Evaluating water deficit and glyphosate treatment on the accumulation of phenolic compounds and photosynthesis rate in transgenic *Codonopsis lanceolata* (Siebold & Zucc.) Trautv. over-expressing γ -tocopherol methyltransferase (γ -tmt) gene

Bimal Kumar Ghimire¹ · Na-Young Son¹ · Seung-Hyun Kim¹ · Chang Yeon Yu² · Ill-Min Chung¹

Received: 6 March 2017 / Accepted: 10 April 2017 / Published online: 29 June 2017
© Springer-Verlag GmbH Germany 2017

Abstract The effect of water stress and herbicide treatment on the phenolic compound concentration and photosynthesis rate in transgenic *Codonopsis lanceolata* plants over-expressing the γ -tmt gene was investigated and compared to that in control non-transgenic *C. lanceolata* plants. The total phenolic compound content was investigated using high-performance liquid chromatography combined with diode array detection in *C. lanceolata* seedlings 3 weeks after water stress and treatment with glyphosate. Changes in the composition of phenolic compounds were observed in leaf and root extracts from transformed *C. lanceolata* plants following water stress and treatment with glyphosate. The total concentration of phenolic compounds in the leaf extracts of transgenic samples after water stress ranged from 3455.13 ± 40.48 to $8695.00 \pm 45.44 \mu\text{g g}^{-1}$ dry weight (DW), whereas the total concentration phenolic compound in the leaf extracts of non-transgenic control samples was $5630.83 \pm 45.91 \mu\text{g g}^{-1}$ DW. The predominant phenolic compounds that increased after the water stress in the transgenic leaf were (+) catechin, benzoic acid, chlorogenic acid, ferulic acid, gallic acid, rutin, vanillic acid, and veratric acid. The total concentration of phenolic compounds in the leaf extracts of transgenic samples after glyphosate treatment ranged from 4744.37 ± 81.81 to $12,051.02 \pm 75.00 \mu\text{g g}^{-1}$ DW, whereas the total concentration of the leaf extracts of non-transgenic control samples after glyphosate treatment was

$3778.28 \pm 59.73 \mu\text{g g}^{-1}$ DW. Major phenolic compounds that increased in the transgenic *C. lanceolata* plants after glyphosate treatment included kaempferol, gallic acid, myricetin, *p*-hydroxybenzoic acid, quercetin, salicylic acid, *t*-cinnamic acid, catechin, benzoic acid, ferulic acid, protocatechuic acid, veratric acid, and vanillic acid. Among these, vanillic acid showed the greatest increase in both leaf and root extracts from transgenic plants relative to those from control *C. lanceolata* plants following treatment with glyphosate, which could affect the 5-enol-pyruvyl shikimate-3-phosphate (EPSP) synthase, an enzyme in the shikimate pathway. We observed enhanced stomatal conductance (gs) and photosynthesis rate (A) in the transgenic plants treated with water stress and glyphosate treatment. The results of this study demonstrated large variations in the functioning of secondary metabolites pathway in response glyphosate and water stress in transgenic *C. lanceolata*.

Keywords *Codonopsis lanceolata* · Glyphosate · Phenolic compound · Photosynthesis · Transgenic

Introduction

Plants constantly fight against biotic and abiotic environmental factors, including chemicals, water stress, and pathogen attack, which affect growth and yield (Bahrani et al. 2010), accumulation of compatible organic solutes (Sanchez-Diaz et al. 2008), and changes in endogenous phytohormones (Perales et al. 2005). Water availability is the most important factor that limits the productive potential of higher plants (Rodriguez et al. 2006; Bray et al. 2000). To survive under conditions of environmental biotic and abiotic stress, plants respond and adapt to these stresses

✉ Ill-Min Chung
imcim@konkuk.ac.kr

¹ Department of Applied Life Science, Konkuk University, Seoul 05025, South Korea

² Bioherb Research Institute, Kangwon National University, Chuncheon 200-701, South Korea

by means of biochemical and physiological processes as well as morphological and developmental changes (Munns 2002). Maintenance of a high antioxidant capacity to scavenge toxic reactive oxygen species (ROS) has been correlated with increased tolerance of plants to a wide variety of environmental stresses (Zaefyzadeh et al. 2009; Chen et al. 2010); phenolic compounds (Usenik et al. 2004). Therefore, globally, the use of resistant genetically engineered crops has increased by >80% (James 2012), contributing immensely to yield, sustainable crop farming, and phytoremediation.

Weeds are a major problem for farmers during crop cultivation, and to control weeds, farmers use a variety of herbicides and weedicides every year. The increased use of herbicides has led to the appearance of tolerant or resistant weeds (Service 2007). Moreover, excessive use of herbicides becomes a major ecological risk when genetically modified organisms are released (GMOs) in the environment (Altieri 2005; Barton and Dracup 2000). Herbicides not only inhibit the growth of weeds, but they also inhibit the growth of useful crops in the field. A number of studies have revealed the influence of herbicide treatment on the phenolic compound composition of the plant, thereby influencing the disease resistance potential of plants (Donnini et al. 2016).

Phytochemicals are found at different levels in many aromatic medicinal plants (Perumalla and Hettiarachchy 2011; Negi 2012; Cowan 1999). In plants, these compounds are related to various functions such as defense against pathogens (Kutchan 2001; Housti et al. 2002). The phenolic profiles of plants may change in response to stress conditions such as those induced by the soil-condition, season, climate, plant component, and other parameters (Marian and Fereidoon 2004). Commercial herbicides exhibit that many different mechanisms of action and several enzymes involved in the biosynthesis of amino acids are the sites of action of herbicides (Duke 1990). Glyphosate (*N*-phosphonomethylglycine) is a systemic post-emergence broad spectrum herbicide, which inhibits EPSP synthase that is involved in the synthesis of aromatic amino acids in the shikimate pathway (Montgomery 1997; Duke et al. 2003). These amino acids are essential for the growth and survival of the plants (Hatcher and Kruger 1997). The physiological effects of glyphosate on plants include reduced protein synthesis, reduced stomatal conductance (Munoz-Rueda et al. 1986), inhibition of photosynthesis and carbon allocation (Geiger et al. 1986), production of ethylene and CO₂ (Abu-Irmaileh et al. 1979), and phenolic compound metabolism (Hernandez et al. 1999). Glyphosate blocks the shikimate pathway, thereby reducing the synthesis of aromatic amino acids (i.e., phenylalanine, tyrosine, and tryptophan) and causes the accumulation of shikimate in affected plant tissues (Lydon

and Duke 1988). Glyphosate has been shown to increase phenylalanine ammonia-lyase (PAL) synthesis in plant and synthesize more flavonoids as a possibly protective reaction in response to oxidative damage (Duke and Hoagland 1978).

Codonopsis lanceolata (Siebold & Zucc.) Trautv. of the family Campanulaceae are mostly found in moist and low mountains and hills of Korea, Japan, and China (Jung et al. 2006). The roots of these plants are known for their various nutritional and medicinal properties and consumed as vegetable in Korea (Kim et al. 2003). In addition, the root of this plant plays a part in the traditional Chinese medicine due to their antioxidant (Oh and Kim 2006), antimicrobial, anti-inflammatory (Li et al. 2007), and immunomodulatory properties (Byeon et al. 2009). It is known to contain many important bioactive chemicals including alkaloids, saponins, vitamins, essential oils, tannins, polyphenolics, and steroids (Ichikawa et al. 2009) which are supposed to be responsible for the various biological activities including caspase-dependent apoptosis activity in humans (Lee et al. 2005), regulatory effects (Lee et al. 2007), lipid peroxidation and anti-aging properties (Batista et al. 2007), and anti-cancer activities, α -glucosidase inhibition, and tyrosinase inhibition (Kim et al. 2009). Previously, we generated transgenic *C. lanceolata* lines (Ghimire et al. 2008), which over-express the γ -*tmt* gene, using *Agrobacterium* mediated transformation. Comparative analyses of the effects of glyphosate and water stress on accumulation of phenolic compounds and photosynthetic rate in transgenic plants in response to over-expression of the γ -*tmt* gene have not been documented. Therefore, in this experiment, we selected transgenic lines to evaluate the glyphosate effects in terms of their total phenolic compounds and photosynthetic rate.

