

Through this observation it appears likely that the variations in the area of the leaf chlorophyll deficiency might be regulated by a second nuclear element in addition to the structural genes in the chromosome. This element inhibits the synthesis of chlorophyll by inactivating the enzyme catalyzing the reaction of chlorophyll synthesis. The time of operation of this element varies from plant to plant in accordance with the cellular environment. For the albino seedlings, the inhibiting element functions when the seeds just start to germinate. For the Class A plants this element starts to function later than that of the albino seedlings. Likewise, the element of Class B plants acts later than that of Class A plants, and that of Class C, later than that of Class B, and that of Class D, later than that of Class C.

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1. Progress towards perennial Zea diploids.

Experiments were reported last year which show that one can make steady progress in increasing the perennial expression of maize-teosinte derivatives at the $4N$ level by the simple breeding technique of repeated mass selection for perennialism. Thus at the 50% maize level, it has been possible to increase the incidence of perennial segregates from an original level of about 0.3 to 0.75 in only 4 cycles of selection. Similarly, the production of basal branches, an attribute of perennialism, was increased from about 4.4 per plant to about 10.0. At the 75% maize level, only two generations of selection have increased the incidence of perennial segregates from 0.0 to about 0.35. Unhappily, either genetic or agronomic investigations of perennialism are difficult, if not meaningless, at the $4N$ level.

The situation is entirely different when working at the diploid level. As outlined, one can easily obtain large populations of maize-perennial teosinte diploids by producing the F_1 triploid generation, backcrossing this triploid to maize, and then intercrossing the resultant array of aneuploids. The chromosome number is rapidly stabilized at 20 because of gametophyte selection for euploidy. Due to the high degree of preferential pairing in the F_1 triploid, the first post triploid generation carries a high theoretical proportion of perennial chromatin, somewhere between 40 and 45%, depending upon certain assumptions.

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Since 4 separate experiments, involving now thousands of putatively perennial segregates derived by as many as 5 successive generations of intensive selection for perennialism or perennial attributes, have completely failed to yield any perennial diploid segregates at all, it is clear that diploidy imposes special difficulties upon the task of reconstructing a genotype which might confer perennialism. One does not even make progress in increasing the expression of perennial attributes, such as basal branch production. Thus in the 2nd post triploid generation, the number of basal branches per plant was 3.3. In the 3rd generation it was 2.9, and in the 4th, 3.1. At the same time production of apparently normal pollen by diploids increased from 88% to 95%, while the net viability of seeds and seedlings increased from 66% to 85%. (The pollen studies noted here represent scores taken from side-by-side outdoor-grown samples. The studies reported last year [MNL 37:8-11] were from greenhouse collections, and were extremely erratic by comparison. It is suggested that the data from outdoor collections should be regarded as valid.) These disappointing experiences with diploids can be explained if perennial teosinte, during its evolution as a tetraploid, has accumulated many mutations, such that if one attempts to rediploidize segments carrying them, an essential function may not be represented, and such "sheltered" tetraploid factors then act as haploid gametophyte or diploid sporophyte lethals. If the loci from perennial teosinte which confer perennialism are linked with such lethals, they will be eliminated inexorably in diploids. Moreover, while the phenotype of the triploid F_1 , and to a lesser extent, the first post triploid generation, look decidedly intermediate between maize and teosinte, the advanced post triploid generations become, in spite of contrarywise selection pressure, extremely maize-like.

It has been possible to rule out the idea that one fails to recover perennialism in diploids because of the absence of tetrasomy by directly doubling with colchicine several of these advanced generation diploids. Thus of nine confirmed and 12 probably newly redoubled tetraploids, none showed the slightest degree of perennialism or increase in the expression of perennial attributes, and all died at the same time as their nondoubled sib mates.

Although an exhaustive study has not yet been made, several diploids having perennial teosinte segments have been examined at pachynema. Since no large, classical rearrangements have been found so far, it seems certain that if they exist, they are certainly not generalized, and cannot explain the general elimination of teosinte characters. The weight of evidence is therefore that one has to deal with subvisible, or "genetic" factors.

As reported last year, only one breakthrough in this otherwise bleak picture has been found. One plant, a trisomic, found in a first post triploid generation, "Clone 85-12," was obtained which although very weak and extremely difficult to maintain, did appear to be perennial. Although it is so far completely self sterile, it does produce pollen about 5% of which stains with IKI. Some of this pollen was used to effect fertilization of perennial teosinte ovules. Of the triploids thus obtained, one was fertilized again by 85-12 pollen. Of the resulting aneuploids, two were saved, with 22 and 24 chromosomes respectively.

These were grown in greenhouse culture, and upon the demise of the first culms, basal and axillary began to produce offsets which could be separated and thence develop into fully normal plants which again produced many fully totipotent basal branches. It is sufficient to say that the 22 chromosome derivative, which has been retained as "Clone A," has a vigorous, fully perennial phenotype and it can be cloned with ease to any desired number of propagules. Out-of-doors it is extremely vigorous, and its basal branches become at least pseudo-rhizomatous. Although it produces about 70% staining pollen, and a multitude of vigorous, 4-rowed distichous ears, it is also nearly self sterile. Only 16 seeds have been obtained from numerous pollinations. Of these, 10 germinated successfully. Two proved to be albinos, while the remaining eight grew to maturity. Three were trisomics, and five were euploids. Even though five of the eight were grown out-of-doors, none showed any perennial tendency or attribute. No basal branches at all were produced under the same conditions in which the sexual parent, Clone A, formed as many as 16 basal branches during one growing season and was still growing strongly when freezing temperatures brought the season to a close. The one ear produced by the sexual derivatives of Clone A was polystichous and the plants were otherwise maize-like. This appears to be the best evidence that loci from perennial teosinte are selectively eliminated in diploids.

While Clone A and 85-12 are nearly self sterile, they may be crossed together, and viable progeny obtained. Of 18 seeds so obtained, 10 grew successfully; one of these appears to be a perennial whose root tips yield perfectly repeatable counts of both 20 and 21 chromosomes.

Clone A may also be crossed with perennial teosinte, and the resulting triploids crossed back to Clone A. From the ensuing aneuploids, one can select those with the lower chromosome numbers for further work. Unlike previous experiences, all such populations at the near-diploid level which were derived ultimately through 85-12 (by way of Clone A) are essentially perennial populations with a few exceptional annuals. Even though as a group these also tend to be strongly self sterile, a few seeds can be obtained by sib pollination.

In summary, the study of pollen abortion, seedling and seed lethality, and inability to transmit teosinte characteristics, including perennialism, at the diploid level or near diploid level, support the idea that perennialism-conferring loci in teosinte are linked with diploid-lethal factors which make perennialism difficult to recover in diploids. Clone 85-12 may have arisen by means of a fortuitous crossover such that a key perennialism locus may be brought at least to a diploid or near-diploid background. The evidence that 85-12 may represent such a specific event is that near diploids derived through it are not only unique in being essentially all perennial, but all possess, in addition, a photoperiodic response such that on long days only terminal inflorescences are produced, while in a short day regime, both terminal and lateral inflorescences appear. In either regime, however, at least Clone A continues to produce totipotent basal branches, and therefore is still perennial even in a short day regime.

Because of the perennial nature of the material, Clone A now represents an easy means to produce any number of additional perennial diploids

and near diploids that could be desired. If the remaining task is essentially one of obtaining fortuitous crossovers in order to place perennialism-conferring loci into maize chromosomes, one then appears to be in the position of having the means by which any degree of pressure may be applied to the problem that it may require.

D. L. Shaver

2. A simple mechanical method of inducing tetraploidy.

Heat shock and colchicine treatments, historically speaking, have yielded a very low percentage of success in producing maize tetraploids. Recently, genetic methods of introducing new chromosomes from diploids into existing tetraploids have been proposed and come into vogue. These methods, involving the genes elongate, asynaptic, and ameiotic, however, require several generations to bring the inducing gene into the background one wishes to tetraploidize before one succeeds in deriving the desired diploid gametes. In an agronomic situation, one eventually faces the problem of linkage of the inducing genes to unfavorable factors. Moreover, the inducing gene itself is introduced into the new tetraploid, where it is then undesirable.

Two experiments have shown that one can easily introduce desired chromosomes from diploids into existing tetraploids by the straightforward procedure of crossing a $4N$ female by the desired diploid. If the resulting shriveled seeds are dissected and the embryo proper is removed and shallowly planted in moist soil, or other situation where the developing embryo can soon persist on its own photosynthate, no difficulty is encountered in growing these triploid embryos. (The dissection procedure was suggested by Ellen Dempsey.)

Triploids produce much pollen in the greenhouse, or out-of-doors on Long Island, but they may not if grown outside in less favorable environments. One can collect this pollen in conventional pollen bags, or by shaking it directly off of the tassels, and then straining it through a stack of U.S. Standard Sieves (W. S. Tyler Co., Cleveland 14, Ohio). A top screen with a mesh size of 149 microns removes clumped pollen and loose anthers. The next screen with a mesh size of 125 microns catches a small proportion of the very largest pollen grains from triploids (and tetraploids). No diploid has yet been found to produce pollen grains which "stay" in this screen. The next screen with 105 micron mesh catches most of the large grains. These were discarded in this experiment. The next screen with 74 micron mesh catches most of the viable small grains, while the last, 53 micron mesh, seems to catch only aborted or dried grains.

The following results were obtained by sipping a population of triploids with the 74 and 125 micron pollen fractions: