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# *IN VIVO* HAPLOID INDUCTION IN MAIZE – PERFORMANCE OF NEW INDUCERS AND SIGNIFICANCE OF DOUBLED HAPLOID LINES IN HYBRID BREEDING

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**ABSTRACT** - In the last three to five years, doubled haploid (DH) lines have increasingly been used in maize (*Zea mays* L.) research and breeding. This became possible by substantial progress in the *in vivo* haploid induction technology. Herein, we describe the development and characteristics of a new induction line, RWS, and discuss quantitative genetic and logistic aspects of the use of DH lines in hybrid maize breeding. – Induction line RWS was derived from an $F_5$ plant of a cross between the Russian induction synthetic KEMS and the French induction line WS14. Kernels with a haploid or $F_1$ embryo can be distinguished by means of the expression of the dominant anthocyanin marker gene *R1-nj*. Misclassification rates based on this marker gene are generally low except for donors carrying anthocyanin inhibitor genes. Reliable estimates of the induction rate were obtained by using tester genotypes with recessive morphological markers. In tests across various induction environments, RWS consistently showed the highest induction rate (8.1% on average) compared to other inducers evaluated herein. – Advantages of using DH lines in hybrid breeding include (1) maximum genetic variance in line *per se* and testcross trials, (2) high reproducibility of early-selection results, (3) high efficiency in stacking targeted gene arrangements and (4) simplified logistics. High cost-savings are possible due to reduced expenses for the selfing program, handling and shipping of seed batches, and for maintenance breeding. Moreover, outstanding DH lines may be protected and commercialized several seasons earlier than lines developed by inbreeding.

KEY WORDS: *Zea mays* L.; Haploid induction; Inducers; Color markers; Effects of donor genotypes and environments; Use of doubled haploids in breeding.

## INTRODUCTION

During the last three to five years, *in vivo* haploid induction has become a widely used tool in maize (*Zea mays* L.) research and breeding. In research, the technology may be used to develop doubled haploid (DH) mapping populations or to analyze linkage disequilibrium and haplotype/trait associations. In breeding, DH lines make possible to increase the efficiency of line development and recurrent selection, and to reduce the effort for line maintenance.

Two modes of *in vivo* haploid induction can be distinguished in maize, leading to maternal and paternal haploids, respectively. The genomes of maternal haploids originate exclusively from the seed-parent plant. Haploid induction in this case is caused by the pollinator parent (COE, 1959). The opposite applies to the induction of paternal haploids, where the pollinator serves as genome donor and the female as inducer (KERMICLE, 1969). In this paper we are dealing with the induction of maternal haploids.

The first haploid maize plant was described by STADLER and RANDOLPH (1929, unpublished; cited in RANDOLPH, 1932). CHASE (1947, 1951) reported a spontaneous haploid induction rate in maize of 0.1% and suggested that haploids could be used for line development in hybrid breeding. However, initially the low spontaneous haploid induction rate did not meet the needs of breeders. A much higher induction rate (up to 2.3%) was detected by COE (1959) in crosses with inbred line Stock6. Further progress was achieved by LASHERMES and BECKERT (1988) who derived inducer line WS14 from a cross between lines W23*ig* and Stock6. This line furnished induction rates of 3-5%. Even higher rates (about 6%) were obtained by SARKAR *et al.* (1994), and SHATSKAYA *et al.* (1994) in progenies of crosses

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between Stock6 and Indian respectively Russian germplasm. Similar results were obtained by CHALYK (1999) with an inducer tracing back to crosses of Stock6 with Moldavian germplasm.

The process(es) leading to maternal haploids in maize are not yet fully understood. WEDZONY *et al.* (2002) studied ovaries of inducer line RWS (see below) during the first 20 days after self-pollination. In about 10% of the embryos the authors found micronuclei of variable size in the cytoplasm of every cell of the shoot primordium. Such micronuclei generally are characteristic for waste chromatin being eliminated from the cell in subsequent cell divisions. In interspecific crosses (wheat × pearl millet, barley × *Hordeum bulbosum*) GERNAND *et al.* (2004) actually could show that the inducer chromosomes degenerate and are fragmented a few days after fertilization. The fragments then coalesce to micronuclei, which are eliminated from the cells within about three weeks. Taken together, the two studies indicate that fertilization and subsequent chromosome elimination might be a major mechanism in maize *in vivo* haploid induction. FISCHER (2004), applying microsatellite markers, observed that a small proportion (1-2%) of haploids obtained with in a broad-based sample of breeding materials carries one, seldomly several paternal chromosome segments. Thus occasionally a minor fraction of the inducer genome may be transferred into the otherwise maternal genome of the haploids.

ROTARENKO and EDER (2003) detected a more than three times higher rate of heterofertilization when using haploid induction line MHI as pollinator compared to a "normal" inbred line. CHALYK *et al.* (2003) found up to 15% aneuploid microsporocytes in line MHI and only about 1% in two inbreds used as checks. These findings indicate that various irregularities appearing between microsporogenesis and fertilization may also be involved in haploid induction.

Segregation studies (LASHERMES and BECKERT, 1988; DEIMLING *et al.*, 1997) and QTL analyses (RÖBER, 1999) demonstrated that *in vivo* haploid induction in maize is a quantitative trait under the control of an unknown large number of loci. Individual QTL explained only small parts of the genetic variation.

A key issue in applying the *in vivo* haploid-induction technique is an efficient screening system for separating the kernels with a haploid embryo from those with a regular diploid $F_1$ embryo. At present this is accomplished by a combination of dominant kernel, embryo, and stem markers (NANDA and CHASE, 1966; CHASE, 1969; RÖBER, 1999; EDER and CHALYK, 2002). GEIGER *et al.* (1994) tested the usefulness of a transgenic herbicide (BASTA) resistance marker. It proved to be absolutely reliable but is very labor intensive since the kernels have to be raised to the seedling stage before the resistance test can be applied. Inducible transgenic markers have not yet been reported.

The spontaneous chromosome doubling rate of maize haploids ranges from 0 to 10% (CHASE, 1969; BECKERT, 1994; DEIMLING *et al.*, 1997; KATO, 2002). Thus, artificial chromosome doubling is necessary for an efficient large-scale application of *in vivo* haploid induction. The highest doubling rates are achievable by immersing 2- to 3-day old seedlings in a colchicine solution as suggested by GAYEN *et al.* (1994). Using an improved version of this method, DEIMLING *et al.* (1997) obtained doubling rates of up to 63%. More recent studies of EDER and CHALYK (2002) with genetically broader materials yielded an average doubling rate of 27%. A gentler method of chromosome doubling was developed by KATO (2002). He treated young haploid plants in the flower formation stage with nitrous oxide gas and obtained high plant survival and satisfactory chromosome doubling rates. However, this method is very laborious and time-consuming and therefore does not allow high-throughput applications.

In the present paper we want to describe the origin, characteristics, and performance of a newly developed induction line (RWS) and to review quantitative genetic, operational, logistic, and economic aspects of using doubled haploid lines in hybrid maize breeding.

## A. DEVELOPMENT AND EVALUATION OF INDUCTION LINE RWS

### MATERIALS AND METHODS

Induction line RWS descends from haploid induction line WS14 (LASHERMES and BECKERT, 1988) and induction synthetic KEMS (SHATSKAYA *et al.*, 1994). Reciprocal crosses between these inducers were advanced by selfing to generation $F_5$, and 65 $F_5$ plants of KEMS × WS14 and 54 $F_5$ plants of WS14 × KEMS were evaluated for haploid induction ability. Line RWS (R = "Russian" inducer KEMS, WS = line WS14) was derived from the best of the former 65 $F_5$ plants.

Induction crosses with $F_1$ and $F_5$ plants of the above two reciprocal crosses were made in autumn 1994 in the greenhouse and in the 1997 field season, respectively. Stock6, WS14, and KEMS served as checks. A single cross between two lines with

the recessive mutant *liguleless* (gene *lg2*; BRINK, 1933), in short "*lglg* tester", and a line carrying various recessive morphological markers developed by Mangelsdorf (WEBER, 1986) for genetic studies, in short "Mangelsdorf's tester", were used as female parents. These two testers allow to unambiguously distinguish between haploid and regular $F_1$ plantlets. The induction rate was calculated as the percentage of haploids in the induction-cross progenies.

The influence of the environment on the induction rate was studied in experiments at Stuttgart-Hohenheim (field 1997, greenhouse spring 1998 and autumn 1998), Gondelsheim/Germany, Upper Rhine Valley (field 1998), Porvenir/Chile and Puerto Vallarta/Mexico (field 1998/99) using RWS and KEMS as inducers and the *lglg* tester and/or Mangelsdorf's tester as donors. Large progenies were studied in each environment (Table 1).

Line RWS is homozygous for the dominant marker gene *R1-nj* (NANDA and CHASE, 1966; NEUFFER *et al.*, 1997) leading to a purple scutellum and a "purple crown" of the aleurone of $F_1$ kernels when crossed with unpigmented donors. These two characteristics are used as embryo and endosperm marker, respectively. In induction crosses, kernels with a haploid embryo and a regular triploid endosperm display an uncolored embryo and a red crown, whereas $F_1$ kernels show pigmentation of both embryo and endosperm. Unintended self-pollination or contamination with pollen from other (unpigmented) germplasm can be detected by a lack of any coloration. However, the expression of the *R1-nj* gene may partly or completely be suppressed by inhibitor genes, e.g. *C1-I*, carried by the female parent (COE and SARKAR, 1964). In such crosses the *R1-nj* marker alone is not sufficient for identifying haploids. Therefore line RWS additionally carries a dominant sun-independent purple-stem marker. In the late seedling stage, this allows to identify the "false positives" among the putative haploids selected by means of the *R1-nj* marker.

To determine the intensity of the scutellum coloration in induction crosses with European and North American breeding materials, inducers RWS and KEMS (the latter also carries the *R1-nj* gene) were crossed with a broad sample of elite single crosses kindly provided by several seed companies (see Acknowledgements). In total 32 dent × dent, 15 flint × flint, and 7 flint × dent singles were tested. In addition, 16 European flint landraces were included in the study. Altogether, 1409 and 1313 ears were pollinated with inducers RWS and KEMS respectively. Scutellum coloration was scored on a 1 to 9 scale (1 = no, 9 = strong coloration). Embryos with scores 1 to 3 were classified as putative haploids. To check the reliability of this classification, all putative haploids and representative samples of putative $F_1$ kernels were grown in the field (Hohenheim 1998 and 1999) until the stem marker became scorable. Data were used to determine the proportion of haploids in the fraction of both putative haploids and putative $F_1$ plants.

All statistical analyses were performed using the program package SAS (SAS Institute, 1988). According to the W-test of SHAPIRO and WILK (1965), the distribution of the induction rate significantly deviated from normality in all data sets. Attempts to transform the data did not succeed. Therefore non-parametric methods (CONOVER, 1980) were used throughout.

## RESULTS

Induction rates obtained in 1994 demonstrate the great progress in the development of novel inducers since Stock6 became available (Table 1). Line WS14 furnished twice and synthetic KEMS seven times more haploids than Stock6. The induction rates of cross WS14 × KEMS and its reciprocal were similar to their midparent value in both generations $F_1$ and $F_5$.

Great differences in induction ability occurred among the $F_5$ plants of the aforementioned cross with several plants transgressing the better parent (Fig. 1). The two best plants in the crosses WS14 × KEMS and KEMS × WS14 had induction rates of 16.5% and 10.9%, respectively. Unfortunately, no selfed seed was obtained from the former. Thus the

TABLE 1 - *Means, 90% confidence intervals (CI), and ranges among individual tester ears for haploid induction rates obtained with various pollinator genotypes (inducers) on a liguleless tester stock, greenhouse Hohenheim 1994 and 1997.*

| Year | Inducer | Offspring tested | Mean | CI | Range |
|---|---|---|---|---|---|
| | | Number | Induction rate (%) | | |
| 1994 | Stock6 | 1156 | 1.04 | 0.43 - 2.08 | 0 - 1.5 |
| 1994 | WS14 | 954 | 1.99 | 1.01 - 3.46 | 0 - 5.1 |
| 1994 | KEMS | 5148 | 6.92 | 6.03 - 7.88 | 1.9 - 13.5 |
| 1994 | $F_1$ (WS14 × KEMS) | 3130 | 3.93 | 3.37 - 4.55 | nd[1] |
| 1994 | $F_1$ (KEMS × WS14) | 2309 | 4.55 | 3.86 - 5.33 | nd |
| 1997 | $F_5$ (WS14 × KEMS) | | | | |
| | – Mean | 21846 | 2.92 | 2.74 - 3.12 | nd |
| | – Best plant | 61 | 16.39 | 9.17 - 26.22 | nd |
| 1997 | $F_5$ (KEMS × WS14) | | | | |
| | – Mean | 28234 | 3.04 | 2.87 - 3.21 | nd |
| | – Best plant | 507 | 10.85 | 8.65 - 13.39 | nd |

[1] Not determined.

latter plant was selected and became parent of line RWS. On average, the $F_5$ plants of crosses WS14 × KEMS and KEMS × WS14 displayed almost identical induction rates.

In all experiments, several plants per female parent were used for the induction crosses, and induction rates were assessed for each ear individually. Considerable differences between ears of a given cross combination were observed in all materials and induction environments (Tables 1 and 2).

Induction crosses on tester *lglg* and Mangelsdorf's tester revealed a clear superiority of inducer RWS over KEMS in all environments (Table 2). On average, the induction rates of RWS and KEMS amounted to 8.12% and 6.05%, respectively. Environmental means for RWS evaluated on tester *lglg* varied between 2.43% (Gondelsheim 1998) and 22.32% (Porvenir 1998/99). No significant differences were found between tester *lglg* and Mangelsdorf's tester when used in the same environment.

The *R1-nj* embryo marker expression permitted to satisfactorily discriminate between maternal haploid and regular $F_1$ embryos in most induction crosses of inducer RWS with dent singles as female parents (donors). In contrast, the misclassification rate was unacceptably high for many flint donors (Fig. 2). The average proportion of verified haploids within the putative haploid fraction amounted to 89.6% in the dent and only 48.0% in the flint group. Yet, many flint genotypes displayed a similarly strong marker expression as the dent group. Flint entries with a low proportion of verified haploids tended towards a high percentage of undetected haploids in the putative $F_1$ fraction ($r = -0.41$, $P = 0.05$). No significant donor × inducer interaction was observed for marker expression and induction rate (data not shown).

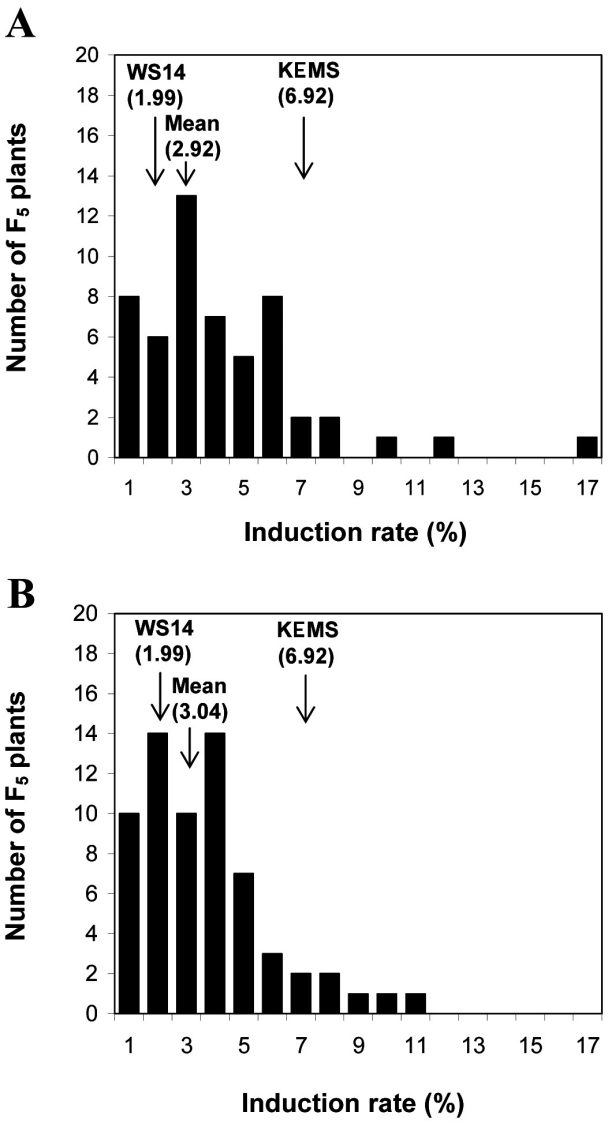



FIGURE 1 - Distribution of haploid-induction rates of 54 $F_5$ plants derived from the cross WS14 × KEMS (**A**) and of 65 $F_5$ plants derived from the cross KEMS × WS14 (**B**) [width of classes: 1%; abscissa values indicate the upper border of each class].

## DISCUSSION

Our results confirm the polygenic basis of *in vivo* haploid induction in maize and demonstrate that the induction rate of line RWS is high enough for routinely applying the technology in breeding and research.

The $F_1$ generation of the cross KEMS × WS14 and its reciprocal did not significantly deviate from the mean induction rate of its parents (Table 1). Thus a cross between two superior induction lines may equally be suited for induction as the parent lines themselves. The use of $F_1$ plants as inducers may be even more advantageous because of their higher vigor, which generally is associated with more abundant pollen shedding. Reciprocal effects do not seem to be important in this regard.

Transgression for induction rate in the $F_5$ populations of the aforementioned reciprocal crosses (Fig. 1) indicate that there is potential for further improvement of this trait if unrelated inducer sources are recombined.

Since anthocyanin inhibitor genes may partially or fully repress the *R1-nj* marker, donors carrying

TABLE 2 - *Means, 90% confidence intervals (CI), and ranges among individual tester ears for the haploid induction rates of inducers RWS and KEMS on tester stocks liguleless* (lglg) *and Mangelsdorf (Mang.) in various induction environments.*

| Environment[1] | Tester | Inducer | Offspring tested | Mean | CI | Range |
|---|---|---|---|---|---|---|
| | | | Number | Induction rate (%) | | |
| HOH97 | *lglg* | RWS | 820 | 7.07 | 5.14 - 9.43 | 0 - 21.1 |
| | | KEMS | 1094 | 6.40 | 4.79 - 8.33 | 0 - 22.6 |
| HOH98a | *lglg* | RWS | 1905 | 6.98 | 5.69 - 8.46 | 0 - 50.0 |
| | | KEMS | 2183 | 5.45 | 4.38 - 6.69 | 0 - 50.0 |
| | Mang. | RWS | 1140 | 7.46 | 5.75 - 9.46 | 0 - 23.2 |
| | | KEMS | 1616 | 5.69 | 4.43 - 7.18 | 0 - 12.2 |
| HOH98b | *lglg* | RWS | 1907 | 6.40 | 5.16 - 7.82 | 0 - 12.1 |
| | | KEMS | 1819 | 2.42 | 1.66 - 3.39 | 0 - 8.4 |
| GON98 | *lglg* | RWS | 1565 | 2.43 | 1.61 - 3.49 | 0 - 8.3 |
| | | KEMS | 1269 | 1.58 | 0.88 - 2.60 | 0 - 9.0 |
| | Mang. | RWS | 407 | 1.23 | 0.32 - 3.19 | 0 - 9.5 |
| | | KEMS | 553 | 0.54 | 0.08 - 1.80 | 0 - 4.6 |
| CHI98/99 | *lglg* | RWS | 905 | 22.32 | 19.2 - 25.7 | 0 - 38.9 |
| | | KEMS | 1620 | 10.43 | 8.73 - 12.3 | 0 - 22.2 |
| MEX98/99 | *lglg* | RWS | 1262 | 12.84 | 10.7 - 15.2 | 0 - 25.0 |
| | | KEMS | 2627 | 9.74 | 8.44 - 11.2 | 0 - 23.8 |
| **Total** | | **RWS** | **9911** | **8.12** | | |
| | | **KEMS** | **12781** | **6.05** | | |

[1] HOH = Stuttgart-Hohenheim; GON = Gondelsheim; CHI = Porvenir (Chile); MEX = Puerto Vallarta (Mexico); a, b = induction crosses performed in spring and summer, respectively.

recessive mutants in the homozygous stage were used as testers (*lglg* and Mangelsdorf's) for assessing induction ability. That way, accurate estimates of the induction rate could be obtained. However, in a breeding program, this approach would not be applicable because few elite donor materials carry suitable recessive marker genes.

Significant variation for induction rate existed between donor genotypes (Fig. 2). However, the range of variation was small compared to that reported in the literature for callus formation and plant regeneration in anther or microspore culture (BÜTER, 1997).

Environments strongly influenced the induction rate. In addition, considerable variation occurred among donor ears of the same induction cross on the same tester in the same experiment (Table 2). Since genetically uniform donors were used, the latter variation might be attributable to small plant-to-plant differences in the flowering state at the day of pollination. SEANY (1954), CHASE (1974), and TYRNOW (1997) observed higher induction rates in case of late pollination, whereas AMAN *et al.* (1981) did not find such an association. Optimizing donor and inducer plant cultivation and choosing a nursery with favorable climatic conditions is likely to reduce environmentally caused variation of induction rates. This may explain that AMAN and SARKAR (1978) and SARKAR *et al.* (1994) did not find significant effects of locations or years on this trait.

The misclassification rate of kernels screened by means of the *R1-nj* embryo marker was acceptable in the dent pool but reached too high values with many flint donors (Fig. 2A). Therefore inhibitors have to be purged in the respective flint (and some dent) materials before the *R1-nj* marker can efficiently be used in breeding practice. Fortunately, screening for color inhibition is easy due to a simple, mostly monogenic inheritance of this trait and can readily be combined with the routine DH line development. The intensity of the scutellum and aleurone coloration in donors without inhibitor gene(s) is similar in dent and flint materials (data not shown). EDER and CHALYK (2002) found an even

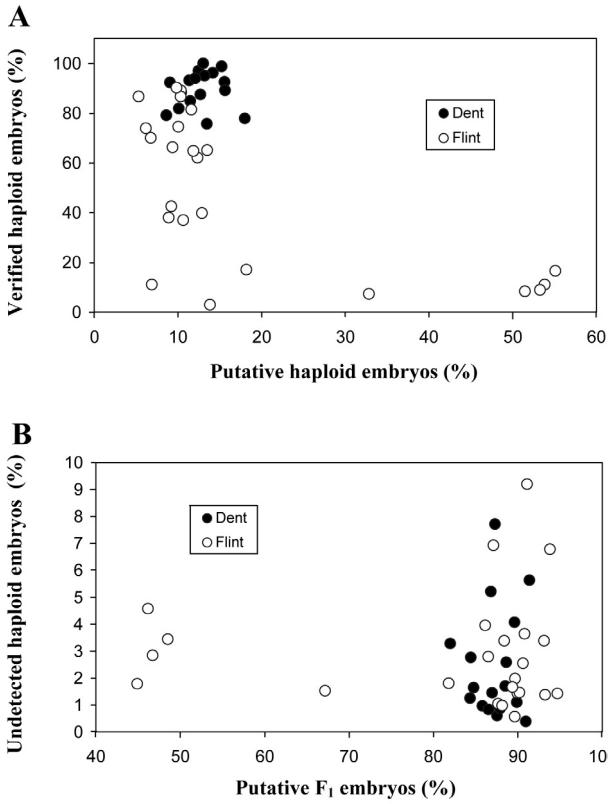



FIGURE 2 - Proportion (%) of verified haploids in the putative (based on the *R1-nj* embryo marker expression) haploid fraction (A) and proportion of undetected haploids in the putative $F_1$ fraction (B) in induction crosses with line RWS on 41 donor genotypes (17 dent singles *versus* 9 flint singles plus 15 European flint landraces), Hohenheim 1998.

more intense scutellum pigmentation in flint than in dent or flint × dent donors.

A stem-color marker in addition to an embryo marker makes possible to remove $F_1$ plants from the putative haploid fraction in early developmental stages in the field. In the adult-plant stage, it is also possible to differentiate haploid from diploid ($F_1$ or $S_1$) plants by means of their distinctive morphology and vigor. However, both screening methods can only be employed after the rather laborious and expensive chromosome doubling procedure. Thus, considerable efforts could be saved by means of a color marker, which is already expressed in the primary root or in the coleoptile. The "purple plant" gene *Pl1* might be suited for this purpose (EDER, 2002, pers. comm.). An alternative option would be an inducible transgenic marker.

## B. SIGNIFICANCE OF DOUBLED HAPLOID LINES IN HYBRID MAIZE BREEDING

### BREEDING SCHEME

The use of DH lines is increasingly replacing the conventional development of inbred lines in commercial hybrid breeding programs. An example of a breeding scheme using *in vivo* haploid induction is presented in Fig. 3. In brief, it comprises the following steps:

(1) Creating new variation by intercrossing selected lines.
(2) *In-vivo* haploid induction in generation $F_1$.
(3) Chromosome doubling of haploid seedlings; transplanting and selfing of DH plants (generation $D_0$).
(4) Evaluation of $D_1$ lines in single-row observation plots and, in parallel, multiplication by selfing.
(5) Evaluation of testcrosses in multi-environment yield trials (two stages).
(6 ff) Build-up of experimental hybrids.

Recombining the best DH lines for starting a new breeding cycle (recurrent selection) may be foreseen after the first and/or second stage of selection for combining ability.

The described breeding cycle requires eight seasons, i.e. four years if an off-season nursery is available. The same period of time is needed for a conventional scheme with combining ability tests in generations $S_2$ and $S_4$. In what follows, we are discussing which other aspects beside cycle length are relevant in comparing DH-line-based and conventional breeding schemes.

#### *Quantitative-genetic aspects*

*Expected gain from selection.* As is well known from quantitative genetics (see e.g. FALCONER and MACKAY, 1996) the expected gain from selection can be described by the following formula:

$$\Delta G = i\, h_x\, \rho_G\, \sigma_y,$$

where $i$ is the selection intensity, $h_x$ the square root of the heritability of the selection criterion, $\rho_G$ the genetic correlation between selection criterion and gain criterion, and $\sigma_y$ the standard deviation of the gain criterion. In long-term breeding programs, the decisive gain criterion for evaluating selection progress is the general combining ability (GCA) of

the improved lines. Test units are, at the beginning of a breeding cycle, the DH lines *per se* and later on their testcrosses.

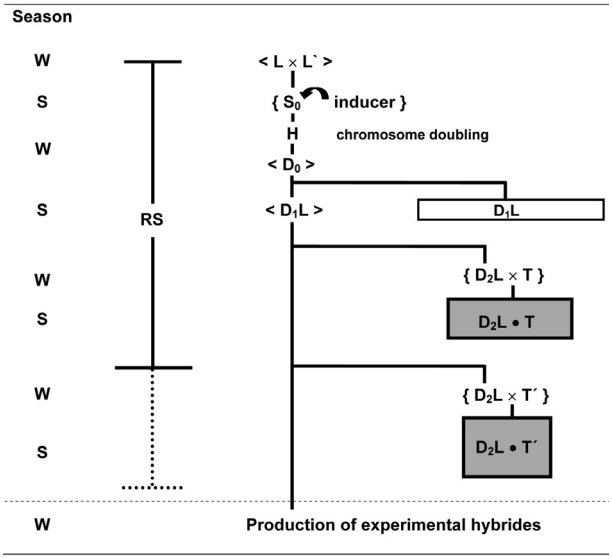
Strong selection (large *i*) leads to a small effective population size and consequently to a loss of genetic variance due to random drift. To keep this loss within certain limits, a minimum number of lines has to be recombined after each breeding cycle. This number depends on the inbreeding coefficient (*F*) of the candidate lines. The number has to be (2 *F*) times larger for inbred lines than for non-inbred genotypes. Assuming that $S_2$ lines ($F = 0.75$) are recombined in conventional breeding, the number of DH lines ($F = 1$) would have to be increased $1 : 0.75 = 1.33$ fold to preserve an equivalent level of genetic variation. This means that selection intensity has to be reduced accordingly when using DH lines.

In contrast to the selection intensity, $h_x$ and $\rho_G$ are increased when using DH-lines. This increase is particularly large in the first testcross stage. Neglecting epistasis, the GCA variance of inbred lines is equal to $^1/_2\ F\ \sigma_A^2$ (FALCONER and MACKAY, 1996), where $\sigma_A^2$ is the additive variance of the base population. Thus the GCA variance of DH lines is $1 : 0.75 = 1.33$ times larger than that of $S_2$ lines. This leads to a better differentiation among the testcrosses and consequently to a higher heritability. SEITZ (2005) compared three sets of $S_2$ and $S_3$ lines each with DH lines derived from the same crosses and evaluated with the same testers in the same environments. On average, the estimated genetic testcross variances for grain yield (bu. / acre) amounted to 50, 94, and 124 for $S_2$, $S_3$, and DH lines, respectively, corroborating the aforementioned theory.

The genetic correlation between selection and gain criterion ($\rho_G$) also increases the more inbred the tested lines are. For example, the correlation between $S_t$ lines and their homozygous progenies for GCA is equal to $\sqrt{F_t}$ whereas for DH lines this correlation is 1. Thus compared with $S_2$ lines, the correlation of DH lines is $1 : \sqrt{0.75} = 1.15$ times stronger.

The genetic standard deviation of the gain criterion ($\sigma_y$) is a population parameter and therefore depends on the base population, i.e. not on the breeding procedure. However, in the long run this is only true if the same upper limit of genetic drift is ascertained in all procedures under comparison.

*Implications of epistatic effects.* Epistatic gene action may positively or negatively affect hybrid maize performance (LAMKEY and EDWARDS, 1999). In most cases, epistatic effects have been reported to cause a decrease in the testcross performance of segregating generations (LAMKEY *et al.*, 1995) or to penalize threeway and double crosses compared to their nonparental single crosses (SPRAGUE *et al.*, 1962; SCHNELL, 1975; MELCHINGER *et al.,* 1986). These effects are commonly referred to as "recombinational loss" and may be explained by a disruption, in meiosis, of coadapted gene arrangements assorted by prior natural and artificial selection. Marker-based analyses of quantitative trait loci (QTL) partially corroborate this hypothesis (STUBER, 1999). To avoid recombinational loss and still offer a chance to select for new positive interactions, a balance between recombination and fixation of gene arrangements is needed. The DH-line approach might offer an optimal way to achieve this goal.




**Abbreviations and symbols:**

| | |
|---|---|
| < > | Crossing or selfing under isolation bag(s). |
| { } | Production of testcross seed in isolation cabin(s) or topcross plot(s). |
| H, DH | Haploid resp. doubled haploid plant. |
| $D_t$, $S_t$ | Plant of doubled haploid generation resp. selfing generation t. |
| L | Line. |
| T | Tester. |
| W, S | Winter (off-season) resp. summer season. |
| RS | Recurrent selection. |
| | Evaluation in single-row plots. |
| | Evaluation in yield trials. |

FIGURE 3 - Flow chart of a hybrid maize-breeding scheme using doubled haploid lines.

***Operational, logistic and economic aspects***

The development of DH lines by *in vivo* haploid induction requires specific skills and equipment for large-scale chromosome doubling, transplanting up-regulated plants to the field, and finally raising and selfing these plants. According to the authors' experience, about 70 - 80% of the haploids survive the colchicine treatment and 20 - 30% of those furnish selfed seed. Thus, in induction crosses with vigorous donor plants, about one to five DH lines emanate from one pollinated ear.

Considerable savings are possible in DH-line development, since no subline production and evaluation is necessary. This does not only allow the breeder to considerably reduce the size of the nursery but also simplifies the logistics of seed exchange between main and off-season programs, since each line has to be shipped only once rather than anew after each further generation of selfing.

Since DH lines are absolutely homozygous and uniform, they ideally meet the requirements for being protected by plant variety rights. This allows to start their commercialization several seasons earlier than in conventional breeding. Furthermore, hardly any maintenance breeding is needed for DH lines whereas inbred lines have to be checked for uniformity even in advanced selfing generations.

Finally, haploid induction is a particularly helpful tool in stacking specific genes in homozygous lines. After genotypes carrying the target genes have been detected (e.g. in early backcross generations), the DH technology allows to fix the respective gene combinations and the genetic background in the shortest possible period of time and with the lowest possible genotyping expenditure.

## GENERAL CONCLUSIONS

Recent progress in the development of haploid induction has made possible the large-scale use of doubled haploids in maize breeding and research. Induction line RWS described in the present paper, has decisively contributed to this success. It excels by a superior induction rate and an efficient embryo/endosperm color marker system for a visual discrimination between kernels with a haploid and those with an $F_1$ embryo. Problems caused by color inhibitors in some donor materials are likely to decline since the practical application of the technology automatically disfavors the respective gene(s). Quantitative genetic theory shows that a greater gain from selection can be expected when using DH lines rather than inbred lines in hybrid maize breeding. In addition, the DH technology offers a number of operational, logistic, and economic advantages. Therefore, the authors consider the achievements in this field as a major breakthrough in maize breeding and genetics.

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