

Upregulation of Isoflavonoids and Soluble Proteins in Edible Legumes by Light and Fungal Elicitor Treatments

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ABSTRACT

Objective: In this study, our working hypothesis was that continuous light and fungal elicitation treatment of legume seedlings would lead to enhanced levels of isoflavonoids and soluble proteins.

Results: Based on short-term light and dark treatments, isoflavonoid (genistein, genistin, daidzein, and daidzin) and soluble protein concentrations were significantly upregulated in the "light" environment compared to the "dark" environment for all edible legume species (kudzu vine, soybean, garbanzo bean, fava bean, mung bean, adzuki bean) that were tested. Kudzu seedlings showed the highest levels of both isoflavonoids and soluble proteins after light-elicited upregulation compared to the other legumes analyzed. All legumes showed less up-regulation of isoflavonoid synthesis when treated with *Phytophthora sojae* fungal elicitor. Oligosaccharide fungal elicitor caused no such upregulation.

Conclusions: The findings in this study show that edible legume seedlings have enhanced levels of isoflavonoids and soluble proteins when they are grown in the light compared to the conventional practice of growing such seedlings in the dark. This will clearly result in significant improvement in their nutritive and medicinal value.

INTRODUCTION

Many legumes in the bean family (Fabaceae) are important sources of isoflavones and soluble dietary protein. Our interest in these compounds derives from the fact that isoflavonoids such as genistein and daidzein are important medicinal compounds (reviewed by Boik, 1996; Duke, 1995). Genistein is a promising anticancer agent that inhibits platelet aggregation, induces apoptosis, inhibits leukotriene production, inhibits DNA topoisomerase II, inhibits angiogenesis, reduces the bioavail-

ability of sex hormones, and induces differentiation in cancer cells (Boik, 1996; Fotsis et al., 1995). Genistein and daidzein both have phytoestrogen activity. Because of their estrogenic activity, these isoflavones are important in the treatment of estrogen-dependent cancers (Kaufman et al., 1997). Daidzein, as well as daidzin, has been shown to inhibit the enzyme, aldehyde dehydrogenase (ALDH-I), and nicotinamideadenine dinucleotide (NAD)-dependent ALDH that catalyzes the oxidation of acetaldehyde, the primary product of alcohol metabolism (Duke, 1995; Keung and Vallee, 1993a,

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1993b). As such, it has been investigated as a treatment for alcoholism.

In the present investigation, we hypothesized that seedlings of selected edible legumes, when provided with continuous illumination, would show increased levels of genistein and daidzein and their glucosyl conjugates, as well as soluble proteins, compared to seedlings grown in the dark. This hypothesis is based on the supposition that light-mediated photosynthetic fixation of carbon dioxide (CO₂) will yield higher levels of isoflavonoids and soluble proteins. Corollary to this hypothesis, we postulated that treatment with fungal (*Phytophthora soja*) cell wall preparations, as well as cell wall oligosaccharides, would have similar upregulating effects. The basis for this reasoning is the fact that when legumes such as soybean (*Glycine max*) are attacked by a fungal pathogen such as *Phytophthora soja*, the legumes increase their synthesis of isoflavonoids through transcriptional upregulation caused by fungal cell wall polysaccharide molecules. In turn, the enhanced levels of genistein in the host plant kill or retard growth of the fungus, and this is the basis for disease resistance in the plant (Dixon et al., 1983).

The primary objective of the present investigation was to determine whether or not continuous exposure to light or transient fungal elicitor treatments could elicit increases in the levels of genistein and daidzein and their glucosyl conjugates as well as soluble proteins in germinating seedlings of selected edible legumes. We show that such treatments do have a positive upregulating effect on synthesis of these isoflavonoids and of soluble proteins in all of the legume seedlings tested, but to the greatest extent, in kudzu seedlings (*Pueraria montana*). Of the two comparisons, light has the greatest upregulating effect. These results have important implications for human nutrition and complementary and alternative medicine.

