **A**. Ooze test; **B**. Pure culture (*R. solanacearum*); **C**. Root wounding in brinjal seedlings; **D**. Root dipping of brinjal seedlings in suspension culture

to each cup containing the inoculated seedlings. In the control group, ten seedlings per genotype were mock inoculated with sterile distilled water and these were monitored for bacterial wilt incidence over 15 days post-inoculation (dpi).

Percentage disease incidence

The total number of plants displaying wilting symptoms out of ten plants per genotype was recorded to calculate the percentage of disease incidence. These observations were recorded 15 days after inoculation and percent disease incidence (PDI) was calculated.

Disease rating

Scoring of BW incidence was done as per the method suggested by (Hussain et al., 2005).

PDI	Scoring	Resistance/Susceptibility
0	0	Highly resistant
1-10	1	Resistant
11-20	2	Moderately resistant
21-30	3	Moderately susceptible
31-40	4	Susceptible
>40	5	Highly susceptible

From the screening experiment based on disease reaction, the genotypes (eight) from all the resistant categories (HR, MR and R) and highly susceptible group (eight) along with four resistant checks (Ponny, Haritha, Surya and Neelima) and susceptible checks (*S. incanum, S. insanum*) were selected. Three week old seedlings of the above mentioned 22 genotypes were analysed for polyphenol oxidase activity, total phenols, reducing sugars, non-reducing sugars, and total sugars on a fresh weight basis from 50 seedlings per genotype at intervals of 12 and 24 hpi. The time interval for phenols and sugars were based on earlier reports of Vanitha et al. (2009). The sample was prepared by uprooting the seedlings and washing with sterile distilled water for conducting the analysis.

Temporal pattern study of enzyme

The temporal pattern was used to assess the polyphenol oxidase (PPO) activity in the RC (Ponny, Haritha, and Surya) and HS checks (*S. incanum* and *S. insanum*). These were inoculated by root dip method as mentioned above. The seedlings were harvested at regular 12-hour intervals from 0, 12, 24, 48, 60 and 72 hpi and they were observed for PPO enzyme activity. A significant difference ($P<0.05$) was noted in all seedlings post-pathogen inoculation compared to the uninoculated genotypes. Upon inoculation, there was an observed rise in PPO (polyphenol oxidase) activity, peaking at the 12-hour mark and reaching its maximum level at 24 hours post inoculation (hpi), followed by a

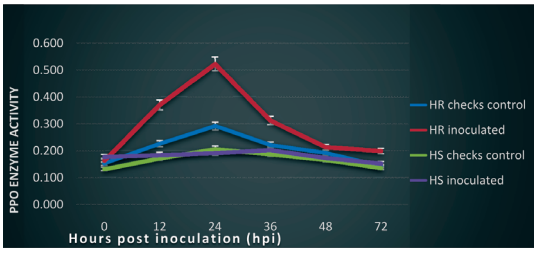

Figure 2: Temporal change in PPO activity in resistant and susceptible brinjal genotypes

subsequent decline, as illustrated in Fig. 2.

Biochemical parameters

Polyphenol oxidase enzyme activity (ΔOD at 495 nm /mg protein / min) in the sample was estimated as per the method suggested by Mishra and Sharma (2012)**.** Total phenol content of leaves was estimated by using Folin-Ciocalteau reagent (Sadasivam and Manickam, 1996) withabsorbance measured at a wavelength of 650 nm. The Anthrone method was used to calculate total sugars in seedling samples (Mahadevan and Sridhar, 1986) at 630 nm absorbance. Dinitro salicylic acid method was used to determine reducing sugars (Mahadevan and Sridhar, 1986) at an absorbance of 620 nm. The absorbance of all biochemical analysis was observed in the Systronics spectrophotometer.

Non-reducing sugars was calculated by subtracting the amount of reducing sugars from total sugars estimated as above.

