JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Accumulation of Genistein and Daidzein, Soybean Isoflavones Implicated in Promoting Human Health, Is Significantly Elevated by Irrigation

JOHN O. BENNETT,[†] OLIVER YU,[‡] LARRY G. HEATHERLY,[§] AND HARI B. KRISHNAN*,[†,#]

Department of Agronomy, University of Missouri, Columbia, Missouri 65211; Donald Danforth Plant Science Center, St. Louis, Missouri 63132; Crop Genetics and Production Research Unit, ARS, U.S. Department of Agriculture, Stoneville, Mississippi 38776; and Plant Genetics Research Unit, ARS, U.S. Department of Agriculture, University of Missouri, Columbia, Missouri 65211

To circumvent drought conditions persisting during seed fill in the mid-south U.S. soybean production region, researchers have developed the early soybean (*Glycine max* [L.] Merr.) production system (ESPS), which entails early planting of short-season varieties. Because soybean supplies a preponderance of the world's protein and oil and consumption of soy-based foods has been associated with multiple health benefits, the effects of this agronomic practice on seed quality traits such as protein, oil, and isoflavones should be investigated. Four cultivars of soybean, two from maturity group IV and two from maturity group V, were planted in April (ESPS) and May (traditional) in a two-year study at Stoneville, MS. Near-infrared analysis of soybean seed was utilized to determine the percentages of protein and oil. Dependent upon variety, the oil content of the early-planted crop was increased by 3−8%, whereas protein was not significantly changed. Visualization of protein extracts fractionated by sodium dodecyl sulfate−polyacrylamide electrophoresis and fluorescence two-dimensional difference gel electrophoresis revealed that early planting did not affect the relative accumulation of the major seed-storage proteins; thus, protein composition was equal to that of traditionally cultivated soybeans. Maturity group IV cultivars contained a higher percentage of oil and a lower percentage of protein than did the maturity group V cultivars, regardless of planting date. Gas chromatographic separation of fatty acids revealed that the percentages of saturated and unsaturated fatty acids were not significantly altered by planting date. Methanol extracts of seed harvested from different planting dates when analyzed by high-performance liquid chromatography showed striking differences in isoflavone content. Dependent upon the variety, total isoflavone content was increased as much as 1.3-fold in early-planted soybeans. Irrigation enhanced the isoflavone content of both early- and late-planted soybeans as much as 2.5-fold. Accumulation of individual isoflavones, daidzein and genistein, was also elevated by irrigation. Because this cultural practice improves the quality traits of seeds, ESPS provides an opportunity for enhancing the quality of soybean.

KEYWORDS: ***Glycine*; isoflavone; seed composition**

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) production in the southern regions of the United States is beset by moisture deficit and high temperatures during reproductive stages of plant development. A system of early planting, utilizing soybeans selected for production in northern regions and shorter growing seasons, was developed to alleviate yield loss because of environmental stresses, especially moisture deficit. These earlier-maturing varieties would enter and conclude critical reproductive stages before the onset of nonoptimal soil moisture, thus enhancing the possibility of increased production. The protocol, known as the early soybean production system (ESPS) (*1*−*5*), has been successful in improving yields in irrigated and nonirrigated soybeans in the mid-south U.S. region (*6*).

Maturity groups, growing regions, and yearly weather variation individually and cumulatively affect characteristics of soybean seed in a manner yet to be elucidated (*7*). Studies

* Address correspondence to this author at the USDA-ARS, 108W Curtis Hall, University of Missouri, Columbia, MO 65211 [telephone (573) 882-8151; fax (573) 884-7850; e-mail KrishnanH@missouri.edu].
[†] Department of Agronomy, University of Missouri.
[‡] Donald Danforth Plant Science Center.
[§] Crop Genetics and Production Research Unit, U.S. Department of Agriculture.
[#] Plant Genetics Research Unit, University of Missouri.

10.1021/jf049133k CCC: $27.50
Published on Web 11/18/2004

designed to determine if a correlation existed between temperature and protein and oil accumulation indicated that higher temperatures diminished oil content and increased protein. When protein and oil data from soybeans grown at different latitudes were analyzed, temperature was implicated as a factor in the distribution of these seed storage compounds. Seeds from the northernmost regions of the test were lower in protein and higher in oil than those grown in areas farther south (*8*, *9*). A similar study compared protein and oil of seed originating from diverse soybean-producing regions. Seeds originating from the northern and western areas of the soybean production locales were higher in oil and lower in protein that those from the southern and eastern regions (*10*). The average day and night temperatures, or mean temperature of each region, was found to be more significant in relation to protein and oil accumulation than maximum or minimum temperatures during the seed-fill stage of plant development (*7*, *11*).

Northern and Southern Uniform Tests for 1948−1998 showed average protein content of soybeans varied significantly between early- and late-maturity groups. Plants adapted for shorter growing seasons in the northern regions produced seed lower in protein than those in the southern regions (*12*). In contrast, the difference in oil content was not as obvious between the two extremes in maturity groups (*12*). Although soybeans harvested from maturity zones 1−3 (northernmost) were lower in protein than those from zone 7 (southernmost), a definitive and incremental increase in protein from cooler to warmer climate was not seen. A significant difference in oil content was not evident among the seeds harvested from these diverse maturity zones (*13*, *14*). Because field conditions present a plethora of variables, experiments in controlled environments have been conducted in an attempt to elucidate the factors affecting protein and oil content of soybean. Data from these experiments indicated that the temperature at the onset of and during seed-fill affected protein and oil content (*11*, *15*).

