



Special Issue: Novel Advances in Agricultural Science and Technology for Sustainable Farming in Tropical Region

Gene Expression Analysis of *ERF15*, *WRKY11*, and *CYP450* in Tobacco Infected with *Ralstonia solanacearum*

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Abstract | Bacterial wilt is one of the destructive infectious diseases in tobacco plants (*Nicotiana tabacum* L.). *Ralstonia solanacearum* Rs is the bacteria responsible for the incidence of the disease. Crop yields can be dramatically reduced in quantity and quality due to attack by this pathogen. Developing cultivars resistant to Rs is a challenge to overcome this problem. Marker-assisted selection (MAS) plays a critical role as a selection tool in the development of high-yielding cultivars. The current study aims to provide basic information regarding the expression of several genes associated with Rs in tobacco. Four tobacco cultivars, namely Hick Broadleaf, Beinhart-1000, Dark-302, and Dark-314, were artificially infected using Rs and subjected to cDNA analysis using end-point PCR in triplication. Results showed that *ERF15*, *WRKY11*, and *CYP450* genes showed no change in expression in the tested tobacco cultivars. Further experiments considering these factors could help elucidate the precise mechanisms governing the three genes expression in response to *R. solanacearum* infection.

Received | July 17, 2024; **Accepted** | February 04, 2025; **Published** | April 02, 2025

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Citation | Sukweenadhi, J., J. Setiabudi, W.D. Savitri and S.C. Kang. 2025. Gene expression analysis of *ERF15*, *WRKY11*, and *CYP450* in tobacco infected with *Ralstonia solanacearum*. *Sarhad Journal of Agriculture*, 39 (Special issue 1): 84-90.

DOI | <https://dx.doi.org/10.17582/journal.sja/2023/39/s1.84.90>

Keywords | Bacterial wilt, Biotic stress, Marker-assisted selection, Resistant cultivar, Tobacco, Virulent



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Introduction

Ralstonia solanacearum Rs is the primary soil-borne pathogen in plants of the Solanaceae family, one of which is tobacco (*Nicotiana tabacum* L.), which causes highly destructive bacterial wilt disease (Garcia *et al.*, 2019). This bacterium has a complex variety of subspecies and has a wide host range up to > 450 species. *R. solanacearum* can cause a significant decrease in the yield and quality of tobacco leaves (Gao *et al.*, 2019). Bacterial wilt

resulted in tobacco mortality in Temanggung of more than 50 % with land productivity of only 60 kg to 150 kg knitted per ha (Dalmadiyo, 1999). The initial symptoms after infection are asymmetrical leaf growth or wilting on one side (Yulianti *et al.*, 2012). The action that is considered adequate for controlling bacterial wilt disease is planting resistant varieties. Therefore, selecting the suitable cultivar selection method determines the success of disease control efforts. Conventional methods that some producers are still applying bring several disadvantages to

the productivity and efficiency of the industry. One of the factors is that, in practice, phenotypic selection tends to depend on environmental conditions, which leads to uncertainty of results. Thus, molecular marker-based selection (MAS) is gaining popularity. This method is based entirely on plant genetic traits without environmental factors, allowing the breeding process to be more precise, faster, and cost-effective.

Previous transcriptomic studies revealed essential genes that regulate tobacco resistance to Rs, namely *ERF15*, *WRKY11*, and *CYP450*. It was shown that *ERF15* is a transcription factor that positively regulates tobacco resistance to Rs (Li *et al.*, 2021). On the other side, *WRKY11* is another transcription factor that has a significant role in the positive regulation of induced systemic resistance (ISR) triggered by Rs. While, *CYP450* was found to play an essential role in increasing the accumulation of phenylpropane derivatives as a resistance effort, especially in the phenylpropane pathway. Based on the relevant references and an adequate database, The current research intends to conduct a preliminary study to analyze the expression of these genes in tobacco under infection with Rs. This study is expected to provide unique expression patterns as a basis for the future development of molecular markers associated with Rs resistance genes.

