Food Chemistry 211 (2016) 347–355

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Metabolite changes in nine different soybean varieties grown under field and greenhouse conditions

K.M. Maria John [a], Savithiry Natarajan [b,**], Devanand L. Luthria [a,*]

[a] Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705, United States
[b] Soybean Genomics and Improvement Laboratory, Plant Sciences Institute, ARS, USDA, Beltsville, MD 20705, United States

ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form 30 March 2016
Accepted 10 May 2016
Available online 10 May 2016

Keywords:
Cultivars
Greenhouse
Metabolites
Multivariate analyses
Soybean

ABSTRACT

Global food security remains a worldwide concern due to changing climate, increasing population, and reduced agriculture acreages. Greenhouse cultivation increases productivity by extending growing seasons, reducing pest infestations and providing protection against short term drastic weather fluctuations like frost, heat, rain, and wind. In the present study, we examined and compared the metabolic responses of nine soybean varieties grown under field and greenhouse conditions. Extracts were assayed by GC-FID, GC-MS, and LC-MS for the identification of 10 primary (amino acids, organic acids, and sugars) and 10 secondary (isoflavones, fatty acid methyl esters) metabolites. Sugar molecules (glucose, sucrose, and pinitol) and isoflavone aglycons were increased but the isoflavones glucoside content decreased in the greenhouse cultivated soybeans. The amino acids and organic acids varied between the varieties. The results show that clustering (PCA and PLS-DA) patterns of soybean metabolites were significantly influenced by the genetic variation and growing conditions.

1. Introduction

Soybeans are one of the major foods consumed in many Asian countries. Soybeans are an excellent source of protein, essential fatty acids, carbohydrates, numerous vitamins, minerals, isoflavones, and fiber. They provide the world's largest source of animal protein feed and the second largest source of vegetable oil. The United States is the leading soybean producer and exporter. Soybean seeds contain high levels of isoflavones exhibiting a wide array of bioactivities such as minimization of postmenopausal symptoms (Messina, 1999; Nakatsu et al., 2014; Pusparini, Yenny, & Hidayat, 2015; Wang & Murphy, 1994).

Soybean based foods such as soy-milk, tofu, soy sauce, soy sprouts, and oil are commonly consumed worldwide. Awareness of the health benefits of soybeans has resulted in an increased demand for soy based food products. Besides their use as food and feed, soybeans have also been recently used in the development of commercial products such as plastics, lubricants, and adhesives (United Soybean Board, 2015). Due to the increased demand for soybeans and their products, there is a distinct need to increase production and improve soybean quality.

Bioactive isoflavones exist in free and conjugated (glucoside, acetyl and malonyl esters) forms in soybeans. Isoflavone glucosides have poor absorbability in the human intestine compared to their corresponding aglycons (Izumi et al., 2000). In fermented soy foods, significant conversion of glucosides to aglycons occurs, hence their absorbability is improved (Baek et al., 2010; Fan, Zhang, Chang, Saito, & Li, 2009; Kang et al., 2011; Kim et al., 2011; Lee et al., 2012; Park et al., 2010; Wu, Wang, Sciarappa, & Simon, 2004). This has resulted in the development of several soybean fermented products like miso, natto, pickled tofu, soy sauce, and doenjang (Jeon, Seo, Shin, & Lee, 2012; Lee, Lee, Jung, & Lee, 2013; Shin & Lee, 2013).

Due to the high economic importance of soybean, a wide array of new varieties are continuously being introduced into the global market (Ha et al., 2013). In addition, the influence of environmental conditions on nutrient profiles are being investigated by researchers across the globe. Greenhouses and other controlled environments have been investigated to improve crop yield, reduce pest damages, extended growing seasons, and provide protections against short term drastic weather fluctuations like frost, heat, rain, and wind. In addition, greenhouses have also been used to study the effect of climate change. Crops like tomatoes (Choi et al., 2014), strawberries (Gündüz & Ozdemir, 2014), and peppers

* Corresponding author at: Food Composition Methods Development Lab, Bldg. 161, Lab 202, BARC (E), 10300 Baltimore Avenue, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705, United States (D.L. Luthria).
** Co-corresponding author.
E-mail address: Dave.Luthria@ars.usda.gov (D.L. Luthria).

http://dx.doi.org/10.1016/j.foodchem.2016.05.055
0308-8146/

(Keyhaninejad, Richins, & O'Connell, 2012) were reported to have improved yield and nutrient content under greenhouse conditions. Whereas in soybeans, the field and greenhouse conditions were studied only for their drought tolerance, disease, and toxicology studies (Dann, Diers, Byrum, & Hammerschmidt, 1998; de Paiva Rolla et al., 2014; Pfleeger, Olszyk, Lee, & Plocher, 2011). However, there is limited research available on understanding changes in the phytochemical content in soybeans grown under greenhouse as compared to the ambient field conditions. Furthermore, only limited varieties have been used in previous studies.

Plant metabolic profiles are directly influenced by genetics, physiological conditions, and the analytical methodologies used. The objective of the current research was to carry out a systematic comparison of the primary and secondary metabolic profiles of nine soybean varieties grown in the field and greenhouse conditions. Furthermore, we investigated application of multivariate analysis to differentiate soybean varieties grown under two conditions (field vs greenhouse).

