

hurricanes. They are especially useful in making mass pollinations where we have been testing mutation rates induced in radiated developing pollen. The tags are all stamped prior to pollination with the data which we wish on the tag. Stamping is done by a "Crown" stamping machine which has 12 bands each containing a complete alphabet and numbers so that any combination can be stamped onto the tag. The tags are 3 1/2 inches by 5/8 inch, large enough for at least 12 letters or figures. The ink used is water-proof since these tags must stay out in the weather. Tags have been exposed continuously to the weather since last summer and the ink has not faded at all. Where a number of pollinations are to be made from the pollen of a single plant the tags can be stamped and hung on the plant desired and they serve as a reminder of how many pollinations are to be made. When the tags are used up the pollinations are complete. They come in five different colors, red, yellow, blue, green and white. Different experiments can be labelled automatically with a different color which makes sorting of the ears at harvest time an easy chore. The plastic tags are wired onto the plant with copper wire. At harvest time a quick jerk cuts through the plastic tag leaving the tag free without the wire. These are then strapped onto the ears with a rubber band. We have been using rubber bands for fastening labels onto hand pollinated ears for about 20 years and they give good results and hardly ever is a band broken. One precaution in using bands on the plastic tags is not to wrap the rubber band too tightly as it might be cut by the plastic tag. The advantage of rubber bands in fastening the tags to the ear is that as the ear shrinks the band also shrinks and remains tight to the ear.

One of the biggest advantages of using prelabelled tags for hand pollinated ears is that it is not necessary to transfer any data from the tag used in the hand pollination which cuts down materially on the errors in labelling the hand pollinated ears. The plastic tags can be obtained from the National Band and Tag Company, Newport, Kentucky.

W. Ralph Singleton

4. Radiation Induced Pre-meiotic Mutation.

A series of experiments was started in 1953 and 1955 to determine the relative sensitivity of the various stages of maize microsporogenesis to gamma radiation. The results reported here are from the 1955 experiment in which plants homozygous dominant for the endosperm characters Su, Y, Sh, and Wx were placed in the Brookhaven gamma field for two day periods where they were radiated for 20 hours each day. The first group of plants was placed in the field 36 days before the pollen was shed. The first group was removed after 40 hours of gamma radiation given at the rate of 50 r per hour, and another group placed in the field. This was continued until the last group to go in the radiation field was shedding pollen when removed from the field. Pollen was collected from all the plants in the experiment on this day and placed on silks of a

multiple recessive tester stock. Ears were harvested and scored for su, y, sh, and wx endosperms.

Nine recessive endosperms were recovered from a population of 18,000 seeds produced from pollen which had been radiated for a two day period between 36 and 14 days before pollen shedding. 7,100 seeds were produced from plants which had received their radiation during the last twelve days of microsporogenesis, and there were 262 mutant endosperms recovered from these seeds.

F₁'s were grown from seeds with mutant endosperms. It was found that of the nine recessive endosperms from the early radiated group five showed the mutant phenotype on the F₁ ears. All these ears were normal with full seed sets. Two of the remaining four seeds did not produce F₁ seedlings, one was lost in the seedling stage to cutworms, and the last produced a normal non-mutant ear. Three of the mutations were from Sh to sh, on chromosome 9, one was from Wx to wx, also on 9, and the last one was from Su to su on chromosome 4. Pollen was not examined from these mutants but transmission of the mutant through the megaspore was apparently normal.

F₁ plants were grown from 130 seeds of the 262 mutant endosperms produced from the pollen radiated during the last 12 days of microsporogenesis. 27 or 20% of the F₁'s showed the same recessive endosperm as the seed which was planted. However, all of these ears were semi-sterile, which would indicate that all of the mutant endosperms produced by radiation of pollen late in the microsporogenesis were the result of chromosomal aberrations.

3.7% of the endosperms scored for the four recessive endosperm characters showed the mutant phenotype in the F₀ when the pollen was radiated during the last 12 days of microsporogenesis. When the mutant types were planted 60% of the resulting F₁'s were semi-sterile while the non-mutant seeds from the same radiation period produced F₁'s of which 30% were semi-sterile.

Since we are more interested in the possibilities of using radiation to produce gene mutations we are more concerned with the five F₁'s showing the mutant phenotype that were produced from pollen radiated early in microsporogenesis. These have not yet been examined cytologically and it is not known whether these are "gene mutations" or simply small deletions. There is ample evidence in the literature that maize chromosomes can carry deletions which are inherited as simple recessive genes.

At this point I should like to do some speculating as to the stages of microsporogenesis during which the five mutants without megaspore sterility could have been produced. Sparrow (Annals of the New York Academy of Science 51: 1508-1540, 1951) showed that the interphase of Trillium is the least sensitive to radiation damage as measured by breakage and states that the effects recovered in the F₁ would be

greatest from stages least sensitive to radiation breakage as damage produced from sensitive stages would be least likely to produce viable F_1 's.

We have some fragmentary data on stage sensitivity in our material as measured by F_0 seed sets. If this is correlated with maize microspore development as described by Kiesselbach (Univ. of Nebraska Agr. Exp. Sta. Res. Bul. 161: 1-96, 1949), it would appear that the three may have been produced during one of the interphases preceding pollen mother cell formation, and the other two may have come from the interphase between meiosis II and the first microspore mitosis.

This is not the first case of radiation induction of pre-meiotic mutation in maize. Dollinger (Maize Genetics Cooperation News Letter 28: 11-12, 1954) reported finding two pre-meiotic mutations. One of these was a mutable involving the A_1 and Sh_2 loci, the other was Bt_2 to bt_2 . Cytological examination of his material revealed no pollen sterility or detectable chromosomal alteration.

Based on these results, it would seem that if one were interested in producing chromosomal aberrations one should radiate mature pollen or the interphase between the first and second microspore division. If one is interested in events inherited as "gene mutations" one should try and hit one of the interphases preceding the pollen mother cell formation in order to use the later division of microsporogenesis to screen out the larger types of chromosomal aberrations.

The control population of 6000 seeds grown for this experiment gave no recessive endosperms and a semi-sterile rate of 2.6% in the F_1 . However, it may be of interest to consider the results of some experiments we did to determine the spontaneous mutation rate in pollen homozygous for Su , Pr , Y , Sh , and Wx . In 1953 an experiment was conducted in which 83,000 seeds were examined. There were 46 recessive endosperms and when these were planted six or 19% showed the recessive character in the F_1 . Of these only one, a Pr to pr , showed full normal seed set, the other 5 were semi-sterile. A control population of 25,000 seeds grown in 1955 gave 35 mutant endosperms in the F_0 and when these were planted five or 16% showed the same phenotype in the F_1 . In this case all of the F_1 's were semi-sterile. It would appear that mutant phenotypes found when dominant pollen is placed on recessive ears results from spontaneous chromosomal aberrations probably produced in the same stages of microsporogenesis which are most sensitive to the production of aberrations from radiation.

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