JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Pseudomonas fluorescens N21.4 Metabolites Enhance Secondary Metabolism Isoflavones in Soybean (*Glycine max*) Calli Cultures

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ABSTRACT: Phytopharmaceuticals are plant secondary metabolites that are strongly inducible and especially sensitive to biotic changes. Plant cell cultures are a good alternative to obtain secondary metabolites, in case effective stimulation can be achieved. In this study, metabolic elicitors from two rhizobacteria able to enhance isoflavone content in soybean seedlings were tested on three different soybean calli cell lines. Results show that metabolic elicitors from *Chryseobacterium balustinum* Aur9 were not effective. However, there are at least two different metabolic elicitors from *Pseudomonas fluorescens* N21.4, one under 10 kDa and another over 10 kDa, that trigger isoflavone metabolism in the three cell lines with different isoflavone content. Elicitors from N21.4 achieved total isoflavone increases up to 29.7% (0.205 mg/g), 64.5% (0.487 mg/g), and 23.4% (0.726 mg/g) in the low-, intermediate-, and high-yield lines, respectively. Therefore, these elicitors have a great potential to enhance isoflavone production in cell cultures for development of functional ingredients.

KEYWORDS: *Soybean calli, elicitation, functional ingredients, isoflavones, rhizobacteria*

INTRODUCTION

Nowadays a healthy diet is a great concern in our society and there is an increasing interest in foods that are naturally rich in bioactive compounds.[1] Also, fortified foods are gaining importance in our diet, and therefore, obtaining good raw materials to prepare them is an interesting topic for plant breeding and agriculture.[2] Moreover, another way to obtain these health benefits is by providing these compounds on food supplements, which appear as a good market opportunity for the pharmaceutical industry.

Phytopharmaceuticals are secondary metabolites synthesized by plants that are key for adaptations to changing environmental conditions and plant communication.[3,4] Therefore, these pathways are strongly inducible[2,5−7] and are especially sensitive to biotic changes, among which are fungi and bacteria. Despite the pathogenic nature of microorganisms, there is a group of free-living nonpathogenic rhizobacteria known as PGPR (plant growth promoting rhizobacteria)[8] that are able to trigger plant secondary metabolism involved in defense, which are, in turn, metabolites with pharmacological interest for human health.[9] These bacteria trigger a physiological state in the plant known as "priming",[10] involving metabolic changes that are evidenced upon pathogen challenge with an improved and more intense defensive response. [11] In view of the above, the use of PGPR to "palliate" changes due to environmental conditions may be an alternative, but still obtaining constant bioactive levels would be conditioned by the growing season. Obtention of phytopharmaceuticals from cell or calli cultures is a reliable alternative, despite the limitation due to the lack of stimuli. However, it could be hypothetically overcome with elicitors delivered in the culture medium.[12−17] Yeast extract or fungal elicitors have been widely used,[18,19] as well as polysaccharides,[20] but elicitors from beneficial bacteria are still in their initial stages within this group.[21]

On the basis of the foregoing, the rationale of this study was to evaluate the effect of bacterial elicitors obtained from two PGPR that have shown ability to trigger defensive responses in soybean seedlings[9] to enhance isoflavone (IF) content in three different soybean lines. In order to develop this study, putative bacterial elicitors released to culture medium were first extracted and separated by molecular weight and then tested at different concentrations in three soybean cell lines with different isoflavone profiles. The objectives of this study were (i) to identify if elicitors are bacterial metabolites released to culture medium, based on changes on isoflavone profiles, and (ii) to understand IF metabolism based on the changes observed on the isoflavone species studied in the three cell lines.

EXPERIMENTAL PROCEDURES

Materials. *Cell Lines.* Three soybean cell lines were used in this study. Two of them were kindly provided by Nestlé Research Center (Switzerland). Both were developed from cotyledons of *Glycine max* (L.) Merr cv. Maple Arrow and exhibit different IF contents; line 13407 shows a low IF concentration and line 13406 shows a high IF concentration.[22] Lines 13406 and 13407 were maintained on the medium described by Gamborg et al.[23] supplemented with sucrose (30 g/L), agar (7 g/L) (Sigma−Aldrich, plant cell culture tested), and 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), pH 6.0. Each cell line was subcultured every 30 days on the medium described above and

Received: August 1, 2012
Revised: October 2, 2012
Accepted: October 5, 2012
Published: October 6, 2012

dx.doi.org/10.1021/jf303334q | J. Agric. Food Chem. 2012, 60, 11080−11087

grown in a Sanyo growth cabinet (MLR-350H) at a light intensity of 32.4 μmol·m^{-2}·s^{-1}, 16/8 h light/dark at 25 °C.

