Theor Appl Genet (1994) 88:429–432

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Relationship between genome size and maturity group in soybean

Received: 17 March 1993 / Accepted: 14 September 1993

Abstract Previous studies have reported a correlation between genome size and relative maturity among plant species. The objective of this study was to determine whether such a relationship exists in soybean. Twenty cultivars, representing maturity groups ranging from 000 to IX, were analyzed using flow cytometric procedures. A 15% difference in genome size was observed ranging from 'BSR 201' at 2.88 pg to 'Maple Presto' at 2.51 pg. A highly significant correlation ($r = 0.55$) was observed between maturity and the genome size of the 20 cultivars.

Key words *Glycine max* · DNA content · Flow cytometry · Maturity

Introduction

Intraspecific variation in nuclear DNA content has been reported for a large number of plant species (Miksche 1971; Price et al. 1980; Laurie and Bennett 1985; Rayburn et al. 1985; Kenton et al. 1986; Watson 1987). Several studies have documented the intraspecific genome size variation observed in soybean (*Glycine max* Merr (L.)). Doerschug et al. (1978) utilized Feulgen reagent to evaluate the DNA content of 11 soybean cultivars and found the DNA content to vary between 1.84 pg and 2.61 pg per 2C nucleus. Bennett (1985) has documented a 50% variation in *Glycine max*. Other researchers have compared genome size variation within the genus *Glycine*. In one study, Hammatt et al. (1991) measured the DNA amounts of 48 species in the *Glycine* genus with flow cytometry procedures. They reported a range in DNA content from 3.80 to 6.59 pg per 4C nucleus, with significant intraspecific variation occurring in *Glycine canescens*. Yamamoto and Nagato (1984) showed a range in DNA content of 3.26 to 5.46 pg in the genus *Glycine*. No correlation between genome size and any plant characteristic has been observed in soybean.

Because DNA amount influences the duration of mitosis (van't Hof 1965; Evans and Rees 1971), it has been proposed that genome size may have an effect on the generation time of an organism (Bennett 1972). Based on this hypothesis, organisms adapted to a shorter growing season would be expected to have less DNA. This relationship has been extensively examined in maize. Rayburn et al. (1985) compared the DNA amount of northern flint maize, US inbred lines, and several Mexican lines and found the northern flint lines to have substantially less DNA. They speculated that this was due to selection for the shorter growing season found in the higher latitudes of North America and for high yield. Rayburn and Auger (1990) and Rayburn (1990) found a significant relationship between genome size and maize lines adapted to different altitudes. Bullock and Rayburn (1991) noted a positive correlation between genome size and effective growing season in these lines. However, Rayburn et al. (1985) reported that when just US inbred lines were analyzed, no significant relationship between growing season and genome size was observed. McMurphy and Rayburn (1991) also observed no significant relationship between genome sizes of 30 commercial corn hybrids and their relative maturity. In both of these cases, the authors concluded that the lack of relationship may be due to the breeding structure of maize in producing both inbred lines and commercial hybrids.

Flow cytometry has been used to determine DNA content in plants (Galbraith et al. 1983) and has been shown to be effective in detecting plant intraspecific DNA content variation (Rayburn et al. 1989; Hammatt et al. 1991). The object of this study was to compare the genome size of 20 soybean cultivars using flow cytometry and to determine whether a relationship exists between genome size and maturity.

Communicated by K. Tsunewaki

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Materials and methods

Twenty cultivars adapted to different maturity zones were selected for this study (Table 1). Cultivars of 000 maturity have the shortest maturity, while cultivars of VII, VIII, and IX have the longest maturity. Seeds of the 20 cultivars were germinated and grown in Terra-Lite® Metro-mix 200 growing medium (Hummert Seed Co., St Louis, Mo.). After 10 days of growth, the plants were placed in the dark for 20–24 h. Nuclei of the soybean plants were isolated as described by Rayburn et al. (1989).

Propidium iodide (PI) was used as a fluorochrome because it has been shown to be an effective stain for estimating DNA content in plants (Arumuganathan and Earle 1991; Michaelson et al. 1991). Nuclei were resuspended in a staining solution consisting of 3% (w/v) polyethylene glycol, 100 µg/ml PI, 9 units/ml RNase A, and 0.1% Triton X-100 in 8 m*M* citrate buffer (pH 7.2). After the nuclei were stained at 37 °C for 20 min, an equal volume of a salt solution, which consisted of the same components as above (excluding RNase) in 0.4 *M* NaCl instead of citrate buffer, was added. Samples were then incubated in the dark for 1 h at 4 °C.

Stained nuclei were analyzed on a Coulter EPICS 751 flow cytometer cell sorter (Coulter Electronics, Hialeah, Fla.). The excitation was provided by a 5-W argon-ion laser. Excitation of the nuclei was at 488 nm with 5000 nuclei per sample being analyzed. Four replicates per cultivar were run.

