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Anther Culturing a Unique Methodology in Achieving Homozygosity

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Authors' contributions

This work was carried out in collaboration between both authors. Author JI designed the study, explain the complete methodology of double haploid production by anther culture technique. Author UY explain the introduction and uses of double haploid in genomics and breeding. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Achieving homozygosity and uniformity has been the goal of breeders for a long time and the task was really time to take and laborious. After performing the selection for 6-7 year breeders were not even confirm about the homozygosity of that genotype. The Double Haploids have made this tedious work easier and more efficient to a great extent. At present, anther culturing is a ground-breaking methodology for plant breeding. Haploids are plants with a gametophytic chromosome number and Double haploid DHs will be haploids that have experienced chromosome duplication. The development of haploids and DHs through gametic embryogenesis permits a solitary advance improvement of homozygous lines from heterozygous guardians, shortening the time required to create homozygous plants in correlation with the current strategies such as quantitative trait loci(QTL) and marker-assisted background selection(MABS) that take lesser time (almost one year) than conventional methods. The anthers of plants are taken and treated with cold temperature. Further, the explant is sterilized with disinfectants such as 70% Ethanol, 20% chlorox and 0.1% $HgCl_2$, dissected in laminar flow and placed on the media. At a specific interval of four-week culture was transferred on the regeneration, shooting and rooting media, and then chromosomes are counted for confirmation. Chromosomes are doubled with colchicine treatment.

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There are a few accessible techniques to acquire haploids such as Pollen culture, Ovary culture and Wide hybridization of which in vitro anther culture is the best and widely utilized. Final, DHs plays important role in product enhancement through genome mapping, quantitative locus investigation, hereditary manipulation and focuses for genome change programs. This review article deals with the complete methodology of double haploid production by anther culture, its effectiveness over other techniques and its uses in breeding programs.

Keywords: Anther culture; haploids; DHs; modern breeding; homozygosity.

1. INTRODUCTION

The varietal development is very tedious and time taking process through old conventional techniques because it includes the steps like screening, development of lines, evaluation, recognition, and crossing for getting highly homozygous lines [1]. Infinite capitals of water, land, and fertilizers, it is very difficult to cope with the major challenges faced by the main food crops e.g. maize, wheat, and rice in limited time. Enhanced production goals can be acquired through modern biotechnological tools and statistical techniques coupled with innovative breeding strategies [2]. The breeding can be accelerated by the use of double haploid production, particularly in corn and barley [3]. DH production has been used in tobacco, barley, canola, wheat, rice and maize [4]. DHs production is referred to as third crucial revolutionary methodology former being a hybrid seed technology and off-season nurseries [1]. The success rate of DHs varies crop to crop and its success depends on the efficiency of the methods used for the production of the haploids.

The plants having a half number of chromosomes in their cells are called haploid plants. Haploids occur naturally in the population but their probability to occur is very less. Naturally, occurring haploid was first found in *Datura stramonium* L. [5]. In maize, first time haploidy was initiated by Chase in 1952 [6]. They can also be produced artificially through methods such as wide hybridization, chromosome elimination, and pollination with mutated pollen (in vivo). This can also be induced through immature gametophyte culturing [7]. Haploids can be subjected to agents such as colchicine and its chromosome number is doubled and it is called doubled haploid (2n=2x). Colchicine is a chemical that is widely used in the transformation of haploids into doubled haploids through chromosomal doubling in vivo and some chemicals such as acenaphthene have also been used for the same purpose in tobacco [1]. The timing and quantity of application of these chemicals have direct consequences on the rate of regeneration and percentage of plantlets [8].