The third cell line was developed from 7-day-old primary roots of *Glycine max* cv. Osumi seedlings according to the procedure described by Bueno et al.[24] with modifications. Seeds were disinfected in 70% ethanol by stirring for 1 min, 5% sodium hypochlorite for 6 min, and five washes with distilled water and were allowed to germinate in 1% European bacteriological agar plates at 25 °C for 7 days. Then, 1 cm long cuts from primary roots were placed on MS2 medium for calli initiation[25] [Murashige and Skoog basal salt mixture (MS), Sigma−Aldrich] supplemented with 13.3 μM 6-benzyladenine (BA), 0.2 μM naphthaleneacetic acid,[26] 2% sucrose, and 0.7% agar (Sigma−Aldrich, plant cell culture tested), pH 6.0. Plates were kept in the dark for 15 days at 25 °C, and with a 16 h photoperiod and light intensity of 32.4 μmol·m^{-2}·s^{-1} for the following 15 days; these conditions were set for all experiments. Calli appeared at the root distal end after 30 days; calli were subcultured every 2 weeks to obtain reproducible pale green-yellowish calli, known as line Osumi.

Bacterial Elicitors. Strains *Chryseobacterium balustinum* Aur9 (CECT 5399) and *Pseudomonas fluorescens* N21.4 (CECT 7620) were used to obtain elicitors, given their ability to trigger secondary metabolism in soybean seedlings[9] and other plant species. Elicitors released to culture medium were used in this study and were obtained from a 24 h liquid culture of each strain as described by Gutiérrez Mañero et al.[21] In summary, cultures (250 mL of nutrient broth, Pronadisa) were started with 1 mL of each bacterial strain in 10 mM $MgSO_4$ buffer, with an optical density (OD) of 1.0 at 600 nm. After 24 h under continuous shaking at 28 °C, culture medium was centrifuged at 4000 rpm at 4 °C for 20 min and then filtered through a 0.2 μm filter to ensure removal of all bacterial debris. This medium was frozen and lyophilized, and then it was suspended in acetone/water (4:1 v/v). The acetone fraction was discarded and the nonsoluble in acetone residue was solubilized in water and centrifuged for 10 min at 4000 rpm at 4 °C to remove any solid debris. Then, the supernatant was filtered through a vivaspin filter (10 kDa molecular mass cutoff); separation was forced by centrifugation at 4 °C for 20 min at 4000 rpm. Two fractions resulted from this process, a fraction with molecules under 10 kDa (small fraction = S) and a fraction with molecules over 10 kDa (large fraction = L). Both were lyophilized before use.

Methods. Nestlé Research Center cell lines were subcultured on plates supplemented with elicitors from strains N21.4 (N) and Aur9 (A). Two potential elicitors were used, one over 10 kDa (large = L) and another under 10 kDa (small = S). Stock solutions of each elicitor were prepared at 1 g/L. The stock solution was added to culture medium (1 or 10 mL) to obtain the final concentration assayed (concentration 1 = 0.01 mg/mL; concentration 2 = 0.1 mg/mL). Elicitors were incorporated into culture medium and autoclaved (30 min 115 °C) and then plated. A nonelicited control was kept in all experiments. Therefore, nine treatments were tested in Nestlé Research Center cell lines. In line Osumi, only bacterial elicitors (S and L) at two concentrations (1 and 2) from N21.4 (N) and control were tested, constituting a total of five treatments.

Each experiment was started from 0.8 cm diameter cell disks. Four disks per plate constituted a replicate, and three replicates were made for each treatment. Plates were kept on a Sanyo MLR-350H growth cabinet at 25 °C, with a 16 h photoperiod with 32.4 μmol·m^{-2}·s^{-1}light intensity. Cell lines 13406 and 13407 were allowed to grow for 15 days, while Osumi cell lines grew for 20 days. After that time, biomass was harvested and isoflavones extracted from 0.5 g of biomass, in 15 mL of 80% methanol as described in Ramos Solano et al.[9]

Isoflavone Determination. Identification and quantification of isoflavones were performed on a Beckman HPLC provided with a two-pump 125 solvent module and a 168 diode array detector. Chromatographic conditions were as follows: UV detection, 262 nm; C18 Phenomenex Luna column (5 μm, 150 mm × 4.6 mm), kept at 30 °C with a Gecko-2000 30−80 °C thermostat; mobile phase, solvent A = water + 0.1% acetic acid, solvent B = acetonitrile + 0.1% acetic acid, with the following gradient: 15−45% B in 40 min and then increased to 100% B in 1 min and remaining at this composition for 9 min, after which it decreased to the initial conditions (15% B) in 1 min and was kept constant for 9 min to restore initial conditions. The flow was set at 1.5 mL/min, and the injection volume was 10 μL. Isoflavone quantification was done by interpolating relative area counts into indirect calibration curves for each, done with a commercial standard.[9]

The indirect calibration curves were constructed with the commercial isoflavones (LC Laboratories) daidzein, daidzin, genistein, genistin, and malonylgenistin.

