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Biological Activity of *Rehmannia glutinosa* Transformed with Resveratrol Synthase Genes

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1. Introduction

The rate of increase of food crop production has decreased due different factors such as global climate change, alteration in use of land, pests, disease, salinity and drought. Food production was said to be inadequate for the increasing world population (WHO, 1996a). Therefore, it is essential to increase food production and distribution in order to meet its demand and free from hunger. Furthermore, transgenic plants can enhance yields, harvesting of crops, reduce dependency on chemical insecticides. Production of transgenic plants can address the global problems such as climate change, deficiency of food and nutrition. Development of transgenic plant involves manipulation or transfer of genes from other organisms which may improve yield, quality, herbicides, and pest or diseases resistant or environmental conditions, increased agricultural productivity and better quality foods. Modification of genetic constitute of plant by inserting transgene enhances nutritional composition of the foods and improve human health and minimizes the use of pesticides and insecticides.

A number of transgenic crop plants has been produced from a variety of crop plants to date with enhanced agronomic characteristics, for example, transgenic tomatoes with improved shelf-life, transgenic fruits and vegetables with delayed ripening time and increased length of storage. Moreover, pest and disease resistance crops have been produced, viz., papaya-ringspot-virus-resistant papaya (Gonsalves 1998), insect resistance cotton, transgenic rice plants that are resistant to rice yellow mottle virus (RYMV) (Pinto et al 1999), improved nutritional contents in the transgenic rice which exhibits an increased production of beta-carotene as a precursor to vitamin A (Ye et al 2000). In addition, technology of transgenic plant production can be used to produce vaccines and bioactive compounds in plants. For example, expression of anti-cancer antibody in rice resulted in production of vaccines against infectious disease from potato (Thanavala et al

1995). *Rehmannia glutinosa* is perennial medicinal plant belongs to the family *Scrophulariaceae*. Its root has long been used in Korea for medicinal purposes such as hemantic, robustness, cardiotonic drug, diabetes treatment, antifebriel and detoxification purposes (Choi et al., 1995). Roots of *R. glutinosa* are usually infected with various pathogens during storage and these infections cause great damage to the roots and impede the intensive farming of the crop (Lim et al 2003).

Resveratrol is found in a limited number of unrelated plants and possess antifungal activity and induction in response to pathogen infection. Resveratrol is well known for its potent antioxidant activity and health-promoting effects, cardioprotection (Ignatowicz and Baer-Dubowska 2001) and reduction of cancer risk have also been observed (Jang et al. 1997; Cal et al. 2003).It can also exert neuroprotective effects by increasing heme oxygenase activity in the brain (Zhuang et al. 2003). The expression of RS transcripts has been associated with an increasedresistance to various fungal pathogens in transgenic tobacco (Hain et al. 1993) and tomato (Thomzik et al. 1997).

The purpose of this report is to better understand the role of transgene to improve the nutrition value of important crops and to evaluate the biological activity of secondary metabolic substances such as resveratrol, SOD, phenolic compounds in *Rehmannia glutinosa* under environmental stress.

2. Transformation of *R. glutinosa* with RS gene

The peanut RS genomic DNA sequence, AhRS3 (GenBank Accession number, AF227963) a polypeptide of 389 amino acid residues, was cloned into the Xba I/Cla I sites of binary expression vector pGA643 under the CaMV35S promoter. This produced a recombinant AhRS3 expression plasmid, pMG-AhRS3. *Agrobacterium tumefaciens* strain LBA4404 harboring the binary vector pMG-AhRS3, which contains the neomycin phosphotransferase gene (npt II) directed by the nos promoter as a selectable marker, was used. A single colony of this strain was grown for 24 h at 28 ± 1 ^{o}C with shaking (150 rpm) in 20 ml of liquid Luria–Bertani (LB) medium (10 g/l tryptone, 10 g/l NaCl, 5 g/l yeast extract, pH 7) containing 100 mg/l kanamycin. The cells were centrifuged for 10 min at 7,000 x *g* at 4 ^{o}C and resuspended in liquid inoculation medium (MS medium with 20 g/l of sucrose) to obtain a final OD_{600} = 1.0 for use in plant infection. The surface-sterilized leaf explants were cultured for 2–3 days on MS medium containing 1 mg/l 6-benzylamino-purine (BAP), 2 mg/l thidiazuron (TDZ), 0.2 mg/l naphthalene acetic acid (NAA), Murashige and Skoog (MS) vitamin, 3% sucrose, and 0.8% agar (pH 5.2). Pre-treated explants were dipped into the Agrobacterium suspension in liquid inoculation medium for 10–15 min, blotted dry on sterile filter paper and incubated in a shoot induction medium (MS medium containing 2 mg/l BAP, 1 mg/l TDZ, 0.2 mg/l NAA, MS vitamin, 3% sucrose, and 0.8% agar at pH 5.2.) in the dark at 23±1 ^{o}C. After co-cultivation for 2 days, the explants were transferred to shoot induction medium containing 50 mg/l kanamycin and 200 mg/l timentin (mixture of ticarcillin disodium and clavulanate potassium) and were transferred to fresh selection medium every 2 weeks. Putative transgenic shoots were regenerated 6–8 weeks after the first sub-culture and were incubated in a growth chamber with a 16 h at 23±1 ^{o}C for 30 days. Putative transgenic

plantlets were then transferred to pots containing autoclaved vermiculite and were grown in the glasshouse.

Transgene-positive T_0 lines were selected by PCR screening. The lines containing RS gene and *npt* II transgene sequences were chosen for the evaluation of biological activities. Transformation of transgene into the plant genome was confirmed by Southern blot analysis.

2.1 Scavenging of DPPH radicals and Inhibition of lipid peroxidation of transgenic *R. glutinosa*

Free radical-scavenging activity was evaluated using trolox as standard antioxidants. The radical-scavenging activity was measured using the stable radical 1,1-diphenyl–2-picrylhydrazyl (DPPH) as previously described (Xing et al., 1996). Various concentrations of the extracts were added to 4 ml 0.004% methanol solution of DPPH. The mixture was shaken and left for 30 min at room temperature in the dark, and the absorbance was measured with a spectrophotometer at 517 nm. The radical-scavenging activity was expressed as a percentage of the absorption of DPPH in the presence and in the absence of the compound. Calculated IC_{50} values indicate the concentration of sample required to scavenge 50% of the DPPH radical. DPPH activity was calculated as

DPPH activity (%) = $(A_{blank} - A_{sample})/A_{blank} \times 100$,

where, A_{blank} is the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Inhibition of lipid peroxidation was determined by measuring thiobarbituric acid-reactive substance production (Buege and Aust 1978).

2.1.1 Measurement of photosynthesis rate

Stomatal conductance (g_s), net CO_2 assimilation rate (A), and the ratio of internal CO_2 concentration (C_i) were measured according to the method described by Ivan et al. (2001) using a portable photosynthesis system (LCA-4; Analytical Development Co., Hoddesdon, Herts, UK) at an air temperature 28 ± 1 °C. For this measurement, ten fully expanded young leaves were selected from the transgenic lines and control plants at the maximum tillering stage. All measurements were performed three times on sunny days between 1000 and 1400 hours on the surface of leaves from August to September under a saturating photosynthetic photo flux density (PPFD) of 1,500 µmol/m/s. Measurements were made at the center of the leaf surface immediately after the CO_2 concentration decrease was stable. Each leaf was allowed to stabilize for 4–6 min before measuring the g_s, C_i, and A.

2.1.2 HPLC analysis

The HPLC analysis was applied using the modified method of Banwart et al. 1985. The mobile phase consisted of solvent A and B. Solvent A contained 98% water and 2% glacial acetic acid in 0.018M ammonium acetate. Solvent B was 70% solvent A and 30% organic solution, the latter being composed of 82% methanol, 16% n-butanol and 2% glacial acetic acid in 0.018M ammonium acetate. Following injection of 20µL of the sample, the flow

rate of the mobile phases was maintained at 1mL min−1. A linear HPLC gradient was employed. The HPLC system consisted of a Young-Lin M930 liquid chromatograph pump and an M720 detector (Young-Lin Instruments Co., Ltd). The column for quantitative analysis was a YMC-Pack ODS-AM-303 (250×4.6mm I.D.), and the UV absorption was measured at 280 nm.

2.1.3 SOD activity

SOD activity of root *R. glutinosa* was measured by the nitro blue tetrazolium (NBT) reduction method (Beyer and Fridovich, 1987). Test tubes containing reaction solution with 3mL of assay buffer, 60 _L of crude enzyme and 30_L of riboflavin were illuminated for 7 min in an aluminium foil lined box containing two 20-W Slyvania Groiux Fluorescent lamps at 25 ◦C. After reaction, the absorbance of the blank solution and reaction solution was measured with a spectrophotometer (Hitachi Ltd., Tokyo, Japan) at 560 nm. SOD activities were calculated as a following equation:

SOD activity (%) = (1 − *A/B*) × 100

A: absorbance of sample; *B*: absorbance of blank.

2.1.4 Paper disc diffusion assay

Bacterial pathogens and fungal strains were grown in liquid medium (micrococcus, nutrient, and YM media) for 20 h to a final concentration of 10^6–10^7 CFU/ml. Aliquots of 0.1 ml of the test microorganisms were spread over the surface of agar plates. Sterilized filter-paper discs (Whatman No. 1, 6 mm) were saturated with 50 µl of the methanol extract at 10,000 ppm and left to dry in a laminar flow cabinet. The soaked, dried discs were then placed in the middle of the plates and incubated for 24 h. Antimicrobial activity was measured as the diameter (mm) of the clear zone of growth inhibition. Negative controls were prepared using the same solvents employed to dissolve the plant extracts.

In order to evaluate morphological and agronomic performance of transgenic *R. glutinosa*, plants of each PCR positive T1 transgenic lines and seed derived control plants were transferred to field containing bed soil and evaluated for morphological characters.

