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Change in ribosomal DNA intergenic spacer-length composition in maize recurrent selection populations. 1. Analysis of BS13, BSSS, and BSCB1

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Abstract Five different ribosomal DNA (rDNA) intergenic spacer-length variants (slvs) were detected among the maize inbreds which were the progenitors of Iowa Stiff Stalk Synthetic (BSSS). One rDNA *Sst*I restriction site polymorphism in the 3′ region of the 26S gene was detected. Nine different rDNA intergenic spacer (IGS) hybridization fragment patterns (assigned letter designations A-I) were observed among the BSSS progenitors. Following 7 cycles of half-sib recurrent selection in BSSS using the Ia13 double cross as a tester, hybridization fragment pattern E became predominant in the population. In contrast, 11 cycles of reciprocal recurrent selection in BSSS with the Iowa Corn Borer Synthetic No. 1 (BSCB1) population resulted in hybridization pattern D becoming predominant. Hybridization pattern E is present in the elite inbreds B14, B37, B73, and B84, which were derived from different cycles of the BSSS half-sib recurrent selection program with Ia13. Hybridization pattern D is present in the elite inbreds B89 and B94, which were derived from different cycles of the BSSS reciprocal recurrent selection program with BSCB1. Therefore, two different forms of recurrent selection on BSSS resulted in different hybridization patterns becoming predominant in the selected populations and present in elite inbreds derived from the populations. These results also suggest that rDNA IGS hybridization fragment patterns D and E, which both have the longest slv detected, may have a selective or adaptive advantage in BSSS materials grown in the Corn Belt.

Key words Ribosomal DNA · Intergenic spacer · Spacer-length variant · *Zea mays* L. · Recurrent selection

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Introduction

Variation in the genetic and molecular organization of ribosomal RNA (rRNA) genes in higher organisms may be associated with adaptive or selective advantages (Cluster et al. 1987; Allard et al. 1990; Sagahai-Maroof et al. 1990). The highly conserved rRNA genes of eukaryotes are organized as a family of tandemly repeated ribosomal DNA (rDNA) arrays (for reviews, see Long and Dawid 1980; Jorgensen and Cluster 1988). rRNA is transcribed from rDNA as a single precursor RNA and is processed into the mature 17S, 5.8S and 26S subunits of the cytosolic ribosome complexes where messenger RNAs are translated into gene products. Each repeat of rDNA (Fig. 1) has a transcription unit coding for 17S, 5.8S and 26S rRNAs and an intergenic spacer (IGS) with transcriptional regulatory elements (for reviews, see Reeder 1989; Sollner-Webb and Mougey 1990). The tandemly repeated rDNA arrays are present at one or a few chromosomal locations in eukaryotes. The copy number of the rDNA repeats varies among maize (*Zea mays* L.) genotypes from approximately 5,000 to 12,000 per 2c nucleus, and these arrays are located at a single nucleolar organizer region (NOR) on chromosome arm 6S (Phillips 1978).

Within the IGS exists another series of tandemly repeated sequences that are referred to as subrepeats (Fig. 1). In higher plants the subrepeats are generally 100–400 base pairs (bp) long and frequently do not vary much in sequence length within a species, but are quite divergent between species. The number of subrepeats in the IGS is often highly variable within plant species, and this variation alters the length of the IGS (for review, see Rogers and Bendich 1987). However, despite considerable sequence and length divergence of the IGS region among eukaryotes, conserved secondary structure of the IGS region appears to be maintained (Baldbridge et al. 1992).

In *Xenopus laevis*, higher numbers of subrepeats in the IGS have been associated with higher levels of

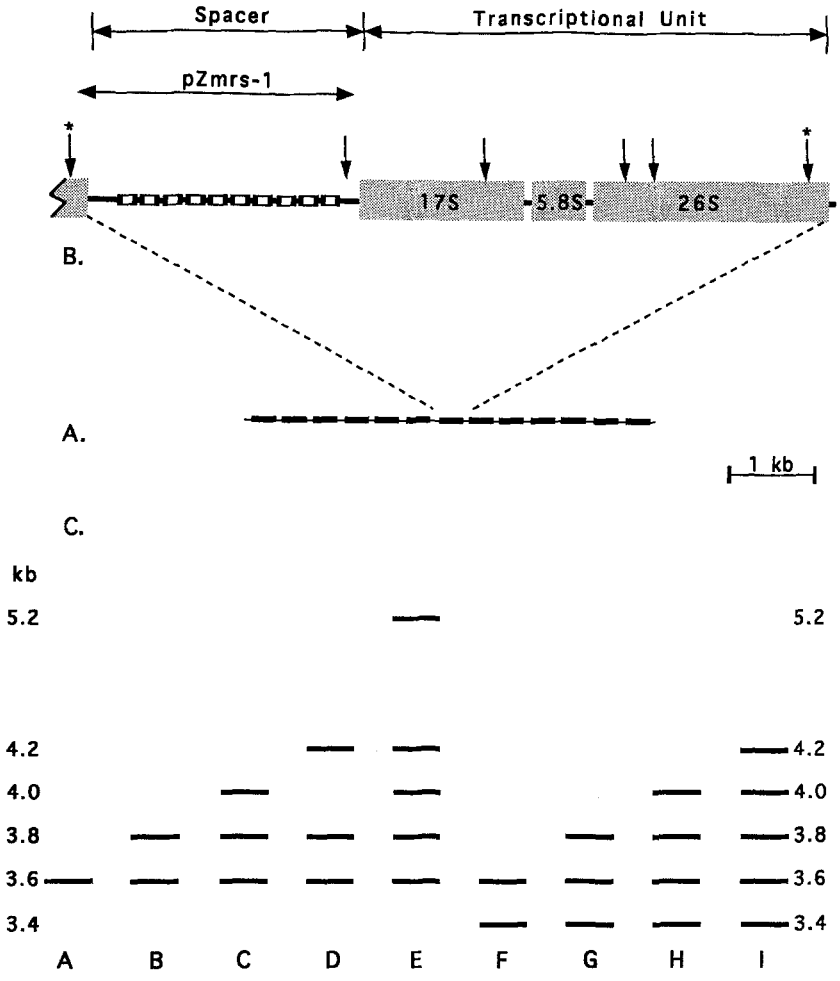

Fig. 1A–C **A** Schematic representation of a tandem array of maize rDNA repeats. **B** Detailed diagram of single rDNA repeat. The transcriptional units coding for the 17S, 5.8S and 26S rRNAs are indicated by the *large filled rectangles*. Subrepeats (approximately 200 bp) in the intergenic spacer are indicated by *small dark unfilled rectangles*. *Sst*I restriction enzyme recognition sites are indicated by *arrows* and the * denotes the *Sst*I recognition site that when absent results in detection of a 5.2-kb hybridization fragment with pZmrs-1 (McMullen et al. 1986). **C** Schematic representation of the different rDNA IGS hybridization fragment patterns detected with pZmrs-1 among the BSSS progenitors to illustrate how the number and organization of different intergenic spacer-length fragments were used to assign designations. (Please note actual distances between fragments are not linear and copy number differences of fragments are not represented)

rDNA transcription in vivo (for review, see Reeder 1984). The subrepeats in *X. laevis* have some homology to the rDNA promoter and have been proposed to serve as enhancers of rDNA transcription. For maize, although DNA sequences within the approximately 200-bp-long IGS subrepeats are not highly homologous to the maize rDNA promoter, the maize subrepeat DNA sequences are organized in a manner that has been hypothesized to function in the enhancement of transcription of rDNA (McMullen et al. 1986; Toloczyki and Feix 1986). In addition to the association of enhanced rDNA transcription with higher numbers of subrepeats in the IGS, there may be DNA sequence differences in specific subrepeats that influence rDNA transcription (Reeder 1989; Allard et al. 1990).

rDNA IGS length variants have served as markers in inheritance studies to assign rDNA allelic designations for barley (*Hordeum vulgare*) (Allard et al. 1990). Natural selection has influenced rDNA allelic frequencies in both wild barley (*H. vulgare* ssp. *spontaneum*) and cultivated barley (*H. vulgare* ssp. *vulgare*) populations. Significant correlations were found between the presence of specific rDNA alleles in wild barley accessions sampled from ecologically diverse habitats in the Middle East and cultivated barley accessions sampled from throughout the world and factors of the physical environment where accessions were collected (Saghai-Maroof et al. 1990). Similarly, the rDNA intergenic spacer-length variant (slv) diversities of wild population accessions of tetraploid wild emmer wheat (*Triticum dicoccoides*) sampled from diverse ecological environments in Israel were significantly correlated with climatic variables associated with the different sampling sites (Flavell et al. 1986). In CCII, a cultivated barley composite cross population which underwent 54 generations of natural selection in the Mediterranean-like Davis, California environment, an rDNA allele associated with adaptation to Mediterranean climate that was infrequent in cycle 0 became predominant in cycle 54. An rDNA allele associated with adaptation to non-Mediterranean climates that was frequent in cycle 0 became infrequent in cycle 54 of CCII (Saghai-Maroof et al. 1984; Allard et al. 1990; Saghai-Maroof et al. 1990).

Directional selection of insect and plant populations has been associated with a change in slv frequencies. In *Drosophila melanogaster*, significantly different slv frequencies developed between two subpopulations selected from a wild base population for fast and slow pre-adult emergence development rate (Cluster et al. 1987). The longer slvs increased in frequency in the fast

subpopulation, and the shorter slvs increased in frequency in the slow subpopulation. In maize, a subpopulation of the open-pollinated variety "Hays Golden" created by mass selection for high grain yield had a significant reduction in the frequency of the shortest slv (Rocheford et al. 1990).

None of the experiments reviewed above involve populations selected for combining ability for grain yield. The goal of the research presented here was to investigate the effect of half-sib recurrent selection for combining ability for grain yield on rDNA IGS hybridization fragment frequencies.

Materials and methods

Genetic materials

The Iowa Stiff Stalk Synthetic (BSSS), which underwent selection for grain yield, maturity, and standability using (1) half-sib selection with the Ia13 double cross as a tester and (2) half-sib reciprocal recurrent selection with the Iowa Corn Borer Synthetic No. 1 (BSCB1), was chosen for evaluation (Hallauer and Miranda 1988). The inbreds chosen by G. F. Sprague to create BSSS (Table 1) were evaluated to identify the rDNA IGS hybridization fragment patterns that were contributed to BSSS cycle 0. The BSSS population was developed in the 1930s by intercrossing 16 inbreds (Sprague 1946). The primary selection criterion was stiffness of stalk, and the secondary selection criterion was combining ability for grain yield. After intercrossing the 16 inbreds, the population was intermated for an uncertain number of generations before it was designated BSSS cycle 0 and used for selection studies (Hallauer and Miranda 1988). The 16 inbreds available for this study consist of 14 of the 16 original progenitor inbreds of BSSS (F1B1-7-1 and C.I. 617 are not available) and the 2 parental inbreds of the missing F1B1-7-1 (Table 1). These 16 available inbreds are designated as the BSSS progenitors, consistent with a previous BSSS study (Messmer et al. 1991).

Half-sib family selection of BSSS was conducted using Ia13, a double-cross hybrid [(L317 × BL349) × (BL345 × Mc401)], as the tester. Approximately 100 half-sib families were evaluated in each cycle, and the top 10 performing families were selected for intermating to develop the next cycle. Seven cycles of half-sib family selection were conducted to produce the population designated BS13 (HT) C7. BSSS cycle 0 and BSCB1 cycle 0 were base populations in a half-sib reciprocal recurrent selection program that involved testing approximately 100 half-sib progenies of the two populations in each cycle, with the top 10 performing families of each population intermated to form the next cycle of selection. These procedures were followed for five cycles and are described by Penny and Eberhardt (1971). The procedures were modified in subsequent cycles and included intermating more than 10 individuals, using S_1 progenies instead of S_0 plants, and changing from half-sib to full-sib selection in cycles 10 and 11 as described in Keeratinijakal and Lamkey (1993). Eleven cycles of these selection procedures resulted in BSSS(R)C11 and BSCB1(R)C11.

A set of elite inbreds derived from different cycles of BS13 (HT) and BSSS (R) was chosen for evaluation by W.A. Russell (Table 1). All genetic materials were kindly provided by W.A. Russell and A.R. Hallauer, Iowa State University.

DNA preparation and chemiluminescent detection of rDNA alleles

Most DNA samples were isolated according to methods described in Rocheford et al. (1990), and some samples were isolated according to methods described in Saghai-Maroof et al. (1984). Approximately 2.5 µg DNA was digested with the restriction enzyme *Sst*I. The two *Sst*I restriction sites that nearly flank the rDNA IGS region (Fig. 1) facilitate detection of IGS-specific length variability. pZmrs-1, the 3.2-kb clone of the maize rDNA IGS (Fig. 1) was used for Southern analyses and was provided by I. Rubenstein, Department of Genetics and Cell Biology, University of Minnesota (McMullen et al. 1986). Chemiluminescent detection of IGS variability was performed for most of the Southern analyses according to published procedures (Kreike et al. 1990; Rocheford and Wallace 1991). A small portion of the Southern analyses were performed according to standard autoradiography procedures (Sambrook et al. 1989).

Designation of rDNA hybridization fragment patterns

In accordance with terminology previously established for barley (Saghai-Maroof et al. 1984; Allard et al. 1990). rDNA IGS hybridization fragments were scored and used to identify slvs and hybridization fragment patterns present in the BSSS progenitors (Figs. 1, 2). The term slv refers to a specific hybridization fragment length associated with intergenic spacer variability. Additionally, a *Sst*I restriction site polymorphism in the 3′ region of the rDNA 26S gene (Fig. 1) was associated with detection of a 5.2-kb hybridization fragment. This fragment consists of the 3.6-kb slv plus a 1.6-kb 26S gene fragment. Collectively, presence of the different slvs and the 5.2-kb hybridization fragment make up the overall rDNA IGS hybridization fragment patterns (Figs. 1, 2). The 5.2-kb fragment was used in the designation of hybridization fragment pattern E, although pattern E is actually unique without inclusion of the 5.2-kb fragment. The loss of the *Sst*I site in the 26S gene technically does not involve the IGS region, but it does involve DNA variation detected by IGS probe pZmrs-1, and the 5.2-kb fragment facilitated scoring of IGS hybridization fragment patterns. Similar to barley (Allard et al. 1990), the rDNA IGS hybridization fragment patterns in maize appear to be inherited as a single unit, but this has not been confirmed with inheritance studies.

Table 1 rDNA intergenic spacer (IGS) hybridization fragment pattern present in maize inbreds used to synthesize Iowa Stiff Stalk Synthetic (BSSS) (Messmer et al. 1991) and in elite inbreds derived from different cycles of two recurrent selection experiments on BSSS

Inbred	Background	IGS Pattern
Progenitors	*Non-Reid group*	
A3G-3-1-3	Unknown	I
CI.540	Illinois Two Ear	E
Ill. 12E	Unknown	H
Ill. Hy	Illinois High Yield	E
LE23	Illinois Low Ear	D
Oh3167B	Echelberger Clarage	D
	Reid Yellow Dent group	
CI.187-2	Krug, Nebr. Reid × Iowa Gold Mine	G
I159	Iodent, Reid Yellow Dent	A
I224	Iodent, Reid Yellow Dent	B
Ind. 461-3	Reid medium (Duddleston no. 461)	F
Ind. AH83	Funks 176A, Reid Yellow Dent	I
Ind. B2[a]	Reid Yellow Dent	G
Ind. Fe2[a]	Reid Early	D
Ind. TR9-1-1-6	Reid Early Dent (Troyer strain)	F
Os420	Osterland 420	G
Wd456	Walden Dent, Reid Yellow Dent	C
Elite BSSS	*Source Population*	
B14	BS13 (HT) Cycle 0	E
B37	BS13 (HT) Cycle 0	E
B72	BS13 (HT) Cycle 3	D
B73	BS13 (HT) Cycle 5	E
B78	BS13 (HT) Cycle 6	E
B84	BS13 (HT) Cycle 7	E
B89	BSSS (R) Cycle 7	D
B94	BSSS (R) Cycle 8	D

[a] Parent of inbred F1B1-7-1

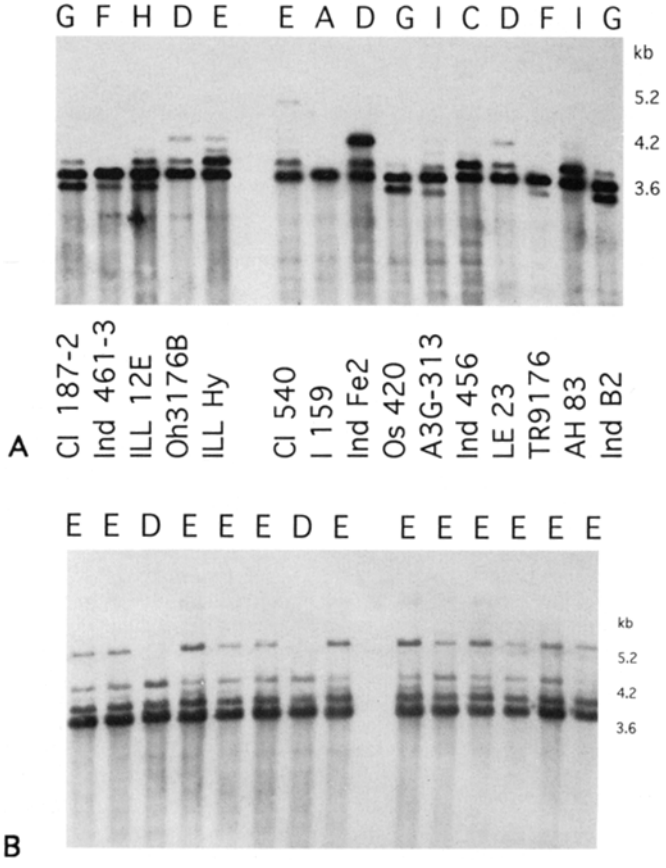

Fig. 2A, B **A** rDNA intergenic spacer (IGS) hybridization fragment patterns detected for the progenitor inbreds of Iowa Stiff Stalk Synthetic with probe pZmrs-1 (McMullen et al. 1986) with designations given in *letters*. Inbred I224 which has pattern **B** is not shown. **B** rDNA IGS hybridization patterns detected with pZmrs-1 for plants sampled from BS13(HT)C7

Determination of rDNA IGS hybridization fragment pattern frequencies

The frequencies of the different rDNA IGS hybridization fragment patterns detected among the BSSS inbred progenitors were used as an approximate estimate for BSSS cycle 0. This was done rather than probing BSSS cycle 0 since: (1) the unique overlapping nature of different hybridization patterns prevented reliable assignment of designations in heterozygous plants, and (2) natural selection during periodic seed increases of BSSS cycle 0 may have influenced hybridization pattern frequencies. Natural selection during cycles of random mating of the BSSS progenitors may have also occurred before these materials were designated BSSS cycle 0. Therefore, this study also includes possible selection effects on BSSS progenitor hybridization pattern frequencies during this initial random mating process. Cycle 0 of BSCB1 was evaluated for rDNA IGS variation because the progenitor inbreds of BSCB1 were not available for analysis at the time this study was performed. Due to the overlapping and very heterogeneous hybridization patterns observed among plants, it was not possible to assign rDNA IGS hybridization fragment pattern designations in BSCB1 cycle 0. Therefore, the frequency of presence of each individual hybridization fragment was determined in plants sampled from BSCB1 cycle 0 and 11. The individual rDNA IGS hybridization fragment frequencies were also determined for the BSSS progenitors, BS13(HT)C7, BSSS(R)C11, and Ia13. (Please note that the 3.4- to 4.2-kb rDNA IGS hybridization fragments are also termed slvs). The numbers of plants used to estimate frequencies were 49 for BS13 (HT) C7, 52 for BSSS (R) C11, 54 for BSCB1(R)C0 and 59 for BSCB1(R) C11. For a sample of 52 plants from a populations, a 0.005 probability exists of missing an allele with a frequency of 0.05 in the population (Falconer 1989).

Statistical analyses

Chi-square analyses for homogeneity were performed to determine whether individual rDNA IGS hybridization fragment frequency differences between populations were significantly different (Steel and Torrie 1980). The standard chi-square calculation for a $2 \times n$ contingency table was used (SAS 1988). Chi-square analyses were not performed between materials whose frequencies were estimated from inbreds (BSSS progenitors, Ia13) versus heterozygous plants (BS13 (HT) C7, BSSS (R) C11, BSCB1 (R) C0, and BSCB1 (R) C11). This was due to overlapping rDNA IGS hybridization patterns frequently preventing the accurate scoring of heterozygotes versus homozygotes. Calculations of the probability that genetic drift effects alone account for hybridization pattern frequency changes are not reported because accurate determination of their frequencies was not possible due to their overlapping nature.

Results and discussion

rDNA IGS variation in BSSS recurrent selection materials

A ladder of slvs differing by approximately 200-bp intervals from 3.4 kb to 4.2 kb and a 5.2-kb hybridization fragment resulting from loss of the *Sst*I restriction site in the 3′ region of the 26S gene were detected (Figs. 1, 2). Collectively, nine different rDNA IGS hybridization fragment patterns (designated A-I) were present among the BSSS progenitors (Fig. 2). The hybridization patterns were similar to those detected previously in maize (Zimmer et al. 1988; Rocheford et al. 1990). The frequencies of the hybridization patterns present among the BSSS progenitors were all relatively low, ranging from 0.06 to 0.19 (Table 2). Therefore, considerable molecular variation in the IGS was present among the BSSS progenitors so that no hybridization pattern predominated but many hybridization patterns were present at low frequencies.

Evaluation of BS13(HT)C7 detected only three different rDNA IGS hybridization fragment patterns. In a population, the observed hybridization fragments are the result of the presence of the homozygous condition for one hybridization pattern or a heterozygous condition for two different hybridization patterns. The hybridization patterns that are most likely present in BS13 (HT)C7 and produced the observed hybridization fragments are A, D, and E. Hybridization pattern E, present in 94% (46/49) of the plants sampled from BS13(HT)C7, was predominant. Hybridization pattern D was present in 2/49 plants, and hybridization pattern A was present in 1/49 plants (Table 2). A luminograph of a sample of plants from BS13(HT)C7 is shown in Fig. 2. Due to the overlapping nature of the hybridization patterns, it was not possible to confidently distinguish an E/E hybridization fragment pattern from an E/A or E/D hybridization fragment pattern. Nevertheless, hybridization pattern E has changed from being present in 2 of 16

Table 2 Frequency of rDNA intergenic spacer (IGS) hybridization fragment patterns (A), rDNA intergenic spacer-length variants (slvs), and the 5.2-kb hybridization fragment (B) for Iowa Stiff Stalk Synthetic (BSSS) materials and corresponding tester materials. (Please note that slvs are not allelic and their frequencies therefore do not add up to one)

A	*Frequency of IGS hybridization fragment patterns*								
	A	B	C	D	E	F	G	H	I
BSSS progenitors	0.06	0.06	0.06	0.19	0.12	0.12	0.19	0.06	0.12
Ia13	0.75			0.25					

B	*Frequency of slvs and 5.2-kb hybridization fragment*					
Kilobases →	3.4	3.6	3.8	4.0	4.2	5.2
BSSS progenitors	0.50	1.00	0.81	0.38	0.44	0.12
BS13(HT)C7	0.00	1.00	0.98	0.94	0.98	0.94
BSSS(R)C11	0.27	1.00	0.94	0.00	0.94	0.00
Ia13	0.00	1.00	0.25	0.00	0.25	0.00
BSCB1(R)C0	0.30	1.00	0.56	0.00	0.30	0.39
BSCB1(R)C11	0.77	1.00	0.90	0.00	0.37	0.56

BSSS progenitors to being present in 46 of 49 BS13(HT)C7 plants, suggestive of a very large increase in frequency. Comparison of the frequency of individual slvs in the BSSS progenitors and BS13(HT)C7 suggested that the 3.4-kb slv may be lost from the population. The frequencies of the 3.8-, 4.0-, and 4.2-kb slvs and the 5.2-kb hybridization fragment were all higher in BS13(HT)C7 than among the BSSS progenitors (Table 2).

Evaluation of BSSS(R)C11 detected three different rDNA IGS hybridization fragment patterns. The hybridization patterns which most likely are responsible for the observed hybridization fragments are D and F. Hybridization pattern D appeared to be homozygous in 38/52 plants, hybridization pattern B appeared to be homozygous in 3/52 plants, and a heterozygote of D/F appeared to be present in 11/52 plants. Hybridization pattern D had become predominant in this population since it was present in 49 of 52 plants. However, the possibility exists that other hybridization patterns are actually present in BSSS(R)C11 but were masked by the D and F hybridization patterns. Comparison of the frequency of slvs in the BSSS progenitors with BSSS(R) C11 suggests that the 4.2-kb slv is higher and the 3.4-kb slv is lower in frequency in BSSS(R)C11 and that the 4.0-kb slv may be lost from the population (Table 2). The 5.2-kb hybridization fragment also appears to be lost from the population.

Evaluation of BSSS tester materials

Although alterations in the rDNA IGS composition of BSSS were associated with two recurrent selection programs, it is important to consider that BSSS plants per se were not evaluated for yield and agronomic performance. Testcross family performance was the criterion used for selecting the superior 10 BSSS plants for intermating to create the next cycle for recurrent selection. The tester populations were therefore evaluated for rDNA IGS composition since the tester contributes one of the two chromosome 6 homologues containing the rDNA repeats present in testcross progeny.

For Ia13, hybridization pattern D was present in one parent and hybridization pattern A was present in three parents. The pattern that became predominant in BS13 (HT)C7 is E, which is not present in the Ia13 tester. The 3.8-, 4.0-, and 4.2-kb slvs and the 5.2-kb hybridization fragment were present at a high frequency (0.94–0.98) in BS13(HT)C7 but absent or present at a low frequency (0.25) in Ia13. Both Ia13 and BS13(HT)C7 had the 3.6-kb slv present in all plants; this slv has been found to be present in almost all maize plants sampled so far (Zimmer et al. 1988; Jupe and Zimmer 1990; Rocheford et al. 1990). These observations suggest that a heterozygous slv composition may be selectively advantageous. Alternatively, since the tester was constant, selection (and/or genetic drift) could only act on the BSSS population, and hybridization pattern E may have had the highest selective value.

For the half-sib reciprocal recurrent selection program the tester, BSCB1, was synthesized from 12 inbreds (Hallauer and Miranda 1988) and is therefore broader based than Ia13. In contrast to the constant Ia13 tester, BSCB1(R) also underwent selection with each cycle of reciprocal recurrent selection of BSSS(R). Comparison of BSCB1(R)C0 and BSCB1(R)C11 revealed that a significant change in overall rDNA IGS hybridization fragment frequencies occurred ($\chi^2 = 47.7$, $P < .001$). This change in IGS hybridization fragment composition was due largely to a significant increase in the presence of the 3.4-kb slv ($\chi^2 = 26.6$, $P < .001$) and 3.8-kb slv ($\chi^2 = 17.0$, $P < .001$), and a nearly significant increase in the 5.2-kb hybridization fragment ($\chi^2 = 3.3$, $P = 0.069$) between BSCB1(R)C0 and BSCB1(R)C11.

Comparison of IGS hybridization fragment frequencies of BSSS(R)C11 with BSCB1(R)C11 was associated with a large chi-square value ($\chi^2 = 110.0$, $P < 0.001$) as was the comparison of BSSS(R)C11 with BSCB1(R)C0 ($\chi^2 = 93.0$, $P < 0.001$). The difference in overall IGS hybridization fragment composition between BSSS(R) C11 and BSCB1(R)C11 was due primarily to differences in the frequency of the 3.4-kb slv ($\chi^2 = 29.0$, $P < 0.001$), the 4.2-kb slv ($\chi^2 = 38.9$, $P < 0.001$) and the 5.2-kb hybridization fragment ($\chi^2 = 41.3$, $P < 0.001$). The differences in rDNA slv frequencies of BSSS(R)C11 and BSCB1(R)C11 also suggest that a heterozygous condition for slvs might be selectively advantageous.

The BSCB1(R) tester population differs considerably from the Ia13 tester since it (1) contains more and different rDNA IGS hybridization fragment variability, (2) underwent a significant change in hybridization fragment composition between cycle 0 and cycle 11, and (3) has a different overall genetic background that also

probably changed during the cycles of selection. Eleven cycles of reciprocal recurrent selection on BSSS (R) with BSCB1 (R) was associated with hybridization pattern D becoming predominant in BSSS (R) rather than hybridization pattern E, which became predominant in BS13 (HT) C7. Thus, the use of a different recurrent selection method and a tester population with a different rDNA IGS hybridization fragment composition than Ia13 resulted in a different hybridization pattern becoming predominant in BSSS.

Evaluation of elite inbreds derived from BSSS

In the sample of elite inbreds derived from different cycles of selection of BS13(HT) and BSSS(R) only rDNA IGS hybridization fragment patterns D and E were found (Table 1). The hybridization pattern present in the elite lines was with one exception (B72) the same pattern that became predominant in the BSSS source population from which the lines were derived. Hybridization pattern E is present in the BSSS progenitors C.I.540 and Ill.Hy, and neither of these inbreds are in the Reid Yellow Dent group (Table 1). Hybridization pattern D is present in the BSSS progenitors LE23 and Oh3167B, which also are not in the Reid Yellow Dent group. Hybridization pattern D is present in Ind. Fe2 (parent of F1B1-7-1), which is in the Reid Yellow Dent group. However, although copy number was not used in the assignment of hybridization pattern designations, pattern D in Ind. Fe2 has a very high-copy number 4.2-kb slv (Fig. 2) and this high-copy number 4.2-kb slv is not present in the elite inbreds. Therefore, the hybridization pattern D present in the elite inbreds likely did not come from Ind. Fe2 but from LE23 or Oh3167B. These interpretations assume that hybridization pattern D or E did not come from the missing C.I.617 progenitor inbred.

The detection of only hybridization patterns D and E in the elite inbreds derived from BS13 (HT) and BSSS (R) suggests a hypothesis that these patterns are associated with a selective or adaptive advantage in BSSS germ plasm grown in the Corn Belt. The strongest evidence may come from elite inbreds B14 and B37, both of which were selected from BSSS cycle 0 and have pattern E. B14 and B37 are historically important inbreds that have been used in the production of high-yielding F_1 hybrids grown in the Corn Belt. The frequency of pattern E among the BSSS progenitors was estimated to be only 0.12. If this same frequency is assumed for BSSS cycle 0, the probability of any two inbreds derived from BSSS cycle 0 having the same pattern E by chance is only 0.014.

Basis for selective or adaptive advantage of maize rDNA slvs

What are the underlying mechanisms that might support a hypothesis of a selective or adaptive advantage for rDNA IGS patterns D and E? Longer rDNA intergenic spacers have been associated with higher transcription rates in *Xenopus laevis* (Reeder 1984) and more rapid development rates in *Drosophila melanogaster* (Cluster et al. 1987). The rDNA IGS patterns D and E, which became predominant in the selected BSSS populations and are present in the elite inbreds selected on superior testcross performance, have longer intergenic spacers. If longer intergenic spacers enhance rDNA transcription in maize, the resulting increase in ribosomes may enable increased synthesis of enzymes and proteins during the grain-filling period or other critical developmental stages of testcross progeny.

The presence of certain DNA sequences in the IGS also has been proposed as a basis for selective or adaptive advantages of slvs in barley (Allard et al. 1990). This hypothesis is supported by the finding that the predominant slvs detected at both *Rrn1* and *Rrn2* in wild and domesticated barley are not the longest spacers present at these two loci. Rather than the length of the IGS providing a selective advantage, there may be specific sequences in the spacer associated with a selective advantage. This may relate to the presence of rDNA IGS pattern A in BS13(HT)C7, pattern F in BSSS(R)C11, and the high frequency of the shorter 3.4-kb slv in BSCB1(R)C11. This question will be addressed by sequencing different slvs.

Specific sequences in the rDNA transcription units may also offer a selective or adaptive advantage (Allard et al. 1990). The different IGS hybridization fragment patterns may simply serve as markers of different rDNA transcriptional unit alleles. Studies on maize and its putative ancestors have demonstrated that restriction endonuclease site variation is more readily detected in the IGS region than the highly conserved rDNA transcriptional units (Zimmer et al. 1988; Jupe et al. 1990). In this study, the 4.2-kb slv was also present in all BSSS plants that showed the 5.2-kb hybridization fragment resulting from loss of the *Sst*I restriction site in the 26S transcriptional unit. The nucleotide change at the *Sst*I restriction site in the 26S transcriptional unit may be present in the same rDNA repeats that have the 4.2-kb slv. The specific nucleotide change in the 26S transcriptional unit causing the *Sst*I site polymorphism may not be associated with a selective advantage. However, the observation of a nucleotide difference in a transcriptional unit co-segregating with a specific slv does provide evidence for IGS length variability serving as a marker for DNA sequence variation in a transcriptional unit.

Sequences flanking the rDNA repeats should be considered in evaluating changes in IGS hybridization pattern frequencies. Sequences linked to the rDNA repeats may be under selection and may influence IGS hybridization fragment frequencies in populations. Evaluation of linkage relationships of single-copy molecular markers flanking different rDNA IGS hybridization fragment patterns in selected populations would address this question. This single-copy molecular

marker information could also be used to estimate genetic drift effects on the chromosome arm 6S region and specific rDNA IGS hybridization fragment patterns since genetic drift effects have been observed in the populations studied in this paper (Smith 1983; Helms et al. 1989; Keeratinijakal and Lamkey 1993). Similarly, the whole genome of both parents of testcross families under evaluation should be considered since loci located throughout the genome of either parent may have effects that indirectly or directly influence selection at the rDNA locus (Clegg et al. 1978; Saghai-Maroof et al. 1984). Development of near-isogenic lines for different rDNA IGS hybridization fragment patterns is in progress, and these lines will be evaluated in hybrid combination with genetically diverse testers.

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