Overexpression of JcWRKY2 confers increased resistance towards *Macrophomina phaseolina* in transgenic tobacco

Mitali Dabi¹ · Parinita Agarwal¹ · Pradeep K. Agarwal¹

Received: 18 August 2020 / Accepted: 14 October 2020 / Published online: 27 October 2020
© King Abdulaziz City for Science and Technology 2020

Abstract

WRKY proteins are plant-specific transcription factors (TFs), and form one of the largest families and are involved in plant development and responses to stress. The salicylic acid (SA) responsive WRKY family auto or cross-regulate the defence stress signalling pathways. In this study, we functionally validated the role of *JcWRKY2* gene from biofuel crop *Jatropha curcas* towards improving resistance to tobacco transgenic against charcoal rot causing necrotrophic fungus, *Macrophomina phaseolina*. The microscopic studies revealed that *JcWRKY2* participated in preventing the spread of infection in transgenic. The generation of H₂O₂ during *M. phaseolina* and combinatorial stress in transgenic induces the expression and activity of antioxidant enzymes. The transcript expression of SA biosynthetic (*NtICS1*) gene, pathogenesis-related (*NtPR-10*) gene and antioxidative enzymes (*NtCAT1* and *NtSOD*) gene revealed that *JcWRKY2* transgenic play a role in SA-mediated, antioxidative enzymes regulation during biotic challenges. The study highlights the potential of *JcWRKY2* as an important regulator for plant biotic stress responses through the SA-dependent pathway.

Keywords *JcWRKY2* · *Macrophomina phaseolina* · Salicylic acid · Tobacco · Transgenic · Reactive oxygen species

Introduction

The sessile nature of plants restricts its escape from unfavorable environmental challenges, including both biotic and abiotic. The biotic factors constitute fungi, bacteria and viruses responsible for plant diseases causing significant losses to plant growth and crop yield. Among the different pathogens, fungi are the primary cause of major agricultural economic losses worldwide. Depending on how the fungi utilize its host for nutrition, the fungal parasites are classified into three groups: Necrotrophs, which kill host cells using their toxins and feed on dead tissue; the biotrophs, which infect different parts of the plant without killing it and utilize the living tissue as nutrition. The third are hemi-biotrophic fungi, an interesting group of fungal pathogens, utilizing

both biotrophic and necrotrophic infections cycles to infect and grow on the host plants. The necrotrophic pathogens are responsible for the major economic crop losses worldwide (Dean et al. 2012).

The plants have developed an advanced immune system to cope with biotic stresses. The host defence involves pattern triggered immunity (PTI), against microbe's infestation, which is initiated by pathogen-recognition receptors (PRRs) that identify microbe-associated-molecular patterns or pathogen-associated molecular patterns (MAMPs or PAMPs). With the recognition of MAMP (PAMP/DAMP), PRRs initiate differential defence responses involving calcium influx, mitogen-activated kinase (MAPK) cascades, reactive oxygen species (ROS) homeostasis, phytohormone, TF/gene regulation. Similarly, the other host defence referred to as effector-triggered immunity (ETI) causes the hypersensitive response (HR), and regulates the gene resistance (R-gene) in response to pathogens.

The phytohormone SA plays an important role in various plant processes including plants innate immunity. SA facilitates local and systemic acquired resistance (LAR and SAR) against biotrophic and hemi-biotrophic pathogens. On microbial infection, the SA biosynthetic pathway is activated, manifesting a broad-spectrum resistance referred as

✉ Parinita Agarwal
parinitaa@csmcni.res.in

✉ Pradeep K. Agarwal
pagarwal@csmcni.res.in

¹ Plant Omics Division, CSIR-Central Salt and Marine Chemicals Research Institute (CSIR-CSMCRI), Council of Scientific and Industrial Research (CSIR), Gijubhai Badheka Marg, Bhavnagar 364 002, Gujarat, India

SAR. SAR is accompanied by activation of pathogenesis-related (PR) proteins, limiting the growth of the pathogen (Glazebrook 2005). Métraux et al. (1990) reported the presence of SA in the phloem sap of cucumber plants during the development of SAR after 7–9 days of infection with necrotrophic fungus *Colletotrichum lagenarium*.