2.2 Results and discussion

2.2.1 Biological activities of transgenic *R. glutinosa*

2.2.1.1 Scavenging of DPPH radicals of transgenic *R. glutinosa*

The free radical scavenging activities of non-transgenic control and transgenic R. glutinosa extracts, α-tocopherol, are presented in Fig.1. A solution of each extract at a concentration of 1.0 mg/ml was prepared. The activities of transgenic sample extracts were between 16.00 and 20.00 µg/ml at 1.0 mg/ml. Most of the transgenic leaves samples showed high antioxidant activity using DPPH as compared to non-transgenic control plants. With regard to RC50 values (the concentration of antioxidant required to achieve absorbance equal to 50% that of a control containing no antioxidants), RS3 transgenic lines showed highest radical-scavenging abilities (RC50 = 16.00 ± 2.00 µm). The DPPH free radical scavenging and

LDL peroxidation activities of trans-3′-H-Rglu and trans-resveratrol isolated from transgenic R. glutinosa evaluated (Fig. 2 & 3). DPPH activity of trans-resveratrol were significantly higher (72 ± 4.5 µm) than trans-3′-H-Rglu (198 ± 6.8 µm). This could be attributed to the higher level of accumulation of resveratrol compounds in the transgenic R. glutinosa (Fig. 4).

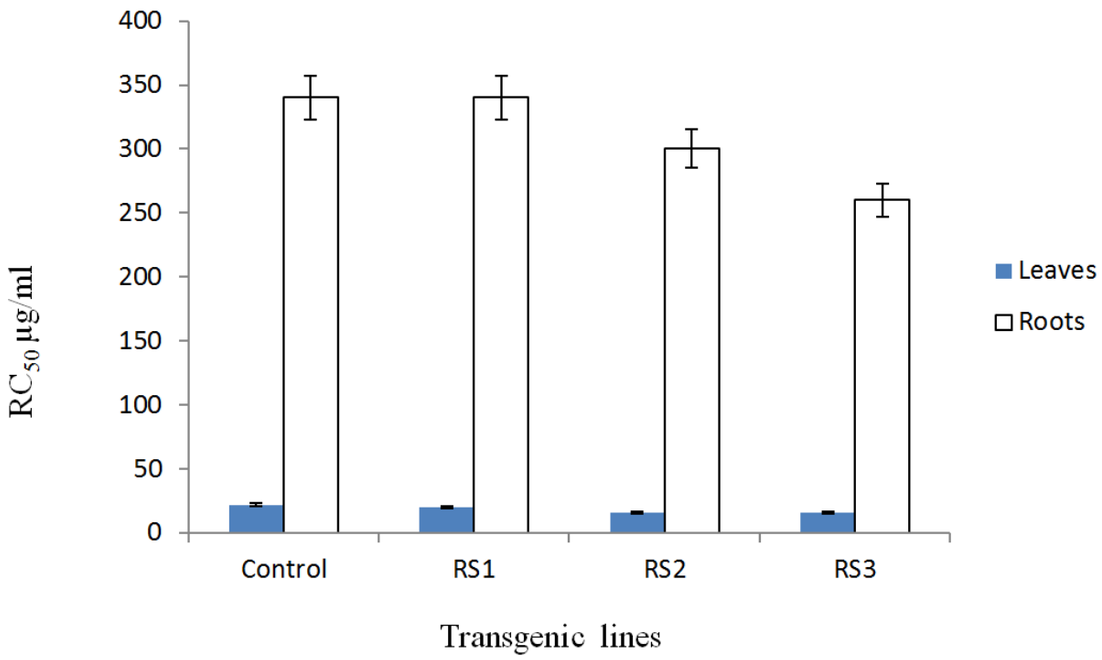


Fig. 1. DPPH free radical scavenging activity of extract in transgenic and non-transgenic *Rehmannia glutinosa*

