Indian Journal of Agricultural Sciences **92** (3): 283–91, March 2022/Review Article
https://doi.org/10.56093/ijas.v92i3.122539

Doubled Haploid Technology in Maize (*Zea mays*): Status and Applications

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Received: 26 November 2020; Accepted: 16 September 2021

ABSTRACT

Maize (*Zea mays* L.) is the third most important staple crop after rice and wheat with enormous diversity and adaptation ability. Hybrid breeding is the most important approach for developing high yielding cultivars in maize. It relies upon the generation of pure inbred lines with desirable traits in quick span to achieve higher genetic gains. Rapidly rising global population and climate change necessitates the development of innovative technologies that can help to safeguard the food security in future. Doubled Haploid (DH) technology is the best approach for rapid development of new inbred lines and has contributed immensely in the rapid generation of inbred lines and hybrid development. In addition, the use of molecular markers with DH technology resulted into mapping of genomic regions for different traits. The recent development in identification of alternative markers for haploid selection and genome editing approaches will further strengthen the DH technology for commercial maize breeding. This review describes important landmarks of maize DH technology, its applications, and recent advances in utilization of emerging technologies, viz. CRIPSR-cas and genomics approaches for DH technology.

Keywords: Chromosome doubling, Colchicine, Doubled haploid, Hybrid, Reverse breeding

There is a need to increase the overall food production by 70% to meet the demands of 9.7 billion population in 2050 and that too under diminishing natural resources like arable land and water. The first green revolution was based on adoption of elite varieties with better management practices followed by adoption of hybrids particularly in cross pollinated crops. Hybrids are best candidates to exploit the heterosis at fullest and achieve rapid genetic gains in maize (*Zea mays* L.). Effective hybrid seed production relies upon the production of established and prolific inbred lines. Enhancement of genetic gains rely mainly on shortening the breeding cycles. Doubled haploid (DH) technology has emerged as the potential technology to boost hybrid seed production through rapid generation of elite inbreds (Prigge *et al.* 2012). It helps in saving the valuable resources and time by rapid attainment of homozygous inbred lines (Prasanna 2012). DH technology starts with the generation of haploids followed by doubling of the chromosomes to attain doubled haploids.

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The first spontaneous haploid in maize was reported by Stadler and Randolph in 1929 and Chase was the first to generate maize DH inbreds through spontaneous parthenogenesis in 1946 (Randolph 1932, Chase 1947). Besides this, the documentation of 0.1% spontaneous haploid induction rate in maize led to the potential utilization of haploids in hybrid breeding (Chase 1951). However, the breeders faced the constraint of very poor spontaneous haploid induction rate. Interestingly in 1959, Coe detected a higher induction rate (up to 2.3%) in crosses with inbred line Stock 6 which later served as the progenitor for subsequently developed inducer lines across the globe. Later, Lashermes and Beckert (1988) also derived inducer line WS14 (3–5% HIR) from a cross between lines W23*ig* and Stock 6. In India, Dr. K R Sarkar carried out significant research at IARI on haploid induction and achieved the haploid induction frequency of about 6% (Sarkar *et al.* 1972). Maize hybrid breeding accelerated in the last decade owing to the remarkable progress in *in vivo* haploid induction technology (Röber *et al.* 2005). However, the success of the DH technology for crop improvement depends upon identification of a suitable inducer, possible introgression of genes responsible for haploid induction, standardization of protocol of *in vitro* methods and chromosome doubling techniques.

Since the origin of DH technology, it has been utilized in diverse crops for derivation of haploids and DH lines. DH mapping population is often used in the construction of genetic maps to identify marker-trait associations, loci/

gene responsible for economically important agronomic traits (Forster and Thomas 2005). Considering the active involvement of various public and private institutions, research labs and commercial seed companies, DH technology in maize has turned to become a mature technology platform. Most multinational companies adopted this technique for development of inbred lines and superior cultivars under multi-environmental trials. The International Maize and Wheat Improvement Center (CIMMYT) has been involved in optimization of DH technology for tropical/subtropical areas through Global Maize Program (GMP) launched in 2007 with partnership of University of Hohenheim, Germany. Some other approaches, viz. marker-assisted selections (MAS), transgenic technology, induced mutagenesis also have the potential to accelerate crop improvement in combination with DH technology (Liu *et al.* 2016). Considering several advantages of DH particularly for 'fast marketing option', it has opened new avenues of benefits for seed industry through reduction of expenses in running breeding operations and accelerating breeding cycles for faster recovery of products.

Fundamental steps in DH Line development (Fig 1)

Step 1: Haploid Induction

Haploids can be derived from either male (androgenesis) or female (gynogenesis) gametophytic cells via *in vitro* methods for haploid induction. The haploids derived from male (microspores/immature anthers) and female (ovaries/ovules) gametophytic cells are known as paternal and maternal haploids, respectively. Although androgenesis has proved a successful technique for DH production in many crop plants, but in maize it achieved little success due to non-responsiveness and dependence on donor genotype, anther stage and pretreatment conditions (Wan and Widholm 1993, Spitko *et al.* 2006). Therefore, *in vivo* haploid induction is a successful method for maize breeding program due to its operational feasibility (Seitz 2005).

In vivo approach of haploid induction includes induction of haploids using parthenogenesis, pollen treatment, inter-specific and intergeneric hybridization, haploidy inducers and directed manipulation of CENH3. The maize DH programme is mainly based on use of *indeterminate gametophyte 1* (*ig1*) mutant and Stock 6-derived lines as inducer lines. The *ig1* mutation was first time reported in Wisconsin-23 (W23) that exhibited about 3% haploid induction rates (HIR) of paternal haploids as compared to 0.1% frequency of maternal haploids (Kermicle *et al.* 1969). Further molecular studies revealed the localization of *ig1* on chromosome 3 which codes for Lateral Organ Boundaries (LOB)-domain protein transcription factors governing lateral organ development (Evans 2007, Husbands *et al.* 2007). Stock 6-derived haploidy inducer lines have been utilized to a larger extent in maize. The desirable attributes of good maternal haploid inducers in maize are: high haploid induction rate (HIR), plentiful pollen production, easy maintenance, good flowering behavior, proper plant height, disease and pest tolerance and wide adaptation. Initially, a spontaneous HIR of 0.1% was reported in maize (Chase 1947, 1951) which was too low to be utilized for practical breeding in maize. But later on, a much higher induction rate (up to 2.3%) was detected by Coe (1959) in crosses with inbred line Stock 6. It later served as the ancestor of all the successively developed inducer lines in maize (Geiger 2009). Crosses attempted between Stock 6 and W23 resulted in derivation of new inducer lines such as RWS, UH400, MHI and PHI exhibiting high haploid induction rate of around 7–16% (Hu *et al.* 2016). CIMMYT has also developed second generation tropicalized haploid inducer line (2GTAILs) with 8–15% HIR (Chaikam 2018). The first study on centromere mediated genome elimination was also tested in maize by using the AcGREENtailswap-CENH3 and AcGREEN-CEHN3 transgenes which can complement the phenotype of CENH3 knockout and knockdown lines in maize, respectively (Kelliher *et al.* 2016). However, HIR in developed inducers was found low as compared to commercial inducers available in maize. Quantitative Trait Loci (QTLs) were also identified for HIR in maize among which *qhir1*, *qhir8,*

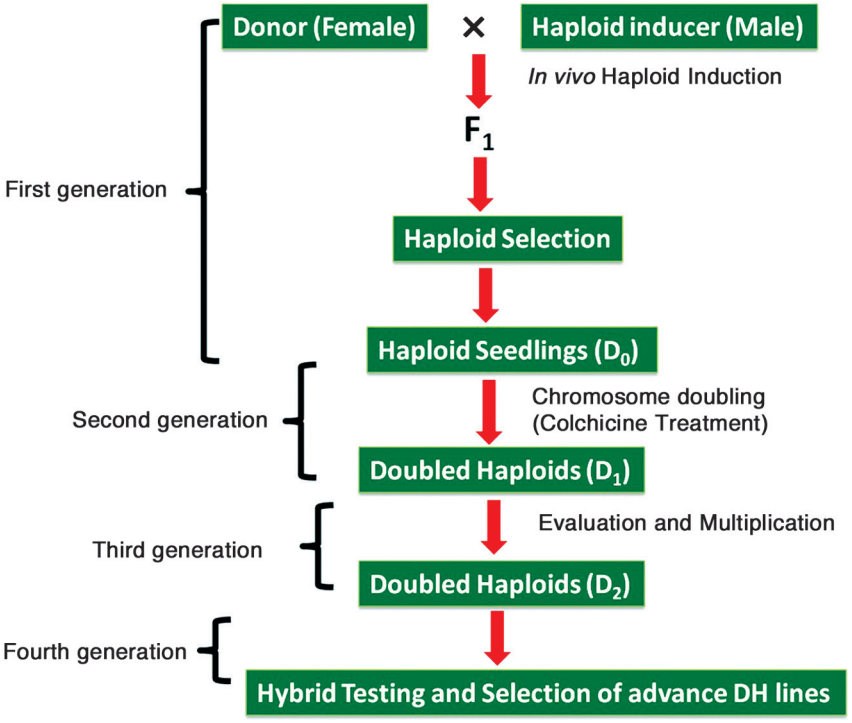

Fig 1 Basic steps for DH line development in maize.

qhir11 and *qhir12* are major. Recent studies on *qhir11* revealed the role of patatin-like phospholipase known as Matrilineal (MTL), PLA1 (Phospholipase A1) and Not Like Dad (NLD) for haploid induction in Stock 6-derived lines (Gilles *et al.* 2017, Kelliher *et al.* 2017, Liu *et al.* 2017). Further investigations suggest that parthenogenesis inducing PsASGR-BABY BOOM-like gene can also be utilized to develop haploid embryos at rate of 25–89% (Conner *et al.* 2017). A list of commercial haploid inducers being utilized for haploid induction in maize breeding programs has been provided in Table 1.

Step 2: Haploid identification or selection

DH technology gained success in maize breeding programmes due to availability of phenotypic seed coloration marker system that helps in direct identification of haploid seeds after harvesting (differentiate diploid seeds). The basis of induction of haploids has been the presence of dominant anthocyanin marker *R1-Navajo (R1-nj)* known as 'red crown' or 'navajo' kernel trait encoded by 'red color' gene *R1*. In the presence of the dominant pigmentation genes *A1* or *A2* and *C2*, *R1-nj* conditions the deep pigmentation of the aleurone layer (endosperm tissue) in the crown (top) region of the kernel. In addition, it causes pigmentation of the scutellum (embryo tissue) (Sarkar and Coe 1966). To be effective, the donor needs to have colourless seeds and the inducer needs to be homozygous for *R1-nj* and the aforementioned dominant pigmentation genes. A kernel resulting from haploid induction has a red crown (regular triploid endosperm) and a non-pigmented scutellum, whereas a regular F_1 kernel displays pigmentation of both the aleurone (outermost layer of endosperm) and scutellum (Geiger 2009). In general, genes that inhibit anthocyanin synthesis are rather rare in dent maize (unlike flint maize) and therefore, anthocyanin marker genes in dent maize mostly are well expressed (Rober 1999). However, this drawback has been negated in the RWS inducer line, which carries a dominant light-independent purple-stem marker. In this case, the inducer is homozygous for the anthocyanin genes *B1 (Booster 1)* and *Pl1(Purple1)* which gives light-independent pigmentation in the coleoptile and root of the F_1 seedlings. Thus, the colourless coleoptiles or root can be identified as haploid type, while the purple coloured ones are diploids at the early development stage (Geiger 2009, Rober *et al.* 2005). A Stock 6-derived inducer CAUHOI (with 2% HIR and higher kernel oil content of 78 g/kg) was developed to identify haploids based on absence of scutellum coloration of *R1-nj* scheme and low embryo oil content (Li *et al.* 2009). The utility of near-infrared spectroscopy has also been explored for differentiating haploids and diploid types (Jones *et al.* 2012). Melchinger *et al.* (2014) reported an alternative method for identification of haploid seeds based on differences arising in oil content stemming from pollination with high oil inducers. Later, Dong *et al.* (2018) devised clustered regularly interspaced short palindromic repeats (CRISPR/cas9) mediated embryo- and endosperm-specific double-fluorescence protein marker for identification of haploids.

Step 3: Chromosome doubling

Seeds of haploid type can't be multiplied or self-fertilized because they are sterile due to the presence of only one set of chromosomes in their cells. Thus, chromosome doubling is necessary to achieve the fertility in these haploid plants. A low rate of genotype-dependent spontaneous doubling has been reported in maize (Geiger *et al.* 2006). Therefore, a protocol with mitotic inhibitor colchicine was developed for efficient artificial chromosome doubling (Gayen *et al.* 1994). Mitotic inhibitors bind to the microtubular protein tubulin and thereby preventing the microtubules from pulling of chromatids towards the poles which in turn results into duplicated haploid genome without cell division. Considering the highly carcinogenic nature of colchicine, other less toxic substrates like herbicides, viz. pronamid, trifluralin, oryzalin for artificial chromosome doubling treatments have been developed (Häntzschel and Weber 2010).

Step 4: Seed multiplication of DH plants

The mitotic inhibitors treated D_0 seedlings produce D_1 seeds under field condition, which represent completely homozygous line. Five percent of the haploid seeds from source germplasm resulted into DH lines. This rate of DH conversion depends upon various factors such as genotype background, accuracy of haploid identification system, chromosome doubling procedure and agronomic practices in the green house as well as field conditions.

Potential Applications of DHs

In recent years, extensive emphasis was laid on shortening of breeding cycles and cost effectiveness. DH technique is an important approach for rapid development of homozygous and homogeneous progenies. It is superior over conventional breeding as it addresses the problem of slow reduction of heterozygosity observed in early segregating generations. DH progenies are homozygous lines and hence serve as good choice for selection and testing under different environmental conditions (Chang and Coe 2009, Liu *et al*. 2016). The various applications of harnessing DH technique have been explained below.

Genetic mapping studies

QTL mapping can be performed with various populations like F_2, backcross generations, RILs and DH. However, as compared to other populations DH most rapidly obtains homozygous population and has edge over F_2 and backcross populations because it provides replicated data over the years and seasons. Therefore, DH is considered as an ideal for mapping population for construction of genetic maps in various crops (Jeffery and Lübberstedt 2014, Liu *et al.* 2016, Wani *et al.* 2018, Choudhary *et al.* 2019, Kumar *et al*. 2019). Comparative QTL analyses of HIR in maize resulted into identification of major QTLs namely *qhir1*, *qhir8, qhir11* and *qhir12* which can be pyramided to increase

Table 1 Detailed list of maize inducer lines for DH line development

Inducer Name	Cross/Source	Origin	HIR (%)	Reference
Stock 6	Mexican corn	Maize Genetics Cooperation Stock Centre, USDA	2.3	Coe 1959
KMS (Korichnevy Marker Saratovsky)	Derived from Coe's Stock 6	Krasnodar Agricultural Research Institute, France, Russia	0.5–3.4	Tyrnov and Zavalishina 1984
ZMS(Zarodyshevy Marker Saratovsky)			2	
EMK (Embryo Marker Krasnodarsky) or ZMK (Zarodyshevy Marker Krasnodarsky)	Based on Coe's Stock 6, PEM (Purple Embryo Marker)		6 to >10	Tyrnov and Zavalishina 1984
WS14	W23ig × Stock 6		3–5	Lasermes and Beckert 1988
KEMS (Krasnador Embryo Marker Synthetic)/ KHI (Krasnodar Haploid Inducer)	Derived from Coe's Stock 6, PEM (Purple Embryo Marker)		6.0–7.9	Shatskaya *et al.* 1994
MHI (Moldovian Haploid Inducer)	KMS × ZMS	Institute of Genetics, Moldova	6.5–7.2	Eder and Chalyk 2002, Rotarenco *et al.* 2010
RWS	KEMS × WS14	University of Hohenheim (UoH), Stuttgart, Germany	8	Röber *et al.* 2005
RWK-76	WS14 × KEMS		9–10	Geiger 2009
RWS/RWK-76	RWS×RWK-76		9–10	Geiger 2009
UH400 (University of Hohenheim 400)	Derived from KEMS		8–15	Chang and Coe 2009
UH600 (University of Hohenheim 600)	-		8.5–12	https://plant-breeding.uni-hohenheim.de/en/84531#jfmulticontent_c167370-4
PK6	Derived from Stock 6, WS14, FIGH1 and MS1334 lines	Diversity et Ecophysiologie des Céréales (GDEC), Clermont-Ferrand, France	6	Eder and Chalyk 2002
HZI1	Derived from Coe's Stock 6	Huazhong Agricultural University, Wuhan, China	>10	Zhang *et al.* 2008a
CAUHOI	Stock 6 × Beijing High Oil population	China Agricultural University	~2%	Li *et al.* 2009
JAAS3 (Jilin Academy of Agricultural Sciences 3)	Stock 6 × M278	Jilin Academy of Agricultural Sciences, China	2.5–15.9	Cai *et al.* 2007
PHI 1 (Procera Haploid Inducer 1)	MHI × Stock 6	Procera Agrochemicals Ltd, & Procera Genetics, Fundulea, Romania	12.1	Rotarenco *et al.* 2010
PHI2			13	
PHI3			14.5	
PHI4			12.8	
TAILs (Tropically adapted inducer lines) like TAIL P1 and TAIL P2	(CML494//(RWS×RWK)//(RWS×RWK) (CML494//(RWS×UH400)//(RWS×UH400)	CIMMYT, Mexico and UoH, Stuttgart, Germany	8–10	Prigge *et al.* 2011
BHI201	RWS, RWK-76 and B73	Iowa State University, USA	12–14	Liu *et al.* 2016
BHI306	RWS, RWK-76, Ames 27451 and PI 340841		11–14	Liu *et al.* 2016
CAU5	UH400	China Agricultural University	10	Xu *et al.* 2013
CAU079	CAUHOI		9	Xu *et al.* 2013
2GTAILs (Lines with highest HIR namely 2GTAIL006 & 2GTAIL009)	TAIL7, TAIL8, TAIL9 and (UH400 × RWSCML269) as inducers & CML 269, CML451, CML495, CML395, CKL05017, CK 05022 as non-inducers	CIMMYT, Mexico	8–15	Chaikam *et al.* 2018

HIR in maize haploid inducers (Prigge *et al.* 2012, Liu *et al.* 2015, Hu *et al.* 2016). DH populations have been utilized for mapping of diverse traits like digestibility, agronomic traits, biotic and abiotic stresses (Table 2). Recently, Zhang *et al*. (2019) identified three major QTLs, viz. *qSD6-2*, *qSD8-2* and *qSBS1-2* that explained more than 10% of the phenotypic variation for stalk lodging associated traits. With the advent of cheaper sequencing platforms, the mapping studies have shifted to the use of SNP markers as cited from most recent studies (Table 2).

Immortalized F_2 (IF_2) populations are developed from different crosses made from RILs or DH populations and have a potential of permanent use in QTL analysis. IF_2 populations have ideal genetic background and are similar to F_2 populations with respect to genetic information, which are used to estimate the additive and dominance effect of QTLs. IF_2 differs from F_2 with respect to replication as unlike IF_2, F_2 cannot be replicated as the single seed represents a single genotype. In maize breeding program, these populations are useful in dissection of genetic basis of grain yield and its components like kernel quality and kernel architecture (Zhang *et al.* 2014).

Reverse Breeding: The reconstitution or derivation of complementary homozygous parental lines from the elite hybrid (through suppression of meiotic recombination process resulting into varied intact chomosomes from each parent) using DH technology is called as reverse breeding (Dirks *et al.* 2009). DH technique relying on the haploid production from a heterozygous individual helps to achieve the haploids. Later on, perfectly complementary homozygous DH progenies representing the parental combination of an elite hybrid can be obtained (Dirks *et al*. 2009, Liu *et al.* 2016). The complementary homozygous DH lines can be crossed in all possible combinations to regenerate or recreate the similar elite heterozygous genotype. Although it has not been carried out in maize, but its success with *Arabidopsis* suggests towards its applicability in other crops (Dirks *et al.* 2009).

Marker Assisted Introgression (Gene stacking)

It is a process of incorporating desirable genes into elite lines using molecular markers to enhance the target trait value in particular genotype. Marker assisted backcrossing (MABC) involves molecular marker-based foreground and background selection strategies to introgress the desired genes into an agronomically important elite line. However, it requires a large number of individuals to stabilize or fix the target gene in the recurrent parent genome. Smaller DH population is required for fixation of particular target gene in the last step of MABC. Recently, Chaikam *et al.* (2018) targeted the marker aided introgression (flaking markers-*bnlg1811* and *umc1917*) of *qhir1* from TAILs to the elite tropical maize inbreds to develop 2GTAILs. One of the introgressed line, 2GTAIL006 was found agronomically superior as well as exhibited an average HIR of 13.1% over TAILs (almost 50% superior over the average of TAILs). Thus, MABC in combination with DH technology can help to execute rapid gene pyramiding (Lübberstedt and Frei 2012).

Hybrid breeding through harnessing wild relatives and landraces: Maize landraces are characterized by broad genetic base for biotic and abiotic stresses, wide geographical adaptation, etc. and hence can be used to improve the genetic background of modern elite cultivars (Choudhary *et al.* 2017). The presence of lethal alleles (masked till homozygous condition) in land races limits their use in crop improvement but DH technology can address this limitation because at haploid level expression of lethal alleles is reduced. Homozygous DH lines contain the fixed allelic variations present in the heterogeneous populations of landraces (Strigens e*t al.* 2013). DH lines created from landraces having high genotypic variance with rapid decay of linkage disequilibrium and absence of population structure can be used for efficient association mapping (Strigens *et al.* 2013).

Plant Varietal Protection

In present scenario, Intellectual Property Rights (IPR) issues are very common in every industry. In this era of growing commercial seed industries, Intellectual Property Protection (IPP) related to Plant Variety Protection (PVP) is gaining importance. The DUS test representing distinctiveness, uniformity and stability has been performed under PVP system of The International Union for the Protection of New Varieties of Plants (UPOV). This test gives the idea about recognizable characters of a particular variety from any other available varieties, which will remain unchanged after its multiplication (UPOV, 2011). The genotypic data of both actual and simulated population created through Single Seed Descent (SSD) and DH process by Smith *et al.* (2008) was examined during initial and later generations. The inheritance of larger blocks of intact parental chromosomes was observed in DH progenies compared to SSD progenies based on simulation data. The study revealed the possibility of selecting DH progenies which are >90% similar to both initial parental hybrids by third generation.

Genome editing and DH

A reliable Haploid identification (HID) method or marker is most important component of DH breeding programme (Geiger 2009). Considering the inefficiency of R1-nj marker to express in certain germplasm there was need to look for an alternative HID marker (Chaikam et al. 2015). Lately, a gene called *Matrilineal (MTL)/ ZmPLA1* has been cloned from maize maternal haploid inducer lines (Kelliher *et al.* 2017, Liu *et al.* 2017). Dong *et al.* (2018) targeted this gene and developed haploid inducer lines using CRISPR/Cas9 system. The inducer lines were then crossed with haploid identification (HID) line (carrying double-fluorescence protein markers) to identify maternal parthenogenesis haploid seeds. The haploid seeds exhibit enhanced green fluorescent protein and DsRED, driven by an embryo-specific promoter (Liu *et al.* 2014)

Table 2 List of QTLS for different traits using DH populations in maize

Cross	Major QTLs (Phenotypic Variation Explained in %)	Traits	Marker type	References
Nongxi531 × Nongxi110	Six QTL for oil (4.34–13.13%), six QTL for protein (5.19–6.66%) and five QTL for starch concentration (4.14–7.85%)	Grain quality, kernel row number	SSR	Zhang *et al.* 2008b
WBB53 × KW4773	One QTL for leaf feeding (25%) and Three QTLs for stalk breakage cumulative 36%)	Stalk breakage, leaf feeding and plant height against European Corn Borer	SSRs and SNPs	Orsini *et al.* 2012
IBM2Syn10-DH (B73 × Mo17)	QTL clusters are located near loci *gln4* and *gln5*, which regulate the activity of glutamine synthetase (5.9–16.5%)	Agronomic and grain quality traits (genetic response to N deficiency)	Genotyping-by-sequencing	Gonzalez-Portilla 2014
AGR_9×GSDCRW-1	21 QTLs (Cumulative 38%)	Node injury against Western Corn Rootworm	SNPs	Hessel 2014
Lim-531 × Lim-789	Few moderate effect QTLs with a maximum of 13.5% for Glu-Rel	Biomass compositional and bioconversion characters	SNP	Torres 2015
B73 × Mo17 (IBM) Syn10	135 QTLs, 18 known functional genes and 25 candidate genes for flowering time and plant height fine-mapped into a 2.21–4.96 Mb interval.	For flowering time and plant height traits	SNPs	Liu *et al.* 2015
Zheng58×Chang7-2	47 QTLs (up to 18.9%)	Stalk associated traits	Illumina Golden-Gate(MaizeSNP3K chip)	Yujie *et al.* 2016
Xianyu335 (PH6WC × PH4CV)	Two major QTLs, *qkl1-2 (*17.8%) and *qkl4-1 (*14.2%)	Kernel length	SNPs	Shi *et al.* 2017
B73 × Mo17 (IBM)	Eight QTLs for germination rate, 11 for seedling length, 13 for mesocotyl length, 15 for plumule length, and 18 for coleoptile length (2.5–7.8%)	Germination ability under deep sowing	SNPs	Liu *et al.* 2017
PH6WC × PH4CV	17 QTLs (Cumulative 35.03%)	Salinity stress	SNPs	Luo *et al.* 2017
15TZ-spring × 15XTS	Two major QTLs, *qED1(*28.3%) and *qCD1* (22.6%)	Ear associated traits like ear diameter	SNPs	Shi *et al.* 2018
IBM Syn10-DH (B73 × Mo17)	Three QTLs namely *qSD6-2* (10.03%), *qSD8-2* (13.73%), and *qSBS1-2* (11.89%)	Stalk lodging associated traits (stalk diameter, stalk bending strength),	SNPs	Zhang *et al.* 2019