Statistical analysis:

The data from both the inoculated and control groups were separately subjected to two-way ANOVA at 12 and 24 hpi.The twenty-two genotypes were grouped into six categories for easier comparison of means for biochemical parameters: highly resistant germplasm (HR), resistant checks (RC), resistant (R), moderately resistant (MR), highly susceptible germplasm (HS), and susceptible checks (SC). These six groups underwent one-way ANOVA, which revealed significant variations in all biochemical parameters among these groups.Both the analysis was done using KAU GRAPES package (Gopinath et al*.,* 2020), as per the completely randomized design of the experiment:

Results and Discussion

Per cent disease incidence (PDI) and rating of brinjal seedlings for BW

For response to BW disease incidence, the brinjal genotypes showed significant variation (Table 2).According to the disease rating scale, the KAU-released varieties Haritha, Ponny, and Surya as well as the local brinjal accessions SM-15 and SM-25 were classed as highly resistant (HR) because they had shown no wilt incidence and did not manifest any wilt signs until two weeks after inoculation which was followed by a period of recovery. Similar magnitude of progression of disease followed by recovery was also observed by Namisy et al. (2019) and Kwon et al. (2021). Accession SM-1 exhibited 10 per cent wilt incidence, was classified as resistant (R), and only displayed very mild wilt symptoms

Table 2: PDI and rating of brinjal genotypes for resistance to BW at seedling stage

Genotypes	PDI (%)	Scoring	Resistance/Susceptibility
SM-1	10(18.43)^{i}	1	R
SM-2	20(26.57)^{hi}	2	MR
SM-3	30(33.21)^{gh}	3	MS
SM-4	40(39.23)^{gh}	4	S
SM-5	20(26.57)^{hi}	2	MR
SM-32	30(33.21)^{gh}	3	MS
SM-33	30(57.10)^{cdc}	3	MS
SM-8	40(36.22)^{gh}	4	S
SM-9	50(42.11)^{fg}	5	HS
SM-10	100(80.78)^{a}	5	HS
SM-11	20(26.57)^{hi}	2	MR
SM-12	10(73.39)^{ab}	5	HS
SM-13	30(33.21)^{gh}	3	MS
SM-14	40(39.23)^{gh}	4	S
SM-15	0^{j}	0	HR
SM-16	30(33.21)^{gh}	3	MS
SM-17	20(26.57)^{hi}	2	MR
SM-18	50(42.12)^{fg}	5	HS
SM-19	40(45.00)^{efg}	4	S
SM-20	70(60.11)^{cd}	5	HS
SM-21	70(53.78)^{def}	5	HS
SM-22	70(53.78)^{def}	5	HS
SM-23	80(60.11)^{cd}	5	HS
SM-24	20(26.57)^{hi}	2	MR
SM-25	0^{j}	0	HR
SM-26	100(80.78)^{a}	5	HS
SM-27	90(67.5)^{bc}	5	HS
Ponny	0^{j}	0	HR
Surya	0^{j}	0	HR
Haritha	10(18.43)^{i}	1	HR
Neelima	20(33.21)^{gh}	2	MR
Mean	37.29		
SE(m)	4.33		
CD	6.13		
CV	16.43		

Transformed values are given in parenthesis

.Incidence of wilt was 20% in the accessions SM-2, SM-5, SM-11, SM-17, SM-24, and the KAU-released hybrid Neelima.The accessions SM-3, SM-13 and SM-16 recorded 30% wilt incidence and were rated as moderately susceptible (MS) in which wilting symptoms began to occur four dpi followed by rapid wilting at five dpi and nearly death of all plants at seven dpi. All susceptible (S) accessions of brinjal (SM-3, SM-13, and SM-16) began to show wilting symptoms five days post inoculation (dpi), followed by a rapid progression of wilting at six dpi, leading to the death of the majority of the plants by eight dpi. Similar results were observed by Singh et al. (2018) and Namisy et al. (2019)The accessions SM-9, SM-10, SM-12, SM-18, SM-20, SM-21, SM-22, SM-23 and wild accessions SM-26, SM-27 were characterized as highly susceptible (HS) as more than 40% of wilt symptoms were observed in these seedlings at two dpi followed by rapid wilting at four dpi and nearly death of all plants occurred by five dpi. Ali et al., 1990 reported similar results in which he reported that the poorest level of resistance was observed in wild relatives S*olanum melongena* var. *insanum, Solanum gilo* and their hybrids with eggplants. For this study, highly susceptible (HS) wild accessions were chosen as susceptible checks, referencing findings from Thomas (2022) and KAU (2019) report. Brinjal's closest wild relatives are *S. insanum* and *S. incanum*, with *S. insanum* regarded as the wild progenitor originating from Asia, while *S. incanum* is predominantly found in dry regions of Africa (Knapp et al., 2013). As was the case in our situation, Kumbar et al. (2017) also observed that some local cultivars and hybrids artificially inoculated at the six-week-old seedling stage planted in wilt sick plot demonstrated longer incubation period for the pathogen in resistant accessions compared to susceptible accessions.The differential reaction in all the genotypes in the present study to the incidence of bacterial wilt may be due to allocation of resources that function in differential defense responses along with growth response in the plants. These defense responses may be passive and active. The passive or pre-existence barriers prevent the

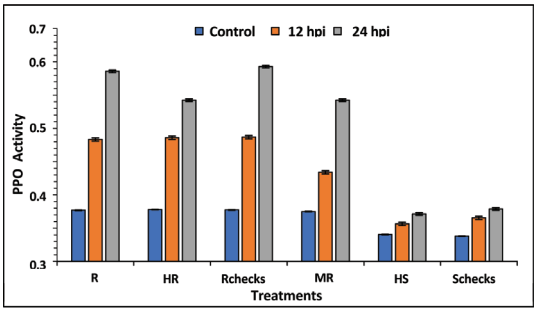

Figure 3. Range of PPO variation at in resistant and susceptible genotypes at 12and 24 hpi

colonization of the pathogen. The active defense mechanisms involve in the generation of hypersensitive response, production of phytoalexins, pathogenesis related proteins/ enzymes phenylalanine ammonia lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO), lipoxygenase (LOX), superoxide dismutase (SOD) and b-1,3 glucanaseetc (Santos et al., 2023). In plants, sugars serve as the main source of energy and C skeletons for all these plant defense responses. Disease resistance is also associated with the presence of phenolic compounds and their synthesis in response to infection (Mandal et al., 2010).

Examination of the mechanism of resistance through biochemical parameters

At 12 and 24 hpi, the R group showed the most significant increase in PPO activity, similar to the RC group, followed by the HR germplasm and MR group compared to their respective control samples (Fig, 3). Additionally, at 24 hpi, the resistant (R) genotypes displayed a substantial 3.5-fold increase in PPO activity compared to the control group.

Table 3. Grouping of resistant and susceptible genotypes

Sl. No.	Categories	Genotypes
1	Highly resistant germplasm (HR)	SM-15, SM-25
2	Resistant (R)	SM-1
3	Moderately resistant (MR)	SM-2, SM-5, SM-11, SM-17, SM-24, Neelima
4	Resistant checks (RC)	Ponny, Haritha, Surya
5	Highly susceptible germplasm (HS)	SM-9, SM-10, SM-12, SM-18, SM-20, SM-21, SM-22, SM-23
6	Susceptible checks (SC)	SM-26, SM-27

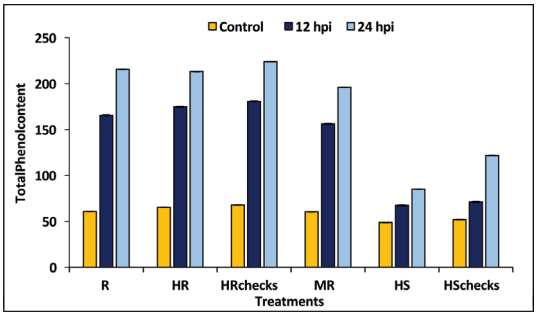

Figure 4. Range of total phenol content in resistant and susceptible genotypes at 12 and 24 hpi

These results indicate that the R, RC, and HR genotypes exhibited a noteworthy boost in PPO activity in response to pathogen inoculation, highlighting their enhanced defense response compared to the control group. There was no significant difference observed in the PPO activity between the HS germplasm and SC at both 12 and 24 hpi.Kavitha and Umesha (2008) as well as Vanitha et al. (2009) reported the temporal induction of PPO activity 12 and 15 hpi in 20 different tomato cultivars. These cultivars exhibited notable differences for the degree of resistance to the BW pathogen. In this study, a notable negative correlation was identified between PPO activity and disease incidence, aligning with the findings of Vanitha et al. (2009). Thilagavathi et al. (2007) similarly reported that oxidative enzymes, like PPO, participate in catalyzing the creation of lignin and other oxidative phenols. These enzymes aid in the development of defensive barriers by modifying cell structure, thus activating the defense system against pathogens.

At 12 and 24 hpi, all brinjal genotypes showed a significant increase in total phenolic content compared to the control. RC had the highest phenolic content, followed by HR, R, and MR germplasm (Fig.4). At 24 hpi, these groups exhibited a threefold increase in total phenolic content compared to the control seedlings. However, the highly susceptible (HS) cultivar showed minimal change in phenolic content between inoculated and control plants. Prakasha and Umesha (2016) reported a 2.7-fold increase in phenolic content in inoculated seedlings of resistant genotypes compared to controls at 24 hpi, but this difference was minor in HS cultivars. Likewise, Vanitha et al.

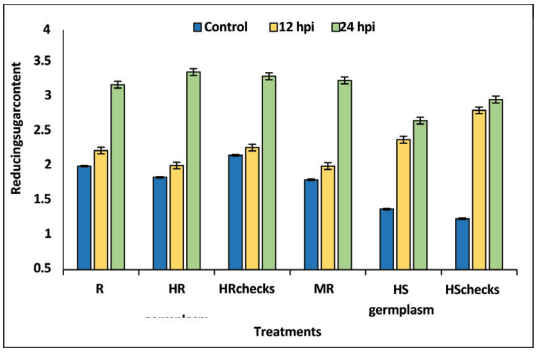

Figure 5. Range of reducing sugars content in resistant and susceptible genotypes at 12 and 24 hpi

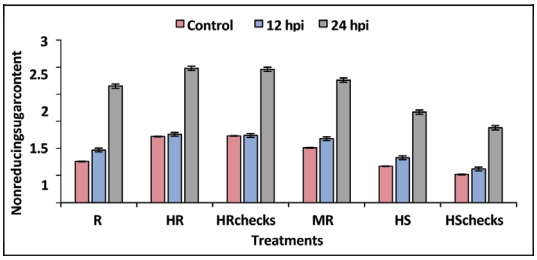

Figure 6. Range of non-reducing sugars content in resistant and susceptible genotypes at 12 and 24 hpi

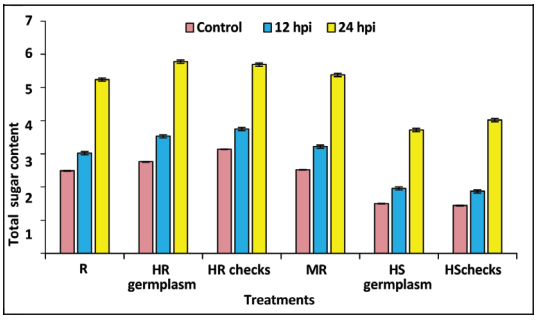

Figure7. Range of total sugar content in resistant and susceptible genotypes at 12 and 24 hpi

Table 4. Correlation analysis between wilt incidence and biochemical parameters

	PPO 12hpi	PPO 24hpi	Phenols 12hpi	Phenols 24hpi	RS 12hpi	RS 24hpi	NRS 12hpi	NRS24hpi	TS 12hpi	TS 24hpi
DI	-0.92**	-0.85**	-0.91**	-0.89**	0.87**	-0.85**	-0.81**	-0.88**	-0.86**	-0.86**

** Significance at 1% level * Significance at 5% level; PPO= Poly phenol enzyme; RS=Reducing Sugars; NRS= Non- Reducing Sugars; TS= Total sugars; DI= Disease incidence.