Materials and Methods

Plant materials

Four tested cultivars are provided by PT. Sadhana (Pasuruan, East Java) includes Hick Broadleaf, Beinhart-1000, Dark-302, and Dark-314. Hick Broadleaf originates from the United States and is known to have a high susceptibility to several diseases, but bacterial wilt has not been reported. Undocumented evidence in the field suggests Hick can suffer from bacterial wilt, so it was used as a positive control in this research. Likewise, Beinhart-1000's resistance to Rs is unknown but it is resistant to many diseases and very rare cases are found in the field so it act as a negative control. Meanwhile, Dark-302 and Dark-314 are developed varieties from PT. Sadhana which will be further investigated regarding its resistance tendency. A 40-day-old seedlings were grown in polybags containing a mixture of compost: cocopeat: soil: husk charcoal (5:4:3:2) at 25 °C to 32 °C with a natural photoperiod of about 12 h and under previously disinfected greenhouse conditions (University of Surabaya). Tobacco 21 d after

transplanting is ready to be used for inoculation. The bacteria *R. solanacearum* used in this study came from two sources: tobacco samples from the Pandaan field owned by PT. Sadhana and pure culture from Center for Standard Testing Instruments for Sweetener and Fiber Plants (*Balai Pengujian Standar Instrumen Tanaman Pemanis dan Serat*, BSIP - TAS), Malang, East Java, Indonesia.

Inoculum preparation

Bacteria from the Pandaan field sample were isolated using Juma (2018) method by first performing bacterial streaming on fresh stem samples from wilted tobacco. The bacterial-rich water was then homogenized, and 100 L was taken to be spread on casein-peptone lecithin polysorbate (CPLP) broth modified with TTC, while the pure culture from BSIP - TAS can be directly subcultured to the media. Incubation was carried out for 48 h at 28 °C. The streak method was then repeated until a single colony was obtained. Several identification steps were carried out, including gram staining, carbohydrate fermentation (glucose, sucrose, maltose, lactose, and dextrose), alcohol hexose oxidation (mannitol and sorbitol), and 16S rRNA sequencing. The biovar determination was carried out according to Schaad (2001). Inoculum preparation was carried out by rejuvenation on modified CPLP agar with TTC for 48 h at 28°C. Virulent colonies were selected and transferred with a sterile tip to the CPLP broth. Then, the suspension was incubated overnight (28 °C, 120 rpm) to reach 108 CFU/mL (0.1 at OD600).

Artificial inoculation

Artificial inoculation was performed with a modification of the method by Gao *et al.* (2019). Inoculation was applied to roots and leaves using a sterile syringe. Inoculum fluid was injected into tobacco polybag media to aid the root penetration. At the same time, leaf infiltration was applied from the abaxial side of the leaves. Tobacco inoculated using sterile distilled water served as a control. Growth conditions were maintained at 28 °C to 30 °C and RH 70 % to 80 %. This stage was carried out in triplication for each test variety, consisting of 10 polybags.

Sample preparation and DNA library construction

The samples that had been collected were immediately stored in liquid nitrogen before processing to maintain the quality of the RNA. The extraction and purification of total RNA from samples were

done using the FavorPrep™ Plant Total RNA Mini Kit (Cat. No. FAPRK 001-1, Favorgen, Taiwan). The process is carried out according to the manufacturer's recommended procedure. cDNA was synthesized using the Reverse Transcription Kit II (Cat. No. RP1400, Smobio, Taiwan). The process was carried out according to the manufacturer's recommended procedure with modification using a 20 µL reaction (Table 1). The cDNA samples were further polymerized using specific primers targeting the *CYP450*, *ERF15*, and *WRKY11* genes with the aid of the T100™ Thermal Cycler (Cat. No. 1861096, Bio-Rad, USA) and Thermal Cycler, 96 well (Cat. No. BT2203, BT Labsystems, USA) instruments. The reaction took place in a mixture of 20 µL consisting of PowerPol 2X PCR Mix (2 µL), cDNA (1 µL), forward PCR primer (1 µL), reverse PCR primer (1 µL), and ddH₂O (2 µL). The *L25* gene was used as an internal reference. This process takes place by triplication. Amplicons were visualized by electrophoresis on 2 % agarose gel using 0.5 times tris/borate/EDTA (TBE) buffer. Electrophoresis was carried out using a voltage of 50 V for 60 min. The electrophoretic gel was visualized using a UV transilluminator (Biometra, Germany), and analysis was performed using the BioDoc analyze software.