Development of plants from the pollen grains (androgenesis) is indicating the totipotency of the plant and is a very successful process. It is acclimatization that is present in the plants and only expresses in the climatic severities [7]. Embryogenesis from unfertilized gametes (female parts) such as ovaries (Gynogenesis) is not very popular as it is a least efficient method but it can be used in the crops where other techniques are not working well [9]. The regeneration of viable plants through pollens has been found in more than 200 species belonging to different families [7]. A lot of modern genetic techniques such as quantitative trait loci and gene association have gained an advantage from the doubled haploids [10]. Anther culturing is preferred for double haploid production because it is an easy and simple method and it allows the experiment to be conducted on a large scale and can be done in different crop species. While other techniques like isolated microspore culture and others require dexterous manpower and complex apparatus as compared to anther culturing [7]. Anther culturing for doubled haploids is effective because a higher number of anthers are produced and the occurrence of doubled haploids is more probable than other methods [11]. Plants, when obtained from anther culturing followed by doubling of chromosome number, have fixed genes (dominant or recessive) and offer ease of selection according to their suitability [12]. Through doubled haploid production not only homozygous lines can be obtained, but the time for obtaining homozygosity is condensed to merely a few months [13]. The efficiency of anther culture for doubled haploid production is dependent on factors such as genotype used, growth conditions, pretreatment of spikes and developmental stages of pollens or anthers [14]. This review explains haploid and double haploid production through anther culturing and the complete procedure for conducting experiments.

2. ANTHER CULTURING

Conventional recombination breeding begins with hybridization between parents take 6-9 cycles and 3-5 years of field evaluation before a breeding line and release as a new variety. In 1968, Niizeki and Oono were produced first haploids by anther culturing in rice [15]. Anther culturing is a suitable technique that fixes homozygosity even in F_1 [16] and haploids improved by colchicine treatment. DHs line can bypass the inbreeding process [7] and has been successfully improved high yield and grain quality [17].

3. HISTORY OF ANTHER CULTURE FOR HAPLOID AND DOUBLE HAPLOID PRODUCTION

In 1921, First time Bergner observed sporophytic haploid in a weed species *Datura stramonium* L. [7]. Haploids importance was recognized in plant breeding and genetic research. Dunwell, 2010 reported the occasional haploids in range of species [18]. All angiosperm crops did not response embryogenesis induction, but wheat (*Triticum aestivum L.),* tobacco (*Nicotiana spp*.), barley (*Hordeum vulgare L*.) and rapeseed (*Brassica napus L.)* are model species to study microspore embryogenesis because of high regeneration efficiency [9] and members of legume family or many woody plants, are remain recalcitrant to this type of in vitro morphogenesis [19] [20]. Researchers developed efficient techniques for haploid and DH production with an increasing number of genotypes and practical experiences of pollen embryogenesis protocols are used [9]. About 300 new varieties have been developed belonging to various families of the plant kingdom [7]. Mostly haploid derived varieties have been developed in following crops, asparagus, barley, Brassica, eggplant, melon, pepper, rapeseed, rice, swede, tobacco, triticale, and wheat. Dunwell [21] reviewed the intellectual property (IP) protection and the patenting system of haploid plants.

4. Anther Collection Stage

Anther culturing efficiency can be improved by correctly identifying the maturity stage of anther in indica rice varieties [22], wheat [23], barley [24] and the bud of chickpea [25]. Development of anther stage different and depends on the genotypes of species. The development stage of pollen is commonly tested in one anther per floral by the acetic-carmine method. The anthers at different stages of development and prepared in acetocarmine staining solution (1% acetocarmine in 45% acetic acid) or DAPI (40, 6-diamidino-2-phenylindole dihydrochloride). To observed under a microscope and to determine the stage of pollen development like in tobacco bicellular stage, in some crops late uninucleate to early-bicellular pollen grains and pollen at uninucleate stage produce mostly haploids plants [7]. Tetrad stage is not suitable for anthers culturing and after first pollen mitosis it falls swiftly, starch deposition begins, sporophytic development stop and macroscopic structures do not form in microspores. At this stage, cold treatment can deviate from normal growth and development. The developmental stage of pollen is important to optimize the anther culture [26,27]. [28] reported a convenient morphological marker that is a distance from flag leaf to penultimate leaf auricle and estimated the maturity stage of pollen.