(2009) observed in their research that inoculating pathogens into tomato plants resulted in the accumulation of phenolic compounds. Resistant (R) cultivars exhibited higher levels compared to susceptible (S) and highly susceptible (HS) cultivars.Total phenolic content was negatively correlated with bacterial wilt (BW) incidence, as also observed in tomatoes by Raj et al. (2006) and Vanitha et al. (2009). Vasse et al. (2005) suggested that infected plants accumulate soluble or cell wall-bound phenols, which possess antibacterial and antioxidant properties crucial for the plant's defense against pathogens. Disease-resistant plants often exhibit resistance to cell wall degradation, a common trait observed in these plants (O'Brien et al., 2012).

After 24 hpi, RC germplasm showed the highest total sugar concentration (including both reducing and non-reducing sugars), followed by HR, R, and MR germplasm (Fig. 5, 6,7). In line with the findings of Lowe-Power et al. (2018) and Hamilton (2019), it was observed that the levels of the reducing sugar glucose in the xylem sap of resistant tomato plants infected with *R. solanacearum* increased by less than two times, while the concentration of trehalose, a non-reducing sugar, increased by 19 times. Similar observations were reported by Liu et al. (2022), indicating that accumulated sugars activate pathogen-resistant genes through sugar signaling. A study conducted by Hamilton (2021) examined bacterial fitness and concluded that the catabolism of sucrose during early infection stages does not contribute to its enhancement. As seen from Table 4, the current research unveiled a significant inverse relationship between disease incidence and the levels of total sugars and non-reducing sugars at both 12 and 24 hpi. However, the correlation between reducing sugars and disease incidence was positive at 12 hpi, while at 24 hpi, it exhibited a negative association. The primary battleground where pathogens compete with host cells for colonization resources, is the cell wall matrix or apoplasm, which is rich in nutrients, including sugars. Additionally, it is a crucial site for sugar signaling in defense and development (Naseem et al., 2017). Pommerrenig et al. (2020) suggested that the Sugar Transport Protein (STP) is likely the primary sugar transporter responsible for reabsorbing apoplasmic sugar to reduce bacterial infection. They also reported that the accumulation of soluble sugars may enhance the plant's natural defenses at the infection site. Matsuoka et al. (2012) utilized sugars such as glucose, proline, and glutamine to manage tomato bacterial wilt. It is evident that a better understanding of sugar metabolism and transport could significantly help in disease resistance breeding.

Phenols and polyphenols, acting as non-enzymatic antioxidants, effectively scavenge reactive oxygen species (ROS) and are commonly found across various plant species, thereby triggering defense mechanisms. Our findings corroborate the significant role of phenolic and polyphenolic compounds in neutralizing free radicals across different eggplant cultivars. Phenols, encompassing flavonoid compounds, contribute to reinforcing the structural integrity of host cell walls through lignin and suberin synthesis, creating physical barriers that impede pathogen proliferation (Ngadze et al., 2012). These flavonoids are mobilized to infection sites, triggering the hypersensitive reaction, an initial defense mechanism leading to programmed cell death, thus limiting pathogen spread (Mierziak et al., 2014).

PPO, a nuclear-encoded defense-related enzyme found in plastids, catalyzes the oxidation of phenols to quinones. Vanitha et al. (2009) observed in tomato cultivars that PPO activity was correlated with changes in total phenol content upon pathogen inoculation, potentially contributing to bacterial wilt resistance.

Trouvelot et al. (2014) extensively reviewed carbohydrates for their significance in plant immunity. Some function as elicitors of plant defenses, while others act as signaling molecules akin to phytohormones. Mono- and disaccharides, like glucose, sucrose or trehalose exemplify this

dual role. They trigger defense responses and regulate defense genes, highlighting their pivotal role in plant immunity. Additionally, sugars possess antioxidant properties, contributing to the scavenging of reactive oxygen species. However, understanding how plants perceive sugars remains a complex area needing further investigation.

Conclusion

The biochemical analysis clearly demonstrates that both PPO activity and total phenols can function as reliable indicators to distinguish between resistance and susceptibility to BW disease in brinjal cultivars.