In addition, pathogen's attack activates HR as a secondary plant defence mechanism. Due to this, ROS including hydroxyl radicals, superoxide radicals, and hydrogen peroxide (H_2O_2) are accumulated. The generation of ROS is often associated with the activity of antioxidant enzymes which in turn scavenge these toxic entities to maintain a cell balance. Furthermore, it is not clear that whether the SA acts upstream or downstream of ROS. Though the self-amplifying feedback loop very well explains the exact relationship between SA and H_2O_2 (Jayakannan et al. 2015).

WRKY TFs constitute one of the largest families of TFs reported in Angiosperms involved defence response (Agarwal et al. 2011). The genome-wide analysis of WRKY family report presence of > 100 members in rice, soybean or poplar, 72 members in *Arabidopsis thaliana*, 35 in *Sellaginella moellendorffii* (Pteridophyte), 80 in pine (gymnosperm) (Bakshi and Oelmüller 2014). They are important TFs up-regulated by SA and facilitate positive and negative regulation of SA-dependent defence signalling responses (Rushton et al. 2010). Resistance towards necrotrophs is genetically complex and comprises the integration of phytohormones including SA, ethylene (ET), jasmonic acid (JA) and abscisic acid (ABA) (Kazans and Lyons 2014).

Macrophomina phaseolina (Tassi) Goid is an important, soil-borne, necrotrophic fungus with wide-spread distribution and infects > 500 different economically important plants. Charcoal rot disease causes major yield loss in many economically important crops (Islam et al. 2012), and causes damage to the root and stalk leading to impaired water, nutrient absorption and translocation. In the present study, we have shown the potential of JcWRKY2 gene in imparting *M. phaseolina* resistance in transgenic tobacco plants via SA-mediated enhanced antioxidative enzyme activity in transgenic.

Materials and methods

Plant growth and stress treatments

To study the *Macrophomina phaseolina* stress tolerance, WT and JcWRKY2 transgenic (L-17, L-23 and L-40, Dabi et al. 2019) T_0 seeds were surface sterilized and germinated on Murashige and Skoog (MS, Murashige and Skoog 1962). The JcWRKY2 transgenic (L-17, L-23 and L-40) were developed by constitutive expression of WRKY TF from *Jatropha curcas* (Dabi et al. 2019). The 15-days old

seedling was transferred to sterile soils containing plastic cups and grown under 16 h light/8 h dark cycles at 25 °C. After acclimatization, well-grown plants were subjected to water, 150 μ M SA, *M. phaseolina*, and combined treatment of SA (150 μ M) and *M. phaseolina* (SA + Macro). *M. phaseolina* was first grown on potato dextrose agar (PDA) for 3–4 days at 25 °C. Then *M. phaseolina* treatment was given by placing four, 20 mm diameter PDA discs with *M. phaseolina* close to the plant at four points 1 cm below. Plants were allowed to grow for 72 h and then the leaves were collected for biochemical and molecular analysis.

In vitro antifungal activity of JcWRKY2 transgenics

To evaluate an antifungal activity in WT and JcWRKY2 transgenic, an intact leaf and leaf extract assay were carried out from in vitro grown plants on MS basal medium as described by Agarwal, (2016). The healthy and fully expanded leaf was kept on 0.8% agar (with 100 mg/mL of carbenicillin) plate and about 2 mm diameter PDA plug containing *M. phaseolina* was placed in the midvein region of the leaf to facilitate infection. Simultaneously, the PDA plug without *M. phaseolina* was also kept as a control. All plates were kept at 28 °C under normal light condition. After 3 dpi (days post inoculation), the leaf surface was observed under stereomicroscope (Leica L2) and photographs were captured at the same magnification. Each experiment was repeated thrice.

For the in vitro study of fungal infection, 10 days-old seedlings of WT and JcWRKY2 tobacco transgenic (L-23 and L-40) were placed in MS medium. Then 10 μ l of microsclerotia suspension (300 ± 20) was spotted at a distance of 3 cm from the root tip and analysed after 48 and 72 h using stereomicroscope (Olympus BX51). Further, 10 μ l of microsclerotia suspension was placed at the cut end of the leaf petiole. The intensity of dead cells (72 h) on leaves were analysed with trypan blue staining and destaining with choral hydrate (Chowdhury et al. 2014).

