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Review on Concept and Impact of Double Haploid Techniques in Crop Improvement

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Abstract
Based on previous studies this review presents about double haploid technology and its role in crop improvement. Double haploids are plants those carry two sets of chromosomes that are created from the haploid plants. Different methods such as androgenesis (microspore or anther), gynogenesis (ovule or ovary) haploid inducer lines and wide crosses are used for developing haploid thereby double haploid. Though various chromosome doubling agents found, colchicine has been widely using. The successes of double haploid production relay on different factors like flower parts development stage, culture media, genotype, donor parent growth condition and haploid detection methods. This technology able shortens breeding cycle or time, complete genetic purity, efficient in genetic study, marker development, mutation and transformation better than traditional way of breeding. Generally, understanding DH technology has important contribution in accelerating breeding program for immediate reaction towards out breaking biotic and abiotic constraints and competitive to world market.
Keywords: double haploid, haploid, crop improvement

1.Introduction

Plant breeding/improving is traditionally a slow process, with the release of new cereal varieties taking up to 12 years as process include developing lines, evaluating and identification, crossing and obtaining true breeding by selfing for longer period (Broughton,1999). With limited natural resources, land and water, and the challenges of a changing climate, the yield of major food crops, maize, rice and wheat, needs to be increased over time. Continued increases in crop performance can be obtained by steeper genetic gains mediated by improved marker technologies, predictive statistics and breeding methodologies (Gerald *et al*., 2013). The challenge for contemporary plant breeding is to not only integrate new traits into our crops, but to accelerate the genetic gain of its breeding programs at the same time, in order to achieve a doubling in speed of yield increase (Begheyn *et al*., 2016).

DH techniques have been, and are being, used to accelerate the breeding programs of a range of crops, most notably maize and barley (Seguí-Simarro, 2015). Double haploids are plants those carry two sets of chromosomes that are created from the haploid pollen grain. It is homozygous at every locus with a potential for having a combination of highly variable phenotypes. Murovec and Bohanec (2012) stated that plants in the regenerated population will carry the fixed gene in a recessive or dominant form that helps to select the more suitable one. Ferrie and Caswell (2011) before doubled haploid plant selection, regeneration of haploid cells is required. The adoption of haploidy in maize breeding programs was initiated by Chase in 1952 as cited by (Asif , 2013). DH technology may be considered the third most important methodological achievement for maize breeding, after hybrid technology and off-season nurseries (Seitz 2004).

The potential of haploidy for plant breeding arose in 1964 with the achievement of haploid embryo formation from in vitro culture of *Datura* anthers (Guha and Maheshwari 1964). Doubled haploid (DH) technology is a valuable tool in modern breeding programs since it allows for the production of completely homozygous lines within a few months and dramatically reduces the time required to establish new cultivars (Wedzony *et al.*, 2009). DH has been applied in more than 200 plant species and is widely used in brassicas and cereals, including wheat, barley, rice and maize (Germana, 2011; Dwivedi *et al*., 2015). In addition, many genomic approaches such as association or QTL mapping benefit greatly from the use of DH populations (Alheit *et al.*, 2011).

Of the different methods available for DH production, microspore embryogenesis (androgenesis) shows the greatest potential due to the abundance of microspores per spike and consequently the higher frequency of DH output as compared to other approaches such as wide crosses (Wurschum *et al*., 2012). Factors limiting the application of microspore culture at a commercial level include the rate of embryogenesis and regeneration, the frequency of albinism among regenerates and the frequency of chromosome doubling required to obtain fertile DH plants (Castillo *et al*., 2009).

The most common doubling agent is colchicine which is traditionally applied in vivo (Kasha *et al.,* 2005). The application of doubling agents, however, not only increases the percentage of chromosome doubling, but also affects the whole androgenetic process. The optimum concentration and time of application for chromosome doubling may have negative effects on embryogenesis and regeneration rate, as well as on the percentage of green plants (Castillo *et al.,* 2009). Like colchicine, acenaphthene vapour also causes chromosome doubling, and it has been used to produce tobacco dihaploids by applying during culture (Burun and Emirolu, 2003). Treatment with an antimitotic herbicide trifluralin has been used in tobacco for doubling chromosome during callus and leaf

mefvein culture (Örçen, 2006).

Generally, in addition to accelerating line development, adoption of the DH technology has several quantitative genetic, operational, and economic advantages (Smith *et al.*, 2008; Geiger 2009). So, for facilitating increased genetic gains per cycle, increased efficiency of the breeding program, and reduced developmental costs it is indispensible to understand the idea and influence of DH in crop improvement.