MATERIALS AND METHODS

Seed sources

Seeds of Japanese Kudzu (*Pueraria montana*) were obtained from Adams-Briscoe Seed Company, Inc., (Jackson, GA). The seeds from this source are treated with a red-colored fungicide

to protect the seedlings from damping-off disease. We strongly advise that seedlings derived from such seeds not be used for human consumption. Seeds of all other legumes tested were obtained from Johnny's Selected Seeds, (Albion, ME); these seeds were not treated with fungicide.

Photoperiod manipulations

Legumes for all experiments were germinated and grown in Bio Set™ seed sprouting units (Fig. 1). Bio Set units were obtained from Johnny's Selected Seeds. Control and experimental environments were simulated in photobioreactors using Precision Scientific Co. Dual Program Illuminated Incubators (Chicago, IL; model no. 818).

To test the effects of light on the synthesis of isoflavonoids and soluble protein in the various legumes, the incubators were programmed in the following manner: control, 16 hours of light and 8 hours of darkness (with 1 hour of light interruption in the middle of the dark period); light, 24 hours of light; dark, 24 hours of darkness. The temperature of the incubators was maintained at 30°C. Light intensity on the Bio Set units was 162 μE·m⁻²·s⁻¹. Seedlings in the Bio Set units were watered twice daily. On emergence of the first true leaves, whole seedlings were harvested, washed with distilled water, and ground into a fine powder using liquid nitrogen. This powder was immediately stored at -80°C prior to extraction and analysis.

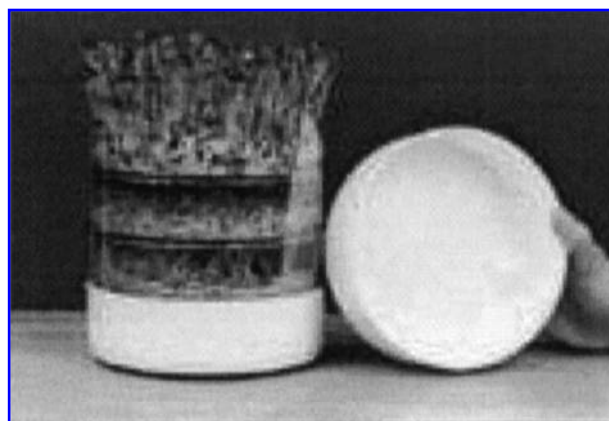


FIG. 1. A Bio Set™ (Johnny's Selected Seeds, Albion, ME) seed sprouting unit.

Fungal elicitor treatments

To test the effects of fungal elicitor on the synthesis of isoflavonoids and soluble protein in the legumes, all legumes were grown in an incubator programmed for 16 hours of light and 8 hours of darkness (with 1 hour of light interruption). Temperature was maintained at 30°C. Light intensity was 162 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Seedlings were watered twice daily. On the emergence of the first true leaves, seedlings were harvested from the Bio Sets and submerged for 30 minutes in either *Phytophthora sojae* or oligosaccharide standard solutions provided by Michael Hahn, B.Sc., M.Sc., Ph.D., Complex Carbohydrate Research Center, USDA, Athens, GA), or distilled water (control). These seedlings were transplanted back to the Bio Sets for continued growth for 4 more days.

After 4 days, whole seedlings for each treatment were harvested, washed with distilled water, and ground into a fine powder using liquid nitrogen. The powder was immediately stored at -80°C prior to extraction, high performance liquid chromatography (HPLC) analysis of isoflavonoids, and spectrophotometric analysis of soluble proteins.

Isoflavonoid extraction

Extraction was carried out as described by Kaufman et al. (Kaufman et al., 1997, 2002). Triplicate samples, 0.5 g each, of the fine powder from each experiment were prepared, two of which were placed in two test tubes containing 4 mL of 80% MeOH. These samples were then vortexed and the supernatant was collected and placed in 15 mL Corex™ centrifuge tubes. This was repeated three times for each test tube. Tubes were then centrifuged for 20 minutes at 27,000g at 22°C in a DuPont Sorvall RC-5 Superspeed refrigerated centrifuge (DuPont Instruments, Newtown, CT). The supernatant was then diluted to a 30% MeOH solution and then run through a Waters Sep-Pak™ Vac C-18 cartridge chromatography column (Waters Corp., Milford, MA). These cartridges were placed on a faucet-type vacuum aspirator apparatus that gently drew the supernatant through the Sep-Pak cartridges. Prior to running extracts through the C-18 cartridges, the cartridges were pre-equilibrated by wash-