5. PREPARATION OF EXPLANT

The experiments can be conducted in the screen house, net houses, greenhouse and field [29]. Most researchers used screen houses [30], net-houses [29] and field [31]. Booting stage (mid uninucleate at reduction division stages) was used for collecting the anthers according to a method of [32]. [33] take panicles of rice with boot leaf sheath and washed with tap water, spread with 70% ethanol, cover with moist tissue paper, kept in a polythene bag and cold shock at 8°C for 8 days. On the day of culture, panicles were sterilized with freshly prepared 0.1% $HgCl_2$ solution for 10 minutes. Again, four times wash with distilled water. [30] cold-treated to panicles at 8-10°C for 7-10 days. After 10 days, sterilized surface with 20% (v/v) chlorox for 20 minutes. Sen and Singh in 2011 took explants at booting stage and wrapped in moist tissue paper, covered with aluminum foil and kept in refrigerator 8-10 days of cold treatment to panicle. Antisepticise sample with 70% alcohol for 2 minutes and 0.1% $HgCl_2$ solution for 8 minutes and many times clean with distilled water. [31] did not wash explant and wrapped panicles in moistened water. Cold pretreatment at 12°C, 15 days in darkness. On the culturing day, panicles sterilized with 94% ethanol and flame sterilized for a few seconds. In wheat, Al-Ashkar 2013 took tillers with spikes and clipped off at surface level and tagged. They were kept in water for 6-8 days at 4°C in a dark environment. Spikes inside flag leaves were sterilized with 20% chlorax solution for 7 min and rinsed 3–4

times in sterile water. In barley, [24] cut spikes with flag leaf from inflorescences node and kept in a beaker containing solution 5.0 mg L^{-1} $CuSo_4.5H_2o$ and 62gL^{-1} mannitol in the dark at 4°C for 4 days. After it removes flag leaf from the spikes and placed in a sterilized petri dish and a small amount (15 mm) kept rising the humidity. In chickpea, Grewal et al. 2009 took buds of different size and store in the plastic container were kept in a refrigerator at 4°C for 3-4 day. Then selected those buds at the uninucleate stage and washed with buffer bleach for 20 minutes. Sterilization of anther and Anther culturing were done in laminar air flow.

6. MEDIA FOR ANTHER CULTURING

Genotypes and composition of media are major source of variation *in-vitro* [34]. Culture medium provides nutrition and also decides the microspores and having an important aspect in androgenesis. Most commonly used media MS [35] and N6 [36] for anther culturing of cereals shown in Table 1. Since 1978, several medium formulations have been reported inducing N6-like Heh-2, Heh-5, SK-3, SK-8, Szechuan medium, Chaleffs R-2 and Medium V shown in Table 1 [37]. In cereals, Low inorganic ammonium nitrogen (NH_4^+) had proved too good for androgenesis and N6 medium containing low NH_4^+ is used for rice anther culture. N6 media with organic ingredients like casein hydrolysate, yeast extracts and coconut water show enhanced androgenic callus induction [15,33].

At late uninucleate or early binucleate stage of microspore development were dissected under sterile conditions. The culture anthers were inoculated onto a modified YP induction medium 1992 and supplements with 5 g/L charcoal, 500 mg/L tryptone, 120 g/L sucrose and 2 g/L gelrite at pH 5.8 and anther were incubated in the dark at 29°C for 4 weeks [38]. [39] used Modified YP

