3. Dominant mutants induced by EMS.

From a population of 3693 F_1 plants produced by treatment of pollen with EMS (method, MNL 45:146), five good dominant mutants were obtained. They included one striped virescent, one dwarf, one yellow striped and two which mimic disease lesions caused by Helminthosporium species. In addition to these five viable mutants, a larger number of dominant inviable cases also occurred, but these could not be propagated and were lost without confirmation. All five have been transmitted through the pollen for at least two generations.

The striped virescent mutant first appears as a nearly white to pale green seedling that gradually changes to a green seedling with white or pale green stripes much like v_5, though the striping may be more extreme. Viability is good, though homozygotes may be too extreme to survive in some cases.

The dwarf mutant is very extreme, rarely growing more than four inches high. Plant parts are small, and internodes are shortened. Some plants produce a few normal anthers which have normal pollen. The mutant does not respond to gibberellic acid.

The yellow striped mutant is not expressed in the seedling, but first appears at the 6-8 leaf stage, when a yellowing of tissue between the veins of the terminal half of all leaves occurs. The appearance is like that of ys, but less extreme. As the plant matures, strong anthocyanin appears on the blade of affected leaves. Viability is good, though plants with extreme expression may be small and weak.

The disease lesion mutants are described under a separate heading below.

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4. Dominant disease lesion mutants.

The first of the disease lesion mutants (designated Les) referred to above appears initially at the 3-leaf seedling stage. One to several irregular, elliptical shaped watery spots (1-3mm x 4-7 mm in size) appear scattered over the surface of the first leaf. In 24 hours these spots develop a necrotic appearance on the top surface of the leaf and sometimes a drop of dark brown exudate on the underneath surface.

Apparently there is a breakdown of cell membranes, allowing some cell fluids to escape and collect in a drop under the lesion. This is followed by an enlargement of the spot and a drying of the drop of exudate to form a lesion with a light center of dead cells surrounded by a dark ring of dried exudate which is in turn surrounded by a region of degenerating necrotic cells. This gives a halo type effect that is typical of lesions from fungal infection. Some lesions remain at this stage, while others continue to spread until the whole leaf becomes withered. This progression of events continues slowly up the plant as other leaves develop and mature. At flowering, a mutant plant may have 3 or 4 fairly sound leaves with each leaf from top to bottom being progressively more withered. The appearance is typical of and practically indistinguishable from the symptoms expressed by a susceptible plant infected with H. maydis, except that the ears produced do not have the hyphae and spore masses usually associated with fungal infections.

The second disease lesion mutant (designated Spt) is first expressed by tiny light spots on the first leaf of a two-leaf seedling. The cells in the spot die abruptly, leaving small, almost white necrotic spots (0.5 mm x 1-3 mm). The spots remain small, and new ones appear on successive leaves as the plant matures. The appearance is typical of the reaction of one type of resistant (hypersensitive) host to the fungus H. maydis. The mutant has very little effect on plant vitality.

Crosses between heterozygotes for the two mutants (+/Spt x +/Les) produced normal plants, plants with lesions, with spots and with both. The phenotype of the double mutant plants was modified, however. Lesions and spots appear on the same leaf, but the lesions were smaller, less deleterious and more frequent, while the spots were larger and less frequent. Subsequent crosses to determine allelism have not given conclusive results because of erratic expression in the progenies of the double heterozygote.

The question of whether these mutants are disease mimics or are cases of susceptibility to some commonly occurring disease-producing organism has been considered. All of the cultures were grown either in fields that were fairly free of Helminthosporium species or in a greenhouse in winter. Segregating sibs and all other cultures were free of

the effect. Crosses were made on several unrelated lines using pollen from mutant plants. In all cases the offspring segregated for mutant and normal types.

To exclude the possibility of a common external infection, kernels of a cross of normal by mutant (for both cases) were washed in 2.63% sodium hypochlorite, soaked in water overnight, and the embryos excised. The embryos were washed again and grown on a sterilized agar medium in testtubes. The mutant phenotype appeared on a number of the cultured seedlings, though not in numbers that would confirm a 1:1 ratio. Efforts to obtain fungal cultures from affected leaves have been unsuccessful.

Based on evidence so far obtained, one would conclude that the mutants are dominant mimics of two states of susceptibility to the fungus H. maydis. However, the striking resemblance of the mutant phenotypes to actual disease lesions raises some doubt.

A number of mutants resembling the large lesion type have been reported. Emerson (Cornell Memoir 70:3-16, 1923) describes a recessive mutant called blotched leaf (bl) which is expressed just before flowering. Simmonds (MNL 24:26-27, 1950) and Hornbrook and Gardner (Radiation Botany 10:113-117, 1971) report similar cases. Ghidoni (Academia Nazionale Dei Lincei, 1973) reports a necrotic lesion mutant linked to wx.

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5. Interactions of the brown-plant anthocyanin factors.

The loci A, A2, Bz, Bz2, and C2 have recessive expressions characterized by partial or complete replacement of anthocyanins by brown pigments in husks, sheaths, cobs, and other plant tissues. Strains homozygous for B and Pl, in intensely pigmented, uniform background were developed. The comparative phenotypic expressions of a, a2, bz, bz2, and c2 now can be described with confidence from several years of tests and observations, along with the effects of these factors in combinations, two at a time. For the combinations, F2 families segregating for two factors were graded and defined without knowledge of genotype. Genotypes were identified individually from testcross results following harvest (the technical assistance of M. D. Murray in the conduct of these tests