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Research Article

Brief History about Success of Anther Culture in Haploid and Double Haploid Plant Regeneration in Crops

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Abstract

To overcome food deficit, food security is very important to feed the world population. For this reason, agricultural scientific research needs to conduct more precisely. Advanced, precise and quick techniques can help to develop crop varieties very effectively. In agricultural research sector haploid and double haploid plant regeneration by anther culture can provide significant results. Many researchers and scientists have been succeeding to release new crop varieties with this method. If this method can combine with other advance research techniques such as genetic engineering, OMICS technology, bioinformatics, improved protocols etc., more desirable results will be found.

INTRODUCTION

A method for the purpose of haploid or double haploid plant regeneration technique is to divide and develop immature pollen/ anther containing microspores into callus or embryonic tissue, that cultured on a fluid or solid nutrient media by maintaining aseptic condition and suitable environment of culture room. Principles of anther culture is production of haploid crops through the use of microspore totipotency, obstruct the normal growth and functioning of microspores into male gametes and microspores systematically turned to a new metabolic pathway for division of vegetable cells. Double haploid (DH) plantlets can be made by a simple way with the treatment of sterilized solution of colchicine (0.4%) for 2-4 days. The diploid plantlets are homozygous because they become double after colchicine treatment with the same number of chromosomes and also genetically stable. The first effort was to develop double haploid crop regeneration was *Brassica* species, based on anther culture [1]. This method is very applicable in plant breeding as well as to fulfill some important objectives (Figure 1, Figure 2).

There are several factors that influence on anther culture such as genotype of donor plants, anther wall- provide the nourishment for development, appropriate culture media- activated charcoal, iron and growth hormones etc., stimulate androgenesis, stages of microspores most efficient in microspore uninucleate phases and for specific species shows best response at mitosis stage, effect of temperature low temperature causes dissolution of microtubules and great effects showed in *Brassica campestris* L. at 35ºC for first 2-3 days, physiological status of donor plant such as water stress, nitrogen requirement etc.

Four essential pathways responsible in which androgenic haploid plants can be produced successfully [2]:

Pathway-I: The microspores of anther firstly divide by

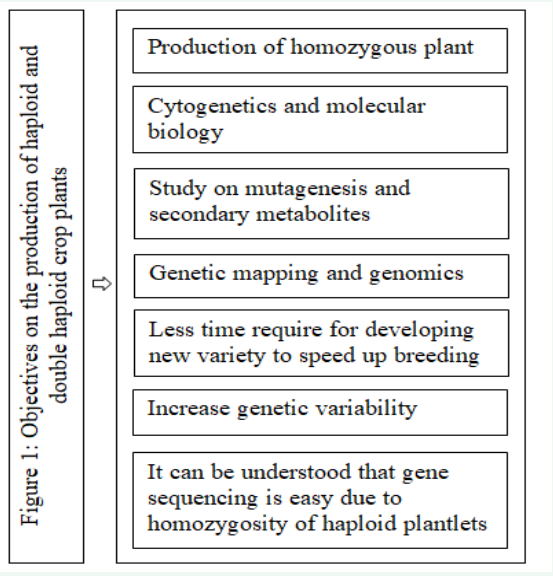

Figure 1

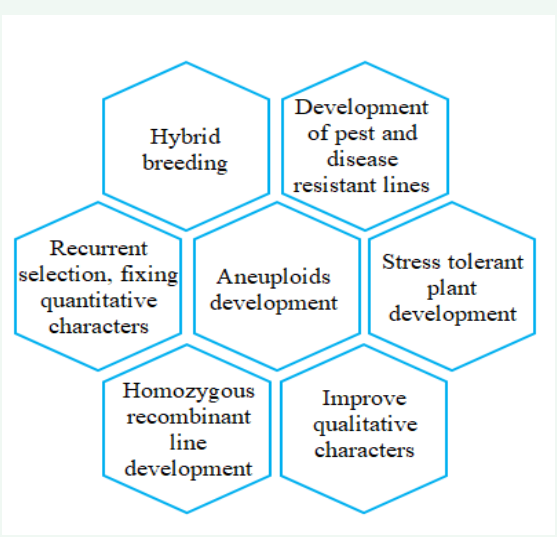

Figure 2 Application of another culture on several breeding techniques.

