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Seed coat color and seed weight contribute differential responses of targeted metabolites in soybean seeds

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ABSTRACT

The distribution and variation of targeted metabolites in soybean seeds are affected by genetic and environmental factors. In this study, we used 192 soybean germplasm accessions collected from two provinces of Korea to elucidate the effects of seed coat color and seeds dry weight on the metabolic variation and responses of targeted metabolites. The effects of seed coat color and seeds dry weight were present in sucrose, total oligosaccharides, total carbohydrates and all measured fatty acids. The targeted metabolites were clustered within three groups. These metabolites were not only differently related to seeds dry weight, but also responded differentially to seed coat color. The inter-relationship between the targeted metabolites was highly present in the result of correlation analysis. Overall, results revealed that the targeted metabolites were diverged in relation to seed coat color and seeds dry weight within locally collected soybean seed germplasm accessions.

1. Introduction

The major components of soybean [*Glycine max* (L.) Merr.] seeds are protein, oil, and soluble carbohydrates which are present up to 70% in dried seeds. The contents of protein range from 36.6% to 50.4% and the contents of oil from 13.4% to 26.4% based on the geographical origins of soybean seeds (Hollung et al., 2005; Lee & Choung, 2011a). The contents of free carbohydrates also vary from 1.7% to 3.7% for stachyose, 0.5–1.8% for raffinose, 2.0–7.2% for sucrose, and 0.1–1.3% for glucose (Choung, 2005; Hollung et al., 2005; Lee & Choung, 2011a). Fatty acid species are mainly composed of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids in soybean seeds (Bellaloui, Smith, Ray, & Gillen, 2009; Choung et al., 2005). Of these fatty acids, linoleic acid is highest in content and following by oleic, palmitic, linolenic, and stearic acids in decreasing amounts (Bellaloui et al., 2009; Choung et al., 2005). Certain minor fatty acid species including myristic (C14:0), palmitoleic (C16:1), arachidic (C20:0), eicosenoic (C20:1), and behenic (C22:0) acids are also present (Nam et al., 2016). As a secondary metabolites, isoflavones, also referred to as phytoestrogens, are highly linked to beneficial health effects in human by reducing numerous diseases, such as cancer and cardiovascular diseases (Adlercreutz & Mazur, 1997), are present in soybean seeds and ranging from 0.7 mg/g to 9.5 mg/g (Charron, Allen, Johnson, Pantalone, & Sams, 2005). A wide range of isoflavone isomers with varying adducts exist, including aglycones, glucosides, acetylglucosides, and malonylglucosides (Kim et al., 2006; Lee & Choung, 2011a, 2011b).

The contents and proportions of metabolites in soybean seeds are highly influenced not only by genetic factors but also by environmental factors (Bellaloui et al., 2009; Hollung et al., 2005; Lee & Choung, 2011a; Shibata et al., 2008). Furthermore, the fluctuation of those compound levels is strongly associated with seed development and maturation stages (Lee, Hwang, Chang, Moon, & Choung, 2013; Lee & Son, 1993; Saldivar, Wang, Chen, & Hou, 2011). Intriguingly, the composition and contents of major and minor metabolites are also affected by seed size, in which the levels of

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lipids, starch, soluble sugars, and soluble proteins, and proportion of palmitate and oleate were relatively higher in large seeds than in small seeds (Guleria, Sharma, Gill, & Munshi, 2008). On the other hand, seed color affects chemical composition in soybean seeds; for example, the levels of dietary fiber and galactose are higher in yellow soybeans than in green soybeans (Redondo-Cuenca, Villanueva-Suárez, Rodríguez-Sevilla, & Mateos-Aparicio, 2007). When considering total sugar contents, the levels of total sugar range from 8.3% to 12.1% but no statistical difference on the content of total sugar in terms of seed coat color exists (Lee & Son, 1993). The levels of daidzein and genistein increase relative to seed size but the level of glycitein does not (Cho et al., 2007). However, seed size had a positive correlation with oleic acid but a negative relationship with linoleic and linolenic acids (Kumar, Rani, Solanki, & Hussain, 2006).

The fluctuation of metabolites in levels and contents is strongly interrelated in soybean seeds. Hartwig, Kuo, and Kenty (1997) reported that oil and protein were strongly inversely related and thus, oppositely related to sucrose. Nitrogen treatment affected the inverse relationship between protein level and oil content but overall there was a strong correlation between seed protein level and seed yield (Ray, Fritschi, & Heatherly, 2006). The contents of protein and oil are negatively affected by increasing drought stress and high air temperature (Dornbos & Mullen, 1992). Seed coat color does not affect the inverse relationship between protein and lipid content (Kim, Lee, Chi, Lee, & Kim, 2007). Also, Hymowitz, Collins, Panczner, and Walker (1972) reported that oil was negatively correlated with protein and stachyose but positively associated with total sugar, sucrose, and raffinose. Protein was negatively related to total sugar, sucrose, and raffinose but positively to stachyose. In contrast, Lee and Son (1993) reported that oil and protein was positively correlated within 1087 colored soybean germplasm accessions. At the same time, total sugar was negatively associated with protein but positively with oil (Lee & Son, 1993). Interestingly, the relationship between protein and oil contents was inversely appeared in whole soybean seed and seed coat (Kim, Chi, Son, Park, & Ryu, 2005). Furthermore, the level of stachyose + raffinose was negatively related with protein in 43 soybean progenies (Wilcox & Shibles, 2001). Within soluble carbohydrates, total sugar was positively correlated with sucrose and raffinose. Sucrose was positively related to raffinose but negatively with stachyose. Levels of raffinose and stachyose are generally inversely correlated (Hymowitz et al., 1972). However, in one study, raffinose was positively correlated with stachyose but negatively with sucrose, which was inversely related with stachyose in soybean lines with a reduced content of raffinose and stachyose and an increased sucrose (Neus, Fehr, & Schnebly, 2005). In another study, raffinose was negatively correlated with maltose, whereas sucrose showed a positive association with total uronic acid (Hollung et al., 2005). Of fatty acid species, the levels of linolenic and linoleic acids have been demonstrated to have an inverse relationship to the oleic acid content, while the contents of palmitic and stearic acids were not affected by temperature (Wolf, Cavins, Kleiman, & Black, 1982). Overall, linoleic acid was shown to be positively related with the level of oleic acid but negatively with linolenic acid, regardless of seed maturity (Carver, Burton, Carter, & Wilson, 1986). However, the relationship between oleic acid and linolenic acid is inconsistent. The content of lipid is more widely distributed among soybean accessions than within wild soybean accessions with higher levels. Interestingly, the relationship between linolenic and oleic acids appears to be inversely associated within two of these accessions (Shibata et al., 2008). Also, the level of oleic acid tended to have opposite correlation with total levels of linoleic and linolenic acids (Gao, Hao, Thelen, & Robertson, 2009). Currently, the soybean breeding program focuses on the reduction in polyunsaturated fatty acids, such as linoleic and linolenic acids, and to increase monounsaturated fatty acids including oleic acid in order to improve nutritional quality and shelf life of soybean oil. Furthermore, it is strongly desirable to reduce saturated fatty acids, for instance, palmitic and stearic acids, in the human diet (Fehr, 2007).

Many studies have reported on the distribution and variation of biochemical or metabolic components and their relationship not only with either environmental factors or genetic factors but also with both factors on the yield and quality of soybean seeds. Of numerous qualitative characteristics of soybean seed, seed coat color may be one of the most important factors to consider for soybean seed quality. It has been reported that the pigmentation on soybean seed coat affects the levels of isoflavones, and the composition and levels of fatty acids (Cho et al., 2013; Lee et al., 2010; Slavin, Kenworthy, & Yu, 2009). Nevertheless, little is known about metabolic or biochemical responses to seed coat color and any physiological characteristics, such as relative seed size within a wide range of soybean seed germplasm accessions. In this study, diverse soybean seed germplasm accessions, which were locally collected with four different seed coat colors, were used to evaluate the effects of seed coat color and seed size (measured by 100 seeds dry weight) on any inter-relationship and variation of targeted metabolites. Therefore, the objectives of this study were to test the hypothesis that the targeted metabolites would be differently affected by seed coat colors and seed dry weight and then, how the fluctuation of these targeted compounds could mediate the inter-relationship or correlation between metabolites within diverse soybean seed germplasm accessions with a different seed coat color. Ultimately, the goal was to provide a more robust biochemical characterization of soybean seeds through further elucidation of metabolic responses to seed coat colors for the soybean breeding program to enhance soybean seed quality and yield.

2. Materials and methods

2.1. Sample collection and preparation

The seeds of 192 Korean soybean germplasm accessions, which were locally collected from 28 locations in Gyeongsangnam-do and Gyeongsangbuk-do provinces, the Republic of Korea (Fig. S1) and were distributed by National Gene Bank, Rural Development Administration (RDA), were used in this study. These soybean seeds [*Glycine max* (L.) Merr.] were cultivated at the experimental field of Department of Functional Crop, National Institute of Crop Science (NICS), RDA in Miryang, Republic of Korea. The soybean seeds were planted on June 18, 2013 with 60×15 cm plant distance, and harvested 7–10 days after physiological maturity from early to middle October, which is equivalent to R7 of soybean reproductive growth stage (Dornbos & McDonald, 1986; Fehr, Caviness, Burmood, & Pennington, 1971). The 4–7–6 kg/10a of $N–P_2O_5–K_2O$ applied the field and insecticide sprayed against pest like soybean moth. After harvested and dried seeds, 100 seeds dry weight was obtained. In terms of the difference of seed coat color, the numbers of black, brown, green, and yellow soybean seed germplasm accessions were 60, 26, 36, and 70, respectively.

All the soybean seed germplasm accessions were dried at 40 °C for 24 h and ground with a micro hammer mill (Culatty AG, Zurich, Switzerland), sieved with a 1.0 mm screen and stored in sealed plastic bags at 4 °C until analyzed for the measurement of soluble carbohydrates, crude protein and oil, isoflavones, and fatty acids.

2.2. Climatic condition

The climatic condition, such as daily minimum/mean/maximum air temperature and daily precipitation from May to October