Table 1. Media used for anther culture

Components	N6 (mg/L)	B5 (mg/L)	He2 (mg/L)	SK1 (mg/L)	Components	MS mg/L
$(NH_4)_2SO_4$	463	134	231	231	$N_2H_4O_3$	1650.000
KNO_3	3535	3125	3181	3180	KNO_3	1900.000
KH_2PO_4	400	0	800	540	KH_2PO_4	170.000
$NaH_2PO_4.H_2O$	0	150	0	0	-	-
$MgSO_4.7H_2O$	185	250	3.5	185	$MgSO_4$	180.690
$CaCl_2.2H_2O$	166	150	166	440	-	-
H_3BO_3	1.6	3	1.6	6.2	H_3BO_3	6.200
$MnSO_4.4H_2O$	22.3	10	22.3	22.3	$MnSO_4.H_2O$	16.900
$ZnSO_4.7H_2O$	8.6	2	1.5	1.5	$ZnSO_4.7H_2O$	8.600
$Na_2MoO_4.2H_2O$	0.25	0.25	0.25	0.25	$MoO_3 \cdot H_2O$	0.213
KI	0.8	0.8	0.8	0.8	KI	0.830
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025	$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025	0.025	0.025	0.025	$CoCl_2 \cdot 6H_2O$	0.025
$FeSO_4.7H_2O$	27.8	27.8	27.8	27.8	$FeH_{14}O_{11}S$	27.800
Na_2EDTA	37.5	37.5	37.5	37.5	Na_2EDTA	37.300
Thiamine-HCl	2.5	10	10	2.5	Thiamine hydrochloride	0.100
Nicotinic acid	2.5	1	0.5	2.5	Nicotinic acid	0.500
Pyridoxine-HCl	2.5	1	0.5	2.5	PyridoxineHCl	0.500
Glycine	2	0	2	2	Glycine	2.000
Inositol	100	100	100	100	myo-Inositol	100.000
2,4-D	1	1	1	1	-	-
NAA	1	1	1	1	-	-
Kinetin	0.5	0.5	0.5	0.5	-	-
Maltose	30	30	30	30	Sucrose	30
Gelrite	2,700	2,700	2,700	2,700	-	-
Yeast Extract	100	0	0	0	-	-
Casein Hydrolysate	0	2002	200	200	-	-
AgNO3	0	8	8	8	-	-
References	[35] [36] [37].					

Table 2. Regeneration and shooting media for haploid plant production

Regeneration median	Composition
MSB0	MSB with 30 g/l sucrose, 5 mg/l BA, 0.2 mg/l NAA, and 6 g/l agar
MSB1	MSB with 40 g/l sucrose, 5 mg/l BA, 0.2 mg/l NAA, and 6 g/l agar
MSB2	MSB with 40 g/l sucrose, 4 mg/l BA, 1 mg/l NAA, 5 mg/l adenine, and 6 g/l agar
MSB3	MSB with 40 g/l sucrose, 4 mg/l BA, 0.5 mg/l NAA, 10 mg/l adenine, and 6 g/l agar
XN	N6 with 40 g/l sucrose, 4 mg/l BA, 1 mg/l NAA, 5 mg/l adenine, and 6 g/l agar
R1	MSB with 40 g/l sucrose, 4 mg/l BA, 1 mg/l NAA, and 6 g/l a
Shooting media	**Composition**
MR1	MSB (1/2 macrosalts content) with 30 g/l sucrose, 1 mg/l IBA, and 6 g/l agar
MR2	MSB (1/2 macrosalts content) with 30 g/l sucrose, 0.5 mg/l IBA, and 6 g/l agar
MR3	MSB (1/2 macro salts content) with 40 g/l sucrose, 0.25 mg/l IBA, 0.25 mg/l NAA, and 6 g/l agar
MR4	MSB (1/2 macro salts content) with 40 g/l sucrose, 0.25 mg/l IBA, 0.5 mg/l NAA, and 6 g/l agar
References	[41]

without plant growth regulator, Modified YP +2 mgl-1kinetin, Modified YP +3 mgl-1kinetin, Modified YP +2 mgl-1kinetin+2 mgl-1 IAA, Modified YP +2 mgl-1kinetin+2 mgl-1 NAA and Modified YP +2 mgl-1kinetin+2 mgl-1, 2,4-Dichlorophenoxyacetic acid (2-4-D) media in their research. [40] used MS medium containing, various concentrations of BAP, Kinetin, NAA (1-Naphthaleneacetic acid) and 2,4-D either alone or in combination. The cultures were maintained at 25±2°C and a 16 hours' photoperiod for four weeks. Different media were used for regeneration and shooting is shown in Table 2.

7. CHROMOSOME COUNTS

Methods for ploidy level determination in haploids and DH plants and can be identified by chromosome counting during meiotic cell division. Root tips or other meristematic tissues can be used for chromosome counting during mitotic division [42]. Chromosome counting during mitotic division is time-consuming and difficult in small chromosomes like *Brassica napus* [43].

8. CHROMOSOME DOUBLING BY COLCHICINE

Haploid plants are short, infertile and sterile due to pollen or egg not able to produce pollen and egg because of the absence of homologous pair in the meiotic division. Chromosome double must be needed for the survival of a haploid plant [44]. Colchicine, an alkaloid found in the seeds of autumn crocus (*Colchicum automnale*). It is an antimitotic agent and used for chromosome doubling, restored the fertility of sterile interspecific, intergeneric hybrids of plants, andro-and gynogenic haploids. Effective colchicine treatments have been reported for several crop plants like wheat, maize, rapeseed, Indian mustard, and watermelon [45]. Plant breeding program needs doubled haploid for hybrid development [44]. Treatment of Colchicine can be applied on post-pollination to the tillering stages of the haploid plants. In wheat and barley, colchicine treatment is normal suggested the 3- to the 4-tiller stage, time 5–8 hours by submerging root system in a colchicine solution having 0.3 ml/l Tween 20, 2% dimethyl sulfoxide (DMSO), 0.1% colchicine, and 10 mg/l of gibberellic acid. Doubling rate can reach 95.6%. The colchicine can be practiced before the seedling stage of haploid plants by using 0.5% colchicine to the rescue medium for 48 h. The colchicine solution (1% colchicine with 100 ppm 2,4-D) can be injected into the uppermost internode of pollinated spikes at 48 and 72 hours after pollination, and the chromosome-doubling rate varies from 33% to 100%. After treatment, the plantlets were washed with running water, transferred to soil and having in a growth chamber at 14– 16°C under 16/8 h day/night before the new tillers emerged. The plants were shifted to normal conditions in a greenhouse. The plant survival rate reached 99% and the chromosome-doubling rate varies 96–98% [5].

9. IMPLEMENTATIONS OF DOUBLED HAPLOIDS

DHs production is a very attractive tool in modern plant breeding. Many methods can be used for

the production of DHs, gynogenesis is used as a substitute method in plants where the issue of male sterility is high [46]. Anther culturing has many advantages over classical techniques in the betterment of the crop plants [47]. Instant fixation of homozygous genes, improved selection, increased genetic variation and early expression of recessive genes are some important benefits of doubled haploids [15]. Following are important uses of DHs explained by Badu et al. [48].

10. USES IN BREEDING CROPS

1. DHs can be used to synthesize the homozygous lines or pure lines in self-pollinated crops and inbred lines in cross-pollinated crops.
2. An earlier release of variety is obtained as it reduces the time for achieving the homozygosity.
3. As there is great similarity so no masking effect of genes and homozygous lines are produced.
4. Greater efficacy is observed because it is easy to combine targeted genes in homozygous line.
5. Selection is done in the first generation of haploids because high selection pressure is applied at this stage.

11. USES IN GENOMICS

Important applications of doubled haploid in the field of genomics:

1. This can be used to recover the recessive genotypes.
2. The occurrence of mutation and its spectrum can be studied through doubled haploids.
3. The great help in the mapping of the population can be taken from doubled haploids.
4. It is reproducible and can be conducted at different locations.
5. The expressivity of a gene can be increased through DHs.
6. Genes can be located and tagged in the DH populations.
7. Traits can be selected through recognition of markers.
8. DH populations also help in Quantitative trait loci because authentic phenotyping of traits can be performed.

Doubled haploids are known for their extreme homozygosity and reduced time required for fixing desirable genes. The time needed can be reduced by 4 years by using DHs as compared with old breeding methodologies [49]. By using DHs, the number of meiotic steps is reduced as compared to filial generations in getting homozygosity. DHs faceless crossing over so they are contributed by the greater portion of the parental DNA than by single seed descent. It is indicating that DHs will inherit a higher segment of DNA from parents than other methods [50]. DHs can be used where higher parental proportion is required. According to Frisch and Melchinger 2007 in quantity of 0.99 quantile SSD (single seed descent) method was able to recover 78% of parental genome,while DHs progeny ensured higher proportion by showing 82% parental contribution. Hence doubled haploids in addition to reducing the time required is also involved in resembling more closely with parents than other methods. It can be referred to as reverse breeding [51].

12. MERITS OF DHS

DHs have revolutionized the modern era of breeding and its success is due to various factors such as application to a wide range of genotypes, ease of conduction and high efficiency. The most important thing is that it saves the time and achieves the homozygosity i.e. performs the action within no time. It can put the march toward better crops at a significantly higher pace. Following are some important merits of DHs [48].

- The capacity to deliver homozygous lines after a solitary round recombination spares a considerable measure of time for the plant reproducers.
- Studies reveal that irregular DH's are equivalent to the chosen lines in pedigree inbreeding.
- Alternate favorable circumstances, incorporate advancement of a substantial number of homozygous lines, effective hereditary examination, and improvement of markers for helpful qualities in considerably less time.
- More targeted advantages incorporate the chance of seed production as an option in contrast to vegetative growth in ornamentals, and in species, for example, trees in which long life cycles and inbreeding depression block normal reproducing techniques, DHs gives new options.
- The acceptance of DH lines in dioecious plants, in which sex is determined by gene

regulation, has an extra favorable position. Such a case is very much concentrated in Asparagus, in which sex dimorphism is controlled by a dominant gene M. Female plants are homozygous for the recessive alleles (mm), while male plants are heterozygous (Mm). Androgenically delivered DH lines are therefore female (mm) or 'super male' (MM). Favorable position of super males is that, when utilized as the pollinating line, every hybrid offspring is male.

13. CONCLUSION AND FUTURE HORIZONS

Haploid and doubled haploid breeding is a very fertile system of modern breeding. It can be the substitute for conventional breeding in which all steps are very laborious and time-consuming in future. Anther culturing is a very effective technique that has been successfully used in many important plant species for the reducing time required to develop important genotypes and achieving extreme homozygosity. The plants like coffee require high time for regeneration such species can be subjected to these techniques and time span can be reduced. Although anther culture is responsive to a wide range of genotypes and gives satisfactory results but many important species are under considerations. Many other mechanisms involved in haploid breeding at the molecular and biochemical level needed to be studied. In addition to this anther culturing needed to be cost-effective, more efficient, practical and satisfying the breeding needs. Hence there is need for improvement of protocols to develop new genotypes. Modern fields of genetics have benefitted the micro embryogenesis. The advancements and research in this field will improve understanding of this process and lead to effective procedures development for embryogenesis in future. A further increasing number of facts and evidence shows the interest of future researchers in anther culture for double haploid production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tefera A. Review on concept and impact of double haploid techniques in crop improvement. Jour. of Natural Sci. Research. 2017;7:23.
2. Gerald N, Frei U and Lubberstedt T. Accelerating plant breeding. Trends in Plant Science. 2013;18(12):667-672.
3. Seguí-Simarro J. Doubled haploidy in model and recalcitrant species. Frontiers in Plant Science. 2015;6.
4. Croser J, Lülsdorf M, Davies P, Clarke, Bayliss L, Mallikarjuna N, Siddique H. Toward doubled haploid production in the fabaceae: Progress, constraints, and opportunities. Critical Reviews in Plant Sci. 2006;25(2):139-157.
5. Niu Z, Jiang A, Hammad A, Oladzadabbasabadi A, Stevens, XUS, Merhoum M, Elias E. Review of doubled haploid production in durum and common wheat through wheat x maize hybridization. Plant Breeding. 2014;133:313–320.
6. Asif M. Progress and opportunities of doubled haploid production. Springer; 2013.
7. Germena M. Anther culture for haploid and doubled haploid production Plant. Cell Tiss. Organ Cult. 2010;104:283–300.
8. Castillo A, Cistué L, Vallés M, Soriano M. Chromosome doubling in monocots. In Advances in Haploid Production in Higher Plants. 2009;329-338.
9. Forster P, Herberle-Bors E, Kasha K, Touraev A. The resurgence of haploids in higher plants. Trends Plant Sci. 2007; 12(8):368–375.
10. Alheit K, Rei J, Maurer H, Hahn V, Weissmann E, Miedaner T, Würschum T. Detection of segregation distortion loci in triticale (x Triticosecale Wittmack) based on a high-density DArT marker consensus genetic linkage map. BMC Genomics. 2011;12(1):380.
11. Wurschum T, Tucker M, Reif J, Maurer H. Improved efficiency of doubled haploid generation in hexaploid triticale by *in vitro* chromosome doubling. BMC Plant Biology. 2012;12(1):109.
12. Murovec J, Bohanec B. Haploids and doubled haploids in plant breeding. In Plant Breeding. In Tech; 2012.
13. Wędzony M, Forster B, Żur I, Golemiec E, Szechyńska-Hebda M, Dubas E, Gotębiowska G. Progress in doubled haploid technology in higher plants. In Advances in haploid production in higher plants, Springer Netherlands. 2009; 1-33.