Materials and methods

Chemicals

All solvents used were of analytical grade. Methanol was obtained from Baker (Phillipsburg, NJ).

Transgenic plant generation and selection

Transgenic *C. lanceolata* were generated by transforming the γ -tocopherol methyltransferase (γ -*tmt*) gene, which encodes key enzymes involved in the final step of tocopherol biosynthesis, according to the *Agrobacterium* mediated method. The vector (pYBI130) was introduced into *Agrobacterium tumefaciens* (LBA 4404). A vector was inserted into leaf explants of *C. lanceolata* plants that contain the neomycin phosphotransferase gene (*npt-II*)

under the control of the nopaline synthase (NOS) promoter as a selectable marker. A construct consisting of γ -*tmt* cDNAs from *Arabidopsis thaliana* under the control of cauliflower mosaic virus (CaMV) 35S promoter and NOS terminator was inserted into the genome of the *C. lanceolata* plants. The obtained regenerants were prescreened using the polymerase chain reaction (PCR) method with specific primers for the neomycin phosphotransferase *npt-II* gene, which were N-1 (5'GAA-GCT-ATT-CGG-CGG-CTA-TGA-CTG-3') as the sense primer and N-2 (5'ATC-GGG-AGC-GGC-GGC-GAT-ACC-CTA-3') as the anti-sense primer. Plants that exhibited a 700-bp (*npt-II*) DNA fragment were used for further selection by means of southern blot analysis. The details of plant transformation, preselection, selection, and transgenic plant analysis have been previously described (Ghimire et al. 2008). The reference specimens were collected from the Plant Biotechnology Department, Kangwon National University, South Korea. The transgenic line (T1, T2, and T3) that showed the highest level of mRNA for the introduced cDNAs was used to investigate the metabolic effects caused by the water stress and glyphosate treatment on the transgenic *C. lanceolata* (Fig. 1).

Fig. 1 **a** PCR analysis from leaves of transgenic *C. lanceolata* using specific primers of amplification of 700 bp *npt-II* gene. Lane 1 molecular marker, lane 2 the plasmid pYBI130 as a positive control, lane 3 the genomic DNA from untransformed plants, lanes 4 and 5 the genomic DNA of T₂ transformants. **b** PCR analysis from leaves of transgenic *C. lanceolata* using specific primers of amplification of the 1070 bp fragment of γ -*tmt* gene. Lane 1 molecular marker, lane 2 the genomic DNA from untransformed plants, lane 3 the plasmid pYBI130 as a positive control, lanes 4–9 the genomic DNA of T₂ transformants

Measurement of photosynthetic rate after water stress and glyphosate treatment

To investigate the physiological significance of water stress and glyphosate treatment, the photosynthetic rate of three *C. lanceolata* transgenic lines was determined and compared to that of non-transgenic control plants. Seeds of wild-type *C. lanceolata* and transgenic plants were obtained from the Bio herb Research Center, Kangwon National University, South Korea and transgenic plants over-expressing the γ -*tmt* gene were previously generated in the Plant Biotechnology Laboratory, Kangwon National University, Korea. The seeds of both control and transgenic *C. lanceolata* were germinated on vermiculite and perlite mixture (3:1) in a plant growth chamber at 25 °C and 80% relative humidity, providing a photoperiod of 16 h light and 8 h dark. After 2 weeks, seedlings were transferred to bigger pots containing vermiculite and soil (3:1) and were maintained in a glass house at $28/18 \pm 2$ °C with $40 \pm 5\%$ humidity and at a PPFD of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ in a green house. When the plantlets contained five leaves, they were individually transplanted in pots (190 × 195 mm) filled with a soil mixture (garden soil, perlite, vermiculite, and sand) and grown in a greenhouse under natural light at day/night temperatures of $28/18$ °C and $40 \pm 5\%$ humidity and at a PPFD of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Water stress applied to both control and transgenic *C. lanceolata* plants. Water stress treatment was initiated by the withdrawal of the water supply (reduced by 60%) compared to control plants. All the plantlets were watered after every 2 days.

Glyphosate were sprayed outside the greenhouse using a backpack sprayer under constant pressure of 2.2 kg f cm^{-2} of CO₂. The plants were grown at $28/18 \pm 2$ °C during glyphosate treatment with humidity of $40 \pm 5\%$ and wind speed of 5–7 km/h. Glyphosate solution was sprayed uniformly on the plants which were in the five-to-seven-leaf stage in the morning at 10:30 using Kwang Sung sprayers, Model no. KS-10-1, South Korea. Care was taken to avoid glyphosate run-off from leaves during plant treatment. The pots were placed in the controlled greenhouse, with temperature ranging from 28 ± 2 °C (day) to 25 ± 2 °C (night) with relative humidity of 70% during s 16/8 photoperiod and the plantlets were irrigated every day. The absence of glyphosate treatment treated as control plant. The experiments were repeated in a completely randomized manner with four pots per replication and were repeated three times at a concentration of 1.3 mg/mL. Data were collected over 2 weeks of spraying transgenic plants with glyphosate. Stomatal conductance (gs), net CO₂ assimilation rate (A), and the ratio of internal CO₂ concentration (C_i) were measured according to the methods 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. All measurements were performed three times during August and September on sunny days between 10:00 and 14:00, on the surface of leaves under a saturating PPFD of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. Measurements were taken at the center of the leaf surface immediately after the decrease in CO_2 concentration was stable. Each leaf was allowed to stabilize for 4–6 min before measurements of g_s , C_i , and A were made. After the completion of physiological analysis, plant sample from all the treatments was harvested for analyzing the biochemical parameters.

Phenolic compound analysis after water stress and glyphosate treatment

The leaves and roots (10 g) from each transgenic line and non-transformed control plants were harvested, dried at room temperature (28 °C), and ground to crude powder. About 2 g of dried crude powder was soaked in 20 ml of 80% methanol for 48 h. The mixture was filtered through No. 1 Whatman filter paper (Schleicher & Schuell, Whatman International Ltd., Maidstone, England) to remove debris. The filtrate was concentrated to dryness under vacuum rotary evaporator at 40 °C. The dried samples were redissolved in 10 mL of 80% HPLC grade methanol solution. An aliquot of each sample was filtered through a 0.45- μm filter unit (TITAN syringe filter nylon membrane) and then analyzed by high-performance liquid chromatography (HPLC). HPLC analysis was carried out on a product from Shimadzu company (Shimadzu Instruments CO., LTD, Japan) with a pump model LC-10AD VP and a detector model SPD-M10A VP (Photodiode Array detector). YMC-Pack ODS-AM-303 (5 μm , 250×4.6 mm I.D) analytical HPLC column was employed for quantitative analyses. The substances being measured during the analytic procedure were monitored and determined at a UV wavelength of 280 nm. Solvent gradient HPLC analysis was applied using the modified method described by Thiruvengadam and Chung (2015). The mobile phase consisted of solvents A and B. Solvent A consisted of 0.1% glacial acetic acid in distilled water and solvent B was 0.1% glacial acetic acid in acetonitrile. The solvent flow rate was 1 mL min^{-1} , and the wavelength of the photodiode array detector (PDA) was 280 nm. The injection volume was 20 μL of the sample, and the linear gradient of the HPLC solvent was as follows: B was increased from 8 to 10% for 2 min, then from 10 to 30% for 25 min, from 30 to 90% for 24 min, and from 90 to 100% for 2 min, and was then maintained at 100% of B for 5 min before returning to the initiation stage. The next sample was injected after 15 min. Genuine standards of 28 phenolic compounds were purchased from Sigma (USA) and Extrasynthese (France) and were dissolved in dimethylsulfoxide (DMSO) and then

used to generate calibration curves. The standard concentrations were obtained at three concentrations, 10, 50, and 100 ppm, and a high linearity of $r^2 > 0.996$ was obtained from each curve. Benzoic acid, caffeic acid, chlorogenic acid, *t*-cinnamic acid, *m*-coumaric, *o*-coumaric acid, *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, homogentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, pyrogallol, resveratrol, rutin, salicylic acid, 5-sulfosalicylic acid, syringic acid, vanillic acid, vanillin, biochanin A, (+) catechin, formononetin, hesperetin, kaempferol, myricetin, naringenin, naringin, and quercetin were each identified by their retention times or by chromatography with other authentic examples, and concentrations were calculated by comparing the peak areas of samples with those of the standards (Table 1).

Statistical analysis

All experiments were repeated at least three times. The data shown represent mean $\pm$ standard deviation. The data were statistically analyzed using analysis of variance (ANOVA), and differences were assessed to be significant at a 5% level of probability ($P < 0.05$).

Results

Accumulation of phenolic compounds after glyphosate treatment

In this study, we treated transgenic *C. lanceolata* with glyphosate and ascertained the phenolic compound concentration in the resistant transgenic lines and control plants. We found quantitative differences in the content of total phenolic compounds between leaf and root samples from transgenic and control plants (Table 2). A significant increase in the total phenolic compound concentration was revealed in leaf extracts from transgenic plants treated with glyphosate, and ranged from 4744.37 ± 81.81 to $12,051.02 \pm 75.00 \mu\text{g/g DW}$ relative to the lower phenolic compound concentration in control plants ($3778.28 \pm 59.73 \mu\text{g/g DW}$). The major phenolic compounds that were increased in leaf extracts from *C. lanceolata* treated with glyphosate were kaempferol, catechin, benzoic acid, ferulic acid, protocatechuic acid, veratric acid, gallic acid, myricetin, *p*-hydroxybenzoic acid, quercetin, *t*-cinnamic acid, and vanillic acid. The most pronounced enhancement was observed in the concentration of quercetin, which ranged from the equivalent of 42.03 to 51.34% of the total phenolic compounds. Biochanin A and *p*-coumaric acid, which were not detected in control plants, were observed in T2 and T3 transgenic lines, respectively. A considerable amount of phenolic acids were observed to decrease in

Table 1 Calibration curves of equation of 28 phenolic compound standards

Sl. no.	Phytochemicals	Abbreviation	Concentration ($\mu\text{g/ml}$) ¹	Linearity (r^2)	Equation
1	Benzoic acid	BE	0.5	0.9995	$y = 7335.1x - 5972.7$
2	Chlorogenic acid	CH	0.5	0.9954	$y = 7335.1x - 5972.7$
3	<i>t</i> -Cinnamic acid	<i>t</i> -C	0.5	0.9994	$y = 169586x - 221,863$
4	<i>m</i> -Coumaric acid	<i>m</i> -C	0.5	0.999	$y = 99520x + 366,589$
5	<i>o</i> -Coumaric acid	<i>o</i> -C	0.5	0.999	$y = 30,379.0x + 146.33$
6	<i>p</i> -Coumaric acid	<i>p</i> -C	0.5	0.985	$y = 10,243.0x - 5998.60$
7	Ferulic acid	FA	0.5	0.9992	$y = 63695x - 165,433$
8	Gallic acid	GA	0.5	0.9994	$y = 35535x - 79,194$
9	Gentisic acid	GE	0.5	0.999	$y = 3183.7x + 496.00$
10	Homogentisic acid	HO	0.5	0.9977	$y = 15919x - 81,841$
11	<i>p</i> -Hydroxybenzoic acid	<i>p</i> -HY	0.5	0.9999	$y = 27919x + 43,418$
12	Protocatechuic acid	PR	0.5	0.9971	$y = 29859x - 174,443$
13	Pyrogallol	PY	0.5	0.999	$y = 3320x + 2150$
14	Resveratrol	RE	0.5	0.999	$y = 31,260.0x - 58.97$
15	Salicylic acid	SA	0.5	1	$y = 15377x + 7783.7$
16	5-Sulfosalicylic acid	SSA	0.5	0.9988	$y = 7115.8x + 21,563$
17	Syringic acid	SY	0.5	0.9997	$y = 62213x + 69,563$
18	Vanillic acid	VA	0.5	1	$y = 34743x + 2786.6$
19	Vanillin	VN	0.5	0.999	$y = 39,731.0x - 3975.80$
20	Veratric acid	VE	0.5	1	$y = 34957x + 15,741$
21	Biochanin A	BA	0.5	0.9979	$y = 42516x + 5293$
22	Catechin	CN	0.5	0.9979	$y = 6810.3x + 29,358$
23	Kaempferol	KA	0.5	0.9994	$y = 32211x - 57,175$
24	Myricetin	MY	0.5	0.9955	$y = 22104x - 195,936$
25	Naringenin	NA	0.5	0.999	$y = 23,238.0x - 787.89$
26	Rutin	RN	0.5	0.999	$y = 34957x + 15,741$
27	Quercetin	QN	0.5	0.9944	$y = 22509x - 213,591$
28	Formononetin	FN	0.5	0.9992	$y = 274000x + 21,300$

transgenic plants, and included salicylic acid. The total phenolic compound concentration decreased in glyphosate treated root extracts of transgenic lines ranging from 1437.96 ± 36.43 to $1748.73 \pm 46.40 \mu\text{g/g DW}$, as compared with control plants ($3020.13 \pm 46.86 \mu\text{g/g DW}$). Similarly, the concentration of myricetin decreased in transgenic roots following treatment with glyphosate as compared to control plants. Significantly increased levels of vanillic acid were revealed in the T2 and T3 transgenic root extracts at 222.00 ± 2.65 and $172.67 \pm 6.43 \mu\text{g/g DW}$, respectively, after treatment with glyphosate as compared to control plants at $6.67 \pm 1.53 (\mu\text{g/g DW})$. Chlorogenic acid and veratric acid, which were not detected in the root of control plants, were observed in root extracts from the T2 transgenic line. Homogenic acid, *p*-coumeric acid, and *p*-hydroxybenzoic acid were not detected in transgenic roots but were present in transgenic leaf extracts of *C. lanceolata* plants.

Accumulation of phenolic compounds after water stress

The effect of water stress on the phenolic compound composition of transgenic *C. lanceolata* and control plants is summarized in Table 3. The results from the present studies revealed that water stress of transgenic and non-transgenic *C. lanceolata* plants caused significant variation in the phenolic compound composition in the root and leaf extracts of transgenic lines as compared to the control plants. The total concentration of phenolic compounds in the leaf extracts of transgenic samples after water stress ranged from 3455.13 ± 40.48 to $8695.00 \pm 45.44 \mu\text{g g}^{-1} \text{DW}$, whereas the total concentration of the leaf extracts of non-transgenic control samples was $5630.45.91 \mu\text{g g}^{-1} \text{DW}$. The predominant phenolic compounds that increased after the water stress in the transgenic leaf were (+) catechin, benzoic acid, chlorogenic

Table 2 Quantities of phenolic compounds from control and transgenic *C. lanceolata* after treating with glyphosate

