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THE USE OF DOUBLED HAPLOID TECHNOLOGY FOR LINES DEVELOPMENT IN MAIZE BREEDING

Abstract – In maize, doubled haploid (DH) technology is used in breeding programs to obtain lines, the primary input for developing new cultivars. This review aims to present the main points related to using this technology in maize, the steps for its application, and the advantages and implications of using DH lines in maize breeding programs.

Keywords: *Zea mays*, inbred line development, haploid inducers, heterosis.

O USO DA TECNOLOGIA DE DUPLO-HAPLÓIDES PARA O DESENVOLVIMETO DE LINHAGENS NO MELHORAMENTO DE MILHO

Resumo - Em milho, a tecnologia de duplo-haploides (DHs) é utilizada nos programas de melhoramento genético para obtenção de linhagens, principal insumo para o desenvolvimento de novas cultivares. O objetivo desta revisão é apresentar os principais pontos relacionados ao uso desta tecnologia em milho, as etapas para sua aplicação e as vantagens e implicações do uso de linhagens DHs em programas de melhoramento de milho.

Palavras-chave: *Zea mays*, desenvolvimento de linhagens endogâmicas, indutores de haploidia, Heterose.

Roberto dos Santos Trindade[1](✉)

[1]Embrapa Milho e Sorgo
E-mail: roberto.trindade@embrapa.br

✉ Corresponding author

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The technology of doubled haploid (DH) lines is used to assist maize breeding programs in obtaining homozygous lines in as little as three generations, unlike the conventional method, which requires a minimum of six generations for the establishment of the line, reducing the time for the development of cultivars (Chaikam et al., 2019). Furthermore, this technique can spontaneously or artificially produce individual haploids followed by genome duplication (Prigge & Melchinger, 2011).

Since the middle of the decade 2000, the DH line production technology has grown in importance in breeding programs of private seed companies, especially in multinational companies, and later in medium-sized companies in both the private and public sectors. Currently, DH technology is the most used method in developing maize lines in competitive breeding programs.

Obtaining DH lines begins with the generation of haploid plants with only the species' primary number of chromosomes (n). Haploid maize plants have reduced vigor and are not viable for reproduction due to the impossibility of a plant undergoing normal reductional meiosis. In contrast, diploid cells have two sets of chromosomes (2n). Therefore, chromosome duplication of the plant is necessary for restoring the diploid number of chromosomes, with consequent recovery, at least in part, of plant fertility (Prasanna et al., 2012). The progeny obtained by the chromosome duplication process is called doubled haploid because it has an exact copy of each chromosome of the haploid plant. Thus, it grants complete homozygosity and the same number of chromosomes as a diploid plant.

The doubled haploid technology reduces the time for obtaining homozygous maize lines from six generations to as little as three. It also reduces the costs and accelerates the introgression of agronomic traits into new cultivars. This pack of advantages has led to seed companies' large-scale application of this technology (Pierre et al., 2011). Consequently, research groups have worked to develop alternative techniques and optimize already existing protocols to obtain doubled haploid lines in maize.

OBTAINING DOUBLED HAPLOIDS IN MAIZE

Weber (2014) lists a series of methodologies for obtaining haploids in maize: i) spontaneous occurrence, ii) interspecific hybridization, iii) late pollination, iv) use of heat, v) induction by chemical treatments, vi) anther or pollen culture (in vitro induction), and vii) in vivo induction. Among these, in vivo haploid induction is currently the most used methoology to obtain DHs of maize commercially, due to its efficiency and the greater ease of implementing it within a breeding program.

The development of haploid maize in vivo requires many steps as haploid inducer genotypes, an efficient system for haploid identification, chromosome duplication protocols, progeny acclimatization in a greenhouse after chromosome duplication, transplanting of the surviving plants

in the field, and self-fertilization of the first generation of DH plants (D0). The main steps for doubled haploid production in maize through in vivo induction are described below.

