Bioassay for the Response of Resveratrol Transgenic Rice Lines to Bacterial and Fungal Diseases

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ABSTRACT Resveratrol is a stilbenoid and phytoalexin produced in response to stresses, such as wounding, and pathogen attacks by bacteria or fungi. Two resveratrol rice lines, Iksan515 and Iksan526, were used to examine resistance against Korean pathogen races for bacterial blight, leaf blast, and brown leaf spot. The screening test for bacterial blight demonstrated an increased susceptibility of both transgenic lines to K1 race, and a more susceptible Iksan515 to K2. Phenotypic evaluation for resistance to brown leaf spot also revealed the susceptibility of Iksan526 to the disease which did not significantly differ from the isogenic variety 'Dongjin', and a slightly more susceptible Iksan515 to the disease compared to check. When the lines were screened with three races (KJ301, KJ101 and KJ133) of leaf blights in the field, both transgenic lines exhibited resistance but at the same level with 'Dongjin'. Our overall findings suggest that foreign phytoalexin resveratrol production in transgenic rice lines transformed with *resveratrol synthase* gene did not confer resistance against fungal and bacterial diseases.

Keywords Transgenic rice, Resveratrol, Bacterial blight, Brown leaf spot, Leaf blast

INTRODUCTION

Resveratrol is a phytoalexin that has antifungal activity and is produced in response to stress, such as pathogens and wounds (Langcake & Pryce 1976). Resveratrols are synthesized via the phenylpropanoid pathway from three molecules of malonyl-CoA and one molecule of coumaroyl-CoA, and this process is catalyzed by stilbene synthase (Rupprich *et al.* 1980). Many *stilbene synthase* (*STS*) genes, including *Vst1*, *Vst2*, *Vst3*, and *StSy* from grapevine (Wiese *et al.* 1994); *PST-1*, *PST-2*, *PST-3*, *PST-4*, and *PST-5* from pine (Preisig-Müller *et al.* 1999); *AhRS* gene from *Arachis hypogea* (Hain *et al.* 1990); and *SbSTS1* gene from *Sorghum bicolor* (Yu *et al.* 2005), have been characterized and transformed into plants to increase resistance against fungal and bacterial diseases.

Some *STS* genes are highly expressed with considerable

amounts of cis- or trans-resveratrol production and their expression is related with resistance against fungi or bacteria in genetically modified (GM) crops. Hain *et al.* (1993) developed transgenic tobacco with *Vst1* and *Vst2*; Stark-Lorenzen *et al.* (1998) and Tian *et al.* (1998) both developed transgenic rice with *Vst1*; and Liang *et al.* (2000) and Serazetdinova *et al.* (2005) developed transgenic wheat with *Vst1/Vst2* and *PSS* gene, respectively. However, some *STS* GM plants produced piceid or failed to produce resveratrol. For instance, the transgenic kiwifruit produced by Kobayashi *et al.* (2000), transgenic white poplar generated by Giorcelli *et al.* (2004) and transgenic strawberry produced by Hanhineva *et al.* (2009) were more susceptible to fungi.

Four hypotheses have been proposed to explain stilbene GM plants that lack resistance or are more susceptible to fungal pathogens. First, a constitutive CaMV35S promoter triggered strong and constitutive stilbene accumulation but

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caused a drastic depletion of the endogenous precursor pools. This affects stilbene production and may interfere with secondary biosynthetic pathways (Hain *et al.* 1990, 1993; Thomzik *et al.* 1997). Second, these GM plants may produce resveratrol glucoside (piceid), which has little or no antifungal activities compared with resveratrol and its dehydrodimer δ -viniferin, which are more effective against fungus (*P. viticola*) zoospores in grapevines (Kobayashi *et al.* 2000; Giorcelli *et al.* 2004; Chang *et al.* 2011). Third, host-pathogen relationships determine the antifungal activity of foreign resveratrol production (Thomzik *et al.* 1997). Finally, the introduction of stilbene genes into plants causes major changes in the biosynthesis of phenolic compounds. Altered metabolite profiles, especially endogenous *chalcone synthase* downregulation, can increase the susceptibility of GM plants against fungal pathogens (Hanhineva *et al.* 2009).

