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ACCELERATED CROP BREEDING USING DOUBLE HAPLOIDS AND DNA MARKERS

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Introduction:

During the twentieth century, the development of inbred maize lines relied almost exclusively on six to eight generations of recurrent selfing and selection to reach the desired level of homozygosity. When the extensive field trials for variety registration are included, it usually takes up to 11–13 years from the time of the initial crosses to the release of new cultivars onto the market. As a result, breeders and geneticists have been eager to adopt methods to speed up the process of inbred line production. In the last, two to three decades, doubled haploid (DH) technology has emerged as an efficient alternative to the traditional method of inbred line development. The technology essentially samples the segregating gametes of the source germplasm, usually a biparental cross or a population, and produces completely homozygous lines in one step. Both in vitro and in vivo methods can be used to develop maize DH lines. However, in vivo methods have proved to be more reliable and efficient in the large-scale production of DH lines and hence are commonly used in maize.

Accelerating crop breeding using doubled haploids, DNA markers, and data management:

The development of crop cultivars by crossbreeding is both time and resource consuming. It takes between 8 to 10 years from the time the cross is made until phenotypically advanced uniform lines are produced. These are then evaluated for at least 3 years to identify potential candidate lines for cultivar release. The continued demand for new cultivars with specific characteristics requires that adopted plant breeding methods accelerate the development of the new cultivars. An offseason nursery reduces the development period of improved populations or advanced lines. This may be further shortened if seeds from the off-season nursery are harvested at near physiological maturity and immediately grown under controlled glasshouse conditions prior to growing under field conditions during the main crop

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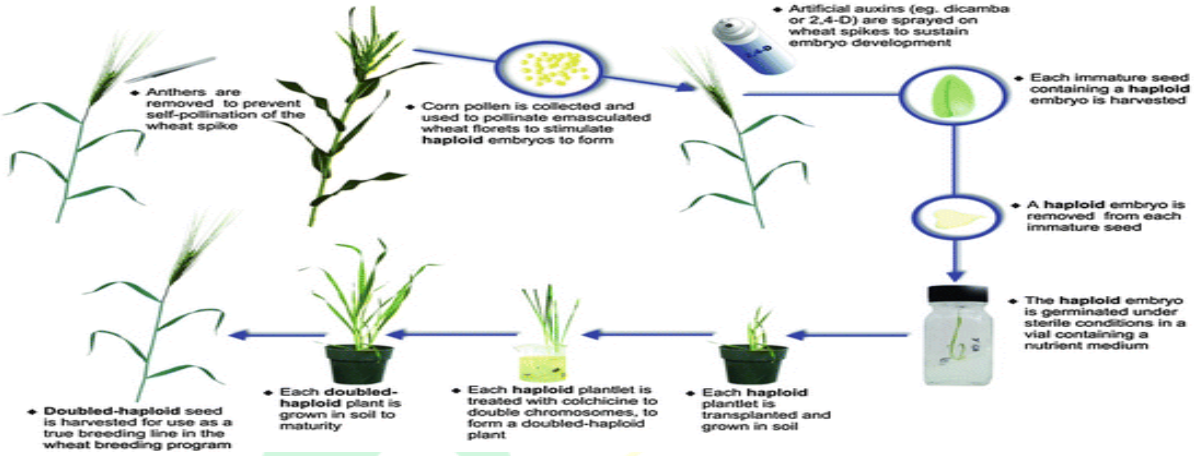
season. At least six generations are needed to advance the lines with acceptable homozygosity levels prior to testing them for agronomic performance.



1. Doubled haploids and marker assisted selection:

Both DH technology and marker-assisted selection (MAS) have independently the potential to shorten cultivar development time. The former requires only two seasons (or one season if microspore engineering and a new breeding technology such as Clustered Regularly Interspaced Short Palindromic Repeats [CRISPR] are employed) to develop homozygous lines (instead of six generations needed ifcrossbreeding is practiced), while the latter has the potential to pyramid many agronomically beneficial alleles simultaneously into improved genetic background and minimize field testing in early generations. MAS can be used as an indirect selection method to speed and increase the precision of the genetic progress, reduce the number of generations, and when integrated into optimized molecular breeding strategies, it can also lower the costs of selection (Dwivedi et al., 2007). Thus, integrating MAS with DH provides new opportunities for the development of improved selection methods that maximize selection gains and accelerate development of crop cultivars (Belicuas et al., 2007). Furthermore, Melchinger et al. (2011) indicated that MAS F2 enrichment –in which F2 homozygous individuals for non-target alleles are discarded and carriers of target alleles are retained in the population– and subsequent MAS for high values of the marker score among DH lines derived from the selected F2 individuals appears to be the best selection method for gene stacking.

2. Genomic selection using DHs:

Genomic selection offers new opportunities for increasing the efficiency of plant breeding programs. In this approach, the genome-wide marker data along with

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phenotyping are used to estimate genomic estimated breeding values (GEBVs) for predicting the performance. In comparison to MAS, which considers only significant marker-trait associations, genomic selection incorporates all marker information, thereby avoiding biased marker effect estimates and capturing more of the variation due to small-effect QTL. Genomic selection uses two types of data sets: a training set and a validation set. A training population with known GEBVs is needed to identify promising germplasm or cultivars with expected genomic value (predicted based on phenotype and GEBVs of the training populations)for making future gains in breeding programs. The training population represents lines that have been phenotyped and genotyped, while the prediction of GEBVs is carried out on lines that have only been genotyped. Several factors contribute to predicting the accuracy of the genomic selection, which include, inter alia, linkage disequilibrium, trait heritability, size of the training populations, number and type of markers, relationship between the training and test populations, and genotype × environment interaction. Simulation studies in maize have clearly shown that response to genomic selection based on DHLs was greater than that of F2 populations or marker-assisted recurrent selection (MARS) with varying numbers of QTL.

3. Establishing haploid induction facilities to support breeding programs:

Many breeding programs worldwide (particularly in the developing world) are limited by the technical knowhow or they lack facilities to apply DH technology for rapid generation of crop cultivars. The major steps involved in production and use of DH technology include haploid induction (in vitro or in vivo), haploid seed identification (using morphological or DNA markers), chromosome doubling of putative haploids (using colchicine treatment), and finally generation of DH seeds to produce DHLs. To date, multinational seed companies have their own facilities employing DH technology in large-scale production of inbred lines for development of hybrid cultivars. For example, DuPont Pioneer claims that just in 2011 more maize inbred lines were generated using DH technology than the total number of maize inbred lines generated in the first 80 years of their breeding program. Likewise, most of the canola or oilseed rape inbred lines that they bred in 2011 were generated through DH technology. Such designated facilities have also come up globally to support public national breeding programs or small-medium private enterprises engaged in seed business. For example, CIMMYT Global Maize Program, in

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collaboration with the Institute of Plant Breeding, Seed Science and Population Genetics of the University of Hohenheim (Germany) has established a state of the art haploid production facility at its experimental station in Agua Fría, Mexico to cater the needs of international maize improvement consortia operating in Asia (IMIC-Asia) and Latin America (IMIC-LA). More recently, CIMMYT has also established a DH facility at the Kenya Agricultural Research Institute in Kibokoto to support both private and public maize breeding programs in Africa. Through these facilities, CIMMYT either provides full or partial service (ranging from developing custom-based haploid inducers to developing DH lines) to breed inbred lines in maize.

Conclusion:

Doubled haploidy is and will continue to be a very efficient tool for the production of completely homozygous lines from heterozygous donor plants in a single step. Since the first discovery of haploid plants in 1920 and in particular after the discovery of in vitro androgenesis in 1964, techniques have been gradually developed and constantly improved. The method has already been used in breeding programs for several decades and is currently the method of choice in all species for which the technique is sufficiently elaborated. Species for which well-established protocols exist predominantly belong to field crops or vegetables, but the technique is gradually also being developed for other plant species, including fruit and ornamental plants and other perennials. It should be mentioned that, in addition to breeding, haploids and doubled haploids have been extensively used in genetic studies, such as gene mapping, marker/trait association studies, location of QTLs, genomics and as targets for transformations. Furthermore the haploid induction technique can nowadays be efficiently combined with several other plant biotechnological techniques, enabling several novel breeding achievements, such as improved mutation breeding, backcrossing, hybrid breeding and genetic transformation.