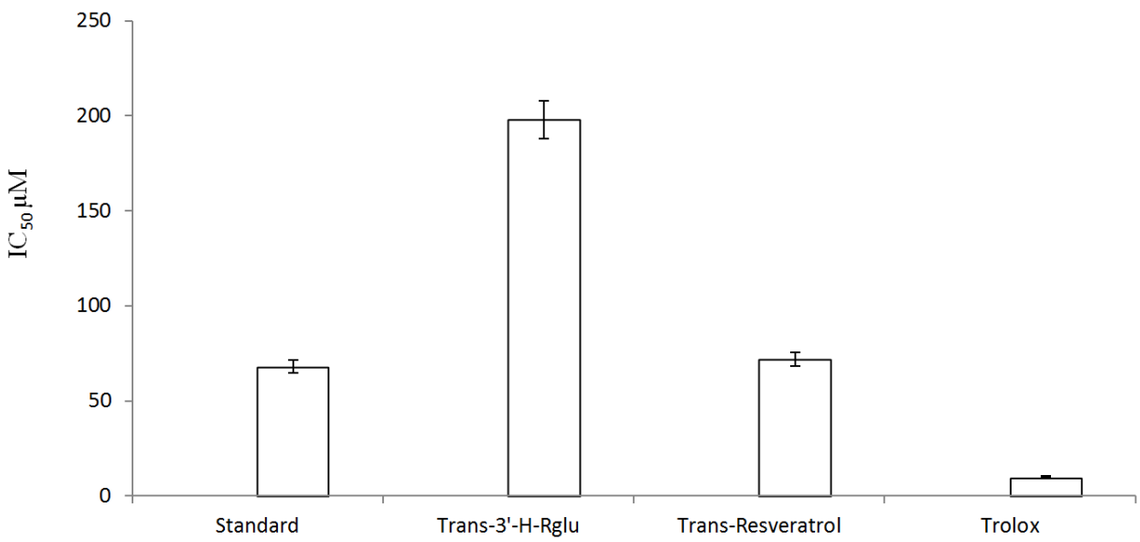


Fig. 2. DPPH scavenging activities of stilbenes isolated from transgenic *Rehmannia glutinosa.*

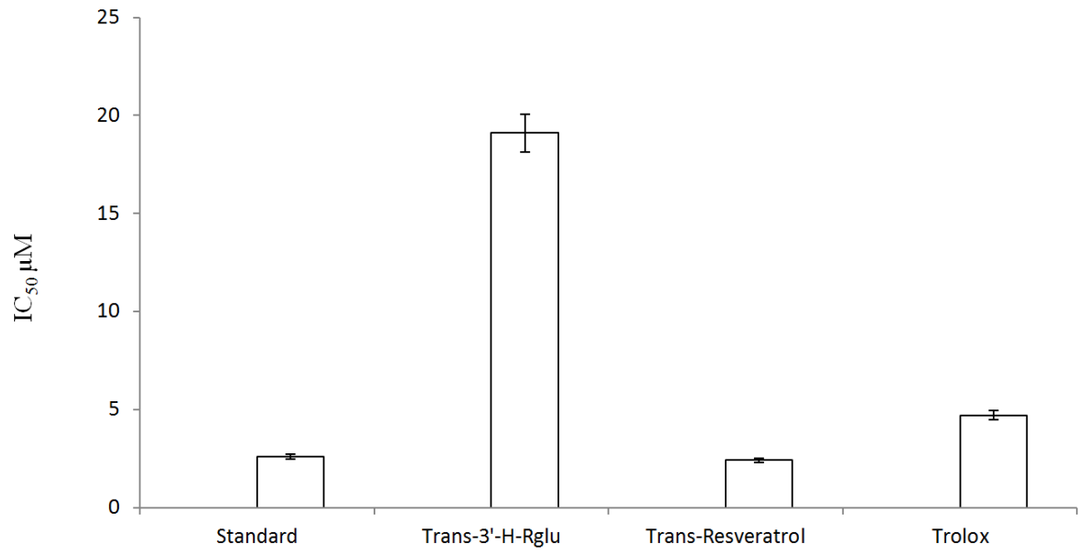


Fig. 3. LDL peroxidation inhibition activities of stilbenes isolated from transgenic *Rehmannia glutinosa.*

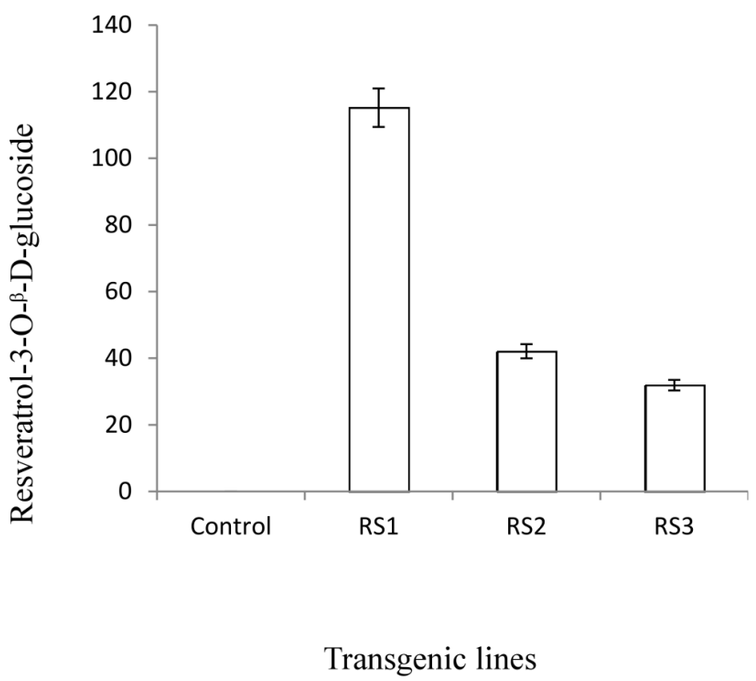


Fig. 4. Accumulation of Resveratrol-3-O-β-D-glucoside levels observed in leaves of RS3-transformed transgenic *Rehmannia glutinosa.*

2.2.2 Superoxide Dismutase (SOD) activity of transgenic *R. glutinosa*

The SOD activities non transgenic plant and transgenic plants (without water stress) were 13.81 and 11.23% respectively. In contrast, the SOD activities non transgenic plant and transgenic plants (with water stress) were 24.59 and 3.8 % respectively (Fig. 5).