Previous studies have demonstrated that the quantity of resveratrol production from GM plants with inserted *stilbene synthase* genes was often much lower than the amounts of piceid and other phenolic compounds that were produced. Moreover, stilbene concentrations and antimicrobial activity were unpredictable. Two studies have been published on transgenic rice with the *stilbene synthase* gene *Vst1* under the control of a pathogen-inducible promoter (Stark-Lorenzen *et al.* 1998; Tian *et al.* 1998). Both GM rices were resistant to leaf blast, but the rice line described by Tian *et al.* (1998) also showed resistance to bacterial blight. However, resveratrol production and concentration were not characterized in either study. Here, we investigated the responses against bacterial blight, leaf blast, and brown leaf spot in two independent transgenic lines Iksan515 and Iksan526 (RS18, Baek *et al.* 2013) that were developed by introducing the *AhSTS1* gene (*RS3*, GenBank accession no. DQ124938) under the control of the ubiquitin promoter into the ‘Dongjin’ commercial rice variety (Baek *et al.* 2013).

MATERIALS AND METHODS

Plant materials and molecular information

Two resveratrol transgenic rice lines Iksan515 and Iksan526 were screened for resistance to bacterial blight, leaf blast, and brown leaf spot. Both lines were generated

by the introduction of the *resveratrol synthase* gene (*AhSTS1*, *RS3*) under the control of the maize *Ubi 1* promoter and the *bialaphos* resistance (*bar*) gene under the control of the *CaMV35S* promoter (Fig. 1A, B). These were inserted into the intergenic regions of chromosome 4 and 12, respectively, with both inverted repeats of two T-DNA insertions.

Transgene expression

Reverse transcription polymerase chain reaction (RT-PCR) and immune-strip tests were performed to confirm *resveratrol synthase* and *bar* gene expression. Total RNA was isolated from rice leaves with TRIzol according to the manufacturer’s instructions (Invitrogen). cDNA was synthesized by using the SuperScript III First-Strand Synthesis System (Invitrogen). The PCR condition were as follows: a predenaturation step at 94°C for 3 min; 28 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extension for 1 min at 72°C; and final extension for 5 min at 72°C. The primer pair for RS was fw-ATGGTGTCTGTGAGTGGAATTCGC and rv-TTATATGGCCACACTGCG and for actin was fw-CTGCTATGTACGTCGCCATC and rv-GTCTCATG GATACCCGCAG.

Bacterial culture and inoculation

Four Korean races (K1, K2, K3, and K3a) of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) were cultured on media containing 5 g yeast extract, 10 g tryptone, 5 g sodium chloride, 5 g sucrose, and 0.5 g MgSO₄ per liter and were allowed to grow at 28°C for 3 to 4 d. The bacterial cells were suspended in sterile distilled water and adjusted to 10⁸ cfu/ml using a spectrophotometer at an OD₆₀₀ value of 0.5.

The bacterial cell suspension was applied to the flag and the top second leaves of each tiller of both transgenic lines and the donor variety at maximum tillering stage in the greenhouse by clipping 2–3 cm from the tip of the leaf using a pair of scissors dipped in the inoculum. Lesion length was measured two weeks after inoculation. Twenty leaves and three replications for each line were used for disease evaluation. Percent disease incidence was calculated according to formula as follows: % Disease severity = Total lesion length/Total leaf length × 100.

Fig. 1. Molecular information for two transgenic rice lines.

A. Transformation vector constructs of two transgenic rice lines. *RS*, *resveratrol synthase* gene; 35S, *CaMV35S* promoter; *ubi1*, maize ubiquitin promoter; B. inserted T-DNA structure for two transgenic lines; C. gene expression of inserted *resveratrol synthase* in rice leaves, as analyzed by RT-PCR; *OsActin* was used as a loading control; D. PAT protein (*bar*) expression tested by immuno strips from young leaves. Two red bands were found for both transgenic lines, indicating successful transformations and *bar* gene expression, compared to single band for the isogenic variety 'Dongjin'.

Evaluation for brown leaf spot resistance

Approximately 30-day-old seedlings of Iksan515, Iksan526 and 'Dongjin' were transplanted in rows at 15 x 30 cm apart in a genetically modified organism (GMO) isolation field at Gunwi, Gyeongbuk province, Korea on June 2, 2011. Field management was done following the standard methods for rice cultivation of Rural Development Administration (RDA, Korea). Thereafter, the plants were observed for the natural infection with brown leaf spot. Disease severity was determined by taking the average number of lesions per leaf area (cm²) of 20 flag leaves, 20 second leaves, and 20 third leaves for each rice line at maturity stage.