Quantification of hydrogen peroxide content

The H_2O_2 content of control and stressed leaves were checked as described by Mukherjee and Choudhuri (1983). The control and stressed leaf tissue were ground in chilled acetone solution. 0.5 mL of 0.1% titanium dioxide in 20% (v/v) H_2SO_4 was added to 2 mL plant extract followed by centrifugation at 6000 rpm for 15 min. After a while, the absorbance of yellow-colored supernatant was taken at A_{415} . The H_2O_2 concentration was estimated against the standard plot.

Determination of antioxidant enzyme activities

The control and stressed leaf samples (2 g) from WT and JcWRKY2 transgenic were extracted in 50 mM phosphate buffer of pH 7, containing 1% polyvinyl pyrrolidone. The homogenate was centrifuged at 15,000 g for 10 min and the supernatant was collected and stored for analysis of antioxidant enzyme activity.

Catalase (EC1.11.1.6)

Catalase enzyme activity was determined following the methods described earlier by Dhindsa et al. (1981). 100 mM phosphate buffer, pH 7.5 and 75 mM H₂O₂, was added to 50 mL enzyme extract. The A₂₄₀ decline, which is an indication of a decrease in H₂O₂ was measured using Epoch spectrophotometer (Biotek, India) and activity was expressed in units.

Superoxide dismutase (EC1.15.1.1)

Superoxide dismutase activity was determined according to the procedures of Dhindsa et al. (1981). Briefly, 100 mM phosphate buffer, pH 7.5, 200 mM methionine, 2.25 mM NBT, 60 mM riboflavin, 3.0 mM EDTA was added to 50 mL of enzyme extract and the reaction mixture was subjected to incubation under bright light (2 × 15 W fluorescent lamp), at room temperature and absorbance was recorded at 560 nm. The log A₅₆₀ was plotted as a function of the volume of enzyme extract (Giannopolitis and Ries 1977). Accordingly, the volume of enzyme extract that corresponds to 50% inhibition was considered as one enzyme unit (Beauchamp and Fridovich 1971).

Real-time PCR analysis of downstream genes

The expression of downstream genes was analyzed, using the cDNA prepared from the control and stressed leaf tissue of the WT and JcWRKY2 T₁ transgenic plants as previously

mentioned in Agarwal et al. (2016). Further, the cDNA served as a template for the analysis of biotic stress-related downstream genes using RT-PCR. The actin gene was used as an internal control. The primer sequences used in the RT-PCR study are given in Table 1.

Statistical analysis

Experiments were performed using minimum of three replicates and the results were obtained as mean ± standard deviation (SD). All the data were subjected to two-way ANOVA with replicates analyzed using Fischer LSD by Infostat software at $P \leq 0.05$. The mean values of treatments that were significantly different from each other are denoted with different alphabets.

Results

Fungal disc assay

The WT and transgenic leaves were inoculated with *M. phaseolina* fungal plugs to study the disease severity. After 48 h of incubation with the fungal disc, the WT leaf inoculated showed a necrotic lesion at the proximal end of the leaf. The infection spreads along the mid-vein on WT leaf, whereas, the infection severity was less in JcWRKY2 transgenic leaves. Darkening of the mid-vein and browning of the leaf was more pronounced in WT as compared to the transgenic (Fig. 1a).