Plant lines Phenolic compounds (μg/g) ^c												
BE	CH	<i>t</i> -CI	<i>p</i> -C	FE	GA	HO	<i>p</i> -HY	PR	SA			
^A CI	307.89 ± 6.54 ^d	182.00 ± 7.45 ^b	71.67 ± 2.08 ^e	0.00 ^a	64.33 ± 3.51 ^c	115.33 ± 3.05 ^c	125.00 ± 4.58 ^b	41.67 ± 1.53 ^c	176.00 ± 2.65 ^d	74.67 ± 3.05 ^f		
^A T1	370.88 ± 6.17 ^e	0.00 ^a	76.33 ± 1.53 ^f	0.00 ^a	163.33 ± 9.45 ^e	89.00 ± 9.0 ^b	120.33 ± 5.51 ^b	327.00 ± 14.53 ^d	142.33 ± 2.52 ^{bc}	33.00 ± 2.00 ^e		
^A T2	561.63 ± 5.74 ^f	1175.00 ± 16.00 ^d	87.33 ± 2.08 ^g	0.00 ^a	173.67 ± 7.09 ^f	293.67 ± 6.81 ^e	377.67 ± 3.21 ^c	24.67 ± 2.52 ^b	382.33 ± 3.86 ^f	0.00 ^a		
^A T3	688.00 ± 10.53 ^g	0.00 ^a	91.00 ± 1.00 ^h	44.35 ± 4.66 ^b	146.33 ± 5.13 ^d	187.00 ± 10.14 ^d	0.00 ^a	784.00 ± 4.58 ^e	326.67 ± 6.03 ^e	77.33 ± 2.08 ^e		
^B CI	184.67 ± 6.51 ^b	0.00 ^a	36.33 ± 1.53 ^c	0.00 ^a	0.00 ^a	61.67 ± 2.08 ^a	0.00 ^a	0.00 ^a	151.00 ± 5.57 ^b	3.50 ± 0.17 ^b		
^B T1	130.33 ± 10.41 ^a	0.00 ^a	41.33 ± 1.55 ^d	0.00 ^a	0.00 ^a	55.67 ± 4.04 ^a	0.00 ^a	0.00 ^a	146.00 ± 3.00 ^c	3.24 ± 0.14 ^b		
^B T2	225.67 ± 12.50 ^c	237.67 ± 6.43 ^c	32.33 ± 2.52 ^b	0.00 ^a	54.33 ± 4.04 ^b	63.00 ± 2.00 ^a	0.00 ^a	0.00 ^a	126.00 ± 2.52 ^a	52.96 ± 2.63 ^d		
^B T3	337.80 ± 2.00 ^c	0.00 ^a	26.33 ± 3.06 ^a	0.00 ^a	0.00 ^a	65.33 ± 4.04 ^a	0.00 ^a	0.00 ^a	130.33 ± 4.51 ^a	4.33 ± 1.53 ^b		
Plant lines Phenolic compounds (μg/g) ^c												
SSA	SY	VA	VE	BCA	CA	KA	MY	RU	QU	Total		
^A CI	10.58 ± 0.59 ^b	0.00 ^a	13.00 ± 2.65 ^b	55.00 ± 3.61 ^c	0.00 ^a	58.81 ± 2.17 ^a	445.33 ± 3.51 ^e	194.00 ± 5.19 ^a	33.00 ± 2.00 ^e	1810.00 ± 5.57 ^d	3778.28 ± 59.73 ^e	
^A T1	0.00 ^a	0.00 ^a	22.00 ± 2.00 ^c	3.50 ± 0.50 ^b	0.00 ^a	0.00 ^a	749.67 ± 13.79 ^f	183.33 ± 7.23 ^a	28.00 ± 2.65 ^d	2435.67 ± 4.93 ^c	4744.37 ± 81.81 ^f	
^A T2	14.68 ± 14.67 ^c	0.00 ^a	158.00 ± 4.00 ^e	118.67 ± 1.53 ^e	22.66 ± 2.52 ^d	1315.55 ± 4.10 ^d	885.67 ± 3.05 ^g	773.33 ± 14.01 ^d	22.400 ± 2.65 ^c	4632.00 ± 6.56 ^f	11,018.93 ± 100.40 ^g	
^A T3	0.00 ^a	0.00 ^a	34.67 ± 1.53 ^d	108.67 ± 2.52 ^d	0.00 ^a	2952.00 ± 7.21 ^f	159.67 ± 2.08 ^d	529.00 ± 1.00 ^e	91.33 ± 6.51 ^f	5831.00 ± 10.00 ^g	12,051.02 ± 75.00 ^h	
^B CI	10.83 ± 0.04 ^b	0.00 ^a	6.67 ± 1.53 ^a	0.00 ^a	12.33 ± 2.52 ^c	2060.67 ± 9.50 ^e	44.00 ± 200 ^b	224.67 ± 14.22 ^b	0.79 ± 0.19 ^a	223.00 ± 3.00 ^b	3020.13 ± 246.86 ^d	
^B T1	0.00 ^a	0.00 ^a	5.83 ± 1.04 ^a	0.00 ^a	8.00 ± 2.00 ^b	927.66 ± 9.07 ^c	0.00 ^a	218.67 ± 12.50 ^b	0.00 ^a	212.00 ± 2.65 ^a	1748.73 ± 46.40 ^c	
^B T2	0.00 ^a	0.00 ^a	222.00 ± 2.65 ^g	5.59 ± 0.37 ^b	7.67 ± 1.53 ^b	0.00 ^a	44.00 ± 2.00 ^b	189.50 ± 9.50 ^a	6.14 ± 0.06 ^b	243.00 ± 6.56 ^c	1509.86 ± 55.31 ^b	
^B T3	0.00 ^a	0.00 ^a	172.67 ± 6.43 ^f	0.00 ^a	6.07 ± 0.89 ^b	211.09 ± 1.80 ^b	96.67 ± 2.08 ^c	183.67 ± 7.57 ^a	0.00 ^a	203.67 ± 2.52 ^a	1437.96 ± 36.43 ^a	

BE benzoic acid, *CH* chlorogenic acid, *t*-*CI* *t*-cinnamic acid, *p*-*C* *p*-coumaric acid, *p*-*C* *p*-coumaric acid, *FE* ferulic acid, *GA* gallic acid, *HO* homogentisic acid, *p*-*HY* *p*-hydroxybenzoic acid, *PR* protocatechuic acid, *SA* salicylic acid, *SSA* 5-sulfosalicylic acid, *SY* syringic acid, *VA* vanillic acid, *VE* veratric acid, *BCA* biochanin A, *CA* (+) catechin, *KA* kaempferol, *MY* myricetin, *RU* rutin, *QU* quercetin

Data in each column with the same letter were not significantly different according to Duncan's multiple comparison test ($P < 0.05$)

^ACI—Leaf of control plant; ^AT1, ^AT2, and ^AT3—leaf of transgenic plant treated with glyphosate

^BCI—Root of control plant; ^BT1, ^BT2, and ^BT3—root of transgenic plant treated with glyphosate

Table 3 Quantities of phenolic compounds from control and transgenic *C. lanceolata* plants after water stress