ing them with MeOH, first 100%, then 80%, and finally 30%. The samples were then eluted from the cartridges using 100% MeOH. The samples containing the isoflavonoids were then air-dried. Next, 1 mL of 80% MeOH was added to the air-dried test tubes. The tubes were then vortexed, covered with Parafilm™ (Pechiney Plastic Packaging Inc., Neenah, WI) and kept in a refrigerator at 4°C until HPLC analysis.

HPLC analysis

Fifteen microliters (15 μL) of each sample were assayed by reverse phase (ODS) HPLC (using a Vydac C18 column, 4.6 \times 250 mm in size) at 280-nm wavelength using a linear gradient from AcCN:H₂O:TFA (20:79.9:0.1) to MeCN:H₂O:TFA (86:13.9:0.1). Genistein, genistin, daidzein, and daidzin concentrations were determined using standard samples of each. These isoflavonoid standards were obtained from Sigma-Aldrich (St. Louis, MO). These methods are cited in Kaufman et al. (Kaufman et al., 1997).

Protein assay

The soluble proteins were extracted from homogenized sprouts in 80% methanol. After filtration through a 0.45- μm filter, the extracts were ready for soluble protein assay. The protein assay method of Bradford (1976) was used. It is based on the proportional binding of the dye, Coomassie blue, to soluble proteins. Within the linear range of the assay (approximately 5 to 25 $\text{mg}\cdot\text{mL}^{-1}$), the more protein present, the more Coomassie dye binds. The protein concentrations of the experimental samples were determined by comparison to a known protein standard: bovine serum albumin (BSA) 1 $\text{mg}\cdot\text{mL}^{-1}$. Absorbance of soluble proteins were assayed quantitatively at 595 nm with a Shimadzu UV160U UV-Visible recording spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD).

Statistical analysis of data

Experiments were repeated at least three times, and the data were analyzed statistically. All results are given as mean \pm standard deviation (SD). Differences between variables were

tested for significance by Student's *t* test. A *p* value of <0.05 was considered to be significant.

RESULTS

Seedlings of six different taxa of edible legumes were subjected to an alteration of environmental and molecular factors in an attempt to upregulate isoflavonoids, their glycosyl conjugates, and soluble proteins. Analyses were conducted on entire seedlings. Of the samples studied, each showed significant increases of the aforementioned compounds following treatments (equal to or greater than a 30% increase) (Tables 1–6). Of special interest are *Pueraria montana* (kudzu) (Table 1A and 1B) and *Cicer arietinum* (garbanzo bean) (Table 3A and 3B). *P. montana* showed the greatest isoflavonoid increases of 80% and 47% after a change in the environmental and biotic parameters, respectively, while *C. arietinum* demon-

strated the highest increases of soluble proteins, namely, 63% and 90%, respectively.

While the fungal elicitor, *P. sojae*, prompted significant increases (of at least 25%) for both isoflavonoids, their glycosyl derivatives and soluble proteins in all species treated, those seedlings treated with oligosaccharide solution show no significant change in concentrations of either soluble proteins or isoflavonoids (Tables 1B–6B). It is possible that the oligosaccharide molecules were not absorbed by the legumes during the treatment period.

The data in Tables 1–6 clearly show that light exposure increases the concentrations of both isoflavonoids and soluble proteins compared to dark control values. Seedlings left in the dark environment show significant decreases in isoflavonoid and soluble protein levels (averaging –23% and –32%, respectively). These decreases are most likely caused by the absence of photosynthetic carbon-fixation in the dark environment. This is the key process in green

TABLE 1A. ISOFLAVONOID CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN KUDZU (*PUERARIA MONTANA*)