Evaluation for leaf blast resistance

Lines of Iksan515, Iksan526 and Dongjin were planted in a row spaced at 10 cm apart. Three rows planted with 'Nagdong' were established beside the test lines as spreader or source of inoculum for natural infection (Fig. 3B). The experiment was setup at Suwon GMO experimental field in July 2012. Overhead irrigation was performed 2-3 times a

Fig. 2. The representative leaf performance of the two resveratrol rice lines Iksan515 and Iksan526 and their isogenic variety 'Dongjin' in response to four Korean races (K1, K2, K3, and K3a) of bacterial blight at 14 d after inoculation. DJ, 'Dongjin'; I515, Iksan515; I526, Iksan526.

day throughout the growing period after sowing. Disease infection was monitored for 40-d-old seedlings. Lesions were scored on a scale of 1 (resistant, small, brown, pin-point-sized specks) to 9 (susceptible, typical leaf blast lesions, 3 mm or longer, infecting more than 75% of the leaf area) in accordance with the Standard Evaluation System (SES, IRRI 2002).

RESULTS

Transgene expression analysis

The T₆ generation lines of resveratrol transgenic rice (Iksan515 and Iksan526) were used for transgene expression profiling. *Resveratrol synthase* gene expression in the young rice leaves was confirmed by RT-PCR for both transgenic lines while it was absent in the isogenic variety 'Dongjin' (Fig. 1C). The selectable marker *bar* gene was stably expressed in the fresh young leaves of both transgenic lines (Fig. 1D) as assessed by immuno-strip assay (PAT). In

a related study, approximately 48 µg/g piceid and less than 1 µg/g resveratrol were detected in the leaves of Iksan515 and Iksan526 (RS18) by high-performance liquid chromatography (HPLC) (Baek *et al.* 2013). The different insertion sites of the two transgenic lines indicate that they are independent lines. T-DNAs of both lines were inserted into intergenic regions of rice chromosomes, suggesting that unintended consequences, such as gene disruption at the insertion site, did not occur.

Transgenic rice lines response to bacterial blight

Both transgenic rice lines and 'Dongjin' were susceptible (lesion length >5 cm) to all four bacterial blight races based on SES disease index. Significant differences between 'Dongjin' and Iksan515 were observed for K1 ($t=5.13^{**}$) at 0.01 and K2 ($t=4.22^{**}$) at 0.05 significance levels, and a significant difference between 'Dongjin' and Iksan526 was noted on response to K1 race ($t=2.56^{*}$) at 0.01 significance level. This indicates that Iksan515 is more susceptible than Iksan526 to K1 and K2 races. Differences

Table 1. Disease severity of transgenic lines and their isogenic variety response to four bacterial blight races (K1, K2, K3, and K3a) of Korea evaluated after 14 days of inoculation in a GMO greenhouse.

Races/lines	Disease severity (% , lesion length / total leaf length)					
	Dongjin		Iksan515	t	Iksan526	t
K1	7.47±1.51 ^a	-	13.37±4.37	5.13 ^{**c}	14.15±2.53	4.22 ^{**}
K2	11.16±1.79	-	18.83±5.14	2.56 ^{*b}	12.14±1.87	0.53
K3	7.00±1.85	-	9.33±4.19	0.64	8.24±2.05	0.77
K3a	8.79±1.19	-	11.55±1.93	1.70	12.44±2.50	1.86

^a Mean ± standard deviation

^b t-test implied significant difference between transgenic lines and Dongjin at the 0.05 level

^c t-test implied significant difference between transgenic lines and Dongjin at the 0.01 level

Table 2. Disease incidence on the flag, second, and third leaves of transgenic lines and their isogenic variety response to leaf brown spot after natural infection in the Gunwi GMO experimental field.