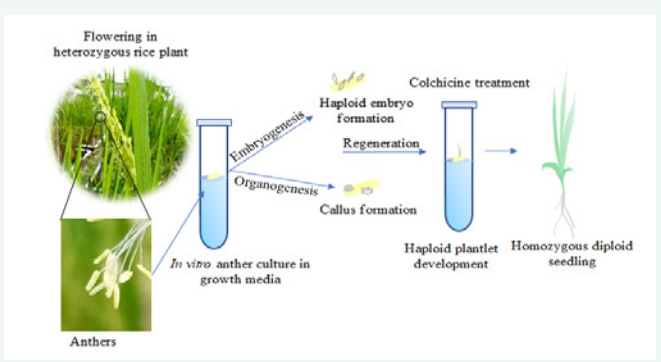

Figure 3 Process of another culture to develop a new homozygous diploid plant.

an equal division and similar daughter cells were involved in saprophytic development but no vegetative and generative cells are not formed.

Pathway-II: The uninucleate microspore divided unequally for vegetative and generative cells formation.

Pathway-III: The pollen embryo formed from the generative cells.

Pathway-IV: The microspores divide asymmetrically and both vegetative and generative cells divide again for contributing saprophyte development.

If we can maintain all of these factors, culture media, aseptic condition and favorable environment for species specific then the pathways can regulate normally (Figure 3).

Historical Overview of Success of Anther Culture in Haploid and Double Haploid Plant Regeneration in Crop Plants:

In 1922, this technique was first discovered by Blakeslee et al. about the existence of haploid regeneration ability that was experimented in a weed species *Datura stramonium* L. [3]. Then the technique developed more precisely for production of haploid by aseptic *in vitro* condition so that it can be used in another type of crop plants [4]. The *Hordeum vulgare* L. was first agricultural crop used for haploid plant regeneration by interspecific wide crossing between 28 chromosomes containing auto-tetraploid cultivated barley *Hordeum vulgare* and tetraploid barley *H. bulbosum,* where selective chromosomes that were eliminated reciprocally due to inability of crossing over between the chromosomes; all progenies with 14 chromosomes were identified in new double monoploid plantlets that was fertile homozyote [5]. Their important aspects of findings are that haploid plantlets can obtain from the cross between the diploid form from any given line of cultivated species by selecting haploids in the progenies of first generation (F_1), also depends on efficient embryo culture techniques with appropriate protocols. Haploid production in similar way between interspecific crosses of *Hordeum* and *Solanum* also possible from the knowledge of their research.

Afterwards, many researchers focus on this strategy for development of new improved crop variety (Figure 4). Forster et al. (2007), explained research which has been established through efficient technologies to modify number of genotypes for the manufacturing of haploids and DH and practical experience with the protocols of anther embryogenesis [6].

By reading two books, editors are Maluszynski et al. (2003) and Touraev et al. (2009) we can understand about some significant agricultural plants and tree species, developed by many researchers about haploid plant regeneration techniques [7,8]. In that books androgenesis in different crops with detailed information, efficient protocols and procedures, their application,

