PROXIMATE COMPOSITION OF MATURE SEEDS FROM SOYBEAN PLANTS GROWN IN DIFFERENT ENVIRONMENTS

T.M. Kuo[1], K. Eskins[2] and R.L. Cooper[3]

[1]Oil Chemical Research and [2]Biomaterials Processing Research, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL 61604 U.S.A. [3]USDA-ARS, Dept. of Hort. and Crop Sci., Ohio Agric. and Develop. Ctr., Wooster, OH 44691 U.S.A.

Abstract

The objectives of this study were to examine the influence of differences in sunlight irradiation on the accumulation of soluble oligosaccharides in mature soybean (*Glycine max* L.) seeds and to determine the correlations between important seed constituents. Plants were grown in field houses covered with plastic filters that varied sunlight irradiation. The most obvious effect of both blue- and red-deficient sunlight was a reduction in the level of stachyose and sucrose that coincided with an increase in raffinose level and decreased oil content. Blue growth house which was deficient in red light spectrum also produced seeds with an increased protein content. Our results derived from environmental conditions in both open fields and under different sunlight irradiation revealed a non-significant correlation between protein level and the level of stachyose or raffinose saccharides. This suggests that it may be possible to develop soybean lines that contain high protein and low raffinose saccharides.

KEYWORDS

Soybean seeds, stachyose, raffinose saccharides, sucrose, oligosaccharides, protein, oil, sunlight spectrum.

INTRODUCTION

Mature soybean seeds contain approximately 40% protein, 20% oil, and 10% water soluble carbohydrates. They are a valuable source of protein and oil. In recent years much attention has also been paid to the major oligosaccharides; sucrose in fermented soya products and raffinose saccharides (stachyose and raffinose) in animal feeds. Soymeals lacking raffinose saccharides would allow poultry to obtain more energy and nutrients from the meal. Thus it is very important to identify any factors that may influence the accumulation of these oligosaccharides in the seed. Environmental growth conditions including light spectral quality can have a profound influence on the chemical composition of soybeans. Howell et al. (1957) reported that blue light elevated the protein content and pink light increased the oil content, but light quality failed to change carbohydrate levels of mature seeds. Britz and Cavins (1993) found that light spectral quality modulated seed fatty acid desaturation. However, these experiments were carried out using plants grown in controlled environmental chambers equipped with artificial light sources. The objectives of this study were to examine the effects of red light and blue light under field conditions, where far-red light was also an important component of irradiance, on the accumulation of soluble oligosaccharides in mature soybean seeds and to determine their relationships with other seed constituents, such as protein and oil.

MATERIALS AND METHODS

Soybean (*G. max* L.) cv. Charleston is a high-yielding and determinate semi-dwarf cultivar adapted to the central Midwest of the U. S. (Cooper et al., 1995) , and it is easily manageable in a confined growth environment. Triplicate samplings (about 20 g each) of mature seeds harvested from an experimental field at Wooster, OH in 1994 were analyzed for the concentrations of protein, oil and soluble oligosaccharides upon arrival in the laboratory in the spring of 1995. The results were compared with those harvested from a field plot and its adjacent growth houses at Peoria, IL in 1995. Planting of Charleston at Peoria consisted of triple replications of three-row individual plots of 1.85 m^2 randomly distributed in a field of about 10.5 m by 20 m. The field was mixed with Osmocote 14-14-14 (Sierra Chemical; Milpitas, CA) during tilling. Spacings for individual plots and rows in each plot were 65 cm, and plants in each row were about 15 cm apart. Plants were watered biweekly with overhead sprinklers. Mature seeds from the center 4 plants of each plot were harvested and pooled for analysis. Planting in growth houses was carried out in 7.6-L (25-cm dia.) pots containing Redi-earth Peat-lite Mix (W. R. Grace; Cambridge, MA) supplemented with Osmocote 14-14-14 (2.1 L/ m^3) and Micro Max micronutritrients (706 g/ m^3; Sierra Chemical). Four pots each containing 3 plants were grown in three growth houses of same size (2.0 m wide by 2.4 m long by 1.8 m high with 2.4 m at roof peak) specially constructed adjacent to the field plot. One growth house was covered with blue Roscolux filter 69 (Rosco; Port Chester, NY) allowing blue/far-red (FR) light transmission, another with red Roscolux filter 19 allowing red/FR transmission, and the third with clear Roscolux filter 00 to allow full spectrum blue/red/FR transmission. The light intensity and day/night temperature fluctuated during daily field conditions. A typical level of light energy in the blue, red and clear houses was 319, 678 and 798 µmol m^{-2} s^{-1}, respectively, to compare with the open field of about 2,000 µmol m^{-2} s^{-1} for unfiltered sunlight. All plants were watered daily and supplemented with Peters 20-20-20 (15 g/L; Grace-Sierra; Milpitas, CA) weekly. Each growth house was equipped with an air-conditioner (its thermostat set at 28-30°C) to provide maximal air exchange from outside and to avoid overheating. Mature seeds harvested from each pot were pooled for analysis.