14. Grauda D, Mikelsone A, Lisina N, Zagata K, Ornicans R, Fokinac O, Lapina L, Rashal L. Anther culture effectiveness in producing doubled haploids of cereals. Latvian Acad. Sci. 2014;68(2014):142-147.
15. Mishra R, Rao G. *In-vitro* androgenesis in rice: Advantages, constraints and future prospects. Rice Science. 2016;23(2):57-68.
16. Tripathy SK, Swain D, Mishra D, Prusty AM, Behera SK, Tripathy P, Chakma B. Elucidation of the genetic basis of anther culture response and its breeding perspective in rice. Euro. Jour. of Biotechn. and Biosc. 2018;6(2):26-30.
17. Mishra R, Rao GJN, Rao RN. Effect of cold pretreatment and phytohormones on anther culture efficiency of two indica rice (*Oryza sativa* L.) hybrids: Ajay and Rajalaxmi. J Exp Biol Agric Sci. 2013;1(2):69–76.
18. Dunwell J. Haploids in flowering plants: origins and exploitation. Plant Biotechnology Journal. 2010;8(4):377-424.
19. Germanà M. Doubled haploid production in fruit crops. Plant Cell, Tissue and Organ Culture. 2006;86(2):131-146.
20. Germana M. Haploid and doubled haploids in fruit trees. In: Touraev A, Forster B, Jain M (eds) Advances in haploid production in higher plants. Springer, Heidelberg. 2009;241–263.
21. Dunwell J. Patents and haploid plants. In: Touraev A, Forster BP, Jain SM (eds). Advances in haploid production in higher plants. Springer, Heidelberg. 2009;97–113.
22. Silva T, Ratnayake W. Anther culture potential of indica rice varieties, Kurulu Thuda and BG250. Trop Agric Res Ext. 2009;12(2):53–56.
23. Al-Ashkar I. Anther culture response and salt tolerance in some wheat genotypes. Annals of Agricultural Sciences. 2013;58(2):139-145.
24. Sriskandarajah S, Sameri M, Lerceteau-Köhler E, Westerbergh A. Increased recovery of green doubled haploid plants from barley anther culture. Crop Science. 2015;55(6):2806.
25. Grewal R, Lulsdorf M, Croser J, Ochatt S, Vandenberg A, Warkentin T. Doubled-haploid production in chickpea *(Cicer arietinum* L.): role of stress treatments. Plant Cell Reports. 2009;28(8):1289-1299.
26. Chaum S, Srianan B, Pichakum A, Kirdmanee C. An efficient procedure for embryogenic callus induction and double haploid plant regeneration through anther culture of Thai aromatic rice (*Oryza sativa* L. subsp. indica). *In-vitro* Cell Dev. Biol. 2009;45(2):171–179.
27. Silva T. Indica rice anther culture: Can the impasse be surpassed? Plant Cell Tiss. Organ. Cult. 2010;100(1):1–11.
28. Bishnoi U, Jain R, Rohilla J, Chowdhury V, Gupta K, Chowdhury B. Anther culture of recalcitrant indica × Basmati rice hybrids. Euphytica. 2000;114(2):93–101.
29. Mohiuddin AK, Karim H, Sultana S. Development of improved doubled-haploids through anther culture of indica rice (*Oryza sativa* L.). Annals of Bio. Resear. 2014;5(10):6-13.
30. Ambarwati A, Somantri H, Utami W, Apriana A, Moeljopawiro S. Rice anther culture to develop double haploid population and blast resistant lines. Jurn. AgroBio. 2011;5(2):71-77.
31. Min J, Sim S, Lee S, Park H, Park GH, Kim M. Comparison of the efficiency to one-step and two-step anther culture in rice (*Oryza sativa* L). Jour. of Agri. and Envi. Sci. 2015;4(1):77-82.
32. Afza R, Shen M, Zapata J, Xie J, Fundi K, Lee S, Eva M, Kodym A. Effect of spikelet position on rice anther culture efficiency. Plant Sci. 2000;153:155-159.
33. Roy B, Mandal B. Anther culture response in indica rice and variations in major agronomic characters among the androclones of a scented cultivar, Karnal local. Afr. Jour. of Biotech. 2005;4(3):235-240.
34. Sen C, Singh P. Anther culture responses in boro rice hybrids. Asian Jour. Off biotech. 2011;3(5):470-477.
35. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physi. Plantarum. 1962;15.
36. Chu C. The N6 medium and its applications to anther culture of cereal crops. In: Proceedings of Symposium on Plant Tissue Culture, Science Press, Peking. 1978;45-50.
37. Kaushal L, Balachandran M, Ulaganathan K, Shenoy V. Effect of culture media on improving anther culture response of rice (*Oryza sativa* L.). Intern. Jour. of Agri. Innov. and Resear. 2014;3(1):2319-1473.
38. Jager K, Bartók T, Ordog V, Barnabas B. Improvement of maize (*Zea mays* L.) anther culture responses by algae-derived

natural substances. South Afri. Jour. of Botany. 2010;76:511–516.
39. Hosseini S, Ismaili A, Mohammadi P. Improvement of maize (*Zea mays* L.) anther culture embryogenesis and direct regeneration by different plant growth regulators and gelling agent. Jour. of Applied Biotech. Reports. 2014;1(1):17-21.
40. Madke S, Cherian J, Badere S. A modified Murashige and Skoog media for efficient multipleshoot induction in *G. arborea* Roxb. Jour. of Forestry Research. 2014;25(3):557−564.
41. Bobkov S. Obtaining calli and regenerated plants in anther cultures of pea. Czech J. Genet. Plant Breed. 2014;50:123–129.
42. Dwivedi S, Anne B, Britt L, Tripathi S, Sharma, Upadhyaya D, Ortiz R. Haploids: Constraints and opportunities in plant breeding. Biotech. Advan. 2014;33:812-829.
43. Weber S, Wilfried L, Friedt W. Efficient doubled haploid production in *Brassica napus* via microspore colchicine treatment *in vitro* and ploidy determination by flow cytometry. Weber-Lühs-Friedt GCIRC-Bulletinpp. 2004;1–6.
44. Das A, Kumar K, Kishor U. Tribhuvan, Das S, Mishra M. Development of haploid and double haploid in fruit crops - A review. Internat. Jour. of Current Microbio. and Applied Sci. 2018;7(5): 2119-2132.
45. Burun B, Emlroglu U. A comparative study on colchicine application methods in obtaining doubled haploids of tobacco (*Nicotiana tabacum* L.). Turk J Biol. 2008; 32:105-111.
46. Bhat J, Murthy G. Factors affecting *in-vitro* gynogenic haploid production in niger (*Guizotia abyssinica* (L. f.) Cass.). Plant Growth Regul. 2007;52(3):241–248.
47. Purwoko B, Dewi I, Khumaida K. Rice anther culture to obtain doubled-haploids with multiple tolerances. AsPac J Mol Biol Biotechnol. 2010;18(1):55–57.
48. Badu M, Tripathy B, Sahu G, Jena A. Role of doubled haploids in vegetable crop improvement. Journal of Pharmacognosy and Phytochemistry. 2017;6(6):384-389.
49. Smith S, Hussain T, Jones E, Graham G, Podich D, Wall S, Williams M. Use of doubled haploids in maize breeding: Implications for intellectual property protection and genetic diversity in hybrid crops. Mol Breeding. 2008;22:51–59.
50. Frisch M, Melchinger A. Variance of the parental genome contribution to inbred lines from biparental crosses. Genetics e Publication; 2007.
51. Dirks G, Van Dun P, Reinink K. Reverse breeding. Intl. Publ. No. WO 03/017753 A2. World Intellectual Property Organisation. Geneva, Switzerland. 2003;83.

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