The restored cms-T versions of most of the inbred lines and the three F_1 hybrids mentioned above were also available and their pollen was tested on CWBM. Pollen from restored cms-S and cms-C versions of some of the inbred lines was also tested. These studies indicate that conversion to male-sterile cytoplasm does not appreciably alter the performance of inbred line pollen on CWBM.

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4. Linkage analysis in the male gametophyte.

As indicated in one of the above reports, we are attempting, through use of the waxy translocation technique, to identify the linkage groups of newly-arisen genic restorers of S male-sterile cytoplasm. The conventional procedure would be laborious as there are no less than six restorers to deal with and it would be necessary to score relatively large testcross progenies for a mature plant trait, male sterility. We propose to simplify the task by taking advantage of the fact that genic restoration of S cytoplasm occurs at the gametophytic level. Since plants with S cytoplasm that are heterozygous for a restorer gene produce equal numbers of normal (Rf) and aborted (rf) pollen grains, it should be possible to obtain at least a preliminary indication of the linkage group for a particular restorer through analysis of iodine-stained pollen samples from plants heterozygous for both the restorer and a particular wx-linked reciprocal translocation.

The procedure involves an initial cross of a plant with S cytoplasm that is heterozygous for a genic restorer, as female parent, with a plant that is homozygous for wx and a particular reciprocal translocation. The male parent in the cross should be in Ml4 background as this inbred line does not restore S. All F_1 offspring should be heterozygous for the translocation and approximately half of these, having received the rf allele from the female parent, should be male-sterile. The remaining half, those carrying the restorer allele from the female parent, should be semi-sterile, with about 25% normal pollen grains. If a particular restorer gene being tested is located on a chromosome other than the two that are involved in the waxy translocation carried by the male parent, blue and red staining normal pollen

grains should occur with equal frequency. If linkage is encountered, however, more than 50% of normal pollen grains should stain blue, the proportion of blue and red being a function of recombination between the restorer and wx loci.

There is some indication that the method described above may be employed successfully. In connection with his Ph.D. thesis study, Dr. Arjun Singh used this method in an attempt to identify the linkage group of the standard restorer, Rf_3. Eighteen homozygous wx-linked reciprocal translocation stocks, involving chromosome 9 with each of the other chromosomes, were involved in these tests. Pollen analysis of 16 of the 18 F_1 heterozygotes had blue:red ratios not significantly deviant from 1:1. Significant deviations from a 1:1 ratio, in favor of the blue-staining class, were encountered in two of three tested plants involving T3-9c, and in all six tested plants involving T2-9b. In the first case, the blue:red ratio was 5:4, in the latter it was 2:1. This preliminary evidence suggests that Rf_3 is located in chromosome 2 or in chromosome 3, probably the former.

We are continuing the effort to locate Rf_3. A number of different wx 2-9 translocation F_1 plants are currently being analyzed and progeny of crosses of these F_1 plants with S male-sterile individuals are now being grown. In addition, F_1 progeny involving crosses of the newly-arisen restorer strains with plants in the wx translocation series are being analyzed in our winter nursery. We anticipate that pollen analysis of these F_1 progenies will, with minimal effort, yield useful information on the linkage characteristics of the new restorers.

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