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Relationships between nuclear DNA content and seed and leaf size in soybean

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Abstract A correlation between genome size and agronomically important traits has been observed in many plant species. The goal of the present research was to determine the relationship between genome size, seed size, and leaf width and length in soybean [*Glycine max* (L.) Merr.] Twelve soybean strains, representing three distinct seed size groups, were analyzed. Flow cytometry was used to estimate their 2C nuclear DNA contents. Data on seed size and leaf size of the 12 strains were obtained from 1994 and 1995 field experiments. Variation of 2C nuclear DNA among the 12 soybean strains was 4.6%, ranging from 2.37 pg for a small-seed strain to 2.48 pg for a large-seed strain. Strain seed size was positively associated with leaf width ($r = 0.92$) and leaf length ($r = 0.93$). Genome size was highly correlated with seed size ($r = 0.97$), leaf width ($r = 0.90$) , and leaf length ($r = 0.93$). The results of our study indicate that there is a significant correlation between genome size and leaf and seed size in soybean. It is possible that selection for greater seed size either leads to, or results from, greater genome size. If so, this relationship might be worth exploring at a more fundamental level.

Key words *Glycine max* · Genome size · Seed size · Leaf size · Flow cytometry

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Introduction

Nuclear DNA content variation has been reported in a number of crop species (Laurie and Bennett 1985; Rayburn et al. 1985; Graham et al. 1994; Lee et al. unpublished data). Doerschug et al. (1978) observed that the DNA content detected by the Feulgen reagent varied from 1.84 to 2.61 pg per 2C nucleus among 11 soybean cultivars. Hammatt et al. (1991) used flow cytometry to show that 4C nuclear DNA content ranged from 3.80 to 6.59 pg within the genus *Glycine*. Bennett (1985) reported a 50% variation of DNA content in *Glycine max*. Graham et al. (1994) observed a highly significant correlation ($r = 0.55$) between maturity and genome size with 20 soybean cultivars, which represent maturity groups ranging from 000 to IX, using flow cytometry.

Nuclear DNA among has been correlated with such characters as nuclear volume, cell volume, mitotic cycle time, and duration of meiosis (Van't Hof and Sparrow 1963; Evans and Rees 1971). Bennett (1976) suggested that variation in nuclear DNA content has a major impact on many plant traits. Because some of these traits contribute to the agronomic yield of the crop, it would be worthwhile to identify the effect of variation in genome size on growth and yield components. Minimal research has been conducted on the relationship between intraspecific genome-size variation and agronomic performance in a major crop species under field conditions. Biradar et al. (1994) reported that growth and yield parameters (e.g., ear or seed weight and seed number per plant) were negatively correlated with nuclear DNA amount in 12 southwestern US maize open-pollinated populations field-tested for agronomic performance. These authors concluded that variation in nuclear DNA content in maize plays an integral role in determining agronomic performance. In soybean, little is known about the relationship between nuclear DNA content and field-based measures of

agronomic traits. The objective of the present study was to examine the relationship between genome size measured by flow cytometry and seed and leaf size measured on 12 soybean strains grown in replicated field tests in 1994 and 1995.

Materials and methods

The 12 soybean strains used in this study included three distinct seed-size groups (see Table 1). Among the 12 strains, four (Saturn, PI 417.339, PI 417.468, and PI 423.894) represent a large-seed size (> 30 g/100 seeds), four (Colfax, Charleston, Lancaster, and Ripley) represent the commercial standard seed size (12–18 g/100 seeds), and four (Mercury, PI 398.374, T208, and T215) represent a small-seed size (< 10 g/100 seeds). Colfax, Charleston, Lancaster, and Ripley are adapted cultivars with a high yield in the mid-western USA. Mercury and Saturn are small- and large-seed cultivars grown in the USA to supply the speciality *natto* and *edamame* markets in Japan. All strains belong to maturity groups II, III, or IV (adapted to north central USA production) with a yellow seed coat. The field experiment was conducted on the East Campus of the University of Nebraska-Lincoln in 1994 and 1995. Planting dates were 7 May 1994 and 17 May 1995 in bordered blocks of 25 seed, with 0.75 m × 0.90 m plots planted with a tractor-drawn mechanical planter. The experimental design was a randomized complete block with five replications for both years. Standard agronomic practices were applied. Plots were harvested when the plants in the plot reached maturity. Harvested seed was air-dried to a seed moisture content of about 8.0%. One-hundred seed samples were drawn from the harvested seed of each plot and weighed to measured seed size (g/100 seeds). The seed-size estimates for each strain (2 years, five replications per year) were averaged to obtain a mean seed size. Leaf width and length (cm) of the 12 soybean strains was measured on the terminal leaflet of the fourth trifoliolate before flowering. Ten random plants per plot and per year were selected and measured. The leaf width and length estimated for each strain (2 years, five replications per year, ten leaf measurements per replicate) were averaged to obtain a mean leaf width and length. The 12 soybean strains were planted again on the East Campus on 24 May 1996 to obtain young leaf tissue for the analysis of nuclear DNA content.