Lesion formation on detached leaf

After 48 hpi (hours post inoculation), the detached leaf of WT, inoculated with the microsclerotial culture develops lesion at the site of inoculation as opposed to transgenic (L-23 and L-40), which showed less dead region around the infection site. Furthermore, trypan blue staining revealed greater damage in WT as compared to transgenic leaf,

Table 1 List of downstream genes studied for transcript regulation in tobacco

Sr. no.	Target gene name	Gene accession number	Primer sequence (5' → 3')	Function
1	NtCAT1	HF564632	F:AGGTACCGCTCATTACACACC R:AAGCAAGCTTTTGACCCAGA	Antioxidative enzyme
2	NtSOD	AF443178	F:TGCAGCTCCACCACCAGAAGCATCATCAGAC R:GGCTACCAACCACCTCGCGGACA	Antioxidative enzyme
3	NtICS1	EU257505	F: CAGTTCTGTTTGCAACCTCC R: GAGTAGGGTGAATGGACGAC	Salicylic acid biosynthesis gene
4	NtPR-10	JQ041907	F::GAGACTGTCTGAAGGTGATGGTGG R: GTGTGGTACTCAGTTGATGTCTTGC	Pathogenesis related gene
5	NtActin	XM016577261.1	F:GATTTGCTGGTGATGATGCTCC R:GTCTCAAACATGATCTGTGTCATC	Actin gene (internal control)

Fig. 1 In vitro fungal assay in WT and transgenic leaves. **a** *Macrophomina phaseolina* disc assay showing infection along the mid vein of leaf in WT and JcWRKY2 transgenic (L-17, L-23, L-40)

and **b** Trypan blue staining of detached leaf from WT and transgenic inoculated with fungus, showing the development of lesion after 72 h. Bar: 0.03 cm

staining of the mid vein in WT, indicates the spread of hyphal threads along the mid vein of the leaf (Fig. 1b).

Formation of microsclerotia and hyphal growth

On analysis, of the periphery of the meristematic region of WT and transgenic roots, a higher number of microsclerotia formation was observed in WT, compared to the transgenic roots, which showed less microsclerotia formation, indicating the higher and mature infection in susceptible tissue of WT. Moreover, the infection continues to spread along the xylem tissue lining till the upper region of WT root. In transgenic microsclerotia were observed loosely attached to the outer meristematic surface (Fig. 2a–c).

After 72 hpi, the growth of fungal hyphae was spread in all directions covering the main root of WT, whereas the hyphal growth was very less dense in transgenic root. The hyphae network showed a preference in the apical meristem region in transgenic whereas it was seen approaching the apical meristem as well as root tip in WT. Similarly, the number of hyphae on the WT root were more as compared to transgenics (Fig. 2d–f).

Oxidative and antioxidative response of transgenic against *M. phaseolina*

During biotic stress, the level of H_2O_2 accumulation indicates the oxidative damage to the plant. With SA treatment,

Fig. 2 In vitro study of *Macrophomina phaseolina* infection. **a** WT root, **b** L-23 transgenic root, **c** L-40 transgenic root. Trypan blue staining of roots showing the difference in the density of microsclerotia formation after 48 h. Behaviour of fungal hyphae in the vicinity of, **d** WT root, **e** L-23 and **f** L-40 transgenic roots as observed after 72 h. Bar: 0.05 cm

WT

L-23

L-40

the H_2O_2 content in WT was higher ($71 \mu\text{mol}/\text{mg}$) than transgenic ($53\text{--}57.3 \mu\text{mol}/\text{mg}$), however, with *M. phaseolina* and combinatorial stress the (SA + Macro) the H_2O_2 content of transgenic was higher as compared to WT, i.e. $70\text{--}93$ and $56\text{--}63.7 \mu\text{mol}/\text{mg}$ FW with *M. phaseolina* and combinatorial stress, respectively (Fig. 3).

The CAT activity was $0.058 \text{ U}/\text{mg}$ in WT and $0.072\text{--}0.083 \text{ U}/\text{mg}$ in transgenic, similarly, the SOD activity was 0.07 in WT and $0.1\text{--}0.2 \text{ U}/\text{mg}$ in transgenic during SA treatment. Under *M. phaseolina* treatment, the CAT and SOD activity was 0.12 and $0.3 \text{ U}/\text{mg}$ in WT, respectively, whereas, CAT and SOD activity in transgenic was

Fig. 3 Quantification of H_2O_2 content of WT and JcWRKY2 transgenic in response to water, SA, Macro and SA + Macro stress. SA salicylic acid, Macro *Macrophomina phaseolina*

0.07–0.08 U/mg and 0.1–0.3 U/mg respectively. In case of combined stress, the activity of CAT enzyme reduced to 0.03 and SOD enzyme reduced to 0.16 U/mg, whereas, the transgenics showed slightly higher activity (Fig. 4a, b).

