MARUMEGH

Kisaan E- Patrika

Available online at www.marumegh.com

 ISSN: 2456-2904

ANTHER CULTURE: OLD AND GOLD TOOLS FOR HAPLOID PLANT PRODUCTION

Ashutosh Singh, Abhishek Kumar and Chethan S.

College of Horticulture & Forestry,

Rani Lakshmi Bai Central Agricultural University, Jhansi, India – 284003

Introduction

Anther culture is one of the important techniques of the plant tissue culture for the production of haploid plants. The aim of anther culture is the regeneration of the haploid plants from the haploid microspore cell under controlled environmental conditions in the artificial culture medium. The technique of anther culture is completely depends of the principle of cellular totipotency of the plant regeneration. However, the production of haploid plants through anther culture under the controlled environmental conditions by exploiting the totipotency of the microspore. Anther culture is also referred as androgenesis, occurs when microspore or pollen shifted from gametophytic to sporophytic pathway of the embryogenesis.

Development of haploid plants by anther culture requires high level of expertise; it also involves the selection of suitable plants as well as proper stage of the anther development. Anther culture or pollen culture for the production of haploid have been reported from more than 250 species of the plant kingdom. Gramineae, Solanaceae, Ranunculaceae, Cruciferae are the most common family of the plant species from which haploid plants are easily raised by anther culture. The haploid production is not a new technique as it was started six decayed earlier, in 1964 by Guha and S.C. Maheshwari confirmed the origin of haploid plantlets from the anther. It was later also successfully reported by Guha and Maheshwari.

Haploid Plants

Most of the haploid derived from pollen or anther culture are observed as sterile because of the single set of the chromosomes in their genome. Doubling of the chromosome number is one of the strategic ways to made fertile and resulting plants are either isogenic diploid or homozygous diploid. Like other plants, such type of homozygous plants shows the normal meiotic segregation. The homozygous diploid developed in such manner must be important in comparsion to the haploid sterile. However, in most of the cases homozygous diploid plants can be used in the genetic study as well as development of the pure lines in the traditional breeding programme.

Haploid production can be achieved by colchicine treatment. Colchicine has been widely used to reduce the duplication of chromosome for the production of haploid plants. Treatment of anther with 0.5 % Colchicine for 24 to 48 hours is sufficient for the haploid plant production. In case of mature haploid plantlet the quantity of colchicine increased from 0.5 % to 4%.

Procedure of Anther Culture: Production of haploids from anther culture is defined as sporophytes with gametophytic chromosome number and has been produced in a variety of plant species using a variety of methods. The parameters recognized for the anther culture areas as follow:

(i) Condition of growth of donor plant
(ii) Genotype of the donor plant
(iii) The pretreatment
(iv) The developmental stage of the anther (or microspore)
(v) The culture medium and the conditions during culture growth.

Haploid Production

The haploid plant production by the anther culture is depends on the developing anthers in the plant flowers at a very critical stage are excised aseptically on a nutrient medium where the microspores within the cultured anther develop into callus tissue that give rise to haploid plantlets through organogenesis or embryogenesis. The lists of successfully produced haploids are given in the table. General procedures for the haploid production using anther culture are given below.

1. The young flower buds are collected and washed under running tap water to remove the dirt.
2. These are then surface sterilized by immersing in 70% ethanol.
3. Then washed in sterile water and transferred into a sterile petridish.
4. With the help of sharp scalpel and using forceps the buds are split open and anther lobes are taken out.
5. One of the anther lobes of each bud is checked by crushing into acetocarmine stain under microscope for the proper stage of microspore development. i.e., just released from the tetrad condition.
6. The filament portions are removed from the selected anther lobes.
7. The damaged anther lobes should be discarded and intact anther lobes are placed into proper media.
8. Incubated at 24°-28°C in dark for 3-8 weeks.
9. The haploid embryos or plantlets develop, come out by bursting the anther lobes.
10. Individually these embryos or plantlets are removed and sub-cultured on suitable media to develop further and root development. Some of the important haploid developed agricultural and horticulture crops mention below in table.

1.	Alfalfa	9.	Corn	17.	Rye
2.	Asparagus	10.	Cucumber	18.	Strawberry
3.	Banana	11.	Grape	19.	Sugarcane
4.	Barley	12.	Peanut	20.	Sunflower
5.	Broccoli	13.	Pepper	21.	Sweet potato
6.	Citrus	14.	Potato	22.	Tomato
7.	Clover	15.	Rapeseed	23.	Triticale
8.	Coffee	16.	Rice	24.	Wheat

Table: List of the commercially developed haploid

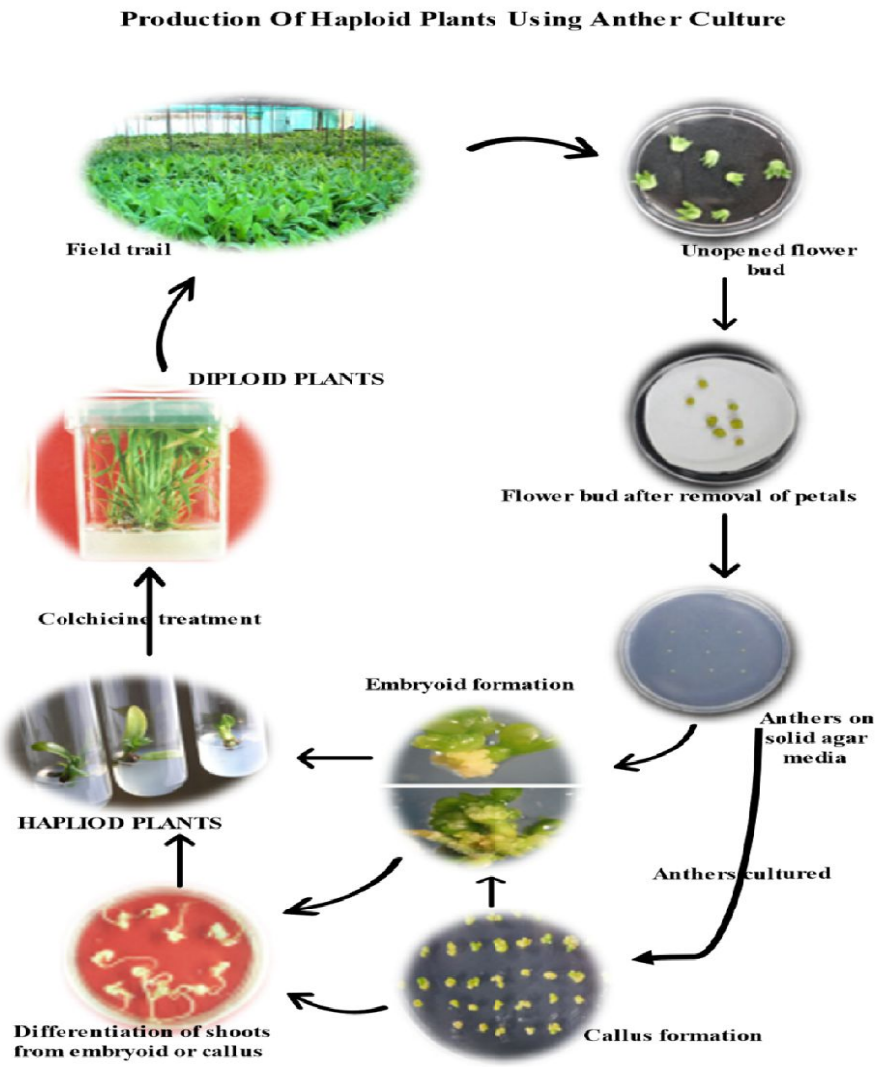

Figure: Diagrammatic representation of haploid production

Advantages of Anther culture

1. Haploids are significant because they carry only one allele of each gene. Thus, any type of mutation is apparent mainly direct screening of recessive mutation is possible.
2. In haploid plant cells plants with lethal genes are easily eliminated from the gene pool.
3. Through haploid culture homozygous diploid or polyploidy plants can be produced, which may be valuable in plant breeding.
4. Production of haploids shortens the time for inbreeding for superior hybrid genotypes.
5. Overcrowding of pollen grain in anther is eliminated and isolated pollen grains are equally exposed to nutrient medium.
6. Unwanted growth of the anther wall and other associated tissue are eliminated.
7. The steps of androgenizes can be observed starting from single cell.
8. Various factor governing androgenizes can be better regulated.
9. Pollen is ideal for uptake, transformation and mutagenic studies as pollens can be uniformly exposed to chemicals and physical mutagens.

Potential Application of Anther Culture

The technique of the anther culture or pollen culture has a remarkable and potential use in the genetic transformation through the production of the haploid plants from the agriculturally important crops. Such type of techniques is also useful in the induction of various resistance traits for the biotic and abiotic stresses. Now days, the anther culture technique of haploid production has become an integral part of the plant breeding through accelerating the production and development of inbred lines in the several crops. Haploid production technology has also been observed in the overcoming seed dormancy and non-viability of the embryo in much number of the horticultural crops.

Genetic maps also provide a framework for the mapping of genes of interest and estimating the magnitude of their effects and aid our understanding of genotype/phenotype associations. DH populations have become standard resources in genetic mapping for species in which DHs are readily available. Doubled haploid populations are ideal for genetic mapping. It is possible to produce a genetic map within two years of the initial cross regardless of the species.

Most of the economic traits are controlled by genes with small but cumulative effects. Although the potential of DH populations in quantitative genetics has been understood for some time, it was the advent of molecular marker maps that provided the impetus for their use in identifying loci controlling quantitative traits. In backcross conversion, genes are introgressed from a donor cultivar or related species into a recipient elite line through repeated backcrossing. A problem in this procedure is being able to identify the lines carrying the trait of interest at each generation. The problem is particularly acute if the trait of interest is recessive, as it will be present only in a heterozygous condition after each backcross. The development of molecular markers provides an easier method of selection based on the genotype (marker) rather than the phenotype. Combined with doubled haploid it becomes more effective.

Conclusion and future perspectives

Here from above mention facts about the anther culture we conclude that this technique is very useful for the haploid production apart from the simplicity of the technique. By the following of the proper procedure of the same technique can have profound breakthrough for the commercial exploitation. However, still it requires the advance tools and techniques for the maximum harness of it's potentially in the field of agricultural biotechnology and molecular biology for strengthen of crop improvement programmes.

References:

Jain, S. Mohan, S. K. Sopory, and R. E. Veilleux. 1996. *In vitro haploid production in higher plants*. Dordrecht: Kluwer Academic Publishers. p.317.

B. Barnabás; B. Obert; G. Kovács 1999. Colchicine, an efficient genome-doubling agent for maize (Zea mays L.) microspores cultured in anthero. *Plant Cell Reports*. **18**(10): 858–862.

Blakelsee, A.F., Belling, J., Farhnam, M.E., and Bergner, A.D.1922. A haploid mutant in the Jimson weed, Datura stramonium. Science **55**:646-647.

Chen, F.Q., D.Prehn, P.M. Hayes, D.Mulrooney, A. Corey, and H.Vivar. 1994. Mapping genes for resistance to barley stripe rust (Puccinia striiformis f. sp. hordei). Theoretical and Applied Genetics. **88**:215-219.

Friedt, W., Breun, J., Zuchner, S., and Foroughi-Wehr, B. 1986. Comparative value of androgenetic doubled haploid and conventionally selected spring barley line. Plant Breeding, **97**:56-63.