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ANTHER DERIVED HAPLOIDS

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Haploids are organisms with a single set of genome (n number). Since last 50 years, production and utilization of anther derived haploids have received much attention of scientists all over the world. Blakeslee in 1922 first reported haploidy in plants. India has done pioneering work in successful production of haploid embryoids. Guha and Maheswari of the Delhi University were the first to report successful production of haploid plantlets by *in vitro* culture of anthers. Nitsch (1969) was the first man to recognize the significance of haploids in crop improvement. Today, plant breeders and cytologists have realised the enormous potentialities of anther derived haploids in breeding programmes as well as in cytological and cytogenetical investigations.

Production of haploids from anther culture.

The first successful production of haploids was reported in *Datura*. Various species of *Nicotiana* has now become the most popular experimental material. Successful anther culture with haploid production has now been reported in several plant species.

Flower buds with anther containing young uninucleate or binucleate pollen are first surface sterilized Squash preparation of the anther could be done to ascertain the stage of the microspore. Individual anthers are then removed and incubated under aseptic condition for 3-6 weeks on a chemically defined media at temperature usually between 24-27° C to yield plantlets. Anthers containing uninucleate microspores have been found more receptive for the production of haploid callus and subsequent differenciation into plantlets. The development of the plantlets from the callus is influenced by the media. When a large number of plantlets are produced from one anther they can be separated and sub-cultured. After 3-6 weeks in the initial medium the plantlets are transferred to a secondary medium. The plantlets after proper development of root systems are transferred to pots.

Factors affecting the production of anther derived haploids

Various factors are associated with the successful production of haploids from anthers, such as genotype of the cultured plants, cultural media, technique, stage of microspore, conditions of anther donor plant, pretreatments adopted, cultural conditions and case of anthers during and after dissection.

Genotype of the cultured species:- The response of anthers placed in culture is influenced by the genotype of the plants. Successful production of haploids from different species is correlated with the genotype of the cultured species. Vyskot and Navak (1974) established significant genotypic effects haploid plantlet production in ten species of *Nicotiana*. It would appear that genotypes differ in the optimum level of concentration of the constitutents of the culture media and now several laboratories are investigating this aspect.

A plant breeder considering the use of anther derived haploids should be reasonably clear that gametic competition does not influence the production of haploid plants or their survival. Nataka and Kurihara (1972) however have reported that pollen competition may not influence genotype of the haploid plantlets.

Not all plantlets derived from anther culture exhibit the haploid chromosome number. Plantlets derived directly from pollen following a true embryo-genetic development are haploid individuals. This is the normal situation in species of *Nicotiana* and *Datura*. But plantlets regenerated from callus may not be true haploids. *Brassica. Oryza, Lycopersicon, Solanum nigrum Petunia, Lolium* etc. come under this category. This situation may be due to the occurence of endomitosis. It is well known that culture media also influence the level of ploidy. Some workers reported that a high concentration of IAA influences the process of endonitosis *Cultural media:-* The need for a proper culture medium for the successful production of plantlets cannot be overemphasized. Nutritional requirements for inducing cell proliferation in pollengrains vary greatly. Most species of tobacco can be cultured in the same type of medium. Sugar, iron and mineral salts are the necessary ingredients for the production of embryoids from pollengrains in tobacco. Coconut milk and plum fruit juice are also effective media in anther culture. Gbo-El-Nil and Hilderbrandt (1973) employed a method which includes a series of media. Their method consisted of an initial medium favouring callus formation, a second medium which favours shoot formation and a third one promoting root production. The culture of isolated pollen, rather than intact anther, is the recent approach.

Microspore stage: Introduction of anther containing microspore to the media at an optimum stage of development is an essential factor for the efficiency of haploid production. Anthers containing uninucleate microspores have been found most receptive for the production

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of haploid plantlets. In some cases anthers with two celled pollengrains also give rise to callus and haploid plantlets. A cytological squash preparation of the anther can be used for ascertaining the stage of the microspore

Conditions of plant supplying anther:- Anthers selected from mother plants maintained in a vigorous and healthy condition provide the best source material. Sunderland (1971) correlated the age of the plant with the efficiency of the anther culture. Tomes and Collins (1976) observed significant effect of the number of days the plant had been in flowering on the haploid plantlet production. It has been observed that anthers from plants grown under high light intensity yielded high output.

Cultural condition:- Sufficient intensity of light is necessary to avoid etiolation of developing plantlets. An increase in the percentage of anthers responding to haploidy induction in culture is reported by Sopery and Maheswari (1976) when the culture was transferred from dark to light conditions. Temperature is also a major factor influencing anther culture experiments. Sunderland (1971) reported that temperature in the range of 25°C favours haploid androgenesis in *Nicotiana*. However, Irikura (1972) reported that a relatively cool temperature in the range of 20°C favours *Solanum* species. Most species can tolerate a medium with a pH value between 5 and 6.