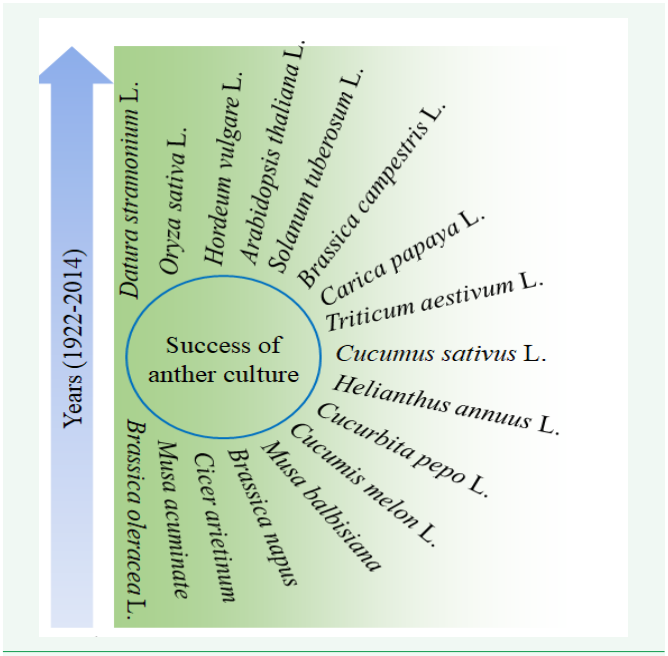

Figure 4 Another culture successfully conducted by several researchers in different years for improving crops.

new advance method, programmed cell death (PCD) and albinism occur during microspore culturing discussed properly. Haploid and double haploid plantlet regeneration process can be done through some useful system such as genetic analysis, gene transformation by microspores of anther, plant physiology and reverse breeding programs on *Arabidopsis thaliana* etc. [9-12]. Detailed information about limitations and prospects of haploid plant breeding can understand from the article by Dwivedi et al. (2015) [13]. Some important crops' QTLs were detected and mapped from DH population [14]. Embryogenic system by anther culture requires half the time than organogenic system via green plant parts successfully discovered in *Triticum aestivum* by Armstrong et al. (1987) [15].

Cereal Crops

Promising information is that haploid regeneration techniques of *japonica* rice crops first developed successfully by the researchers Niizeki and Oono (1968) [16]. Both haploid and double haploid plantlets of salt tolerant *indica* rice cultivars also efficiently developed by anther culture using *in vitro* aseptic condition the genotypes known as Pokkali and Nona Bokra, which could be outstanding materials for future breeding of rice [17]. Applying maltose in culture media and alternating temperature 30°C/20°C increases the efficiency of *indica* cultivars that was recalcitrant. Transgenic wheat has been retrieved from a research using microspore transformation techniques, which was homozygotic, double-haploid [12]. Constrictions and future prospects for successful androgenesis of rice crops are discussed in a recent review paper [18]. Some released double haploid rice varieties also listed in this article that are high yielding, maturation capacity is very quick, resistant from diseases (blast, sheath rot, sheath blight and bacterial infection), short life cycle, well forming grain quality, resistant to brown plant hopper and yellow stem borer insects as well as stress tolerant against high salt concentration, high temperature or drought and cold or frost.

Vegetable Crops

Some important procedures developed successfully to recover disease resistant melon (*Cucumis melon* L.) cultivars that were susceptible by several viruses [19]. It has been observed that powdery mildew in melons infected by the fungus of *Sphaerotheca fuliginea* and this problem can be manifested at the haploid level [20]. Some factors also responsible for successful plant regeneration of *Cucumus sativus* explained by Przyborowski and Nlemirowicz-Szgzytt (1994) [21]. Research on anthers of 34 double haploids clones of Potato (*Solanum tuberosum*) conducted by Sopory and Rogan (1976) and showed successfully induction of pollen division and embryoid formation [22]. Among haploid and diploid plantlets, mostly haploid plantlets found by the influence of different gamma (γ) rays wave length and genotypes on the induction of anthers to embryoid on *Cucurbita pepo* L. [23]. *Brassica oleracea* var. *botrytis* also developed efficiently [24]. In their research the embryos formed that was genotype dependent and the techniques also useful for genetic research.

Oilseed Crops

In *Brassica campestris* anther culture, Keller and Armstrong (1979) suggested that high temperature treatment at 35°C for 1-3 days before culture at 25°C responsible for significant successful plantlet regeneration, encouraging embryogenesis and haploid plant development [1]. *Brassica napus* also widely cultivated variety improved by *in vitro* mutation using anther culture to overcome any contamination by *Sclerotinia sclerotiorum* [25]. Different doses of chemicals enhanced microspore embryogenesis of *B. napus* very significantly by maintaining 30°C temperature [26]. Parthenogenesis that induced by irradiated pollen also used for double haploid production of *Helianthus annuus* L. that was agronomically useful, high fertility and highly resistant to downy mildew disease [27].