Table 1: PCR program for expression analysis.

Steps	Temp. (°C)	Time	No. of cycles
Pre-denaturation	94	2 min	1
Denaturation	94	30 sec	28
Annealing	L25	30 sec	
	ERF15		
	WRKY11		
	CYP450		
Extension	72	30 sec	
Post-extension	72	1 min	1
Hold	4	∞	1

Data analysis

The intensity of the amplicon band is interpreted through the ImageJ application with calibration and the help of ROI manager tools using a black inverted image. Meanwhile, non-parametric statistical analysis was completed using the Kruskal Wallis test through the GraphPad Prism 8 application.

Results and Discussion

From planting on selective media, both inoculum

sources from BSIP-TAS and PT. Sadhana gives the appearance of a single, circular colony with a pink core and rough edges and a wet, glossy cream color (Figure 1). This characteristic indicates that the colonies are virulent and suitable for inoculation (Juma, 2018). Microscopic observations and Gram staining showed the shape of small, Gram-negative straight rods. The two sources of inoculum were then given abbreviations: RsB (from BSIP - TAS) and RsS (from self-work isolation on the Pandaan field).

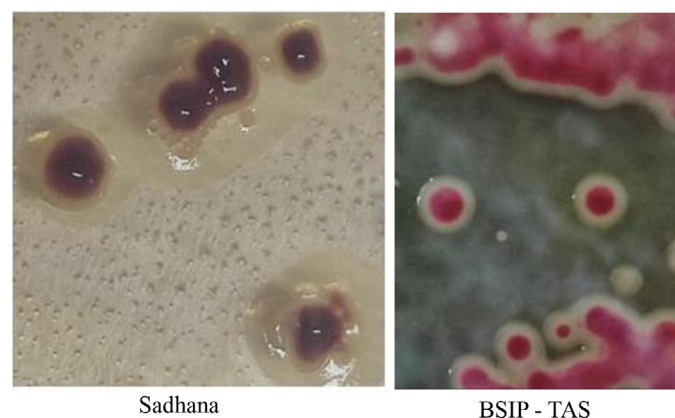


Figure 1: Virulence test on selective media.

Biochemical confirmation showed that both inoculum sources produced acid from glucose, but only RsB could produce gas. *R. solanacearum* has been reported to give positive results on the glucose test and gas production as an aerobic bacteria (Pawaskar et al., 2014). From the sucrose test, RsB was favorable for acid and gas production, which indicated the characteristics of *R. solanacearum*. Meanwhile, RsS was unable to use sucrose. Through the biochemical type test (biovar), RsB was able to use all tested sugars and hexose alcohols as carbon sources, while RsS did not ferment dextrose, lactose, and sorbitol and was negative for gas production in other test compounds (Table 2). Based on these results, RsB is most likely from biovar 3. On the other hand, RsS cannot be ascertained regarding the status of the biovar owned.

Table 2: *Ralstonia solanacearum* biovar test.

Carbon source	Inoculum source	
	BSIP - TAS	Sadhana
Dextrose	+	-
Mannitol	+	+
Sorbitol	+	-
Lactose	+	-
Maltose	+	+

Notes: + = able to take carbon source; - = unable to take carbon source.

Based on the results of the kinship analysis using the neighbour-joining method, the relatively high similarity to the 16S rRNA of tobacco *R. solanacearum* (generally classified in race 1) was shown by the two isolates, namely Balittas 1 and Sadhana 1 (Figure 2). To find out more, the analysis continued by comparing races related to other plant hosts, namely race 2 (Musaceae) and race 3 (potato). Appendix 6 shows the closeness of the two isolates to representatives of race 2 compared to other races. Race 2 pathogenicity is very limited to Musaceae and has never been reported to infect tobacco cultivars.