Regulation of downstream genes in response to *M. phaseolina* stress

To study the role of JcWRKY2 in biotic stress, the expression of various downstream genes i.e. antioxidative enzymes (*NtCAT1* and *NtSOD*), pathogenesis-related protein (*NtPR-10*) and SA biosynthesis gene (*NtICS1*), were analysed. With SA treatment, the *NtCAT1* transcript showed a similar accumulation of 0.8 and 1.0–1.2-fold in WT and transgenic, respectively (Fig. 5a). Under *M. phaseolina* stress, the expression was higher in WT (1.4-fold) than transgenic, however, the combinatorial stress (SA + Macro) showed increased transcript accumulation in transgenic as compared to WT (Fig. 5a). The *NtSOD* gene, with SA stress, showed increased expression in WT (1.5-fold), as compared to transgenic lines which was 0.6–0.9-fold. The similar expression trend was observed in *M. phaseolina* treatment. The transgenic showed transcript accumulation of 2.3–2.9-fold and the WT showed 1.1-fold expression (Fig. 5b). The transcript level of *NtICS1* gene, which is associated with SA biosynthesis, was observed to be significantly higher in transgenic (2.2–3.1-fold) as compared to WT (0.9-fold) with SA treatment. The same pattern was observed in combinatorial treatment, with WT and transgenic showing 0.6-fold and 2.4–3.3-fold expression, respectively (Fig. 5c). The *NtPR-10*, pathogenesis-related gene, showed increased expression in transgenic with all the treatments (Fig. 5d). The transcript expression was 0.9–1.4-fold, 2.6–2.9-fold and 2.3–2.6-fold

with SA, Macro and combinatorial stress, respectively (Fig. 5d).

Discussion

M. phaseolina, fungus with a wide host range induces charcoal rot, in different economically important food and oil crops. The genetic diversity of *M. phaseolina* has been studied using PCR-based molecular markers (Su et al. 2001; Purkayastha et al. 2006; Das et al. 2008). In sesame quantitative trait loci associated with charcoal rot resistance caused by *M. phaseolina* has been identified using simple sequence repeat (SSR) markers (Wang et al. 2017). To protect crop damage by *M. phaseolina* infection different management methods are employed, including bio-control agent, botanicals, chemicals and fungicides etc. However, utilization of agronomic practices or use of fungicides has shown only limited success, therefore, a sustainable strategy needs to be deployed for improving the charcoal/collar rot disease resistance. Thus, genetic engineering needs to be employed for developing plants with improved *Macrophomina* resistance. The *Chill1* gene from *Oryza sativa* confers *Macrophomina* tolerance in transgenic *Corchorus olitorius* (Majumder et al. 2018). Earlier, our group has shown the involvement of *JcPR-10a* gene from *Jatropha curcas* towards reducing *M. phaseolina* susceptibility via its RNase activity and cytokinin binding potential (Agarwal et al. 2016).

The WRKY TFs form a co-ordinated network for auto and cross regulation of different signalling pathways towards conferring biotic resistance. The WRKY70 TF mediates SA-dependent resistance during plant-pathogen interactions (Li et al. 2006). AtWRKY70, acts as SA-dependent activator and JA-regulated repressor of defence genes (Li et al. 2006).

Fig. 4 Antioxidative enzyme activity of WT and JcWRKY2 transgenic with water, SA, Macro and SA + Macro stress. **a** CAT and **b** SOD. Values are represented as mean $\pm$ SE ($n=3$) and marked with different alphabets to indicate significant difference at $P \leq 0.05$ probability. SA salicylic acid, Macro *Macrophomina phaseolina*

It acts downstream of a NPR1 in SA signalling pathway (Wang et al. 2006). Further it regulates basal defence and R gene-mediated resistance against *Hyaloperonospora parasitica* (Knoth et al. 2007). Ectopic overexpression of AtWRKY33, enhanced resistance against two necrotrophic fungal pathogens (Zheng et al. 2006). AtWRKY41 modulates the crosstalk between the SA and JA hormone pathways by constitutive expression of the PR-5 gene and reduced expression of the PDF1.2 gene in Arabidopsis. Accordingly, AtWRKY41 overexpression imparts resistance towards *Pseudomonas syringae* but reduces resistance towards *Erwinia carotovora* (Higashi et al. 2008). OsWRKY13, rice WRKY gene activates SA synthesis and responsive genes and suppressed the JA synthesis and responsive genes (Qiu et al. 2007). The tobacco transgenic overexpressing GhWRKY44 showed improved resistance bacterial pathogen *Ralstonia solanacearum* and fungal pathogen *Ralstonia solani* via induction of pathogenesis-related genes (PR-1,

PR-2, PR-5) and NPR1 for SA signalling, and the PR-4 gene for JA signalling (Li et al. 2015). The OsWRKY13 binds to its own promoter and also to the target genes of the SA- and JA-dependent signalling pathways (Qiu et al. 2007).

The fungal tolerance as depicted by leaf assays of WT and JcWRKY2 transgenic suggest the role of JcWRKY2 in imparting resistance towards *M. phaseolina*. After a few hours of infection, WT root tissue showed severe infection with dense microsclerotia on the root tip region and murky hyphae growth completely entangling the WT roots. In the WT root, the hyphae preferentially grew towards the root by covering it than away from it while in transgenic the hyphae growth was not covering the roots and observed parallel to root length. The observed phenomena indicate the chemotrophic growth pattern of *M. phaseolina*, which further can be linked with the reports of other mycorrhizal fungi (Jamal et al. 2010). Furthermore, in the trypan blue staining assay of in vitro leaves, the WT leaf showed more concentrated

Fig. 5 Relative expression of downstream genes in WT and JcWRKY2 transgenic with SA, Macro and SA + Macro stress. **a** *NtCAT1*, **b** *NtSOD*, **c** *NtICS1* and **d** *NtPR-10*. Values are represented as mean $\pm$ SE ($n=3$). SA salicylic acid; Macro, *Macrophomina phaseolina*

dead cell region around the infection site as compared to transgenic. The difference in fungal tolerance pattern of WT and transgenic is may be due to the internal salicylic acid signalling through resulted in systemic acquired resistance. Similarly, CaWRKY27 positively regulates the resistance towards *R. solanacearum* infection via regulation of SA-, JA- and ET-hormone signalling pathways (Dang et al. 2014). In addition, TaWRKY70 provides resistance to *Puccinia striiformis*, by modulation of SA and ET-hormone signalling (Wang et al. 2016).

The transcript level of *NtSOD* and *NtCAT1* was increased in JcWRKY2 transgenic under *M. phaseolina* stress, contribute to the enhanced tolerance. Further, the expression of *NtPR-10* was highly increased with *M. phaseolina* and combinatorial stress. Likewise, OsWRKY6 enhanced resistance with increased expression

of the downstream OsPR10a gene (Choi et al. 2015). OsWRKY67 also confers bacteria blight resistance by the induction of PR1a and PR10 genes and the activation of the SA signalling pathway (Liu et al. 2018). SolyWRKY41 protein could interact with ICS1 protein by recognizing and binding W-box to regulate pathogen and stress response (Wildermuth et al. 2001). The elevated transcript level of SA biosynthesis gene, *NtICS1*, in transgenic suggests the involvement of SA during *M. phaseolina* tolerance. In summary, our study demonstrates that JcWRKY2 triumphantly regulate resistance against *M. phaseolina* through SA signalling involving maintenance of ROS homeostasis. Our study demonstrates that JcWRKY2 triumphantly regulate resistance against *M. phaseolina* fungus through SA signalling.