Fruit Crops

Assani et al. (2003) has been reported the haploid and some diploid plant regeneration way from a banana species (*Musa balbisiana*), this species also resistant to various diseases. In their research, they observed the callus induced by anther, mostly in the uninucleate phase as well as genotype dependent and about 7.9% anthers developed into embryos [28]. About 523-megabase genome sequence and analysis of double haploid *Musa acuminate* species also reveals banana species improvement genetically [29]. Anther culture of *Carica papaya* was effective in a uninucleate phase callus induction, and was found large number of embryoids when it was cultivated without growth hormones with MS media containing 03% sucrose [30].

Pulse Crops

Cicer arietinum L. was developed successfully through anther culture. For developing embryos of chickpea, the researchers firstly applied some specific stress treatments (cold stress, electric shock, centrifugation, high osmotic pressure), then electroporation treatment enhanced root formation in plantlet culture media. After that they select both haploid and double haploid plantlet by flow cytometric analysis of calli during early regeneration stages. Several important research strategies, progress, constraints and opportunities towards the double haploid production in Fabaceae can be found from the article of Croser et al. (2006) [31].

To validate and increase accuracy for improving the above discussed crops with anther culture in further research, some advanced breeding techniques is indispensable for the researchers (Figure 5) [32-36].

CONCLUSION

For considering food safety in present and also considering future aspect we need to develop more appropriate technology for the plants that are recalcitrant, which have no success in the recovery of haploids or double haploids plant regeneration. We know that advance molecular methods for gene manipulating such as gene transformation, CRISPER/Cas9 genome editing tool, marker assisted selection, and omics technology can be applied with the anther culture techniques for developing new variety because the fundamental mechanisms in all living organisms are regulated by gene of genome. Combining these approaches will help us develop variety successfully. For genomics and

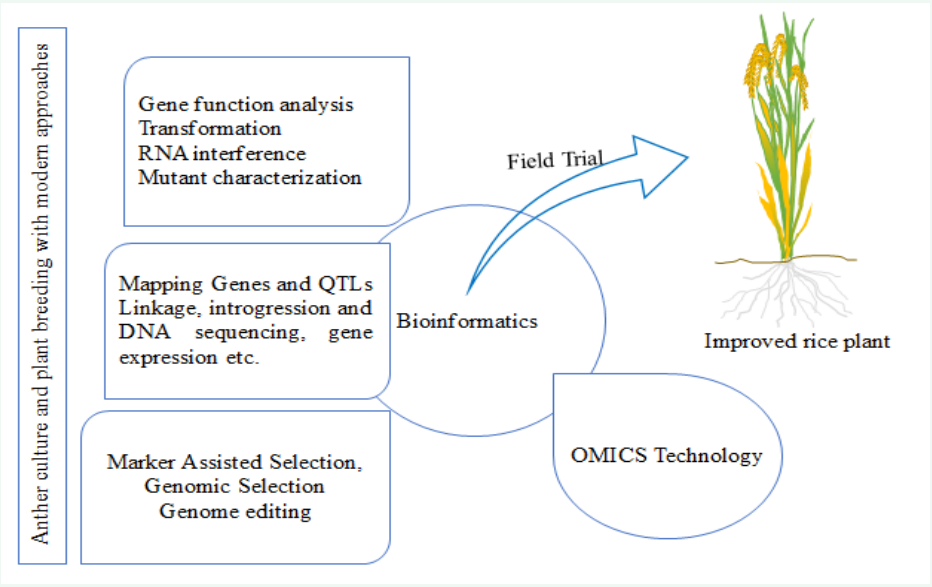

Figure 5 **Advanced breeding techniques to develop a new crop variety after developing haploid or double haploid plant through another culture.**

cytogenetics study, haploid crops of homozygous lines are very useful. If it can be done, we can get more useful crop variety that is more efficient. Improved protocols need to develop for more efficiency and more frequent plantlet development successfully during haploid and diploid plant regeneration. In applied plant breeding both haploid and double haploid plant regeneration techniques are very reasonable way to accomplish various research goals.

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Conflicts of Interest

The author declares that no interest of copyright violation and financial support.

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