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Haploids and Doubled Haploid Technology Application in Modern Plant Breeding

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Abstract: Plant breeding, genetics, and genetic engineering all benefit from the use of doubled haploids technology. For genetic mapping of complicated phenotypes, the doubled haploid technique is a useful tool. To make doubled haploids, haploid cells (which are genetically unstable in the first place) can duplicate their genome at any point during their growth, resulting in diploid cells that don't require any additional therapies. The use of doubled haploidy in breeding is influenced by a variety of circumstances. Doubled haploids (DHs) are exploited in a variety of ways, depending on available technologies and species. In horticultural crops that are perennial in nature, outcrossing with inbreeding depression, or have high economic value where breeding time is critical, doubled haploids are gaining popularity as a powerful approach for enhancing genetic gain per cycle. For various reasons, the current methods for producing haploids and doubled haploids are primarily focused on the rapid generation of pure lines to speed hybrid seed production or CMS conversion, as well as the production of di-haploids to simplify breeding operations, such as in the case of potatoes. These approaches involve using methods based primarily on in vitro culture, in vivo induction of haploid development, or a combination of the two to make haploid embryos in vivo and then rescue them in vitro. Generally the aim of this review paper is to assess the technology of haploids, double haploids and their role in modern crop improvement program.

Keywords: Haploids, Double Haploids, Plant Breeding, Crop, Genetics

1. Introduction

When haploid cells undergo chromosome doubling, a genotype known as a doubled haploid (DH) is generated. Plant breeding relies heavily on the artificial creation of doubled haploids [1]. For self-pollinating species like wheat, barley, and rapeseed, as well as cross-pollinating species like maize, the DH technology has been widely used in breeding projects around the world. In a single generation, these techniques allow entirely homozygous lines to be generated from heterozygous parents. Advanced homozygous lines are the end products of self-pollinating species breeding programs, whereas the main goal of open pollinating species breeding programs is to increase testcross performance and select superior hybrids [2].

In a number of basic research investigations as well as practical research, doubled haploids (DH) have proven to be a beneficial tool [3]. The output of meiosis is haploid gametes, which have half of a complete set of chromosomes (2n) in a cell with a full set of chromosomes (2n) (n). Haploid cells are formed from pollen, egg cells, or other gametophyte cells, and a doubled haploid cell is created through induced or spontaneous chromosomal doubling, which can then be developed into a doubled haploid plant. The haploid cells are monoploid if the initial plant was diploid, and the doubled haploids may be referred to as doubled monoploids. Dihaploid organisms developed from tetraploids or hexaploids are frequently referred to as tetraploids or hexaploids (the doubled dihaploids are tetraploid or hexaploid, respectively) [4].

In perennial crops with short mating cycles, doubled haploidy is a potent tool for generating genetic improvement that leads to economic gains [5]. Traditional inbreeding takes six generations to achieve approximately 100 percent homozygozity, whereas doubled haploidy takes only one. Plants derived from tetraploid crop plants could be valuable in breeding initiatives involving diploid crop cousins [6].

Individuals with DHs have a diploid genome derived from

a haploid set of chromosomes that has been duplicated, resulting in all of their loci having the identical alleles. They are 100% homozygous individuals and are an important tool for basic and applied research, as well as breeding programs, where they are frequently employed as pure lines for hybrid seed production [3]. In plant breeding, the ability to develop homozygous and homogenous lines is a critical time limitation. Homozygous and homogeneous lines can be created in two generations rather than five or more utilizing doubled haploids (DHs) [7]. Other benefits include lower cultivar production costs, more precise phenotypic trait evaluation, effective deletion of unwanted genes, and trait fixing in haploids via marker-assisted selection, resulting in more efficient use of molecular markers and trait combinations.

The employment of DH technology in the breeding of the primary self-pollinated grain species wheat and barley, as well as maize and canola, has become standard because the success of breeding programs is determined by genetic gain per unit time [8]. Homozygosity is accomplished in one generation using double haploid production techniques, removing the need for multiple generations of self-pollination. Particularly in biennial crops and crops with a long juvenile stage, the time savings are significant. Haploidy may be the only means to develop inbred lines in self-incompatible species, dioecious species, and species that suffer from inbreeding depression due to self-pollination [9].

The role of DH in the breeding process is largely determined by the mechanism of reproduction used by the plant. They might be final cultivars in self-pollinated species, or they can be utilized as parental lines in hybrid development or test-crosses in cross-pollinated species [10]. In self-pollinated species, the fundamental breeding system begins with crosses of desired genotypes, resulting in hybrids having both parents' chromosome sets. Recombinations permit new gene combinations during gamete development, which are fixed throughout the process of doubled haploid production.

As a result, doubled haploids are completely homozygous recombinant products of parental genomes. They can be propagated as true breeding lines, allowing for large-scale agronomic performance testing throughout time. Because recessive alleles are fixed in one generation and directly expressed, full homozygozity increases the effectiveness of selection for both qualitative and quantitative traits [11]. Understanding DH technology has a significant impact on speeding up breeding programs in order to respond quickly to biotic and abiotic restrictions [12]. Generally the aim of this review paper is to assess the technology of haploids, double haploids and their role in modern crop improvement program.

