DOI:10.1021/jf900833k

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Engineering Flax with the GT Family 1 *Solanum sogarandinum* Glycosyltransferase SsGT1 Confers Increased Resistance to *Fusarium* Infection

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The aim of this study was to engineer a flax with increased resistance to pathogens. The approach was based on the recent analysis of the *Solanum sogarandinum*-derived glycosyltransferase (UGT) protein, designated SsGT1 (previously called 5UGT). On the basis of enzyme studies, the recombinant SsGT1 is a 7-*O*-glycosyltransferase, the natural substrates of which include both anthocyanidins and flavonols such as kaempferol and quercetin. Because flavonoids act as antioxidants and glycosylation increases the stability of flavonoids, it has been suggested that the accumulation of a higher quantity of flavonoid glycosides in transgenic plants might improve their resistance to pathogen infection. Flax overproducing SsGT1 showed higher resistance to *Fusarium* infection than wild-type plants, and this was correlated with a significant increase in the flavonoid glycoside content in the transgenic plants. Overproduction of glycosyltransferase in transgenic flax also resulted in proanthocyanin, lignan, phenolic acid, and unsaturated fatty acid accumulation in the seeds. The last is meaningful from a biotechnological point of view and might suggest the involvement of polyphenol glycosides in the protection of unsaturated fatty acids against oxidation and thus improve oil storage. It is thus suggested that introduction of SsGT1 is sufficient for engineering altered pathogen resistance in flax.

KEYWORDS: Flax; glycosyltransferase; flavonoids; *Fusarium*; *Linum usitatissimum* L.

INTRODUCTION

Flax is grown for commercial use in over 30 countries of the world. In Poland, flax is an important industrial fiber and oil crop. Flax diseases caused by fungal pathogens are the primary factor limiting plant production. Serious yield losses are reported due to pathogen infection. One of the ways that plants use for protection against pathogens is production of various secondary metabolites, including polyphenols, which are a broad and diverse group that includes flavonoids, phenolic acids, phenols, lignans, and tannins. All of those compounds are present at high levels in flaxseed. These compounds possess multiple biological activities and can act in planta as antimicrobials, antioxidants, photoreceptors, and visual attractors (*1*, *2*). The great diversity of these compounds results from the many possible modifications, such as glycosylation by glycosyltransferases, acylation by acyl transferases, hydroxylation by hydroxylases, and methylation by methyl transferases (*3*).

Glycosylation (i.e., conjugation to a sugar moiety) is often the last step in the biosynthesis of a number of secondary plant products including flavonoids, cyanohydrins, steroidal alkaloids, and saponins (*4*, *5*). The ubiquitous glycosyltransferase family members (UGTs) catalyze the transfer of a nucleotide-diphosphate activated monosaccharide unit (glucose, rhamnose, galactose, xylose, rutinose, and neohesperidose) to an acceptor molecule (*6*). Glycosylation often leads to changes in both the activity of the acceptor molecule and its subcellular localization (*4*). In plants in which a variety of UGTs were detected, they were found to control diverse functions, including anthocyanin metabolism regulation, auxin metabolism modulation, and an unknown function induced by methyl jasmonate and salicylic acid (*7*). It is known that glycosylation of low molecular weight compounds such as flavonoids (i.e., anthocyanins) makes the molecules more stable, increases their solubility in the vacuole, and can enhance the pathogen resistance of plants (*8*).

To date, glycosyltransferases have been found in all living organisms (prokaryotes, eukaryotes, *Archaea*, and viruses). UGT sequences have been classified into 75 families according to sequence similarity, the existence of a consensus sequence, and the catalytic specificity; family 1 contains the most sequences (*8*). Family 1 UGTs, identified on the basis of a 44 amino acid C-terminal consensus motif (PSPG), are encoded by 120 UGT

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 Published on Web 07/08/2009

genes in *Arabidopsis*. UGTs have been isolated and characterized from many plant sources including cell suspensions, seedlings, flower parts, and roots; however, they were not detected in fruits (*9*). Flavonoid glycosyltransferases, including those that catalyze O-glycosylation at ring positions 3, 5, 7, and 4′, generally have optimum pH values in the alkaline range (*2*, *10*, *11*) and have a molecular mass ranging from 45 to 60 kDa (*12*). Most of these plant enzymes are soluble and, therefore, thought to be cytosolic (*12*).

It is well-known that almost all of the anthocyanidins (except the 3-deoxy types) are glycosylated at the 3-hydroxyl position, and the anthocyanins are usually glycosylated at the 5-hydroxyl position (*13*). Anthocyanidin 3-*O*-glycosyltransferase (3UGT) has so far been described from many plant species, unlike anthocyanidin 5-*O*-glycosyltransferase (5UGT). Anthocyanidin 5-*O*-glycosyltransferase was detected for the first time in *Silene dioica* by Kamsteeg et al. (*14*). To date, the enzyme has also been found and analyzed from flowers of *Iris ensata*, *Perilla frutescens*, *Verbena hybrida*, *Petunia hybrida*, and *Torenia hybrida* (*10*).

The glycosyltransferase from *Solanum sogarandinum* was recently cloned and analyzed (*15*, *16*). Potato plants overexpressing the SsGT1 (5UGT) gene accumulated a high quantity of anthocyanidin diglucoside. Further detailed analysis of the enzyme revealed its broad substrate specificity. The highest activity was detected against kaempferol and peonidin (*17*). Hence, it was concluded that the enzyme from *S. sogarandinum* modifies a broad spectrum of phenylpropanoid compounds and that kaempferol and anthocyanidin are the best targets for enzyme action.

In this paper, the effects of overexpressing SsGT1 (previously called 5UGT) in flax plants were examined and related to pathogen resistance. It was expected that the overexpression of SsGT1 might give a higher resistance of flax plants to *Fusarium* infection, that is, to the major fungal pathogen infecting flax plants, causing the greatest decline in yield.

MATERIALS AND METHODS

Plant Material. Flax seeds (cv. Linola 947) were obtained from the Flax and Hemp Collection of the Institute of Natural Fibers, Poland. The transgenic and control plants were grown in tissue culture and were cultivated in a greenhouse under a 16 h of light (21 °C)/8 h of darkness (16 °C) regimen. The plants were grown in soil in individual pots and were watered daily. For analysis, the control and selected transgenic plants were grown in a field, and seeds were harvested 3 months after the transfer of the tissue-cultured plants to the soil. Plant selection was done on the first generation of plants; all further analyses were performed on second-generation plants.

Transgenic Plant Construction and Selection. Two-week-old cotyledon and hypocotyl explants were transformed. For transformation, we cloned cDNA coding SsGT1 (EMBL/GenBank accession no. AY033489) from *Solanum sogarandinum* into *Bam*HI/*Sal*I restriction sites of pBiAR/NAP vector in the sense orientation under a seed-specific napin promoter (EMBL/GenBank, accession no. J02798) and OSC terminator.

The vector was introduced into *Agrobacterium tumefaciens* strain C58C1:pGV2260. *A. tumefaciens*-inoculated explants were subsequently transferred to a callus induction and shoot regeneration medium (*18*). The 77 transformants were preselected by Polymerase Chain Reaction (PCR) and then selected by means of Western blot analysis. PCR was carried out with the use of specific primers for the neomycine phosphotransferase (*nptII*) gene (forward, CCGACCTGTCCGGTGCCC; reverse, CGCCACACCCAGCCGGCC) and glycosyltransferase gene (forward, GTCCTCTTGGTGACATTTCCCACAC; reverse, TGAGGAAATGCCACCACAGGT ACAC) on genomic DNA isolated from 3-week-old tissue-cultured plants as a template. Those transgenic plants that contained the predicted *npt II* (475 bp fragment) and SsGT1 (1100 bp fragment) gene products were further analyzed. The final selection was carried out by means of Western blot analysis. The protein extracts prepared from the flax seeds of six preselected transformants were run on a 12% SDS−polyacrylamide gel and blotted electrophoretically onto nitrocellulose membrane (Schleicher and Schuell). Following transfer, the membrane was incubated with blocking buffer (5% dry milk) and then with an antibody against SsGT1-recombinant protein (1:2000 dilution). Alkaline phosphatase-conjugated goat anti-rabbit IgG was used as a secondary antibody at 1:1500 dilution. The efficiency of transformation was 7.8%.

Evaluation of Flax Resistance to *Fusarium culmorum* and *Fusarium oxysporum* Infection. Flax seeds were immersed in 96% ethanol for 1 min and then washed three times with sterile water and placed on a plate. After 7 days of growth on MS medium, the seedlings were inoculated by placing them on medium with *F. culmorum* or *F. oxysporum*. Four pieces of 1 cm square inoculum of fungi were cultured for 7 days at 18 °C on potato−dextrose−agar (PDA) medium; 10−14 days after transfer, the number of infected flax seedlings (roots and hypocotyls) was counted and expressed as a percentage of the total seedlings used for the experiment (*19*). The experiment was carried out in three repetitions for three times.

HPLC Analysis of Flavonoid Glycoside Content in the Transgenic Flax Plants. The materials used in this study were crushed using a laboratory mill. One gram of flax seeds was extracted with 7 mL of 35% aqueous methanol containing 1 g/L L-ascorbic acid as an antioxidant, for 18 h at 20 °C in glass screw-capped vials, and then sonicated for 15 min. Next, the samples were centrifuged (5 min, 19000*g*), and the clear supernatant was injected onto a HPLC column. Analyses of flavone and flavonol derivatives were carried out on a Merck-Hitachi L-7455 liquid chromatograph with a diode array detector (DAD) and quaternary pump L-7100 equipped with a D-7000 HSM multisolvent delivery system (Merck-Hitachi, Tokyo, Japan) and an L-7200 autosampler. Separation was performed on a Synergi Fusion RP-80A 150 × 4.6 mm (4 μm) Phenomenex (Torrance, CA) column. The oven temperature was set to 20 °C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). The program began with a linear gradient from 0 to 25% B at 36 min, followed by washing and reconditioning of the column. The flow rate was 1.0 mL/min, and the runs were monitored at the following wavelengths: flavone at 340 nm and flavonol derivatives at 360 nm.

Determination of the Total Anthocyanin Glycoside Content. Fifteen milligrams of seeds was extracted with 1 mL of methanol/HCl (95:5, v/v) in an ultrasonic bath for 30 min. The extract was centrifuged at 14000*g* for 10 min. Two dilutions of the sample were performed: first, 100 μL of the supernatant was mixed with 900 μL of 0.025 M potassium chloride buffer, pH 1.0, and then, 100 μL of supernatant was mixed with 900 μL of 0.4 M sodium acetate buffer, pH 4.5. The solution was allowed to stand at room temperature for 15 min, and then the absorbance at 510 and 700 nm was measured, which allowed for haze correction. The results were reported as cyanidin-3-*O*-glucoside equivalents (*20*).

Secoisolariciresinol Diglucoside (SDG) Extraction and Measurement. A 0.25 g sample of flax seeds was extracted three times with 1.5 mL of 80% methanol (v/v) for 10 min at 80 °C. Prior to extraction, the seeds were defatted with hot hexane. The extracts was centrifuged and evaporated to near dryness at 40 °C under vacuum. The extract was then resuspended and subjected to alkaline hydrolysis (1 mL, 0.3 M aqueous sodium hydroxide) for 2 days at room temperature followed by neutralization using 2 M hydrochloric acid (*21*). The extract was analyzed on a Waters Acquity UPLC system with a 2996 PDA detector, using Acquity UPLC column BEH C18, 2.1 × 100 mm, 1.7 μm. The mobile phase was A = acetonitrile/B = 20 mM ammonium formate, pH 3, in a gradient flow: 1 min, 10%/90% A/B, 2−6 min gradient to 40%/60% A/B, and 7 min gradient from 40% to100% A with a 0.4 mL/min flow rate. The compound was measured at 280 and 320 nm.

Evaluation of Proanthocyanin Content. For measurements 15 mg of seeds was used. Proanthocyanins were hydrolyzed with 1 mL of *n*-butanol/HCl (95:5, v/v) and 33 μL of a 2% (w/v) $NH_4Fe(SO_4)_{21} \cdot 12H_2O$ in 2 M HCl for 40 min at 95 °C. The extract was centrifuged at 14000*g* for 10 min, and the supernatant was used for proanthocyanin content evaluation. Proanthocyanin detection was carried out by measuring absorption at 540 nm, and proanthocyanin content was expressed as catechin equivalents (*22*).

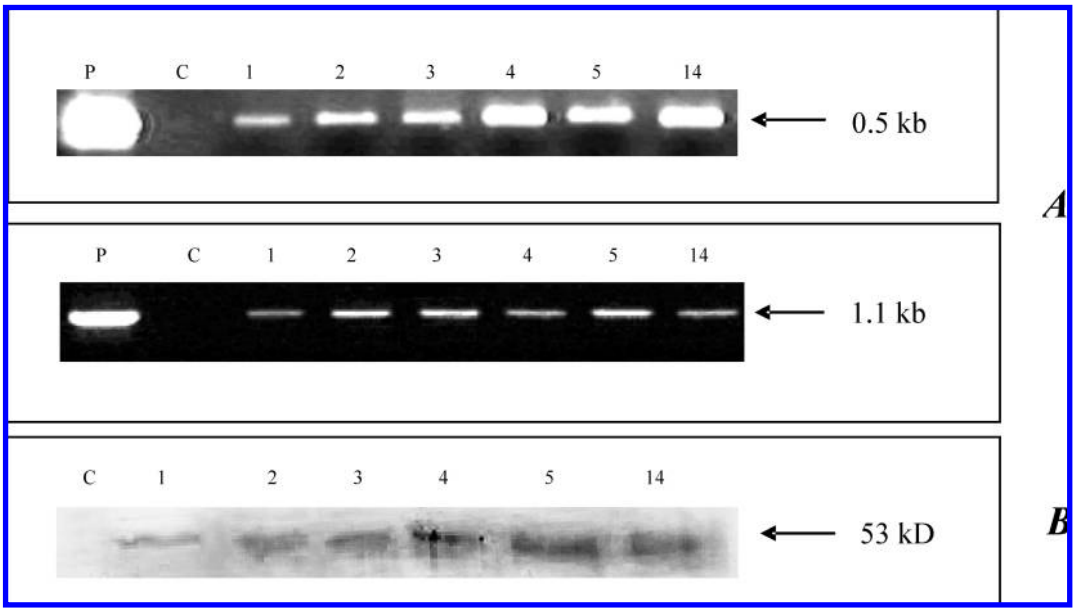

Figure 1. (**A**) Agarose gel electrophoresis of the *npt II* gene (upper panel) and SsGT1 gene (bottom panel) PCR product (500 and 1100 bp, respectively). The *npt II* and SsGT1 genes were amplified with the use of specific primers; genomic DNA isolated from tissue-cultured flax plants was used as a template. C, negative control (nontransformed plant); P, positive control (plasmid containing a *npt II* gene, upper panel; plasmid containing a SsGT1 gene, bottom panel); the different transgenic lines are numbered. (**B**) The transgenic plant protein extract isolated from the seeds was run on a 12% SDS−polyacrylamide gel and blotted electrophoretically onto nitrocellulose membrane (Schleicher and Schuell). Following transfer, the membrane was incubated with blocking buffer (5% dry milk) and then with antibody against SsGT1 recombinant protein (1:2000 dilution). Alkaline phosphatase-conjugated goat anti-rabbit IgG was used as a secondary antibody at 1:1500 dilution. C, nontransformed plant; the different transgenic lines are numbered.

Determination of Fatty Acid Content in Flaxseeds. Methyl esters of fatty acids (FAMEs) were prepared from seeds by extraction with 1 mL of 1 N HCl in methanol and incubation at 80 °C for 90 min. Then 1 mL of 0.9% NaCl and 1 mL of hexane were added to the extract, mixed, and centrifuged at low speed (3 min at 100 rpm). The hexane phase was collected; the lipids were concentrated in a N_2 stream and stored at −20 °C. The methyl esters were quantited by gas chromatography, using pentadecanoic acid as an internal standard (*15*).

Statistical Analysis. Data on the parameters measured were statistically elaborated using ANOVA and the Laven test, followed by the RIR Tukey or Kruskal−Wallis test. All calculations were carried out using the Statistica 7.1 software package (StatSoft Polska, Poland).

RESULTS

Transgenic Plant Generation and Selection. The transgenic plants expressing glycosyltransferase cDNA (SsGT1) from *S. sogarandinum* under the control of the seed-specific napin promoter were generated as described under Materials and Methods. The obtained plants were prescreened via the PCR method with specific primers for the neomycin phosphotransferase and SsGT1 genes (**Figure 1A**). Plants that yielded the expected 475 bp (*npt II*) and 1100 bp (SsGT1) DNA fragments were used for further selection by Western blot analysis (**Figure 1B**). Of the several positive transgenic lines, those with the highest SsGT1 protein contents (U2, U3, U4, U5, and U14) were used for phenotype and infection analysis, but for further chemical analysis only the lines U4 and U5, highly resistant against *Fusarium*, were selected.

Phenotype Analysis. There were no visible differences in leaf shape and size or petal and seed color between the transformed and nontransformed plants. However, the obtained transgenic plants had a higher yield of seeds than the control plants. The transgenic plants produced more (94% on average) seeds per plant than the nontransformed plants (**Table 1**), mostly as a result of transgenic plants producing more seed bags per plant as the numbers of seeds in a bag and the weight of a single seed changed only slightly. Lines U2, U3, U4, U5, and U14, respectively, produced 80, 99, 79, 84, and 131% more seeds per plant than the control plants. The fresh weight of a single seed was significantly higher (ca. 11%) than that for the control only in case of one transgenic line, U5.

Resistance to *F. culmorum* and *F. oxysporum* Infection. The main goal of this study was to investigate the physiological significance of the increased contents of the glycoside derivates of flavonoid for the transgenic flax plants; of greatest interest was their role in protection against pathogen infection. It was expected that the increase in the contents of glycosides of flavonoids might give rise to a higher resistance to pathogen infection as with the recently obtained potato plants with overexpression of glycosyltransferase (*23*).

Indeed, resistance to *F. culmorum* and *F. oxysporum* was significantly higher in the transgenic plants than in the control plants (**Figure 2**). Resistance of the transgenic plants to *F. oxysporum* increased by about 73% and that to *F. culmorum* by about 83%. In the case of *F. oxysporum*, the most resistant transgenic line was U5, which exhibited about an 89% increase in resistance to this pathogen. In the case of *F. culmorum*, the best line was U4, which was 98% more resistant than the control plant. All of the data were statistically analyzed as described under Materials and Methods.

HPLC Analysis of the Flavonoid Content in Transgenic Flax Plants. Because the recombinant enzyme appears to display activity toward flavonols and anthocyanidins, we measured the levels of these compounds in transgenic flax. Methanol extracts from seeds of the two most resistant transgenic lines (U4 and U5) overexpressing the SsGT1 enzyme exhibited increased kaempferol and quercetin glycoside contents. The kaempferol glycoside levels, respectively, increased by 191 and 104% in seeds from U4 and U5 plants, and the quercetin glycoside level increased by 156% in seeds from the U4 line and by 74% in seeds from the U5 line relative to the levels for the control plants. The data are presented in **Figure 3**.

Table 1. Yield of Flax Seeds from the Control and Transgenic Lines[a]

line	FW of seeds per plant (g)	FW of seeds per bag (mg)	no. of seeds per bag	FW of single seed (mg)
control	4.28 ± 0.31*	42.04 ± 2.92	8.00 ± 0.40	5.26 ± 0.28
U2	8.03 ± 1.01*	38.23 ± 4.82	7.63 ± 0.74	5.01 ± 0.29
U3	8.88 ± 0.78*	45.77 ± 4.03	8.21 ± 0.57	5.58 ± 0.25
U4	7.99 ± 0.51*	44.87 ± 2.89	8.22 ± 0.57	5.46 ± 0.18
U5	8.22 ± 1.25*	42.15 ± 6.43	7.19 ± 1.03	5.87 ± 0.18*
U14	10.29 ± 0.72*	47.87 ± 3.35	8.42 ± 0.64	5.69 ± 0.26

[a] The mean value (n = 20) ± SD is presented, P < 0.05. *, statistically significant

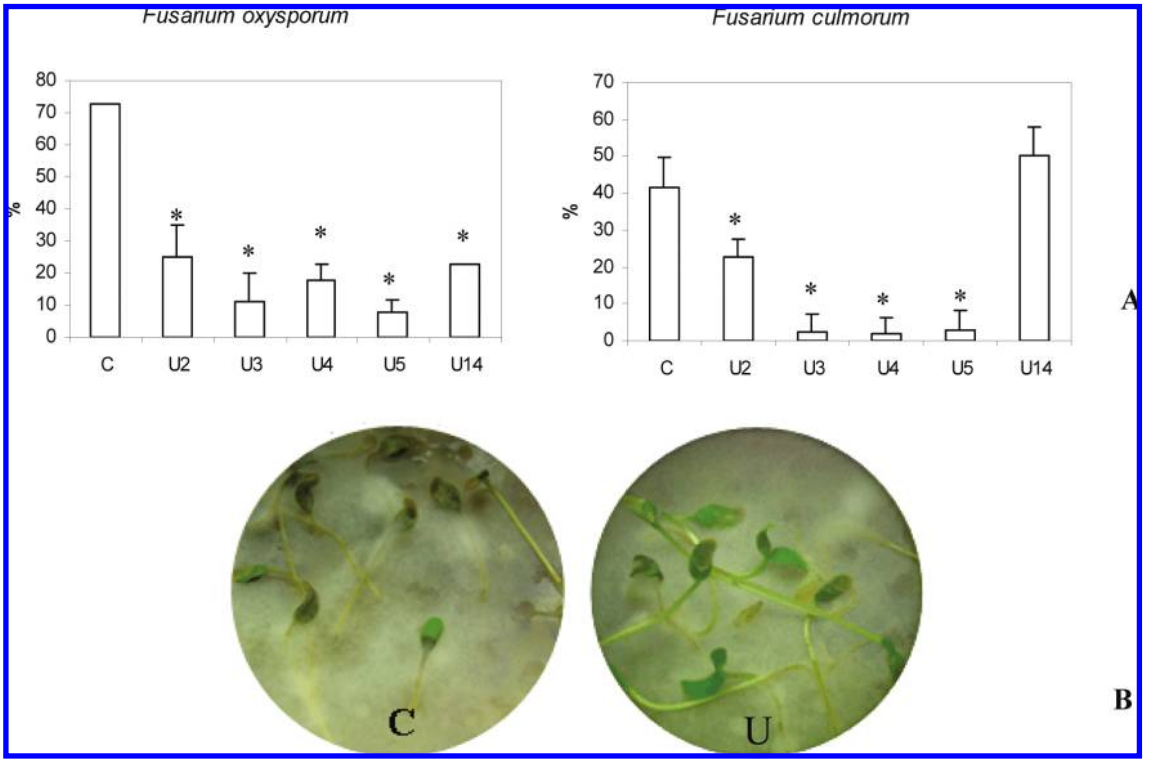

Figure 2. (**A**) Evaluation of resistance of the control (C) and the transgenic lines (numbered) to *F. culmorum* (right) and *F. oxysporum* (left) by the mycelium method. Seven-day-old flax seedlings were transferred onto fungal culture grown for 7 days at 18 °C on potato−dextrose−agar (PDA) medium; 10−14 days later the number of infected flax seedlings (roots and hypocotyls) was counted and the result expressed as a percentage of total seedlings used for the experiment. The mean value (n = 20) ± SD is presented, P < 0.05; * statistically significant. (**B**) Flax seedlings (7 days old) transferred onto fungal culture and grown for 10−14 days (C, control; U, transgenic line).

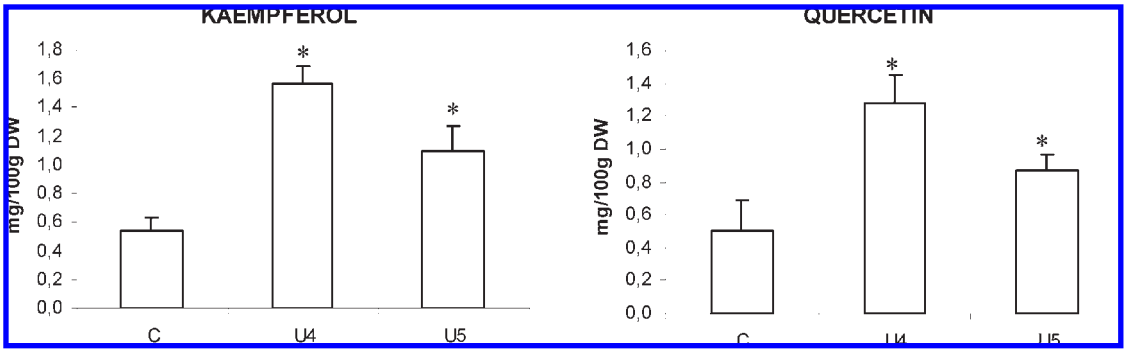

Figure 3. HPLC determination of the kaempferol and quercetin glycoside content in seeds from the control (C) and the transgenic flax plants (numbered). Flavonoids were separated on a Synergi Fusion RP-80A 150 × 4.6 mm (4 μm) Phenomenex (Torrance, CA) column. The mean value (n = 3) ± SD is presented, P < 0.05; * statistically significant.

The significant relationship between the kaempferol and quercetin glycoside contents and resistance to *F. oxysporum* in the transgenic plants was assessed. The calculated correlation coefficient was −0.82 for kaempferol and −0.77 for quercetin. Similarly, the very high correlation coefficient between resistance to *F. culmorum* and kaempferol and quercetin glycoside contents (−0.90 and −0.86, respectively) was noted.

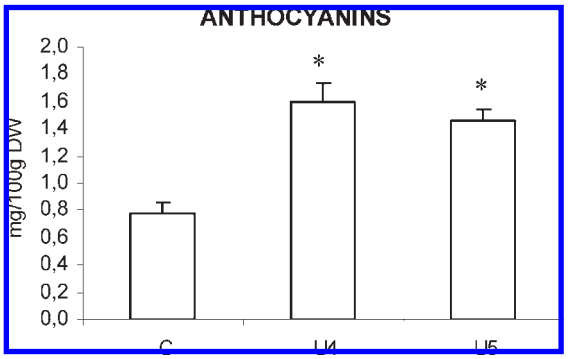

Figure 4. Anthocyanin content in seeds from control (C) and transgenic flax (numbered). The mean value ($n = 3$) ± SD is presented, $P < 0.05$; * statistically significant.

Total Anthocyanin Measurements. The total anthocyanin contents in plants were measured quantitatively via the pH-differential method (*20*). Anthocyanins undergo a reversible structural transformation at different pH values, and this feature was used for the quantitative spectrophotometric method. The colored oxonium form predominates at pH 1.0, in contrast to the colorless hemiketal form at pH 4.5.

The total anthocyanin content in the seeds from the U4 and U5 transgenic plants was increased, respectively, 104 and 86% over the control value. The calculated correlation coefficients between the anthocyanin level and the plants' resistance were −0.95 for *F. oxysporum* and −0.99 for *F. culmorum*.

Thus, the data are suggested to confirm that SsGT1 has activity toward flavonol and anthocyanin in the transgenic plants and that an increased level of the glycoside derivative of flavonoids resulted in efficient plant protection against pathogen (**Figure 4**).

Analysis of Proanthocyanin Contents in Seeds. It was shown that proanthocyanins determine seeds' agronomic and nutritional properties (*24*). It is suggested that the coordinated action of glycosyltransferase and dihydroflavonol reductase-like enzyme converts leucoanthocyanin to proanthocyanin (*25*). Thus, the proanthocyanin content in transgenic plant was measured. The amount of proanthocyanins in seeds from transformant increased from about 67% in line U5 to 98% in line U4 (**Figure 5**).

Analysis of Phenolic Acid and Lignan Contents. Because of broad substrate specificity of overproduced glycosyltransferase, it was speculated that various compounds of the phenylpropanoid pathway could be accumulated and might participate in antioxidative potential of seeds (*24*). Thus, we have also measured the content of the diphenolic compound, SDG, the major component of lignans, and lignan- bound phenolic acids. We have found a 2-fold increase of secoisolariciresinol diglucoside in transgenic plants (from 30.8 to 42.4 mg/g of FW) in comparison to control plants (18.35 mg/g of FW). We also observed a dramatic increase of ferulic acid content from −0.51 μg/g of FW for control plants and to between 21.5 and 23.86 μg/g of FW depending of transgenic line. The level of *p*-coumaric acid was only slightly decreased from 91.02 μg/g of FW for control plants to between 83.8 and 85.2 μg/g of FW for transgenic plants (**Figure 6**). There is a high positive correlation (0.89) between SDG level and the resistance of flaxseeds against *Fusarium* infection, and this coefficient was even higher for phenolic acids (0.96). Thus, it is suggested that the levels of lignans and phenolic acids affect pathogen resistance to the seeds from transgenic lines.

Lipid Composition of Transgenic Flaxseed. The significant increase in antioxidant compounds in the transgenic seeds may protect the unsaturated fatty acids, which are abundant essential compounds of flax oil but easily undergo peroxidation. It was

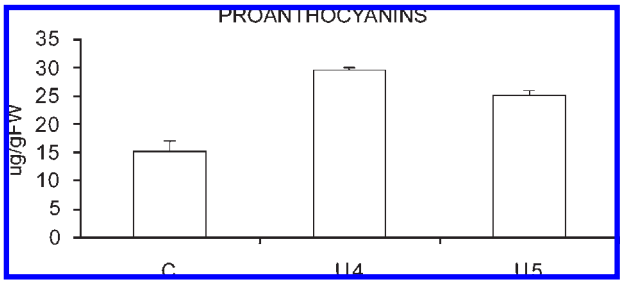

Figure 5. Proanthocyanidyn content in seeds from control (C) and transgenic flax (numbered). The mean value ($n = 3$) ± SD is presented, $P < 0.05$.

shown previously that plant extracts with increased flavonoid content can protect oils from oxidation as shown by TBRS assay (*17*). Therefore, it was of great interest to see if such protection can occur in vivo and influence oil composition. The flaxseed oil was examined by gas chromatography equipped with a flame ionization detector. The increase in unsaturated 18:1 (11−14%), 18:2 (9.5−24.7%), and 18:3 (23.7−54.5%) fatty acids in both transgenic lines was detected. The highest increase was measured in line U4 (**Table 2**). Thus, the increase in antioxidative constituents of seeds was accompanied by significant changes in fatty acid composition; however, at this point it is impossible to say that this is a direct result of increased flavonoid content.

DISCUSSION

The engineering of plants with increased resistance to fungal pathogens has recently emerged as a powerful method. The approach that has been widely used for this purpose includes plant transformation with genes coding for plant pathogenesis-related (PR) constituents, genes encoding antimicrobial peptides, pathogen-derived genes, and genes associated with defense responses. It is also recognized that activating lignin synthesis in infected plants resulted in plant protection against pathogenicity (*26*).

Several genes that confer resistance to pathogens have been successfully introduced into plants and found to protect them from diseases (*27*). The approach is especially useful in plants that have a limited content of endogenous resistance genes. However, it should be pointed out that in several cases, certain antipathogenic genes show restricted taxonomic functionality. For instance, the *RPS2* gene from *Arabidopsis* does not function in transgenic tomato (*27*). Therefore, the most promising approach in generating a broad spectrum of resistant plants is to engineer elicitors and secondary metabolites that confer resistance to different kinds of pathogens. Because the initial pathogen infection process occurs at the plant surface, the latter are meaningful. For instance, phenylpropanoids inhibit the activity of fungal hydrolytic enzymes, and in onion, resistance to *Colletotrichum cercinous* is related to catechol and protocatechuic acid. In potato tubers, resistance to *Streptomyces scabies* is related to chlorogenic acid. It is also significant that engineering plants with increased phenylpropanoid compound content has tremendous promise for the improvement of the diet and health of the consumer. In several studies, the beneficial role of flavonoids for human health has been shown, and they have been linked to anticancer benefits and the reduction of heart disease (*28*).

