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The Use of the Monoploid Method for the Production of Homozygous Diploid Lines of Corn

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THE USE OF THE MONOPLOID METHOD FOR THE PRODUCTION OF HOMOZYGOUS DIPLOID LINES OF CORN

by

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INTRODUCTION

Monoploid individuals have been observed to occur in several species of plants at varying, though usually low, frequencies. Spontaneous or artificially induced doubling of the chromosome complement of cells in sectors of the pistillate and staminate meristems of the monoploid plants greatly increases the chances for successful self-fertilization. The homozygous diploid lines so produced are the genetic equivalents of advanced generation inbred lines.

This procedure has certain advantages over the customary practice of self-pollination for a number of generations to produce inbred lines. The frequency of occurrence of zygotes, homozygous for certain desirable genes, in the progeny of an individual heterozygous for the genes in question, will be the square of the frequency of occurrence of the gametes carrying the desired genes. The monoploid method fixes the genetic systems of individual gametes in testable, reproducible form. The resultant sporophytes are completely homozygous and should prove advantageous for the study of certain breeding problems.

These homozygotes would offer an opportunity for the study of heterosis in a new light resulting in a clearer explanation of hybrid vigor from the genetical point of view. The comparison of related populations of lines derived by the doubling of monoploids and lines obtained by inbreeding and selection would be expected to yield information as to the effectiveness of breeding methods in excluding deleterious genes.

The diploids derived by this method could also be utilized for the study of experimental designs in the field and for determining the residual variability due to environmental factors.

Testing of the monoploids themselves for certain agronomic characteristics, such as disease resistance, followed by backcrossing the plants so tested to the source stock should permit the development of heterozygous stocks with high frequencies of desired genes.

The development of a practical method of breeding corn through the utilization of homozygous lines obtained from the doubling of monoploids will depend upon the obtaining of a reasonably high rate of monoploids from any particular stock, representing desirable gametes for corn production, and on the successful doubling of such monoploids. This study is an investigation of various aspects of these problems.

REVIEW OF THE LITERATURE

The literature on parthenogenesis, haploidy, and colchicine techniques is rather extensive and a complete review is beyond the scope of the present study. Therefore, only that portion of the literature concerned with the more pertinent aspects of the problem which have been investigated in the higher plants has been selected for review. Also, little of the literature dealing with the cytogenetic aspects of the problem has been included herein.

The terminology of the particular author has, in all cases, been retained. Certain portions of the review dealing with allopolyploid species, such as those of the genus Nicotiana, will present data and observations concerning haploid plants which are not monoploids. These papers have been included for review because of the similarity of the basic processes and problems to those of species where the haploid plants are true monoploids.

Haploidy was first cytologically proved and reported by Blakeslee, Belling, Farnham and Bergner (1) in the genus Datura. With increasing knowledge and refinement of techniques in the fields of genetics and plant breeding, numerous instances of the occurrence of haploids in the higher plants have been reported. Their occurrence has been noted in the following genera, among others: Capsicum, Crepis, Datura, Fragaria, Gossypium, Hordeum, Lilium, Lycopersicum, Nicotiana, Oenothera, Solanum, Sorghum, Triticum, and Zea.

In all genera, the detection of the parthenogenetic haploids is possible in hybrid progenies of intra- or interspecific and intergeneric crosses primarily by their resemblance to the maternal type in contrast to the paternal type where dominance is involved. The opposite situation

exists where androgenetic haploids are distinguished by their resemblance to the paternal type in contrast to the maternal type.

With few exceptions the frequency of occurrence of haploids has been very low. Between 1931 and 1937, Satina, Blakeslee, and Avery (40) isolated 73 Datura haploids from a population of 410,000 individuals, a frequency of about 1:5,600. Johansen (25) reported an unusual frequency of 1:100 from one commercial lot of barley. Harland (22) found a rate of 1 haploid among 3,000 to 4,000 plants of sea-island cotton. Jones and Longley (26) discovered 11 haploid plants among approximately 300,000 rice plants. Katayama (27) has observed them to occur at a frequency of 0.48 per cent in Triticum monococcum. Smith (41), however, worked with the same species but found a frequency of 0.1 per cent or 1:1,000.

Observations by Randolph (39) have indicated that the frequency of the parthenogenetic development of the egg nucleus in maize under the stimuli of pollination and normal endosperm development is of the order of one in two thousand, or 0.05 per cent.

More recent investigations, however, have shown that perhaps the frequency may more closely approximate one in one thousand. Chase (4) reported obtaining 43 monoploids from 38,684 seedlings, a frequency of 1:900. Einset (12) found that progenies from trisomic maize plants pollinated by normal diploids gave a frequency of monoploids of 1:958. Frequencies have varied, however, from 1:100 in a diploid multiple recessive tester used by Stadler (unpublished) and reported by Chase (4) to 0:14,648 obtained by Fortuno (16) among progenies of five open-pollinated maize varieties.

The euploid reduction of chromosome numbers that produces haploid sporophytes has been observed to be accompanied by certain changes in

morphology that are characteristic of the monoploid condition. These effects, as would be expected, are a reversal of those which have been widely observed to occur as a result of euploid increases of chromosome numbers. The addition of a complete genome has been noted to bring about an increase in size of nuclei, cells, and some organs, as well as prolonged vegetative growth periods and certain physiological alterations. These effects have been reported by Elmsweller and Ruttle (13), Kostoff (28), Noggle (36), and others.

The most direct change resulting from the initiation of the monoploid condition is that of a reduction in the size of cells and nuclei. Kostoff (28) studied the ratio of both linear and volume dimensions of cells of haploid and diploid forms of three species of Nicotiana and Crepis capillaris. Root cells, stomatal cells, and pollen mother cells were measured during the course of the study. The least amount of change occurred in the linear dimension of pollen mother cells of N. sylvestris where the cells of the diploid form were 131 per cent larger than the cells of the haploid form. The data on the three species of Nicotiana were further substantiated by the same author in a latter paper (29).

Gates and Goodwin (18) noted that the cells of a haploid Oenothera rubricalyx were smaller than those of the diploid form. Similarly, Gains and Aase (17) found that haploid pollen mother cells of Triticum compactum humboldtii were smaller than those of the diploid plants. Emerson (14) reported that the mean diameters of nuclei of pollen mother cells in the diploid and haploid forms of Oenothera franciscana were 12.9 and 9.6 microns, respectively.

Navashin (34), who worked with a polyploid series of Crepis capillaris, found a strong positive correlation between the chromatin mass as measured by the length of the chromosomes in the meristem cells

of root tips as they appeared in metaphase and the cell volumes. He noted that the cell areas and cell volumes of the haploid form of this species were not quite half as large as those of the diploid form.

The work of Wettstein (45) and Kostoff (29) has demonstrated that the morphological alterations of haploids and experimental autopolyploids are ultimately due to alterations in cell size. Haploids usually appear as more delicate mane-forms of the diploids with a reduction in size of various organs and plant parts.

Smith (41) observed that the leaves of haploid Triticum monococcum plants were narrower and shorter than were leaves of the diploid plants. Haploid Crepis capillaris plants were shown to resemble reduced diploids by Hollingshead (23) but they differed somewhat in leaf shape.

Chase (5) noted that a morphological characteristic of maize monoploids could be used to distinguish them from diploid plants. The first seedling leaf of monoploids was found to be less than one-half as long as the comparable leaf of diploid seedlings of the source stock in almost all instances. Fortuno (16) has also noted an altered leaf appearance in haploid maize plants.

Effects similar to these have been reported in various species by Blakeslee, Belling, Farnham and Bergner (1), Brown (2), Christensen and Bamford (6), Emerson (14), Gates and Goodwin (18), Lamm (30), Lindstrom and Koos (31), McCrey (32), Nordenskiold (37), and Tometorp (43).

A great many of the haploids observed in plants have arisen among hybrid progenies of interspecific or intergeneric crosses. For this reason, it has been held by various workers, as for example, Kostoff (29), that hybridization is one of the more effective methods for the production of haploids.

By crossing Nicotiana rustica by Solanum nigrum, Petunia violacea, Lycopersicum esculentum, Atropa Belladona, and Datura stramonium, Ivanov (24) attempted to study the effectiveness of hybridization in the production of haploids. Only one viable seed was obtained and it was a parthenogenetic diploid Lycopersicum esculentum.

In Triticum monococcum, Smith (41) observed 49 haploids among 2,478 hybrids (2 per cent) after crossing with T. aegilopoides and 7 among 8,700 individuals (0.08 per cent) from naturally selfed progenies.

Haploidy was observed in Oenothera by Davis and Kulkarni (9) without employing crossing, while Emerson (14), Gates and Goodwin (18), and Catcheside (3) found haploids in the same genus after interspecific hybridization. Davis (8) failed to induce the development of haploids in Oenothera Lamarckiana by pollinations involving wide crosses.