Handling of anthers during and after dissection is a very critical step in the entire proceedings.

Most haploids produced by anther culture are sterile and hence maintenance of the haploid line for the production of seed and further genetic manipulation of the lines requires the establishment of diploid condition. Diploid condition can be achieved in three ways (1) as stated earlier a low percentage of haploid plants revert to diploid condition spontaneously (ii) doubling of chromosome can be easily done by colchicine treatment. [This can be done either by treating the leaf axils or by immersing of the plantlets in colchicine solution. (Nataka and Tanaka, 1961; Burk, 1972)] and (iii) by *in vitro* application of colchicine in hayloid callus cultures.

Economic utilization

The anther culture offers greater opportunity to plant breeders as a rapid method of producing fertile and completely homozygous lines which can be used directly in crop improvement programme. Collins and Legg (1975) compared the anther derived doubled haploid lines with conventionally derived lines and found the variation among haploid lines comparable in magnitude to that observed among conventionally derived lines.

Melchers and Habib (1970) enumerated the potential use of anther derived haploids in plant breeding. Their suggestions included induced mutagenesis, determination of genetic ratios and the development of breeding lines with specific characteristics such as combination of several dominant genes. Doubled haploid lines offers a definite advantage over the conventional backcross method of breeding which is mostly employed in transfer of genes for qualitative characters. With the use of doubled haploid lines the time required for a routine back cross programme can be drastically reduced. Moreover, it offers and increased probability of retaining the character under transfer and more rapid stabilization of the transferred genetic material in homozygous form at the end of the back cross programme. Another advantage of anther derived haploids is that with smaller population the breeder can determine phenotypic ratios. For example a monohybrid F_2 phenotypic ratio becomes a 1:1 ratio instead of a classical 3:1 ratio and the F_2 ratio for duplicate factor inheritance become 3:1 instead of 15,1. This is because the investigator is actually dealing with a gametic ratio (Collins and Legg 1975). Homozygous diploid plants for special characters can be produced from anther derived haploids by introducing the gene for the desired character and doubling the chromosome compliment of haploids. Recently this technique has been employed to study the phage mediated transfer of genes controlling galactose metabolism from the bacterium *Escherichia coli* in the haploid tissue obtained from anthers of tomato (Doy, 1973). This technique offers immense scope for transfering nitrogen fixing genes to higher plants.

Selection of induced and spontaneous mutants from anther culture are means to create useful and new genetic variability. Haploids can play a major role in the identification and selection of auxotropic mutants for elucidation of biochemical pathways, in researches to select resistance for antibiotics' base-analogues and herbicides, in selection for adaptation against environmental stress, and for selection for metabolic overproduction.

Thus anther culture offers a rapid and economic, as well as efficient, method for producing homozygous lines and developing novel varieties. The breeder can be reasonably certain that the lines he has selected are homozygous when obtained by doubling the chromosome compliment of haploid plants. Anther derived haploids can also be employed in induced mutagenesis, genetic transformation, development of specialised cytogenetic stock etc which all are important in broadening the genetic base and crop improvement.

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RIDLEY CENTENNIAL AWARD FOR Dr. B. C. SEKHAR

The third Ridley Centennial Award was presented to Tan Sri Dr BC Sekhar, for his outstanding contribution to the natural rubber industry over many years. The Award was established by the Malaysian Rubber Producers' Council in 1977. The presentation of the award to Tan Sri Dr. Sekhar came the day after his retirement as Controller of Rubber Research of the Malaysian Rubber Research and Development Board. His distinguished career in the natural rubber industry started in 1949, when, having graduated from New Delhi University and Michigan University, USA, he joined the Rubber Research Institute of Malaysia as an assistant chemist. In 1966 he was appointed Director of the Institute and in 1974 Controller of Rubber Research and Chairman of the Malaysian Rubber Research and Development Board. In the 1970s he was a leading proponent of technically specified rubber, resulting in the now well-established Standard Malaysian rubber scheme. Since then his enthusiasm has made its mark in many areas, including price stabilization through the International Natural Rubber Organisation, the Malaysian Government's Dynamic Production Policy, and the effective co-ordination of natural rubber research through the International Rubber Research and Development Board (IRRDB). In conferring the Ridley Centennial Award the Minister of Primary Industries, Dato' Paul Leong, said that Tan Sri Dr Sekhar was 'another man of rubber with similar pioneering spirit and vision worthy of Ridley's memory.'