2. Literature Review

2.1. History of Double Haploids

Since the discovery of haploids in many species, the efficiency of selection for both qualitative and quantitative traits has enhanced due to perfect homozygozity. Blakeslee et al. provided the first report of the haploid plant in Datura stramonium in 1992 [11]. Guha and Maheshwari (1964) later devised an anther culture procedure for the laboratory production of haploids. Wide crossing has been used to produce haploids in barley (Kasha and Kao, 1970) and tobacco (Burk et al., 1979) [13]. The most sensitive species for doubled haploid production are tobacco, rapeseed, and barley. Over 250 species have now been studied using doubled haploid techniques [14].

Since Generating doubled haploids, where the haploid embryo is rescued cultured and the chromosome is doubled through the use of colchicine or other mitotic inhibitors, such as nitrous oxide gas and some herbicides, the efficiency of selection for both qualitative and quantitative characters has increased due to complete homozygozity [15]. The haploid plant's chromosome counts are doubled in small sectors when a mitotic inhibitor is applied to the apical meristem. Seeds are normally produced by the duplicated sections. These seeds are pure line cultivars with doubled haploids. Androgenesis is the most favoured approach for doubling haploid generation among the aforementioned ways [16].

2.2. Production of Double Haploids

In vivo or in vitro, doubled haploids can be created. Parthenogenesis, pseudogamy, or chromosomal removal after broad crossing produce haploid embryos in vivo [17]. The haploid embryo is saved, cultivated, and chromosome-doubling results in haploids with doubled chromosomes. Gynogenesis (ovary and flower culture) and androgenesis are two in vitro approaches (anther and microspore culture). The recommended procedure is androgenesis. Wide crossing is another means of making haploids. Wide crossing with related species can create haploids in barley and tobacco. Interspecific pollination is a feasible method for obtaining seed-derived haploids of N. tabacum that can be used as a replacement or complement to anther culture [14]. For many crop species, the creation of doubled haploids (DH) has become a vital tool in sophisticated plant breeding institutes and commercial firms. However, as a result of the development of new, more efficient, and less expensive large-scale production methods, doubled haploids are now being used in less advanced breeding programs [18].

Anther culture is the most widely used method for generating DHs. It is technically straightforward, requiring only the following steps: (1) flower bud collection, (2) anther isolation from flower buds, (3) inoculation and in vitro culture in an agar-based culture medium, (4) embryo isolation, (5) plant regeneration, and (5) regenerant analysis [3]. Microspore embryogenesis can also be produced utilizing anther-derived microspore. Because a phase of microspore isolation and inoculation into liquid medium must be included in the process, it is more complicated than anther culture. Furthermore, due to the lack of anther tissues, effective microspore growth and development is solely dependent on medium composition. Thus, in order to establish an effective microspore growth procedure, medium

composition must include all of the ingredients required by microspores and be tailored to the unique characteristics of each species' microspores [19]. DHs will be acquired from all diploid regenerants. With all of these benefits in mind, isolated microspore cultivation is the method of choice in materials with well-established methods [3].

2.3. Uses of Double Haploids in Plant Breeding

In advanced breeding operations of various agricultural species, induction and regeneration of haploids followed by spontaneous or induced chromosomal doubling are extensively employed approaches [10]. The role of DH in the breeding process is largely determined by the mechanism of reproduction used by the plant. More than 290 variations have previously been issued (http://www.scri.ac.uk/assoc/COST851/Default.htm). They have been effectively employed for commercial cultivar production of species such as asparagus, barley, Brassica juncea, eggplant, melon, pepper, rapeseed, rice, tobacco, triticale, and wheat. In comparison to traditional approaches, which only achieve partial homozygozity, DH technology allows totally homozygous plants to be grown in one generation, saving several generations of selfing [14].

2.3.1. Mapping Quantitative Trait Loci

Genes with minor but cumulative effects control quantitative features. DH has been used to find loci that control quantitative traits [20]. It is because of their true breeding tendency and the ease with which enormous numbers can be created. Genes control the majority of economic features, with minor but cumulative effects. Although the use of DH populations in quantitative genetics has long been recognized, it was the introduction of molecular marker maps that spurred their use in discovering loci that affect quantitative traits [21]. Because the effects of quantitative trait loci (QTLs) are minor and impacted heavily by environmental factors, accurate phenotyping with repeatable trials is required. Because of their actual breeding nature and the ease with which they may be created in vast numbers, this is conceivable with doubled haploidy organisms. 130 quantitative traits in nine crop species have been mapped using DH populations. For QTL detection, a total of 56 DH populations were employed [22].

2.3.2. Backcross Breeding

Genes from a donor cultivar or similar species are introgressed into a recipient elite line in backcross conversion [23]. The introduction of molecular markers has made selection based on genotype (marker) rather than phenotype easier. Conventional approaches become more effective when paired with twofold haploidy selection. A recipient parent is crossed with a donor line and the hybrid (F1) is backcrossed to the recipient in marker assisted backcross conversion [24]. Backcrossing the resulting generation (BC1) till the required genotypes are obtained is repeated until the desired genotypes are obtained. The combination of twofold haploidy and a molecular marker reduces the time it takes for homozygous lines to emerge [14].

