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# Method to Evaluate Pollen Viability of Upland Cotton: Tests with Chromosome Translocations

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## ABSTRACT

**Cytogenetically induced semisterility can provide a useful phenotype to facilitate many sorts of genetic and cytogenetic manipulations that otherwise require meiotic analyses. Most chromatin deficiencies are not pollen-transmissible in Upland cotton (*Gossypium hirsutum* L.) due to reduced viability. A quick and reliable technique for the assessment of cotton pollen fertility has not been established. A modified fluorochrome reaction (FCR) method, using fluorescein diacetate, was evaluated for application as a pollen viability/fertility indicator by scoring fluorescence of pollen from normal and reciprocal chromosome translocation cytotypes grown in greenhouse and field environments. Analyzed materials included 44 different true-breeding translocation homozygotes, respective F1 heterozygotes, and three different cytogenetically analyzed BC1F1 families. Translocation heterozygotes consistently produced higher frequencies (12.3–38.8%) of less intensely fluorescing pollen grains than homozygotes (0.4–6.4%) or normals (0.4–4.0%). Results were concordant with cytogenetic expectations and meiotic analyses, indicating that these and perhaps other cytogenetically aberrant types of cotton can be discerned phenotypically on the basis of percent pollen viability, thereby facilitating their detection and use as cytogenetic tools.**


In diploid and highly diploidized polyploid plant species, cytogenetic aberrations typically induce sporophytic semisterility due to the production and segregation of genetically unbalanced gametophytes whose capacity to function normally is reduced or absent (Burnham, 1956). This semisterility can often be evaluated by staining procedures that assess pollen quality, and used as a means to classify phenotypically certain sporophytic cytotypes, e.g., chromosome translocations in soybean, *Glycine spp.* (Palmer et al., 1987). Usefulness is maximal when cytogenetically aberrant types are morphologically indistinct and require laborious meiotic analysis for their detection and subsequent use as cytogenetic tools.

Most cytogenetic deficiencies in Upland cotton ($2n = 4x = 52$) are not pollen-transmissible, indicating that chromatin deficiencies adversely affect microgametophytic viability/fertility. Numerous stains and techniques have been tested as a means to accurately assess cotton pollen fertility. These include nuclear stains such as lactophenol and acetocarmine (Aslam et al., 1964; Sarvella, 1964; Douglas, 1968; Barrow, 1983); cytoplasmic stains such as potassium iodide (Sarvella, 1964; Mehetre et al., 1980); differential stains such as Alexander's stain (Mehetre et al., 1980; Barrow, 1983); various other stains and dyes such as acid fuchsin, crystal violet, cotton blue, eosin, and orange G (Sarvella, 1964; Mehetre et al., 1980); vital stains such as tetrazolium salts (Aslam et al., 1964; Sarvella, 1964; Barrow, 1983); and both in vivo and in vitro pollen germinability (Mehetre et al., 1980; Barrow, 1983). Most of these pollen staining procedures satisfactorily discriminated between living and dead pollen, and among pollen grains of known male-sterile and male-fertile types, but were not reliably effective in the classification of functional pollen grains with reduced viability and thus tended to overestimate sporophytic fertility (Barrow, 1983). Most dyes and stains merely reveal the nutritional state or maturity of the microgametophyte, i.e., pollen quality, so stainability of a pollen grain may not correlate well with its ability to function and effect fertilization. Pollen germinability tests have been reported to provide reasonable estimates of cotton pollen fertility (Barrow, 1983), but they require a good deal more preparation and time than do staining procedures.

The assessment of pollen viability, i.e., the competence of an individual of a given pollen population to deliver male gametes to the embryo sac (Heslop-Harrison et al., 1984), could be the most reliable and practical method to determine the fertility of cotton pollen. The fluorochrome reaction (FCR) method of Heslop-Harrison and Heslop-Harrison (1970) infers pollen viability by evaluating the integrity of the plasmalemma of the vegetative cell of the microgametophyte by testing for the presence and activity of several esterase enzymes known to be essential for normal gametophytic functions. Active esterases that are associated with fully functional cell walls hydrolyze the fluorescein diacetate ion, causing it to become photo-excitable at ultra-violet wavelengths around 460 nm (Heslop-Harrison and Heslop-Harrison, 1970; Heslop-Harrison et al., 1984; Shivanna and Heslop-Harrison, 1981).

Gould et al. (1986) have shown that anucleate protoplasts of cotton hydrolyze fluorescein diacetate. The purpose of this study was to develop an FCR method with fluorescein diacetate as a vital stain to rapidly and reliably distinguish viable and nonviable pollen grains of cotton. The objective was to determine if the relative frequencies of fluorescing pollen grains would reliably correlate to the cytogenetic constitution of the parental sporophytes.

## MATERIALS AND METHODS

Modified solutions based on the original FCR method (Heslop-Harrison and Heslop-Harrison, 1970; Shivanna and Heslop-Harrison, 1981) were visually evaluated in terms of pollen wall integrity and intensity of fluorescence by observing stained pollen grains from several known chromosome translocation homozygotes and heterozygotes. Special attention was given to achieving maximal differential fluorescence (strong vs. weak intensity) among pollen grains of translocation heterozygotes. Pollen from inbred line Texas Marker no. 1 (TM-1) served as a control. Normally, near 100% of TM-1 pollen fluoresced brightly, but pollen fixed in

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FAA or heat-killed by incubation at 80 °C for 1 h did not fluoresce.

The FCR method modified to produce optimal results was achieved with the following solutions and procedures. Stock solution one (SS1) consisted of 1.75 *M* sucrose, 3.23 m*M* $H_3BO_3$, 3.05 m*M* $Ca(NO_3)_2$, 3.33 m*M* $MgSO_4$, 1.98 m*M* $KNO_3$, and distilled $H_2O$ to volume. High sugar and salt concentrations were required to prevent osmotic bursting and induce a maximal fluorochrome reaction, respectively. Stock solution two (SS2) consisted of 7.21 m*M* fluorescein diacetate (Sigma Chemical Co., St. Louis, MO, Lot 53F-5022) dissolved in acetone. Stock solutions were stored in the refrigerator for up to 4 wk. The working solution was prepared hourly by incrementally adding 8 to 12 drops of SS2 into 10 mL of SS1 until the composite solution became slightly milky.

Specimens were prepared by collecting flowers at anthesis, generously dusting a slide with pollen, adding two to three drops of the working solution, and gently applying a coverslip. Slides were usually observed about 2 min after specimen preparation in order to allow fluorochrome uptake and the reaction to occur. For all experimentation, pollen counts of at least 500 grains per flower from two flowers per plant were made using a Zeiss Universal II microscope equipped with epifluorescence, barrier filter LP520, and exciter filter BP450-490 (blue), at a magnification of 125×.

The utility of this tailored FCR method for classifying sporophytic cytotypes was appraised by a series of three experiments. All analyzed materials were obtained from the U. S. Cotton Cytogenetics Collection maintained at the Texas Agricultural Experiment Station, College Station, TX.

In Exp. 1, the frequencies of differential pollen fluorescence, i.e., viable vs. nonviable pollen grains, were determined for five normal (NN) TM-1 plants, and paired sets (three plants each) of three different reciprocal chromosome translocation homozygotes (TT) and their respective F1 heterozygotes (NT). The translocations involved terminal interchanges among chromosome arms 7L, 11R, 12L, 12R, and 19R. These 23 plants were vegetatively propagated and evaluated in the field during the summer of 1986, in the greenhouse during the winter of 1986–1987, and again in the field during the summer of 1987. Plant identities were coded so that pollen viability counts were performed and plants were classified as fertile or semisterile without knowledge of the cytotype. Experiment 1 was conducted as an initial test to determine the efficiency of classification and also to test for flower-to-flower, plant-to-plant, and environmental variation. Experiment 1 was designed as a two-factor (environment and cytotype) factorial experiment with sampling (plants) and subsampling (flowers). An analysis of variance was computed on arcsin transformed percent nonviable pollen (% sterility) data to identify sources of variation. A mixed model was used with cytotype considered as a fixed effect; and environment, plants, and flowers within plants considered as random effects (Steel and Torrie, 1980). Mean percentages of nonviable pollen and standard errors were computed separately for NN, and NT and TT cytotypes of each chromosome translocation line for each environment.

Experiment 2 was conducted to discern which chromosome translocation heterozygotes of cotton exhibited a subnormal viability frequency to render them phenotypically classifiable. Using known NT-TT pairs, pollen viability frequencies were determined for at least one plant each of 44 different simple reciprocal chromosome translocation lines. Experiment 2 also was conducted as a blind test. Plants were vegetatively propagated and analyzed in the greenhouse during the winter of 1986 to 1987 and in the field during the summer of 1987. Two environments were used in Exp. 2 to monitor consistency of phenotypic classification, not to test variation of viability frequencies. Mean percentages of nonviable pollen and standard errors were computed for NT and TT cytotypes of each chromosome translocation line separately for each environment.

Experiment 3 was a test of the FCR method in which 29 plants of a segregating backcross-population were analyzed independently by meiotic analyses and pollen viability. Three small BC1F1 families were created by backcrossing the three known NT's incorporated in Exp. 1 as pollen parents to NN seed parents. Pollen viability and meiotic analyses were performed on plants grown either in the greenhouse during the winter of 1986–1987, or in the field during the summer of 1987. Meiotic metaphase I chromosome configurations of 26 bivalents (26II) vs. 24 bivalents and a quadrivalent (24II + IV) were used to indicate which plants were NN or NT cytotypes, respectively. The mean percentages of nonviable pollen and the standard errors were determined on a per plant basis, and any plant with a high incidence of nonviable pollen (>12%) was classified as an NT cytotype.

Table 1. Analysis of variance on percentages of nonviable pollen (arcsin transformed) for TM-1 (NN) and three paired chromosome translocation homozygous (TT) and heterozygous (NT) lines of Upland cotton grown in three environments.

| Source | df | MS | *F* |
|---|---|---|---|
| Cytotype | 6 | 0.265 | 111.99** |
| NT vs. TT (7L–12R) | (1) | 0.236 | 116.67** |
| NT vs. TT (11R–12L) | (1) | 0.561 | 264.46** |
| NT vs. TT (12R–19R) | (1) | 0.628 | 301.22** |
| NN vs. TT (all 3) | (1) | 0.001 | 0.43 |
| Environment | 2 | 0.015 | 21.82** |
| Env. × cytotype | 12 | 0.002 | 5.13** |
| Plants w/in cytotype | 16 | 0.0007 | 1.51 |
| residual (flowers w/in plants) | 101 | 0.0004 | |

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

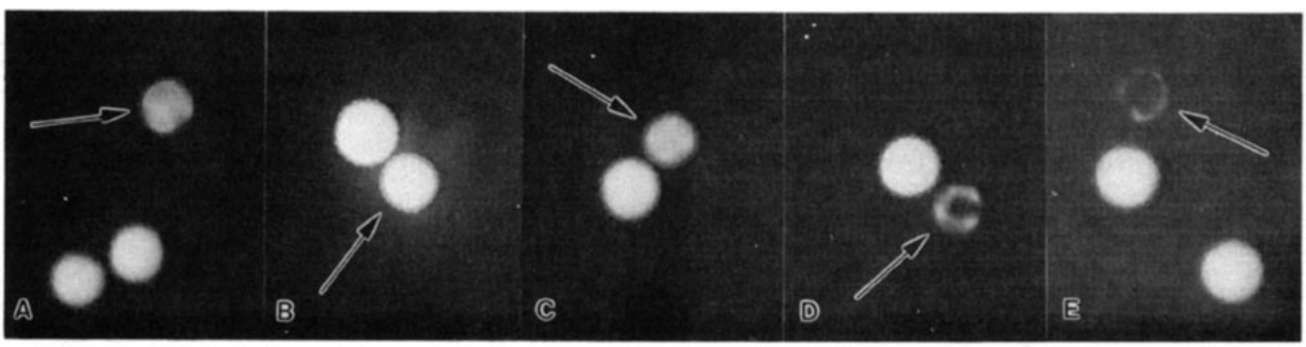


**Fig. 1. Pollen fluorescence of Upland cotton as observed when stained with fluorescein diacetate (FCR method), ca. 225×. Nonviable pollen grains are indicated by arrows (A–E). Nonviability was interpreted as a decrease in fluorescence intensity (A, C, D, E), smaller relative size (B), or both (C, D, E).**

Results of the two classification methods were compared at the end of the experiment.

## RESULTS AND DISCUSSION

### *Pollen Stainability*

The FCR method revealed variability in pollen morphology as well as fluorescence (staining) intensity. This indicated that a systematic categorization that incorporated both factors was necessary for consistency of classifying pollen as viable or nonviable. The largest pollen grains were fully engorged with starch, fluoresced bright luminescent green, and were considered fully viable (fertile). The nonviable (sterile) category harbored an array of pollen types, including pollen grains that (i) were small but fluoresced brightly, (ii) were large or small and fluoresced dimly, (iii) fluoresced unevenly with lighter and darker areas, or (iv) did not fluoresce at all (Fig. 1).

In all three experiments, NN and TT cytotypes consistently produced pollen populations with near 100% viability and were deemed fully fertile. The nonviable class of pollen of these cytotypes consisted almost entirely of smaller pollen grains that exhibited no visible fluorescence. In contrast, NT cytotypes produced the aforementioned array of viable and nonviable pollen types. Variability of pollen types produced from NT cytotypes was expected because meiotic products from adjacent-1 and adjacent-2 chromosome disjunctions will yield four kinds of duplication-deficient gametes, and gametophytic consequences of the different genetic imbalances will not be uniform (Burnham, 1962). Numerical nondisjunction, malorientation, or mispairing leading to aneuploidy may also contribute to the variety of pollen types (Brown et al., 1981; Ray, 1984).

### *Experiment 1*

The analysis of variance for Exp. 1 (Table 1) indicated that environment as well as cytotype were important sources of variation in pollen viability values as determined by the FCR method. No significant ($P > 0.05$) variation in pollen viability values was apparent among plants within cytotypes or between flowers within plants, revealing phenotypic consistency within a given cytotype. Variation among the seven cytotypes was significant ($P < 0.01$), indicating that large differences in percentages of nonviable pollen existed among cytotypes. Orthogonal contrasts revealed that variances due to NT vs. respective TT cytotypes were significant ($P < 0.01$) and collectively accounted for nearly all variation due to cytotype, whereas little variance arose from differences among TT and NN cytotypes. Variation due to environments was significant ($P < 0.01$) and the environment × cytotype interaction was also significant ($P < 0.01$), suggesting that pollen viability estimates among and within cytotypes were dissimilar in heterogeneous environments. However, the relative magnitudes of the mean square values for cytotype, environment, and their interaction implied that cytotype was the only meaningful factor. Due to the precision associated with the large number of experimental units in Exp. 1 the analysis of variance was overly conservative and somewhat too sensitive.

Values of the minima, maxima, means, and standard errors (Table 2) illustrated that the variation in percentages of nonviable pollen due to environment, interaction, and sampling were relatively small in comparison to that due to cytotype, i.e., between NT and TT or NN cytotypes. Mean percentages of nonviable pollen from NT cytotypes were at least threefold that from NN and TT cytotypes. Standard errors for all cytotypes were less than 2% in all three environments. No environmental trend was evident, and phenotypic switching (based on pollen viability) among cytotypes did not occur.

Mean percentages of nonviable pollen from NT cytotypes (Table 2) varied among the different chromosome translocations and were less than 50%, the frequency expected assuming a theoretical 1:1 ratio of alternate:adjacent chromosome disjunctions. Variation among NT cytotypes was expected because their pollen would include duplications and deficiencies for different blocks of genes, for which the adverse effects of genetic imbalance on gametophytic viability would

**Table 2. Percentages of nonviable pollen for TM-1 (NN) and three pairs of chromosome translocation homozygotes (TT) and heterozygotes (NT) of Upland cotton grown in three environments.**

| | Cytotype | | | | | | |
|---|---|---|---|---|---|---|---|
| | TM-1 | 7L–12R(1043)† | | 11R–12L(6–5M) | | 12R–19R(9–5M) | |
| Environment/statistics | NN | TT | NT | TT | NT | TT | NT |
| | % | | | | | | |
| Field 1986 | | | | | | | |
| Minimum | 0.8 | 1.8 | 19.5 | 2.4 | 23.7 | 1.8 | 31.6 |
| Maximum | 2.2 | 4.6 | 26.6 | 6.4 | 31.3 | 6.0 | 35.7 |
| $\bar{X}$ | 1.6 | 3.5 | 23.0 | 4.8 | 28.3 | 3.9 | 32.5 |
| SE | 0.2 | 0.4 | 1.2 | 0.6 | 1.1 | 0.7 | 0.6 |
| Greenhouse 1986–1987 | | | | | | | |
| Minimum | 0.4 | 0.4 | 13.5 | 1.0 | 25.7 | 1.1 | 21.3 |
| Maximum | 1.5 | 1.7 | 16.8 | 1.6 | 34.9 | 3.9 | 34.3 |
| $\bar{X}$ | 1.0 | 0.9 | 15.2 | 1.4 | 29.7 | 2.3 | 28.6 |
| SE | 0.1 | 0.2 | 0.5 | 0.1 | 1.3 | 0.4 | 2.1 |
| Field 1987 | | | | | | | |
| Minimum | 0.4 | 0.6 | 12.3 | 1.8 | 18.2 | 1.5 | 21.2 |
| Maximum | 2.2 | 2.6 | 19.9 | 3.6 | 30.1 | 3.0 | 33.1 |
| $\bar{X}$ | 1.3 | 1.6 | 16.0 | 2.7 | 23.9 | 2.1 | 26.6 |
| SE | 0.1 | 0.3 | 1.3 | 0.3 | 1.6 | 0.2 | 1.8 |

† Specific translocations are identified according to the chromosome arms involved; line designations for homozygotes are given parenthetically.

Table 3. Mean percentages of nonviable pollen and standard errors for pairs of chromosome translocation homozygotes (TT) and heterozygotes (NT) of Upland cotton grown in two environments.

| | Environment | | | |
|---|---|---|---|---|
| | Greenhouse 1986–1987 | | Field 1987 | |
| Translocation line | TT | NT | TT | NT |
| | % | | | |
| 1L–2R (IVa)† | 2.6 ± 0.6 | 15.3 ± 1.4 | 2.9 ± 0.5 | 16.1 ± 1.1 |
| 1L–2L (DP–30) | 3.2 ± 0.5 | 17.1 ± 1.2 | 3.7 ± 0.6 | 14.6 ± 0.9 |
| 1L–3L (2935) | 4.5 ± 0.5 | 19.8 ± 1.3 | 4.6 ± 0.6 | 19.1 ± 1.1 |
| 1L–7L (5–4c) | 3.1 ± 0.3 | 15.9 ± 0.8 | 2.2 ± 0.4 | 17.8 ± 1.1 |
| 1L–8L (2775b) | 2.5 ± 0.5 | 27.2 ± 1.5 | 2.9 ± 0.5 | 25.6 ± 1.2 |
| 1L–14L (2780) | 1.6 ± 0.4 | 20.8 ± 1.1 | 1.5 ± 0.4 | 18.1 ± 1.1 |
| 1L–20R (4669) | 2.2 ± 0.5 | 17.0 ± 1.2 | 3.4 ± 0.5 | 18.8 ± 1.1 |
| 1R–16R (4672) | 2.2 ± 0.5 | 25.7 ± 1.2 | 2.0 ± 0.6 | 31.1 ± 1.2 |
| 2L–3L (1059) | 1.6 ± 0.3 | 19.2 ± 0.9 | 4.8 ± 0.7 | 14.8 ± 1.0 |
| 2L–6R (7–2B) | 1.7 ± 0.2 | 19.3 ± 1.1 | 2.2 ± 0.5 | 14.8 ± 0.9 |
| 2L–9R (8B–3) | 4.1 ± 0.6 | 19.0 ± 1.2 | 4.0 ± 0.6 | 14.3 ± 1.1 |
| 2R–3L (IV1) | 2.6 ± 0.3 | 24.1 ± 1.2 | 1.1 ± 0.3 | 20.0 ± 1.2 |
| 2R–8R (1039) | 2.6 ± 0.6 | 22.4 ± 1.1 | 1.5 ± 0.4 | 19.3 ± 1.2 |
| 2R–14R (2B–1) | 3.3 ± 0.2 | 15.7 ± 1.0 | 2.2 ± 0.4 | 18.3 ± 1.6 |
| 3L–6L (4010) | 0.9 ± 0.5 | 34.6 ± 1.5 | 3.1 ± 0.5 | 31.0 ± 1.4 |
| 3L–19L (E–20–7) | 5.1 ± 0.6 | 22.9 ± 1.1 | 2.6 ± 0.3 | 15.8 ± 1.3 |
| 3R–5R (8–5Gb) | 4.4 ± 0.4 | 22.8 ± 0.8 | 3.4 ± 0.6 | 25.5 ± 1.3 |
| 3R–9R (8–30–5) | 1.9 ± 0.5 | 17.1 ± 1.2 | 3.3 ± 0.7 | 20.0 ± 1.0 |
| 4L–5R (IV2) | 4.9 ± 0.5 | 14.2 ± 1.3 | 3.3 ± 0.6 | 13.0 ± 1.0 |
| 4L–19R (10–5ka) | 1.3 ± 0.3 | 17.6 ± 0.9 | 2.2 ± 0.4 | 19.5 ± 1.2 |
| 4R–15L (1040) | 2.7 ± 0.4 | 23.2 ± 1.1 | 3.5 ± 0.5 | 28.8 ± 1.0 |
| 5R–12R (SL–18) | 2.2 ± 0.4 | 18.9 ± 1.1 | 2.7 ± 0.5 | 19.0 ± 1.2 |
| 5R–23R (2775a) | 1.2 ± 0.3 | 34.9 ± 1.7 | 1.7 ± 0.4 | 38.8 ± 1.4 |
| 6L–14L (AZ–7) | 3.1 ± 0.4 | 17.0 ± 1.1 | 2.5 ± 0.5 | 14.7 ± 1.1 |
| 7L–12R (1043) | 4.4 ± 0.4 | 14.1 ± 0.9 | 2.4 ± 0.5 | 18.5 ± 1.2 |
| 7L–18R (4659) | 3.3 ± 0.3 | 14.2 ± 0.7 | 4.4 ± 0.6 | 13.1 ± 1.1 |
| 7R–11R (1052) | 3.6 ± 0.5 | 15.1 ± 0.9 | 5.0 ± 0.7 | 14.2 ± 1.1 |
| 7R–21R (2790) | 4.1 ± 0.6 | 26.5 ± 1.2 | 4.0 ± 0.6 | 32.8 ± 1.4 |
| 8R–19R (2778) | 2.2 ± 0.3 | 21.3 ± 1.2 | 4.9 ± 0.3 | 30.0 ± 1.3 |
| 9R–20L (2772) | 2.9 ± 0.5 | 15.3 ± 0.9 | 2.9 ± 0.5 | 14.0 ± 0.9 |
| 9R–25? (2870) | 1.1 ± 0.2 | 19.2 ± 1.1 | 3.6 ± 0.6 | 16.0 ± 1.1 |
| 10R–19R (1626) | 3.9 ± 0.4 | 22.2 ± 1.2 | 2.2 ± 0.3 | 23.2 ± 1.2 |
| 11L–15L (1058a) | 3.2 ± 0.4 | 26.9 ± 1.1 | 4.4 ± 0.6 | 27.3 ± 1.3 |
| 11R–12L (6–5M) | 1.7 ± 0.3 | 30.1 ± 1.5 | 2.4 ± 0.5 | 27.0 ± 1.3 |
| 11R–17R (1316) | 6.1 ± 0.6 | 15.5 ± 1.0 | 5.2 ± 0.7 | 18.1 ± 1.2 |
| 12R–19R (9–5M) | 3.0 ± 0.5 | 33.6 ± 1.2 | 3.2 ± 0.5 | 29.6 ± 1.3 |
| 14R–24R (2781) | 2.0 ± 0.3 | 25.1 ± 1.3 | 2.8 ± 0.5 | 26.7 ± 1.2 |
| 14?–26? (A–21–8) | 5.0 ± 0.6 | 13.6 ± 1.0 | 5.4 ± 0.7 | 14.5 ± 1.1 |
| 15R–16L (8–5Ga) | 3.7 ± 0.4 | 22.0 ± 1.1 | 2.4 ± 0.5 | 23.0 ± 1.2 |
| 15R–20R (SL–15) | 2.4 ± 0.3 | 16.5 ± 1.1 | 3.3 ± 0.3 | 18.4 ± 1.2 |
| 19L–21R (E–22–13) | 5.0 ± 0.5 | 27.2 ± 1.5 | 5.1 ± 0.4 | 25.8 ± 1.3 |
| 20R–21L (7–3F) | 4.6 ± 0.3 | 24.3 ± 1.2 | 2.0 ± 0.3 | 23.3 ± 1.1 |
| 20L–22R (DP–4) | 1.7 ± 0.2 | 17.2 ± 0.9 | 2.9 ± 0.5 | 23.1 ± 1.2 |
| 20R–25R (2791) | 3.6 ± 0.4 | 19.4 ± 1.0 | 2.0 ± 0.3 | 26.2 ± 1.3 |

† Specific translocations are identified according to the chromosome arms involved; line designations for homozygotes are given parenthetically.

differ (Burnham, 1956). Pairing configurations, orientations, and patterns of disjunction differ among translocation lines and also contribute to this variability (Brown et al., 1981; Ray, 1984). That the observed frequencies of nonviability for NT cytotypes were less than 50% might be attributed to the polyploid nature of upland cotton, which presumably allows for genetic buffering and some microgametophytic tolerance of aneuploidy (Menzel and Brown, 1954), and/or the preponderance of alternate disjunctions (Brown et al., 1981; Ray, 1984).

*Experiment 2*

Pollen viability data between the 44 pairs of TT and NT cytotypes evaluated by the FCR method in Exp. 2 yielded results analogous to Exp. 1. The data demonstrated (Table 3) that NT cytotypes consistently produced higher frequencies of nonviable pollen grains than their respective TT cytotypes, which were uniformly viable and indistinguishable. Mean percentages of nonviable pollen for NT cytotypes were always at least threefold that of respective TT cytotypes, and standard errors for all cytotypes were less than 2%. Environmental effects were apparent, but no phenotypic switching among cytotypes or environmental trend was noted. As in Exp. 1, mean percentages of nonviable pollen for NT cytotypes varied among translocations, whereas variation within translocations among environments was small. Therefore, the FCR method reliably evaluated pollen viability for many different kinds of duplication-deficiencies in cotton.

Table 4. Comparison of cytogenetic analyses with pollen viability analyses for classification of chromosome translocation heterozygous (NT) and normal (NN) cytotypes within a backcross population of Upland cotton grown in the field 1987.

| | Cytogenetic analysis | | Pollen viability analysis | |
|---|---|---|---|---|
| Origin | Meiotic configuration | Cytotype inferred | Mean % nonviable pollen ± SE | Cytotype inferred |
| NN (inbred line) × NT 7R–12L (1043)† | | | | |
| Plant 1 | 24II + IV | NT | 19.8 ± 1.2 | NT |
| 2 | 24II + IV | NT | 19.0 ± 1.2 | NT |
| 3 | 26II | NN | 1.9 ± 0.4 | NN |
| 4 | 24II + IV | NT | 20.8 ± 0.9 | NT |
| 5 | 26II | NN | 1.4 ± 0.4 | NN |
| 6 | 24II + IV | NT | 16.2 ± 0.8 | NT |
| 7 | 24II + IV | NT | 19.7 + 1.1 | NT |
| 8 | 26II | NN | 2.9 ± 0.5 | NN |
| 9 | 24II + IV | NT | 26.6 ± 1.3 | NT |
| NN (inbred line) × NT 11R–12L (6–5M) | | | | |
| Plant 1 | 24II + IV | NT | 23.6 ± 1.3 | NT |
| 2 | 24II + IV | NT | 20.1 ± 1.1 | NT |
| 3 | 26II | NN | 1.8 ± 0.4 | NN |
| 4 | 24II + IV | NT | 25.2 ± 1.2 | NT |
| 5 | 26II | NN | 3.0 ± 0.5 | NN |
| 6 | 26II | NN | 2.4 ± 0.5 | NN |
| 7 | 24II + IV | NT | 27.0 ± 1.2 | NT |
| 8 | 26II | NN | 3.4 ± 0.5 | NN |
| NN (inbred line) × NT 12R–19R (9–5M) | | | | |
| Plant 1 | 24II + IV | NT | 32.1 ± 1.4 | NT |
| 2 | 24II + IV | NT | 31.5 ± 1.4 | NT |
| 3 | 24II + IV | NT | 30.1 ± 1.3 | NT |
| 4 | 24II + IV | NT | 37.4 ± 1.5 | NT |
| 5 | 24II + IV | NT | 35.7 ± 1.4 | NT |
| 6 | 26II | NN | 4.0 ± 0.6 | NN |
| 7 | 24II + IV | NT | 28.3 ± 1.4 | NT |
| 8 | 26II | NN | 1.4 ± 0.4 | NN |
| 9 | 24II + IV | NT | 29.1 ± 0.9 | NT |
| 10 | 26II | NN | 1.7 ± 0.4 | NN |
| 11 | 26II | NN | 2.4 ± 0.5 | NN |
| 12 | 24II + IV | NT | 29.1 ± 1.2 | NT |

† Specific translocations are identified according to the chromosome arms involved; line designations for homozygotes are given parenthetically.

*Experiment 3*

Experiment 3 was conducted to determine if the FCR method could be used to identify NT cytotypes vs. NN sibs in a segregating backcross population, typical of those used for genetic mapping of marker genes or translocation breakpoints. Meiotic analysis is the method routinely used to identify the genetically informative NT cytotypes (Fig. 2). A reliable pollen staining technique would provide an expeditious alternative.

Twenty-nine BC1F1 plants were evaluated in Exp. 3 (Table 4). Eleven plants formed 26 bivalents (26II)

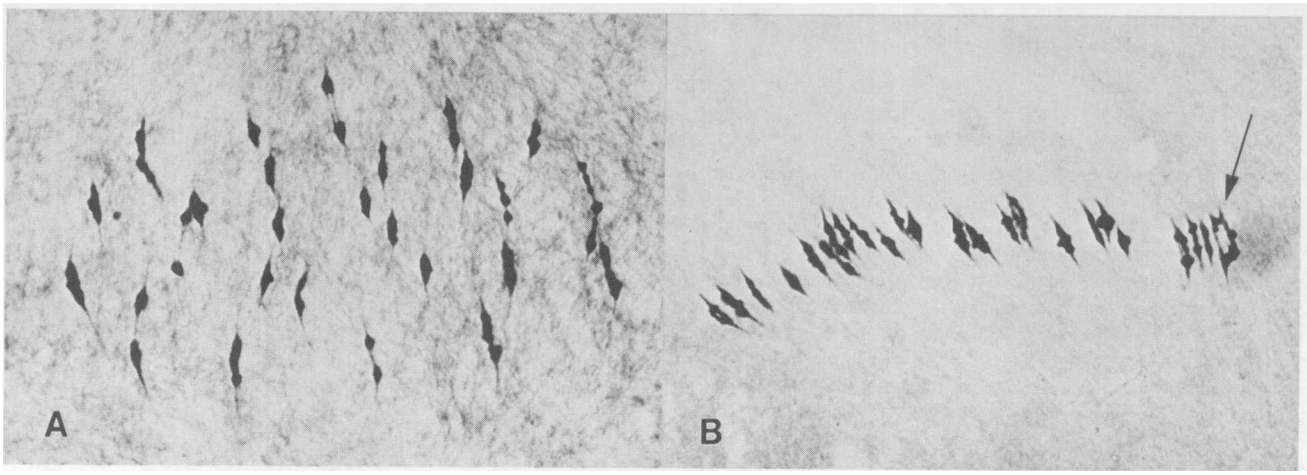


**Fig. 2. Pollen mother cells of Upland cotton in meiotic metaphase I, ca. 1000×. Normal plants were observed to have 26 bivalents (A), whereas chromosome translocation heterozygotes typically exhibited 24 bivalents and a quadrivalent (arrow) (B).**

at meiotic metaphase I, and had greater than 97% viable pollen. These were classified as NN cytotypes by both analyses. The remaining 18 plants modally formed 24 bivalents and a quadrivalent (24II + IV) at metaphase I, and had less than 82% viable pollen. Both analyses classified these as NT cytotypes. Thus, the resolution of cytotype by pollen viability analysis vs. meiotic analysis was concordant for all 29 plants.

## CONCLUSION

The modified FCR method proved to be an effective tool for the evaluation of pollen viability/fertility in a manner that provided a phenotype that accurately distinguished chromosome translocation heterozygotes from homozygotes or normals. In contrast, Aslam et al. (1964) reported that tetrazolium vital staining techniques failed to distinguish pollen within several translocation lines of cotton.

Classification methods that circumvent time-consuming meiotic analyses could have a tremendous impact on cotton genetics by facilitating the use of existing cytogenetic tools. The extensive chromosome translocation collection of cotton incorporates 58 different simple reciprocal translocations (plus several complex types) that involve 25 of 26 chromosomes, 43 of 52 chromosome arms, and whose chromosome breakpoints are mapped (Menzel et al., 1985). The practical accessibility of the translocation collection alone increases the number of mapped genetic markers in cotton almost threefold. Cytogenetic manipulations involving translocations also will be facilitated. For instance, it is now feasible to develop isolines carrying the translocations, and to derive therefrom isogenic segmental duplication-deficient cytotypes. If other chromatin-deficient cytotypes such as monosomics, monotelodisomics, and segmental duplication-deficiencies could be classified by vital staining pollen with the FCR method, their utility in genetic studies could be greatly enhanced as well. Douglas (1968) reported that six primary monosomics of cotton showed significantly higher frequencies of abortive pollen grains relative to a standard inbred line when stained with acetocarmine. Generally, the FCR method seems to have wider applicability and better resolution than existing techniques for assessment of cotton pollen fertility.

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