Similarly, Satina, Blakeslee, and Avery (40) have reported that the frequency of haploids in Datura from selfed progenies does not differ from that in progenies from inter- and intraspecific crosses.

The first two Datura haploids obtained by Blakeslee, Belling, Farnham, and Bergner (1) were from a group of abnormally appearing individuals secured in an attempt to induce chromosomal irregularities by the application of cold as a stimulus.

Randolph (38) found a haploid in Zea in material treated by heat during the course of an experiment designed primarily to test the effects of heat treatments on the fertilization process.

According to Kostoff (29), the experiments with temperature led Haberlandt (21) to believe that necrohormones from dying cells in the neighborhood of the egg served to stimulate its parthenogenetic development. However, Gustafsson (20) rejected the idea of the stimulus

of the necrohormone. He believed that the development of the egg-cell was, in most cases, autonomic and was not determined by any stimulation from dying cells in close proximity to it.

Mechanical stimulation of cells and nuclei in the ovules without fertilization was attempted by Davis (8) in an effort to induce parthenogenesis. Some 200 ovaries of Oenothera Lamarckiana were pricked and slashed without success.

Chemical treatments to induce parthenogenesis were attempted by Van Overbeek, Conklin, and Blakeslee (44). A non-viable pseudoembryo consisting of several hundred cells derived from the inner layer of the integument was produced by the injection of naphthaleneacetic acid into ovaries of Datura stramonium. Several other substances injected in combination with auxin failed to induce the formation of viable seeds.

Smith (41) found that by delaying pollination in Triticum monococcum the per cent of haploids in the progenies was increased markedly. By permitting 3, 6, and 9 days to lapse between the opening of the first florets and emasculation, he obtained haploid frequencies of 1.1, 5.4, and 20.6 per cent, respectively.

According to Smith (41), Stadler (unpublished) had not increased the frequency of haploids in maize by delaying pollination.

Fortuno (16) found that the frequency of the occurrence of haploids in maize was not increased by delayed pollinations for periods of eight or twelve days.

Chase (4,5) obtained similar evidence that delayed pollination of itself is not a sufficient explanation for the wide variations of frequency of haploidy recorded in maize.

The frequency of monoploid development in maize was found by Chase (4,5) to be largely dependent upon the seed stock and pollen parent used.

Certain stocks when used as seed parents tended to produce significantly higher frequencies of monoploids than other stocks, irrespective of the pollen parent used, and similarly certain stocks when used as pollen parents tended to produce higher frequencies of monoploids than others, irrespective of the seed parents involved. Thus, the actual incidence of monoploids obtained in the progeny of any particular cross was found to be influenced by both parents in spite of the fact that the pollen parent contributed none of its genes to the monoploid plant. Diploids derived from monoploids were found to be particularly favorable parthenogenetic stocks.

The artificial production of haploids by X-ray treatment of the female organs or pollen cells has been practiced rather extensively, but the results obtained by the various workers do not appear to be uniform nor conclusive.

The first haploids derived by X-ray treatment of pollen were obtained by Stadler (42) in corn (Zea mays).

Katayama (27) located 2 haploids among 38 individuals (5.26 per cent) produced by spikes of Triticum monococcum X-rayed during meiosis. When mature pollen was irradiated and applied to normal pistils many more haploids (17.58 per cent) were derived. When irradiated pistils were pollinated with normal pollen, no haploids were obtained. Katayama concluded that the sperm nuclei from irradiated pollen mother cells sometimes degenerated after entering the ovule, without fusing with the egg, but their presence stimulated the egg to develop parthenogenetically.

Kostoff (29) interpreted Katayama's observations as definite proof that pollinations with X-rayed pollen greatly increases the frequency of haploids but assumed that the frequency would vary with the strain of the species used.

According to Fortuno (16) Yefeikan and Vasiljev (47) also studied the effects of X-ray treatment of pollen on haploid frequency in 8 Triticum species and varieties. They found 2 haploids among 35 seeds obtained (5.7 per cent), one a T. persicum var. fuliginosum, and the other a T. dicoccum var. rufum.

An androgenic haploid exhibiting all the recessive characters of the male parent was obtained by Gerassimova (19) from the progenies of Crepis tectorum flowers treated with X-rays and pollinated with normal pollen.

Following X-ray treatment of pollen, Ivanov (24) located four Nicotiana rustica haploids. The variety humilis when crossed with the variety texana, its pollen subjected to a dose of 17,000 r., produced one haploid among 17 plants (i. e., a frequency of 5.9 per cent). Three others were obtained among 21 plants (i. e., a frequency of 14 per cent) following the reciprocal cross with the humilis pollen subjected to a dose of 26,000 r.

Smith (41) found that X-ray treatment of Triticum monococcum did not increase the frequency of haploidy under the conditions of his experiment.

Haploids have been observed to occur in relation with polyembryony in various species. One of the more extensive studies of twin embryos was conducted by Harland (22) using Sea Island and Upland types of cotton and the hybrid Gossypium hirsutum x G. barbadense. He found 20 twin-embryo seeds among several thousand seeds of the Sea Island strain V. 135 in which one of the embryos was usually much smaller than the other. From these he was able to raise 14 haplo-diploid pairs to maturity. He also obtained a haplo-diploid twin from the Upland cotton (G. hirsutum) and observed cases of twin embryos in strains of mixed

G. hirsutum - G. barbadense ancestry, at least one of which was a haplo-diploid twin pair. He provisionally concluded that not only a genetic basis existed that controlled the frequency of occurrence of haploids in different strains, but that the degree of fertility of the haploids was also a function of genetic constitution.

Cooper (7) studied the incidence and relative development of twin embryos, one diploid and one haploid, in seven species of Lilium at early stages of embryogenesis. The seven species included the following: Lilium Martagon, L. regale, L. superbum, L. elegans, L. Henryi, L. Hansoni, and L. longiflorum. He observed 102 instances of developing haplo-diploid twin pairs from 9,500 ovules studied. One haplo-triploid twin pair was also obtained by him from the cross, Nicotiana glutinosa x N. tabacum. As a result of his study, Cooper concluded that the embryo arising from the synergid develops slowly and usually disintegrates at an early stage of development.

In Datura, Satina, Blakeslee, and Avery (40) found 5 twin pairs, one of which was a haplo-diploid pair. Christensen and Bamford (6) obtained 7 haploids from nearly 200 twin seedlings of Capsicum annuum var. World Beater. Similar twin pairs have been found in the following species: Solanum chaucha x S. tuberosum (Lamm, 30), Hordeum distichum (Tomstorp, 43), Secale sp. (Nordenskiold, 37), and Zea mays (Chase, 4).

Instances in which both members of the twin pair were haploids have also been reported. Twin seedlings, both of which were haploids, were obtained by Müntzing (33) from the autotetraploid Dactylis glomerata. A set of maize monoploid twins was discovered and reported by Ford (15).

Thus it may be seen that four types of twin pairs have been reported: double haploid, haplo-diploid, haplo-triploid, and double diploid.

Studies have been conducted on the origin of polyembryony. The work by Cooper (7) has previously been mentioned. After an investigation of polyembryony in Alnus rugosa, which reproduces by apomixis, Woodworth (46) believed that embryos may arise from a diploid egg, formed following omission of the reduction division and by nucellar budding, or development of the antipodals, synergids, or the endosperm.

Nielsen (35) concluded that multiple embryos of Poa pratensis may arise from: (1) the egg and a synergid of an embryo sac carrying the reduced chromosome number, one member of the egg apparatus developing parthenogenetically because of the stimulative effect of fertilization of the other; (2) two embryo sacs in which the eggs (or the egg of one and the synergid of the other) carrying the gametophytic chromosome number had been fertilized; (3) the unfertilized eggs, or from an egg and a synergid of embryo sacs that arose by somatic apospory; (4) from eggs (or the egg and a synergid) of embryo sacs developed through somatic apospory, one forming a diploid embryo and the other a tetraploid by somatic doubling of the dividing zygote; (5) two egg cells or from an egg and a synergid that arose by somatic apospory one of which was fertilized, the other developing parthenogenetically; and (6) from the fertilized egg derived from a reduced macrospore and from an egg of an aposporous embryo sac. These studies are of interest as possibly indicating the mode of origin of single haploid embryos.

Monoploids are frequently sterile due to the absence of homologous chromosomes and the resultant failure of synapsis. The degree of sterility is usually quite high but has been found to vary with the species. Blakeslee, et. al. (1) found that haploid Datura stramonium plants were 80 per cent pollen sterile. A haploid wheat plant obtained by crossing

Triticum compactum humboldtii by *Aegilops cylindrica* was reported to be 99.8 per cent self sterile by Gains and Aase (17). Similar data have been reported by Harland (22), Kostoff (29), and others.

Spontaneous somatic chromosome doubling of sectors of the floral parts and the production of diploid progenies following selfing has been reported. Because of this phenomenon, Lindstrom and Koos (31) were successful in selfing haploid *Solanum Lycopersicum* plants. Hollingshead (23) was quite successful in selfing a haploid *Crepis* plant for the same reason.

Spontaneous somatic diploidization in monoploid maize plants occurred frequently enough to permit Chase (5) to obtain progenies from 10 per cent of untreated monoploids following self-fertilization.

Since it is highly desirable that monoploids produce selfed progenies, colchicine techniques have been employed in an attempt to increase the frequency of somatic doubling of the chromosome number. A review of the development, action, and methods of the colchicine technique is not regarded as necessary since this agent has become rather widely used for this purpose. An excellent review of the literature of the subject has been published by Dermen (10) and a listing of the literature has been compiled by Eigsti (11).

Chase (5) indicated that the injection of 0.05 per cent colchicine and 10 per cent glycerine in an aqueous solution into the scutellar node of young monoploid maize seedlings appeared promising as a method for increasing the rate of doubling and reducing the degree of self-sterility.

Chromosome doubling has been found to occur quite frequently in the roots of haploids of some species. Thus, Kostoff (29) listed the per cent of diploid and chimeral roots of the haploid plant of *Nicotiana Langsdorffii*, *N. triplex*, *N. glutinosa*, *N. tabacoum*, and *Crepis capillaris*

as being 2 per cent, 25 per cent, 24.4 per cent, 36.6 per cent, and 63.6 per cent, respectively. Therefore, it is apparent that errors in estimating the frequency of the occurrence of haploids may be incurred where chromosome counts of root tip cells are used for positive identification of putative haploids.

The various studies on haploid plants may be summarized as follows:

(1) haploids have been observed to occur in many species of plants but usually at a very low frequency;

(2) haploid plants are usually smaller and have a more delicate appearance than comparable diploid plants;

(3) X-rays, hybridization, and abnormally low or high temperatures have been suggested as increasing the rate of the appearance of haploids;

(4) mechanical stimulation, chemical treatments, and delayed pollination have not been found to increase the rate of the appearance of haploids in most instances;

(5) haploids have been found to occur in polyembryonic seeds; and

(6) haploids are usually highly sterile but some doubling of chromosomes in both reproductive and vegetative tissue occurs and this may be increased by colchicine treatments.

MATERIALS AND METHODS

The pollen parents carrying unusual dominant marker gene systems selected for use in this study were an elite inbred line, 38-11 (A Pu_1 Pu_2), a brown line (a B Pl R), and a single cross hybrid, brown x purple (A B Pl R).

The maternal stocks used as monoploid source material were of varied origin. Four elite inbred lines that have been developed by conventional breeding methods and four inbred lines developed by the monoploid method were utilized in an attempt to compare the monoploid frequencies in progenies of inbred lines developed by the two methods.

The four standard inbred lines used were 38-11, WF9, W22, and N6. The inbred lines developed by the monoploid method were H73, H212, H214, and H226. Two single cross hybrids involving three of the latter lines were also used.

An open-pollinated variety, Shoup's Krug, and the first and second cycle synthetics developed from it by recurrent selection procedures were used as maternal parents to study the effect of delayed pollination upon the frequency of monoploid occurrence. These maternal source varieties were also used to study the effect of the recurrent selection breeding method upon monoploid frequency.

Marked seed obtained from Dr. S. S. Chase, Iowa State College, was also tested during the course of the study. This material was Early Synthetic x N; mixed dents, mostly of Stiff Stalk Synthetic origin, x V; mixed three and four way crosses, all of Stiff Stalk Synthetic origin, x V; and (H214 x H14) x Q-AB.

The standard method of controlled cross-pollination was used in making source x marker crosses. Delayed pollinations, designated as

late in the summarized results, were made by pollinating the seed parent after the tips of the extruded portion of the silks were completely desiccated. These ears were checked at the time of harvest and any bearing kernels in a position other than on the proximal half of the cob were discarded.

The marked kernels were germinated in an unlighted germinator at 28° C. and examined each day for the appearance of the marker phenotype on the seedling roots. All seedlings exhibiting the marker characteristic were discarded at each examination. Classification was completed, with most of the material, within seven days after the material entered the germinator.

At the time of the first classification, a check was also made for kernels resulting from accidental self- or cross-pollinations. Since the pollen stocks most frequently used carried the genes for purple aleurone (A_1 A_2 A_3 C R i Pr) as well as the plant marker genes, kernels not showing the aleurone marker phenotype were discarded.

Root tips for cytological study were taken from each putative monoploid seedling after the screening process was completed and killed in Formal Acetic Alcohol or 3:1 Alcohol-Acetic acid solution. Chromosome counts were made after the root tips were squashed and stained with aceto-carmine or propino-carmine. Colchicine treatment was carried out before the seedlings were potted by injection into the scutellar node of an aqueous solution of colchicine plus 10 per cent glycerine. Concentrations of 0.05, 0.10, and 0.15 per cent colchicine were used.

The putative monoploid seedlings were transferred to the greenhouse after treatment for morphological observation. The length and width of the first seedling leaf was measured after the leaf was fully extended.

The upper surface of this leaf was further used for microscopic determination of the size and number of stomata. Stomatal size was measured by the use of an ocular micrometer at a magnification of 440X. Stomata counts were made by determining the number of stomata in a row parallel to the direction of veination of the leaf across the maximum diameter of the microscopic field at 440X.

RESULTS

Frequency of Monoploids

Monoploid plants were found to occur at relatively low frequencies in all of the material tested. The mean frequency of monoploidy, as shown in Table 1, was 0.82 per thousand plants tested. The mixed three and four way crosses, all of Stiff Stalk Synthetic origin, two of the inbred lines developed by the monoploid method, H75 and H226, and the four inbred lines developed by long-time selfing, WF9, 38-11, W22, and M6, gave frequencies considerably higher than the mean frequency for all material tested when used as seed stocks. The frequency of 2.45 monoploids per thousand plants of the progeny of the cross H75 x (Brown x Purple) is significant at the one per cent level as measured by the Chi-square test for goodness of fit to the mean frequency for all material tested. The frequency of 1.23 monoploids per thousand plants of the progeny of the cross involving the mixed three and four way crosses, all of Stiff Stalk Synthetic origin, x V is significant at the five per cent level as measured by the same test.

The data were further classified into four groups according to origin of the seed parents. The four groups were: (1) inbred lines developed by long-time selfing, (2) inbred lines developed by the monoploid method and crosses involving these lines, (3) material originating from Stiff Stalk Synthetic, and (4) material originating from the open-pollinated variety, Shoup's Krug. The last group mentioned produced monoploids at a frequency considerably lower than the mean frequency for all material tested. This difference is significant at the one per cent level of probability as measured by the Chi-square test for goodness of fit.

Table 2 presents the frequency data for the open-pollinated Krug variety and the first and second cycle synthetic varieties developed from it by recurrent selection procedures. The monoploids are classified as originating in progenies resulting from early or late pollinations. The differences in frequencies of monoploidy are not significant at the five per cent level of probability.

A portion of the material, that involving 38-11 as the pollen parent, was discarded before the study was completed because of the lack of expression of the marker characteristic. A relatively large proportion of the progeny did not exhibit the purple plumule characteristic of 38-11 at any time before germination or after seven days of germination. Both of the maternal parents for which data on the progenies were recorded, Table 3, are yellow dent strains. Both crosses differed significantly (P less than 0.01) in the number of progeny not exhibiting the purple plumule character at any time. The frequency of monoploidy was not determined for these crosses.

Twin embryos were not found to be reliable sources of monoploids. Fifteen twin embryos were noted during the course of this study. Both individuals of the twin pair were proven to be diploid in fourteen of these. The remaining twin embryo was of the double monoploid type.

Table 1. Summary of the frequency of occurrence of monoploids in the dent stocks tested.

Seed Parents	Pollen Parents	Number of Progeny	Number of Monoploids	Frequency per Thousand
Early Synthetic	N	9,100	3	0.33
Mixed dents, mostly of Stiff Stalk Synthetic origin	V	12,500	10	0.80
Mixed three and four way crosses, all of Stiff Stalk Synthetic origin	V	30,000	37	1.23*
H214 x B14	Q-AB	2,700	1	0.37
Shoup's Krug O.P.	Brown x Purple	10,000	4	0.40
Shoup's Krug Syn. I	Brown x Purple	10,000	3	0.30
Shoup's Krug Syn. II	Brown x Purple	10,000	4	0.40
Shoup's Krug Syn. II	Brown	5,000	1	0.20
W79	Brown x Purple	3,000	4	1.33
W8-11	Brown x Purple	1,700	3	1.76
W22	Brown	3,000	4	1.33
N6	Brown	3,000	4	1.33
H73	Brown x Purple	4,946	12	2.43**
H212	Brown x Purple	4,025	3	0.75
H214	Brown x Purple	1,800	0	0.00
H225	Brown x Purple	3,050	4	1.31
H225 x H212	Brown	3,000	1	0.33
H225 x H214	Brown	3,000	0	0.00
Totals		119,821	98	0.82

* Significant Chi-square value at the 5% point.
** Significant Chi-square value at the 1% point.

Table 2. Number and frequency per thousand kernels of monoploids obtained from 5000 kernel samples of Shoup's Krug O.P., Shoup's Krug Syn. I, and Shoup's Krug Syn. II when pollinated by (Brown x Purple) at early and late dates with respect to development of the pistillate inflorescence.

	Shoup's Krug O. P.		Shoup's Krug Syn. I		Shoup's Krug Syn. II	
	Number	Frequency	Number	Frequency	Number	Frequency
Early	2	.400	2	.400	2	.400
Late	2	.400	1	.200	2	.400
Means	2.0	.400	1.5	.300	2.0	.400

Table 5. A test of the expression of the 38-11 marker characteristic when embryos were classified through the pericarp and after seven days of germination.

Seed Parents	Total Progeny	Plumule color apparent		No expression
		Through Pericarp	After Germination	
		Per Cent	Per Cent	Per Cent
Gr15-1 x Gr4-2	3,055	51.9	42.0	6.1
Krug Syn. I	1,174	51.2	28.4	20.4

Mortality Rates

Seedling mortality rates were quite high in all of the material studied. Table 4 presents the mortality rates for putative monoploid Stiff Stalk Synthetic seedlings with and without colchicine treatment. Chromosome counts were not made on this material until the plants were moved from the greenhouse to the field. Therefore, the chromosome number of the plants dying prior to this time are not known. Differences between the mortality rates due to treatments are not significant at the five per cent level of probability as measured by the Chi-square test of goodness of fit to the mean frequency over all treatments. Likewise, the difference between the mortality rates of the plants that were not injected and the injected group is not significant as measured by the same test. All probability values obtained were 0.1 or larger.

The mean mortality rates of monoploid seedlings from the eleven seed parents listed in Table 5 for the three levels of concentration of colchicine used are not significant at the five per cent level of probability.

Table 4. Mortality rates of Putative monoploid seedlings from mixed three and four way crosses, all of Stiff Stalk Synthetic origin x V parentage.

Treatment		Total number of seedlings	Number of seedlings not surviving	Mortality % of total
None		139	21	15.1
Colchicine 1/ injected into scutellar node	0.00%	12	4	33.3
	0.05%	30	6	30.0
	0.10%	10	1	10.0
	0.15%	10	1	10.0
Totals		191	33	

1/ Colchicine at the concentrations indicated and 10 per cent glycerine in an aqueous solution.

Table 5. Mortality of monoploids from some dent stocks following injection of colchicine at the indicated concentrations into the scutellar nodes of the young seedlings.

Seed Parent	0.05%		0.10%		0.15%	
	No. Treated	No. Died	No. Treated	No. Died	No. Treated	No. Died
Shoup's Krug O.P.	2	2	1	0	1	1
Shoup's Krug I	1	0	1	1	1	0
Shoup's Krug II	2	0	2	1	1	1
WF9	1	0	2	0	1	0
38-11	1	0	-	-	2	0
W22	2	0	1	0	1	0
N6	2	1	2	1	-	-
H73	6	2	4	2	2	1
H212	-	-	2	2	1	0
H225	2	0	1	0	1	0
H225 x H212	1	0	-	-	-	-
Totals	20	5	16	7	11	3
Mortality (Percent)	25.0		43.8		27.3	

Fertility of Monoploids

The majority of the monoploid plants obtained throughout the course of this study were completely sterile. Some plants had anthers extruded from within the glumes, but in no case were there more than fifteen pollen containing anthers produced on a plant and in most instances where pollen was produced it was from one to three anthers. There was a marked tendency for the monoploids, regardless of their origin, to be protandrous.

The data on the fertility of a group of monoploids of like origin are presented in Table 6. Colchicine treatment appeared to increase the percentage of plants producing selfed seed, but the difference between the colchicine treated group and the plants receiving no treatment is not significant at the five per cent level of probability as measured by the Chi-square test. No consistent trend was found for the different levels of concentration of colchicine.

The mean number of kernels developed per fertile plant was higher in the non-injected group than in the group which received injections. An average of less than one kernel of selfed seed per plant was obtained over all treatments, while for the fertile plants the mean number of seeds ranged from 0 to 7 per plant.

Table 6. Fertility of selfed monoploids of mixed three and four way crosses, all of Stiff Stalk Synthetic origin x V parentage.

	Treatment				
	None	Colchicine 1/ %			
		0.00	0.05	0.10	0.15
Number of plants	26	4	5	2	2
Fertile plants %	11.5	25.0	40.0	0.0	50.0
Mean number of kernels per fertile plant	7.0	4.0	1.0	0.0	2.0
Mean number of kernels per plant treated	0.8	1.0	0.4	0.0	1.0

1/ Colchicine at the concentrations indicated and 10 per cent glycerine in an aqueous solution injected into the scutellar node.

Leaf Characters

An attempt was made to determine morphological characters of the first seedling leaves that could be used as a rapid method of distinguishing between monoploid and diploid seedlings. The data obtained for the characters studied are presented in Tables 7, 8, and 9 and in Figures 1, 2, 3, 4, and 5. The maternal and paternal parent seedlings were not injected with colchicine whereas the progeny were injected. The parental seedling leaf characters, therefore, are not directly comparable to those of the progeny but are included to point out the differences which were found when the injection treatment was imposed upon the progeny.

The leaf data for progeny and parental seedlings of the crosses involving the Krug sources as the seed parents are presented in Table 7. In general, the injected progeny, both monoploid and diploid, had shorter and narrower leaves than the non-injected parents. The leaves of the progeny were broader with respect to their length than were the leaves of the seedlings of the parental stocks. The stomata of the first seedling leaves of the parental plants were larger and farther apart than were those of the leaves of the progeny. The data on the crosses involving the four elite inbred lines, Table 8, and the inbred lines developed by the monoploid method, Table 9, follow the trends illustrated by the Krug material.

The frequency distributions of the seedling leaf characters of the progeny which did not exhibit the marker characteristics are compared in Figures 1, 2, 3, 4, and 5. The monoploid distributions are based upon data from thirty-five seedlings and the diploid distributions upon data from twenty-four seedlings. The seedlings originated

from the crosses listed in Tables 7, 8, and 9. The leaf characters studied were quite variable in both populations. There was little difference in the ranges of the two types of progeny and the modal classes are not widely separated in any of the five distributions.

Table 7. Summary of the means and standard errors of characters of the first seedling leaf of parents, and monoploid and diploid progenies not exhibiting the marker characteristics from crosses involving the open-pollinated variety, Krug, and the first and second cycle synthetics derived from it. 1/

	leaf length mm.	leaf width mm.	ratio of length to width	stomata size u	stomata number in field at 440 X
Krug O. P. x (Brown x Purple)					
Maternal parent	65.8 ± 5.19	15.8 ± .97	4.18 ± .30	42.0 ± .91	1.53 ± .13
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	31.5 ± 6.50	9.5 ± 1.50	3.28 ± .16	27.6 ± 1.80	3.50 ± .17
Monoploid progeny	35.0	10.0	3.50	25.8	3.33
Krug I x (Brown x Purple)					
Maternal parent	68.8 ± 2.65	17.0 ± .22	4.05 ± .14	39.7 ± 3.37	2.47 ± .20
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	45.8 ± 4.96	12.5 ± .86	3.70 ± .14	32.2 ± 3.50	3.17 ± .22
Monoploid progeny	28.5 ± 6.50	10.5 ± .50	2.75 ± .75	22.0 ± .00	4.16 ± .17
Krug II x (Brown x Purple)					
Maternal parent	67.8 ± 1.98	15.4 ± .75	4.43 ± .19	47.8 ± 11.10	1.80 ± .27
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	- - -	- - -	- - -	- - -	- - -
Monoploid progeny	30.0 ± 8.00	9.0 ± 1.00	3.48 ± 1.28	26.9 ± 2.47	3.44 ± .15
Krug II x Brown					
Maternal parent	67.8 ± 1.98	15.4 ± .75	4.43 ± .19	47.8 ± 11.10	1.80 ± .27
Paternal parent	57.2 ± 1.58	11.8 ± .38	4.86 ± .14	36.1 ± 1.39	2.20 ± .16
Diploid progeny	- - -	- - -	- - -	- - -	- - -
Monoploid progeny	30.0	10.0	3.00	25.8	5.33

1/ Progeny received colchicine treatment.

Table 8. Summary of the means and standard errors of characters of the first seedling leaf of parents, and monoploid and diploid progenies not exhibiting the marker characteristics from crosses involving four elite inbred lines, developed by standard self-pollination procedures. 1/

	leaf length mm.	leaf width mm.	ratio of length to width	stomata size u	stomata number in field at 440 X
WF9 x (Brown x Purple)					
Maternal parent	40.0 ± 4.34	16.2 ± .38	2.48 ± .28	34.6 ± 2.49	2.73 ± .20
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	- - -	- - -	- - -	- - -	- - -
Monoploid progeny	30.0 ± 4.51	12.5 ± .50	2.41 ± .12	25.7 ± 2.14	4.00 ± .58
38-11 x (Brown x Purple)					
Maternal parent	81.2 ± 1.96	15.2 ± .20	5.34 ± .15	36.8 ± 1.17	2.26 ± .06
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	45.0 ± 4.36	10.7 ± .88	4.35 ± .79	31.9 ± 2.47	3.00 ± .19
Monoploid progeny	33.3 ± 2.03	12.0 ± .58	2.80 ± .03	25.7 ± 2.14	3.22 ± .22
W22 x Brown					
Maternal parent	55.2 ± 2.61	13.6 ± .24	4.07 ± .24	33.1 ± 1.17	2.80 ± .08
Paternal parent	57.2 ± 1.58	11.8 ± .38	4.86 ± .14	36.1 ± 1.39	2.20 ± .16
Diploid progeny	- - -	- - -	- - -	- - -	- - -
Monoploid progeny	33.0 ± 1.08	9.5 ± 1.04	3.59 ± .40	25.8 ± 3.00	3.16 ± .10
M6 x Brown					
Maternal parent	62.0 ± 2.32	12.6 ± .24	4.92 ± .16	34.6 ± 1.48	2.27 ± .12
Paternal parent	57.2 ± 1.58	11.8 ± .38	4.86 ± .14	36.1 ± 1.39	2.20 ± .16
Diploid progeny	- ? -	- - -	- ? -	- ? -	- ? -
Monoploid progeny	34.5 ± 14.50	11.5 ± .50	2.95 ± 1.13	23.9 ± 5.80	3.34 ± .34

1/ Progeny received colchicine treatment.

Table 9. Summary of the means and standard errors of characters of the first seedling leaf of parents, and monoploid and diploid progenies not exhibiting the marker characteristics from crosses involving four inbred lines developed by the monoploid method and two single cross hybrids of three of these lines. 1/

	leaf length mm.	leaf width mm.	ratio of length: to width	stomata size u	stomata number in field at 440 X
H73 x (Brown x Purple)					
Maternal parent	60.0 ± 1.64	14.8 ± .38	4.07 ± .18	37.5 ± 1.39	2.07 ± .16
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	29.6 ± 2.12	9.0 ± .73	3.46 ± .40	26.2 ± 1.09	2.08 ± .27
Monoploid progeny	28.6 ± 1.44	9.6 ± .44	3.05 ± .24	27.8 ± 1.38	2.67 ± .23
H212 x (Brown x Purple)					
Maternal parent	34.8 ± 1.09	10.4 ± .24	3.26 ± .16	39.0 ± .28	2.67 ± .33
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	27.5 ± 11.50	8.5 ± .50	3.33 ± 1.55	33.2 ± 7.35	3.66 ± .33
Monoploid progeny	19.0 ± 3.00	7.0 ± 2.00	2.82 ± .38	29.4 ± .00	3.66 ± 1.34
H214 x (Brown x Purple)					
Maternal parent	47.2 ± .73	17.0 ± .10	2.78 ± .04	39.0 ± 2.22	2.53 ± .25
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	44.5 ± 2.50	10.5 ± .50	4.26 ± .44	29.4 ± .00	3.50 ± .83
Monoploid progeny	- - -	- - -	- - -	- - -	- - -
H225 x (Brown x Purple)					
Maternal parent	39.0 ± 2.32	13.8 ± .20	2.82 ± .14	39.7 ± 3.17	2.53 ± .76
Paternal parent	69.2 ± .49	12.2 ± .20	5.68 ± .12	34.6 ± 1.48	2.27 ± .24
Diploid progeny	18.0 ± 1.00	10.7 ± .33	1.69 ± .04	24.5 ± 1.26	3.89 ± .62
Monoploid progeny	20.0 ± 1.08	9.0 ± .71	2.28 ± .82	26.7 ± .90	3.17 ± .17
(H225 x H212) x Brown					
Maternal parent	45.3 ± 1.50	13.6 ± .24	3.34 ± .76	45.6 ± 1.28	2.67 ± .24
Paternal parent	37.2 ± 1.28	11.8 ± .38	4.26 ± .14	36.1 ± 1.39	[illegible] ± .16
Diploid progeny	- - -	- - -	- - -	- - -	- - -
Monoploid progeny	25.0	8.0	3.12	25.8	3.00

1/ Progeny received Colchicine treatment.

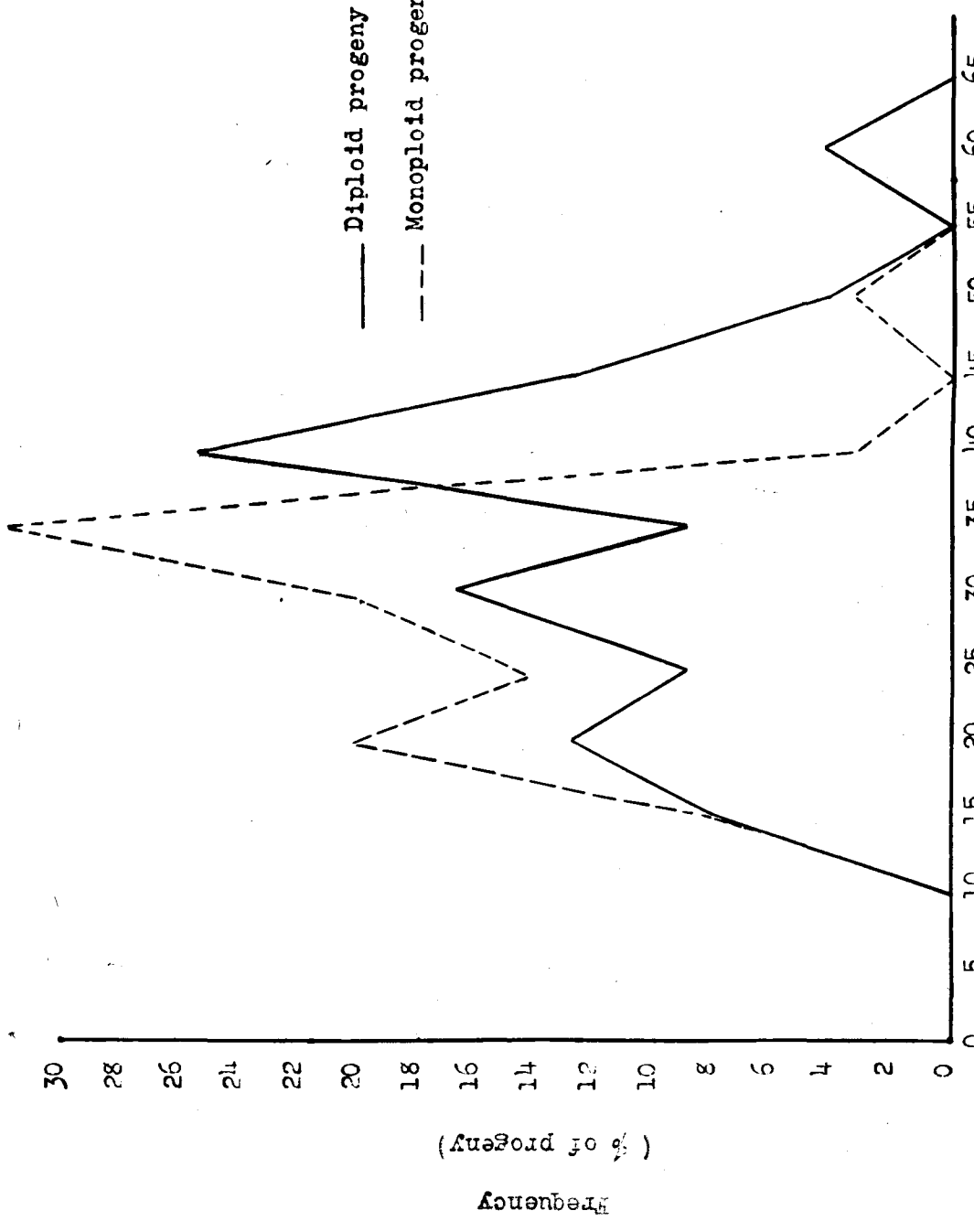


Figure No. 1. Frequency distribution of the length of the first seedling leaf of monoploid and diploid progeny not exhibiting the marker characteristics.

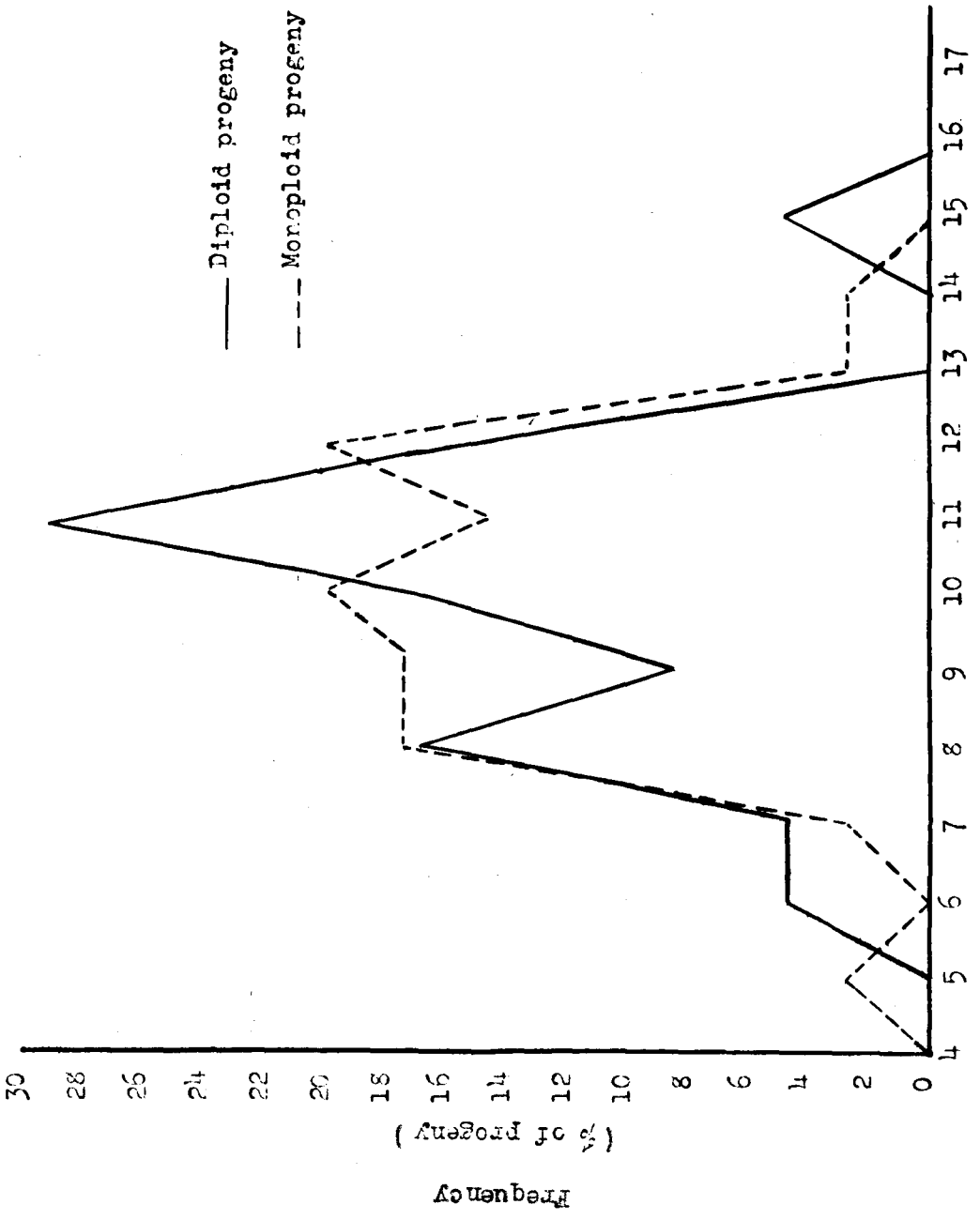


Figure No. 2. Frequency distribution of the width of the first seedling leaf of monoploid and diploid progeny not exhibiting the marker characteristics.

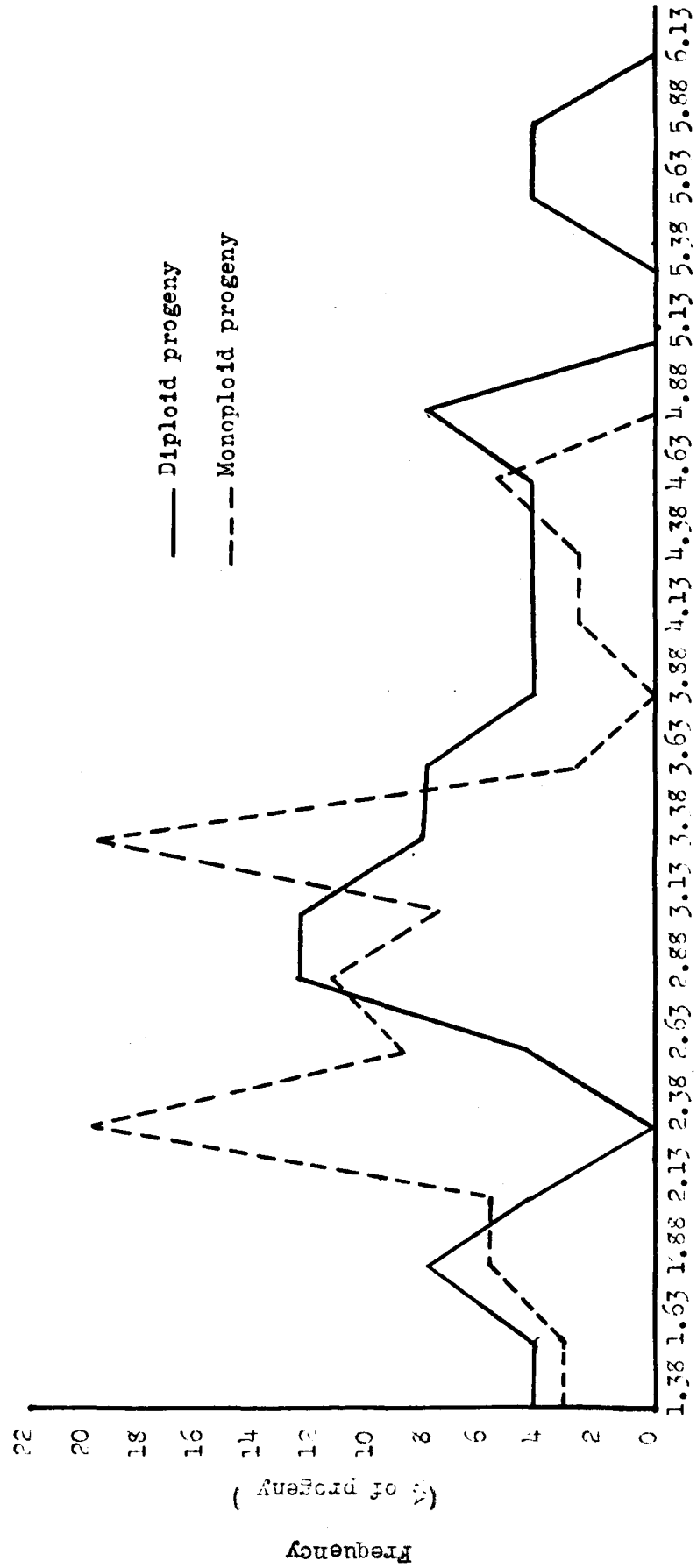
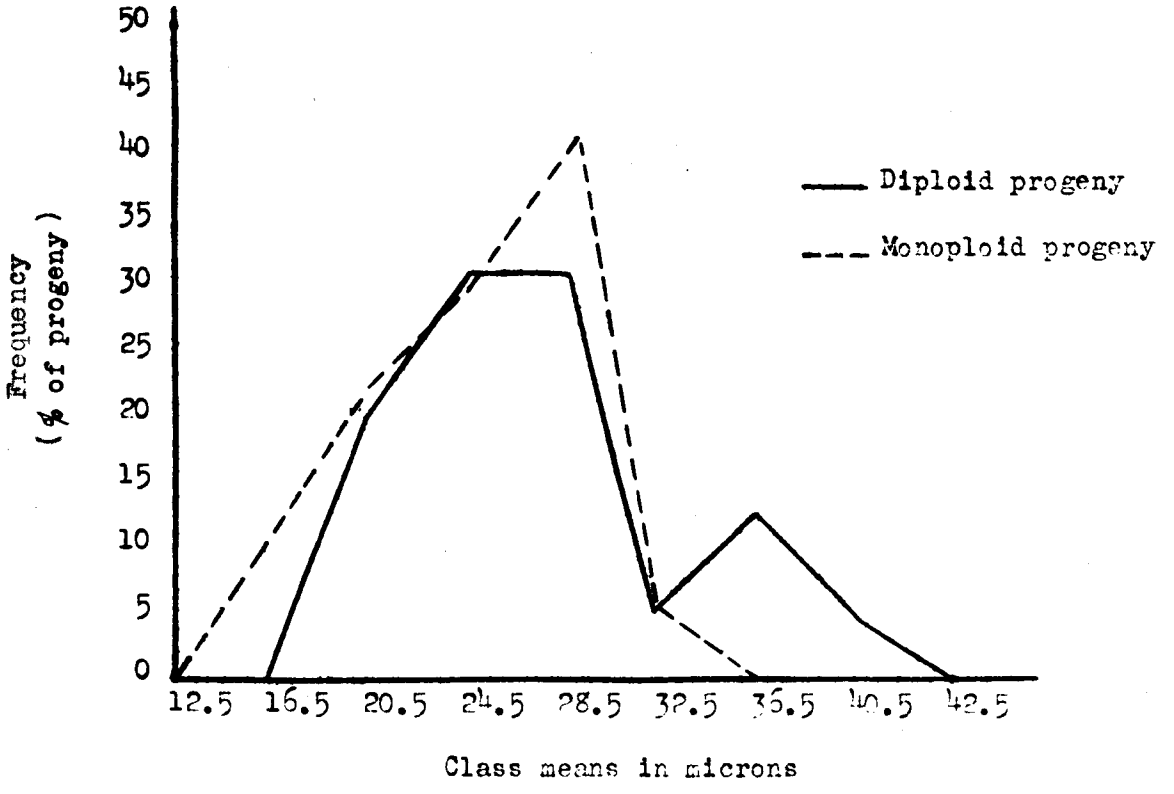