2.3.3. Bulked Segregant Analysis (BSA)

In marker aided breeding, bulked segregant analysis is a prominent technique. The genotypes at the two extreme ends produce two bulks once the population is screened for a trait of interest. The presence or absence of molecular markers is checked [25]. In a double haploid population, the genotypes at the two extreme ends form two bulks, allowing for precise and repeatable testing. In bulked segregant analysis, double haploid populations are widely used. In rapeseed and barley, this approach has been used [26].

2.3.4. Genetic Maps

For species where double haploids are widely available, double haploid populations have become standard resources in genetic mapping. For genetic mapping, doubled haploid populations are optimal [27]. Genetic maps are critical for deducing evolution patterns and syntenic links across species by understanding the structure and organization of genomes. It is used to explore genotype/phenotype relationships by providing a framework for mapping of genes of interest and assessing the size of their effects. In every type of species, a genetic map can be produced in double haploid populations within two years of the initial cross [28]. The development of molecular marker maps in eight crop species was greatly aided by double haploid populations [21].

2.3.5. Genetic Studies

Haplotype populations can be used to calculate genetic ratios and mutation rates. A tiny doubled haploid (DH) population was used to show that chromosome 5H contains a dwarfing gene in barley. Another study looked at the segregation of a variety of markers in barley [29].

2.3.6. Genomics

Despite the fact that quantitative trait loci research has yielded a wealth of information on gene locations and magnitudes of effects on a variety of traits, identifying the genes involved has remained a challenge. Because of the low resolution of quantitative trait loci analysis [14], this is the case. The establishment of recombinant chromosomal substitution lines or stepwise aligned recombinant inbred lines [30] would be a solution to this challenge. Backcrossing is utilized to achieve the required level of recombination, and genetic markers are employed to detect desired recombinant chromosomal substitution lines in the target region, which can be fixed by doubling haploidy. In a map created from a doubled haploid population, molecular markers were shown to be connected to significant genes and quantitative trait loci for resistance to rice blast, bacterial blight, and sheath blight.

2.3.7. Elite Crossing

Traditional breeding procedures are sluggish, taking 10-15 years to establish a cultivar. Another issue is the inefficiency of early generation selection due to heterozygozity. Doubled haploids can overcome these two drawbacks, allowing more elite crossings to be examined and selected in less time [14].

2.3.8. Cultivar Development

Most species demand uniformity in their cultivated lines, which may be easily achieved through twofold haploid production [31]. Doubled haploids can be employed in cultivar creation in a variety of ways. The doubled haploid lines can be published as cultivars, employed as parents in hybrid cultivar development, or used more indirectly to create breeder lines and conserve germplasm [7]. There are over 100 direct DH cultivars of barley [32]. There are already roughly 300 doubled haploid derived cultivars in 12 species worldwide, according to published literature. Because of the creation of procedures for 25 species, the importance of DHs in plant breeding has risen dramatically in recent years [33]. The potential for ornamental production is being aggressively investigated [14]. Doubled haploidy already plays a key role in hybrid cultivar production of vegetables.

2.4. Advantages of Doubled Haploid Breeding

Plant breeders save a lot of time by being able to develop homozygous lines after just one cycle of recombination. Random DHs have been found to be comparable to selected lines in pedigree inbreeding [1]. Other benefits include the rapid generation of a large number of homozygous lines, fast genetic analysis, and the rapid development of markers for relevant features [34]. In ornamentals, seed propagation as an alternative to vegetative multiplication is possible, and in species such as trees, where long life cycles and inbreeding depression limit standard breeding strategies, twofold haploidy presents new options [14].

2.5. Challenges in Doubled Haploid Breeding

The DH population's fundamental disadvantage is that selection cannot be enforced on the population [35]. However, in traditional breeding, selection can be carried out for numerous generations, improving desirable characteristics in the population. Some plants are aneuploids and others are mixed haploid-diploid types in haploids grown from anther culture [36]. The cost of developing tissue culture and growing facilities is another disadvantage linked with double haploidy. Overuse of doubled haploidy in breeding material may diminish genetic variation. As a result, before using twofold haploidy in breeding programs, various considerations must be considered [37].

3. Conclusions

Most plant genera currently have double haploids protocols thanks to technological advancements. In just a few decades, the number of species capable of doubling haploidy has risen to 250. With the gradual removal of species from the recalcitrant category, response efficiency has improved as well. As a result, plant breeding will be more efficient. In plant breeding operations, double haploids have had a substantial impact on reducing time, labor, and expense. In order to conserve rare alleles that may become relevant in future breeding efforts, breeders must maintain a fair amount of variety. Doubled haploidy is a very efficient strategy for producing totally homozygous lines from heterozygous donor plants in a single stage, and it will continue to be so.

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