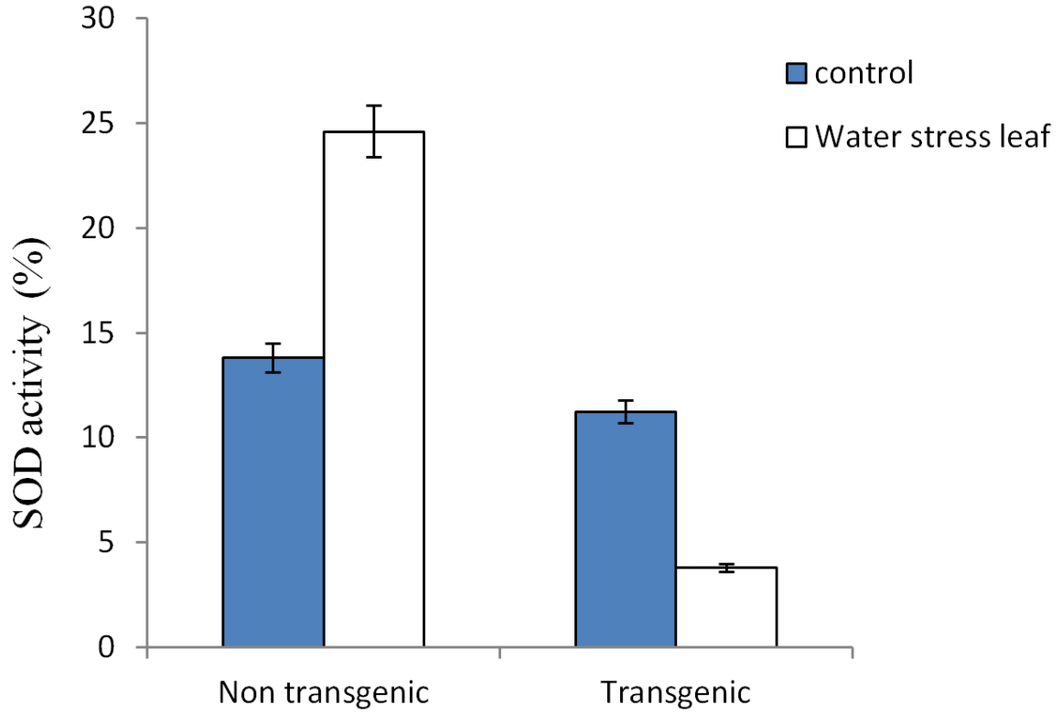


Fig. 5. SOD activities of stilbenes isolated from transgenic *Rehmannia glutinosa.*

2.2.3 Phenolic compound analysis of transgenic *R. glutinosa*

The quantitative analysis of phenolic compounds of non-transgenic and transgenic *R. glutinosa* extract performed using HPLC is given in Table 1. We found quantitative differences in total phenolic compounds between transgenic and control plants stem and root samples under hyper irrigation treatment (Table 2).

Lines	Plant parts	Hyd[1]	Chl[2]	Cat[3]	Caf[4]	Syr[5]	Sal[6]	Cou[7]	Fer[8]	Hes[9]	Nar[10]	Hyr[11]	Cin[12]	Que[13]	Nan[14]	Total
		ug/g														
Control	S	9.12	37.3	1.33	13.25	25.68	0.25	97.19	67.02	54.11	52.02	19.07	82.77	0	0	412.69
	R	4.13	0	0	0	2.19	0	7.21	12.35	6.71	0	53.45	98.53	26.39	0	210.94
RS1	S	3.71	34.13	1.14	0.25	20.81	3.35	90.84	76.28	47.66	55.17	16.99	185.22	0	0	497.72
	R	1.48	0	0	0	10.21	0	18.04	8.53	3.23	0	14.42	198.69	14.57	0	269.18
RS2	S	0.25	29.77	0.25	0.25	11.26	0.25	74.26	83.06	34.41	176.93	73.53	83.69	0	0	537.88
	R	9.48	0	0	0	4.84	0	7.75	7.41	0	10.96	23.88	114.2	19.99	0	189.01
RS3	S	0.25	43.08	0.25	0.25	12.48	0.25	117.69	60.2	48.63	84.61	43	100.91	0	0	468.27
	R	14.61	6.47	0	0	10.02	0	23.32	17.95	0.77	0	24.18	264.23	19.57	0	360.01

S: shoot, R: root, Hyd[1]: *p*-hydroxybenzoic acid, Chl[2]: Chlorogenic acid, Cat[3]: Cathechin, Caf[4]: Caffeic acid, Syr[5]: Syringic acid, Sal[6]: Salicylic acid, Cou[7]: *p*-coumeric acid, Fer[8]: Ferulic acid, Hes[9]: Hesperidin, Nar[10]: Narigen, Hyr[11]: Hyricetin, Cin[12]: trans-cinnamic acid, Que[13]: Quercitin, Nan[14]: Narigenin.