The most aggressive infecting flax pathogen is *F. oxysporum* (*29*). Its invasion involves an oxidative burst (oxidative radicals), inducing lignin synthesis that provides a physical barrier to the pathogen, on the one hand, and results in plant tissue lesion, on the other hand. It is thus suggested that the overproduction of antioxidative constituents in the plant might be useful in protecting them against infection. Data published so far support the thesis that antioxidants could be part of a

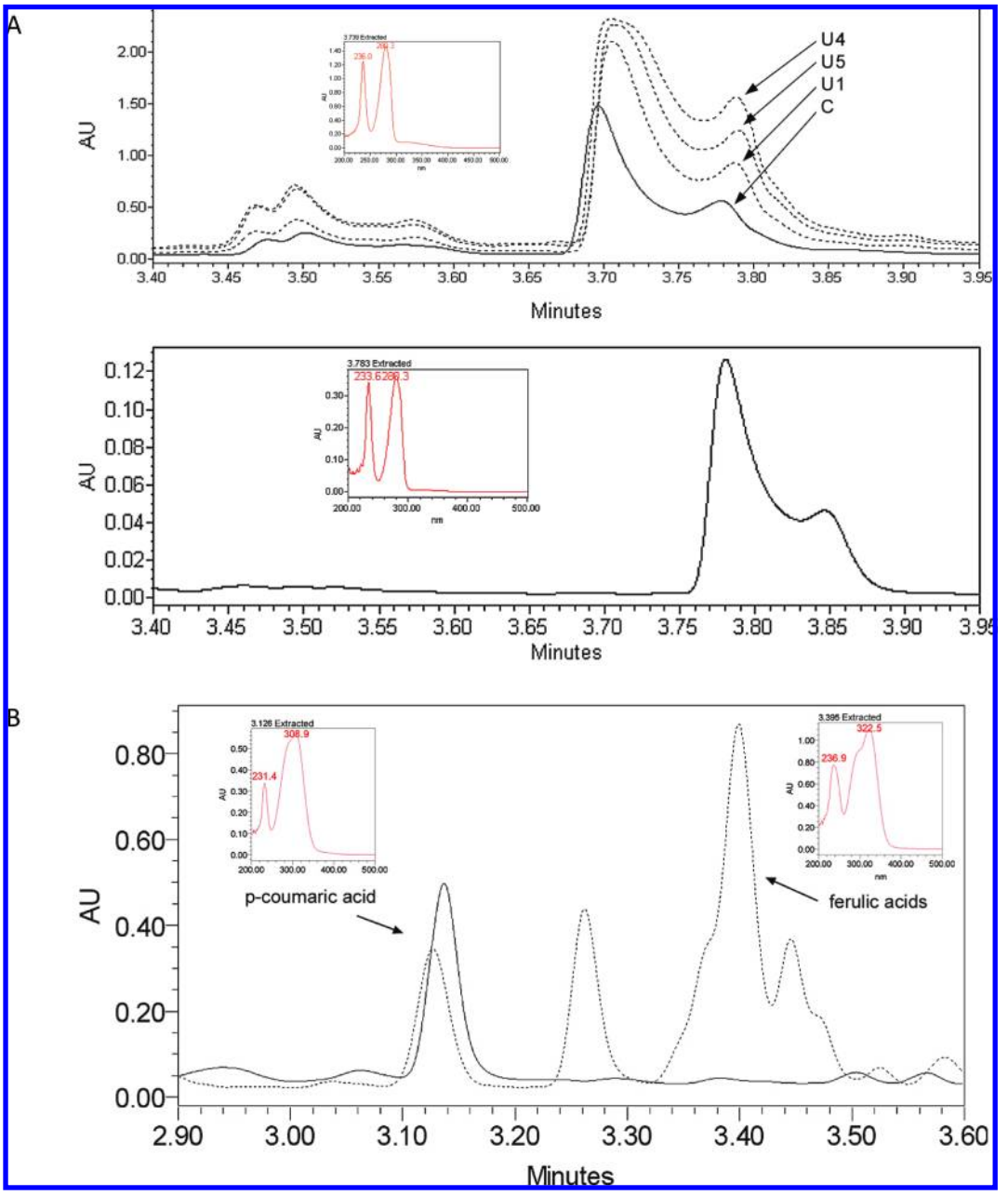

Figure 6. UPLC chromatograms of a crude extracts of flaxseed (**A**) 280 nm chromatogram, SDG identification (plant extract, upper panel; SDG standard, lower panel); (**B**) 320 nm chromatogram, phenolic acids identification (dotted line, transgenic plants; solid line, flax control plant). The mean value ($n = 20$) ± SD is presented, $P < 0.05$.

Table 2. Oleolate (18:1), Linoleate (18:2), and Linolenate (18:3) Contents in the Seeds of Control and Transgenic Plants[a]

	C 18:1 (μg/g of FW)	C 18:2 (μg/g of FW)	C 18:3 (μg/g of FW)
control	182.38 ± 55	887.96 ± 199	20.37 ± 6
U4	202.65 ± 36	1107.63 ± 121	31.49 ± 6.5
U5	208.51 ± 15	973.16 ± 105	25.2 ± 6.8

[a] The mean value ($n = 20$) ± SD is presented, $P < 0.05$.

constitutive pool of compounds responsible for plant resistance to biotic and abiotic stresses. To increase the pool of antioxidants, we recently overexpressed the key genes controlling flavonoid biosynthesis in flax. When overproduced in flax, the enzymes CHS, CHI, and DFR brought about a 71% increase in seed yield and an increase (5-fold) in the antioxidant level. The increased antioxidant capacity of seeds resulted in higher (25%) protection against *Fusarium* infection. The obtained data suggest that by increasing the plant antioxidative status, the defense mechanism against the pathogen might be improved (*15*).

In this paper we used glycosyltransferase overproduction as an approach to increase the pool of antioxidants. It was shown that viruses, wounding, or salicylic acid (SA) treatment rapidly induces glycosyltransferases (*30*). However, no data on any role of glucosyltranferases in flax plant defenses against fungi were available.

Thus, the aim of this study was to analyze the impact of SsGT1 overexpression on flax resistance to pathogen infection and on plant yield and to compare the effect of this overexpression to that of CHS, CHI, and DFR (*15*). The overexpression of the glycosyltransferase gene in seeds resulted in a significant increase (60%) in the transgenic plant resistance to *Fusarium* infection. The resistance of these transformants was even higher than that of

transformants overproducing flavonoid compounds. The resistance of SsGT1 overproducing plants against *Fusarium* infection was >40% higher than that of plants enriched with flavonoids (*15*). Therefore, it can be suggested that the stabilization of phenolic compounds by glycosylation is more effective in plant protection against *Fusarium* infection than flavonoid overproduction. Moreover, it could be inferred that the primary reason for higher transgenic flax resistance was flavonoid glycoside accumulation. We assessed the very high correlation coefficients between kaempferol, quercetin, and anthocyanin glycoside contents and resistance to *Fusarium*, which relates to an important function of the glycosylation process in pathogenesis. More importantly, glycosylation did not result in yield decline, which can sometimes be observed in plants with significant overproduction of flavonoids (*31*).

Besides flax protection against pathogen infection, our effort has been directed toward genetically improving the health effect of flaxseeds. Flaxseeds are a rich source of lignans with beneficial health effect. It is reported that they lower the risk of cardiovascular diseases and protect consumers against different types of cancer such as breast and colon cancer (*32*−*34*). Lignans are macromolecules, and large-scale isolation of lignans revealed the presence of other phenolic compounds such as coumaric, ferulic, and sinapic acids (*35*, *36*), flavonoid herbacetin, and flavonol kaempferol (*35*, *36*). Although the main components of the macromolecule are identified, the mechanism of its formation is not yet known. Recently, we have shown that SsGT1 revealed broad substrate specificity, modifying flavonoids and phenolic acids as well (*17*). It was thus interesting to detect lignan macromolecule composition from transgenic flax and compare it to the control. A complete saponification of lignan macromolecule revealed a 30-fold increase in SDG and a 40-fold in ferulic acid; the coumaric acid content was only slightly decreased when compared to control. There was, however, no detectable quantity of kaempferol or herbacetin.

Our effort has been also directed toward genetically improving the market properties of flax oil. Flaxseed is a rich source of PUFA with beneficial health effects. However, the market value of flaxseed oil is limited by the perishability of PUFA, which are highly susceptible to peroxidation. Thus, increased levels of antioxidants in seeds could be an important regimen for fortifying the fatty acids against oxidation.

Because phenylpropanoid compounds could protect lipids against peroxidation and have hydroxy radical scavenging activity (*37*, *38*), we have analyzed the fatty acid compositions in seeds from control and transgenic plants. Transgenic seeds showed increases in unsaturated fatty acid (18:1, 18:2, 18:3) levels, thus suggesting a highly positive effect of overproduced glycosylated compounds on PUFA level in protection against free radicals. Interestingly, oil enriched with oleic acid (18:1) lowers the risk of coronary heart disease (*39*).

It is suggested that the increase in antioxidant capacity may result in an increase of plant growth (*40*) and protection against infectious agents (*2*). Transgenic flax plants were visually indistinguishable from the control plants but were characterized by higher yields of seeds, higher numbers of seeds per bag, and higher fresh weight of seeds per plant when compared to the control. Resistance to fungi was also a characteristic feature of transgenic plants.

In conclusion, we found that transgenic flax overexpressing cDNA encoding the SsGT1 showed increased phenolic content. The consequence is the modification of fatty acid composition in seeds and a significant increase in the component level of lignan macromolecule. What is most important is the significant increase in resistance to pathogen that was achieved accompanied by flax yield. Because transgenic flax showed remarkably increased resistance to *Fusarium* infection, we suggest that glycosylated phenylpropanoid compounds are principal components of flax defense against *Fusarium* infection.

ABBREVIATIONS USED

CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroflavonol reductase; IC_{50}, half-maximal inhibitory concentration; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; UGT, uridine diphosphate-glucosyl transferase.

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Received October 6, 2008. Revised manuscript received June 23, 2009. Accepted June 23, 2009. This study is supported by Grants 2P06A 02029, PBZ-MNiI-2/1/2005, and N302 017 31/1809 from the Ministry of Education and Sciences.