Phenolic compounds (μg/g)										
BE	CH	t-CI	p-C	FE	GA	HO	p-HY	PR	SA	
^A CI	159.33 ± 3.05 ^c	691.67 ± 3.05 ^e	37.67 ± 1.53 ^c	36.33 ± 2.52 ^b	75.67 ± 1.53 ^c	56.33 ± 2.08 ^c	134.67 ± 1.53 ^c	0.00 ^a	150.67 ± 5.13 ^{bc}	555.00 ± 5.55 ^g
^A T1	315.00 ± 3.61 ^d	1005.00 ± 3.61 ^f	681.33 ± 6.66 ^f	0.00 ^a	163.33 ± 1.55 ^e	82.00 ± 2.65 ^e	115.00 ± 2.64 ^b	23.00 ± 2.00 ^b	0.00 ^a	211.67 ± 2.08 ^c
^A T2	312.00 ± 2.65 ^d	3066.33 ± 1.53 ^h	31.00 ± 1.00 ^b	495.00 ± 3.61 ^c	96.00 ± 2.69 ^d	67.33 ± 2.08 ^d	0.00 ^a	134.33 ± 1.53 ^d	151.00 ± 1.00 ^b	1293.00 ± 2.64 ^h
^A T3	454.00 ± 2.65 ^f	1548.67 ± 6.43 ^g	44.00 ± 2.65 ^d	0.00 ^a	72.00 ± 2.00 ^c	80.00 ± 2.00 ^e	114.33 ± 2.08 ^b	74.33 ± 2.52 ^c	155.00 ± 1.00 ^c	383.00 ± 2.64 ^f
^B CI	43.33 ± 3.22 ^a	250.00 ± 5.57 ^c	24.67 ± 3.21 ^a	0.00 ^a	255.00 ± 1.00 ^f	51.33 ± 1.53 ^b	0.00 ^a	0.00 ^a	0.00 ^a	173.00 ± 2.67 ^b
^B T1	323.00 ± 2.65 ^e	455.67 ± 5.451 ^d	53.33 ± 2.08 ^e	0.00 ^a	94.33 ± 3.51 ^d	115.00 ± 2.65 ^f	190.67 ± 5.03 ^d	25.00 ± 2.64 ^b	212.00 ± 2.65 ^e	258.67 ± 11.84 ^e
^B T2	62.33 ± 1.53 ^b	0.00 ^a	27.00 ± 2.00 ^{ab}	0.00 ^a	54.00 ± 2.61 ^a	57.12 ± 2.08 ^c	0.00 ^a	0.00 ^a	0.00 ^a	4.00 ± 0.50 ^a
^B T3	65.33 ± 4.04 ^b	223.67 ± 2.52 ^b	36.67 ± 2.81 ^c	0.00 ^a	65.33 ± 3.51 ^b	0.00 ^a	0.00 ^a	0.00 ^a	165.00 ± 3.00 ^d	233.67 ± 1.52 ^d
Phenolic compounds (μg/g)										
SSA	SY	VA	VE	BCA	CA	KA	MY	RU	QU	Total
^A CI	0.00 ^a	26.00 ± 1.80 ^c	16.00 ± 1.50 ^b	4.00 ± 1.00 ^b	0.00 ^a	28.83 ± 0.76 ^a	462.33 ± 7.77 ^f	214.00 ± 1.00 ^b	8.00 ± 1.00 ^c	2974.33 ± 5.11 ^g
^A T1	0.00 ^a	23.66 ± 2.52 ^b	35.00 ± 2.50 ^c	47.33 ± 1.52 ^c	0.00 ^a	48.14 ± 1.01 ^b	105.00 ± 3.61 ^e	280.67 ± 2.52 ^c	64.00 ± 1.00 ^c	3455.13 ± 40.48 ^c
^A T2	0.00 ^a	21.33 ± 1.53 ^b	251.67 ± 2.08 ^e	0.00 ^a	0.00 ^a	160.33 ± 2.52 ^d	96.00 ± 2.65 ^d	252.00 ± 6.08 ^d	22.00 ± 1.50 ^d	426.67 ± 1.52 ^f
^A T3	0.00 ^a	2.00 ± 0.22 ^a	39.00 ± 1.80 ^c	44.67 ± 1.53 ^d	0.00 ^a	32.66 ± 2.08 ^a	623.67 ± 9.02 ^g	307.00 ± 3.61 ^f	7.67 ± 0.57 ^c	4713.00 ± 2.64 ^h
^B CI	0.00 ^a	167.33 ± 2.08 ^d	7.00 ± 1.10 ^a	43.33 ± 1.52 ^d	0.00 ^a	384.67 ± 4.04 ^f	0.00 ^a	213.67 ± 3.21 ^b	4.00 ± 1.00 ^b	234.67 ± 3.51 ^b
^B T1	0.00 ^a	0.00 ^a	385.33 ± 6.43 ^g	0.00 ^a	0.00 ^a	106.67 ± 3.79 ^c	103.00 ± 3.00 ^{de}	324.00 ± 2.65 ^g	7.00 ± 1.12 ^c	363.00 ± 2.65 ^e
^B T2	0.00 ^a	0.00 ^a	180.67 ± 4.55 ^d	0.00 ^a	0.00 ^a	204.67 ± 4.04 ^e	64.67 ± 3.51	0.00 ^a	0.00 ^a	223.33 ± 2.08 ^a
^B T3	0.00 ^a	0.00 ^a	294.67 ± 3.21 ^f	18.67 ± 1.55	0.00 ^a	592.33 ± 6.51 ^g	42.33 ± 1.53 ^b	236.00 ± 2.00	0.00 ^a	263.33 ± 2.08 ^d

BE benzoic acid, CH chlorogenic acid, t-CI t-cinnamic acid, p-C p-coumaric acid, FE ferulic acid, GA gallic acid, HO homogentisic acid, p-HY p-hydroxybenzoic acid, PR protocatechuic acid, SA salicylic acid, SSA 5-sulfosalicylic acid, SY syringic acid, VA vanillic acid, VE veratric acid, BCA biochanin A, CA (+) catechin, KA kaempferol, MY myricetin, RU rutin, QU quercetin

Data in each column with the same letter were not significantly different according to Duncan's multiple comparison test ($P < 0.05$)

^ACI—Leaf of control plant; ^AT1, ^AT2, and ^AT3—leaf of transgenic plant treated with water deficit

^BCI—Root of control plant; ^BT1, ^BT2, and ^BT3—root of transgenic plant treated with water deficit

Table 4 Photosynthetic performance of transgenic and non-transgenic (control) *C. lanceolata* plants treated with glyphosate after 21 days

Plant lines	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	gs (cm s^{-1})	C _i ($\mu\text{mol mol}^{-1}$)
C1	2.28 $\pm$ 0.12 ^d	0.05 $\pm$ 0.001 ^b	377.15 $\pm$ 4.29 ^a
T1	5.07 $\pm$ 0.11 ^b	0.05 $\pm$ 0.005 ^b	308.50 $\pm$ 4.94 ^c
T2	5.44 $\pm$ 0.31 ^a	0.06 $\pm$ 0.004 ^a	277.83 $\pm$ 2.06 ^d
T3	3.91 $\pm$ 0.950 ^c	0.05 $\pm$ 0.004 ^b	348.30 $\pm$ 2.301 ^b

A photosynthetic rate, gs stomatal conductance, C_i CO₂ concentration

Plant lines: C control plant, T1, T2, T3 transgenic plants

The data represent the mean of 8–10 independent determinations $\pm$ SD. Data in each column with the same letter were not significantly different according to Duncan's multiple comparison test ($P < 0.05$)

Table 5 Photosynthetic performance of transgenic and non-transgenic (control) *C. lanceolata* plants under water stress after 21 days

Plant lines	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	gs (cm s^{-1})	C _i ($\mu\text{mol mol}^{-1}$)
C1	2.09 $\pm$ 0.69 ^d	0.05 $\pm$ 0.004 ^b	384.97 $\pm$ 3.92 ^a
T1	4.67 $\pm$ 0.91 ^a	0.05 $\pm$ 0.001 ^b	286.90 $\pm$ 4.31 ^d
T2	4.07 $\pm$ 0.31 ^b	0.05 $\pm$ 0.002 ^b	321.60 $\pm$ 3.83 ^c
T3	3.58 $\pm$ 0.66 ^c	0.06 $\pm$ 0.001 ^a	342.80 $\pm$ 6.65 ^b

A Photosynthetic rate, gs stomatal conductance, C_i CO₂ concentration

Plant lines: C Control plant, T1, T2, and T3 transgenic plants

The data represent the mean of 8–10 independent determinations $\pm$ SD

Data in each column with the same letter were not significantly different according to Duncan's multiple comparison test ($P < 0.05$)

acid, ferulic acid, gallic acid, rutin, vanillic acid, and veratric acid. The most pronounced enhancement was observed in the concentration of quercetin (54.20% of the total phenolic compounds). *p*-Hydroxybenzoic acid, which were not detected in control plants, were observed in the leaf extracts of T1, T2, and T3 transgenic lines, respectively (Table 3). Similarly, kaempferol, which were not detected in control plants, were observed in the root extracts of T1, T2, and T3 transgenic lines, respectively. A considerable amount of phenolic acids were observed to decrease in transgenic plants, and included homogentisic acid and syringic acid. The total phenolic compound concentration increased in water stress leaf and root extracts of transgenic lines compared to control plants.

Measurement of photosynthesis rate after water stress and glyphosate treatment

The physiological effects of water stress and glyphosate treatment on the transgenic *C. lanceolata* investigated by measuring the photosynthesis rate (A), stomatal conductance (gs), and CO₂ concentration (C_i) and compared them to those of the non-transgenic control plants (Table 4). In the glyphosate treatment experiment, the aerial parts of transgenic plants sprayed with glyphosate revealed higher

A (3.91 $\pm$ 0.950 to 5.44 $\pm$ 0.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and gs (0.5 $\pm$ 0.004 to 0.06 $\pm$ 0.004 cm s^{-1}) relative to the glyphosate treated control plants 2.28 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.05 cm s^{-1} , respectively). In this study, we observed gs and A in the transgenic plants treated with water stress after 21 days relative to those observed in the non-transgenic control plants (Table 5). In the water stress experiment, transgenic plants grown under greenhouse conditions showed higher A (3.58 $\pm$ 0.66 to 4.67 $\pm$ 0.91 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and gs (0.5 $\pm$ 0.001 to 0.06 $\pm$ 0.001 cm s^{-1}) relative to water stressed control plants (2.09 $\pm$ 0.69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.05 $\pm$ 0.004 cm s^{-1} , respectively).

Discussion

This study investigated the physiological effects of glyphosate on the phenolic compound composition and photosynthesis rate of transgenic *C. lanceolata* plants. We found that transgenic lines of *C. lanceolata* had a higher photosynthesis rate (A), stomatal conductance (gs), and elevated phenolic concentration following glyphosate treatment. A high level of phenolic acid suggests that alternative biosynthesis of phenolic compounds was activated by glyphosate treatment due to inhibition of

5-enolpyruvylshikimate-3-*p*-synthase. Glyphosate has been shown to reduce/inhibit the production of phenolics and other phytochemical responses for disease resistance (Greaves and Sargent 1986). However, in this study, we observed increased levels of total phenolic compounds in transgenic leaves treated with glyphosate. After glyphosate treatment, the levels of gallic acid, *t*-cinnamic acid, and quercetin increased in the transgenic leaf extracts, whereas vanillic acid increased in the root extracts of transgenic *C. lanceolata* plants treated with glyphosate. Some of the phenolic compounds that were not present initially in the transgenic *C. lanceolata* plants were observed in transgenic *C. lanceolata* plants treated with glyphosate. This result indicates that some of these phenolic compounds are produced only after glyphosate treatment. However, in transgenic roots treated with glyphosate, we observed a decrease in the total phenolic compound concentration relative to that observed in the control plants. Several phenolic compounds (chlorogenic acid, ferulic acid, and veratric acid) that were not detected in control plants were found in transgenic plants (T2) sprayed with glyphosate. The presence of these compounds in transgenic lines indicates that genetic or chemical disruption of the metabolic pathway of phenolic compounds can lead to an increase in the phenolic compound concentration and in glyphosate resistance. In a similar study, an increase in phenolic compounds such as hydrobenzoic acid after glyphosate treatment was reported by Hernandez et al. (1999). However, a number of studies reported decreased phenolic compound concentration after glyphosate treatment in plants such as soybean, lupine, ryegrass, and pigweed (Lydon and Duke 1988; Marchiosi et al. 2009). In this study, the concentration of gallic acid increased in leaf extracts of transgenic plants following treatment with glyphosate. Since glyphosate is known to block EPSP synthase (Duke 1988), which is an enzyme in the shikimate pathway, the increase in gallic acid concentration observed in the presence of glyphosate indicates that an alternative pathway might exist for the synthesis of gallic acid. Ossipov et al. (2003) indicated that the most possible alternative biosynthesis pathway for gallic acid during glyphosate treatment is from dehydroshikimic acid. Consistent with our result, Becerra-Moreno et al. (2012) in their study reported increased shikimic acid (SA) and chlorogenic acid content following treatment with glyphosate.

We evaluated the metabolic response to water stress in transgenic *C. lanceolata* plants. In the present study, introduction of the γ -*tmt* gene in *C. lanceolata* plants resulted in an increased photosynthesis rate following water stress. In a previous study, we generated transgenic *C. lanceolata* plants by over-expressing the γ -*tmt* gene, which had increased levels of α -tocopherol and antioxidant property (Ghimire et al. 2008, 2011). It is believed that α -

tocopherol protects pigments and proteins in the photosynthetic pigments (Kanwischer et al. 2005; Maeda et al. 2008; Matrige et al. 2008; Semchuk et al. 2009). It is likely that the higher α -tocopherol concentration in transgenic *C. lanceolata* plants infers increased protection of these organelles from the effect of water stress. Another possible reason for the higher photosynthesis rate could be decreased levels of reactive oxygen species (ROS) in transgenic *C. lanceolata* plants due to the higher antioxidant property of α -tocopherol (Ghimire et al. 2011). Moreover, in the previous study, we found that ROS scavenging enzymes were up-regulated in transgenic *C. lanceolata* plants indicating that the γ -*tmt* gene is potentially involved in oxidative stress in transgenic *C. lanceolata* plants (Seong et al. 2009). It seems likely that the higher ROS scavenging activity of α -tocopherol can withstand the water stress. Moreover, it has been argued that tocopherol protects the cellular machinery, and can thus improve plant defense responses (Espinoza et al. 2013). The author reported a delay in leaf senescence and reduced oxidative leaf damage in tobacco plants due to increased α -tocopherol levels as a result of drought-induced over-expression of a Homogentisate phytyltransferase (VTE2.1) gene. Gajewska and Sklodowska (2007), and Collin et al. (2008) reported that increased levels of tocopherol confer enhanced tolerance to plants against drought. The tocopherol content correlates positively with tolerance to water deficit in different species (Guo et al. 2006; Munne-Bosch et al. 1999) and genes in the tocopherol pathway might respond differently to different environmental stresses (Kanwischer et al. 2005; Abbasi et al. 2007). Moreover, transgenic plants with higher levels of tocopherol outperform the wild-type plants in the prevention of oxidative damage to the membrane and photosynthetic apparatus during drought treatment (Liu et al. 2008). It has been shown that α -tocopherol contributes to carbohydrate metabolism and photoassimilate transport (Maeda et al. 2006), provides stability and protection of phenylpropanoid compounds in plants (Yao et al. 2015), and plays an important role in adaptive mechanisms to environmental conditions (Loyola et al. 2012). On the other hand, the level of α -tocopherol is increased in response to a variety of abiotic stresses in photosynthetic plant tissues (Noctor 2006). Therefore, it is likely that an increased concentration of α -tocopherol and phenolic compounds has a direct influence on the increased photosynthetic rate in transgenic *C. lanceolata* plants during water stress. Some of phenolic compounds are efficient endogenous antioxidants (Larson 1988), can scavenge ROS, and can prevent chloroplasts from being damaged (Ye et al. 2000). In particular, we observed increased in the catechin, benzoic acid, chlorogenic acid, ferulic acid, gallic acid, rutin, vanillic acid, and veratric acid in water stressed transgenic

plant. In the similar studies, higher level of phenolic compound was reported under water stressed condition (Hameed et al. 2013; Ayaz et al. 2000; Abreu and Mazzafera 2005; Hura et al. 2009; Kirakosyan et al. 2004). It has been shown that water stress increase the levels of ROS in plant cells (Smirnov 1993). Hura et al. (2009) indicated that increase in ferulic acid act as photoprotector/or as a ROS scavenger, thus a reliable indicator of the resistance to drought stress. Thus, in this study, it is possible that increased phenolic compounds in the transgenic plant with water stressed containing higher concentration of ferulic acid could gain more water stress tolerance by protecting photosynthetic apparatus relative to control plants. However, Cheruiyot et al. (2007), Sánchez-Rodríguez et al. (2011), Yildiz-Aktas et al. (2009) observed degradation of phenolic compound concentration during water stress relative to control plants. The result indicates that water deficit affected the physiological and biochemical properties of transgenic *C. lanceolata* by regulating the synthesis of secondary metabolites. Hence, it is possible that a higher concentration of phenolic compounds in the transgenic plants contributed to the higher photosynthetic activity observed in water stress transgenic plants. However, the decline in photosynthetic rate and stomatal closure has been reported during water stress (Flexas et al. 2009; Ghannoum 2009). Therefore, it remains to be determined whether increased levels of phenolic compounds and α -tocopherol have a direct effect on increased water stress resistance in transgenic *C. lanceolata* plants.

Acknowledgements The authors express their gratitude to the 2015 KU Brain Pool of Konkuk University for supporting this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Abbasi AR, Hajirezaei M, Hofius D, Sonnewald U, Voll LM (2007) Specific roles of α - and γ -tocopherol in abiotic stress responses of transgenic tobacco. *Plant Physiol* 143:1720–1738
- Abreu IN, Mazzafera P (2005) Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant Physiol Biochem* 43:241–248
- Abu-Irmaileh BE, Jordon LS, Kumamoto J (1979) Enhancement of CO_2 and ethylene production and cellulase activity by glyphosate in *Phaseolus vulgaris*. *Weed Sci* 27:103–106
- Altieri MA (2005) The myth of coexistence: why transgenic crops are not compatible with agro-ecologically based systems of production. *B Sci Technol Soc* 25:361–371
- Ayaz FA, Kadioglu A, Turgut R (2000) Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setosa* (Rosc.) Eichler. *Can J Plant Sci* 80:373–378
- Bahrani MJ, Bahrami H, Haghighi AAK (2010) Effect of water stress on ten forage grasses native or introduced to Iran. *Grassl Sci* 56:1–5
- Barton JE, Dracup M (2000) Genetically modified crops and the environment. *Agron J* 92:797–803
- Batista ES, Costa AGV, Pinheiro-Santana HM (2007) Adição da vitamina E aos alimentos: implicações para os alimentos e para a saúde humana. *Revista de Nutricao* 20:525–535
- Becerra-Moreno A, Benavides J, Cisneros-Zevallos L, Jacobo-Velázquez DA (2012) Plants as biofactories: glyphosate-induced production of shikimic acid and phenolic antioxidants in wounded carrot tissue. *J Agric Food Chem* 60:11378–11386
- Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. In: Gruissem W, Buchanan B, Jones R (eds) *Biochemistry and molecular biology of plants*. American Society of Plant Physiologists, Rockville, pp 1158–1249
- Byeon SE, Choi WS, Hwang EK, Lee J, Rhee MH, Park HJ, Cho JY (2009) Inhibitory effect of saponin fraction from *Codonopsis lanceolata* on immune cell-mediated inflammatory responses. *Arch Pharmacol Res* 32:813–822
- Chen Q, Zhang M, Shen S (2010) Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (*Helianthus tuberosus* L.). *Acta Physiol Plant* 33:273–278
- Cheruiyot EK, Mumera LM, Ngetich WK, Hassanali A, Wachira F (2007) Polyphenols as potential indicators for drought tolerance in tea *Camellia sinensis* L. *Biosci Biotechnol Biochem* 71:2190–2197
- Collin VC, Eymery F, Genty B, Rey P, Havaux M (2008) Vitamin E is essential for the tolerance of *Arabidopsis thaliana* to metal-induced oxidative stress. *Plant, Cell Environ* 31:244–257
- Cowan MM (1999) Plant products as antimicrobial agents. *Clin Microbiol Rev* 12:564–582
- Donnini S, Tessarin P, Ribera-Fonseca A, Foggia MD, Parpinello GP, Rombolà AD (2016) Glyphosate impacts on polyphenolic composition in grapevine (*Vitis vinifera* L.) berries and wine. *Food Chem* 213:26–30
- Duke SO (1988) Glyphosate. In: Kearney PC, Kaufman DD (eds) *Herbicides: chemistry, degradation and mode of action*, vol 3. Marcel Dekker Inc., New York, pp 1–70
- Duke SO (1990) Overview of herbicide mechanisms of action. *Environ Health Perspect* 87:263–271
- Duke SO, Hoagland RE (1978) Effects of glyphosate on metabolism of phenolic compounds I. Induction of phenylalanine ammonia-lyase activity in dark-grown maize roots. *Plant Sci Lett* 11:185–190
- Duke SO, Baerson SR, Rimando AM (2003) Herbicides: glyphosate. In: Plimmer JR, Gammon DW, Ragsdale NN (eds) *Encyclopedia of agrochemicals*. Wiley, New York
- Espinoza A, Martina AS, Lopez-Climent M, Ruiz-Lara S, Gomez-Cadenas A, Casaretto JA (2013) Engineered drought-induced biosynthesis of α -tocopherol alleviates stress-induced leaf damage in tobacco. *Plant Physiol* 170:1285–1294
- Flexas J, Baron M, Bota J, Ducruet JM, Galle A, Galmes J, Jimenez M, Pou A, Carbo MR, Sajani C, Tomas M, Medrano H (2009) Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandieri*-*V. rupestris*). *J Exp Bot* 60(8):2361–2377
- Gajewska E, Skłodowska M (2007) Relations between tocopherol, chlorophyll and lipid peroxides contents in shoots of Ni-treated wheat. *Plant Physiol* 164:364–366
- Geiger DR, Kapitán SW, Tucci MA (1986) Glyphosate inhibits photosynthesis and allocation of carbon starch in sugar beet leaves. *Plant Physiol* 82:468–472
- Ghannoum O (2009) C4 photosynthesis and water stress. *Ann Bot* 103:635–644