After assessing resistance responses and crucial biochemical characteristics, the screening studies for bacterial wilt (BW) incidence identified eight genotypes that exhibit resistance to the disease. These genotypes include R accession SM-1, MR accessions SM-2 and SM-5 from North Kerala, MR accessions SM-11, SM-17, and SM-24, as well as HR accessions SM-15 and SM-25 from NBPGR. Following the evaluation of agronomic and yield traits, these accessions are candidates for potential inclusion in upcoming breeding programs.

Acknowledgement

The authors are obliged to Department of Plant Breeding and Genetics of Kerala Agricultural University, Padannakkad for financial support, NAHEP-CAAST lab and Department of Plant Pathology for technical support.

References

Alam, I. and Salimullah, M. 2021. Genetic engineering of eggplant *(Solanum melongena* L.*)*: Progress, controversy and Potential. *Hortic.* 7(78): 1-3.

Ali, M., Quadir, M.A., Okubo, H. and Fujieda, K., 1990. Resistance of eggplant, its wild relatives and their hybrids to different strains of *Pseudomonas solanacearum*. *Sci. Hort.*, *45*(1-2), pp.1-9.

Bainsla, N.K., Singh, S., Singh, P.K., Kumar, K., Singh, A.K. and Gautam, R.K., 2016. Genetic behaviour of bacterial wilt resistance in brinjal (*Solanum melongena* L.) in tropics of Andaman and Nicobar Islands of India. *Amer. J. Plant Sci.* 7 (2)-333-338.

Cao, B.H, Lei, J.J., Wang, Y. and Chen, G.J. 2009. Inheritance and identification of SCAR marker linked to bacterial wilt-resistance in eggplant. *African J. Biotech*. 8(20): 5201- 5207.

Hamilton, C. D., Steidl, O. R., MacIntyre, A. M., Hendrich, C. G. and Allen, C. 2021. *Ralstonia solanacearum* depends on catabolism of myo-inositol, sucrose, and trehalose for virulence in an infection stage–dependent manner. *Mol. Plant-Microbe Interactions* 34(6): 669-679.

Hussain, M.Z., Rahman, M.A. and Bashar, M.A. 2005. Screening of brinjal accessions for bacterial wilt caused by Ralstonia solanacearum. *Bangladesh J. Bot.*, *34*(1): 53-58.

KAU [Kerala Agricultural University]. 2019. Research Report 2019 [on-line]. Available:https://kau.in/sites/default/files/documents/research_report_2019.pdf [04January2022].

Kavitha, R. and Umesha, S., 2008. Regulation of defense-related enzymes associated with bacterial spot resistance in tomato. *Phytoparasit.* 36: 144-159.

Kumbar, S., Narayanankutty, C. and Sainamole, K. P. 2021. Evaluation of eggplant rootstocks for grafting eggplant to improve fruit yield and control bacterial wilt disease. *Eur. J. Plant Pathol.* 161: 73–90.

Kwon, J. S., Nam, J. Y., Yeom, S. I., and Kang, W. H. 2021. Leaf-to-whole plant spread bioassay for pepper and *Ralstonia solanacearum* interaction determines inheritance of resistance to bacterial wilt for further breeding. *Int. J. Mol. Sci.*22(5): 2279.

Liu, Y. H., Song, Y. H., and Ruan, Y. L.2022. Sugar conundrum in plant–pathogen interactions: roles of invertase and sugar transporters depend on pathosystems. *J. Exp. Bot.* 73(7): 1910-1925.

Lowe-Power, T. M., Hendrich, C. G., von Roepenack-Lahaye, E., Li, B., Wu, D., Mitra, R., Dalsing, B. L., Ricca, P., Naidoo, J., Cook, D., Jancewicz, A., Masson, P., Thomma, B., Lahaye, T., Michael, A. J. and Allen, C. 2018. Metabolomics of tomato xylem sap during bacterial wilt reveals *Ralstonia solanacearum* produces abundant putrescine, a metabolite that accelerates wilt disease. *Environ. Microbiol.* 20(4):1330-1349.