Objective

- To review and understand the idea and influence of double haploid technology in crop improvement
- To inspire breeders with this DH technology and simplify their path to develop crops variety.

2.Literature Review

2.1 Haploid production Methods Double haploid

Begheyn *et al.* (2016) reported that depending on species and protocol availability, as well as efficiency in terms of investments and yields different methods can be used for the production of haploid or DH plants. Of these methods, anther and microspore culture and chromosome elimination techniques (bulbosum technique and maize technique) appear to be the most effective and most popular (Hussain *et al*., 2012).

Dwivedi *et al*. (2015) observed that except its technically more challenging isolated microspore culture (IMC) is preferred over anther culture (AC) because of its higher efficiency and has been routine in barley, tobacco (Nicotiana tabacum L.) and rapeseed (Brassica napus L.) breeding for some time. Response to in vitro DH induction is highly genotype-dependent and colchicine may be needed for chromosome doubling.

Ari *et al*. (2016) carried out research on Comparison of different androgenesis protocols for doubled haploid plant production in ornamental pepper (*Capsicum annuum L*.) using 48 genotype and culturing anther on MS and B5 media and shed-microspore cultured on Nitsch and Nitsch(NN) medium. Accordingly, they reported that the shed-microspore culture protocol was superior to both semisolid anther culture protocols and suggested it could be used effectively for the development of DH lines in ornamental pepper breeding programs.

Tadesse *et al.* (2013) investigated that anther culture and wheat × maize cross are the most commonly used induction methods in wheat. Niroula and Bimb (2009) reported that superiority of wide crosses over other techniques includes higher efficacy (2–3 times more efficient for green plant production than anther culture), simplicity, less genotype dependent response, less gametoclonal variation, and being less time consuming. Niu *et al.* (2014) also reported that wide cross of wheat with maize followed by embryo rescue and chemical chromosome duplication is an efficient method in DH wheat (Triticum aestivum L.) plants production. Bakhshi *et al.* (2017) evaluated the production of doubled haploid wheat lines using various methods of wheat and maize crossing (classic or normal method, detached tiller method and intermediate) and found three lines for heat-tolerant wheat varieties.

Davies and Sidhu (2017) described method of generating oat DHs using oat × maize pollination, followed by dicamba spray, embryo rescue, and colchicine treatment.

An in vivo maternal haploid inducer is typically the standard method in maize because it has been the most successful (Liu *et al.,* 2016). Prasanna *et al.* (2012) described that haploid inducers are specialized genetic stocks which, when crossed to a diploid (normal) maize plant, result in progeny kernels in an ear with segregation for diploid (2n) kernels and certain fraction of haploid (n) kernels due to anomalous fertilization. Haploid inducer lines are routinely used to obtain an average of 10% haploid kernels on the seed parent and treated with colchicine to obtain DHs plants of maize breeding (Geiger and Gordillo, 2009).

Prigge *et al*. (2011) reviewed that a number of haploid inducer lines with high HIR have been derived over the years. Accordingly, they indicated that temperate inducers like UH400 (developed at University of Hohenheim), RWS (Russian inducer), and RWS × UH400 were successfully employed for haploid induction. The same scholar pointed out that CIMMYT Global Maize Program in collaboration with University of Hohenheim, Germany have been developed tropically adapted inducer lines (TAILs; with 8–10% HIR). Dicu and Cristea (2016) utilized inducer such as stock 6, MHI and Procera haploid inducers (PHI) as indicted in table 1 for developing haploid, double haploid and hybrids of maize.

Kelliher *et al.* (2017) found that haploid induction in maize is a post zygotic character attributed to a frame-shift mutation in MATRILINEAL (MTL) gene which is phospholipase specific to the sperm cell cytoplasm. Moreover, they reported that MTL mutation or editing results 6.7% haploid induction rate because of membrane composition or lipid profiles alteration there by delay pollen germination and tube growth. HIR relies not only on haploid inducers but also on maternal genetic background. Accordingly, two QTL on chromosome 1(*qmhir1*) and chromosome 3 (*qmhir2*), which highly affect haploid induction from the maternal side were reported (Wu *et al.,* 2014).