Malonyldaidzin was identified by high-performance liquid chromatography/electrospray ionization ion-trap mass spectrometry (HPLC/ESI-ITMS) [Agilent 1100/Esquire 3000 (Bruker)]. Chromatographic conditions were the same as those specified for HPLC-diode array with the following modifications: The flow was set at 1.5 mL/min but with a split of 1/100, and the injection volume was 50 μL. Experimental conditions were as follows: scan 50−700 m/z; polarity positive; capillary 4000 V; skim 1, 43.2 V; skim 2, 6.0 V; cap exit offset, 77.2 V; cap exit, 120.4 V; nebulizer, 10 psi; dry gas, 6 L/min; dry temp, 300 °C. The calibration curve of malonyldaidzin was the same as that of daidzein. Because the malonyl group does not contain an ultraviolet chromophore, it was hypothesized that the absorption properties of the β-glucoside structures at 262 nm should not be modified by a malonyl conjugation and that their response factor depended only on their molecular weight. [27]

Statistical Analyses. To evaluate elicitor effects on isoflavone content, one-way analysis of variance was performed. When differences were significant, the least significant differences (LSD) posthoc test was also performed[28] with the software Statgraphics plus 5.1 for Windows.

RESULTS

Malonyldaidzin Identification. Malonyldaidzin was identified by HPLC/ESI-ITMS. Ionic traces corresponding to daidzein and malonyldaidzin are shown in Table 1. An ionic chromatogram and the corresponding mass spectrum of malonyldaidzin are presented in Figure 1, where the molecular ion (M + H$^+$) m/z = 503 and the ionic fragment (M + H$^+$) m/z = 255 originated by the cleavage of the glycosidic bond can be identified.

Table 1. Malonyldaidzin and Daidzein Retention Times by HPLC-Diode Array and HPLC/MS and the Corresponding Ionics

	retention time (min)		ionics		
	HPLC- diode array	HPLC/MS	M	M + H	M + Na
daidzein	14.7	11.60	254	255	277
malonyldaidzin	9.6	11.64	502	503	525

Isoflavone Content of Nonelicited Cell Lines 13407, 13406, and Osumi. Normal growth rates were detected in all three cell lines under all treatments. Total isoflavone content on line 13407 was lowest (0.158 mg/g), intermediate on line Osumi (0.296 mg/g), and highest on line 13406 (approximately 0.6 mg/g). Lines 13406 and 13407 showed higher daidzein derivatives, while genistein derivatives were more abundant in line Osumi, accounting for 68% of total isoflavones (Table 2). Furthermore, conjugated species predominated on line Osumi and 13406 while aglycons predominated in the low isoflavone content line 13407 (Figure 2). Isoflavone contents after elicitation are presented for each line separately.

Effects of Elicitor Application on Isoflavone Content in 13407 Cell Line. In line 13407 (Figure 3), elicitors (L and S) from N21.4 at the lowest concentrations significantly increased total isoflavones (+29.7% over control), based on a significant increase in the daidzein family (Figure 3A). No

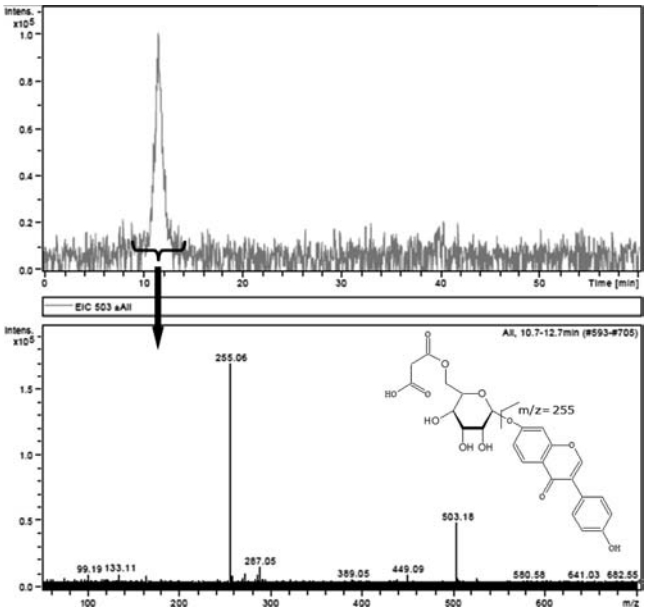

Figure 1. Ionic chromatogram at m/z = 503 and the corresponding mass spectrum.