On each day, two samples of the cultivar 'Burlison' were prepared and set at fluorescence intensity channel 100. The remaining samples were then analyzed, and a genome size relative to 'Burlison' was determined with the results being expressed as arbitrary units (AU). Corn inbred 'W22' was also included in the analysis in order to estimate soybean cultivar genome size. Since 'W22' has 5.35 pg of DNA per 2C nucleus (McMurphy and Rayburn 1991), comparison of the G1 fluorescence channel of 'W22' relative to that of 'Burlison' allowed an estimation of the genome size of 'Burlison'. Pearson's correlation coefficient was run between genome size and numerical maturity group, i.e., 000, 00, ..., IX. This was possible due to the maturity group rating representing the actual maturity.

Results and discussion

When 'W22' and 'Burlison' were compared, the mean of the G1 peak of 'Burlison' was 48% less than that of 'W22' (Fig. 1). Since the genome size of 'W22' has been established to be 5.35 pg, the genome size of 'Burlison' is approximately 2.78 pg. This value is well within the reported range for soybean cultivars (Bennett 1985).

Highly significant differences in the genome sizes of the 20 cultivars were detected (Table 1). DNA content ranged from 2.51 pg for 'Maple Presto' to 2.88 pg for 'BSR 201', a 15% difference. These DNA amounts are also well within the DNA amounts reported by Bennett (1985), but the variation is somewhat less than that reported by Doerschug et al. (1978). It is likely that this discrepancy resulted from the varying modes of action of the fluorochromes used in these different studies. Since PI is an intercalating fluorochrome, chromosome structure affects DNA binding. Chromosome structure has been shown to decrease the sensitivity of detecting intraspecific DNA content variation in maize when the fluorochrome PI is used (Rayburn et al. 1992). This is

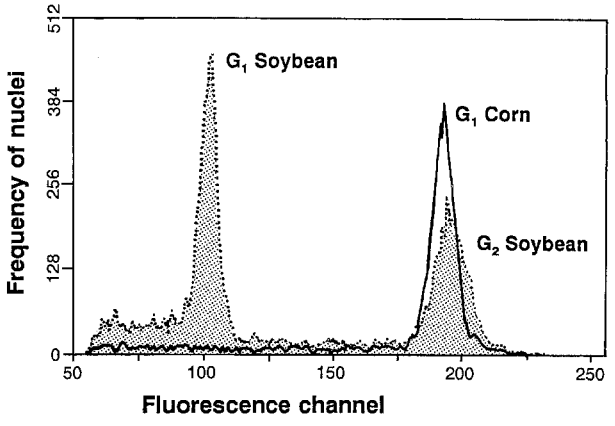

Fig. 1 Overlaid histograms of maize inbred line 'W22' (*open*) and soybean cultivar 'Burlison' (*shaded*)

Table 1 Nuclear DNA content of the 20 soybean cultivars used in the statistical analyses

Cultivar-maturity group	Mean ± SD[a]	Picograms per 2C nucleus	Duncan's grouping[b]
BSR 201 -II	103.37 ± 0.12	2.88	A
Hardee -VIII	102.65 ± 1.26	2.86	AB
Jupiter -IX	101.34 ± 1.23	2.83	ABC
Jack -II	101.21 ± 1.50	2.82	ABC
Hartwig -V	101.11 ± 0.85	2.82	ABC
Bell -I	100.97 ± 1.63	2.81	BC
Aojia -III	99.84 ± 2.39	2.79	CD
Resnick -III	99.66 ± 2.08	2.78	CDE
Gnome 85 -II	99.22 ± 1.15	2.77	CDEF
Maple Ridge- 000	98.45 ± 1.27	2.75	DEFG
Sioux- 000	98.08 ± 1.59	2.74	DEFGH
Agazzi- 0	97.99 ± 0.73	2.73	DEFGH
Crockett- VII	97.47 ± 1.19	2.72	EFGHI
Hood- VI	97.27 ± 1.75	2.71	FGHI
Pando- 000	97.11 ± 1.06	2.71	FGHI
Dawson- 0	96.87 ± 1.60	2.70	GHI
McCall- 00	96.06 ± 0.60	2.68	HI
Corsoy 79- II	95.99 ± 0.59	2.68	HI
Amsoy- II	95.51 ± 1.33	2.66	I
Maple Presto- 000	90.00 ± 1.21	2.51	J

[a] SD, Standard deviation
[b] Genotypes with same letter are not significantly different at the $P = 0.05$ level

due to condensed heterochromatin not being easily accessible to intercalating fluorochromes such as PI. In spite of this, our data paralleled that reported by Doerschug et al. (1978) in that the three cultivars common to both studies ('Amsoy', 'Pando', and 'Sioux') had similar relative rankings in terms of DNA amounts.