HAPLOID INDUCTION

The haploid induction is the initial step for obtaining doubled haploid maize lines. A source genotype from which one intends to extract new lines is crossed with a haploid inducer, seeking to obtain several haploid seeds that will pass through chromosome duplication to generate new DH lines. Haploid inducers are genotypes (lines, hybrids, or varieties) that can induce, in a particular proportion, the formation of seeds with haploid embryos, with a genetic constitution based on the genome of the source genotype.

In maize, two in vivo induction of haploidy processes are known: androgenesis and gynogenesis. In androgenesis, the haploid inducer is the female parent, receiving pollen from the source genotype in its silks. However, when the reproductive nucleus of the pollen grain arrives at the embryonic sac and unites with the oosphere, a haploid embryo develops, containing only genes derived from the source genotype (male parent). Kermicle (1969) found that haploidy induction by androgenesis is controlled by the mutant recessive gene *ig*1 (indeterminate gametophyte), responsible for embryological anomalies, such as anucleated oospheres. After fertilization, by the reproductive nucleus of the pollen grain, they form haploid embryos with the mother plant's cytoplasm and the male parent's genes.

In gynogenesis, the inducer is used as the male parent. In this system, when the reproductive nucleus of the pollen grain of the gynogenetic inducer enters contact with the oosphere of the source genotype within the embryonic sac, there is induction of mitotic division of the oosphere in a haploid embryo, carrying genes only of the female parent. This process is the most used for obtaining doubled haploid lines. Furthermore, it has a higher rate of induction and practicality of use than androgenesis since the source genotypes are used as female parents. Thus, pollination for induction of haploidy can be performed in isolated lots, with detasseling of the source populations, maintaining only the pollen coming from the maternal inducer in the field. In addition, tropicalized gynogenetic inducers are already available, facilitating this technology in countries like Brazil. Thus, in this review, emphasis will be given to that methodology.

Geiger (2009) cites two possible hypotheses to explain the gynogenesis process: I) Gynogenesis occurs by fusing a defective inducer nucleus of the pollen grain with the oosphere within the embryonic sac. After that, the chromosomes of this defective nucleus degenerate during subsequent cell divisions, while a second nucleus unites with the polar nuclei, forming the triploid endosperm. II) Gynogenesis occurs because one of the reproductive nuclei of the pollen grain is incapable of fertilizing the ovule, leading to the differentiation of the egg cell in a haploid embryo by parthenogenesis. Another

reproductive nucleus of the pollen grain unites with the polar nuclei, forming the endosperm.

All maize lines currently used as haploid inducers are derived from the Stock 6 line, which induces the formation of gynogenetic haploids with a 3% frequency. Another line, the W23 line, generates androgenetic haploids with a frequency between 1% and 3% (Prasanna et al., 2012). The inducers developed at the beginning of the twenty-first century have obtained rates above 10% in the induction of haploid seeds (Forster et al., 2007; Rotarenco et al., 2010; Kebede et al., 2011; Prigge et al., 2011; Mowers et al., 2018).

The gynogenetic haploidy process in maize can be induced by manual crosses or open pollination in isolated blocks (inducer nursery). The decision regarding what type of cross to use depends on diverse factors, such as I) characteristics of the inducer, such as plant height, pollen production, agronomic performance, and induction rate; II) the need for isolation, in time and space, of the induction nursery concerning other nearby fields; III) the need for staggering the inducers sowing (split); IV) number of source populations used in the production of DHs; V) the number of haploids planned; and VI) availability of laborers for carrying out manual crosses, and other field activities.

In the case of haploid induction by open pollination in nurseries, Prasanna et al. (2012) recommend that for each inducer row, there be two source genotype rows (1:2 proportion), with the weekly staggering of sowing of the inducer in four to five dates, spaced at every seven days from sowing. The climatic conditions during the induction period should also be considered. Factors such as temperatures, relative humidity, water deficit and light, among other factors, can affect pollen production, reducing the number of haploid seeds obtained. Thus, recommendations are to adjust the induction of haploids to the seasons and locations with more suitable climatic conditions.

The success in haploidy induction depends on several other factors, such as the choice of the location for setting up the induction field (with flat topography, seeking to facilitate mechanization), good natural fertility, and no history of the occurrence of diseases. Other factors to be considered are suitable soil management and tillage practices and effective control of pests, diseases, and weeds.

In a recent study, Trampe et al. (2022) showed that the potential of different genotypes for haploid induction is a quantitative trait, strongly affected by environmental conditions. In this study, 247 F2:3 families derived from a biparental cross between elite lines were induced to haploidy in three locations by crossing with the maternal inducer BHI Bulk, identifying four QTLs (Quantitative trait loci). Together, the QTLs explain 37.4% of phenotypic variance but do not have a stable response in all the environments, indicating the effects of the QTL × environment interaction for haploid induction potential in maize.

IDENTIFICATION OF HAPLOIDS

In identifying positive haploids in maize, a non-destructive, easily applied, and accurate methodology is necessary to evaluate a large number of samples in a short time.

Nanda and Chase (1966) developed a system for identifying haploids in maize using the expression of the *R1–navajo* (*R1-nJ*) gene, which codifies the purple color through biosynthesis of anthocyanin in the endosperm and embryo of maize seeds. In this system, embryos with white color and a purple endosperm are haploids, having genes only from the source genotype. They can be selected for chromosome duplication to obtain DH lines. However, seeds with a purple embryo and purple endosperm are diploids, resulting from the effective crossing of the source genotype with the inducer, and they should be discarded. Furthermore, a third category should also be discarded, formed by seeds in which expression of the *R1-nj* gene is inhibited, making haploid identification impossible (Figure 1).

The system based on the *R1-nj* gene has been widely used to identify haploid maize due to its ease of use. However, visual inspection of each seed is laborious and time-consuming, requiring trained laborers for efficient selection. Another factor to be considered is the genetic variability in the expression of the *R1-nj* allele. Although the *R1-nj* gene has dominant expression, its effect can be suppressed by anthocyanin inhibitor genes in the source genotype (Prasanna et al., 2012; Chaikam et al., 2015).

As described below, new methodologies have been tested for increasing acuity in selecting haploids and doubled haploids.

Near-infrared spectrometry (NIR): This technique uses electromagnetic radiation with a 750-2500 nm wavelength frequency. When combined with multivariate calibration methods, it allows qualitative and quantitative evaluation in a rapid and non-destructive way and without using reagents (Jones et al., 2012). For example, in haploid seeds, Gustin et al. (2020) found an increase in protein content, and the seeds were classified for oil content by skNIR (single-kernel near-infrared reflectance spectroscopy). Through near-infrared spectroscopy combined with partial least squares-discriminant analysis (PLS-DA), Simeone et al. (2019) obtained the correct classification of the seeds and the plants with 100% efficiency as haploid seeds, as well as haploid, diploid, and doubled haploid plants in different maize genotypes using leaf samples. NIR proved rapid, simple, non-destructive, and reliable in this classification compared to the visual method.

Cytogenetic evaluations: despite its high cost per analysis, flow cytometry is the "gold standard" for confirmation of haploid induction in various studies based on quantifying DNA content (Couto et al., 2013, 2015b). Couto et al. (2015a), using flow cytometry as the method for comparing the efficiency of two methodologies of chromosome duplication in maize, found that the genetic background of

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Figure 1 – Categories of seeds for identification of haploids in maize using the expression of the R1-nj gene: A) positive haploids, with purple pigmented endosperm but with non-pigmented embryo; B) diploids, with purple pigmented endosperm and embryo; and C) inhibition, in which expression of the R1-nj gene is inhibited, preventing the identification of the haploid plants.

the germplasm affects haploid induction, though not the chromosome duplication rate in different protocols.

Molecular markers: once parametrized with reference genomes, molecular markers can be used as tools to identify haploids in maize. Belícuas et al. (2007), using microsatellite markers (SSR) to identify haploids in tropical germplasm, found that among 462 seeds identified as haploids, only four individuals identified as were true putative haploids. In an experiment for developing new haploid inducers, SSR markers were used to confirm the haploid's androgenetic nature (Couto et al., 2013). In an experiment for developing new haploid inducers, SSR markers were used to confirm the haploid's androgenetic nature (Couto et al., 2015b).

Oil content in the grain: Li et al. (2009) validated a haploid inducer with xenia effect of high grain oil content, called CAUHOI. Seeds with oil content above 48.40 g/kg were identified as putative haploids. The same strategy was used for Melchinger et al. (2014), using the inducer line UH600, with high grain oil content.

Leaf anatomy: Choe et al. (2012) evaluated the potential for using the length of the stomatal guard cells as a parameter to distinguish false positives of doubled haploids of maize. Also, Souza et al. (2018) used morphoanatomical traits of the guard cells, the number of stomata, and the microgametogenesis for identifying haploid, doubled haploid, and diploid genotypes of maize.

Grain weight: Smelser et al. (2015) studied the applicability of the weight of haploid and diploid kernels of maize as a parameter for differentiating these two classes. In that study, it was found that the efficiency of the methodology was dependent on the genotype under evaluation.

Morphological traits in newly germinated seedlings: Chaikam et al. (2017) evaluated the use of root and coleoptile length and the number of lateral roots for differentiation between haploid and diploid seedlings in maize. In another study, the dominant purple pigmentation trait of roots of haploid inducers in maize allowed correct identification of positive haploids in the seedling stage, increasing the efficiency of the development process of DH lines (Chaikam et al., 2016).

Some researchers join in vitro techniques, such as the recovery of embryos and selection of haploids in the initial stages of development, through the anthocyanin expression in the embryo, for later chromosome duplication. This technique involves higher cost and training of the team, but it can be associated even with the *in vivo* methodology to obtain DHs.

CHROMOSOME DUPLICATION

Chromosome duplication is a process in which copies of each chromosome present in haploid cells of maize are generated. In the case of maternal or gynogenetic haploids, these chromosomes come only from the female parent.

Since haploid cells contain only half the number of chromosomes of a species, the homologous chromosome pairing at the time of meiotic divisions does not occur, thus, implying the abortion of gametophyte development (Prasanna et al., 2012). Consequently, haploid plants tend to be sterile. Therefore, through correct chromosome duplication, obtaining a copy of each chromosome in haploid cells, fertility is restored, allowing self-fertilization of the plants to produce seeds of completely homozygous lines.

For chromosome duplication, haploid seeds selected in the induction step pass-through treatment with antimitotic agents cause the ruptures of the fibers of the spindle apparatus during the cell division, impeding the separation of the homologous chromosomes (Prigge & Melchinger, 2011). Consequently, although the chromosomes duplicate, their migration to the poles is impeded, resulting in a cell with the number of chromosomes duplicated. Thus, each one of the ten maize chromosomes present in the haploid cell is copied, but its division is impeded, forming a new nuclear membrane surrounding a nucleus that comes to have 20 chromosomes. This organism is then called a doubled haploid because its cells have two identical copies of each chromosome.

Chromosome duplication requires antimitotic agents, allowing the genome of many haploids to be duplicated with minimal losses by toxicity. The product most applied for this purpose is colchicine, an alkaloid extracted from the species *Colchicum autumnale* L. (autumn crocus), which can impede the formation of the spindle apparatus during cell division through rupturing the tubulin fibers.

One of the methodologies available for chromosome duplication is described in Prigge and Melchinger (2011). The protocol consists of immersing 2mm of the tip of the hypocotyl

of seedlings in a 0.06% colchicine solution for 8 hours at ambient temperature. After the treatment, the seedlings are washed three times and grown in trays or pots containing a substrate in a greenhouse or screened enclosure for 15 to 25 days for later transplanting in the field. Other protocols (Deimling et al., 1997; Couto et al., 2015a; Vanous et al., 2017) involve the treatment of newly germinated seedlings, indicating that the efficiency of duplication tends to be greater and that the process, in general, is facilitated in the initial stages of maize development.

Nevertheless, due to the high degree of toxicity and high cost of colchicine as a reagent, alternative products have been tested and used for chromosome duplication; in this group of products are herbicides such as trifluralin, oryzalin, and pronamide (Wan et al., 1991; Häntzschel & Weber, 2010), nitrous oxide (Kato, 2002), and other products. All these protocols inhibit the mitosis process by inducing the chromosome duplication of the haploid cell.

Another relatively recent line of research is based on the fact that the response potential of haploids to chromosome duplication is a quantitative trait, and this research has raised the possibility of spontaneous duplication in maize haploids (Chaikam et al., 2019; Molenaar et al., 2019; Boerman et al., 2020; Trentin et al., 2020; Trampe et al., 2022). In this context, studies show that the selection of individuals that have the potential of reversing the haploid state to diploid, that is, that can naturally restore fertility, may favor programs for the development of DH lines through introgression of this trait in source populations.

Molenaar et al. (2019) performed three recurrent selection cycles to increase the values of spontaneous chromosome duplication in maize and found heritability values on the population level of 0.91, though with variation from 0.11 to 0.19 on the individual level, showing the work in the process for selection of this trait. In the same study, additive and epistatic effects were predominant, with substantial gains regarding male fertility in haploid plants. Furthermore, De la Fuente et al. (2020) evaluated the effects of general combining ability above 17% and specific combining ability above 25% for spontaneous chromosome duplication in maize.

Other studies have identified genomic regions associated with spontaneous chromosome duplication and restoration of male fertility in the haploid maize (Yang et al., 2019; Ma et al., 2018; Chaikam et al., 2018; Ren et al., 2020). Boerman et al. (2020), reviewing the data from different authors, highlighted approximately 36 different QTLs linked with spontaneous chromosome duplication dispersed throughout the maize genome. These studies show that the restoration of male fertility through chromosome duplication in maize is an inheritable trait. Thus, this trait is subject to mapping and incorporation in the elite germplasm in maize breeding programs to reduce the dependence on mitosis inhibitors for DH production.

POST CHROMOSOME DUPLICATION MANAGEMENT

Plants that pass through the chromosome duplication process are called generation D0 (Prasanna et al., 2012), the first generation after chromosome duplication. In this step, it is imperative to identify diploid hybrids that were mistakenly selected as haploids during the haploid induction process due to problems in anthocyanin expression in the embryo. These diploids stand out by their high vigor compared to D0 plants and should be eliminated to prevent them from contaminating these D0 plants with undesirable pollen (Trindade et al., 2019).

Fragile plants characterize D0 lines, with low stature and minor production of pollen and seeds; ears with few style stigmas are common, and tassels with male-sterile parts. Consequently, one more generation of self-fertilization is necessary to obtain D1 lines that are uniform, with totally fertile tassels, are more stable, and have better agronomic performance, which allows the production of a more significant number of seeds.

After obtaining lines of the D1 generation, it is possible to evaluate the lines *per se*, eliminating those with undesirable traits. It is also possible to evaluate them in progeny tests (testcross) in the same way as lines obtained by the conventional method, aiming at selecting DH lines that provide better combining ability for obtaining superior cultivars. Due to their greater vigor and uniformity, the D1 lines selected and the subsequent generations (D2, D3…) are used for breeding purposes as parental lines of commercial hybrids.

The fragility of D0 lines requires extreme care in their management and self-fertilization to prevent plant losses. It is recommended that after the chromosome duplication process and triple washing, the seedlings be placed in trays or containers with an adequate substrate, receiving slow-release fertilizers; thus, the plants are not burned, and fungicide and insecticide treatment. The D0 seedlings, after treatment, should be kept in a greenhouse or screened enclosure for acclimatization for approximately 15 to 25 days until reaching the third or fourth leaf stage (V3/V4) for recovery from the colchicine treatment before transplanting in a permanent location. In the same way as in the induction field, proper soil management and tillage practices and efficient control of pests, diseases, and weeds are essential in growing the D0 population.

As the D0 seedlings have small ears and low pollen production, and some parts of the tassels are even sterile, the use of strategies to obtain seeds through self-fertilization is necessary. Therefore, in addition to careful isolation of ears to avoid contamination from undesirable pollen, it is crucial to perform self-fertilizations repeatedly on the same plant, whenever possible, to ensure the most significant number of seeds, and use pollination bags adapted to the reduced size of the tassels. Equally, careful handling of the reproductive structures during the pollination operations and cleaning of hands to avoid contamination by undesirable pollen, among other measures, are

necessary.

EFFICIENCY OF THE PROCESS OF OBTAINING DOUBLED HAPLOIDS

The efficiency of the process of producing DH lines depends on the mean induction rate observed for haploid inducers when crossed with a series of source genotypes, on the amount of pollen produced by the inducers, on the total number of crosses made between the inducer and source populations, on the absence of anthocyanin inhibitors in the source genotypes, on the effectiveness of the chromosome duplication process, and on care in management and self-fertilization of the D0 population (Trindade et al., 2019).

For success in producing DH lines on a commercial scale, Prasanna et al. (2012) recommend minimum rates of 6% for haploid induction and 5% for the chromosome duplication process. For example, considering these rates for the production of 100 DH lines of a determined source population that generates 250 seeds per ear, approximately 134 crosses would be necessary, which would result in 33500 seeds, of which 2010 (6%) of the seeds would be haploids. Of these 2010 haploid seeds, around 100 seeds (5%) would be duplicated successfully, resulting in D0 lines at the end of this process.

These estimates agree with Röber et al. (2005), who highlight that, on a commercial scale, approximately one to five DH lines are obtained from each source population. In this context, to ensure success in DHs production, Chang and Coe (2009) recommend that the efficiency of the process should be raised to values of 12% for frequency of induction and 30% for chromosome duplication.

APPLICATIONS OF THE DH TECHNOLOGY IN MAIZE BREEDING

Geiger and Gordillo (2009) describe a method for maize breeding using the doubled haploid technology, which may have variations for each work strategy but has the following steps: i) generation of genetic variability by crosses between selected genotypes, ii) haploid induction, iii) identification of haploids and chromosome duplication to obtain D0, iv) self-fertilization of D0 to obtain DH lines, v) visual evaluations of lines *per se* together with seed multiplication, and vi) development and evaluation of testcross hybrids obtained from crossing DH lines with testers of appropriate heterotic groups (different and complementary).

Introduction of the doubled haploid line technology in a breeding program includes three steps: 1) establishment of protocols in which different methodologies are tested for the production of DHs, aiming at the most suitable pipeline for the program; 2) implementation of the doubled haploid technology in gradual substitution of the conventional process of line production, according to the demands and capacity of the breeding program; and 3) application of the DH line technique in taking advantage of assets, that is, obtaining cultivars with more excellent added value through more rapid introgression of

traits of interest in the elite germplasm. Although these steps include cyclical standard processes in maize breeding, the particular aspects of each program (work strategy, infrastructure, periods for making crosses, evaluation of hybrids, and others) should be evaluated. Thus, it is recommended that breeders analyze the processes used in their programs and gradually introduce DH technology in their work in a phased manner so that this process results in tangible gains in the production of new elite lines.

Application of the doubled haploid line technology is used on a commercial scale mainly by companies in the private sector, and various authors discuss the possible impacts, advantages, and limitations of the technology of obtaining doubled haploid lines (Mayor & Bernardo, 2009; Pierre et al., 2011; Prasanna et al., 2012; Weber, 2014; Krchov & Bernardo, 2015). Among the doubled haploid technology applications in plant breeding, the following stand out:

I) It accelerates the acquisition of new cultivars, where the volume of DHs and the speed at which they can be obtained in a short space of time favor the use of this technology in elite germplasm;

II) It better uses and maintains the elite genetic base or active germplasm banks (AGBs) in maize breeding programs;

III) Combining classic or genetic modification methods with marker-assisted selection facilitates the introgression of traits of interest;

IV) Populations of mapping for marker-assisted selection can be acquired. In this case, as the doubled haploid lines represent the genetic variability inherent to the source genotype from which they are derived and have total homozygosity, DHs can be used as populations for mapping, increasing the accuracy of the estimation of quantitative traits and the genotype × environment interaction when evaluated in different locations. However, due to homozygosity, only two genotypes are possible for each locus (AA and aa), which impedes the estimation of parameters linked to the dominance of the mapped loci (Schuster & Cruz, 2008).

V) It allows training, validation, and selection populations to be obtained for wide genomic selection.

VI) The doubled haploid line technology in recurrent selection programs can accelerate the acquisition of superior synthetic varieties and hybrids in each breeding cycle and breeding of source populations.

FINAL CONSIDERATIONS

The doubled haploid technology has the great advantage of making it possible to obtain completely homozygous lines rapidly. That way, the genetic value of the DH lines, in terms of combining ability, occurs at the highest degree of inbreeding, which results in greater accuracy in selecting superior genotypes for traits of interest. In this case, the genetic value estimated in DHs corresponds to the real potential of the lines and not an estimate of what would be its mean potential in advanced generations, as obtained

in progeny tests in an early generation obtained by the conventional process. Mowers and Foster (2020) compared DH lines with lines from six to eight self-fertilization cycles and found more significant genetic variance and greater yield of hybrids derived from DH lines.

The DH technology makes it possible to obtain many lines, making it essential to evaluate them concerning combining ability (Riedelsheimer et al., 2012). Furthermore, lines with high values of combining ability contribute to the production of elite hybrids since the additive effects can be transmitted from the parental lines to the hybrids (Wang et al., 2020). However, with the application of the DH technology for obtaining lines, the opportunity of selection for the elimination of undesirable traits in intermediate phases is lost, as occurs in the Pedigree method. Nevertheless, these difficulties tend to be minimized by choosing better sources to extract DHs with a high frequency of favorable alleles, such as backcrosses for elite parents or S1 plants selected for desirable traits.

A crucial issue in generating double haploid lines in maize is from which generation or type of source population will be obtained. Considering that haploid induction in maize can be carried out from any population, the practice most used has been obtaining lines from F1 hybrids, in which the parents are duly selected according to the interests of the program (Röber et al., 2005; Melchinger et al., 2011). This approach accelerates the process of obtaining inbred lines, thus, saving time and resources.

Bernardo (2009), reviewing diverse authors, indicates that in segregating generations, such as F2 and S1, the possibilities of recombination of genes would be more significant, which would compensate for the investment of resources required by the advancement of populations for other generations, due to the greater probability of obtaining individuals with superior combinations of genes. Consequently, in segregating populations, there is an increase in genetic variability, which allows the selection of individuals in early generations (Bordes et al., 2006, 2007). Another possibility is that this more significant variability allows combinations of superior genes and that this is reflected in the combining ability of doubled haploid lines when obtained from segregating generations (Bernardo, 2009).

However, the supposed effect of narrowing the genetic base by the type of source population may preserve superior allele combinations accumulated over time when this recombination of genes occurs from elite maize lines (Bernardo, 2009). In addition, as the breeding program advances in obtaining DHs, the tendency is for the best DH lines to be used in crosses within each heterotic group to produce new sources for the extraction of lines. This recycling process will eliminate the most deleterious alleles since in the D1 generation, the doubled haploid lines with very low vigor or that bear undesirable traits are eliminated.

Another great advantage of the doubled haploid technology is the possibility of integration among processes, such as wide

genomic selection and DH technology. That way, more significant genetic gains per unit of time can be achieved, considering that selection can be carried out without the need for evaluation of all the generations in the field and that with the genotyping of new doubled haploid lines, all the additive genetic variability can be exploited because the lines are entirely inbred. Furthermore, dominance effects can also be exploited through appropriate genetic-statistical models joined with phenotypic data of hybrids in the training populations.

All the above shows the potential of the doubled haploid line technology as an auxiliary tool of great value in maize breeding programs and that the technique is evolving with the development of new protocols and deepen research in the different steps for its application (haploid induction, haploid selection, chromosome duplication, and post-induction management). Although the use of the technique is expanding in modern maize breeding programs, investments in research are still necessary, intending to reduce costs and increase the viability of the DH technology in smaller maize breeding programs.

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