In addition to the major seed-storage compounds, soybeans also contain isoflavones, which serve a variety of biological functions. The principal isoflavones of soybean seeds, daidzein, genistein, and glycitein, are synthesized from the phenylpropanoid pathway and stored as glucosyl- and malonyl-glucosyl conjugates in vacuoles. Accumulation of these compounds in soybean is cultivar-dependent and influenced by environmental conditions during the seed fill (*16*−*20*). Cool temperatures during the onset and duration of seed fill have been shown to increase the isoflavone content of the soybean severalfold (*21*).

Because hectareage of soybeans produced under the ESPS protocol is considerable, the quality traits of the resulting crop should be evaluated. This study was designed to ascertain whether crops grown under the ESPS regimen met or exceeded quality traits expected and to assay the effect of the system on the accumulation of isoflavones. Because a portion of the hectareage in the mid-south is irrigated, we also determined the effect of this practice on protein, oil, and isoflavone accumulation.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Field studies were conducted in 2002 and 2003 at the Delta Research and Extension Center near Stoneville, MS (latitude 33° 26′ N). The pH at the study site ranged from 6.5 to 7.7. Soil tests indicated phosphorus and potassium levels were adequate, thus needing no supplementation (*22*, *23*). Nonirrigated (NI) and irrigated (IRR) experiments were conducted in the same location each year. A randomized complete block design with four replications was used each year. Cultivars were randomly assigned to plots within each of two planting date blocks on both NI and IRR sites in 2002 and remained in the same location for 2003. Two cultivars representing maturity group IV (MG IV), 4403 and 4891, and two from maturity group V (MG V), 5701 and 9594, were chosen for this study. Seeds were treated with mefenoxam [(*R*)-2-{2,6-(dimethylphenyl)-methoxyacetylamino}propionic acid methyl ester] fungicide (Syngenta Corp., Wilmington DE) at 0.11 g of active ingredient per kg of seed prior to seeding each year. Irrigation was applied when soil water potential at the 30-cm depth decreased to approximately −50 kPa. The plots were irrigated through physiological maturity of each cultivar.

Near-Infrared Reflectance Spectroscopy (NIR) of Seed Protein and Oil. An aliquot consisting of approximately 40−50 whole seeds from each replication of each treatment was divided into three portions and assayed for protein and oil content using an Infratech 1255 food and feed analyzer NIR spectrophotometer (Tecator AB Hoganas, Sweden). The average value of three readings was taken as the protein and oil contents of that replication and treatment.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). A 15-mg aliquot of finely ground seed was extracted with 1.0 mL of a solution containing 125 mM Tris-HCl buffer, pH 6.8, 4% sodium dodecyl sulfate (w/v), 20% glycerol (v/v), and 0.03 mM bromophenol blue. After removal of cellular debris by centrifugation (5000*g*, 15 min), the supernatant containing the total seed protein was transferred to new microcentrifuge tubes and combined with 50 μL of 2-mercaptoethanol. Samples were heated in a boiling water bath for 5 min and cooled on ice until used. SDS-PAGE (*24*) was conducted at room temperature on a 13.5% resolving gel (w/v) at 20 mA using a Hoefer SE 260 minigel apparatus (Amersham Bioscience, Piscataway, NJ). Gels were stained overnight with Coomassie Blue R-250. After destaining in 50% methanol/10% glacial acetic acid (v/v), gels were preserved in 10% glacial acid (v/v) prior to visualization. The relative content of seed protein components, separated by SDS-PAGE, was determined by computer-assisted densitometry using the Gene Wizard System (Syngene, Cambridge, U.K.).

Fluorescence 2-D Difference Gel Electrophoresis (DIGE). Dry soybean seed (1 g) was ground to a fine powder and extracted with 2.5 mL of buffer (0.1 M Tris-HCl, pH 8.8, 10 mM EDTA, 0.4% 2-mecaptoethanol, 0.9% sucrose). The suspension was mixed with an equal volume of Tris-HCl-buffered phenol (pH 8.8), stirred for 30 min at 4 °C, and then centrifuged (5000*g*, 10 min, 4 °C). The phenolic phase was decanted into a new tube and the aqueous phase re-extracted with 2.5 mL of buffered phenol. Phenolic phases were combined and proteins precipitated by mixing 5 volumes of 0.1 M ammonium acetate with 100% methanol chilled to −20 °C. After overnight storage at −20 °C, the proteins were precipitated from suspension by centrifugation (20000*g*, 20 min, 4 °C). The protein pellet was washed twice in 0.1 M ammonium acetate in methanol, twice in ice-cold acetone, and finally a single wash in cold 70% ethanol. Prior to electrophoresis, the pellet was dispersed in 1 mL of isoelectric focusing buffer [8 M urea, 2 M thiourea, 2% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 2% Triton X-100, 50 mM dithiothreitol (DTT), 0.5% ampholytes pH 3−10]. A 50 μg aliquot of the protein was labeled with 200 pmol of either Cy3 or Cy5 fluorescent dye and kept on ice for 30 min. The nucleophilic labeling reaction was quenched by adding 10 mM lysine. Fluorescently labeled proteins were combined and applied to immobilized pH gradient (IPG) strips by active rehydration at 50 V for 12 h, at 500 V for 1 h, at 1000 V for 2 h, and at 8000 V for 1 h. Strips were equilibrated for two-dimensional electrophoresis in SDS-PAGE buffer (50 mM Tris-HCl, pH 6.8, 6 M urea, 30% glycerol, 5% SDS) to which 2% DTT was added. Equilibration was repeated, supplementing the buffer with 2.5% iodoacetamide. After a rinse in SDS-PAGE running buffer, the strips were placed on an 11−17% acrylamide gradient gel and overlaid with agarose solution (60 mM, Tris-HCl, pH 6.8, 60 mM SDS, 0.5% agarose, 0.01% bromophenol blue). Following electrophoretic separation, the gels were imaged using the FLA-5000 fluorescent image analyzer (Fuji Photo Film Co., Tokyo, Japan).