Figure No. 3. Frequency distribution of the ratio of length to width of the first seedling leaf of monoploid and diploid progeny not exhibiting the marker characteristics.

Figure No. 4. Frequency distribution of the size of the stomata of the first seedling leaf of monoploid and diploid progeny not exhibiting the marker characteristics.



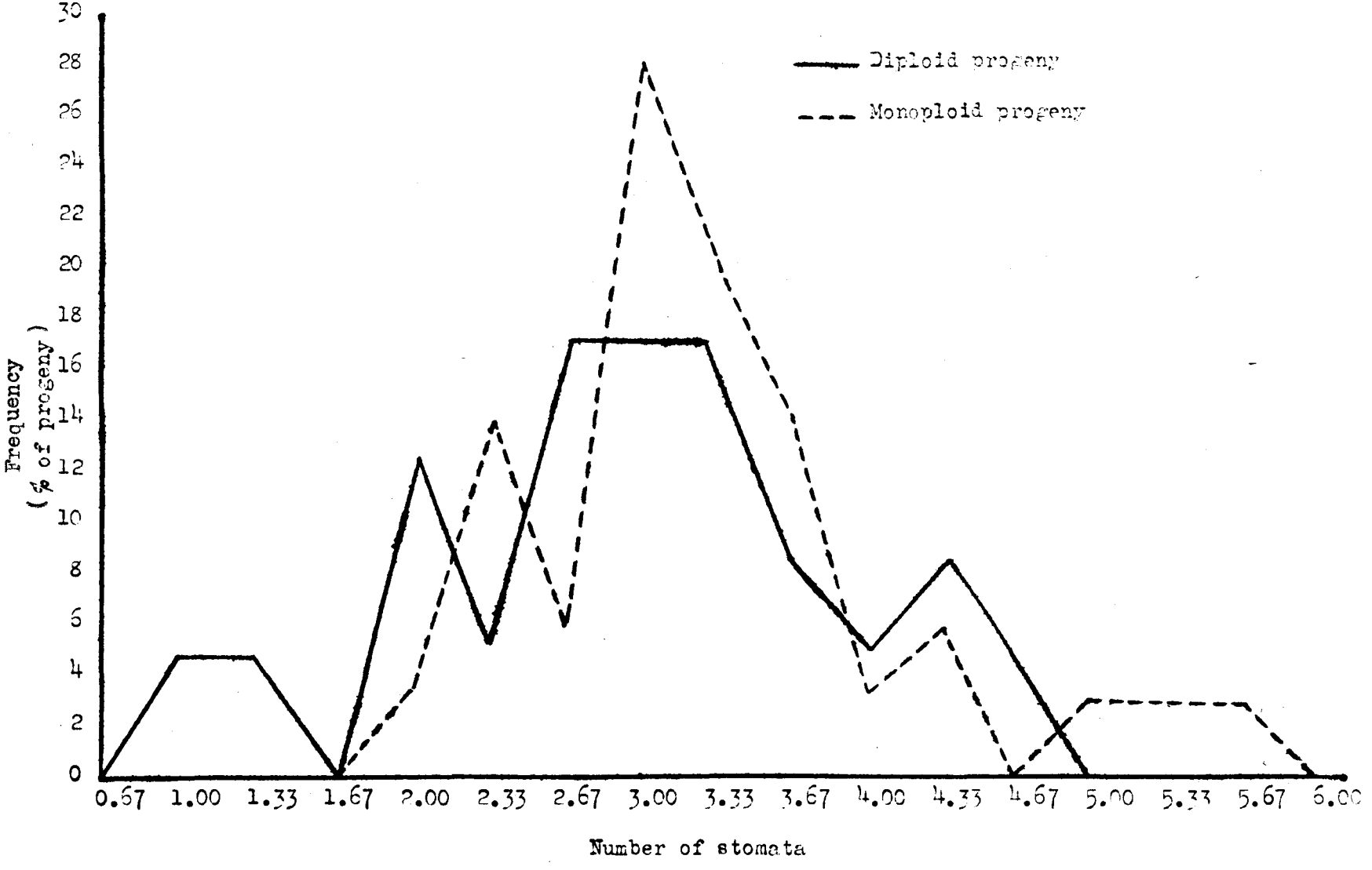


Figure No. 5. Frequency distribution of the number of stomata of the first seedling leaf of monoploid and diploid progeny not exhibiting the marker characteristics. Class values based upon the means of three counts across the diameter of the microscopic field at a magnification of 440 X.

DISCUSSION AND CONCLUSIONS

The frequency of the occurrence of monoploids, although quite low does not appear to be the main factor limiting the utilization of this method for the establishment of inbred lines for use in a breeding program. It is unfortunate that of the source material included in this study, that from which a plant breeder would be more interested in obtaining inbred lines produced monoploids at a significantly lower frequency than the other sources. Any technique whereby the frequency of monoploidy in the open-pollinated varieties could be increased would greatly enhance the value of the method for application in a breeding program. Neither delayed pollination nor the agronomic selection practiced in the development of the first and second cycle Krug synthetics increased the frequency of monoploidy over that of the original variety pollinated when the pistillate inflorescences were fully developed.

The low frequency of occurrence of monoploids in the open-pollinated varieties may perhaps be circumvented by backcrossing lines which are known to have a high frequency to the variety and recovering monoploids from the backcross progenies. This would, however, impose the necessity of growing at least two additional generations to provide source material and would correspondingly decrease the desirability of the method.

It appears that any seedling progeny not exhibiting the marker characteristics should be retained until mature for positive identification of all monoploids. Four of the first fifty-one chromosome determinations made on monoploid seedlings during the course of this study were in error as judged by the morphology and fertility of the mature plants. All of the errors were in classifying plants as diploids when they were in reality monoploids. This may be an indication of the frequency at which doubling of the chromosome number

occurs in somatic tissue of monoploid corn seedlings.

The high mortality rate of the monoploid seedlings and their low fertility are the major problems inherent in the monoploid method as used in this study. The low frequency at which monoploids were found to occur would not prohibit extensive use of the method in a breeding program if it were possible to obtain selfed seed on all or nearly all monoploids detected.

It is evident that 0.05, 0.10, and 0.15 per cent colchicine injections are not adequate chromosome doubling treatments. Concentrations other than these, different methods of application, or other chemicals may prove more satisfactory. The injection method of application severly injured some of the seedlings subjected to it. Although this injury was not reflected by a significant increase in the mortality rate, it would seem desirable to use a method of application which would result in less damage to the seedlings.

While it is quite easy to distinguish monoploid from diploid plants on the basis of the morphology and fertility of the mature plants, it was not possible to distinguish between them in the seedling stage under the conditions of this experiment. If a morphological characteristic can be found which would permit rapid and positive identification of monoploid seedlings, it would be extremely valuable. The elimination of the time consuming and exacting task of making chromosome counts of the putative monoploid seedlings would permit the screening of much larger populations. If it were to be of any marked benefit, such a morphological characteristic would necessarily have to be evident before treatments to increase the fertility of monoploids were applied. Such a characteristic may not exist naturally, however, it may be possible to induce its development by the differential

reaction of monoploids and diploids to some type of treatment.

At least three generations are required for the establishment of inbred lines by the monoploid method. These generations are the following: (1) the generation necessary to produce the marked F_1 seed, (2) the generation in which the monoploids are detected, grown to maturity and selfed, and (3) a diploid generation to assure an ample supply of selfed seed. Tests of the combining abilities of the lines established by the monoploid method cannot begin until at least one generation of homozygous diploids have been produced. The net saving in time in the production of tested pure breeding lines from untested material will not, therefore, be as great as may seem apparent upon superficial examination of the method.

The monoploid method, in its present stage of development, does not appear to be an efficient substitute for long-time selfing in any extensive search for inbred lines to replace those presently used in hybrid combinations. The lines developed by the monoploid method may, however, be valuable in certain special genetic and breeding studies. If the frequency of the occurrence of monoploids can be greatly increased or the fertility level increased or if the inbred lines established from monoploids prove to be greatly superior agronomically than those established by long-time selfing, the method will undoubtedly assume increasing importance as a breeding technique.

SUMMARY

Approximately one hundred twenty thousand individual plants representing the progenies of eighteen crosses of corn were examined for monoploid sporophytes. Monoploids were found to occur at a mean frequency of 0.82 per thousand seedlings screened. Significant differences in the frequency of the occurrence of monoploids were found. Delayed pollination and limited agronomic selection did not increase the frequency of monoploidy in the open-pollinated variety, Krug, and its derivatives.

The high mortality rate and low self-fertility level of monoploids were found to be the main factors limiting the use of the monoploid method for extensive use in establishing inbred lines of corn. Injections of 0.05, 0.10, and 0.15 per cent colchicine solutions into the scutellar nodes of the young monoploid seedlings did not significantly increase their self-fertility.

Length of the first seedling leaf, leaf width, the ratio of leaf length to leaf width, stomata size, and stomata frequency were not found to clearly differentiate monoploid from diploid seedlings.

The monoploid method, as used in this study, was not found to be superior to the long-time selfing method now in use for the establishment of large numbers of inbred lines from previously untested material.

LITERATURE CITED

1. Blakeslee, A. F., Belling, J., Farnham, M. E., and Bergner, A. D. A haploid mutant in the jimson weed, Datura stromonium. Science 55:646-647. 1922.

2. Brown, Meta Suehs. Haploid plants in sorghum. Jour. Heredity 34:163-166. 1943.

3. Catcheside, D. G. The chromosomes of a new haploid Oenothera. Cytologia 4:68-113. 1932.

4. Chase, Sherret S. Monoploid frequencies in a commercial double cross hybrid maize, and in its component single cross hybrids and inbred lines. Genetics 34:328-332. 1949.

5. _____Production of homozygous diploids of maize from monoploids. Jour. Amer. Soc. Agron. 44:263-267. 1952.

6. Christensen, Hilde M., and Bamford, Ronald. Haploids in twin seedlings of peppers, Capsicum annuum L. Jour. Heredity 34:99-104. 1943.

7. Cooper, D. C. Haploid-diploid twin embryos in Lilium and Miectiana. Amer. Jour. Bot. 30:408-413. 1943.

8. Davis, Bradley Moore. Some attempts to obtain haploids from Oenothera Lamarckiana. Amer. Nat. 65:233-242. 1931.

9. _____, and Kulkarni, C. G. The cytology and genetics of a haploid sport from Oenothera franciscana. Genetics 15:55-80. 1930.

10. Dermen, H. Colchicine polyploidy and technique. Bot. Rev. 6:599-635. 1940.

11. Eigsti, O. J. Colchicine bibliography. Lloydia 10:65-114. 1947.

12. Einset, J. Chromosome length in relation to transmission frequency in maize trisomes. Genetics 28:349-364. 1943.

13. Elmsweller, S. L., and Ruttle, M. L. Induced polyploidy in floriculture. Amer. Nat. 75:310-326. 1941.

14. Emerson, S. H. The reduction division in a haploid Oenothera. La Cellule 39:159-165. 1929.

15. Ford, Lee. A cytogenetic study of a set of maize monoploid twins. Genetics 37:580. 1952.

16. Fortuno, J. Velez. Unpublished M. S. thesis. University of Missouri. Columbia, Missouri. 1948.

17. Gaines, E. F., and Aase, H. C. A haploid wheat plant. Amer. Jour. Bot. 13:373-385. 1926.

18. Gates, R. R., and Goodwin, K. M. A new haploid Oenothera with some considerations on haploidy in plants and animals. Jour. Genetics 23:123-156. 1930.

19. Gerassimova, H. Experimentell erhaltene haploide Pflanze von Crepis tectorum L. Planta 25:696-702. 1936.

20. Gustafsson, Ake. Studies on the mechanisms of parthenogenesis. Hereditas 21:1-111. 1935.

21. Haberlandt, G. Zur Zytologie und Physiologie des weiblichen Gametophyten von Oenothera. Sitzb. preuss. Akad. Wiss. 7:33-47. 1927.

22. Harland, S. C. Haploids in polyembryonic seeds of sea island cotton. Jour. Heredity 27:229-231. 1936.

23. Hollingshead, Lillian. A cytological study of haploid Crepis capillaris plants. Univ. California Publ. Agri. Sci. 6:107-134. 1930.

24. Ivanov, M. A. Experimental production of haploids in Nicotiana rustica. Genetica 20:295-397. 1938.

25. Johansen, Donald A. Haploids in Hordeum vulgare. Nat. Acad. Sci. Proc. 20:98-100. 1934.

26. Jones, Jenkin W., and Longley, A. E. Sterility and aberrant chromosome numbers in Caloro and other varieties of rice. Jour. Agr. Res. 62:361-399. 1941.

27. Katayama, Yoshiwo. Haploid formation by X-rays in Triticum monococcum. Cytologia 5:235-237. 1934.

28. Kostoff, Dontcho. Directed heritable variations conditioned euploid chromosome alterations. Jour. Genetics 36:447-468. 1937.

29. _____ The problem of haploidy. Biblio. Genet. 13:1-148. 1941.

30. Lamm, Robert. Notes on a haploid potato hybrid. Hereditas 24:391-396. 1938.

31. Lindstrom, E. W., and Koos, Katharine. Cyto-genetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. Jour. Bot. 18:398-410. 1931.

32. McCrey, Francis A. Another haploid Nicotiana tabacum plant. Bot. Gaz. 93:227-230. 1932.

33. Müntzing, Arne. Characteristics of two haploid twins in Dactylis glomerata. Hereditas 29:134-140. 1943.

34. Navashin, M. Chromatin mass and cell volume in related species. Uni. California Publ. Agr. Sci. 6:207-230. 1931.

35. Nielsen, Ethar L. Breeding behavior and chromosome numbers in progenies from twin and triplet plants of Poa pretensis. Bot. Gaz. 108:26-40. 1946.

36. Noggle, G. R. The physiology of polyploidy in plants. I. Review of the literature. Lloydia 9:153-173. 1946.

37. Nordenskiold, Hedda. Studies of a haploid rye plant. Hereditas 25: 204-210. 1939.

38. Randolph, L. F. Some effects of high temperature on polyploidy and other variation in maize. Proc. Nat. Acad. Sci. 28:222-229. 1932.

39. _____, and Fischer, H. E. The occurrence of parthenogenetic diploids in tetraploid maize. Proc. Nat. Acad. Sci. 25:161-164. 1939.

40. Satina, S., Blakeslee, A. F., and Avery, A. G. Balanced and unbalanced haploids in Datura. Jour. Heredity 28:193-202. 1937.

41. Smith, Luther. Haploidy in einkorn. Jour. Agr. Res. 73:291-301. 1946.

42. Stadler, L. J. The experimental modification of heredity in crop plants. Sci. Agr. 11:557-572. 1931.

43. Tometorp, Gosta. Cytological studies on haploid Hordeum distichum. Hereditas 25:241-253. 1939.

44. Van Overbeek, J., Conklin, Marie E., and Blakeslee, A. F. Chemical stimulation of ovule development and its possible relation to parthenogenesis. Amer. Jour. Bot. 28:647-656. 1941.

45. Wettstein, F. Morphologie und Physiologie des Formwechsels der Moose auf genetischer Grundlage I. Zeitschr. Induck. Abstamm. u. Vererb. 33:1-236. 1924.

46. Woodworth, Robert H. Cytological studies in the Betulaceae. III. Parthenogenesis and polyembryony in Alnus rugosa. Bot. Gaz. 89:402-409. 1930.

47. Yefeikin, A. K., and Vasiljev, B. I. Artificial induction of haploid durum wheats by pollination with X-rayed pollen. Bull. Appl. Bot. Genet. Plant Breeding. Ser. II, No. 7: 39-45. 1935.