Chen *et al.* (2010) described that ovule culture (gynogenesis) also used mainly in species recalcitrant to androgenesis for example in sugar beet (*Beta vulgaris* L.), onion (*Allium cepa* L.), and some tree species. Similarly, they pointed out that gynogenesis method of haploid induction efficiency is much lower due to the smaller number of ovules available per flower. However, crops like onion consists three carpels per floret and each with two egg

cells which makes ovule culture more attractive.

Table 1 Main characteristics of the initial and PHI inducers

Inducer	planting-flowering days	plant height cm	Haploid inducing rate (%)	source
stock 6	60	158	1.2	
MHI	65	192	7.2	
PHI-1	55	151	12.1	Procera Genetics Ltd Company, Romania
PHI-2	60	198	13.0	"
PHI-3	70	180	14.5	"
PHI-4	65	200	12.8	"

Dicu and Cristea (2016)

2.2 Agents and stage of chromosome doubling

Prasanna *et al.* (2012) described that although colchicine is the most commonly used mitotic inhibitor, there are many other types that can be used and are less toxic, which include herbicides such as pronamid, APM, trifluralin, and oryzalin. These alternatives have likely not become standardized due to conflicting findings. For instance, these herbicides have been shown to be effective chromosome doublers (Hantzschel and Weber, 2010). In gynogenic embryos of onion trifluralin, oryzalin or APM produced higher doubling efficiencies than colchicine reported by Grzebelus and Adamus 2004 (as cited by Castillo *et al.* (2009). Other studies however, show that doubling rates for these herbicides were not significantly different than spontaneous doubling rates (Vanous, 2011). The drug inhibits spindle function during mitosis and disturbs normal polar segregation of sister chromatids to form a restitution nucleus (Tadesse *et al.,* 2013).

Haploid genome doubling rate of trifluralin treatment in *B. napus* was 85.7%, for colchicine 74.1% and for oryzalin 66.5%, compared to only 42.3% spontaneously was reported by KliMa *et al.,* 2008 (as cited by Ren *et al.,* 2017). Melchinger *et al.* (2016) indicated that APM combined in an optimum dosage with pronamide has similar rates of genome doubling as colchicine in maize.

Tadesse *et al*. (2013) pointed that chromosome doubling at 3-5 tiller capping technique or 3-4 leaves stages by the immersion and anther cultured in colchicine containing media is possible. However, colchicine at early stage of plant may result toxicity and also increase frequency of an aneuploids among in vitro chromosome-doubled wheat haploids.

Castillo *et al*. (2009) reported that chromosome doubling agents incorporated in the induction medium of anthers, microspores or callus and in the regeneration medium of embryos at early stages of gametic embryogenesis is better than to plants. Thus, the authors pointed that application to plant/seedling stage require high concentration and solution volume, high rate of mortality, and production of chimeric plants, that can lead to production of low seed set and therefore requiring an additional growth cycle for seed multiplication.

Wurschum *et al*, (2012) observed that the concentration and time of colchicine treatment at the seedling stage affect the rate of double haploid production. Accordingly, the authors found that the highest values range from 56.2% to 66.7% for 1 mM and 5 mM concentrations and incubation times of 48 h and 72 h. Yuan *et al.* (2015) reported that optimal chromosome doubling of the haploids was obtained with a solution of 0.2% colchicine for 9–12h or 0.4%colchicine for 3–9h for cabbage and 0.05%colchicine for 6–12h for broccoli.

2.3 Factors in double haploid production

Kumari (2009) and Seguí-Simarro(2010) stated that different factors such as donor plant growing conditions, stress pre-treatment, medium composition and culture conditions all influence the embryo induction rates, number of regenerated plants and, the ratio between green and albino regenerants in turn able impact success of double haploid breeding .

2.3.1 Effect of culture media and genotype

Al-Ashkar (2013) investigated on media and genotypes effect in anther culture response and salt tolerance using five bread wheat genotype (Line-A, Gemmeiza-7, Gemmeiza-11, Misr-1, and Misr-2) in which line-A crossed with the rest and also used as line and four culture media type (NPB99 with maltose-A_1 and sucrose-A_2, N6 with maltose-A_3 and sucrose A_4 but no PAA as in NPB99) and obtained highly significant difference for genotype, media and their interaction. This scholar reported that the five wheat genotypes were different in their response (callus induction, plant regeneration and green plant regeneration) according to the medium used. For instance, the percentage of anthers that developed calli ranged from 4.67% for the cross (Line-A, Gemmeiza-7) to 9.42% for the cross (Line-A · Misr-1) among the genotypes across the four media compared to the parental Line-A, which gave 7.67%.