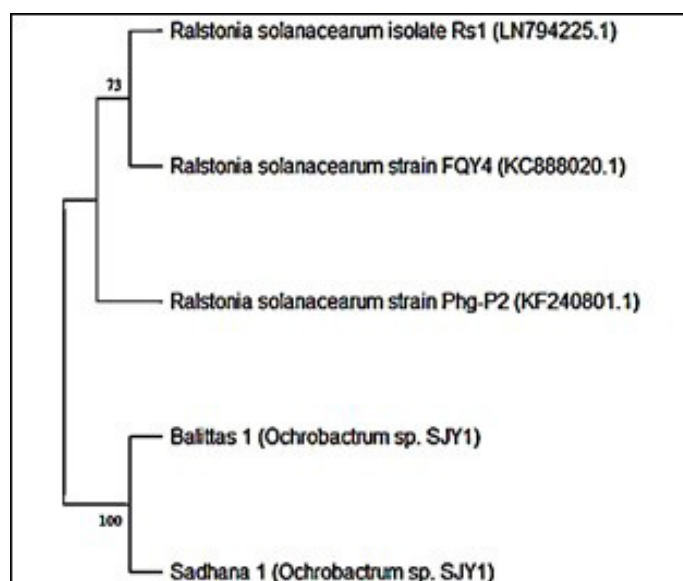


Figure 2: The phylogenetic tree between *Rs* strains of different races and *Rs* isolates.

Several infection trials have been conducted at different times using two previously identified inoculum sources. Young tobacco aged 10 wk after sowing / 3 wk after transfer to the ground is used because the immune response is mature and in ideal physiological conditions. Inoculation with Sadhana isolate did not give the appearance of symptoms of bacterial wilt after 7 d. Physical tobacco was still fresh, with green leaves and stems, as in Figure 3. Meanwhile, with the inoculum from BSIP - TAS, the incidence of disease did not appear phenotypically after 3 d (Figure 4), even if incubation had been continued for up to 7 d. This event may illustrate the phenomenon of non-host resistance, which occurs between *Rs* race 2 and the tested tobacco. However, no characteristic features such as a hypersensitive response/HR (necrosis) were observed, especially in the inoculation area. This can be explained by the possibility that the pathogen concentration is insufficient to induce HR symptoms and instead triggers “priming” events that reinforce

HR prevention (Katawczik *et al.*, 2016).



Figure 3: Hick Broadleaf after inoculation (7 DAI) using Sadhana isolate: control (left) and inoculated (right).



Figure 4: Hick Broadleaf, Beinbart-1000, Dark-314, and Dark-302 (left-right) after inoculation (3 DAI) using isolates of BSIP-TAS.

The wounding process before inoculation has been demonstrated by researchers and has been shown to increase the penetration of pathogens into plant tissues (Li *et al.*, 2021). However, in this study, the wounding option was not an alternative as wounding itself was explicitly reported to trigger ethylene production that affects *ERF* expression (Nishiuchi *et al.*, 2002). This event was also observed in other studies related to the activation of the *WRKY* and *CYP450* family genes through injury (Koo *et al.*, 2011; Wang *et al.*, 2014). So, the wound will only result in a bias in gene expression, so it is not representative of the infectious condition. The sample collection in the form of leaves is preferred over roots because there are reports that root samples provide low repeatability of expression patterns (Kiba *et al.*, 2007). Genes related to pathogen attacks will predominantly be expressed in the roots at an unstable rate, so leaves may be more natural in describing *Rs*-tobacco interactions. Young leaves are also an option for obtaining high-quality RNA and minimal contaminants. As in older tissues, the cell wall becomes more difficult to destroy, and the potential presence of secondary metabolites can inhibit the extraction of genetic material from cells, thereby reducing RNA recovery.

Ethylene response factors (*ERF*) are plant-specific

transcription factors and one of the most prominent family members. Studies found that *ERF15* overexpression triggered by *P. syringae* pv. tomato and *Botrytis cinerea* significantly increased resistance in *Arabidopsis* (Zhang *et al.*, 2015). Other findings suggested an early transcriptional response of genes related to the flavonoid pathway, including *ERF15*, in *N. tabacum* infected with Rs after 3 h of immersion inoculation (Gao *et al.*, 2019). This report shows that *ERF15* could be one of the front lines in tobacco's early response to Rs. This fact is confirmed by another who also observed a marked difference in *ERF15* expression in the tested Rs-resistant tobacco (Pan *et al.*, 2021; Li *et al.*, 2021). In contrast, in this study, changes in *ERF15* gene expression did not occur during the early incubation periods in each tobacco variety (Figure 5).

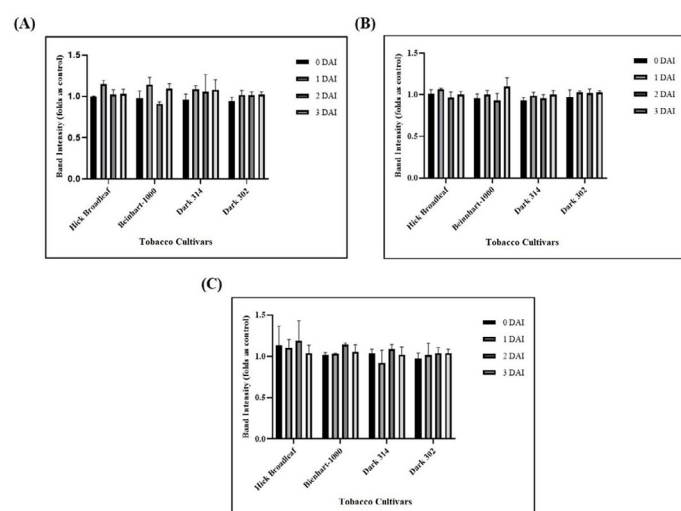


Figure 5: Relative expression of several genes associated with Rs in tobacco: (A) *ERF15*, (B) *WRKY11*, and (C) *CYP450*.

On the other hand, the same case is observed in *WRKY11*. *WRKY* is one of a large family of regulatory proteins in plant metabolism that are related to resistance to pathogens. A study revealed that *WRKY11* is involved as an activator in the positive regulation of flavonoid-related genes such as *MYB12*, *FLS*, *GT5*, and *UGT*, which causes an increase in tobacco defence against biotic stress (Wang *et al.*, 2014). Other research also found that bacterial wilt infection in resistant varieties will trigger the accumulation of flavonoids, inhibiting the colonization of Rs in the tissue and strengthening resistance (Li *et al.*, 2021). However, the current result again showed no change in *WRKY11* gene expression.

Cytochrome P450 (*CYP450*) is the most prominent family of plant enzymes involved in biosynthesis many

defence secondary metabolites. Some studies prove *CYP450* has decreased the expression of Rs infection at high soil moisture (Huang *et al.*, 2021). It was also said that under these conditions, the production of the hormone gibberellins increased, and there was a correlation with the increased susceptibility of plants to Rs. At the same time, Li *et al.* (2021) reported a significant increase in *CYP450* expression at 3 hsi and 7 hsi, indicating a positive involvement of this gene in defence against Rs. Figure 5 did not show any changes in *CYP450* expression.

In general, the expression of *ERF15*, *WRKY11*, and *CYP450* genes did not change in this study. This can be caused by several factors, namely the compatibility of pathogen-host interactions. Based on the results of molecular identification, the tested pathogens were not closely related to *R. solanacearum* race 1, which was virulent to tobacco. When viewed from the disease-triangle concept, it is impossible for an interaction that leads to the emergence of a disease reaction by the host when there is a mismatch of molecular components with the pathogen. The methods of inoculation and sampling used in our study might differ from those in other research. Variations in inoculation techniques and sampling times can influence gene expression results. Gao *et al.* (2019) demonstrated that different inoculation methods and environmental conditions could affect gene expression outcomes. Furthermore, the environmental conditions under which our experiments were conducted, including soil composition, humidity, and temperature, may differ from those in other studies. These factors can significantly influence gene expression. Lowe-Power *et al.* (2016) highlighted that the salicylic acid and abscisic acid pathways might dominate the early response of tobacco to Rs infection, potentially suppressing the expected changes in gene expression. The compatibility between the pathogen and the host is crucial. Our study used *Ralstonia solanacearum* isolates that may not be fully virulent to the tested tobacco varieties. This could explain the lack of changes in gene expression. Pan *et al.* (2021) and Li *et al.* (2021) have shown that genetic responses can vary significantly depending on pathogen-host compatibility. Crosstalk between different signaling pathways in response to biotic stress can affect gene expression outcomes. The activation of salicylic acid and abscisic acid pathways might suppress the changes in *ERF15*, *WRKY11*, and *CYP450* expression (Lowe-Power *et al.*, 2016; Pan *et al.*, 2021).