Soybean nuclei were isolated from young leaves obtained from the 1996 field-grown soybeans by the chopping method as described by Arumuganathan and Earle (1991). Leaf materials (about 20 mg) from individual plants were put together with internal control barley leaf tissue (about 30 mg) into a plastic Petri dish containing 0.5 ml of ice-cold nuclear isolation buffer [10 mM $MgSO_4 \cdot 7H_2O$, 50 mM KCl, 5 mM Hepes, 3 mM dithiothreitol, 0.25% Triton X-100, and 100 μg/ml propidium iodide (PI)]. The isolated nuclear suspension was filtered through a 80-μm nylon mesh, incubated on ice for 30 min, and then analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.). Three independently derived nucleus samples were obtained for each strain. Each sample was analyzed twice (i.e., a total of six replicates per each strain). Approximately 2000 G_1 nuclei were analyzed per strain. The DNA content of each strain was calculated based on an internal standard, which was the 2C DNA content (10.24 pg) of the barley strain NE 86954 (Lee et al. 1997). Correlations between genome size, seed size, and leaf width and length were calculated with the Proc Corr procedure of SAS.

Results and discussion

The phenotypic traits of the 12 strains, including the 2-year strain means for seed size (g/100 seeds) and leaf width and length (cm), are summarized in Table 1. These 12 strains had served as parents in the Nebraska soybean breeding project for the purpose of developing high-yielding cultivars with large and small seed sizes. The seed-size range within the four large, four medium, and four small seed strains was 31–37, 12–17, and 6–8 g/100 seed, respectively. The DNA content values of each strain were based on the mean fluorescence intensity of the G_1 nuclei. A typical flow cytometric histogram showing the relative PI fluorescent intensity of soybean versus the barley standard is presented in Fig. 1.

Significant differences were observed in the genome sizes of the 12 soybean strains (Table 1). Strain DNA content and strain seed size were related, ranging from a low DNA content of 2.37 pg for the small seed strain T215 to a high DNA content of 2.48 pg for the large seed-size strain PI423.894. In percentage terms, this is a 4.6% difference between the DNA contents. The mean DNA contents of the large, medium and small seed-groups of strains were significantly different at 2.46 pg, 2.40 pg and 2.38 pg, respectively. These 2C nuclear DNA contents are consistent with the DNA amount of 4.70 pg per 4C nucleus reported for the soybean cultivar ESSEX by Hammatt et al. (1991), but are somewhat larger than the 4C value of 3.70 pg calculated for soybean by Doerschug et al. (1978). Graham et al. (1994) recently reported that DNA amounts among 20 soybean cultivars ranged from 2.51 to 2.88 pg per 2C nucleus within 20 soybean cultivars. Variance in the DNA amounts observed among these different studies probably reflect differences in the genotypes used or the varying modes of action of the fluorochromes employed by the researchers.

The mean DNA contents of the three seed-size groups were significantly different and a Duncan's multiple range test also revealed significant differences among the DNA content of the strains *within* each of three different seed-size groups. For example, the small-seed strains T208 and T215 had smaller DNA contents than Mercury and PI398.374, and the medium-seed strains Colfax and Charleston had smaller DNA contents than Lancaster or Ripley.

Correlation coefficients were calculated between leaf width, leaf length, seed size, and genome size (Table 2). These correlations approach unity, indicating a highly significant relationship among these traits. Large-seed strains had large leaf width and leaf length, and *vice-versa* (Table 1). Cell size and cell number have a direct bearing on plant and organ size. In soybean, large-seed size is typically associated with large plant organs, including not just the expected large cotyledons but also flowers and leaves. Humphries and Wheeler (1963) reported that there was general agreement that cell number was the main determinant of leaf size and that cell size was relatively unimportant. Guldan and Brun (1985) suggested that much of the difference in the seed size of soybean was due to differences in the number of cells in the cotyledon. Swank et al. (1987) reported that

Table 1 Phenotypic characteristics of the 12 soybean strains used in this study including the means and standard deviations in their genome size, seed size, leaf width, and leaf length

Strain	Maturity group	Phenotypic descriptor (1234 56[a])	Seed size[b] (g/100 seeds)	Leaf size[b]		Relative PI fluorescent intensity[c]	2C nuclear DNA content (pg)[d]	Duncan's grouping[e]
				Width (cm)	Length (cm)			
Large-seed strains								
Saturn	III	DWGTnYY	31.36 ± 3.86	7.38 ± 1.10	10.02 ± 2.06	202.31 ± 0.56	2.46 ± 0.01	B
PI417.339	IV	DWGBrYY	36.26 ± 3.52	7.51 ± 1.18	11.19 ± 1.99	201.75 ± 0.56	2.45 ± 0.01	C
PI417.468	IV	DPGBrYY	36.29 ± 6.35	7.11 ± 1.21	10.26 ± 1.85	202.07 ± 0.47	2.46 ± 0.01	BC
PI423.894	IV	DWGBrYY	37.47 ± 4.19	7.87 ± 1.16	11.64 ± 1.85	203.99 ± 0.56	2.48 ± 0.01	A
Means			35.34 ± 5.03	7.47 ± 1.19	10.80 ± 2.04	202.53 ± 0.54	2.46 ± 0.01	
Medium-seed strains								
Colfax	II	DWGTnYBf	17.84 ± 1.26	7.04 ± 1.00	8.62 ± 1.25	197.22 ± 0.49	2.40 ± 0.01	E
Charleston	III	DPTTnYBl	15.28 ± 1.43	6.37 ± 0.90	9.29 ± 1.71	197.10 ± 0.47	2.40 ± 0.01	E
Lancaster	III	DPTTnYBl	16.13 ± 1.05	6.36 ± 0.87	9.40 ± 1.10	197.89 ± 0.54	2.41 ± 0.01	D
Ripley	IV	DPGTnYBl	12.54 ± 1.24	6.27 ± 0.91	9.30 ± 1.31	197.88 ± 0.49	2.41 ± 0.01	D
Means			15.44 ± 2.28	6.51 ± 0.97	9.15 ± 1.38	197.52 ± 0.50	2.40 ± 0.01	
Small-seed strains								
Mercury	II	DPGTnYBf	8.85 ± 0.41	4.94 ± 0.84	8.28 ± 1.54	196.22 ± 0.49	2.39 ± 0.01	F
PI398.374	IV	DPGB1YBf	7.47 ± 1.15	5.33 ± 1.00	8.14 ± 1.42	196.28 ± 0.55	2.39 ± 0.01	F
T208	IV	DPGTnYBf	6.13 ± 0.43	5.30 ± 0.98	8.61 ± 1.60	195.37 ± 0.46	2.38 ± 0.01	G
T215	IV	DPGB1YBf	6.78 ± 0.88	5.17 ± 0.91	8.03 ± 1.56	195.05 ± 0.53	2.37 ± 0.01	G
Means			7.30 ± 1.27	5.18 ± 0.94	8.25 ± 1.54	195.73 ± 0.51	2.38 ± 0.01	

[a] Phenotypic descriptor codes: 1 *I*ndeterminate, *D*eterminate; 2 flower color, *P*urple or *W*hite; 3 pubescence color, *T*awny or *G*ray; 4 pod color, *B*rown or *T*a*n*; 5 seed-coat color, *Y*ellow; 6 hilum color, *Y*ellow, *Buf*f and *B*lack
[b] Means computed from 1994 and 1995 field test data
[c] Mean and standard deviation of the relative PI fluorescence intensity (channel number) of G_1 nuclei were calculated from two flow cytometric runs made on each of three independent isolations of nuclei from each strain
[d] Mean and standard deviation of the 2C nuclear DNA content were calculated from the relative PI fluorescent intensity, using barley DNA (NE86954, 10.24 pg/2C, Lee et al. 1997) as the internal control
[e] Means with the same letters are not significantly different at the $\alpha = 0.05$ probability level

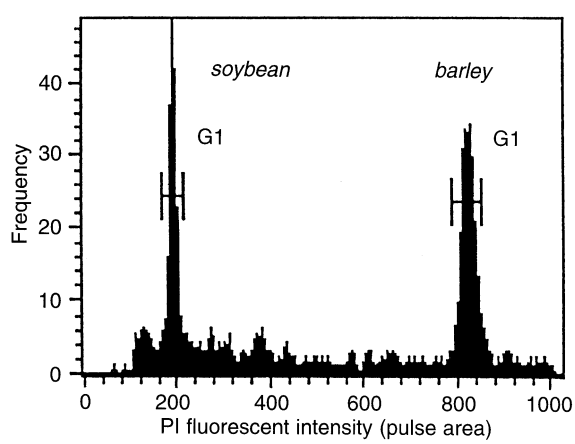

Fig. 1 Flow cytometric histogram showing relative DNA content based on relative PI fluorescent intensity (channel number). Linear PI fluorescence intensity of G_1 nuclei was used for the calculation of DNA content. Barley DNA was used as an internal control

larger seed in soybean was associated with a larger number of cotyledon cells, but also noted that increased cell size contributed positively to genotypic differences in seed size. We have not determined whether the seed-size difference between our three groups of strains is due to differences in cell number, cell volume, or both.