Table 2. Isoflavone Concentrations[a]

cell line	daidzein family (mg/g)	genistein family (mg/g)	total (mg/g)
13407	0.094 ± 0.009	0.063 ± 0.005	0.158 ± 0.013
13406	0.411 ± 0.015	0.178 ± 0.002	0.588 ± 0.016
Osumi	0.095 ± 0.009	0.201 ± 0.016	0.296 ± 0.023

[a]Total isoflavone concentrations and those from daidzein and genistein families in the three cell lines assayed are shown. Data are the average ± standard error (n = 3).

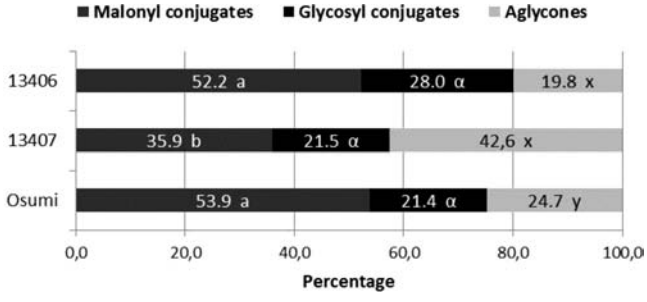

Figure 2. Percentage of malonyl-conjugated, glycosyl-conjugated, and aglycon isoflavones in the three cell lines assayed. Numbers indicate the average (n = 3). Different letters indicate the existence of significant differences according to LSD test ($p < 0.05$) between the different cell lines on (a, b, c) malonyl-conjugated, (α, β, γ) glycosyl-conjugated, and (x, y, z) aglycon isoflavones.

effects were detected under elicitors from Aur9 (Figure 3B). However, changes in isoflavones species upon elicitation revealed that increases detected for N21.4 elicitors at low doses were due to a significant increase in daidzein conjugates (Figure 4A).

Effects of Elicitor Application on Isoflavone Content in 13406 Cell Line. When these elicitors were delivered to line 13406 (high yield) (Figure 5), large and small elicitors at high concentration (NS2 and NL2) from N21.4 (Figure 5A) increased total isoflavones, although it was only significant for small elicitors (+23.4% over control); changes were due to the

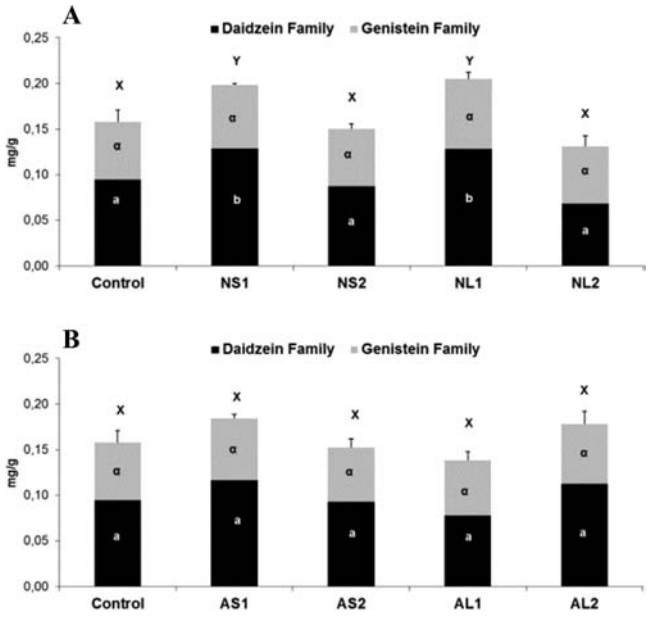

Figure 3. Total isoflavone concentration (milligrams per gram) and concentrations from the daidzein and genistein families in calli from 13407 cell line, untreated (controls) and treated with the two fractions (L and S) at the two assayed concentrations (1 and 2) from (A) N21.4 (labeled N) and (B) Aur 9 (labeled A). Data are the average ± standard error (n = 3). Standard error in the figure corresponds to total isoflavones. Different letters indicate significant differences among treatments according to LSD test ($p < 0.05$): (x, y, z) total isoflavones; (α, β, γ) genistein family; (a, b, c) daidzein family.

increase on the daidzein family (+30% over control) and it was detected on aglycons (Figure 6A). Interestingly, despite the decrease it is worth mentioning that the genistein family is increased by 21.3% in calli elicited with N21.4 small elicitors at the low concentration (Figure 5A) and is due to malonylgenistin (Figure 6A). Large elicitors at high concentration account for 11% increases over nonelicited samples. Only large elicitors from Aur9 at low concentration caused a significant decrease in total isoflavones; no other fraction was effective (Figure 5B).

Effects of Elicitor Application on Isoflavone Content in Osumi Cell Line. The experiment performed in line Osumi was carried out only with elicitors from N21.4, on the basis of its better performance in Nestlé Research Center cell lines.

Small elicitors from N21.4 triggered isoflavone content in line Osumi, while large elicitors were not effective (Figure 7). Small elicitors at concentration 2 (NS2) significantly increased total isoflavone content (up to 52.3%), based on an increase in the daidzein family. Moreover, the lower concentration of these elicitors (NS1) caused a significant increase on total isoflavones (+64.5%), due to an increase in the daidzein family that tripled isoflavone levels in controls.

Finally, changes in the chemical species of isoflavones were studied (Figure 8). Increases detected under NS1 and NS2 were due to increases in daidzein conjugates. Although NS1 significantly increased genistein conjugates, the increase was not detected when the genistein family was considered as a whole (Figure 8).

DISCUSSION

The increasing demand for functional foods calls for new and effective sources to obtain functional ingredients,[2] and this

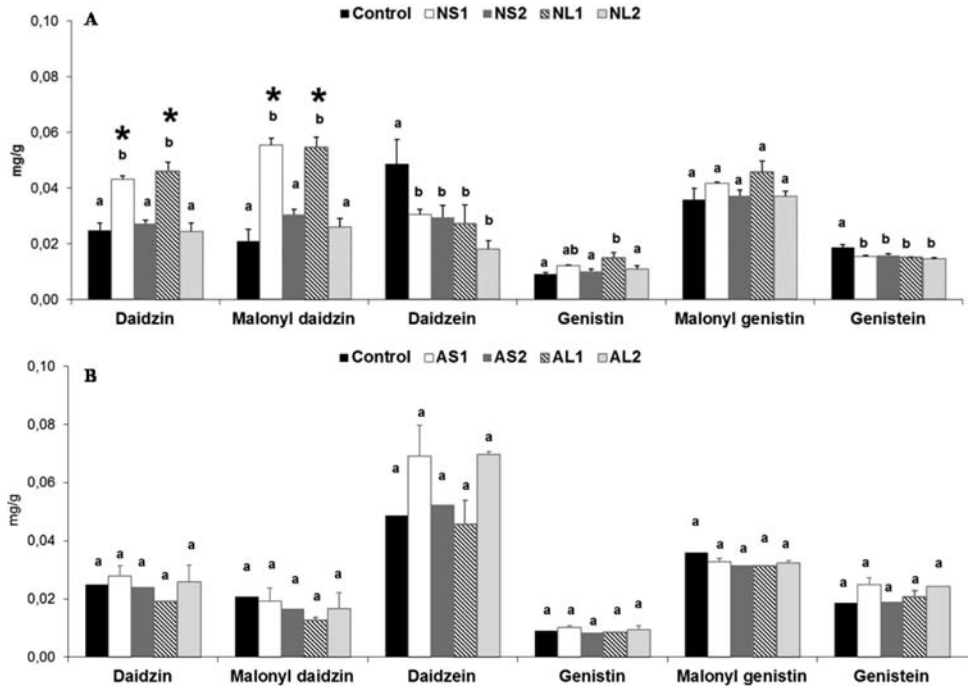

Figure 4. Concentration of each isoflavone species (milligrams per gram) in calli from 13407 cell line, untreated and treated with the two fractions (L and S) at the two assayed concentrations (1 and 2) from (A) N21.4 (labeled N) and (B) Aur 9 (labeled A). Data are the average ± standard error (n = 3). Different letters indicate significant differences among treatments according to LSD test (p < 0.05); asterisks indicate the most relevant results.

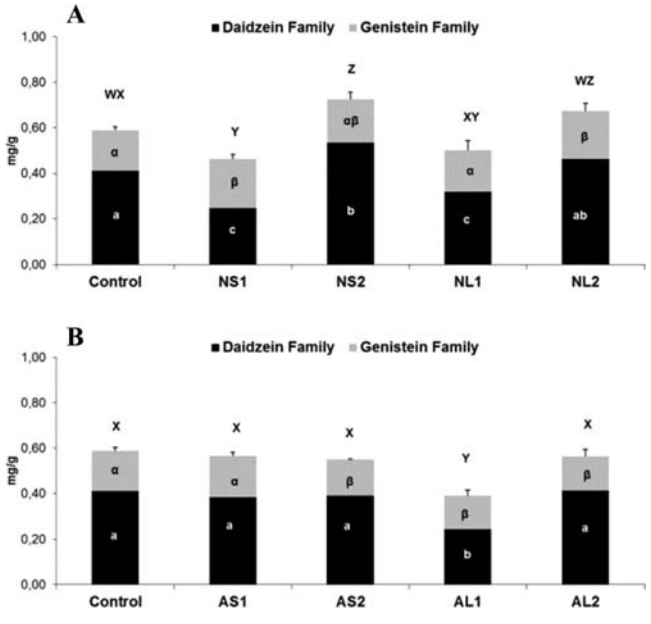

Figure 5. Total isoflavone concentration (milligrams per gram) and concentrations from the daidzein and genistein families in calli from 13406 cell line, untreated (controls) and treated with the two fractions (L and S) at the two assayed concentrations (1 and 2) from (A) N21.4 (labeled N) and (B) Aur 9 (labeled A). Data are the average ± standard error (n = 3). Standard error in the figure corresponds to total IF. Different letters indicate significant differences among treatments according to LSD test (p < 0.05): (x, y, z) total IF; (α, β, γ) genistein family; (a, b, c) daidzein family.

demand shows in the great development of biotechnological procedures to obtain functional foods or ingredients for fortified foods. To overcome low yields, due to the lack of stimulating factors that trigger secondary metabolism in calli cultures, metabolic elicitors from two bacterial strains able to trigger isoflavone metabolism in *Glycine max* seedlings[9] have been used to improve isoflavone contents in cell lines, focusing on development of an economically feasible and profitable procedure to obtain isoflavones.

The use of elicitors for cell cultures is an emerging tool with great potential to increase levels of bioactive compounds.[29] It has been shown that plant growth regulators affect cell cultures,[30] and the use of biotic elicitors is a challenging approach that has already shown good results. These elicitors are delivered as extracts or fragments to mimic triggering of defensive responses.[21,30−32] Yeast extracts increase saponins in *Panax gingseng* cell cultures;[33] the fungal cell wall derivatives chitin and chitosan trigger secondary metabolite production in *Pueraria candolei* cell cultures;[34] and polysaccharides of different origins have also shown their ability to trigger secondary metabolites in different plant species such as *Vitis vinifera*.[20] As a matter of fact, microbial cell wall polysaccharides are frequently effective elicitors,[29,35−37] although other bacterial metabolites that are not necessarily structural molecules cannot be ruled out. The innovative part of the work presented here is the elicitors, a unique material obtained from beneficial strains isolated from the rhizosphere. Hence, putative elicitors from strains N21.4 and Aur9 were extracted from bacterial culture medium, targeting water-soluble elements released to culture medium after 24 h growth. In order to ensure that effects were due to bacterial elicitors and not to culture medium composition,[21] putative elicitors were also extracted from culture medium without bacterial growth and tested on calli cultures. As a general rule, putative elicitors from control culture medium negatively affected isoflavone contents (data not shown); in those cases in which an increase was shown, effects of elicitors widely overcame these values.

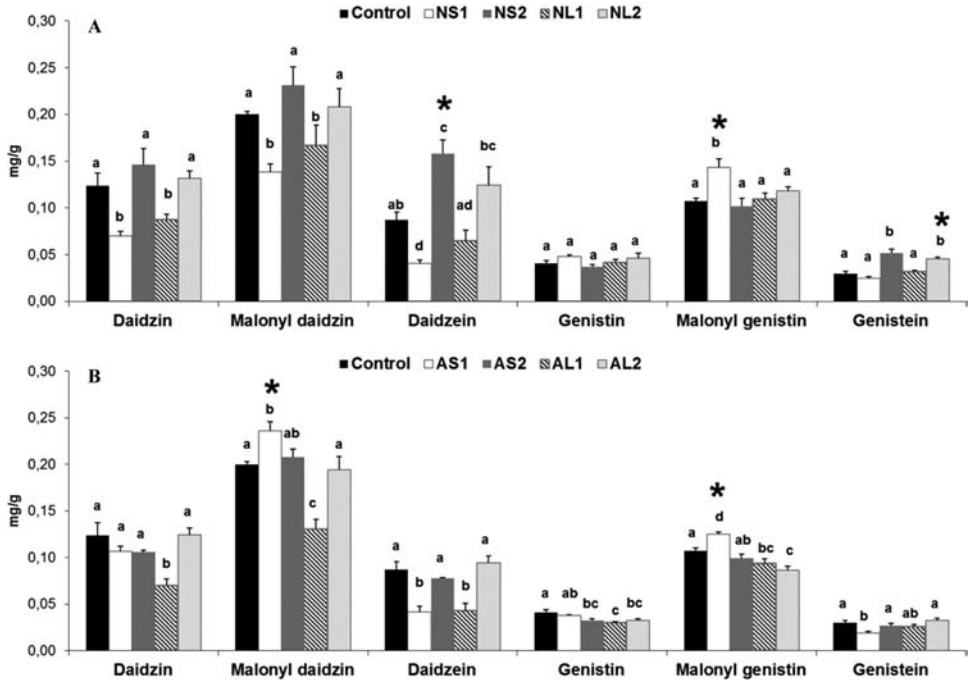

Figure 6. Concentration of each isoflavone species (milligrams per gram) in calli from 13406 cell line, untreated and treated with the two fractions (L and S) at the two assayed concentrations (1 and 2) from (A) N21.4 (labeled N) and (B) Aur 9 (labeled A). Data are the average ± standard error (n = 3). Different letters indicate significant differences among treatments; asterisks indicate the most relevant results.

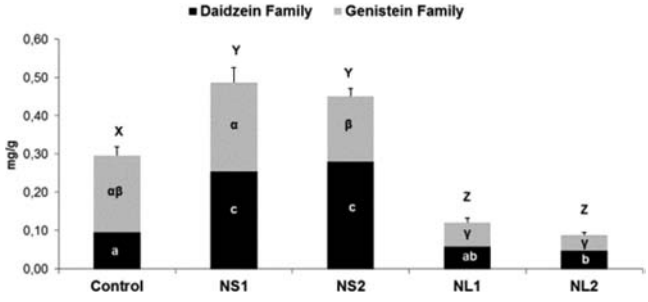

Figure 7. Total isoflavone concentration (milligrams per gram) and concentrations from the daidzein and genistein families in calli from Osumi cell line, untreated (controls) and treated with the two fractions (L and S) at the two assayed concentrations (1 and 2) from N21.4 (labeled N). Data are the average ± standard error (n = 3). Standard error in the figure corresponds to total IF. Different letters indicate significant differences among treatments.

Despite the effectiveness of both PGPR to increase isoflavone content on soybean seedlings, only elicitors from N21.4 were effective on calli cultures. Therefore, among elicitors from N21.4, water-soluble bacterial metabolites released to culture medium can be confirmed, while elicitors from Aur9 are not of this nature, consistent with previous results showing the structural nature of Aur9 elicitors.[37] Hence Aur9 elicitors were not assayed on Osumi cell line.

The rationale of the study involves using different cell lines on the basis of the different genetic material to unravel isoflavone metabolism, and with this purpose, the two cell lines from Nestle research center were used. However, specificity between plant and bacteria has been reported in the interaction process, and the two bacterial strains under study had only shown positive results on soybean seedlings var. Osumi, so a third cell line was developed and tested in order to obtain information in the same plant material (var. Osumi) and to extend the potential of elicitors to other plant materials. The

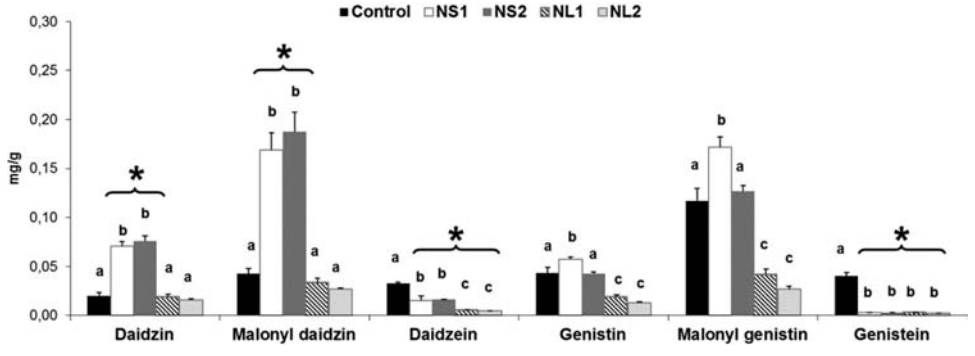

Figure 8. Concentration of each isoflavone species (milligrams per gram) in calli from Osumi cell line, untreated and treated with the two fractions (L and S) at the two assayed concentrations (1 and 2) from N21.4 (labeled N). Data are the average ± standard error (n = 3). Different letters indicate significant differences among treatments; asterisks indicate the most relevant results.

two cell lines from Nestle Research Center showed different isoflavone contents despite having a common origin.[22] Differences occurred not only in amount but also in their chemical forms; interestingly, Osumi showed higher contents of genistein family and malonyl conjugates were most abundant (Figure 2 and Table 2), revealing that storage forms are favored as in the case of the high isoflavone content line (13406) (Figure 2). Based on the differences on the plant material, the response of the three lines to the biotic elicitors was different as expected. Interestingly, elicitors from N21.4 were effective on all three cell lines, confirming the low specificity of this strain. It should be highlighted that the different composition of isoflavone chemical forms (Table 2) among the three lines may result in a benefit for health improvement, since intestinal absorption has a strong individual component, limiting health benefits.[38]

According to the literature, there is a threshold population density to trigger defensive responses in plants.[39] Consistently, not only the type of elicitor but also its concentration affected isoflavone contents in the cell lines. In the low isoflavone content line (13407), both elicitors from N21.4 delivered at low concentration effectively enhanced total isoflavones based on increases in the daidzein family. Among isoflavones, daidzein is considered as a phytoanticipin since it is directly transformed in pterocarpans, the real phytoalexins with a contrasted role on plant defense;[2] supporting these data, other biotic elicitors have shown their ability to increase aucuparin, the major phytoalexin in *Sorbus aucuparia*, in cell cultures.[40] Most phenolic compounds are synthesized as malonyl conjugates and stored in the vacuoles.[41]Therefore, accumulation of malonyldaidzin represents the most effective way to store defense compounds for immediate use upon stress challenge. These results in line 13407 are consistent with the *primed* state described for plants[10,42] but in nondifferentiated cells. Also, the fast accumulation of aglycons from malonyl derivatives upon elicitor application for further transformation on pterocarpans, reported by Barz and Mackenbrock[43] and Farag et al.,[44] supports these findings. This hypothesis holds true also for line 13406 (Figure 5A), in which the elicitor at low dose triggers mobilization of conjugates, resulting in a decrease in total isoflavone,[43] while higher concentrations mimic a stronger stress signal that stimulates de novo synthesis coupled to aglycon increases instead of mobilizing stored reserves (Figure 6A). In view of this, and considering that daidzein is a phytoanticipin, the immediate precursor of phytoalexins, effects of N21.4 metabolic elicitors can be summarized in two stages: First, there is a low-intensity response that results in accumulation of malonyl derivatives, for further mobilization upon stress challenge (*priming*) (NS1 and NL1). Second, a very intense response that may show either a decrease in total isoflavones due to conjugate mobilization (NS2 and NL2, as shown in Figure 5 and 6 artwork or the opposite, an increase in total isoflavones due to stimulation of synthesis de novo coupled with aglycon increase (line 13406, NS2 and NL2 treatments) (Figures 5A and 6A). This hypothesis is also supported by results from line Osumi, which is only responsive to small metabolic elicitors from N21.4 (NS) that also trigger accumulation of conjugated forms, glycosylated and malonylated (Figure 8A). These results are consistent with the different responses triggered by fungal elicitors in *Eschscholzia californica*, leading to accumulation of benzylisoquinolein alkaloids. Roos et al.[45] reported two signaling pathways depending on elicitor concentration, a jasmonate-dependent pathway associated with high elicitor concentrations and a jasmonate-independent pathway triggered by low elicitor concentrations in which the vacuole is directly involved.

These findings evidence the differential sensitivity of the two cell lines to metabolic elicitors of N21.4 and suggest that it may be a receptor-mediated process, based on saturation at high concentration reported in line 13407.[37] Furthermore, it is evidenced that *Pseudomonas fluorescens* N21.4 has at least two different metabolic elicitors, different from the already-reported elicitors for this bacterial species,[46] and supports the notion of the complex defensive networks in plants that are responsive to many different bacterial determinants, constituting a robust mechanism to ensure plant survival.[46,47]

Beyond the physiological changes discussed above, these elicitors may be used as a tool to produce isoflavones for food supplements or food fortification. Increases reported for the low- and intermediate-yield lines are striking compared to that of the high-yield line, in calli. However, the 23.4% increase in the latter represents 0.726 mg/g total isoflavones, overcoming the greater increases in the lower- and intermediate-yield cell lines (29.7% and 64.5%, respectively) that account for 0.205 and 0.487 mg/g total isoflavones, respectively.

In conclusion, the present work has shown that metabolic elicitors from *P. fluorescens* N21.4 are effective bacterial determinants that trigger secondary isoflavone metabolism in soybean cell lines. This strain is able to release at least two metabolic elicitors of different sizes that contribute differently to trigger isoflavone metabolism depending on the cell line. The daidzein pathway is affected in all cell lines and the small elicitors are able to trigger also the genistein pathway only in line 13406, which already had high genistein contents. Therefore, these bacterial determinants from *P. fluorescens* N21.4 can be used to effectively trigger isoflavone metabolism in calli cultures to obtain isoflavones to prepare fortified foods or food supplements and may be further extended to cell cultures for higher yields.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge financial support from AGL2009-08324, AGL2009-13487-C04-04 (Subprograma AGR), CAM (S2009/AMB-1511), and Fundación Universitaria CEU San Pablo. We would like to thanks Nestlé Research Center for kindly provide soybean cell lines.

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