Fatty Acid Methyl Ester (FAME) Analysis of Fatty Acid Components of Soybean Oil. An aliquot of three seeds was selected from each replication of treatment. Seed coats were cracked and seeds placed in a test tube containing 1 mL of hexane/chloroform/methanol (8:5:2 v/v/v). After an overnight extraction, 150 μL of the solution

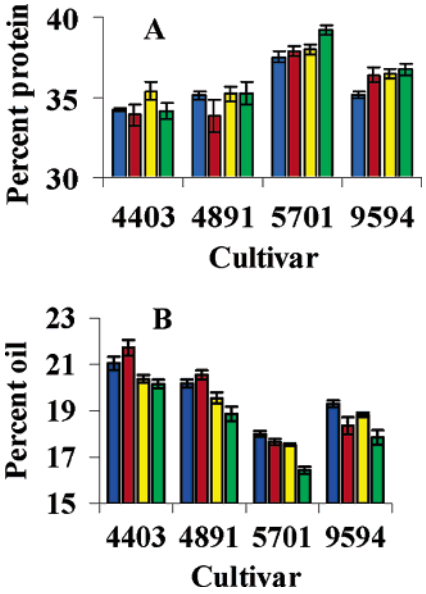

Figure 1. Percent oil and protein as influenced by planting date and irrigation. Analysis of seed by near-infrared spectroscopy was conducted to determine the percentages of seed oil and protein from each cultivar, as a response to planting date and irrigation. Treatments are represented by colored bars: blue, early-planted, irrigated; red, early-planted, nonirrigated; yellow, late-planted, irrigated; green, late-planted, nonirrigated. Standard deviation is indicated by vertical bars in each data set.

was pipetted into a vial containing 75 μL of sodium methoxide−methanol/petroleum ether/ethyl ether solution (1:4:2 v/v/v) for fatty acid methylation. The methyl esters of the fatty acids were separated on a 30 m × 0.5 μm AT-Silar capillary column (Alltech, Deerfield, IL) used in conjunction with an Agilent 6890 gas chromatograph (Agilent, Palo Alto, CA). Fatty acid esters in the eluate were detected by flame ionization. Standards of each esterified fatty acid analyzed, palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) (Matreya, State College, PA), were used for calibration. Each compound was reported as the normalized percentage of the total fatty acid content.

Isoflavone Extraction and Analysis. Approximately 1 g of soybean seed from each cultivar was ground to a fine powder using a General Electric seed grinder (model 5XBG008, New York). The powder was extracted with 4 mL of 80% methanol at 80 °C. After centrifugation (5000*g*, 15 min), the supernatant was filtered using 0.45 μm polytetrafluoroethylene (PTFE) Acrodisc syringe filters (Gelman Laboratory, Portsmouth, U.K.). Samples were analyzed by reverse-phase HPLC on a System Gold high-performance liquid chromatography (HPLC) system (Beckman Coulter, Fullerton, CA) using a Luna C18 (*2*), 5 μm, 4.6 × 150 mm column (Phenomenox, Torrance, CA). Separation and elution were accomplished employing an 18 min linear gradient initiated with 20% methanol/80% 10 mM ammonium acetate (v/v) (pH 5.6) and completed with 100% methanol at a flow rate of 1 mL/min. Detection of the metabolites was accomplished by photodiode array following published procedures (*25*, *26*). Identification and quantification of each isoflavone component were based on available standards (Indofine Chemical Co., Somerville, NJ).

RESULTS

Early Production System and Irrigation Effects on Protein and Oil. Protein and oil are the principal storage compounds of soybean. To ascertain if the ESPS affects the contents of these macromolecules, seed aliquots were collected from field plots and analyzed by NIR spectroscopy. Protein content of soybeans from ESPS was comparable to that of traditionally planted crop (**Figure 1A**). Irrigation of early- and late-planted soybeans did not influence the protein content (**Figure 1A**). Oil

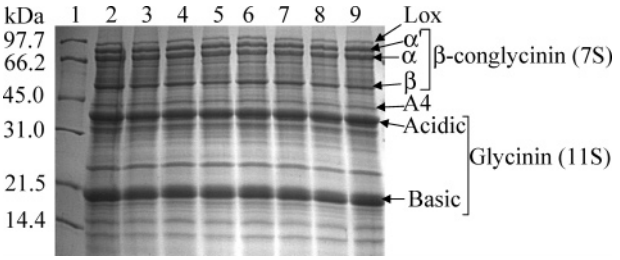

Figure 2. Effect of planting date and irrigation on accumulation of seed proteins. Total seed proteins were fractionated by 13.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and visualized by Coomassie blue: lane 1, molecular weight marker; lanes 2–5, seed proteins of cv. 5701; lanes 6–9, seed proteins of cv. 9594; lanes 2 and 6, early-planted, irrigated; lanes 3 and 7, early-planted, nonirrigated; lanes 4 and 8, late-planted, irrigated; lanes 5 and 9, late-planted, nonirrigated. Molecular masses in kDa are indicated on the left.

content of seed from the MG IV cultivars 4403 and 4891 was increased by 8 and 9%, respectively, in the early-planted crop, whereas MG V cultivar 5701 showed a 7% increase in oil. Irrigation did not significantly alter the oil content of the MG IV cultivars from either planting date, but did affect oil accumulation in the MG V cultivars. Irrigated early- and late-planted cv. 9594 showed a 5% increase in oil, and late-planted cv. 5701 demonstrated a 7% increase in oil (**Figure 1B**). MG V cultivars contained a higher percentage of protein and a lower percentage oil than the MG IV cultivars regardless of treatment (**Figure 1**).

Influence of Early Planting and Irrigation on Seed Protein Composition. Because the total protein content was not affected by planting date and irrigation, the question as to whether the relative abundance of seed storage polypeptides had been affected by the ESPS regimen was addressed. Total seed protein was extracted from an aliquot of seed representing each treatment, separated by SDS-PAGE, and stained with Coomassie blue. Visual observation of the gels revealed no obvious differences in the seed protein profiles. The relative contents of α′- (72 kDa), α- (70 kDa), and β- (52 kDa) subunits of β-conglycinin (7S) were not affected by irrigation or planting date. Acidic (40 kDa) and basic (20 kDa) subunits of glycinin (11S) were found in equal abundance in all treatments. The accrual of lipoxygenase (94 kDa), which is present in significant quantities in soybean, was similar in all treatments (**Figure 2**). Comparison of gels using computer-assisted densitometry revealed similar accretion of the seed storage proteins (data not shown). Because one-dimensional gels indicated that protein profiles did not vary among treatments, more sensitive, two-dimensional fluorescence difference electrophoresis (*27*) was performed to detect subtle variations in protein content (**Figure 3**). Seed proteins from nonirrigated and irrigated plants were labeled with Cy3 and Cy5 fluorescent dyes, respectively. Relative contents of individual proteins were determined by spot colors of the superimposed images. If the resulting color was yellow, the particular protein accumulated equally in the two treatments being compared. A red image spot indicated more protein in seed from nonirrigated plants, whereas a green image spot noted seed protein from irrigated plants was present in greater amount. Mostly, the yellow color was visible in gel image overlays, supporting the premise that planting date and irrigation did not significantly affect protein profile (**Figure 3**). However, a few green fluorescent spots were seen, indicating that accumulation of these proteins was enhanced in seeds from irrigated plants (**Figure 3**). Two prominent green spots with an apparent molecular mass of 52 kDa and isoelectric points

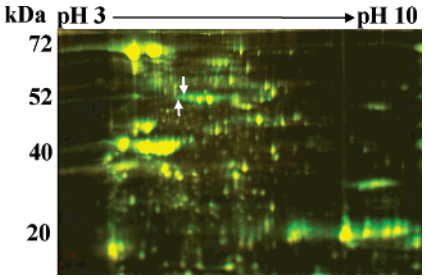

Figure 3. Comparison of seed protein profiles by difference gel electrophoresis (DIGE). Seed proteins from irrigated and nonirrigated plants of soybean cultivar 4403 were labeled with Cy3 and Cy5, respectively, mixed, and then subjected to 2-D DIGE. The gel was scanned at emission wavelengths specific for each dye, and the resulting images were overlaid and visualized using the FLA-5000 laser analyzer. Arrows point to the β-subunit of β-conglycinin.

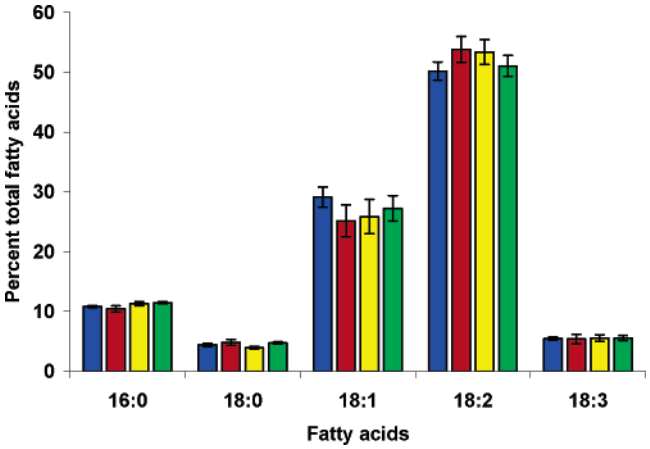

Figure 4. Distribution of fatty acids in seed. Fatty acids were separated and quantitated by gas chromatography, and the amount of each is reported as a percentage of the total. Treatments are represented by colored bars: blue, early-planted, irrigated; red, early-planted, nonirrigated; yellow, late-planted, irrigated; green, late-planted, nonirrigated. Standard deviation is indicated by vertical bars in each data set.

comparable to that of the β-subunit of β-conglycinin were found, suggesting the content of this polypeptide was elevated in the seed from irrigated soybeans (**Figure 3**, shown by arrows).

Fatty Acid Partitioning Affected by Planting Date and Irrigation. Soybean oil contains five fatty acids, palmitic, stearic, oleic, linoleic, and linolenic, which differ considerably in their physical properties. Altering the relative concentrations of these compounds changes the characteristics of the oil, thus making it suitable for specific uses. Oils that contain more oleic and less linoleic and linolenic acids have higher oxidative stability, making them suitable for both food preparation and industrial processes. High linolenic acid content imparts predisposition to oxidation, rendering this soybean oil useful in applications requiring fast-drying oils. The fatty acid components of soybean oils were examined to ascertain whether the ESPS regimen affected the relative contents of these compounds. Quantitative analysis of methylated esters using gas chromatography indicated that the accumulated percentage of fatty acid was consistent; however, subtle differences did occur in the profiles (**Figure 4**). Neither planting date nor irrigation significantly affected the fatty acid contents of MG IV or MG V.

Isoflavone Content Increased by Irrigation. Environmental conditions during the reproductive stages of soybean are known to affect the isoflavone content of the seed. Because plants under the ESPS regimen will enter the reproductive stage at a different

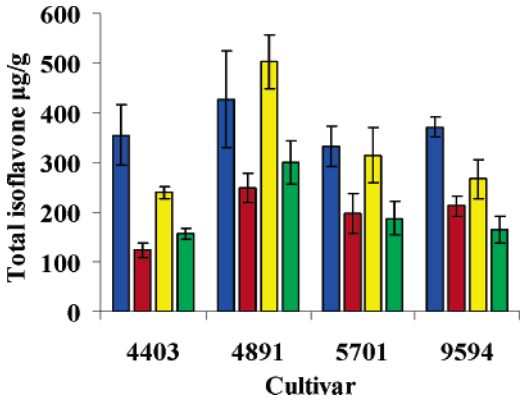

Figure 5. Assimilation of total isoflavone in soybean seed. High-performance liquid chromatography was utilized to determine accrual of isoflavones in seed as affected by planting date and irrigation. Treatments are represented by colored bars: blue, early-planted, irrigated; red, early-planted, nonirrigated; yellow, late-planted, irrigated; green, late-planted, nonirrigated. Standard deviation is indicated by vertical bars in each data set.

time, and ostensibly different environmental conditions, than those planted according to traditional protocol, the effect on isoflavone content was examined. After methanol extraction, isoflavones and their respective conjugates were individually quantified by HPLC and the total metabolite content was determined. Results indicated that the isoflavone contents of the early-planted crop and late-planted crop were comparable. The early planting of MG IV cv. 4403 contained 123 $\pm$ 16 μg/g isoflavone and the late planting, 157 $\pm$ 11 μg/g of the metabolite. Representing the MG V cultivars, early-planted 5701 seed contained 213 $\pm$ 20 μg/g isoflavone, and the late-planted seed contained 193 $\pm$ 63 μg/g isoflavone (**Figure 5**). In contrast, irrigation consistently and significantly increased the seed isoflavone content. When irrigated, seed from cv. 4403 contained 355 $\pm$ 60 μg/g isoflavone, whereas that from nonirrigated plants had 123 $\pm$ 16 μg/g total isoflavone (**Figure 5**). The MG V 9594 plants that were irrigated produced seed containing 370 $\pm$ 20 μg/g isoflavone, wheres seed from nonirrigated plants contained 213 $\pm$ 20 μg/g of the metabolite. Irrigated early-planted MG IV and V cultivars showed 2.8- and 1.7-fold increases in isoflavone content, respectively, whereas the same late-planted cultivars each exhibited a 1.6-fold increase (**Figure 5**). The response of individual compounds, daidzein and genistein, to planting date and irrigation was similar to that of total isoflavone accumulation, with the exception of glycitein, which showed an increase only in the irrigated early-planted MG IV cultivars (**Figure 6**).

DISCUSSION

Epidemiological studies have shown a reduced risk of cancer, heart disease, and other chronic illnesses in populations that consume soybeans and soy products (*28*). Studies have indicated the isoflavones may be partially responsible for the health benefits associated with soybean consumption (*29*, *30*). Results from this study asserted that irrigation dramatically increased the isoflavone content of soybean seed. The mechanism by which this increase was facilitated is unknown. In soybean, isoflavones are principally found in roots and seeds; however, the metabolite has also been isolated from leaf and stem tissue (*31*, *32*). Ostensibly, the increase in isoflavone content, as the result of irrigation, could arise solely from increased synthesis in the seed components or possibly be translocated from distal production sites. Previous papers suggest flavanoids that ac-

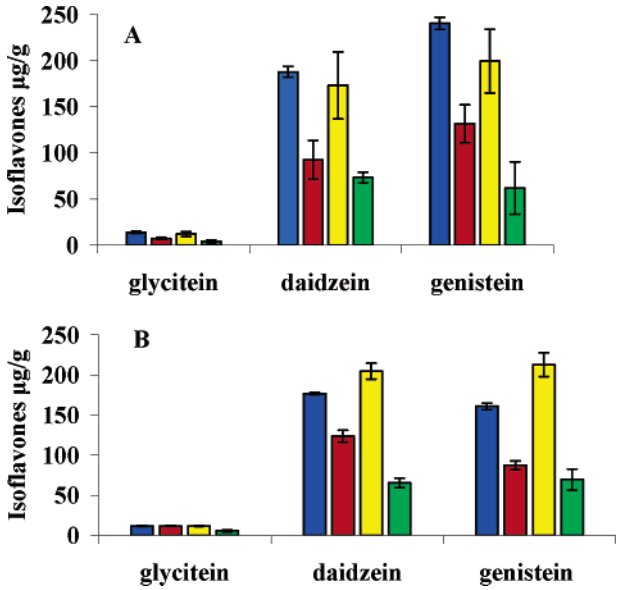

Figure 6. Accumulation of isoflavone components as influenced by planting date and irrigation. Isoflavones glycitein, daidzein, and genistein were separated and quantitated by high-performance liquid chromatography using designated standards. Depicted in (**A**) are the isoflavone contents of MG IV cv. 4403 and in (**B**), those of MG V cv. 9594. Treatments are represented by colored bars: blue, early-planted, irrigated; red, early-planted, nonirrigated; yellow, late-planted, irrigated; green, late-planted, nonirrigated. Standard deviation is indicated by vertical bars in each data set.

cumulate after ultraviolet irradiation and pathogenic induction are produced by the cells directly exposed to the environmental stimuli rather than being transported from cells of other tissues (*33*, *34*). However, recent work suggests that although seeds are the principal site of isoflavone synthesis, some accumulation is due to transport from other plant organs including maternal tissues (*35*). In the seed tissue, isoflavone synthase has been shown to be expressed only in embryos and seed coats, and not in the developing cotyledons, suggesting the majority of the isoflavones in the cotyledons are transported from other tissues (*36*, *37*). Translocation of glucosinolate from leaf to seed in *Brassica napus* (L.) (*38*, *39*) and similar transport of plant alkaloids provide a precedent for movement of the isoflavones through the vascular system. Soybean-pod exudates collected from the juncture of the marginal veins have been found to contain isoflavones, indicating putative vascular transport. In addition, soybean embryos have been shown in vitro to assimilate exogenous isoflavones (*35*), thus demonstrating the possibility of movement from source to sink tissue. Clearly, the mechanisms underlying the effects of irrigation on isoflavone accumulation require further investigation.

Results presented in this study indicate that protein and oil contents of ESPS-produced soybeans are comparable or enhanced with respect to a crop grown under the traditional regimen. Although the protein content of MG IV and MG V cultivars did not vary significantly with planting date, oil accumulation was higher in early-planted MG IV cultivars. Field studies have shown that oil content is affected by temperature during seed fill, but protein levels do not correlate with temperature during this period (*7*). Because the temperatures during seed fill of early- and late-planted crops in this two-year study were similar (*40*), the increased content of oil in MG IV cultivars could be attributed to genetic makeup of these cultivars (*41*) as both MG IV cultivars had higher oil content than the MG V cultivars regardless of treatment. Irrigation in the mid-south region is a management practice that can be utilized where water resources are readily available. Previous studies have shown protein and oil accumulation do not respond to irrigation (*42*, *43*). Results from our study are consistent with the previous work with respect to protein, but irrigation did increase the oil content in late-planted MG IV and early- and late-planted MG V cultivars. Alleviation of possible moisture deficit during the reproductive stages is a possible reason for the increase in oil content. Planting dates and irrigation appeared to have a subtle effect on fatty acid composition in the current study. For example, an increase or decrease in oleic acid content was countered by a reciprocal change in polyunsaturated fatty acids. This could be related to changes in the activity of the desaturase enzyme, which is known to be influenced by both temperature (*44*) and light quality (*45*).

Soybeans planted according to the ESPS have shown a significant yield advantage in the mid-south growing region (*2*). In this study, we demonstrate that protein and oil contents of the crop are comparable to those of soybeans grown under traditional cultural practices. When irrigated, both ESPS and traditionally planted soybeans consistently reveal a minimum 1.5-fold increase in isoflavone content. Because isoflavone consumption has been attributed to reduced incidence of certain types of cancer and other chronic illness, producing soybean cultivars that accumulate higher isoflavone levels under a variety of growing conditions is desirable. Determination of the biochemical means by which irrigation improves isoflavone content could aid in the development of cultivars that have an enhanced and uniform content of the metabolite.

ACKNOWLEDGMENT

We thank Dr. Larry Darrah for critical reading of the manuscript, Dr. Michiyo Matsuno and Laura Walker of the Danforth Center and Dr. Brian Mooney, University of Missouri Protein Core Facility, for their technical assistance.

LITERATURE CITED

(1) Bowers, G. An early soybean production system for drought avoidance. *J. Prod. Agric.* **1995**, *8*, 112−119.
(2) Heatherly, L.; Spurlock, S. Yield and economics of traditional and early soybean production system (ESPS) seedings in the midsouthern United States. *Field Crops Res.* **1999**, *63*, 35−45.
(3) Heatherly, L. Early soybean production system (ESPS). In *Soybean Production in the Mid-south*; Heatherly, L., Hodges, H., Eds.; CRC Press: Boca Raton, FL, 1999; pp 103−118.
(4) Popp, M.; Keisling, T.; McNew, R.; Oliver, L.; Dillon, C.; Wallace, D. Planting date, cultivar, and tillage system effects on dryland soybean production. *Agron. J.* **2002**, *94*, 81−88.
(5) Kane, M.; Steele, C.; Grabau, L.; Mackown, C.; Hildebrand, D. Early-maturing soybean cropping system: Protein and oil content and oil composition. *Agron. J.* **1997**, *89*, 464−469.
(6) Heatherly, L. Soybean irrigation. In *Soybean Production in the Mid-south*; Heatherly, L., Hodges, H., Eds.; CRC Press: Boca Raton, FL, 1999; pp 119−142.
(7) Piper, E.; Boote, K. Temperature and cultivar effects on soybean seed oil and protein concentrations. *J. Am. Oil Chem. Soc.* **1999**, *76*, 1233−1241.
(8) Cherry, J.; Bishop, L.; Hasegawa, P.; Leffler, H. Differences in the fatty acid composition of soybean (*Glycine-max*) seed produced in northern and southern areas of the USA. *Phytochemistry* **1985**, *24*, 237−242.
(9) Breene, W.; Lin, S.; Hardman, L.; Orf, J. Protein and oil content of soybeans from different geographic locations. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1927−1931.
(10) Hurburgh, C.; Brumm, T.; Guinn, J.; Hartwig, R. Protein and oil patterns in USA and world soybean markets. *J. Am. Oil Chem. Soc.* **1990**, *67*, 966−973.