and an endosperm-specific promoter (Kalla *et al.* 1994), respectively. The embryo- and endosperm-specific double-fluorescence protein marker can be effectively used in identification of haploid in both mature seeds and young embryo (Dong *et al.* 2018). This *ZmMTL(ZmPLA1)* gene is conserved across cereal crops and hence can be used in different crops. CRISPR-Cas9 directed targeting of first exon of the phospholipase in haploid inducer lines resulted into enhancement of haploid induction by 2% (Liu *et al.* 2017). Similarly, transcription-activator-like effector nuclease (TALEN)-mediated deletions nearby the site of the 4-bp insertion in Stock 6-derived lines enhanced haploid induction up to 12% (Kelliher *et al.* 2017). Recently, a new approach known as Haploid-Inducer Mediated Genome Editing (IMGE) has been devised for developing genome-edited haploids using CAU5 HI line carrying the CRISPR/Cas9 cassette for ZmLG1or UB2 as pollinator and B73 as female. Hence the emerging genome/gene-editing technologies will further strengthen DH programmes in maize (Wang *et al.* 2019, Kumar *et al.* 2020).

Conclusion and future prospects

DH technique is now yielding real and tangible results in both basic and applied biology owing to its various merits such as single step based rapid development of homozygous lines, perfect compliance of developed lines with DUS criteria for variety protection, reduced expenses for maintenance in breeding program, facilitating marker assisted selection, reverse breeding, genome-wide selection and genome editing techniques. Progress and promotion for DH production based maize breeding can be cited from the establishment of centralized maize DH facility at Kiboko (Kenya) by the collaboration of CIMMYT and Kenya Agricultural and Livestock Research Organization (KALRO) and DH facility at Bengaluru, India by collaborating with the Asian institutes. In recent years, there has been a shift from conventional methods of inbred development to DH technology derived lines in commercial maize breeding programs. In the US DeKalb640, a double cross (B14 × H2167/H2386 × H2389) involving 3 DH lines and one conventional inbred was the first accepted commercial hybrid with tolerance to high planting density (Chang *et al.* 2009). Maize research and breeding can be improved through combining DH technique with hybrid breeding, backcross breeding, genetic mapping, identification of QTLs, transgenic, induced mutagenesis and functional genomics. There is a further scope for identification of suitable inducer lines as well as introgression of genes for haploid induction from temperate to tropical maize as evident from generation of 2GTAILS. Future emphasis should be given on application of centromere mutations to produce haploid plants and identification of alternative markers for haploid selection. Haploid induction can also be combined with mini-chromosome for introducing multiple genes into elite lines. Further, "Super Haploid" inducers can also be developed which are capable of generating haploids with spontaneous haploid genome doubling capability. The integration of DH technology with MAS and GS offers new insights to minimize breeding cycles and maximize genetic gains. The future targets should rely on deeper understanding of the haploid induction mechanism and fine tuning of protocols for CRISP/cas9 for their effective use in enhancing the haploid induction rate in maize.

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