Protein and oil was determined by near-infrared transmittance spectroscopy. The soluble carbohydrate fraction of the defatted meal was prepared and quantitated by HPLC as described previously (Kuo et al., 1990), except that the ground meal was defatted with pentane/ hexane (50-50 mix) in a Butt-type extractor for 6 h. Data acquisition and integration were carried out by using Thermo Separation Products (San Jose, CA) PC1000 System Software (Ver. 3.0.1). An evaporative light scattering detector, Model MK IIA (Varex; Burtonville, MD) was used to monitor the HPLC elution with water containing 10 mg/ L of calcium acetate as the mobile phase.

RESULTS AND DISCUSSION

The mean values for the concentrations of stachyose, raffinose, stachyose plus raffinose, sucrose, stachyose plus raffinose plus sucrose, oil, and protein of mature seeds harvested from three growth houses and two different fields are reported in Table 1. Differences in values were analyzed statistically, and the analysis of variance (ANOVA) showed that growth location was a significant source of variations for seed constituents studied ($p<0.01$). Specific contrasts of means were used to test: Ohio versus Illinois field; field (i.e., Ohio and Illinois) versus the clear house; field versus the red house; and field versus the blue house. These contrasts were tested because plants grown in specially constructed field houses, unlike experimental conditions under artificial light sources, were subjected to the natural variations of filtered sunlight intensity with one major difference being light spectral quality. Stachyose concentration was similar between two fields, but it differed significantly between field and each growth house ($p<0.05$) (column 2, Table 1). In both red and blue growth houses, where the sunlight irradiation was deficient in the respective blue and red light spectra, stachyose decreased substantially. Despite a concomitant increase in raffinose level, total concentration of stachyose and raffinose in seeds from the color

houses was still significantly lower than that from the field ($p<0.05$). Seeds from the clear growth house had a similar effect. The results suggest that both sunlight intensity and spectral quality can affect the accumulation of raffinose saccharides in the seed. On the other hand, sucrose concentration decreased only in the blue house (column 5, Table 1). Taking all major soluble oligosaccharides (i.e., stachyose, raffinose and sucrose) together into consideration, however, seeds from both red and blue houses contained significantly lower concentrations of these oligosaccharides than seeds grown in the field ($p<0.05$) (column 6, Table 1). There were no differences between two fields and between field and the clear house, indicating that light spectral quality might play a more important role than light intensity in affecting the total amount of these soluble oligosaccharides in the mature seed. Analysis of contrasts of means showed that both protein and oil conc. differed significantly in seeds from two fields, and oil in all three growth houses increased significantly, whereas protein concentration only differed (with an increase) in the blue house. In an experiment using entirely artificial lights devoid of far-red light spectrum, Howell et al. (1957) also observed that blue light increased the protein level, and that light quality had no effect on the total carbohydrate level in the seed. They also concluded that pink light increased the level of oil in the seed, whereas our results showed that light spectral quality had no such an effect. It is not known if the discrepancy is the result of a far-red light effect.