Conclusions and Recommendations

This study aimed to provide basic information on the expression of several genes associated with *Ralstonia solanacearum* (Rs) in tobacco (*Nicotiana tabacum* L.). Specifically, this study focused on *ERF15*, *WRKY11*, and *CYP450* genes. The researchers aimed to understand the role of these genes in the defense response of tobacco plants against Rs, which causes bacterial wilt disease. Investigation on *ERF15*, *WRKY11*, and *CYP450* gene expression patterns in different tobacco varieties during Rs infection. By analyzing gene expression at the mRNA level using PCR techniques, the researchers aimed to determine if there were any changes in the expression of these genes in response to Rs infection. The findings from this study are expected to provide valuable insights into the molecular mechanisms underlying tobacco resistance to Rs. The information obtained may serve as a basis for developing molecular markers associated with Rs resistance genes in future tobacco varieties. Ultimately, this study aims to contribute to developing more effective strategies for breeding and selecting resistant tobacco varieties, which can help reduce the damaging effects of bacterial wilt disease caused by Rs.

Acknowledgements

This work was supported by the Ministry of Education, Culture, Research and Technology, Republic of Indonesia (155/E1.1/KS.06.02/2022) and PT. Sadhana.

Novelty Statement

The study aims to characterize the resistance of new tobacco cultivars, Dark 302 and Dark 314, to *Ralstonia solanacearum* (Rs) infection through transcriptomic analysis. It investigates the expression of critical genes associated with tobacco defense against Rs (*ERF15*, *WRKY11*, *CYP450*). The research aims to provide foundational information for future marker-assisted selection methods for tobacco breeding and contribute to understanding the molecular mechanisms underlying tobacco resistance to Rs. It also offers potential insights for developing improved strategies to combat bacterial wilt disease in tobacco crops.

Author's Contribution

Johan Sukweenadhi: Conceptualized and designed

the study, elaborated the intellectual concept, performed the literature search, manuscript review, manuscript revision, and guarantor.

James Setiabudi: Carried out experimental studies, performed literature searches, data acquisition, statistical analysis, and manuscript preparation.

Wina Dian Savitri: Elaborated the intellectual content and manuscript review.

Se-Chan Kang: Elaborated the intellectual content and manuscript review.

Conflict of interest

The authors have declared no conflict of interest.

References

- Dalmadiyo, G., 1999. Integrated disease control Prosiding Semiloka Teknologi Tembakau. Balittas, Malang, pp. 14-30.
- Gao, W., R. Chen, M. Pan, W. Tang, T. Lan, L. Huang, W. Chi and W. Wu. 2019. Early transcriptional response of seedling roots to *Ralstonia solanacearum* in tobacco (*Nicotiana tabacum* L.). Eur. J. Plant Pathol., 155(2): 527–536. <https://doi.org/10.1007/s10658-019-01788-x>
- Garcia, R., K., Jim and T., Lindsey. 2019. *Ralstonia solanacearum* species complex: A quick diagnostic guide. Plant Health Progr., 20: 7-13. <https://doi.org/10.1094/PHP-04-18-0015-DG>
- Huang, M., H. Xing, Z. Li, H. Li, L. Wu and Y. Jiang. 2021. Identification and expression profile of the soil moisture and *Ralstonia solanacearum* response CYPome in ginger (*Zingiber officinale*). PeerJ., 9: 1–22. <https://doi.org/10.7717/peerj.11755>
- Juma, P., 2018. Guide on isolation, culture and inoculation of bacterial wilt (*Ralstonia solanacearum*).
- Katawczik, M., M.T. Tseng and A.L. Mila. 2016. Diversity of *Ralstonia solanacearum* populations affecting tobacco crops in North Carolina. Tobacco Sci., 53: 1–11. <https://doi.org/10.3381/15-047>
- Kiba, A., M. Maimbo, A. Kanda, H. Tomiyama, K. Ohnishi and Y. Hikichi. 2007. Isolation and expression analysis of candidate genes related to *Ralstonia solanacearum* tobacco interaction. Plant Biotechnol. J., 24(4): 409–416. <https://doi.org/10.5511/plantbiotechnology.24.409>