Leaf position/lines	Number of lesions / leaf area (cm ²)					
	Dongjin		Iksan515	t	Iksan526	t
Flag leaf	0.21±0.10 ^a	-	0.63±0.25	2.27 ^{**b}	0.38±0.13	1.47
The second leaf	0.20±0.12	-	0.36±0.25	0.87	0.20±0.14	0.00
The third leaf	0.34±0.08	-	0.64±0.40	1.05	0.35±0.20	0.07
Means of upper three leaves	0.25±0.12	-	0.54±0.33	7.12 ^{**}	0.31±0.18	2.03

^a Mean ± standard deviation

^b t-test implied significant difference between transgenic lines and Dongjin at the 0.01 level

in the severity of leaf blight caused by K3 and K3a races were not statistically significant between 'Dongjin' and the two transgenic lines, although the later tend to be more susceptible over time compared to 'Dongjin'. However, it should be noted that high standard deviations (± 4.37 for K1, ± 5.14 for K2) and relatively large ranges between the investigated individuals (9.0–17.74 for K1, 13.69–23.97 for K2) of the bacterial blight severity for Iksan515 was the likely reason for significant differences ($t = 5.13^{**}$ for K1, $t = 2.56^*$ for K2) compared to the response of 'Dongjin'.

Transgenic rice lines response to brown leaf spot

The flag leaf of 'Dongjin' and Iksan515 exhibited reactions to brown leaf spot which were significantly different to

each other ($t = 2.27^{**}$) at 0.01 significance level. In addition, Iksan515 showed more infected lesions on the second and third leaves compared to Iksan526. However, Iksan526 responses to brown leaf spot were not significantly different to Dongjin on the flag leaf, second leaf, and third leaf (Table 2). More lesions were observed on the flag leaves of Iksan515 (Fig. 3A) compared to Iksan526 and 'Dongjin' which both displayed the same number. Overall, the brown leaf spot response of Iksan515 was significantly different ($t = 7.12^{**}$) from 'Dongjin', but not to Iksan526. The severity (number of lesions/leaf area (cm^2)) of Iksan515 was the most highly variable among the treatment genotypes with values ranging from 0.38–0.88 on flag leaf, 0.11–0.61 on the second leaf, and 0.24–1.04 on the third leaf,

Fig. 3. Evaluation for brown leaf spot and leaf blast by natural infection in the Gunwi and Suwon GMO experimental fields, respectively. A. flag leaves of the transgenic rice lines and their isogenic variety 'Dongjin' in response to brown leaf spot in the Gunwi GMO experimental field; B. layout of 'Dongjin', Iksan515, Iksan526, and spreader rows of leaf blast (seedlings) by natural infection at the Suwon GMO experimental field and the evaluation criteria for resistance, moderate resistance, and susceptibility; C. evaluation for leaf blast in the case of the transgenic rice lines Iksan515 and Iksan526 and their isogenic variety 'Dongjin'.

compared to ‘Dongjin’ which had corresponding value of 0.11–0.31, 0.08–0.32 and 0.26–0.42, and to Iksan526 which also revealed 0.25–0.51, 0.06–0.34 and 0.15–0.55 severity score, respectively. This may have probably caused the significant difference ($t = 7.12^{**}$) observed at 0.01 significance level between Iksan515 and ‘Dongjin’.

Transgenic rice lines response to leaf blast disease

The transgenic lines and ‘Dongjin’ showed resistance (Scale = 1) to leaf blast which was highly incomparable to the susceptible cultivar ‘Nagdong’ (Scale = 9, Fig. 3C). Although we did not observe significant differences in the response between transgenic lines and the isogenic variety to blast races influencing Suwon rice production such as KJ301, KJ101, and KJ133. Likewise, in the present study Dongjin showed higher resistance (scale = 1) to leaf blast compared when the screening was done in Suwon region where it got only moderate resistance (scale = 5). This is possibly due to the environmental conditions of the GMO experimental field in Gunwi such as low humidity and temperature.

DISCUSSION

We found out that production of the secondary metabolite phytoalexin resveratrol induced by *resveratrol synthase* gene insertion into the transgenic rice lines Iksan515 and Iksan526 did not improve the resistance to bacterial blight and brown leaf spot. Both transgenic lines exhibited showed resistance to rice blast but at level comparable to the isogenic variety ‘Dongjin’. Most studies on transgenic crops overexpressing *stilbene synthase* reported increased resistance against bacterial and fungal diseases (Hain *et al.* 1993; Stark-Lorenzen *et al.* 1997; Tian *et al.* 1998; Hipskind *et al.* 2000; Liang *et al.* 2000; Coutos-Thevenot *et al.* 2001; Zhu *et al.* 2004; Lim *et al.* 2005; Serazetdinova *et al.* 2005; Liu *et al.* 2011). However, no resistance or increased susceptibility conferred by *stilbene synthase* gene transformation has been reported in transgenic tomato, kiwifruits, white poplar, or strawberry (Thomzik *et al.* 1997; Kobayashi *et al.* 2000; Giorcelli *et al.* 2004; Hanhineva *et al.* 2009). Here, we present the contradictory conclusions from previous

studies about piceid production and concentrations, host-pathogen relationships, desirable promoters, and alternations of biosynthesis pathways.