Xynias *et al*. (2014) evaluated factors affecting doubled haploid plant production via maize technique in bread wheat and reported that culture media influence both haploid and double haploid production.

Javed *et al.* (2012) suggested the interactions of genotypes, culture media, and culture temperatures play important roles in the activation of recalcitrant genes for callus induction and regeneration.

Microspores are sensitive to fructose from sucrose in media and maltose has been reported to be a superior source of carbohydrate than sucrose for androgenesis in several species, including cereals (Sen *et al*., 2011). Park *et a.* (2013) also conducted correlation analysis of maltose concentration in media and the frequency of albino plants and suggested that the application of the maltose minimizes the frequency of albino plants from anthers.

Previous study indicated that AgNO3 is known to interfere with ethylene action and form complexes which inhibit ethylene responses in anther culture program. Faruq *et al.* (2014) also reported that the addition of the same compound to the medium improves callus induction and plant regeneration in indica rice.

Mishra *et al.* (2015) evaluated callus induction, callus regeneration, green plant regeneration and albino plant regeneration frequencies of four hybrids rice namely CRHR5, CRHR7, JKRH401 and JKRH405 using three media with different nutrition composition namely N6, MO19 and SK-1 at India. Accordingly, reported that CRHR5 and CRHR7 had responded well on N6, JKRH405 responded well on MO19 while JKRH401 responded well on SK-1 and indicated the effect of genotype and media on developing double haploid.

2.3.2 Male and female flower development stage

Depending species the optimum microspore stage that can reprogramme the microspores from gametophytic to sporophytic pathway appears to differ. Asif (2013) stated that efficiency of microspore culture is dependent on plant age and pollen stage at which the floral organs are collected from donor plants for microspore isolation. Similarly, pointed that greater androgenic response has been noticed if microspore isolation is done with the floral organs that emerge first than those appearing in the later life cycle of donor plants.

Mishra *et al.* (2016) reported the difficulty of culturing the older stages of pollen due to problem of differentiation into a male gametophyte. Besides, they described that the response of anthers at the tetrad stage is not good at all, and it falls sharply after the first pollen mitosis. At this stage, starch deposition begins, but no sporophytic development and subsequently no macroscopic structures form in the microspores and need cold pre-treatment. Kaushal *et al*. (2014) observed that cold pre-treatment at 12 ºC for 5 d give the best performance for callus induction and plant regeneration in 13 indica rice genotypes.

Xynias *et al*. (2014) also reported that embryo production from bread wheat x maize hybridization was highest from embryos rescued at 12–14 day after pollination.

Asif (2013) indicated that female gametes or flower buds for gynogenesis should be collected before anthesis (pollen shedding). However, the collection can be made at any time in case of a male sterile or self-incompatible species. Sato *et al*. (2002) reported that bicellular microspore stage with two equal nuclei is one of the most crucial phase to induce embryogenesis in *B . rapa*.

Fan *et al*.(1988) pointed out that DAPI and Acetocarmine stains for observation under an optical microscope have been extensively employed in tissue culture studies to identify the accurate microspore stage prior to their collection or before using for isolation to induce embryogenesis. Lantos *et al.* (2016) used an Olympus CK-2 inverted microscope to check the microspore developmental stages and collected donor tillers when the microspores were at early- and mid-uninucleate stages.

2.3.3 Growth conditions of donor plants

Davies and Sidhu (2017) developed double haploid of oat using wide crossing (oat x maize) and indicated that the growth condition (fertilizer, growth regulator, temperature, container and light intensity) of donor plant (both oat and maize) was important for the success of double haploid production.

Asif (2013) pointed out that effectiveness of methods in double haploid production enhanced to a greater extent as the donor plant is free of insects, pests, diseases, absence of nutrient and water deficiencies and environmental stresses like temperature, humidity, and photoperiod. Moreover, stated that donor plants that are planted under controlled conditions (green house, glass house or control chambers) provide better embryogenic response than plants grown under field conditions.

2.4 Detection of haploid and double haploid

Haploids can be distinguished from diploids based on phenotypic markers or differences in plant characteristics at the adult stage (Xu *et al*., 2013; Wu *et al*., 2014). Melchinger *et al.* (2016) indicated that identification of haploids at the seed or early seedling stage is better for large scale production of DH lines, as chromosomal doubling with mitotic inhibitors applied at early seedling stage.