Acknowledgements CSIR-CSMCRI Communication No.-PRIS 130/2019. The financial assistance from Department of Science and Technology (DST)-WOS A and Council of Scientific and Industrial Research (CSIR), New Delhi, India is duly acknowledged. MD is supported by UGC-RGNF and AcSIR for Ph.D. enrolment.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

References

- Agarwal P, Reddy MP, Chikara J (2011) WRKY: its structure, evolutionary relationship, DNA-binding selectivity, role in stress tolerance and development of plants. *Mol Biol Rep* 38:3883–3896. <https://doi.org/10.1007/s11033-010-0504-5>
- Agarwal P, Dabi M, Sapara K, Joshi P, Agarwal P (2016) Ectopic expression of JcWRKY transcription factor confers salinity tolerance via salicylic acid signalling. *Front Plant Sci* 7:1541. <https://doi.org/10.3389/fpls.2016.01541>
- Bakshi M, Oelmüller R (2014) WRKY transcription factors: Jack of many trades in plants. *Plant Signal Behav* 2:27700. <https://doi.org/10.4161/psb.27700>
- Beauchamp C, Fridovich I (1971) Superoxide dismutase improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 1:276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8)
- Choi C, Hwang SH, Fang IR et al (2015) Molecular characterization of OsWRKY6 that bind to the W-box like element 1 of OsPR10a promoter and confer reduced susceptibility to pathogen. *Korean Soc Plant Pathol* 2:136. <https://doi.org/10.1111/nph.13516>
- Chowdhury S, Basu A, Chaudhuri TR, Kundu S (2014) In-vitro characterization of the behaviour of *Macrophomina phaseolina* (Tassi) Goid at the rhizosphere and during early infection of roots of resistant and susceptible varieties of sesame. *Eur J Plant Pathol* 2:361–375. <https://doi.org/10.1111/nph.13516>
- Dabi M, Agarwal P, Agarwal PK (2019) Functional validation of JcWRKY2, a group III transcription factor towards mitigating salinity stress in transgenic tobacco. *DNA Cell Biol* 38:1271–1298. <https://doi.org/10.1089/dna.2019.4895>
- Dang F, Wang Y, She J et al (2014) Overexpression of CaWRKY27, a subgroup IIe WRKY transcription factor of Capsicum annuum, positively regulates tobacco resistance to Ralstoniasolanacearum infection. *Physiol Plant* 3:397–411. <https://doi.org/10.1111/ppl.12093>
- Das IK, Fakrudin B, Arora DK (2008) RAPD cluster analysis and chlorate sensitivity of some Indian isolates of *Macrophomina phaseolina* from sorghum and their relationships with pathogenicity. *Microbiol Res* 2:215–224. <https://doi.org/10.1016/j.micres.2006.05.006>
- Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Foster GD (2012) The Top 10 fungal pathogens in molecular plant pathology. *Mol Plant Pathol* 13:414–430. <https://doi.org/10.1111/j.1364-3703.2011.00783.x>
- Dhindsa RS, Plumb-Dhindsa P, Thorpe TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J Exp Bot* 1:93–101. <https://doi.org/10.1093/jxb/32.1.93>
- Giannopolitis CN, Ries S (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol* 2:309–314. <https://doi.org/10.1104/pp.59.2.309>
- Glazebrook J (2005) Contrasting mechanisms of defence against biotrophic and necrotrophic pathogens. *Annu Rev Phytopathol* 43:205–227. <https://doi.org/10.1146/annurev.phyto.43.040204.135923>
- Higashi K, Ishiga Y, Inagaki Y (2008) Modulation of defense signal transduction by flagellin-induced WRKY41 transcription factor in *Arabidopsis thaliana*. *Mol Genet Genomics* 279:303–312. <https://doi.org/10.1007/s00438-007-0315-0>
- Islam MS, Haque MS, Islam MM (2012) Tools to kill: genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. *BMC Genomics* 1:493. <https://doi.org/10.1186/1471-2164-13-493>
- Jamil M, Charnikhova T, Verstappen F, Bouwmeester H (2010) Carotenoid inhibitors reduce strigolactone production and Strigahermonthica infection in rice. *Arch Biochem Biophys* 504:123–131. <https://doi.org/10.1016/j.abb.2010.08.005>