First, a number of transformation experiments designed to produce resveratrol-3-glucoside (piceid), resveratrol derivatives, or no resveratrol in GM kiwifruit and strawberry transformed with the *stilbene synthase* gene resulted to an increased susceptibility or no resistance against *Botrytis cinerea* (Kobayashi *et al.* 2000; Hanhineva *et al.* 2009). At that time no studies have been conducted yet comparing the antifungal activity of piceid and resveratrol. Schouten *et al.* (2002) studied the laccase *Bclcc2* gene from *Botrytis cinerea* and found that it converts resveratrol into more toxic compounds and induces self-intoxication to protect the grapevine (*Vitis*) from *Botrytis cinerea* infection. This contradicts the previous theory that resveratrol detoxifies. However, *Bclcc2* laccase was only expressed in the presence of resveratrol or tannins, not piceid. Resveratrol exerts antifungal activity against grapevine pathogens, but resveratrol seems to be more than a final product (Chang *et al.* 2011). Resveratrol can be further metabolized into oxidative δ -viniferin that is even more effective against *P. viticola* zoospores or into the ineffective glycoside piceid, which does not show any toxic activity against *P. viticola* zoospores even at concentration more than 100 μ M (390 μ g/ml), at least 5 times that of resveratrol and 10 times that of δ -viniferin. White poplar was transformed with a *stilbene synthase* (*StSy*) gene from grapevine by Giorcelli *et al.* (2004), and a total of 615.2 μ g/g trans- and cis-isomers piceid was detected in the fresh weight (FW) transformants, but no resistance against the pathogen *Melampsora pulcherrima* was observed.

However, piceid was also identified at a concentration of 0.5–20 μ g/g FW in the leaves of transgenic alfalfa transformed with *RS* under the control of *CaMV35S* promoter by Hipskind *et al.* (2000) and transgenic papaya with *VstI* insertion under the control of the pathogen-inducible promoter *VstI* at a concentration of 54 μ g/g by Zhu *et al.* (2004). Both plants showed resistance to the fungal pathogens *Phoma medicaginis* and *Phytophthora palmivora*. Moreover, Liu *et al.* (2011) overexpressed a *resveratrol synthase* gene (*PcRS*) in transgenic *Arabidopsis*, which led to the accumulation of 93.31 to 183.73 μ g/g (FW) trans-piceid

and resulted in enhanced resistance against the fungal pathogen *C. higginsianum*. These studies indicate that low concentrations of piceid can confer resistance against fungal pathogens to some extent. It is unclear whether these findings suggests that foreign piceid has antifungal activity or if a different mechanism is at work in grapevine due to the existence of endogenous piceid.

Second, antifungal activity of foreign resveratrol may depend on the relationships between hosts and pathogens. Thomzik *et al.* (1997) transferred two genes from the grapevine coding for *stilbene synthase* into tomato. The accumulation of phytoalexin trans-resveratrol was detectable after fungal inoculation and resulted in a significant increase in the resistance of transgenic tomato to *Phytophthora infestans* but no significant increase in resistance to *Botrytis cinerea* and *Alternaria solani*. Therefore, they assumed that the foreign phytoalexin resveratrol only affected host-pathogen relationships, such as those involving the biotrophic or hemibiotrophic pathogen *P. infestans* and necrotrophic pathogens such as *Alternaria* and *Botrytis*, all of which were less sensitive to the presence of resveratrol. However, transgenic tobacco, rice, alfalfa, wheat, *Arabidopsis*, and *Rehmannia glutinosa* showed resistance to necrotrophic pathogens, but transgenic poplar showed no resistance to the biotrophic pathogen *Melampsora pulcherrima*, suggesting no direct relationship between foreign phytoalexin resveratrol and the pathogen type.