The R1-nj (Navajo) anthocyanin color marker is widely used for haploid identification and all currently used haploid inducers have R1-nj gene (Melchinger *et al*., 2015). Thus, both endosperm and embryo of diploid seeds display this pigmentation while haploid seeds show pigmentation only in the endosperm, with the embryo remaining white (Giselly *et al*., 2013). Vanous *et al.* (2017) used another anthocyanin marker observed as red/purple coloration of roots, Pl1 (Purple1) as haploid showed colorless or white roots, whereas diploids display red/purple roots. However, depending on the genetic background of source genotype, the genetic background of the haploid inducer and environmental factors the expression of the R1 - nj color marker can vary significantly

(Kebede *et al*., 2011; Prigge *et al*., 2011). Chaikam *et al*. (2015) also reported that dominant color inhibitor genes (C1-I) complete inhibition to R1-nj was shown as frequently occurring (~30%). Thus, it is important to associate the marker with other methods, such as morphological (seed length, width and thickness in mm) and molecular markers (Giselly *et al.*, 2013).

Liu *et al*. (2016) reported that presence of other methods like oil content (nuclear magnetic resonance-NMR), near-infrared spectroscopy, and seed weight to identify haploid and diploid of mature kernels and suggested that the oil content is the most reliable and accurate as stored in embryo, haploid kernels will contain less oil as compared to diploid kernels. Wang *et al.* (2016) reported that oil content method utilized by pollinating donor genotype with high oil inducer lines.

Mishra *et al*. (2015) characterized double haploid of rice hybrids via ploidy analysis (chromosome counts to determine ploidy level), morpho-agronomic, Physico-chemical (hulling, milling, grain length and width, amylose content) was conducted. Accordingly, they stated that haploid plants were fully sterile and pollen grains small in size, with short stature and small glumes, while doubled haploids were fully fertile, medium the size of pollen and fully filled pollen grains were viewed under the microscope. Yuan *et al.* (2015) also indicated that cabbage and broccoli haploid plant growth potential is weaker and smaller size, flower bud smaller, flatter without pollen and stamens are missing, or the stamen development is not normal.

2.5 Impact of double haploid

2.5.1 Breeding cycle

Four major steps: (1) production of haploids by in vivo maternal haploid induction, (2) selection of haploid kernels or seedlings, (3) genome doubling by application of a doubling agent to haploid seedlings, and (4) self-fertilization of successfully doubled haploids (Vanous *et al*. 2017).

Maximum genetic variance in line per se and testcross trials, high reproducibility of early-selection results, high efficiency in stacking targeted gene arrangements and simplified logistics are some of advantages of using DH lines in hybrid that reported by Rober *et al*, 2005 (as cited by Dicu and Cristea, 2016).

Rahman and Jimenez (2016) reported that in classical plant breeding program eight inbreeding (selfing) generation is required to get an almost complete homozygous plant (99.2%) for triat of interest while double haploid result perfect homozygosity (100%) of all traits in only one generation. The double haploid lines not only reduce the breeding cycle, but also increase the selection efficiency, and reduce the activity to maintain the breeding lines through selfing and selection (Rober *et al*., 2005).

DHs have been released directly as cultivars in barley, wheat, rapeseed, rice (Oryza sativa L.), and other crops, or used as parents of F1 hybrids of vegetables and maize, in order to benefit from hybrid vigour (heterosis) (Birchler *et al.*, 2010; Niu *et al.*, 2014; Mishra *et al.,* 2016).

