Young flower buds on intact plants or on cuttings may be exposed to various mutagens in either a gaseous or aqueous state. Material for cytological studies may be fixed within 24 hours after treatment; pollen abortion in mature flowers may be observed with peaks at 5 to 7 and 16 to 20 days after treatment, reflecting injury induced during microspore mitotic and meiotic stages respectively; loss of reproductive integrity of stamen hairs reaches a maximum at about 14 days; and somatic mutations and morphological changes in petals and stamen hairs may be scored throughout a 10- to 20-day post-treatment period; stamen hairs (17) and haploid pollen tubes (95) provide excellent material for chromosome analysis.

Various other genera and species have also been used to detect somatic mutation and morphological changes in petals and stamen and should be equally useful in chemical mutagen studies (table 1). Specific-locus method (waxy locus) in pollen.—The waxy locus in maize, barley, and rice determines the type of starch which is synthesized in the triploid endosperm and in the haploid pollen grain. In the case of the pollen grain the phenotype is determined by its own genotype and not by the genotype of the plant. The dominant wx pollen grains stain blue with an iodine-potassium iodide stain while recessive wx pollen grains stain a reddish-brown color (73, 74) because wx pollen lacks the enzyme required in the last step of starch formation. Since the wild type is wx, the frequency of induction of ww can be assayed in millions of pollen grains relatively easily and quickly. Furthermore, the phenotype appears in the treated generation, and does not require the time necessary to obtain an M2 generation. This technique was used in barley by Eriksson (23) who irradiated plants homozygous for wx and analyzed the frequencies of reversions from the waxy phenotype to the wild type, and Baldi, (3) who studied the spontaneous back mutation rate at this locus in rice. The frequency of intra-cistron recombination may also be measured with this technique by crossing two ww mutants of independent origin and collecting and staining pollen from the F1 as done by Briggs and coworkers (6, 7) for EMS- and radiation-induced mutations. This is a very simple and rapid technique for detecting even very low frequencies of induced mutations in higher plants.

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