Third, pathogen-inducible promoters used to control stilbene synthase genes are favorable to confer GM plants with pathogen resistance. Three types of promoters consisting of constitutive promoter CaMV35S (transgenic alfalfa, kiwifruit, poplar, *rehmannia glutinosa*, strawberry, and *Arabidopsis*), pathogen-inducible promoters Vst1 (transgenic tobacco, tomato, rice, wheat, and papaya) and PR10 (grapevine), and floral filament-specific promoter fl1 (strawberry) have been used to control stilbene gene expression in previous studies. The CaMV35S promoter often triggered strong and constitutive stilbene accumulation followed by a drastic depletion of the endogenous precursor pools. In contrast, the pathogen-inducible promoter appears to be a promising way to maintain precursor pool levels as suggested by Delaunoy *et al.* (2009), allowing strong stilbene accumulation at the infection site. At least three

studies were done involving transformation of the stilbene genes under the control of the CaMV35S promoter which enhanced resistance to fungal pathogens (Hipskind *et al.* 2000; Lim *et al.* 2005; Liu *et al.* 2011). But, no resistance to *Botrytis cinerea* and *Alternaria solani* was reported for transgenic tomato under the control of pathogen-inducible promoter Vst1 (Thomzik *et al.* 1997). Therefore, different promoters may not provide a reasonable explanation on susceptible responses against pathogens by foreign stilbene resveratrol or piceid transformation.

Finally, alternations of phenolic compounds up- and downstream of *resveratrol synthase* upset the balance of the phenylpropanoid pathway. Increased phenolic compounds and decreased flavonol and *chalcone synthase* transcript levels led to increased susceptibility to grey mold fungus in transgenic strawberry (Hanhineva *et al.* 2009). Furthermore, altered seed coat pigmentation and significant reductions in anthocyanin levels were observed in transgenic *Arabidopsis*, but *chalcone synthase* was not downregulated (Liu *et al.* 2011). These two studies proposed that additional *stilbene synthase* activities limited the supply of precursors for *chalcone synthase*, which caused unbalanced flavonoid biosynthesis. Piceid production, low stilbene concentrations, constitutive promoter activity, and stilbene biosynthesis pathway alterations are possible reasons for the lack of antifungal activity in connection with foreign stilbene gene expression. Likewise, phytoalexin stilbene transformations seem to result in unpredictable stilbene production, expression levels, and antifungal activities.

In the present study, *resveratrol synthase* gene (*RS3*) was transformed into rice under the control of maize *ubi1* promoter, and expression was confirmed in both transgenic lines. HPLC confirmed that Iksan 526 contained approximately 48 µg/g trans-piceid and less than 1 µg/g resveratrol in leaves (Baek *et al.* 2013). Similar concentrations of resveratrol and trans-piceid were also reported for Iksan515. Except for leaf blast, Iksan515 showed much susceptible responses to K1 and K2 races of bacterial blight and brown leaf spot compared to the isogenic variety ‘Dongjin’. A highly variable severity of both diseases in Iksan515 lines suggests that individual segregation or phenotypic mutations occurred during the rice tissue culture. Taken together, resveratrol rice lines transformed with *resveratrol synthase* gene did

not show increased resistance to the three pathogens investigated in this study. Based on the data discussed above, the use of the constitutive promoter *ubi1*, relatively low production of resveratrol and piceid ($48 \pm 1 \mu\text{g/g}$), and the alterations of phenolic acids or competition for the precursors of *resveratrol synthase* and *chalcone synthase* probably resulted in increased susceptibility to bacterial and fungal diseases for both transgenic lines Iksan515 and Iksan526. In addition, foreign gene introduction and chromosome rearrangement during tissue culture may have upset the chromosome balance and contributed to unpredictable foreign stilbene transformation. To further define the up- and downregulation of enzymes that is crucial to resveratrol production in the phenylpropanoid pathway, such as *phenylalanine ammonia-lyase* (*PAL*), *cinnamate 4-hydroxylase* (*C4H*), *4-coumarate CoA ligase* (*4CL*), and *chalcone synthase* (*CHS*), as well as *resveratrol synthase* (*RS*), future studies should employ real-time PCR to assess both transgenic lines at different time points after pathogen inoculation.

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