Table 1. Distribution of major phenolic compounds in control and transgenic plants transformed by resveratrol synthase in *R. glutinosa* under hyper irrigation.

The average total concentrations of phenolic compounds in control plant stem and roots were 412.69 and 210.94 µg/g dry weight (DW), respectively; in comparison, transgenic stem and

root samples had higher concentrations of 468.27–537.88 and 189.01–360.01µg/g DW, respectively. The phenolic compounds that increased in the transgenic lines were *p*-hydroybenzoic acid, *p*-coumaric acid, ferulic acid, narigenin, trans-cinnamic acid, chlorogenic acid. Similarly, we found quantitative differences in total phenolic compounds between transgenic and control plants stem and root samples under pathogen treatment (Table 2). The average total concentrations of phenolic compounds in control plant stem and roots were 364.58 and 181.20 µg/g DW, respectively; in comparison, transgenic stem and root samples had higher concentrations of 555.00–919.16 and 312.70–677.26 µg/g DW, respectively.

Lines		Hyd[1]	Chl[2]	Cat[3]	Caf[4]	Syr[5]	Sal[6]	Cou[7]	Fer[8]	Hes[9]	Nar[10]	Hyr[11]	Cin[12]	Que[13]	Nan[114]	Total
		ug/g														
Control	S	7.08	5.75	0	0	0	0.25	0.25	22.8	0	293.04	26.14	0	0	22.11	364.58
	R	21.4	14.28	0	9.82	0	7.78	5.73	12.53	0	0	33.23	94.47	17.66	0	181.2
RS1	S	27.98	39.58	2.57	0	53.45	22.22	28.97	195.6	0	195.35	56.84	0	0	0	555
	R	8.97	10.64	0	0	21.45	0	10.21	6.93	0	3.35	33.91	211.4	20.16	0	318.05
RS2	S	35.27	78.8	1.18	0.25	60.42	240.9	38.61	137.3	15.97	245.74	38.84	6.87	0	133.01	919.16
	R	0	0	0	0	36.87	0	9.04	4.25	8.37	0	8.35	223.25	22.58	0	312.7
RS3	S	0	0	0	0	0	0	153.91	353.5	25.04	97.34	47.42	0	0	0	677.26
	R	30.54	14.61	0	7.01	0	6.02	46.33	21.62	9.93	0	26.56	464.94	19.7	0	602.11

S: shoot, R: root, Hyd[1]: *p*-hydroxybenzoic acid, Chl[2]: Chlorogenic acid, Cat[3]: Cathechin, Caf[4]: Caffeic acid, Syr[5]: Syringic acid, Sal[6]: Salicylic acid, Cou[7]: *p*-coumeric acid, Fer[8]: Ferulic acid, Hes[9]: Hesperidin, Nar[10]: Narigen, Hyr[11]: Hyricetin, Cin[12]: trans-cinnamic acid, Que[13]: Quercitin, Nan[14]: Narigenin.

Table 2. Distribution of major phenolic compounds in control and transgenic plants transformed by resveratrol synthase in *R. glutinosa* under infected pathogen (*Fusarium oxysporum*).

2.2.4 Antimicrobial activities transgenic *R. glutinosa*

Antimicrobial activities of the non-transgenic and transgenic plants were assessed by a paper disc diffusion assay. The results indicated variation in the antimicrobial properties of the resveratrol-3-O-B-D glucoside and resveratrol extracted from the transgenic *R. glutinosa* (Table 3). In general, the resveratrol was more effective than the resveratrol-3-O-B-D glucoside against all the microbes tested. The strongest inhibitory effect was against *E. coli* and *S. typhimurium* at concentration of 1 mg/ml. Antimicrobial activity in plant extracts depends not only on the presence of phenolic compounds, but also on the presence of various secondary metabolites (Gordana et al., 2007). These observations suggest that the antimicrobial activity of transgenic *R. glutinosa* enhanced by the RS3 genes transformed into the *R. glutinosa* genome. However, other phenolic acid-like phenols are thought to contribute to plant defences against pests and pathogens (Awika & Rooney, 2004).

Compounds	Clear zone (mm)							
	Conc. (ppm)	*P. jadinii*	*C. albicans*	*S. aureus*	*B. subtilis*	*K. pneumonia*	*E. coli*	*S. typhimurium*
Resveratrol -3-O-B-D-glucoside	20000	11	10.8	10.6	10.1	9.8	12.4	12.7
Resveratrol	20000	13.7	12.8	12.9	14.2	11.5	19.8	18.6

Table 3. Antimicrobial activity of stilbenes compounds isolated from transgenic *R. glutinosa.*

2.2.5 Morphological characterization of transgenic *R. glutinosa*

Phenotypic differences were observed within the different transgenic lines and between the transgenic and non-transgenic control plants (Table 4). However, there were no apparent differences in terms of root length and root diameter. Significant differences in root weight were observed between transgenic and non-transgenic lines and showed reduced weight over control plants.