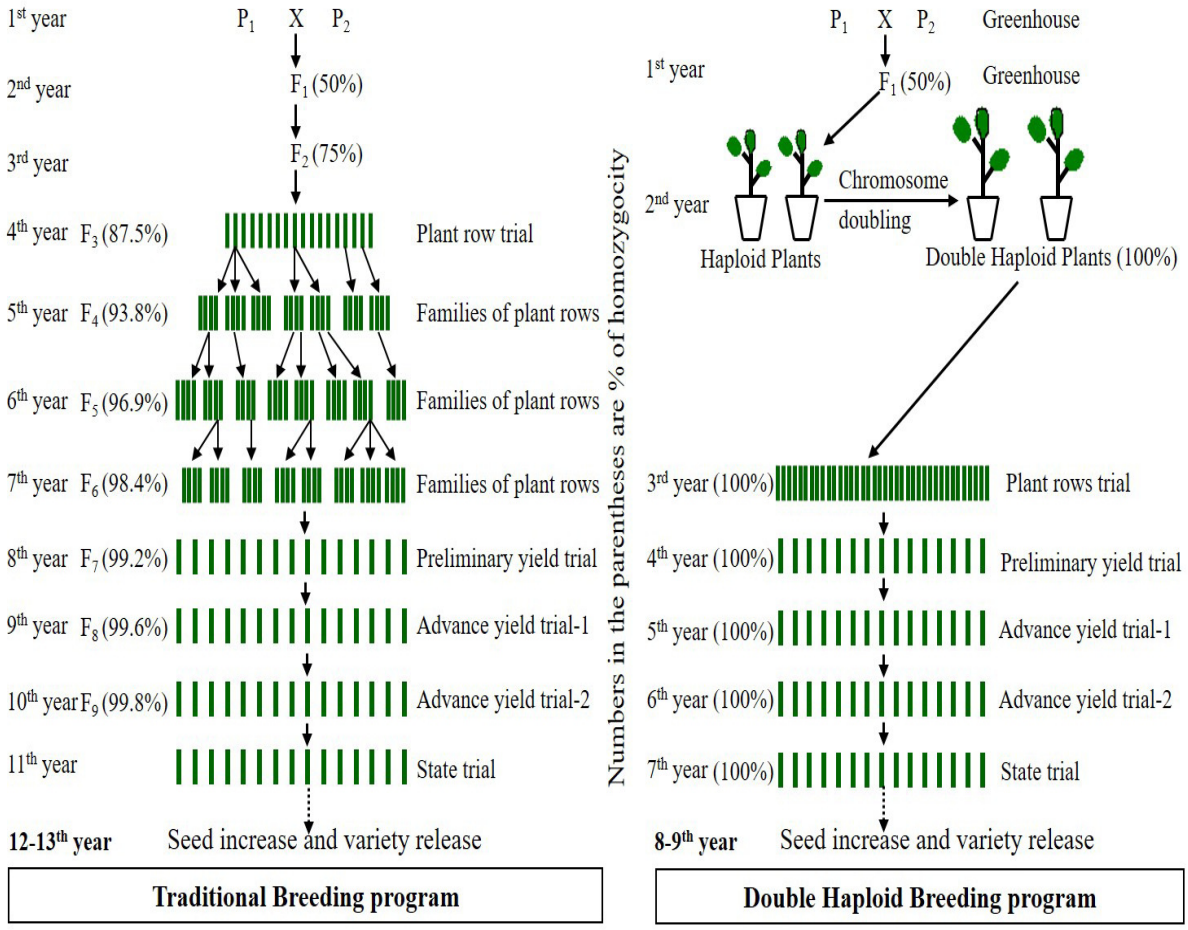
Prigge (2012) described that completely homozygous maize DH lines can be produced in only two cropping seasons that is taken for haploid inducing and chromosome doubling treatment as opposed to 6–10 seasons using the traditional method of recurrent self-pollination. Moreover, they reported that lines able to display complete genetic variance at the beginning of the selection procedure, thus simplifying selection of superior genotypes; higher genetic variance results in higher heritability of lines per se and testcross evaluations, improving testing precision and no residual heterozygosity.

Grain quality has always been an important consideration in rice variety selection and development (Babu *et al*, 2013). Xa and Lang (2011) suggested that anther culture technique offers great opportunity for accelerating breeding progress and improving grain quality characters. Siddique (2015) reported that DH lines of rice are more viable and more than 100 rice breeding lines or varieties and several anther derived lines have been developed in China and India, Japan, South Korea, Hungary and USA, respectively. For saline areas of Bangladesh DH lines from the crosses involving salt-tolerant rice lines developed and identified as DH line AC-1 at International Rice Research Institute (IRRI) (Thomson *et al*., 2010). Purwoko *et a.* (2010) reported that the use of anther culture in upland rice breeding program by producing DH lines tolerant to aluminum stress, shade and blast resistance.

Melchinger *et al.* (2017) observed that the importance of DH technology in creating immortalized genetic units from genetic resources to make them amenable to crop improvement. The scholars reported that thousands of landraces are stored in seed banks as “gold reserve” for future use in plant breeding but they represent heterogeneous populations and their utilization is hampered. Finally, they indicated that libraries of DH lines will make genetic resources accessible to crop improvement by linking molecular inventories of seed banks with meaningful phenotypes.

Barkley and Chumley (2012) stated that double haploid technology greatly accelerated time to market for new wheat varieties and faster genetic gains in wheat yield thereby reducing up to five years. Thus, reduction in varietal development time has significant economic impacts on the wheat breeding program, by costs saving in reduced development time and increased revenues resulting from the earlier release of a new, higher-yielding variety.