Reduction in DNA amounts in maize has reportedly occurred due to selection for populations adapted to shorter growing seasons while maintaining high yields (Rayburn et al. 1985; Bullock and Rayburn 1991). This reduction in DNA content has been related to a decreased mitotic cell-cycle time (Bennett 1972) and is thought to result in plants that reach flowering in a shorter time interval (Rayburn et al. 1985). In soybean, cultivars are grouped according to their general area of adaptation. This grouping consists of the 000 type, which is the earliest maturing and adapted to the higher latitudes, to the X type, which is the latest-maturing type and is adapted to low latitudes (Poehlman 1983).

In this study, soybean genome size was significantly correlated with maturity ($r = 0.55$, $P > 0.01$). The genome size of three cultivars of the early-maturity group 000 ('Maple Presto', 'Sioux', and 'Pando') all had relatively low estimates of genomic DNA (2.51, 2.70, and 2.74, respectively) (Table 1), supporting the hypothesis of Rayburn et al. (1985) that selection for early maturity and high yield reduces nuclear DNA content. However, there were exceptions to the general trend. Group II cultivars 'BSR 201' and 'Jack' had high amounts of DNA (2.88 and 2.82, respectively), and the later-maturing cultivars 'Hood' and 'Crockett' had low DNA amounts. Despite these exceptions, it appears that small genome size may be required for early-maturing cultivars. However, selection pressure on genome size for the later-maturing cultivars may be somewhat relaxed.

Although the positive correlation between genome size and maturity observed in this study is similar those mentioned in other reports (Laurie and Bennett 1985; Rayburn et al. 1985; Kenton et al. 1986), Our study is the first to report a relationship within plant cultivars being used at present. In both the inbred lines used by Rayburn et al. (1985) and the commercial hybrids used by McMurphy and Rayburn (1991) a disruption between genome size and maturity was observed. McMurphy and Rayburn (1991) concluded that breeding for improved maize may have disrupted the evolutionary trend of reducing genome size as time to maturity decreases. In the case of soybean, a recently cultivated crop in the New World, extensive hybridization has not occurred since pedigrees of individual cultivars generally trace back to plant introductions of similar maturity. Consequently, selection in soybean apparently has not had the widespread disrupting effect on genome size as has occurred in maize. In addition, the significant correlation between maturity group and genome size also reflects a significant correlation between latitude and genome size as well.

However, the fact that some early-maturing cultivars had high DNA amounts suggests that hybridization is beginning to have an effect on soybean genome size. 'Jack' and 'BSR 201' (Maturity group II) are examples of this type of hybridization since the pedigrees of both include 'CNS', an ancestor of many late-maturing cultivars (Lohnes and Bernard 1991). If as predicted 'CNS' has a large genome size, this type of hybridization would be expected to increase the amounts of genomic DNA in earlier-maturing cultivars with 'CNS' in their pedigree. We anticipate that additional hybridization will further reduce the relationship between genome size and maturity in soybeans as in the case in corn. An exception to this may be the early-maturing cultivars (Maturity group 000 through Maturity group 0) that require low amounts of DNA to reach maturity.

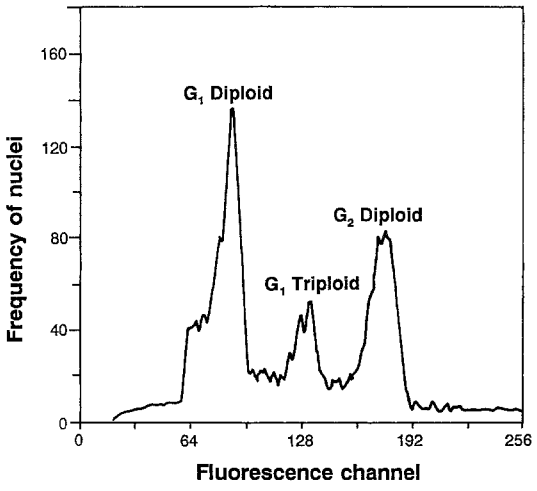

Fig. 2 Histogram representing the nuclei distribution from stems of three 'Maple Presto' plants. The largest G1 peak observed had the diploid DNA amount, while the smaller G1 peak had the triploid DNA amount

In one analysis of 'Maple Presto' two G1 peaks were observed with the second G1 being 0.5 times higher than that of the first G1 peak (Fig. 2). The second G1 peak corresponds to a 3C, triploid, DNA amount. This could result from the fertilization of an unreduced egg with a normal haploid pollen nucleus. This sample apparently contained two diploid plants and one triploid plant. The triploid peak was not considered in the statistical analysis of this cultivar.

The results presented here show that there is a significant variation in genome size in soybean. This variation is related to the maturity of the cultivar, with earlier-maturing lines having smaller genome sizes. This work indicates that the relationship between genome size and maturity is being disrupted by hybridization during the development of modern soybean cultivars.

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