*Euphytica* **79**: 13–18, 1994.



# Properties of maternal haploid maize plants and potential application to maize breeding

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Received 1 March 1993; accepted 11 July 1994

*Key words:* *Zea mays*, haploid induction, maternal haploids, inducer line ZMS, maize

## Summary

Presented are the results of a two-year study of haploid maize plants in the field. The haploids were produced with the aid of inducer line ZMS. In total, 604 and 1030 haploids were obtained and studied in the first and second years, respectively. Tassels of haploid plants were found to be almost completley sterile. Fertility of ears was studied by pollinating them with the pollen from diploid inbred lines, the cross resulting in almost all of the haploid ears carrying kernels. On average 27.4 kernels per ear of haploid plant were obtained in the first year of study and 26.3 in the second. These gave rise to normal diploid plants. This property allows genotypes selected at the level of haploid plants to be involved in breeding process. Unusual plants were found among haploids, phenotypically resembling homozygous lines. It was assumed that the plants had resulted from spontaneous chromosome doubling in haploids. The results of comparative studies of progenies of unusual plants and inbred lines derived from the same synthetic population are presented.

## Introduction

The production of haploid maize (*Zea mays* L.) plants on a grand scale became possible owing to the development of high-efficiency inducer lines (Tyrnov & Zavalishina, 1984). These lines allow haploids to be derived with a frequency of up to 3 and more percent practically for any genotype. The dominant marker genes A, C, R-nj:cudu carried by the inducer line ZMB (Zarodyshevy Marker Saratovsky) enable haploids to be identified among mature seeds, resulting in high producibility of this line.

The production of effective haploid-inducing lines, used as males, became possible following Coe's (1959) report. The author has demonstrated that haploid plants occur among the progeny of maize line Stock 6 at a frequency of 3.2%. Lashermes & Beckert (1988) made an attempt at genetically analyzing the haploid-inducing capacity of Stock 6. They found the trait most probably to be heritable and controlled by a small number of genes.

In parallel with these *in vivo* studies, an increasingly successful *in vitro* production of maize haploids and doubled haploids has been achieved (Pauk, 1985; Wan et al., 1988; Bentolila et al., 1992). The future possibility of obtaining haploid plants from anthers will be an important alternative method for inducing haploids from unfertilized egg cells.

There are some advantages to be gained from involving haploids in the breeding process. One typical advantage is the rapid production of homozygous lines. For these to be produced by traditional methods, five to seven selfings are usually required. Use of haploids takes much less time to produce homozygous lines (Chase, 1951).

No less important a merit is the simplification of allelic interactions in haploids. This, (a) removes the overdominating effects preventing selection of useful additive genes and additive × additive epistatic effects for improving synthetic populations and developing new lines, (b) allows elimination of harmful, lethal and semilethal recessive genes from breeding material. It has been shown, using the silkworm as an example, that elimination of harmful recessive alleles from a line increases its viability and performance (Strunnikov et al., 1983).

The segregation ratio in haploids is of considerable interest to the breeders. Nei (1963) has shown the advantage of this over the corresponding parameter in diploids. Differences in the segregation formulas result in the fact that favourable gene recombinations can be identified in haploids using substantially smaller samples of plants. This is of fundamental importance, particularly when dealing with quantitative traits. In connection with this, it is of interest to mention the work of Jansen (1992) who has provided tables listing minimum numbers of doubled haploid lines that guarantee the breeder retains the desired genotypes with a given probability.

Furthermore, haploid plants allow the mutant genes to be identified readily if they are not lethal. In same cases, mutant genes could present a potential for breeding.

How can haploid maize plants be involved in the breeding process? Some properties of haploid plants considered in the present paper may help answer this question.

*Table 1.* Results of pollinating haploid maize plants with pollen from diploid lines

| No | Parameter | Number of plants | | Percent | |
|---|---|---|---|---|---|
| | | 1991 | 1992 | 1991 | 1992 |
| 1 | Haploids pollinated with pollen from diploid lines | 72 | 162 | 100 | 100 |
| 2 | Number of ears with kernels | 68 | 158 | 94.4 | 97.5 |
| 3 | Number of barren ears | 4 | 4 | 5.6 | 2.5 |

*Table 2.* Number of kernels in ears of haploid plants resulting from pollination with pollen from diploid lines

| No | Parameter | 1991 | 1992 |
|---|---|---|---|
| 1 | Average number of kernels per ear | 27.4 | 26.3 |
| 2 | Maximum number of kernels per ear | 127 | 107 |

## Materials and methods

The experimental scheme included three general parts: a) estimation of haploid plants fertility; b) estimation of the frequency of spontaneously doubled haploids appearance; c) comparison of doubled haploid lines with inbred lines.

