Distribution of Fatty Acids in Newly Developed Tissues of Soybean Seedlings

Krishna Hari Dhakal¹, Yeon-Shin Jeong², Tae-Joung Ha³, In-Youl Baek⁴, Young-Keun Yeo³, and Young-Hyun Hwang¹*

¹Division of Plant Biosciences, Kyungpook National University, Daegu, 702-701, Korea
²Institute of Agriculture Science and Technology, Kyungpook National University, Daegu 702-701, Korea
³School of Food Science and Biotechnology, Kyungpook National University, Daegu 702-701, Korea
⁴Department of Functional Crop, Legume & Oil Crop Division, NICS, RDA, Milyang, 627-803, Korea

Abstract : The objective of this study was to determine the fatty acid composition of newly developed tissues of germinated soybean seeds. Five soybean accessions with varied fatty acid composition were allowed to germinate in sand under greenhouse conditions. Seedlings were picked up after 4, 6, 8, 10 and 12 days of germination and freeze dried. The fatty acid composition of the newly developed tissues was analyzed by gas chromatography. Significant variation in fatty acid composition was observed between accessions, days of germination, and variety × day of germination in whole and the cotyledons. In the case of newly developed five tissues, significant variation in fatty acid composition were observed between days of germination except oleic acid for root, hypocotyl and epicotyl stem and except stearic acid for hypocotyl and unifoliate leaves while all the parameters were significantly different for accession. Significant interactions of accession and days of germination were observed for palmitic, linoleic and linolenic acid in all tissues; only for oleic acid in hypocotyl, epicotyl and unifoliate leaves; and only for stearic acid in root, hypocotyl, epicotyl and unifoliate leaves. During germination, the fatty acid composition of newly developed tissues changed dramatically but whole seedlings and cotyledons changed slightly. These tissues contained five major fatty acids as found in original seeds, but compositions were totally different from that of the seed: higher in palmitic, stearic and linolenic acid and lower in oleic and linoleic acid. New tissues conserved their fatty acid compositions regardless of genotypic variation in the original seeds.

Key words : Fatty acid composition, Newly developed tissues, Seedling, Germination, Soybean

INTRODUCTION

Soybean (Glycine max L.) is the leading oilseed crop produced and consumed in the world today. Current world production of soybean far exceeds that of any other edible oil seeds. Soybean represented 56% of the world’s vegetable oilseeds production in 2008. It has been dominant oilseed produced since 1960 (Smith and Huysker, 1987). The fatty acid composition of soybean oil is often not considered ideal in terms of oil functionality and oxidative stability. Industrial hydrogenation is as effective as plant breeding in altering the fatty acid composition of oil, but trans fatty acids produced during hydrogenation are related to risk of developing heart diseases (Willet and Ascherio, 1994). So, plant breeding is increasingly becoming a priority approach in modifying the fatty acid composition of soybean oil, particularly when combined with biotechnology. To alter the fatty acid composition of soybean oil by genetic manipulation, it is necessary to know how the fatty acids in the soybean plant are metabolized. It is known that soybean plant stores oil in its seeds which are synthesized during seed development and then used as carbon and energy sources during seed germination (Murphy, 1990). Previous investigators (Brown et al., 1962; Singh et al., 1968; Liu and Brown, 1996; Dhakal et al., 2009) studied changes in fatty acid composition during seed germination. Simmons and Quackenbush (1954) studied the sequence of formation of fatty acid in the developing soybean seeds and reported that the radioactivities were found to appear in the fatty acids in the following order oleic, saturated, linoleic and...
linolenic acid. The presence of the highest specific activity in oleic acid was interpreted to suggest that oleic acid was the precursor for the other fatty acids. Dutton and Mounts (1966), investigated the mechanism of desaturation of fatty acids in photosynthesizing flax, soybean and safflower plants at seed setting stage and observed that oleic acid was the first to acquire radioactivity, which subsequently appeared in linoleic and linolenic acid. The objective of this study was to determine the fatty acid composition in newly developed tissues of germinated soybean seeds.

MATERIALS AND METHODS

Seed Materials and Germination

Five soybean accessions Pungsannamulkong, Cheongjakong, Eunhakong, KGL12072, and KGL12234, with varying fatty acid composition were selected. Among five soybeans, Pungsannamulkong, Cheongjakong and Eunhakong had normal fatty acid composition while KGL12072 and KGL12234 had high oleic acid and low linolenic acid, respectively. All soybeans were allowed to germinate in sand under greenhouse conditions. After 4, 6, 8, 10 and 12 days of germination, about 50 seedlings were picked up. Cotyledons and newly developed tissues were then separated from individual seedlings by using pair of scissors. Tissue samples of the same type from different seedlings were combined. Whole seedlings, cotyledons and newly developed tissues were freeze dried and ground into powder by a dry mill. New tissues included root, hypocotyl stem, epicotyl stem, unifoliate leaves, and trifoliate leaves. A portion of sample powder (about 0.5 g) of whole seedling, cotyledon, and tissues was used for fatty acid analysis. Similarly, other intact seeds from each variety were also ground together and a portion of the whole powder (about 0.5 g) was used for fatty acid analysis of the whole seed.

Fatty Acid Analysis

For oil extraction, each sample (0.5 g) was taken in a test tube and 10 ml of hexane was poured into it. The samples were then placed in a shaking incubator (150 rpm) at 50°C for two days. The clear supernatant was transferred into another test tube. Hexane was evaporated by passing air in evaporating unit. The extracted oil (0.15 mL) from each sample was placed in a screw-capped vial, and 5 ml of methylation solution (H₂SO₄ : MeOH : toluene = 1 mL : 20 mL : 10 mL) was added. The sealed vial was heated on a water bath (100°C) for 60 min, and allowed to cool at room temperature. Then 5 mL of water was added and shaken. The mixture separated into two layers, and the upper layer was taken by Pasteur pipette and dried by using anhydrous sodium sulfate for 5 min. Then 1 µL of taken sample was directly injected to the GC using automatic sampler (Agilent 7683B). An Agilent 7890A gas chromatography with a flame ionization detector (FID) and 0.32 mm i.d × 25 m HP-FFAT capillary column was used. The oven temperature was raised from 150°C (1 min. holding) to 230°C at a constant rate of 2.5°C per minute. The injector and detector temperature were kept at 250°C and 230°C, respectively. The carrier gas was nitrogen at a flow rate of 1 mL per min., and the split ratio at the injector port was 50:1.

Statistical Analysis

Analysis of variance (ANOVA) and multiple mean comparisons were performed using the general linear model (GLM) by Statistical Analysis System (SAS 9.1) to identify significant treatment effects and interactions. Differences among mean values were determined using Least Significant Difference and DMRT at $P \leq 0.05$. Data were analyzed as two factorial completely randomized design with two replications.

RESULTS AND DISCUSSION

Fatty acid composition of six soybean accessions

There was a large variation in fatty acid composition in seeds of five soybean accessions (Table 1). Pungsannamulkong, Cheongjakong, and Eunhakong had normal fatty acid composition. The ranges was 10.5–12.0%, 3.7–4.3%, 16.0–24.4%, 51.8–59.8%, and 7.5–8.8% for palmitic, stearic,