Isoflavonoids	Environmental factors			Biotic factors (fungal elicitor)		
	Control	Light	Dark	Control	<i>P. sojae</i>	Oligosaccharide
Daidzein	906.5 ± 80.2	1,350.3 ± 132.5	800.1 ± 45.2	750.0 ± 25.2	955.3 ± 75.9	744.2 ± 88.1
Genistein	256.1 ± 21.5	432.1 ± 25.2	184.3 ± 7.5	250.6 ± 14.0	302.3 ± 25.0	275.3 ± 35.2
Genistin	140.0 ± 13.3	655.0 ± 80.5	0	175.3 ± 15.1	503.5 ± 11.9	180.5 ± 10.0
Daidzin	222.9 ± 15.9	302.5 ± 20.9	145.3 ± 8.5	211.3 ± 25.3	274.3 ± 38.5	224.1 ± 11.4
Total	1525.5 ± 130.9	2739.9 ± 259.1	1129.7 ± 101.2	1387.2 ± 129.6	2035.4 ± 151.3	1424.1 ± 144.7
Percent of control (%)	100	180	74	100	147	103

^aThree independent experiments, *n* = 3; values are means ± standard deviation (SD).

TABLE 1B. SOLUBLE PROTEIN CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN KUDZU (*PUERARIA MONTANA*)

Control	Environmental factors		Biotic factors (fungal elicitor)		
	Light	Dark	Control	<i>P. sojae</i>	Oligosaccharide
31,007.3 ± 2536.3	40,125.0 ± 2805.1	25,055.3 ± 1555.0	28,055.0 ± 2000.6	35,155.5 ± 2057.1	20,577.8 ± 1853.1
Control (%)	Percent of control (%)	Percent of control (%)	Control (%)	Percent of control (%)	Percent of control (%)
100	130	81	100	125	

^aThree independent experiments, *n* = 3; values are means ± standard deviation (SD).

TABLE 2A. ISOFLAVONOID CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN SOYBEAN (*GLYCINE MAX*)

Isoflavonoids	Environmental factors			Biotic factors (fungal elicitor)		
	Control	Light	Dark	Control	P. sojae	Oligosaccharide
Daidzein	45.3 ± 3.2	55.8 ± 4.4	32.3 ± 2.2	40.1 ± 4.5	48.2 ± 4.1	38.1 ± 3.8
Genistein	55.3 ± 4.1	68.2 ± 5.9	48.6 ± 3.9	44.9 ± 3.3	49.1 ± 8.2	42.6 ± 4.1
Genistin	22.1 ± 1.1	31.6 ± 2.2	17.2 ± 2.7	15.0 ± 0.7	19.6 ± 5.1	16.2 ± 1.9
Daidzin	11.5 ± 0.7	15.3 ± 0.9	6.5 ± 0.4	8.0 ± 0.3	13.3 ± 0.9	7.6 ± 0.5
Total	134.2 ± 9.1	170.9 ± 13.4	104.6 ± 9.2	108.0 ± 8.8	130.2 ± 18.3	104.5 ± 10.3
Percent of control (%)	100	128	78	100	120	96

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 2B. SOLUBLE PROTEIN CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN SOYBEAN (*GLYCINE MAX*)

Control	Environmental factors		Control	Biotic factors (fungal elicitor)	
	Light	Dark		P. sojae	Oligosaccharide
18,055.3 ± 912.3	22,200.3 ± 1001.5	15,509.0 ± 798.6	16,838.3 ± 1205.7	21,555.0 ± 1855.5	17,000.0 ± 1504.3
Control (%)	Percent of control (%)	Percent of control (%)	Control (%)	Percent of control (%)	Percent of control (%)
100	123	86	100	128	101

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 3A. ISOFLAVONOID CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN GARBANZO BEAN (*CICER ARIETINUM*)

Isoflavonoids	Environmental factors			Biotic factors (fungal elicitor)		
	Control	Light	Dark	Control	P. sojae	Oligosaccharide
Daidzein	1.5 ± 0.1	2.5 ± 0.6	0.9 ± 0.2	1.3 ± 0.1	2.3 ± 0.7	0.9 ± 0.1
Genistein	64.2 ± 5.2	88.7 ± 5.5	55.5 ± 4.1	59.0 ± 4.5	73.1 ± 4.9	58.1 ± 5.7
Genistin	30.0 ± 3.0	38.6 ± 3.6	25.1 ± 2.9	26.6 ± 1.9	28.0 ± 1.8	26.3 ± 1.3
Daidzin	0.0	0.0	0.0	0.0	0.0	0.0
Total	95.7 ± 8.3	129.8 ± 9.7	81.5 ± 7.2	86.9 ± 6.5	103.4 ± 7.4	85.3 ± 7.1
Percent of control (%)	100	135	84	100	118	98

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 3B. SOLUBLE PROTEIN CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN GARBANZO BEAN (*CICER ARIETINUM*)

Control	Environmental factors		Control	Biotic factors (fungal elicitor)	
	Light	Dark		P. sojae	Oligosaccharide
14,506.3 ± 1005.3	23,660.0 ± 2125.2	10,502.3 ± 859.7	11,330.1 ± 968.6	21,513.3 ± 2500.5	12,587.9 ± 950.8
Control (%)	Percent of control (%)	Percent of control (%)	Control (%)	Percent of control (%)	Percent of control (%)
100	163	72	100	190	111

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 4A. ISOFLAVONOID CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN FAVA BEAN (*VICIA FABA*)

Isoflavonoids	Environmental factors			Biotic factors (fungal elicitor)		
	Control	Light	Dark	Control	P. sojae	Oligosaccharide
Daidzein	25.2 ± 2.0	36.9 ± 2.6	20.0 ± 1.5	20.1 ± 2.5	32.0 ± 2.6	18.1 ± 1.8
Genistein	31.0 ± 2.0	48.5 ± 3.6	27.6 ± 3.5	25.5 ± 3.4	28.4 ± 2.8	27.3 ± 1.6
Genistin	17.9 ± 1.1	36.7 ± 2.5	8.3 ± 0.6	14.1 ± 0.9	19.7 ± 1.1	13.4 ± 1.7
Daidzin	14.6 ± 2.1	22.1 ± 1.5	7.0 ± 0.4	11.1 ± 0.9	13.3 ± 0.8	9.0 ± 0.9
Total	88.7 ± 7.2	144.2 ± 10.2	62.9 ± 6.0	70.8 ± 7.7	103.4 ± 7.3	67.8 ± 6.0
Percent of control (%)	100	162	71	100	145	96

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 4B. SOLUBLE PROTEIN CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN FAVA BEAN (*VICIA FABA*)

Control	Environmental factors		Control	Biotic factors (fungal elicitor)	
	Light	Dark		P. sojae	Oligosaccharide
26,359.0 ± 2600.2	35,692.1 ± 2561.3	15,360.2 ± 977.4	22,145.3 ± 1501.1	29,365.1 ± 2651.4	24,366.0 ± 1548.7
Control (%)	Percent of control (%)	Percent of control (%)	Control (%)	Percent of control (%)	Percent of control (%)
100	135	58	100	133	110

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 5A. ISOFLAVONOID CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN MUNG BEAN (*PHASEOLUS AUREUS*)

Isoflavonoids	Environmental factors			Biotic factors (fungal elicitor)		
	Control	Light	Dark	Control	P. sojae	Oligosaccharide
Daidzein	36.2 ± 2.5	58.2 ± 4.5	21.6 ± 2.1	30.0 ± 2.3	39.0 ± 3.9	34.1 ± 2.5
Genistein	45.5 ± 3.6	66.3 ± 5.5	40.3 ± 5.6	38.6 ± 2.5	51.0 ± 4.0	42.5 ± 6.6
Genistin	25.6 ± 1.1	36.9 ± 3.4	21.9 ± 2.7	23.7 ± 1.9	26.7 ± 1.5	24.0 ± 5.1
Daidzin	29.7 ± 2.3	41.3 ± 3.6	20.7 ± 1.1	27.5 ± 3.6	38.1 ± 3.6	31.2 ± 2.0
Total	137.0 ± 9.5	202.7 ± 17.0	104.5 ± 11.5	119.8 ± 10.3	154.8 ± 13.0	131.8 ± 16.2
Percent of control (%)	100	148	77	100	129	110

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 5B. SOLUBLE PROTEIN CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN MUNG BEAN (*PHASEOLUS AUREUS*)