- Jayakannan M, Bose J, Babourina O, Rengel Z, Shabala S (2015) Salicylic acid in plant salinity stress signalling and tolerance. *Plant Growth Regul* 1:25–40. <https://doi.org/10.1007/s10725-015-0028-z>
- Kazan K, Lyons R (2014) Intervention of phytohormone pathways by pathogen effectors. *Plant Cell* 26:2285–2309. <https://doi.org/10.1105/tpc.114.125419>
- Knott C, Ringler J, Dangl JL, Eulgem T (2007) Arabidopsis WRKY70 is required for full RPP4-mediated disease resistance and basal defense against *Hyaloperonospora parasitica*. *Mol Plant Microbe Interact* 2:120–128. <https://doi.org/10.1094/mpmi-20-2-0120>
- Li J, Brader G, Kariola T, Palva ET (2006) WRKY70 modulates the selection of signalling pathways in plant defence. *Plant J* 46:477–491. <https://doi.org/10.1111/j.1365-3113X.2006.02712.x>
- Li J, Wang J, Wang N, Guo X, Gao Z (2015) GhWRKY44, WRKY transcription factor of cotton, mediates defense responses to pathogen infection in transgenic *Nicotiana benthamiana*. *Plant Cell Tissue Organ Cult* 1:127–140. <https://doi.org/10.1007/s11240-014-0688-9>
- Liu Q, Li X, Yan S et al (2018) OsWRKY67 positively regulates blast and bacteria blight resistance by direct activation of PR genes in rice. *BMC Plant Biol* 1:257. <https://doi.org/10.1186/s12870-018-1479-y>
- Métraux JP, Signer H, Ryals J, Ward E, Wyss-Benz M et al (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250:1004–1006. <https://doi.org/10.1126/science.250.4983.1004>
- Majumder S, Datta K, Sarkar C, Saha SC, Datta SK (2018) The development of *Macrophomina phaseolina* (Fungus) resistant and glufosinate (Herbicide) tolerant transgenic jute. *Front Plant Sci* 10:9–920. <https://doi.org/10.3389/fpls.2018.00920>
- Mukherjee SP, Choudhuri MA (1983) Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. *Physiol Plant* 2:166–170. <https://doi.org/10.1111/j.1399-3054.1983.tb04162.x>
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* 3:473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Purkayastha S, Kaur B, Dilbaghi N, Chaudhuri A (2006) Characterization of *Macrophomina phaseolina*, the charcoal rot pathogen of cluster bean, using conventional techniques and PCR-based molecular markers. *Plant Pathol* 1:106–116. <https://doi.org/10.1111/j.1365-3059.2005.01317.x>
- Qiu D, Xiao J, Ding X et al (2007) OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signalling. *Mol Plant Microbe Interact* 5:492–499. <https://doi.org/10.1094/MPMI-20-5-0492>
- Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. *Trends Plant Sci* 5:247–258. <https://doi.org/10.1016/j.tplants.2010.02.006>
- Su G, Suh SO, Schneider RW, Russin JS (2001) Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*.

- Phytopathology 2:120–126. [https://doi.org/10.1094/PHTO.2001.91\(2\).pp.120](https://doi.org/10.1094/PHTO.2001.91(2).pp.120)
- Wang D, Amornsiripanitch N, Dong X (2006) A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. PLoSPathog 2:1042–1050. <https://doi.org/10.1371/journal.ppat.0020123>
- Wang J, Tao F, An F et al (2016) Wheat transcription factor TaWRKY70 is positively involved in high-temperature seedling plant resistance to *Puccinia striiformis* f. sp. tritici. Mol Plant Pathol 5:649–661. <https://doi.org/10.1111/mpp.12425>
- Wang L, Zhang Y, Zhu X, Zhu X, Li D, Zhang X, Gao Y, Xiao G, Wei X (2017) Development of an SSR-based genetic map in sesame and identification of quantitative trait loci associated with charcoal rot resistance. Sci Rep 1:1–8. <https://doi.org/10.1038/s41598-017-08858-2>
- Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 409:562–565. <https://doi.org/10.1038/35107108>
- Zheng Z, Qamar SA, Chen Z, Mengiste T (2006) Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. Plant J 4:592–605. <https://doi.org/10.1111/j.1365-3113.2006.02901.x>