The synthetic population SA created for breeding purposes was used as parental material. Seeds of this synthetic population were sown in a space-protected nursery in 1990. Prior to flowering, tassels were removed from the resulting plants. The pollen parent used was the inducer line ZMS described by Tyrnov & Zavalishina (1984).

The resulting seeds were grouped according to marker gene expression. The dominant allele of gene R-nj:cudu carried by line ZMS was expressed simultaneously in the endosperm and in the embryo. In the crown of the kernel, the aleurone and the scutellum were stained violet by anthocyan (Coe et al., 1988). Classed as haploids were the seeds with the R-nj:cudu gene expressed in the endosperm but not in the embryo. The seeds in which gene R-nj:cudu was expressed in both the embryo and the endosperm were classed as diploids. The following year, the haploids were sown in a field plot using an ordinary seeder. Management practices used with haploid plants were the same as those with diploid plants. Studies of haploid plants were carried out in 1991–1992. Measurements were performed upon the completion of flowering. Statistical treatment of the data obtained was carried out using standard algorithms.

## Results and discussion

Tyrnov & Zavalishina (1984) have shown that line ZMS induces maternal haploids with a frequency ranging from 0.55 to 3.43%. An average of about 2% of kernels with haploid embryos were obtained in our previous experiments using the same inducer (Chalyk & Ostrovsky, 1993). The high inducing ability of the ZMS line enabled the production of a great number of haploid plants from synthetic population SA. A total of 604 haploid plants were produced in 1991 and 1030 haploids in 1992.

*Fertility of haploid plants.* The majority of haploid plants were found to be without anthers in their tassels during flowering and to exhibit almost complete male sterility. This poses considerable difficulties in using haploids for breeding purposes. The presence of some anthers carrying fertile pollen in a small proportion