Mahadevan, A. and Sridhar, R. 1986. *Methods In Physiological Plant Pathology (*3rd Ed.). Sivakami publications, Chennai, pp. 192-193.

Mandal, S. M., Chakraborty, D. and Dey, S. 2010.

Phenolic acids act as signaling molecules in plant-microbe symbioses. *Plant Signaling Behav.* 5(4): 359-368.

Matsuoka, T. K., Toyota, K., Sato, K., Masuda and Kuroda, T. 2005. Suppressive mechanisms of used pumice to bacterial wilt of tomato. *Soil Microorg.,* 59: 15–20.

Mierziak, J., Kostyn, K. and Kulma, A., 2014, Flavonoids as important molecules of plant interactions with the environment. Molecules, 19: 16240-16265.

Mishra, B. B. and Sharma, A. 2012. Purification and characterisation of polyphenol oxidase (PPO) from eggplant (*Solanum melongena)*. *J. Food Chem*. 134(4): 1855-1861.

Ngadze, E., Icishahayo, D., Coutinho, T.A. and van der Waals, J.E., 2012, Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. *Plant Dis*., 96:186–192.

Naseem, M., Kunz, M., and Dandekar, T. 2017. Plant–pathogen maneuvering over apoplastic sugars. *Trends Plant Sci*. 22: 740–743.

O'Brien, J.A., Daudi, A., Butt, V.S. and Paul Bolwell, G., 2012. Reactive oxygen species and their role in plant defence and cell wall metabolism. *Planta*, *236*, pp.765-779.

Pommerrenig, B., Müdsam, C., Kischka, D. and Neuhaus, H. E. 2020. Treat and trick: common regulation and manipulation of sugar transporters during sink establishment by the plant and the pathogen. *J. Exp. Bot*. 71: 3930–3940.

Prakasha, A. and Umesha, S. 2016. Biochemical and molecular variations of guaiacol peroxidase and total phenols in bacterial wilt pathogenesis of *Solanum melongena*. *Biochem. Anal. Biochem.* 5(3): 1-7.

Raj, N. S., Sarosh, B. R. and Shetty, H. S. 2006. Induction and accumulation of polyphenol oxidase activities as implicated in development of resistance against pearl millet downy mildew disease. *Funct. Plant Biol.* 33: 563–571.

Sadasivam, S. and Manickam, A. 1996. Biochemical Methods (3rd edn). New Age International (P) Ltd., New Delhi. 2(4): 272p.

Santos, C. and Franco, O. L. 2023. Pathogenesis-Related Proteins (PRs) with Enzyme Activity Activating Plant Defense Responses. *Plants (Basel)*. 12(11):2226.

Singh, N., Phukan, T., Sharma, P. L., Kabyashree, K., Barman, A., Kumar, R., Sonti, R. V., Genin, S. and Ray, S. K. 2018. An innovative root inoculation method to study *Ralstonia solanacearum* pathogenicity in tomato seedlings. *Phytopathol.* 108(4): 436-442.

Thilagavathi, R., Saravanakumar, D., Ragupathi, N., and Samiyappan, R. 2007. A combination of biocontrol agents improves the management of dry root rot (*Macrophominaphaseolina*) in greengram. *Phytopathol. Mediterranean* 46: 157–167.

Thomas, A. 2022. Morphological and biochemical characterization of brinjal (*Solanum melongena* L.) genotypes. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur 179p.

Trouvelot, S., Héloir, M.C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., Combier, M., Trdá, L., Daire, X. and Adrian, M., 2014. Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Frontiers Plant Sci*., 5, p.592.

Vanitha, S. C., Niranjana, S. R. and Umesha, S. 2009. Role of phenylalanine ammonia lyase and polyphenol Oxidase in host resistance to bacterial wilt of tomato. *J.Phytopathol.* 157: 552-557.

Vasse, J., Fray, P. and Trigalet, A. 2005. Microscopic infection of intercellular infection and protoxylem invasion of tomato roots by *Pseudomonas solanacearum. Mol. Plant microbe Inter.* 8: 241-251.