This experiment was performed to determine efficient treatment procedures with Myleran based on physiological and genetic effects (detected from leaf sector analysis and mutants obtained). Apparent mutations have been obtained; however they have not met the criteria of Stadler (1946), nor have they been checked for correspondence or contamination. However, contamination should not be a problem, since the field was isolated from other maize and the treated stock was dominant for the genes that were analyzed.

Apparently Myleran is not nearly as efficient as ethyl methanesulfonate in producing mutations (Amano and Smith, in manuscript). Solubility may be a factor affecting its mutagenic efficiency. However, certain treatments are rather effective in producing leaf sectors and aleurone color mutations. Also, these data indicate that still more effective treatments can be devised.

R. W. Briggs

BROOKHAVEN NATIONAL LABORATORY*
Upton, New York
Biology Department
and
TEXAS A and M UNIVERSITY
College Station, Texas

1. Further progress in perennialism of Zea.

A. Diploids. A continuation of the work of selective breeding in the Clone A family of perennial clones (MNL 38: 17-21) has resulted in the production of several 20 chromosome derivatives which can be cloned and apparently maintained indefinitely with careful handling. While they do not breed true for perennialism upon selfing, they are much more fertile than the parental Clone A. Moreover, it is the first time that factors needed for a minimal expression of perennialism have been shown to be transmitted by near diploids (though this transmission of course occurs rather readily by triploids). This indicates

*Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

that progress is being made in incorporating needed genes into gametophyte-viable chromosomes. Since these derived 20 chromosome perennials are maize-like, resembling maize-teosinte derivatives having more than 75% maize heredity, and produce polystichous ears, it seems fair to describe them as prototype perennial maize. Unlike Clone A, ear production does occur on long days, but on such a regime, ear formation is delayed and the organ is suppressed in size, but is competent to produce seed. Perennialism is maintained because basal branches retain totipotency. Propagation is facilitated by the production of an annulus of adventitious roots by each branch near the point of attachment to the parental plant.

Further study of Clone A derivatives partially confirms the idea that this group of material, as proposed previously (MNL 38: 17-21) is homozygous recessive for a major perennialism locus. When two "late" white maize inbreds were outcrossed to Clone A, backcrossed, and selfed, "clean" segregations were noted for the Clone A long-day phenotype, namely delayed tassel formation, and a failure of, or abortive, ear formation. This confirms the idea that Clone A has derived by means of an event whereby a major perennial teosinte locus has been successfully diploidized and is normally transmissable when inserted into 2n maize. However, this expression of the factor, provisionally named the "pe" gene, could not be identified when inserted into a white sweet corn, among five BC_1 S_1 progenies. Hence the locus is either background-dependent, or a 3% chance has "come off." Since none of the maize progenies displaying the "pe" phenotype were perennial, because they lacked basal branching, it seems possible to support the working hypothesis that perennialism displayed by the Clone A family is attributable to a major gene plus an unknown number of loci which confer basal branching and a favorable "background." Additional work has been initiated to gain further information on the nature and background dependency of "pe". It is recognized that "pe" may represent a close linkage rather than a "point" gene. It would obviously be difficult to locate its map position if available genetic stocks did not supply an effective "background."

Many massive experiments failed to produce perennial near-diploids (Genetics 50: 393-406 and unpublished results). Even though in this disappointing work derivatives often showed high degrees of branching once the first culms became florally induced, all succeeding basal branches lost totipotency, as in

pure maize. The entire Clone A family, which finally lead to success in isolating 20 chromosome perennials, was derived from a single exceptional plant. This plant and its Clone A derivatives differed markedly from all previous material in that once a basal branch was produced, it remained totipotent; hence most Clone A family near-diploids were perennial.

On the assumption that pe acts to confer totipotency upon basal branches, this "gene" was combined with the gt gene discovered by E. G. Anderson. One hundred and thirty-three seedlings, expected to segregate 15:1 for the double recessive phenotype were seeded in the greenhouse in early April, and transplanted out-of-doors in early May. Grassy tiller segregates soon cut off the first generation of tillers which in most cases ended determinately in abnormal female inflorescences in maize-like fashion. In 8 of the 133 plants, however, the "grassy tillers" grew luxuriantly, and reached a size superior to that of the main stalk. Both the first culm and the first generation tillers produce normal, fertile tassels, and polystichous, fertile ears, though these were tassel-tipped and delayed in timing. First-rank tillers soon produced a new generation of tillers. Either first or second generation tillers could be removed and easily propagated by virtue of their production of adventitious roots near the point of attachment to the parental culm. Both continued to produce further tillers after such cloning. Unfortunately, however, the third and succeeding vegetative generations were no longer indeterminant, but showed a progressively suppressed and preinduced growth habit. As of early October, the fourth vegetative generation had been produced. Four of the most vigorous of these were lifted and brought to A & M where a long day regime was initiated in a futile attempt to restore normal growth aspect to the fifth vegetative generation. As of January, 1965, only one is still alive, now on its sixth vegetative generation, but is almost certainly now dying.

While these "two gene" gt/gt pe/pe clones of essentially pure maize were not perennial, at least under the conditions described, they were possibly more perennial-like than any maize observed thus far. As of early October, the double recessive segregates described above were perfectly green, including the first culms, bearing mature ears, while their sib mates were almost completely dead. It is possible that the progressive suppression of the third and succeeding vegetative generations may have related

to a short day effect imposed by the advancing fall season, and that these two-gene clones might be perennial in a continuous regime of long days. This possibility is being tested. Moreover, the unexpected production of near-normal ears by these clones may have related to an unfortunate choice of a maize background marginal for the expression of pe. This possibility is being tested by transferring the two-gene system into a maize background in which the expression of pe is "clean." Moreover, additional syntheses are being made in which other maize loci, such as id, which confer attributes of perennialism, are being added to the gt-pe combination. Nevertheless, it appears that gt-pe maize is unique. One obvious use for it would be as a near-perennial "base" recurrent parent from which to extract pure-breeding perennial diploids from perennial teosinte or from Clone A family derivatives. Another might be as a simple modification in a seed field pollen parent to delay and spread pollen production without delayed planting. Since both gene modifications are recessive, resulting seed production would have a normal phenotype. Another might be as an evergreen-proliferative type of maize where mature ears could be harvested from a field which would then serve as a source of succulent pasturage until freezing weather.

B. Tetraploids. Rapid progress continues in breeding for perennialism and maize-likeness at the 75% maize level. While most segregates produce four-rowed ears, the frequency of polystichous ears was greater in the fourth generation of mass selection than in the third. Moreover, the incidence of plants showing production of rhizomes during the juvenile growth phase has also increased markedly. The ease with which this level of results was obtained is greatly surprising, in view of the fact that the project was begun with the expectation that it would be a 20 to 50 year task. It appears rather that if the proper effort could be supported, practical 4n maize perennial varieties could be achieved within five years. The perennial expression in general is enhanced by environmental factors which induce vegetative vigor in maize, such as cool temperatures, long days, high light levels, adequate levels of plant food, and uncrowded growing space. The expression is depressed by the opposite conditions, which are often to be found in the greenhouse. In pot culture, polystichy is depressed, as in depauperate maize, and field-polystichous segregates are often distichous in pot culture. Ear formation is greatly depressed by long days, even though tassel formation

and dehiscence proceeds. There does not, however, appear to be set ear-inducing photoperiod, but rather the threshold appears to be intrinsically set differently in each plant by genetic factors. Thus at Brookhaven, ears harvested from perennial plants on October 7 ranged in maturity stage from complete maturity to only milk stage. Basal branch formation is inhibited by "age" after a juvenile flush of activity. This inhibition is relieved by further aging of the first culms, and renewed basal growth occurs at the time maturity of seed is reached on the first culms. In marginally perennial plants, such renewed growth is often preinduced, and the branchlets, usually subtended by their own annulus of adventitious roots, can be removed and cultured, and these propagules may then terminate in a fertile female inflorescence. Axillary buds of such propagules, however, may develop as fully indeterminant culms. Hence marginally perennial plants have often shown an alteration of totipotency among the axillary buds of successive vegetative generations. Basal activity can be stimulated at any time by removing older culms. The perennial expression thus appears to be intimately associated with auxin-level controlling factors and photoperiod responses.

C. Winter hardiness. A small population of 4n perennials is being observed for winter hardiness at College Station. A small fraction still persists as of late January. Hardiness appears to have several components: 1) "resistance" to preinduction during the long fall inducing photoperiod before frost, 2) resistance to the shock of hard freeze-backs, 3) maintenance of activity by deeply placed buds, and 4) resistance to freeze injury by rhizomes, and relative frost resistance of foliage. The appearance of marked differences in the overwintering population suggests that a great deal of selection progress could be made. The often observed frost resistance of maize plants having a high sugar content suggests incorporation of C103 genes into perennial material as another active approach. As of now, several plants have an excellent chance to survive the winter.

D. L. Shaver*

Present Address: Department of Plant Sciences, Texas A & M University, College Station, Texas.