Table 2 Correlation between leaf width (cm), leaf length (cm), seed size (g/100 seeds), and genome size (pg per 2C nuclear DNA Content)

Item	Leaf length	Seed size	Genome size
Leaf width	0.88*	0.92*	0.90*
Leaf length	–	0.93*	0.93*
Seed size		–	0.97*

* All correlations were significant at the $\alpha < 0.001$ probability level

In our study, genome size was a good predictor of both leaf and seed size (Fig. 2). However, it should be noted that while large differences in genome size clearly predicted the covariate differences in seed and leaf size (Fig. 2), the prediction becomes less reliable when the genome-size difference between two strains is very small but still repeatable (e.g., compare the strains Charleston and Lancaster for DNA content and seed size).

The positive correlations between genome size, seed size, leaf width, and length seen in this study are similar to those observed in other species by Bennett (1972)

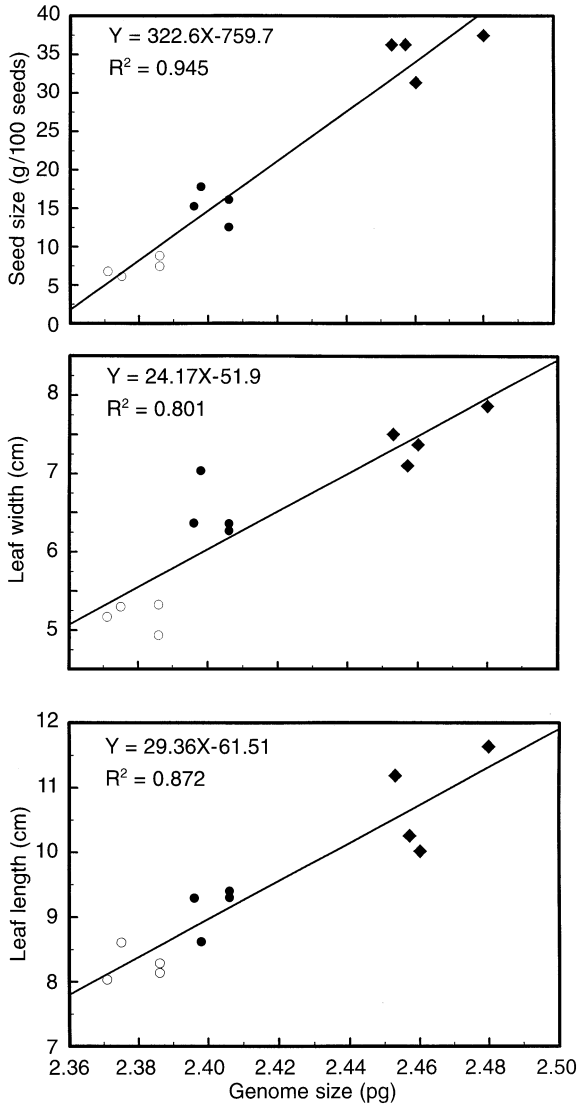

Fig. 2 Relationship between soybean genome size (2C nuclear DNA content) and seed size (top panel), leaf width (middle panel), and leaf length (bottom panel). Symbols represent the soybean strains with large seed (*solid diamond*), small seed (*open circle*), and medium seed (*solid circle*). All regression values (b) were significant at a $\alpha = 0.001$ probability level

and Jones and Brown (1976). Bennett (1972) reported significant positive relationships between DNA amount and seed weight in *Allium* and *Vicia*. Similar positive relationships were seen in *Crepis* (Jones and Brown 1976). However, Biradar et al. (1994) observed a negative correlation between maize genome size and agronomically important yield parameters, such as ear or seed weight and seed number per plant. A similar negative association of genome size and several yield parameters, such as seed weight, was observed in *Senecio* species (Lawrence 1985). The genotypic range in seed size in these prior studies was not as large as that in our study, which could account for the differing results. Graham et al. (1994) observed that, in 20 soybean cultivars representing maturity groups ranging from 000 to IX, soybean genome size was significantly correlated with maturity ($r = 0.55$, $P > 0.01$). We found no association of genome size and maturity in our study, but our strain maturity range was much smaller. The degree of correlation between genome size and agronomically important phenotypic characters may vary depending on the plant species, the phenotypic characters of interest, and the environmental conditions. Specht and Williams (1993) reported that soybean breeders has steadily increased seed size over 75 years of sustained genetic improvement efforts. They estimated the rate of change in seed size as about 0.1 g/100 seed per year. Given the results of our study, which revealed a significant correlation between genome size and seed and leaf size in soybean, it is possible that soybean breeder's selection for greater seed size either led to, or resulted from, greater genome size. If so, this relationship might be worth exploring at a more fundamental level.

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