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade chemicals (methanol, and acetonitrile) were purchased from Burdick and Jackson (Muskegon, MI, USA). Derivatizing agents methoxyamine hydrochloride, pyridine, and N,O-*bis* (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) were purchased from Sigma Aldrich (St. Louis, MO, USA). Individual standards for the primary and secondary metabolites were purchased from Sigma Aldrich (St. Louis, MO, USA) and Nu-Chek Prep, Inc. (Elysian, MN, USA).

2.2. Soybean samples grown in field and greenhouse

Nine different soybean varieties (PI#549032, #549046, #157434, #548488, #88788, #555397, #636694, #632431, #639740) from United States Department of Agriculture (USDA) germplasm collection were selected for this study. Detailed information for the nine varieties (all soybean genotypes including wild type) is presented in Table 1. The varieties were cultivated under two distinct conditions, field (FI) and greenhouse (GH), during the period of June and July, 2014 at USDA facility in Beltsville, Maryland. For field experiments, the soybean seeds were planted using a randomized complete block design with three replications (hillplots were spaced at 91 cm between hills in rows spaced from 61 to 76 cm with five seeds per hill). Seeds were harvested at maturity from each hillplot. The average temperature during the growing season in the field was 71 °F with natural photoperiod. For greenhouse experiments, the photoperiod (grown in 16 h light/8 h dark for the first 2 months, then 12 h light/12 h dark to initiate flowering) and temperature (72 °F) conditions were maintained as similar as possible to those in the field by computer control. Five seeds of each of the genotypes were planted in individual 6-quart pots that were filled with moistened Potting Mix (Canadian sphagnum moss, perlite, vermiculite, dolomitic limestone, starter fertilizer, trace elements and a wetting agent). Soil from the field was mixed with the potting mix to provide *Bradyrhizobium japonicum* inoculation similar to that present in the field to permit normal nodulation and nitrogen fixation. After a month, Osmocote 14-14-14 solid fertilizer was applied. During second month, Bloom Booster fertilizer was applied to enhance the reproductive stages (flowering and pod formation). The pods were collected at full maturity (R9) and stored at 4 °C until analyzed.

2.3. Sample preparation for primary metabolite analysis

Ground soybean samples (100 mg) collected from the field and greenhouse were individually extracted with 1 ml of methanol:water:chloroform (2.5:1:1, v:v:v) using ultrasonic assisted extraction for 10 min. The extracts were centrifuged at 10,000 rpm for a period of 10 min and the supernatants were collected. This extraction procedure was repeated two additional times and the collected supernatants were pooled and evaporated to dryness using a speedvac. The resulting pellet was derivatized as follows: samples were treated with 200 µl of methoxyamine hydrochloride in pyridine (20 mg/ml) followed by a 90-min incubation at 35 °C with methyloximation. N,O-*bis* (trimethylsilyl) trifluoroacetamide (BSTFA, 100 µl) containing 1% trimethylchlorosilane (TMCS), and then incubated at 40 °C for 30 min to accelerate the rate of the silylation reaction (Maria John, Jung, Lee, Kim, & Lee, 2013). Two replicate samples were collected for each cultivar under both growing conditions (greenhouse and field). Three extractions and analyses were carried out for each soybean sample, resulting in six analyses for each variety under a single growing condition.

2.4. Primary metabolite analysis by gas chromatography–mass spectrometry (GC-MS)

Analysis of samples was performed using an Agilent GC-MS system (7890A) with an autosampler (Agilent 7693) equipped with a HP-5MS capillary column (30 m length × 0.25 mm i.d. × 0.25 µM film thickness-Agilent J&W GC column). The injector temperature was 250 °C, and the injection volume was 1 µl. The oven temperature was programmed as follows: 80 °C for 2 min, then ramped to 300 °C at a rate of 10 °C/min, and held at 300 °C for 3 min. The transfer line temperature was set at 250 °C. The ionization potential was set at −70 V (electron energy) with a source temperature of 200 °C. The detector voltage was 1450 V and the mass range was set at 50–600 *m*/*z* with an acquisition rate of 10 spectra per second. Two replicate samples were collected for each variety under both growing conditions (greenhouse and field). Three extractions and analyses were carried out for each soybean sample, resulting in six analyses for each variety under a single growing condition.

Table 1
Soybean cultivars analyzed for amino acids, fatty acids, and isoflavones.

S.No	USDA accession #	Genotypes	Origin/information	Maturity group	Field grown	Greenhouse grown
1	PI# 549032	Wild soybean (*G.soja*)	China	III	FI 1	GH 1
2	PI# 549046	Wild soybean (*G.soja*)	China	IV	FI 2	GH 2
3	PI# 157434	Soybean Landraces	S. Korea	IV	FI 3	GH 3
4	PI# 548488	Soybean Landraces	Heilongjiang, China	V	FI 4	GH 4
5	PI# 88788	Soybean Landraces	China	III	FI 5	GH 5
6	PI# 555397	Soybean Bred for Seed Traits	Maryland, USA	IVS	FI 6	GH 6
7	PI# 636694	Soybean Bred for Seed Traits	Maryland, USA	IVS	FI 7	GH 7
8	PI# 632431	Soybean Bred for Seed Traits	Ohio, USA	IV	FI 8	GH 8
9	PI# 639740	Soybean Bred for Seed Traits	Illinois, USA	IV	FI 9	GH 9