Line	Root length (cm)	root diameter	Root weight
Control	24.3	20	330.1
RS1	20.3	14	142
RS2	23.6	18	226.3
RS3	21.9	15	159.5
RS4	21.7	17	273.1

Table 4. Growth characteristics of transgenic *R. glutinosa.*

2.2.6 Analysis of catapol content of transgenic *R. glutinosa*

The catapol contents and composition in subterranean parts of the transgenic lines and non-transformed plants were investigated using HPLC (Fig. 6). Overexpression of RS3 gene significantly increased the catapol, compared to that of wild type *R. glutinosa.*

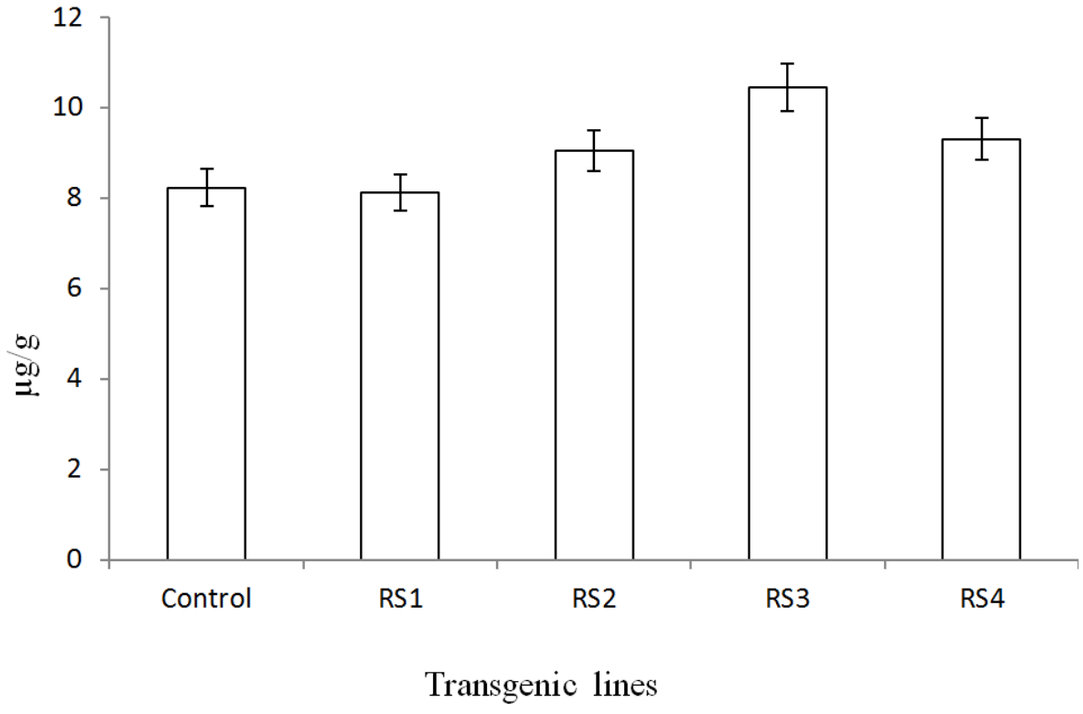


Fig. 6. Content of catapol in root of *R. glutinosa*

2.2.7 Effect of the photosynthesis rate in transgenic *R. glutinosa*

To compare the effect of RS3 gene overexpression on the photosynthesis rate and yield of transgenic and control plants, we measured stomatal conductance (gs), CO_2 concentration (CI), and photosynthesis rate (A) and found significant differences in these factors between transgenic and control plants (Table 5). The photosynthesis rate increased progressively with increasing CO_2 concentration. Photosynthesis rate of both non-transgenic and transgenic plants reduced by the increased duration of dry stress, being much lower at 15 days. Comparatively, transgenic lines showed higher photosynthetic control plants. Therefore, it is very possible that the higher level of RS3 gene in the transgenic plant is responsible for its enhanced photosynthetic performance.

Treatment	Days	Photosynthetic rate					
		Non-transgenic plant			Transgenic plant		
		A (µmol $m^{-2}s^{-1}$)	gs (µmol $m^{-2}s^{-1}$)	Ci (ppm)	A (µmol $m^{-2}s^{-1}$)	Gs (µmol m-2s-1)	Ci (ppm)
Control-		14.63 ± 0.1	0.29 ± 0.0	241.67 ± 0.85	17.42 ± 0.14	0.27 ± 0.02	212.47 ± 0.17
	3D	9.68 ± 0.06	0.11 ± 0.01	195.8 ± 3.14	17.1 ± 0.06	0.32 ± 0.0	223.98 ± 1.88
Dry stress	9D	3.63 ± 0.14	0.02 ± 0.0	45.52 ± 5.92	14.39 ± 0.04	0.23 ± 0.0	220.13 ± 1.24
	15D	1.13 ± 0.01	0.0	215.82 ± 0.78	1.69 ± 0.31	0.0	194.13 ± 4.21

A: Photosynthetic rate; gs: Stomatal conductance; Ci: Intercellular; CO_2concentration.

Table 5. Photosynthetic rate of non-trangenic and transgenic *R. glutinosa* under water stress.

It can be concluded that introduction of RS3 gene into the *R. glutinosa* genome resulted into increased production of stilbenes compounds that enhances the biological activity of plants. Increased in the resveratrol compounds further enhanced the disease resistance capacity of plant. This may cause beneficial effects on human and plant defence system.

3. References

Anwart WL, Porter PM, Granato TC, Hassett JJ (1985) HPLC separation and wavelength area ratios of more than 50 phenolics and flavonoids. J. Chem. Ecol. 11: 383–395.

Awika J M and Rooney LW (2004). Sorghum phytochemical and their potential impact on human health. Phytochemistry, 65: 1199–1221.

Beyer Jr, WE, Fridovich I, (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161: 559–566.

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol. 52: 302–310.

Cal C, Garban H, Jazirehi A, Yeh C, Mizutani Y and Bonavida B (2003) Resveratrol and cancer: chemoprevention, apoptosis, and chemo-immunosensitizing activities. Curr. Med. Chem. Anti-Canc. Agents. 3: 77–93.

Gonsalves D (1998) Control of papaya ringspot virus in papaya: A case study. *Annual Review of Phytopathology*, 36: 415-437.

Gordana S C, Jasna MC, Sonja MD, Tumbas VT, Markov SL, and Dragoljub DC (2007) Antioxidant potential, lipid peroxidation inhibition and antimicrobial activities of Satureja montana L. Subsp. kitaibelii extracts. International Journal of Molecular Sciences, 8: 1013–1027.

Hain R, Reif HJ, Krause E, Langebartels R, Kind H, Vornam B, Wiese W, Schmelzer E, Schreier PH, Stocker RH and Stenzel K (1993) Disease resistance results from phytoalexin expression in a novel plant. Nature 361: 153–156.

Ivan Y, Tsonko T, Violeta V, Katya G, Peter I, Nikolai T, Tatyana P (2001) Changes in CO_2 ssimilation, transpiration and stomatal resistance of different wheat cultivars experiencing drought under field conditions. Bulg J Plant Physiol 27:20–33

Ignatowicz E and Baer-Dubowska W (2001) Resveratrol, a natural chemopreventive agent against degenerative diseases. Pol. J. Pharmacol. 53: 557–569.

Jang M, Cai L, Udean GO, Slowing KV, Thomas CF, Beecher CWW, Fong HHS, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC and Pezzuto JM (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 218–220.

Lim JD, Yun SJ, Lee SJ, Chung IM, Kim MJ, Heo K and Yu CY (2004) Comparison of resveratrol contents in medicinal plants. Korean J. Medicinal Crop Sci. 12: 163–170.

Pinto Y M, Kok RA and Baulcombe DC (1999) Resistance to rice yellow mottle virus (RYMV) in cultivated African rice varieties containing RYMV transgenes. *Nature Biotechnology* 17:702–707.

Thanavala Y, Yang Y, Lyons P, Mason HS, Arntzen C (1995). Immunogenicity of Transgenic Plant-derived Hepatitis B Surface Antigen. Proc. Nat. Acad. Sci. USA. 92: 3358-3361.

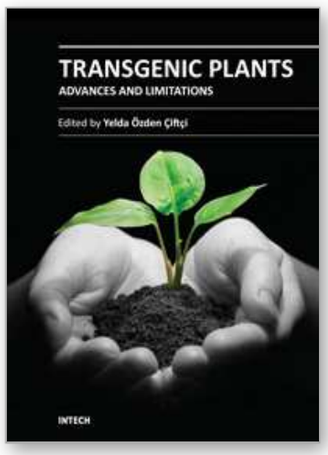
Thomzik JE, Stenzel K, Stocker R, Schreier PH, Hain R and Stahl DJ (1997) Synthesis of a grapevine phytoalexin in transgenic tomatoes (Lycopersicon esculentum Mill.) conditions resistance against Phytophthora infestations. Physiol. Mol. Plant Pathol. 51: 265–278.

WHO.: 1996a, Micronutrient Malnutrition: Half theWorld's Population Affected,World Health Organization, 13 Nov. 1996 no. 78, pp. 1–4.

Xing Q, Kadota S, Tadata T, Namba T (1996) Antioxidant effect of phenylethanoids from *Cistanche deserticola.* Biological and Pharmaceutical Bulletin. 19: 1580-1585.

Ye X, et al (2000) Engineering the provitamin A (beta carotene) biosynthesis pathway into (carotenoid-free) rice endosperm. Science, 287, 303-305.

Zhuang H, Kim YS, Koehler RC and Dore S (2003) Potential mechanism by which resveratrol, a red wine constituent, protects neurons. Ann. New York Acad. Sci. 993: 276–286.



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Development of efficient transformation protocols is becoming a complementary strategy to conventional breeding techniques for the improvement of crops. Thus, Transgenic Plants - Advances and Limitations covers the recent advances carried on improvement of transformation methods together with assessment of the impact of genetically transformed crops on biosafety. Each chapter has been written by one or more experienced researchers in the field and then carefully edited to ensure throughness and consistency.

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