Figure 1: *A comparison of a standard time for a cultivar release using traditional and double haploid breeding methods*



The numbers within the bracket is the percentage of homozygosity (Rahman et al., 2016).

2.5.2 Genetic study and molecular marker development

Rahman and Jimenez (2016) reviewed that traditionally bi-parental crosses segregate down generation while double haploid technology could fix the genotypic recombinations for the homozygosity of many loci in a single generation. Thus, double haploid lines are used with high efficiency for genetic study of quantitative traits using a smaller population. Zhang *et al.* (2014) pointed that out-crossing crop plants like bulb onion, the complications of heterozygosity can be solved by using homozygous double haploids. Double haploid (DH) lines have been developed in bulb onion and have proved useful for various genetic and genomic studies (Duangjit *et al*., 2013). Khosa *et al.* (2016) developed a transcriptome dataset using double haploid CUDH2107 as reference line from Cornell University (US) to provide more genomic resources for the Allium research community via understanding the genetic and molecular basis of various traits. Accordingly, they found genes encoding for the persistant tapetal cell1 (PTC1) and male meiocytedeath1 (MMD1) protein regulating tapetal development and pollen formation and responsible for defect at meosis II .

Mishra *et al*. (2016) reported that as QTLs effects are small and highly influenced by the environmental factors, accurate phenotyping with replicated trials required and this is possible via doubled haploids because of their true breeding nature and convenience of producing large numbers. Xu *et al*. (2011) stated that QTLs linked to sheath blight resistance were detected by using DH populations of Maybelle, an American japonica rice variety, and Baiyeqiu, a Chinese indica rice landrace. Park *et al.* (2014) found that two QTLs affecting yield and yield components by using a DH population of Cheongcheong (indica) and Nagdong (japonica). The same DH population used to map four QTLs related to amylose content, two QTLs related to protein content and two QTLs associated with lipid content for rice quality analysis (Lee *et al*, 2014).

King and Baten (2015) reported that among brassica mapping populations currently listed on the website 70% are DH population while 30% are other population. DH population preferred due to immortality (maintained over many years by selfing without genetic loss) and has very simple pattern of segregation (1:1), which is of great value if the localization of QTLs for some complex traits is considered (Cegielska-Taras *et al.,* 2015).

Rahman and Jimenez (2016) indicated that double haploid has major advantage in using a dominant

marker(RAPD or AFLP) to select 100% homozygous dominant plants from segregating population, where in the F2, the dominant trait segregates as one-third homozygous dominant and two-third heterozygous dominant in the population. Thus, they stated that the success rate of using a dominant marker for selecting homozygous dominant plants in the F_2 populations only 33 %.

Ren *et al.* (2017) pointed out that combination of marker assisted selection and DHs offers new opportunities for increasing genetic gain and shortens the time required to cultivar breeding and have been successfully used to accelerate resistance breeding in cereal crops. Marker assisted selection (MAS) was used for the selection of resistance genes, and DH technology was used for the generation of homozygous lines. Wessels and Botes (2014) developed a series of DH wheat lines containing rust resistance genes.

2.5.3 Mutagenesis and transformation

Ferrie and Mollers (2011) described that conventional mutagenesis has been used for many years to generate variation, but it is time consuming in regards to detection and evaluation of the mutants as well as risk of production of chimeras or the loss of a desired trait. However, haploid microspores are an ideal system for conducting mutagenesis studies as millions of microspores can be isolated and cultured in a small space and any DNA change made at the haploid single cell stage will be homozygous in the doubled haploid plant facilitating identification of recessive and dominant mutants. Wu *et al.* (2012) also reported that DH systems are important for mutant selection, due to the ease of selection and fixation of mutations and the desired recombinants, especially when quantitative traits are concerned.

Combining mutagenesis and transformation studies with DH techniques significantly reduces the time and costs required to obtain modified homozygotes for genotype-phenotype validation, as well as generate and fixate genetic variation (Shen, 2015).

Microspores of tobacco, rapeseed, wheat, and barley are exploited in transformation and mutagenesis programs, in order to fix mutations and transgenes in a single step through subsequent DH induction (Brew-Appiah *et al*., 2013; Shen *et al*., 2015). Markedly smaller populations are required to obtain genotypes with multiple homozygous transgene inserts or mutations when DH induction is used instead of self-fertilization (Lubberstedt and Frei, 2012).