(11) Gibson, L.; Mullen, R. Soybean seed composition under high day and night growth temperatures. *J. Am. Oil Chem. Soc.* **1996**, *73*, 733−737.
(12) Yaklich, R.; Vinyard, B.; Camp, M.; Douglass, S. Analysis of seed protein and oil from soybean Northern and Southern Region Uniform Tests. *Crop Sci.* **2002**, *42*, 1504−1515.
(13) Grieshop, C.; Fahey, G. Comparison of quality characteristics of soybeans from Brazil, China and the United States. *J. Agric. Food Chem.* **2001**, *49*, 2669−2673.
(14) Grieshop, C.; Kadzere, C.; Clapper, G.; Flickinger, E.; Bauer, L.; Frazier, R.; Fahey, G. Chemical and nutritional characteristics of United States soybeans and soybean meals. *J. Agric. Food Chem.* **2003**, *51*, 7684−7691.
(15) Wolf, R.; Cavins, J.; Kleiman, R.; Black, L. Effect of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acids and sugars. *J. Am. Oil Chem. Soc.* **1982**, *59*, 230−232.
(16) Eldridge, A.; Kwolek, W. Soybean isoflavones effect of environment and variety on composition. *J. Agric. Food Chem.* **1983**, *31*, 394−396.
(17) Kudou, S.; Fleury, Y.; Welt, D.; Magnolato, D.; Uchida, T.; Kitamura, K. Malonyl isoflavone glycosides in soybeans seeds *Glycine max* (Merrill.). *Agric. Biol. Chem.* **1991**, *55*, 2227−2233.
(18) Wang, H.; Murphy, P. Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. *J. Agric. Food Chem.* **1994**, *42*, 1674−1677.
(19) Hoeck, J.; Fehr, W.; Murphy, P.; Welke, G. Influence of genotype and environment on isoflavone contents of soybean. *Crop Sci.* **2000**, *40*, 48−51.
(20) Lee, S.; Ahn, J.; Kim, S.; Kim, J.; Han, S.; Jung, M.; Chung, I. Variation in isoflavone of soybean cultivars with location and storage duration. *J. Agric. Food Chem.* **2003**, *51*, 3382−3389.
(21) Tsukamoto, C.; Shimada, S.; Igita, K.; Kudou, S.; Kokubun, M.; Okubo, K.; Kitamura, K. Factors affecting isoflavone content in soybean seeds: Changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. *J. Agric. Food Chem.* **1995**, *43*, 1184−1192.
(22) Varco, J. Nutrition and fertility requirements. In *Soybean Production in the Mid-south*; Heatherly, L., Hodges, H., Eds.; CRC Press: Boca Raton, FL, 1999; pp 53−70.
(23) Heatherly, L.; Elmore, R. Managing inputs for peak production. In *Soybean Improvement, Production, and Uses*, 3rd ed.; Specht, J., Boerma, R., Eds.; Agronomy Monograph 16; American Society of Agronomy: Madison, WI, 2004; pp 451−536.
(24) Laemmali, U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.
(25) Yu, O.; Jung, W.; Shi, J.; Crose, R.; Fader, G.; McGonigle, B.; Odell, J. Production of the isoflavones genistein and daidzein in non-legume dicot and monocot tissues. Isoflavone accumulation is related to activity of the phenylpropanoid pathway. *Plant Physiol.* **2000**, *124*, 781−793.
(26) Yu, O.; Shi, J.; Hession, A.; Maxwell, C.; McGonigle, B.; Odell, J. Metabolic engineering to increase isoflavone biosynthesis in soybean seed. *Phytochemistry* **2003**, *63*, 753−763.
(27) Unlu, M.; Morgan, M.; Minden, J. Difference gel electrophoresis: A single gel method for detecting changes in protein extracts. *Electrophoresis* **1997**, *18*, 2071−2077.
(28) Cassidy, A.; Faughnan, M. Phyto-oestrogens through the life cycle. *Proc. Nutr. Soc.* **2000**, *59*, 489−496.
(29) Messina, M. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* **1999**, *70*, 439S−450S.
(30) Watanabe, F.; Uesugi, S.; Kikuchi, Y. Isoflavones for prevention of cancer, cardiovascular diseases, gynecological problems and possible immune potentiation. *Biomed. Pharmacother.* **2002**, *56*, 302−312.
(31) Ho, H.; Chen, R.; Leung, L.; Chan, F.; Huang, Y.; Chen, Z. Difference in flavanoid and isoflavone profile between soybean and soy leaf. *Biomed. Pharmacother.* **2002**, *56*, 457−461.
(32) Romani, A.; Vignolini, P.; Galardi, C.; Aroldi, C.; Vazzana, C.; Heimler, D. Polyphenolic content in different plant parts of soy cultivars grown under natural conditions. *J. Agric. Food Chem.* **2003**, *51*, 5301−5306.
(33) Asthana, A.; McCloud, E.; Berenbaum, M.; Tuveson, R. Phototoxicity of Citrus jambhiri to fungi under enhanced UV-B radiation: Role of furanocoumarins. *J. Chem. Ecol.* **1993**, *19*, 2813−2830.
(34) Siegrist, J.; Muhlenbeck, S.; Buchenauer, H. Cultured parsley cells, a model system for the rapid testing of abiotic and natural substances as inducers of systemic acquired resistance. *Physiol. Mol. Plant Pathol.* **1998**, *53*, 223−238.
(35) Dhaubhadel, S.; McGarvey, B.; Williams, R.; Gijzen, M. Isoflavanoid biosynthesis and accumulation in developing soybean seeds. *Plant Mol. Biol.* **2004**, *53*, 733−743.
(36) Jung, W.; Yu, O.; Lau, S.; O'Keefe, D.; Odell, J.; Fader, G.; McGonigle, B. Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes. *Nat. Biotechnol.* **2000**, *18*, 208−212.
(37) Subramanian, S.; Xu, L.; Lu, G.; Odell, J.; Yu, O. The promoters of isoflavone synthase genes respond differentially to nodulation and defense signals in transgenic soybean roots. *Plant Mol. Biol.* **2004**, *54*, 623−639.
(38) Gijzen, M.; McGregor, I.; Seguin-Swart, G. Glucosinolate uptake by developing rapeseed embryos. *Plant Physiol.* **1989**, *89*, 260−263.
(39) Chen, S.; Halkier, B. Characterization of glucosinolate uptake by leaf protoplasts of *Brassica napus*. *J. Biol. Chem.* **2000**, *275*, 22955−22960.
(40) Carle, R.; Ranney, C.; Shanklin, R. Weather data summary for 1964−1993 Stoneville, MS. *MAFES Tech. Bull. 201*; 1995.
(41) Miller, J.; Fehr, W. Direct and indirect recurrent selection for protein in soybeans. *Crop Sci.* **1979**, *19*, 101−106.
(42) Matheny, T.; Hunt, P. Effects of irrigation and sulfur application on soybeans *Glycine max.* cultivar Bragg grown on a Norfolk loamy sand typic paleudult. *Commun. Soil Sci. Plant Anal.* **1981**, *12*, 147−160.
(43) Sweeney, D.; Long, J.; Kirkham, M. A single irrigation to improve early maturing soybean yield and quality. *Soil Sci. Soc. Am. J.* **2003**, *67*, 235−240.
(44) Cheesbrough, T. Changes in the enzymes for fatty acid synthesis and desaturation during acclimation of developing soybean seeds to altered growth temperature. *Plant Physiol.* **1989**, *90*, 760−764.
(45) Britz, S.; Cavins, J. Spectral quality during pod development modulates soybean seed fatty acid desaturation. *Plant. Cell Environ.* **1993**, *16*, 719−725.

Received for review May 28, 2004. Accepted October 5, 2004. Product names are necessary to report factually on available data; however, the University of Missouri and the USDA neither guarantee nor warrant the standard of product, and the use of the name by the University of Missouri or the USDA implies no approval of the product to the exclusion of others that may be suitable.

JF049133K