Correlation coefficients between each of the seed constituents studied are reported in Table 2. Stachyose was negatively correlated with oil, whereas the negative correlation between stachyose and protein was non-significant. Total major raffinose saccharides (stachyose plus raffinose) and protein or oil also had a non-significant correlation. There was a significant negative correlation between sucrose and oil, and between protein and oil, but non-significant correlation between sucrose and protein. From an economic standpoint, it is important to obtain soybeans with a greatly reduced amount of raffinose saccharides because soymeals with low raffinose saccharides would allow farm animals, especially poultry, to gain more energy and nutrients from the feed. Hymowitz et al. (1972) reported a positive and significant (0.41) correlation between stachyose and protein from the results of field-grown soybean seeds, and indicated difficulties for breeders to obtain soybeans low in stachyose content while maintaining a high percentage of protein. Recently, Hartwig et al. (1996) found a non-significant correlation between stachyose (0.11) or stachyose plus raffinose (-0.05) and protein in a study consisting of 40 soybean genotypes grown in a field at Stoneville, MS. Our results, which included both open field conditions and different sunlight spectral effects, support the observations of Hartwig et al. (1996); this is to further suggest that it is possible to develop high protein soybean lines containing low amounts of raffinose saccharides.

ACKNOWLEDGMENTS

We wish to thank Renee Tucker, JoAnne Toohill and Henry Bene for technical assistance, the staff of the Analytical Chemistry Support Unit for analyzing protein and oil, and Dr. Terry Nelsen for statistical analysis and helpful discussion.

REFERENCES

Britz, S.J. and J.F. Cavins. 1993. Spectral quality during pod development modulates soybean seed fatty acid desaturation. Plant, Cell and Environment **16**: 719-725.

Cooper, R.L., R.J. Martin, S.K. St. Martin, A. Calip-DuBois, R.J. Fioritto and A.F. Schmitthenner. 1995. Registration of 'Charleston' soybean. Crop Sci. **35**: 593.

Howell, R.W., O.A. Krober and F.I. Collins. 1957. The effect of light quality on growth and composition of soybeans. Plant Physiol. **33** (Suppl.): 8.

Hartwig, E.E., T.M. Kuo and M.M. Kenty. 1996. Seed protein and its relationship to soluble sugars in soybeans. Crop Sci. In press.

Hymowitz, T., F.I. Collins, J. Panczner and W.M. Walker. 1972. Relationship between the content of oil, protein, and sugar in soybean seed. Agron. *J*. **64**: 613-616.

Kuo, T.M., D.C. Doehlert and C.G. Crawford. 1990. Sugar metabolism in germinating soybean seeds -- Evidence for the sorbitol pathway in soybean axes. Plant Physiol. **93**: 1514-1520.

Table 1

Seed constituents of 'Charleston' soybean grown in different environments[1].

Location	Sta	Raf	Sta+Raf	Suc	Sta+Raf+Suc	Oil	Protein
1	44.4 [ab]	12.2 [b]	56.6 [b]	62.4 [a]	119.0 [a]	17.6 [c]	42.2 [a]
2	49.3 [a]	15.8 [a]	65.1 [a]	57.0 [ab]	122.1 [a]	19.8 [b]	39.0 [c]
3	39.6 [bc]	13.8 [b]	53.5 [b]	59.9 [ab]	113.4 [ab]	20.4 [ab]	40.1 [b]
4	36.0 [c]	17.2 [a]	53.2 [b]	55.8 [b]	109.0 [b]	20.9 [a]	40.1 [b]
5	38.1 [c]	17.1 [a]	55.2 [b]	46.0 [c]	101.2 [b]	20.8 [a]	42.0 [a]

[a, b, c, d] Different letters within a column indicate significant ($p<0.05$) differences.

[1] Location 1: field at Wooster, OH; 2: field at Peoria, IL; 3: clear growth house; 4: red growth house; 5: blue growth house. Locations 1 and 2 had three replicated samples, and 3, 4 and 5 had four replicated samples. Concentrations of soluble oligosaccharides are expressed as mg/g dry wt., and oil and protein as percent dry wt.. Sta=stachyose, Raf=raffinose, Suc=sucrose.

Table 2

Corrections between seed constituents of 'Charleston' soybean grown at five different locations as shown in Table 1.

	Raffinose	Sta+Raf	Sucrose	Sta+Raf+Suc	Oil	Protein
Stachyose	-0.22	0.92***	0.40*	0.84***	-0.47**	-0.20
Raffinose		0.17	-0.65***	-0.36*	0.65***	-0.10
Sta+Raf			0.14	0.71**	-0.22	-0.24
Sucrose				0.80**	-0.43*	-0.35
Sta+Raf+Suc					-0.44*	-0.39*
Oil						-0.38*

*, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively.