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correlation of the TBA scores of the F_2 plants with their linolenic acid contents. There was considerable variation in linolenic acid content among plants with similar TBA scores (Table 1). In Cross 1, the single individual with a TBA score of 1 had a greater linolenic acid content than 31 of the other plants in the population. The TBA scores of the F_2 plants with the least linolenic acid contents were 2.5 in Cross 1, and 1.0 and 1.5 in Cross 2 (Table 2).

The association between TBA scores and linolenic acid contents observed in this study indicated that the TBA test cannot be used as a substitute for GC analyses in evaluating soybean seed samples. However, it may be a useful method to rapidly eliminate genotypes with excessively high linolenic acid content before GC analysis. The maximum acceptable score for a group of lines would be determined by rating a random sample of the lines by the TBA test and evaluating the same samples by GC analysis. The maximum acceptable score would include the majority of the lines with an acceptable linolenic acid content. For example, if an acceptable linolenic acid content in our study was 30 g kg^{-1} or less, the maximum acceptable TBA score would be 2.5 (Tables 1 and 2).

The TBA test can be conducted more rapidly and at a lower cost than the GC analysis. The rate at which the TBA test can be conducted is primarily limited by personnel. In our laboratory, four people working together can analyze about 150 samples per hour with the TBA test. One person identifies the samples and counts the seed into the template. The second person assists in counting seed and crushes the samples. The third person treats the oil spots on the filter paper, and the fourth person scores the samples. Samples selected by the TBA test are analyzed by the GC. The rate at which the linolenic acid content can be measured is limited primarily by the number and type of GC available. A GC with dual analysis columns and an automatic injector can analyze a sample about every 3 min. Two people are needed to prepare the samples for the GC.

Table 2. Seven soybean plants (10%) in each cross with the least linolenic acid content and their TBA scores.

Cross no.	Plant number	Linolenic acid content	TBA
		g kg^{-1}	score†
1	466028	26	2.5
1	466007	28	1.5
1	466081	30	2.5
1	465067	30	2.5
1	465070	31	2.5
1	465022	31	1.5
1	465061	31‡	1.5
LSD (0.05)		7	1.5
2	468076	29	1.0
2	468087	29	1.5
2	468034	30	1.0
2	467019	30	2.5
2	468017	30	1.5
2	468003	30	2.5
2	467052	31‡	2.5
LSD (0.05)		7	1.2

† Score of 1 was equal to the color of the parent A5 and a score of 5 was equal to the color of the high-yielding parent cultivar.
‡ Plants were ranked for linolenic acid content measured to 1 g kg^{-1}.

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POLLEN FERTILITY OF SOME SIMPLE AND COMPOUND TRANSLOCATIONS OF COTTON

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Abstract

Pollen semisterility can serve as a useful criterion for detecting cytogenetic deficiencies and heterozygosity of cytogenetic aberrations in many diploid crops. A modified form of the fluorescein diacetate-based fluorochrome reaction method was recently developed and found to allow reliable detection of cytogenetically induced pollen semisterility of cotton (*Gossypium hirsutum* L., $2n = 4x = 52$) simple reciprocal translocation heterozygotes. We report the pollen fertility levels of plants either homozygous (TT) or heterozygous (NT) for 20 translocations, including 16 two-chromosome, three three-chromosome, and one four-chromosome translocations. Fertility ranged from 90 to nearly 100% for TT and from 52 to 81% for NT plants, indicating that TT and NT cytogenetic types in testcross populations that are segregating for a single translocation should be distinguishable on the basis of pollen fertility. Thus, all 45 breakpoints of the screened translocations are of potential use as genetic markers. These and previously published results demonstrate collectively that all identified translocations of *G. hirsutum* are amenable to pollen analysis by fluorescence microscopy, even though the species is a disomic tetraploid.

THE GENETIC MAP of cotton includes 65 gene loci governing various traits (Endrizzi et al., 1984). Twelve of 16 linkage groups have been assigned to

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specific chromosomes, and two others have been assigned to a genomic group, A or D. Menzel and Brown (1978a) and Brown (1980) reported 58 simple reciprocal translocation stocks. These stocks, as well as four complex translocation stocks (including three three-chromosome translocations and one four-chromosome translocation), are maintained in our laboratory. The breakpoint positions have previously been delimited to specific chromosomes and, in most instances, to specific chromosome arms. Genetic positions of most of the translocation breakpoints have been mapped cytogenetically (Menzel and Brown, 1978a,b; Menzel et al., 1985). The translocations collectively involve 25 of the 26 chromosomes and 43 of the 52 arms of *G. hirsutum*.

The translocation breakpoints offer 129 potential genetic markers on 25 of the 26 chromosomes. However, their usefulness as markers has been limited by our inability to reliably classify cytogenetic types of segregating populations by means other than meiotic analysis, which is expensive and time-consuming. The fluorochrome reaction (FCR) method (Heslop-Harrison and Heslop-Harrison, 1970; Heslop-Harrison et al., 1984) was recently modified for use on cotton pollen, and its effectiveness demonstrated on 43 identified simple translocations and one translocation (TT14-26) of unconfirmed identity (Gwyn and Stelly, 1989), raising the number of mapped genetic markers (Endrizzi et al., 1984) in cotton by 86, i.e., ~130%. Environmental and environment × genotype effects on pollen fertility were found to be significant, but minute relative to effects of the genotype (i.e. translocation homozygosity vs. heterozygosity).

In the present work we tried to extend the number of translocations shown amenable to the FCR method, so as to further increase the number of usable genetic markers in this important crop species.

Materials and Methods

Sixteen translocation homozygotes (Table 1) of the Cotton Cytogenetics Collection, maintained at Texas A&M University, were selfed and crossed in 1987 with TM-1, the genetic and cytogenetic standard for *G. hirsutum* (Kohel et al., 1970), to produce seed homozygous or heterozygous respectively, for each translocation. Progeny families were phenotypically screened in a field at College Station, TX, to verify identity, after which two representative plants were cut back and transplanted to the greenhouse for pollen analysis during the winter of 1988–1989. Four additional translocations (Table 1) were selfed and crossed during 1988, and pollen of field-grown progenies screened during the summer of 1989.

Pollen samples (collected from two flowers of one or two plants of each cytogenetic type) were analyzed for pollen fluorescence by methods described previously (Gwyn and Stelly, 1989), using an Olympus (Lake Success, NY) Vanox-S fluorescence microscope. Pollen were classified while viewing at 100×.

Data were analyzed using SYSTAT 4.0 and graphed with SYGRAPH 1.1 software (Systat, Inc. Evanston, IL). Data from the greenhouse and field experiments were treated similarly, but not pooled. Both experiments were designed as factorial experiments: e.g. for the greenhouse, 16 translocation lines and two cytogenetic conditions for each line (TT vs. NT), assuming fixed effects for the translocation line and cytogenetic condition. Variance of pollen infertility was analyzed for nontransformed and transformed (arcsine of the square root of the incidence of sterile grains) data.

Results and Discussion

The analyses of variance for raw and transformed pollen sterility data from the greenhouse gave similar results, so only the statistics for raw data are cited below. Results from analyses of variance were essentially identical for data from the field and greenhouse, so only the latter are discussed specifically. Statistically significant ($P < 0.001$) sources of variation included effects of the cytogenetic lines ($F = 12.65$; 15 df/32 df), the cytogenetic conditions ($F = 2705$; 1 df/32 df), and their interaction effects ($F = 20.44$; 15 df/32 df). Effects of the cytogenetic condition (TT vs. NT) dwarfed all other sources of variation in pollen sterility, as observed by Gwyn and Stelly (1989).

Concordance was extremely high between sterility estimates for the two flowers of each genotype, the maximum difference observed being 8.3%. Variation between flowers within genotypes contributed less than 2% of the sum of squared deviations from the mean. Comparisons made below are therefore based on the means of the two flowers for each genotype (i.e, approximately 1000 pollen grains). Assuming a common binomial probability, the standard errors of the mean percentages of sterility (s) and fertility (f) would be $[(sf)/1000]^{0.5}$ (Mather, 1951), which is in the range of 0 to 2% sterility for each cytogenetic type. Average pollen fertilities of TT and NT types were 95 and 67%, respectively (range = 90–98% for TT, and 52–81% for NT). Marked differences in fertility existed between TT and NT types for each translocation (Fig. 1).

Considering only data from the greenhouse-grown plants, fertility of the two-chromosome reciprocal translocation heterozygotes averaged 66.7%, whereas the three-chromosome and four-chromosome trans-

Table 1. Codes, line designations, and breakpoint sites of 20 cotton chromosomal translocations, homozygotes and heterozygotes of which were subjected to pollen fertility analysis during 1988–1989.

Code	Line†	Breakpoint sites‡
5–11–21	2779	5–11–21
2–11–13–24	4655	2–11–13–24
2–19–23	DP6	2–19–23
1–2–3	VI_1	1–2–3
19R–24R	2786	19R–24R
15R–16Rb	2767	15R–16R
15R–16Ra	8–5Ga	15R–16R
14L–23	2777	14L–23
13R–19R	2925	13R–19R
11R–17R§	1316	11R–17R
10L–21L§	4675	10L–21L
10R–11R	2785	10R–11R
9R–17Rb§	6340	9R–17R
9R–17Ra	1036	9R–17R
8R–19R	5–5B	8R–19R
6L–10R	Z9–9	6L–10R
6L–7L	1048	6L–7L
5L–9L	C14–3	5L–9L
2R–8Rb	1058b	2R–8R
1R–16Ra	2770	1R–16R

† Line identifications as listed by Endrizzi et al. (1984).
‡ Breakpoints assigned according to personal communication (1986) from the late Dr. M.Y. Menzel; R = right arm and L = left arm, if known.
§ Heterozygotes and homozygotes were screened only in the field during 1989.

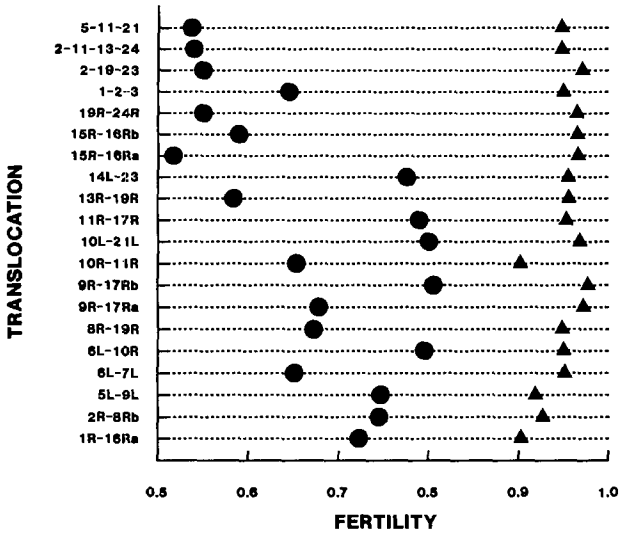

Fig. 1. Average percentages of fully fluorescent pollen in cotton, from paired translocation homozygotes (triangles) and heterozygotes (circles), following treatment with a fluorescein-diacetate solution. Translocation codes indicate the chromosomal (number) and arm (L vs. R) positions of breakpoints; translocations involving the same chromosomes are distinguished by trailing *a* or *b*; lines are identified in Table 1.

locations collectively averaged 56.8%; the difference is significant ($P < 0.05$, 14 df), assuming a common variance. The discrepancy would have been greater if data from the field-grown plants had been included. The expected fertility of the complex translocations is lower than that of reciprocal two-chromosome translocations, in that the former are expected to form chromatin-deficient meiotic products more frequently.

The overall percentages of sterility observed by Gwyn and Stelly (1989) were lower than we found in this study: 3.0 vs. 4.9% for translocation homozygotes and 21.1% vs. 33.2% for translocation heterozygotes. The overall differences between the present and previous results might reflect idiosyncrasies of the specific sets of translocations screened. Alternatively, the differences may have arisen because the pollen data were collected by different individuals. The criteria used to classify pollen are applied in part subjectively, so pollen classifications necessarily vary somewhat by researcher. We note a marked difference in observed infertility for the one translocation heterozygote screened in both studies, NT15R-16Ra; Gwyn and Stelly (1989) observed 22.5% infertility, compared with 48.3% in this study. It appears that in optimal application of the technique, classifying plants of a given segregating family should be done by a single individual, or a machine. Data collected by more than one individual might need to be standardized by appropriate transformation on a per-person basis.

Results presented here and by Gwyn and Stelly (1989) demonstrate that the fluorescein diacetate-based FCR method can be used to distinguish translocation homozygotes from heterozygotes for all of the known translocations in the cytogenetics collection of cotton. However, not all cotton chromosomal translocations may be so amenable to the FCR technique. Most of the breakpoints of the translocations in the Cotton Cytogenetics Collection are genetically close to their respective centromeres (Menzel et al., 1985), possibly because only translocations that had high frequencies of easily detected multivalents were characterized and eventually integrated into the collection (M.Y. Menzel, 1986, personal communication). Given the positions of the breakpoints, segmentally duplication-deficient (DpDf) microspores and microgametophytes from the translocation heterozygotes must typically involve duplications and deficiencies of entire arms or relatively large segments of chromosome arms. Heterozygosity for certain translocations, especially those involving breakpoints in terminal regions, would not be expected to lead to high levels of FCR-detectable pollen sterility, provided that the DpDf gametes lacked no genes that are critical to metabolism or gametophytogenesis. Thus, it would be inappropriate to conclude that the FCR technique will reveal heterozygosity of all cotton translocations. Translocations involving distal breakpoints that exist among the wild *G. hirsutum* cottons (Endrizzi, 1966) may provide ample means to verify this limitation of the FCR technique. On the other hand, our results indicate that the FCR technique will permit facile detection of most if not all primary monosomics, monotelodisomics, translocation-derived duplication-deficient (segmental trisomic-monosomic), and tertiary monosomic plants in segregating families. Thus, the FCR technique can be expected to facilitate chromosomal manipulations involving all other cytogenetic tools.

Circumstantial data indicate that pollen sterility associated with sporophytic translocation heterozygosity results largely from segmental nullisomy (deficiency) rather than segmental disomy (duplication). Extra chromosomes of trisomic and tetrasomic cotton were long ago found to be pollen transmissable (M.Y. Menzel, 1984, personal communication; J.E. Endrizzi, 1989, personal communication; Endrizzi, et al., 1963), indicating that hyperaneuploid pollen of cotton is often functional and competitive with euploid pollen. In contrast, deficiencies for whole chromosomes and chromosome arms are typically not pollen-transmissible in cotton (Endrizzi et al., 1984), and pollen fertility of monosomic sporophytes is impaired (Douglas, 1968). The inviability of many types of DpDf microgametophytes suggests that intergenomic genetic redundancy between the A and D genomes does not allow for segmental nullisomy of arms or large parts of arms.

The FCR technique makes it possible to use the 129 identified translocation breakpoints as genetic markers. Taken collectively with 65 mapped gene loci (Endrizzi et al., 1984), the total number of chromosomally mapped markers now stands close to 200. Perhaps more important is that the chromosome translocation breakpoints provide markers on 25 of the 26 chromosomes and 43 of the 52 arms; mapped gene markers have previously been available for only about half of the chromosomes. A translocation involving Chromosome 26 has not yet been identified, but a known linkage group exists in this chromosome, and a monotelodisomic stock, deficient for one copy of the long

arm of chromosome 26, is available (Endrizzi et al., 1984). Thus, one or more usable cytogenetic and/or genetic markers is now established for each of the 26 chromosomes of cotton.

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