2.6 Progress of double haploid technology

Now a day a number of multinational companies use this technology widely in obtaining doubled-haploid lines for market introduction of the new maize hybrids improved in terms of their main characters via tackling the current challenge of climate changes causing the expansion of desertification phenomenon and aggravating the draught in soil and the atmospheric heat (Dicu and Cristea, 2016). Chen and Li (2009) also reported that DH technology is well known to be an efficient and reliable means of advancing maize breeding programs and highly utilized worldwide.

Doubled haploid breeding through anther culture has emerged as an exciting and powerful tool, and a convenient alternative to conventional techniques for crop improvement (Purwoko *et al*, 2010). Mishra *et al.* (2016) reported that presently more than 250 plant species and hybrids have been regenerated using anther culture technique, which include major cereals such as rice, wheat, maize, barley and a range of economically important trees, fruit crops and medicinal plants.

Germana, (2011) pointed that recent increasing number of reports on gametic embryogenesis and haploid and DH production is evidence of the great interest in this useful breeding tool and fascinating research field. Studies on the molecular basis of microspore embryogenesis have profited from the development of advanced genomic, transcriptomic, proteomic and these tools will likely (and hope- fully) result in the identification of many interesting genes involved in microspore reprogramming and embryogenesis in the near future

The review by Liu *et al*. (2016) provides an extensive list of uses for DH technology, which include: rapid development of inbred lines, marker-assisted backcrossing, gene stacking, population improvement, exploitation of genetic resources, among many others.

Khakwani *et al*. (2015) pointed that CIMMYT has provided a comprehensive protocol to develop maize DH-lines and following this will surely report several doubled haploid lines as first DH line AH1 reported in Pakistan. Currently, haploid induction technique efficiently combined with several other plant biotechnological techniques, enabling several novel breeding achievements, such as hybrid breeding, improved mutation breeding, reverse breeding, and genetic transformation.

Bumagat, (2014) indicated that DH facility for Africa has been established by the International Maize and Wheat Improvement Center (CIMMYT) in partnership with the Kenya Agricultural Research Institute (KARI) on a 20 hectare land in KARI-Kiboko station. Besides, the facility established with financial support from Bill and Melinda Gates foundation and aimed to produce at least 100,000 DH maize lines per year by 2016, thus, strengthening maize breeding programs in Africa and improving breeding efficiency.

3. Conclusion

As to conclusion the DH technology plays an important role in the field of plant breeding, genetics and genetic engineering. The ability to shorten the breeding cycles and production of complete homozygous plants makes the technology best for cultivar development, back crossing, genome mapping, QTL mapping, gene identification, gene discovery and transgenic plant development. The technology not only offers an opportunity to speed up traditional breeding methods, but allows greater flexibility in that it can be applied at any generation, allowing rapid response to changing market demands. It also provides great opportunities in improving the grain quality and its nutritional value to overcome the malnutrition problem.

Various studies indicated that deployment of double haploid in breeding programs must be practical, cost efficient, satisfy breeding objective, and produce marketable cultivars. The technology has downside such as reduction of genetic variation which may be better preserved in heterozygous lines and challenge to develop more genotype independent methods.

Though most of multinational companies use this technology widely, no any significant works and literatures found on double haploid production in Ethiopia rather through informal communication with Ambo highland maize breeding program information obtained that the program currently sending limited number of materials to CIMMYT for double haploid line production.

Generally, understanding DH technology has important contribution in accelerating breeding program for immediate reaction towards out breaking biotic and abiotic constraints.

4.Prospects

It is unquestionable that DH technology together with other biotechnological tools like MAS plays an important role in breeding program. It is indicated that haploid induction for DH production will be conducted through different methods, but to be efficient it is better to develop genotype independent protocol for various economically important crops. In in vivo maize haploid induction it is not clear that whether due to single fertilization or chromosome elimination and better understanding of it will improve haploid induction system. Previous research found different chemical for chromosome doubling and they are on use today, but it is also better to study for non-toxic, less cost and effective doubling agents.

Crops like coffee, fruit crops and tree take longer time to generate lines, develop variety and release to farming community and because of this it is better to adopt DH technology. In developing country like, Ethiopia where the biotechnology is at its young stage better to exploit the experience of developed country and use their effective protocol for production of DH lines.

Generally, team spirit is very crucial as different principles are present in different institute. Accordingly, professionals at research institute as well higher education those who have experience and skill breeding and biotechnology should have to work closely to be competitive in the world market via using cutting age technologies.

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