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Changes occurring in compositions and antioxidant properties of healthy soybean seeds [*Glycine max* (L.) Merr.] and soybean seeds diseased by *Phomopsis longicolla* and *Cercospora kikuchii* fungal pathogens

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ABSTRACT

Changes in the compositions (isoflavone, protein, oil, and fatty acid) and antioxidant properties were evaluated in healthy soybeans and soybeans diseased by *Phomopsis longicolla* and *Cercospora kikuchii*. The total isoflavone content (1491.3 μg/g) of healthy seeds was observed to be considerably different than that of diseased seeds (*P. longicolla*: 292.6, *C. kikuchii*: 727.2 μg/g), with malonlygenistin exhibiting the greatest decrease (726.1 → 57.1, 351.9 μg/g). Significantly, three isoflavones exhibited a slight increase, and their structures were confirmed as daidzein, glycitein, and genistein, based on their molecular ions at *m/z* 253.1, 283.0, and 269.1 using the negative mode of HPLC-DAD-ESI/MS. The remaining compositions showed slight variations. The effects against 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radicals in healthy seeds were stronger than the diseased soybeans, depending upon the isoflavone level. Our results may be useful in evaluating the relationship between composition and antioxidant activity as a result of changes caused by soybean fungal pathogens.

1. Introduction

In recent years, the consumption of phytochemical-rich foods, including crops, fruits, vegetables, and edible plants, has been associated with the prevention of various chronic diseases (Amin, Kucuk, Khuri, & Shin, 2009; Salma Khanam, Oba, Yanase, & Murakami, 2012). Among numerous sources, the soybean [*Glycine max* (L.) Merr.] is one of the most important crops because of its beneficial effects on human health, including the prevention of cancer, coronary heart disease, and osteoporosis and its antioxidant properties (Kumar, Rani, Dixit, Pratap, & Bhatnagar, 2010; Scheiber, Liu, Subbiah, Rebar, & Setchell, 2001). Moreover, this species has been widely used in food and industrial applications, due to its high protein and oil concentrations. Many studies have described that the pharmacological activities of soybean are related to its contents of phytochemicals (isoflavone, anthocyanin, phenolic acid, and saponin), protein, and oil (Astadi, Astuti, Santoso, & Nugraheni, 2009; Kalogeropoulos et al., 2010). Specifically, the major phytochemicals, isoflavones, play essential roles in preventing human diseases, due to their antiatherosclerotic, antioxidant,

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and anticancer properties (Antony, Clarkson, Hughes, Morgan, & Burke, 1996). It is well-established that soybean isoflavones have aglycone (daidzein, genistein, and glycitein), glucoside, glucoside malonate, and glucoside acetylate groups (Cho et al., 2013). The content and distribution of twelve individual isoflavones in four forms exhibit remarkable differences based on the cultivar, geographic region, and environmental factors (Hoeck, Fehr, Murphy, & Welke, 2000; Lee, Yan, Ahn, & Chung, 2003). We have recently reported that the isoflavone contents differed significantly according to the storage condition and soybean seed coat colour (Cho et al., 2013; Lee & Cho, 2012). Protein, oil, and fatty acid are also considered valuable precious nutritional components owing to their potential health-promoting effects (Lin, Meijer, Vermeer, & Trautwein, 2004). Based on the above background, researchers have persistently focused on this species for the development of valuable dietary supplements, functional foods, and pharmaceuticals.

Soybean is influenced by various factors including planting date, irrigation, year, germination, harvest time, and environment effects (Mengistu & Heatherly, 2006; Mengistu, Smith, Bellaloui, Paris, & Wrather, 2010). Unfortunately, this crop is usually attacked by fungal infections during cultivation, and post-harvest (Mengistu & Heatherly, 2006; Svetaz et al., 2004; Upchurch & Ramirez, 2010). Of the various pathogens, Phomopsis seed infection and purple seed stain were the cause of the most serious soybean diseases due to the reduction of the quality and yield of seeds (Pioli, Benavídez, Morandi, & Bodrero, 2000). These above symptoms are well known as infections of *Phomopsis longicolla* and *Cercospora kikuchii* pathogens (Mengistu & Heatherly, 2006; Upchurch & Ramirez, 2010). Soybean seeds infected by *P. longicolla* appear shriveled, elongated, cracked, and often chalky white and seeds infected by *C. kikuchii* are discoloured with pink, pale purple, and dark purple (Chen, Lyda, & Halliwell, 1979; Kulik & Sinclair, 1999; Spilker, Schmitthenner, & Ellett, 1981). In particular, it is well documented that *P. longicolla* was the major fungal pathogen species with the highest isolation in pods, stems, leaves, seeds, and roots of natural plants from hot and humid environments (Bellaloui, Mengistu, Fisher, & Abel, 2012). Furthermore, the previous research evaluated that soybean seeds infected by *P. longicolla* had lower phenolic compounds than healthy seeds (Bellaloui, Mengistu, & Zobiole, 2012). Although many studies have evaluated the isoflavone, protein, oil, and fatty acid contents, as well as the beneficial health properties of the soybean, there are few reports examining the variation of the composition and the antioxidant activities by fungal pathogens. Thus, our research was designed to evaluate the components and radical scavenging capacities of healthy and diseased seeds as important information on the nutritional quality of the soybean.

The primary purpose of the present work was to investigate and compare the contents of four different components (isoflavone, protein, oil, and fatty acid) of healthy soybean seeds and soybean seeds diseased by *P. longicolla* and *C. kikuchii* pathogens. In addition, we evaluated for the first time the variations of antioxidant properties against DPPH and ABTS radicals caused by fungal pathogens.

2. Materials and methods

2.1. Plant material and chemicals

The soybean cultivar (cv. Daemangkong) was developed by the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Korea. This cultivar was grown in the experimental field of the NICS, Milyang, Gyeongnam, in 2011. After harvesting, the healthy seeds were dried under natural light and stored at −40 °C until analysis. The seeds diseased by *P. longicolla* and *C. kikuchii* pathogens were also collected in the same region (Fig. 1).

Analytical grade water, acetonitrile, and acetic acid were purchased for HPLC analysis from J.T. Baker (Phillipsburg, NJ, USA). For quantitative analysis, isoflavone aglycone and glucoside standards were isolated from soybean seeds, as reported in our earlier study (Lee & Cho, 2012). The remaining isoflavones, acetyl and malonyl glucosides, were purchased from Fujicco Co. (Ltd. Nacalai Tesque Inc., Kobe, Japan). Fatty acid standards including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) methyl esters were obtained from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), butylated hydroxytoluene (BHT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and potassium persulphate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). A genomic DNA Extraction Kit was purchased from Intron Biotechnology (Suwon, Korea). All other reagents were of analytical grade and were purchased from Sigma Chemical.

2.2. Instruments

To measure antioxidant activity, UV–vis absorption spectra were measured on a Beckman DU650 spectrophotometer (Beckman Coulter, Fullerton, USA). Isoflavone contents were analysed using an Agilent 1100 series (Boeblingen, Germany) with a quaternary pump, Agilent 1100 series diode-array detector, and 1100 well plate autosampler. The mass data of isoflavone aglycones were obtained using an Equire 4000 LC/MS system (Bruker Daltonick GmbH, Bremen, Germany). The protein and oil contents were determined using a B-339 Auto Kieldahl analyser (Buchi, Schweiz) and a BUCHI B-811 Extraction System (Buchi, Schweiz). Fatty acid content was analysed using a gas chromatograph (Agilent 7890A series, Boeblingen, Germany).

2.3. Isolation and 26S rRNA sequence analysis of fungal isolates

Two fungi strains were isolated from soybean seeds in the experimental field of the NICS, Milyang, Gyeongnam (Fig. 1). For isolation of the total genomic DNA, two isolates were prepared by inoculating a loop of fungal mass into a 250 ml Erlenmeyer flask containing 50 ml of PDB and then incubating at 25 °C with shaking at 150 rpm for 5 days. Filtered mycelia were freeze-dried and were then subjected to DNA extraction using a Genomic DNA Extraction Kit. The extracted DNA was then used as a template for PCR to amplify 26S rRNA. The PCR amplification, ligation, transformation, plasmid purification, and nucleotide sequence were confirmed as

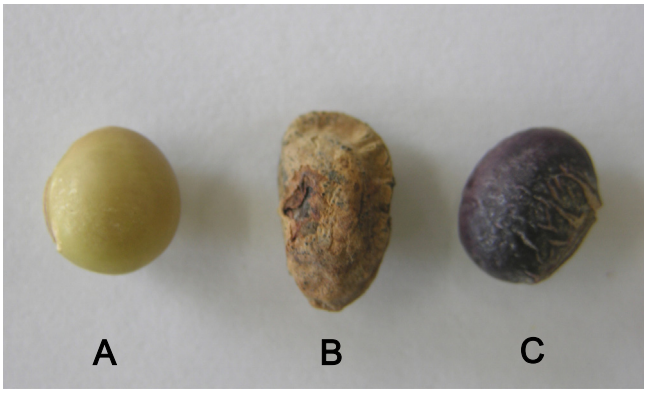

Fig. 1. Healthy and infected soybean seeds (cv. Daemangkong): (A) healthy seeds; (B) diseased seeds (*P. longicolla*); (C) diseased seeds (*C. kikuchii*).