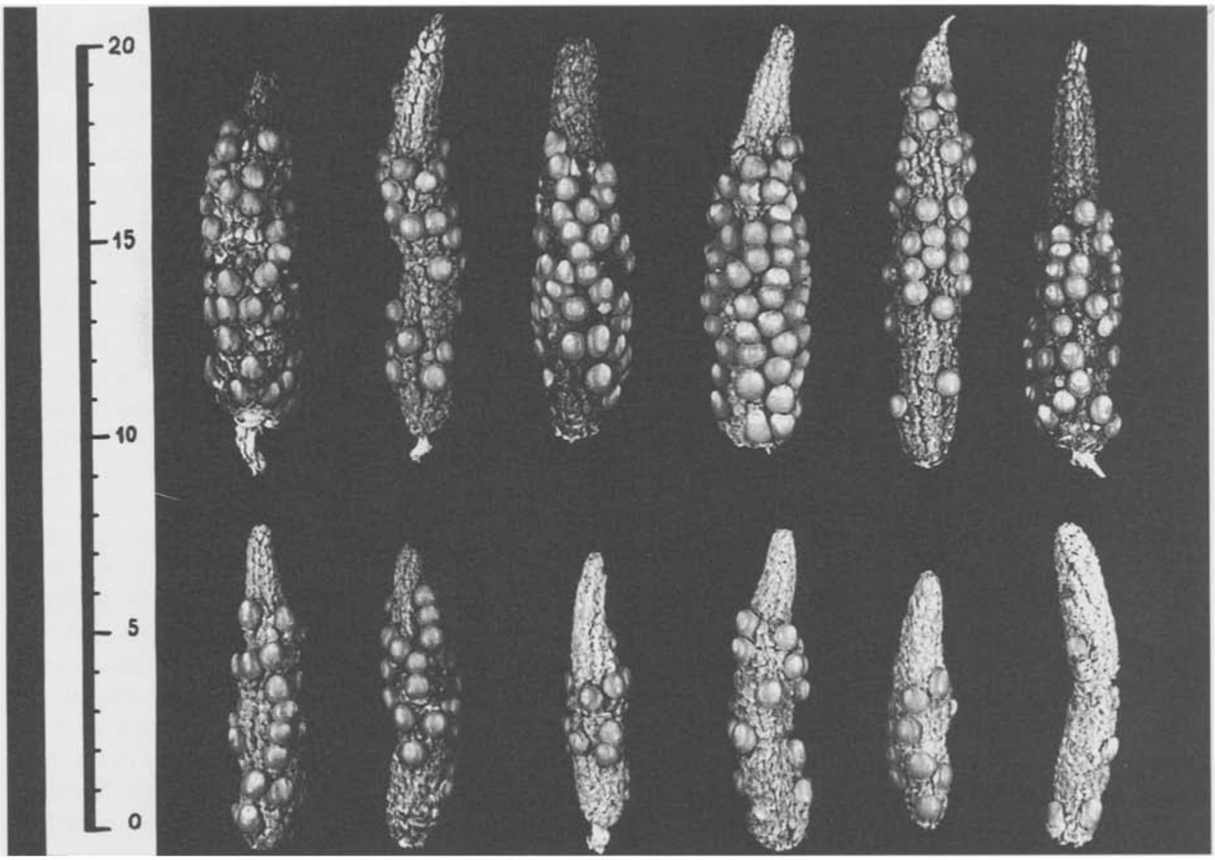


*Fig. 1.* Ears of haploid plants pollinated with pollen from diploid lines.

of haploid plants is unlikely to be an important factor even for selfing these. However, female gametophyte fertility studies have yielded encouraging results.

In order to assess the fertility of female gametophyte, some haploid plants were pollinated with a pollen mixture. A total of 72 haploids were used in 1991 and 162 haploids in 1992. Pollen was taken from diploid inbred lines derived previously from population SA. The cross resulted in haploid ears carrying kernels. It should be noted that almost all of the pollinated ears carried kernels. As few as four plants were without any kernels in 1991 and again in 1992 (Table 1). This result is considerably superior to that reported by Chase (1969). In his experiments, only about one-third of monoploids studied set some seed upon outcrossing.

The resulting reasonably high mean number of kernels per ear appeared to be somewhat unexpected. On average, 27.4 kernels per ear were obtained in 1991 and 26.3 in 1992. The highest number of kernels in an ear was 127 in 1991 and 107 in 1992 (Table 2). It may be summised that seeds from haploid plants can be obtained with a fair degree of success. Some ears from haploid plants are shown in Fig. 1.

Seeds harvested from haploid plants were sown in a field plot the following year. These gave rise to normal diploid plants which hardly differed from plants of the initial population, SA. The only difference was that the progeny of haploid plants exhibited lower growth rates at early developmental stages than did the plants from the initial population. However, no significant differences were observable by the time of flowering. This suggests that pollination of haploid ears with normal, viable pollen, results in the formation of normal seeds with diploid embryos. Moreover, the seeds are formed in numbers which are quite sufficient for breeding work. This is the first property of haploid maize plants which is worth to be emphasized. It allows, despite the nearly complete male sterility, various genes and gene combinations selected at the

*Table 3*. Frequency of unusual plants among haploids

| No | Item | Number of plants | | Percent | |
|---|---|---|---|---|---|
| | | 1991 | 1992 | 1991 | 1992 |
| 1 | Total number of plants planted as haploids | 604 | 1030 | 100 | 100 |
| 2 | Frequency of unusual plants | 16 | 34 | 2.6 | 3.3 |
| 3 | Unusual plants selfed | 10 | 32 | 1.3 | 3.1 |

*Table 4*. Standard deviations of plant height for inbred lines and progeny of unusual plants obtained from the same synthetic SA

| Rank of line | Standard deviation | |
|---|---|---|
| | Inbred lines | Progeny of unusual plants |
| 1 | 5.7 | 7.2 |
| 2 | 6.8 | 7.9 |
| 3 | 7.2 | 8.1 |
| 4 | 7.3 | 9.4 |
| 5 | 9.5 | 9.8 |
| 6 | 10.0 | 9.9 |
| 7 | 11.0 | 10.7 |
| 8 | 12.4 | 10.8 |
| 9 | 12.9 | 10.9 |
| 10 | 19.5 | 12.1 |

level of haploid plants to be effectively involved in the breeding process.

*Spontaneous doubling of haploids*. Another feature of haploid maize plants which is of considerable breeding value is spontaneous chromosome doubling. Khoklov et al. (1976) have shown diploid cells to be present in almost any tissue of haploid maize plants. Their mean number was found to be 0.42% of the 17800 cells analyzed. The maximum of diploid cells was found to be 1.23%. The authors suggest that this results from spontaneous chromosome doubling. These findings may explain the appearance among our haploids of unusual plants. It should be noted that these plants displayed themselves spontaneously without being treated with any chemical or physical factors, as well as with no application of any special pollinations. These plants showed no resemblance to hybrid (synthetic SA × ZMS) and heterosis was absent. Nor were they haploids. Their phenotype was that of homozygous lines. By way of explanation, it can be suggested that unusual plants resulted from spontaneous diploidization which occurred at early ontogenetic stages. At any rate, fertility of the above plants was normal and the majority of them were selfed. The ears resulting from selfing confirmed the assumption that these represented lines. Some of these ears are shown in Fig. 2. In 1991, the number of such plants was 16 and in 1992 it went up to 34, which constituted 2.6% and 3.3%, respectively (Table 3). This data is comparable with the maximum rate of spontaneous chromosome doubling in cells of haploid plants (1.23%) reported by Khokhlov et al. (1976).

In order to ascertain whether the unusual plants indeed were homozygous, the following experiment was carried out. The progeny produced by selfing 10 plants, which presumably were spontaneously doubled haploids, were sown in a field plot. The adjacent area was sown with 10 inbred lines previously derived from population SA by ordinary five selfings. One of the most informative estimators of the degree of homo- or heterozygosity of the plants under study is the degree of uniformity of their progenies resulting from selfing. Table 4 lists standard deviations for the character 'plant height'. These standard deviations have been ranked. The data obtained indicate that there were no significant differences in variation patterns between inbred lines, on the one hand, and progenies of unusual plants, on the other. This reduces the probability that the unusual plants occurring among haploids were hybrids (synthetic SA × ZMS). It is very unlikely that the unusual plants resulted from heterofertilization. If that had been the case, the resulting plants would have been heterozygous, with high variation observed in their progeny. At any rate, the variation would have been more pronouonced than that of the inbred lines. That, however, was not the case (Table 4). The possibility of a mechanical contamination is also ruled out since the seeds used for sowing were only those with dominant marker genes from the pollen parent (ZMS) expressed in their endosperms. The progeny of the unusual plants lacked dominant marker genes (Fig. 2).

Thus, the following three properties of the unusual plants can be distinguished: (a) they lack heterosis, (b) the uniformity of their progeny does not differ from that of inbred lines, and (c) they lack dominant marker genes of the pollen parent (ZMS). This suggests that

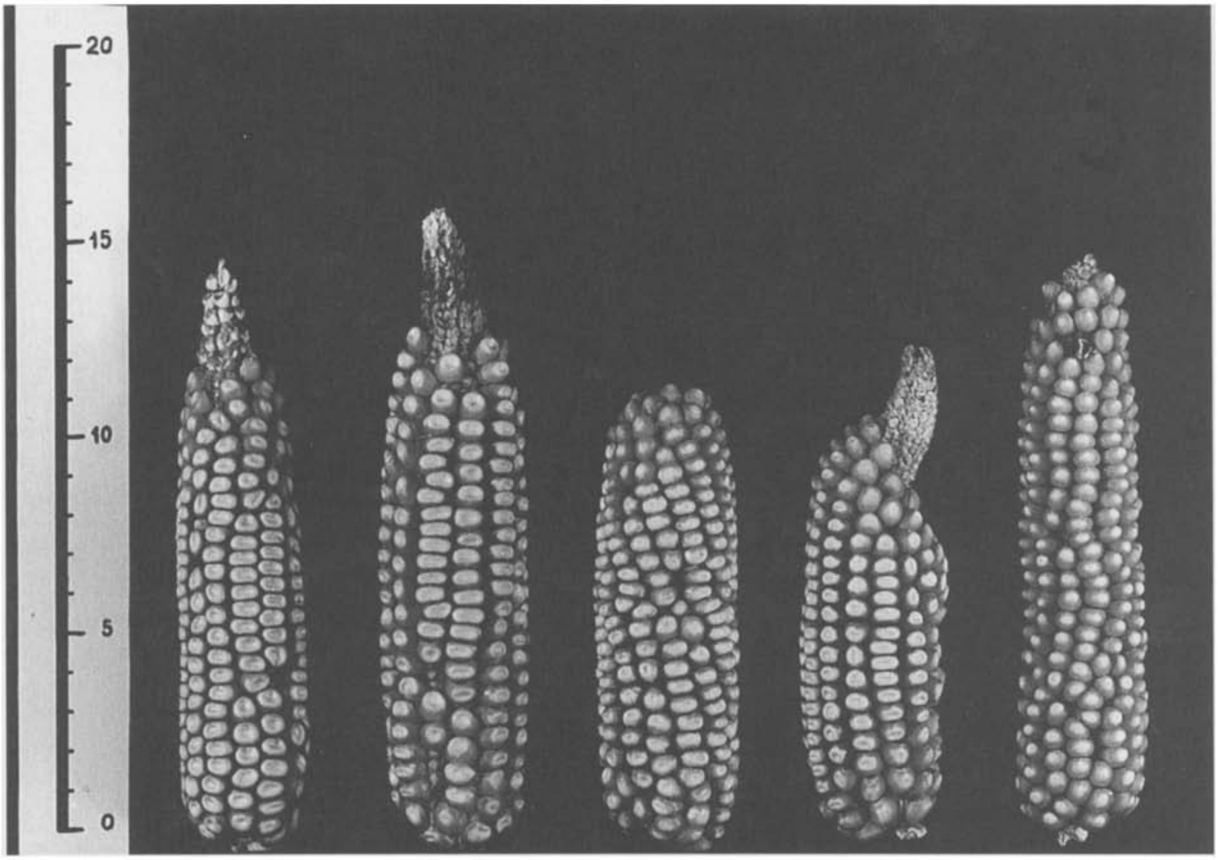


*Fig. 2.* Ears from selfed plants which are presumably spontaneously doubled haploids.

the most plausible explanation here is that plants with phenotypes of homozygous lines result from spontaneous chromosome doubling in maternal haploids. Therefore, the progeny by selfing such plants are here referred to as doubled haploid lines.

*Comparison of doubled haploid lines with inbred lines.* Table 5 lists plant and ear trait values as averaged over a group of 10 inbred lines and over 10 doubled haploid lines, derived from the same synthetic population SA. In general, doubled haploid lines were inferior to inbred lines with respect to many of the traits measured. The differences were statistically significant for such traits as the height of the ear position, leaf width, internode number, etc. It is worth noting that the inbred lines were being established over a period of five years. During all this time there was continuous selection for the most desirable genotypes. Therefore, in terms of breeding, the inbred lines were quite favourable.

*Table 5.* Trait means for 10 inbred lines and 10 doubled haploid lines, derived from the same synthetic population (SA)

| Traits | | Inbred lines | Doubled haploid lines | Student's t value |
|---|---|---|---|---|
| Plant height | cm | 141.8 | 132.5 | 1.63 |
| Ear position | cm | 39.1 | 28.6 | 3.25* |
| Tassel length | cm | 36.3 | 33.4 | 1.80 |
| Leaf length | cm | 60.3 | 58.4 | 0.53 |
| Leaf width | cm | 8.3 | 7.4 | 3.31* |
| Number of nodes | no. | 10.8 | 9.7 | 3.17* |
| Number of tassel branches | no. | 6.5 | 4.4 | 2.68* |
| Ear length | cm | 13.6 | 12.4 | 1.92 |
| Ear diameter | cm | 3.9 | 3.2 | 4.83* |
| Number of ear rows | no. | 13.7 | 12.1 | 2.77* |

* Differences are significant at 5% level.

As far as the doubled haploid lines were concerned, they represented the genetic constitution of population SA. Two of these were in no respect inferior to the inbred lines and were rather promising for breeding. Six lines exhibited satisfactory performance, two were very poor. This appeared to be due to the fact that the possible process of chromosome doubling proceeds spontaneously regardless of whether or not the embryo contains useful genes. The properties of the doubled haploid lines are therefore entirely dependent on the parental stock used.

The following conclusion can be drawn from the present study. Use of the ZMS inducer line enables maternal haploid maize plants to be produced in large numbers. The application of these for breeding purposes encounters difficulties resulting from almost complete sterility of tassels. The female gametophyte, however, functions quite satisfactorily. This allows the genotypes selected at the level of haploid plants to be involved in further breeding work. Among the haploids studied, plants occurred which appear to have resulted from spontaneous chromosome doubling at early ontogenetic stages. Progenies of these plants did not differ in the degree of uniformity from inbred lines.

The above properties of haploid plants suggest their possible applications in breeding work.

## Acknowledgements

The author is deeply grateful to Mr. G.K. Lakhman for his invaluable assistance in preparing the English version of this manuscript.

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