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HAPLOIDY IN MAIZE *(ZEA MAYS* L.*)* BREEDING AND RESEARCH

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Abstract

A haploid is a plant that contains a gametic chromosome number (n). They can appear spontaneously in nature or as a result of different induction techniques. For maize (Zea mays L.), in situ induction of maternal haploids, results by using a selected inducing genotype (line). The first inducer line was Stock 6 discovered by Coe in 1959, with an induction rate of 2.3%. Over the years through hybridization and selection the induction rate improved. A homozygous line is obtained by crossing the inducer line with an initial material, selecting the resulted haploids, doubling the number of the chromosomes and performing a self-pollination. Using this technique in maize breeding allows a reduction in the time needed to obtain a homozygous line. Also, this technique can be incorporated in different breeding schemes, like recurrent selection. One of the best inducer at this moment is PHI with an induction rate of 12-15%.

Key words: *Zea mays L., haploid, induction rate, homozygous line, breeding schemes, PHI.*

INTRODUCTION

Haploidy is the phenomenon by which the chromosome number of somatic cells is reduced by half, so haploids are plants that contain a gametic chromosome number (n). Haploid plants can spontaneously appear in nature or they can be the result of various induction techniques (Murovec et al., 2012). However, spontaneous occurrence is a rare event and therefore of limited practical value. Thus, various induction techniques have been studied and improved. For maize (*Zea mays* L.) the most used induction technique is *in vivo* induction of maternal haploids. This technique is based on the use of special genotypes that have the characteristic to induce haploids. Two hypotheses have been proposed about the mechanisms of maternal haploid induction. The first hypothesis supported by Wedzony et al. (2002) states that one of the two sperm cells coming from the inducer line pollen is defective but still capable of fusing to the egg cell. During cell division, the chromosomes from the inducer parent deteriorate and are eliminated from the primordial cells. The second hypothesis supported by Chalyk et al. (2003) states that one of the two sperm cells is unable to fuse with the egg cell. As a result of this phenomenon, haploid embryogenesis is activated. The second sperm cell then fuses with the central cell. The resulted haploids are small, present low plant vigor and are sterile. In order to propagate them, their fertility must be restored. This can be obtained by spontaneous doubling of the chromosomes or by induced doubling. The resulted doubled haploids (DH) are completely homozygous and homogeneous (Chalyk et al., 2003).

Production of doubled haploids. Producing DH lines typically requires four steps: (i) inducing haploids by crossing heterozygous plants with a haploid inducer; (ii) identifying haploid kernels through morphological markers; (iii) chromosome doubling of haploids by colchicine treatment; and (iv) selfing to obtain seeds of DH lines (Melchinger et al., 2005; Seitz, 2005). While the use of DH lines in maize breeding was first proposed nearly 60 years ago (Coe, 1959), with the use of the first recognized inducer line, Stock 6, with a haploid induction rate of 2.3%, the routine production of maize DH lines became possible only upon the development of haploid inducers that lead to a high frequency of haploids (Seitz, 2005).

(i) DH lines are typically induced among F_1 plants, but recent studies suggest that F_2 plants

are a better material for haploid induction (Bernardo, 2009).
(ii) The *R1-nj* marker gene (purple scutellum and a "purple crown" of the aleurone) is widely used for the screening of haploids in dry seeds (Gordillo et al., 2010; Rotarenco et al., 2010).

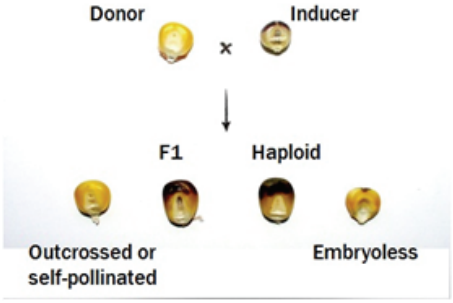

Figure 1. Haploid identification, kernel marker R1-nj (Rotarenco et al., 2010)

However, the expression of this gene has a strong female influence: sometimes the screening of haploids might be very confusing or even impossible, especially in those cases when there are inhibitor genes (*C1-I)* in females (common for flint maize) (Gordillo et al., 2010; Rotarenco et al., 2010).
In this case, the combination of *B1* and *Pl1* marker genes (sunlight – independent purple pigmentation in plant tissues) allows haploids to be identified by the lack of anthocyanin coloration in seedlings (Rotarenco et al., 2010).

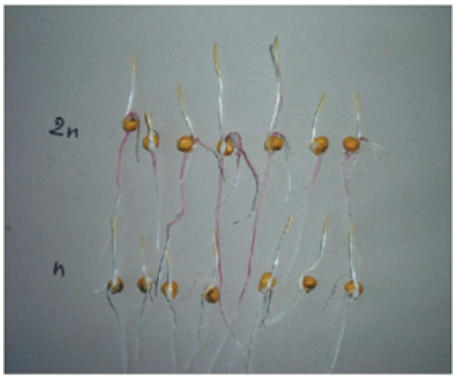

Figure 2. Haploid identification, root markers - combination of B1 and Pl1 (Rotarenco et al., 2010)

(iii) Chromosome doubling can occur naturally or artificially. Spontaneously the doubling rate is around 3% (Deimling et al., 1997). Thus, for a practical use, artificial doubling is required. Colchicine treatment is the most used procedure for chromosome doubling. The colchicine mechanism of action consists in stopping cell division during mitosis. As a result, the anaphase and telophase doesn't occur, so after metaphase the number of chromosomes is doubled (Nebel, 1937).
Presently, one of the most known and used chromosome doubling method is the Deimling procedure (Deimling et al., 1997).
(iv) After chromosome doubling, the resulted DHs are self-pollinated to obtain DH lines.
Applications of doubled haploids in maize breeding. Through the use of DH technology, completely homozygous plants can be obtained in two generations, compared with the conventional methods were multiple generations of self-pollination are required to obtain a partially homozygous plant (Gordillo et al., 2010; Melchinger et al., 2005; Picard et al., 1994; Rober et al., 2005; Rotarenco et al., 2010; Seitz, 2005). The efficiency of selection for qualitative and quantitative characters is increased since the recessive alleles are expressed due to the complete homozygousity. Doubled haploids can be used in a recurrent selection scheme, were after multiple cycles of crossing, DH production and selection, an improvement of the population is expected due to recombination and selection (Bouchez et al., 2000; Gallais, 2009; Gordillo et al., 2010). Another use of DH technology would be in mutation breeding. The homozygousity enables the fixation of mutations in the first generation after mutagenic treatment (Murovec et al, 2012).
Alternatively to the DH technology, pure haploid plants can be used for breeding and research purposes. This is a possibility caused by their ability to produce normal kernels after pollination with the pollen from diploid plants. (Chalyk et al., 2001; Rotarenco et al., 2012).

MATERIALS AND METHODS

The initial material was two haploid inducers lines: MHI with a haploid-inducing rate of 6 to 8% (Chalyk, 1999) and Stock 6 with a haploid-

inducing rate of 1 to 2% (Coe, 1959). Selection for desirable characteristics and traits was carried out in the progeny of the hybrid MHI × Stock 6. The desirable characteristics were high haploid inducing rate anthocyanin marker genes, a good pollen production and improved agronomic traits.

RESULTS AND DISCUSSIONS

For the use of DH technology in maize breeding and research is necessary a haploid inducer with a high haploid - inducing rate, with anthocyanin marker genes, with a good pollen production and with improved agronomic traits. Thus, Procera Genetics started, in 2005, breeding for an improved haploid inducer containing all the characteristics mentioned above.

In generation $F_{5,}$ 9 new inducers were identified. By phenotype and other characteristics, they were divided into four groups and called Procera Haploid Inducers (PHI).

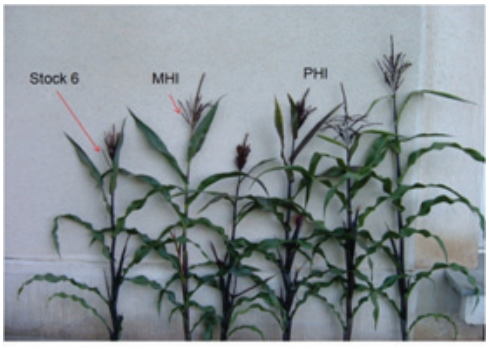

Figure 3. Initial and PHI inducers, (from left to right) Stock 6, MHI, PHI-1, PHI-2, PHI-3 and PHI-4 (Rotarenco et al., 2010)

Haploid-inducing rates were almost twice as high in the PHI inducers as in the best initial inducer MHI (Rotarenco et al., 2010). In 2011 and 2012, haploid-inducing rate estimates of generations F_7 and F_8 confirmed the high haploid-inducing efficiency of the new PHI inducers (Figure 4 and 5).

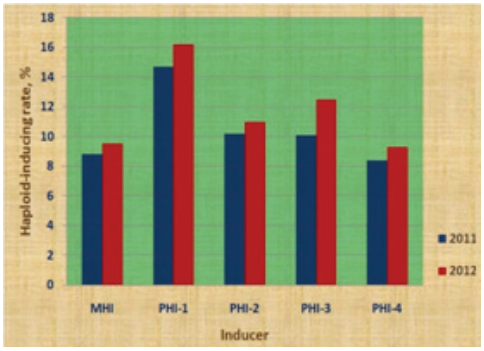

Figure 4. Haploid-inducing efficiency of five lines in crosses with the donor B73 x Mo17

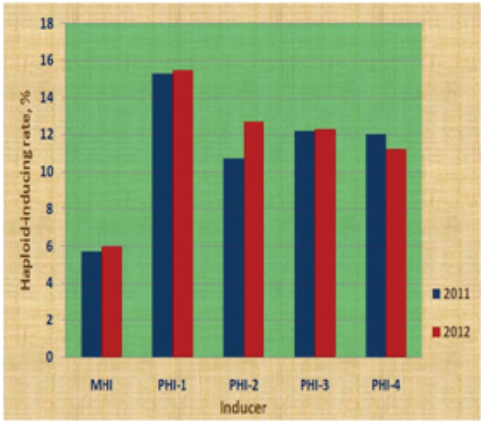

Figure 5. Haploid-inducing efficiency of five lines in crosses with the donor A619 x A464

The expression of the main grain color marker gene, *R1-nj,* was improved in the PHI inducers (Figure 6).

Additionally, the PHI inducers possess a combination of two marker genes, *Pl1* and *B1,* allowing haploids to be identified at the stage of 4-day-old seedlings and among mature plants (Figure 7 and 8).

The inducers also excelled in good pollen shedding and seed set. In addition, improved agronomic performance (plant height, tassel size, logging resistance etc.) was achieved by selection in 2011 and 2012 (Figures 9, 10, 11, and 12).

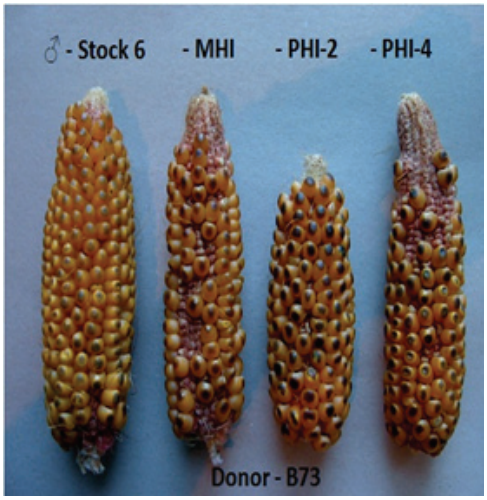

Figure 6. Embryo and endosperm marker – the R1-nj gene

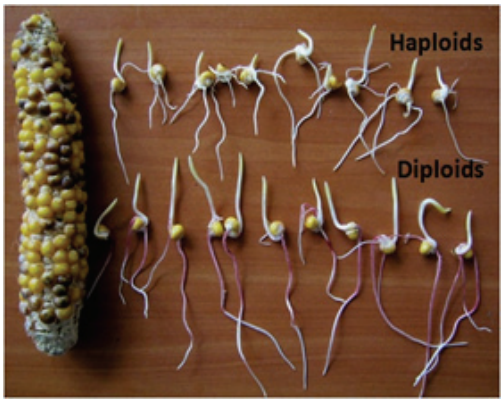

Figure 7. Root marker - the Pl1 and B1 genes

Figure 8. Stalk marker - the Pl1 and B1 genes

Figure 9. PHI-1

Figure 10. PHI-2

Figure 11. PHI-3

Figure 12. PHI-4

CONCLUSIONS

DH technology allows reducing the time and the expenses in maize breeding and increasing the efficiency of selection procedures significantly. The new inducers (PHI) can significantly increase the efficiency of *in vivo* haploid induction. Also we can say that haploid induction is a rather complex phenomenon which requires further studies. In conclusion, we suggest that haploids and DHs should widely be used in breeding and research projects.

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