Control	Environmental factors		Control	Biotic factors (fungal elicitor)	
	Light	Dark		P. sojae	Oligosaccharide
20,014.3 ± 2012.4	25,390.0 ± 1521.5	14,522.1 ± 895.7	18,225.3 ± 1052.3	23,618.3 ± 2500.6	19,000.3 ± 1689.8
Control (%)	Percent of control (%)	Percent of control (%)	Control (%)	Percent of control (%)	Percent of control (%)
100	127	73	100	130	104

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 6A. ISOFLAVONOID CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN ADZUKI BEAN (*PHASEOLUS ACUTIFOLIUS*)

Isoflavonoids	Environmental factors			Biotic factors (fungal elicitor)		
	Control	Light	Dark	Control	P. sojae	Oligosaccharide
Daidzein	26.3 ± 2.5	45.0 ± 4.1	9.6 ± 1.0	22.0 ± 1.7	26.3 ± 2.4	20.0 ± 1.9
Genistein	56.3 ± 4.2	70.2 ± 6.3	33.2 ± 2.3	44.1 ± 3.6	54.3 ± 3.6	43.2 ± 4.1
Genistin	27.0 ± 1.6	32.1 ± 2.7	16.3 ± 1.3	21.3 ± 1.7	25.0 ± 1.9	19.0 ± 2.3
Daidzin	16.6 ± 1.1	20.0 ± 1.5	0.2 ± 0.1	10.0 ± 0.9	12.0 ± 0.6	9.9 ± 0.8
Total	126.2 ± 9.4	167.1 ± 14.6	59.3 ± 4.6	97.4 ± 7.9	117.6 ± 8.5	92.1 ± 9.1
Percent of control (%)	100	132	47	100	122	95

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

TABLE 6B. SOLUBLE PROTEIN CONCENTRATIONS ($\text{mg} \cdot \text{kg}^{-1}$ DRY WEIGHT BIOMASS)^a IN ADZUKI BEAN (*PHASEOLUS ACUTIFOLIUS*)

Control	Environmental factors		Biotic factors (fungal elicitor)		
	Light	Dark	Control	P. sojae	Oligosaccharide
23,106.4 ± 2333.3	35,390.3 ± 3500.2	11,369.2 ± 1501.1	20,000.3 ± 2085.3	23,505.6 ± 2310.4	18,506.2 ± 1204.7
Control (%)	Percent of control (%)	Percent of control (%)	Control (%)	Percent of control (%)	Percent of control (%)
100	153	50	100	118	93

^aThree independent experiments, $n = 3$; values are means \pm standard deviation (SD).

plants that is responsible for the synthesis of secondary metabolites, such as isoflavonoids, and primary metabolites, such as soluble proteins (Stafford, 1990).

DISCUSSION

Our data and methods have demonstrated that upregulation of levels of isoflavonoids and soluble proteins in leguminous seedlings is not only possible, but also, easy to accomplish by simply germinating the legume seeds in the light rather than in the dark as is usually done. As an alternative to mung bean, soybean, fava bean, adzuki bean, or garbanzo bean sprouts, kudzu sprouts may offer a far better source of genistein and daidzein isoflavonoids (compare Tables 1–6) and soluble proteins. This may be of some importance to vegetarians, and certainly, as a complementary and alternative medicine modality. It is important to stress that roots be included in legume seedlings that are

harvested because they contain the highest levels of isoflavonoids (Kaufman et al., 1997). Furthermore, these leguminous plants are intended to be eaten as sprouts, rather than as fully grown plants.

CONCLUSIONS

In the future, studies should be designed to test the effects of isoflavonoids derived from edible leguminous seedlings on patients in well-designed double-blinded clinical trials. Integrating isoflavonoid-based treatments into mainstream medicine could prove to be highly beneficial to patients, particularly because isoflavonoid therapy is relatively inexpensive compared to the currently prescribed cancer drugs, and there are few adverse side-effects.

In countries where proper medical care is not easily accessible, legume-based diets can be a great asset to a community. Our results have shown that light-elicited upregulation of iso-

flavonoids and soluble proteins is easily accomplished and can significantly improve the protein and isoflavonoid contents of various edible legumes.

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