- Ghimire BK, Seong ES, Lim JD, Heo K, Kim MJ, Chung IM, Juvik JA, Yu CY (2008) Agrobacterium-mediated transformation of *Codonopsis lanceolata* using the γ -TMT gene. *Plant Cell Tissue Organ Cult* 95:265–274
- Ghimire BK, Seong ES, Goh EJ, Kang EY, Ahn JK, Yu CY, Chung IM (2011) Improving antioxidant activity in transgenic *Codonopsis lanceolata* plants via over-expression of the γ -tocopherol methyltransferase (γ -tmt) gene. *Plant Growth Regul* 63:1–6
- Greaves MP, Sargent JA (1986) Herbicide-induced microbial invasion of plant roots. *Weed Sc* 34:50–53
- Guo J, Liu X, Li X, Chen S, Jin Z, Liu G (2006) Overexpression of VTE1 from *Arabidopsis* resulting in high vitamin E accumulation and salt stress tolerance increase in tobacco plant. *J Appl Environ Biol* 12:468–471
- Hameed A, Goher M, Iqbal N (2013) Drought induced programmed cell death and associated changes in antioxidants, proteases, and lipid peroxidation in wheat leaves. *Biol Plant* 57:370–374
- Hatcher DW, Kruger JE (1997) Simple phenolic acids in flours prepared from Canadian wheat: relationship to ash content, color, and polyphenol oxidase activity. *Cereal Chem* 74:337–343
- Hernandez A, Garcia-Plazaola JI, Becerril JM (1999) Glyphosate effects on phenolic metabolism of nodulated soybean (*Glycine max* L. Merr.). *J Agric Food Chem* 47:2920–2925
- Housti F, Andary C, Gargadennec A, Amssa M (2002) Effects of wounding and salicylic acids on hydroxycinnamoylmalic acids in *Thunbergia alata*. *Plant Physiol Biochem* 40:761–769
- Hura T, Hura K, Grzesiak S (2009) Leaf dehydration induces different content of phenolics and ferulic acid in drought-resistant and sensitive genotypes of spring triticale. *Z Naturforsch C* 64(1–2):85–95
- Ichikawa M, Ohta S, Komoto N, Ushijima M, Kodera Y, Hayama M, Shirota O, Sekita S, Kuroyanagi M (2009) Simultaneous determination of seven saponins in the roots of *Codonopsis lanceolata* by liquid chromatography-mass spectrometry. *J Nat Med* 63:52–57
- Ivan Y, Tsonko T, Violeta V, Katya G, Peter I, Nikolai T, Tatyana P (2001) Changes in CO₂ assimilation, transpiration and stomatal resistance of different wheat cultivars experiencing drought under field conditions. *Bulg J Plant Physiol* 27:20–33
- James C (2012) Global Status of Commercialized Biotech/GM Crops: 2012, ISAAA Briefs No. 44 (International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY)
- Jung SW, Han AJ, Hong HJ, Choung MG, Kim KS, Park SH (2006) α -Glucosidase inhibitors from the roots of *Codonopsis lanceolata* Trautv. *Agric Chem Biotechnol* 49:162–164
- Kanwischer M, Porfirova S, Bergmuller E, Dormann P (2005) Alterations in tocopherol cyclase activity in transgenic and mutant plants of *Arabidopsis* affect tocopherol content, tocopherol composition, and oxidative stress. *Plant Physiol* 137:713–723
- Kim HI, Kim HH, Hong EY, Yun JS, Yun T, Hwang JK, Lee CH (2003) Breeding of tetraploid in *Codonopsis lanceolata* (Sieb. et Zucc.) Trautvetter by colchicine treatment. *Plant Resour* 6:227–232
- Kim SS, Ha JH, Jeong MH, Ahn JH, Yoon WB, Park SJ, Seong DH, Lee HY (2009) comparison of biological activities of fermented *Codonopsis lanceolata* and fresh *Codonopsis lanceolata*. *Korean J Med Crop Sci* 17(4):280–285
- Kirakosyan A, Kaufman P, Warber S, Zick S, Aaronson K, Bolling S, Chang SC (2004) Applied environmental stresses to enhance the levels of polyphenolics in leaves of hawthorn plants. *Physiol Plant* 121:182–186
- Kutchan TM (2001) Ecological arsenal and developmental dispatcher. The paradigm of secondary metabolism. *Plant Physiol* 125:58
- Larson RA (1988) The antioxidants of higher plants. *Phytochemistry* 27(4):969–978
- Lee KW, Jung HJ, Park HJ, Kim DG, Lee JY, Lee KT (2005) β -D-Xylopyranosyl-(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl echinocystic acid isolated from the roots of *Codonopsis lanceolata* induces caspase-dependent apoptosis in human acute promyelocytic leukemia HL-60 cells. *Biol Pharm Bull* 28:854–859
- Lee YG, Kim JY, Lee JY, Byeon SE, Hong EK, Lee J, Rhee MH, Park HJ, Cho JY (2007) Regulatory effects of *Codonopsis lanceolata* on macrophage-mediated immune responses. *J Ethnopharmacol* 112:180–188
- Li JP, Liang ZM, Yuan Z (2007) Triterpenoid saponins and anti-inflammatory activity of *Codonopsis lanceolata*. *Pharmazie* 62(6):463–466
- Liu X, Hua X, Guo J, Qi D, Wang L, Liu Z, Jin Z, Chen S, Liu G (2008) Enhanced tolerance to drought stress in transgenic tobacco plants overexpressing VTE1 for increased tocopherol production from *Arabidopsis thaliana*. *Biotechnol Lett* 30:1275–1280
- Loyola J, Verdugo I, Gonzalez E, Casaretto JA, Ruiz-Lara S (2012) Plastidic isoprenoid biosynthesis in tomato: physiological and molecular analysis in genotypes resistant and sensitive to drought stress. *Plant Biol* 14:149–156
- Lydon J, Duke SO (1988) Glyphosate induction of elevated levels of hydroxybenzoic acids in higher plants. *J Agric Food Chem* 36:813–818
- Maeda H, Song W, Sage TL, DellaPenna D (2006) Tocopherols play a crucial role in low-temperature adaptation and phloem loading in *Arabidopsis*. *Plant Cell* 18:2710–2732
- Maeda H, Sage TL, Isaac G, Welti R, DellaPenna D (2008) Tocopherols modulate extra plastidic polyunsaturated fatty acid metabolism in *Arabidopsis* at low temperature. *Plant Cell* 20:452–470
- Marchiosi R, Ferrarese MLL, Bonini EA, Fernades NG, Ferro AP, Ferrarese-Filho O (2009) Glyphosate-induced metabolic changes in susceptible and glyphosate-resistant soybean (*Glycine max* L.) roots. *Pestic Biochem Phys* 93:28–33
- Marian N, Fereidoon S (2004) Extraction and analysis of phenolics in food. *J Chromatogr A* 1054:95–111
- Matrige M, Ksas B, Rey P, Havaux M (2008) Tocotrienols, the unsaturated forms of vitamin E, can function as antioxidants and lipid protectors in tobacco leaves. *Plant Physiol* 147:764–778
- Montgomery DC (1997) Design and analysis of experiments, 4th edn. Wiley, New York
- Munne-Bosch S, Schwarz K, Alegre L (1999) Enhanced formation of alpha-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. *Plant Physiol* 121:1047–1052
- Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell Environ* 25:239–250
- Munoz-Rueda A, Gonzalez-Murua C, Becerril JM, Sanchez-Díaz MF (1986) Effects of glyphosate [*N*-(phosphonomethyl)glycine] on photosynthetic pigments, stomatal response and photosynthetic electron transport in *Medicago sativa* and *Trifolium pratense*. *Physiol Plant* 66:63–68
- Negi PS (2012) Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. *Int J Food Microbiol* 156:7–17
- Noctor G (2006) Metabolic signaling in defence and stress: the central roles of soluble redox couples. *Plant Cell Environ* 29(3):409–425
- Oh HS, Kim JH (2006) Characterization of pPhysiological functionalities of *Codonopsis lanceolata*, *Cornus officinalis* S. et Z, and their mixtures. *J Exp Biomed Sci* 12:393–398
- Ossipov V, Salminen JP, Ossipova S, Haukioja E, Pihlaja K (2003) Gallic acid and hydrolysable tannins are formed in birch leaves from an intermediate compound of the shikimate pathway. *Biochem Syst Ecol* 31:3–16
- Perales L, Arbona B, Gomez-Cadenas A, Cornejo MJ, Sanz A (2005) A relationship between tolerance to dehydration of rice lines and

- ability for ABA synthesis under stress. *Plant Physiol Biochem* 43:786–792
- Perumalla AVS, Hettiarachchy NS (2011) Green tea and grape seed extracts—potential applications in food safety and quality. *Food Res Int* 44:827–839
- Rodriguez M, Canales E, Borroto CJ, Carmona E, Lopez J, Pujol M, Borrás-Hidalgo O (2006) Identification of genes induced upon water-deficit stress in a drought-tolerant rice cultivar. *Plant Physiol* 163:577–584
- Sanchez-Diaz M, Tapia C, Antolín MC (2008) Abscisic acid and drought response of Canarian laurel forest tree species growing under controlled conditions. *Environ Exp Bot* 64:155–161
- Sánchez-Rodríguez E, Moreno DA, Ferreres F, Rubio-Wilhelmi MM, Ruiz JM (2011) Differential responses of five cherry tomato varieties to water stress: changes on phenolic metabolites and related enzymes. *Phytochemistry* 72:723–729
- Semchuk NM, Lushchak OV, Falk J, Krupinska K, Lushchak VL (2009) Inactivation of genes, encoding tocopherol biosynthetic pathway enzymes, results in oxidative stress in outdoor grown *Arabidopsis thaliana*. *Plant Physiol Biochem* 47:384–390
- Seong ES, Ghimire BK, Goh EJ, Lim JD, Kim MJ, Chung IM, Yu CY (2009) Overexpression of the γ -TMT gene in *Codonopsis lanceolata*. *Biol Plant* 53:631–636
- Service RF (2007) A growing threat down on the farm. *Science* 316:1114–1117
- Smirnoff N (1993) The role of active oxygen in the response to water deficit and desiccation. *New Phytol* 125:27–58
- Thiruvengadam M, Chung IM (2015) Selenium, putrescine, and cadmium influence health-promoting phytochemicals and molecular-level effects on turnip (*Brassica rapa* ssp. *rapa*). *Food Chem* 173:185–193
- Usenik V, Mikulic Petkovsek M, Solar A, Stampar F (2004) Flavanols of leaves in relation to apple scab resistance. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 111:137–144
- Yao Y, You J, Ou Y, Ma J, Wu X, Xu G (2015) Ultraviolet-B protection of ascorbate and tocopherol in plants related with their function on the stability on carotenoid and phenylpropanoid compounds. *Plant Physiol Biochem* 90:23–31
- Ye ZZ, Rodriguez R, Tran A, Hoang H, de Los Santos D, Brown S, Vellano RL (2000) The developmental transition to flowering repress ascorbate peroxidase activity and induces enzymatic lipid peroxidation in leaf tissue of *Arabidopsis thaliana*. *Plant Sci* 158:115–127
- Yildiz-Aktas L, Dagnon S, Gurel A, Gesheva E, Edreva A (2009) Drought tolerance in cotton: involvement of non-enzymatic ROS-scavenging compounds. *J Agron Crop Sci* 195:247–253
- Zaefyzadeh M, Quliyev RA, Babayeva SM, Abbasov MA (2009) The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. *Turk J Biol* 33:1–7