- Koo, A.J.K., T.F. Cooke and G.A. Howe. 2011. Cytochrome P450 CYP94B3 mediates catabolism and inactivation of the plant hormone jasmonoyl-L-isoleucine. *Proc Natl Acad Sci U.S.A.*, 108(22): 9298–9303. <https://doi.org/10.1073/pnas.1103542108>
- Li, Y., L. Wang, G. Sun, X. Li, Z. Chen and F. Feng. 2021. Digital gene expression analysis of the response to *Ralstonia solanacearum* between resistant and susceptible tobacco varieties. *Sci. Rep.*, 11(1): 3887. <https://doi.org/10.1038/s41598-021-82576-8>
- Lowe-Power, T.M., J.M. Jacobs, F. Ailloud, B. Fochs, P. Prior and C. Allen. 2016. Degradation of the plant defense signal salicylic acid protects *Ralstonia solanacearum* from toxicity and enhances virulence on tobacco. *mBio.*, 7(3): 1–12. <https://doi.org/10.1128/mBio.00656-16>
- Nishiuchi, T., K. Suzuki, S. Kitajima, F. Sato and H. Shinshi. 2002. Wounding activates immediate early transcription of genes for ERFs in tobacco plants. *Plant Mol. Biol.*, 49(5): 473–482. <https://doi.org/10.1023/A:1015553232309>
- Pan, X., J. Chen, A. Yang, Q. Yuan, W. Zhao, T. Xu, B. Chen, M. Ren, R. Geng, Z. Zong, M. Zhuwen, H. Zhenrui and Z. Zhenchen. 2021. Comparative transcriptome profiling reveals defense-related genes against *Ralstonia solanacearum* infection in tobacco. *Front. Plant Sci.*, 12: 1–13. <https://doi.org/10.3389/fpls.2021.767882>
- Pawaskar, J., M.S. Joshi, S. Navathe and R.C. Agale, 2014. Physiological and biochemical characters of *Ralstonia solanacearum*. *Int. J. Agric. Sci.*, 1(6): 2348–3997. <https://www.researchgate.net/publication/273059476>.
- Schaad, N.W., 2001. Initial identification of common genera. In: (eds. N.W. Schaad, J.B. Jones and W. Chun). *Laboratory guide for identification of plant pathogenic bacteria*. 3rd Edition. APS Press, St Paul, Minnesota, pp. 1–6.
- Wang, X., Y. Yan, Y. Li, X. Chu, C. Wu and X. Guo. 2014. GhWRKY40, a multiple stress-responsive cotton WRKY gene, plays an important role in the wounding response and enhances susceptibility to *Ralstonia solanacearum* infection in transgenic *Nicotiana benthamiana*. *PLoS One*, 9(4). <https://doi.org/10.1371/journal.pone.0093577>
- Yulianti, T., N. Hidayah and S. Yulaikah. 2012. Resistance of eight local Bondowoso tobacco cultivars to three important pathogens (*Ralstonia solanacearum*, *Pectobacterium carotovorum*, and *Phytophthora nicotianae*). *J. Litt.*, 18: 89–94. <https://doi.org/10.21082/jlitri.v18n3.2012.89-94>
- Zhang, H., L. Huang, Y. Dai, S. Liu, Y. Hong, L. Tian, L. Huang, Z. Cao, D. Li and F. Song. 2015. Arabidopsis AtERF15 positively regulates immunity against *Pseudomonas syringae* pv. tomato DC3000 and *Botrytis cinerea*. *Front. Plant Sci.*, 6: 1–13. <https://doi.org/10.3389/fpls.2015.00686>