MICROSPORE CULTURE OR POLLEN CULTURE

 Isolated pollen grains when cultured in vitro gives rise to haploid embryos or callus and this approach is called pollen culture. Pollen may be isolated either by squeezing or float culture of anthers.

Squeeze culture

 About 50 anthers may be placed in 20 ml of medium and squeezed with a glass rod; the solution is filtered through a nylon mesh of suitable pore size and centrifuged at 500 – 800 RPM for 5 minutes. The pollen pellet is collected washed twice and suspended at a final density of 103 – 104  pollen /ml

Float culture:

 Excised anthers are floated on a shallow liquid medium in petridishes; the anthers dehisce in a few days releasing their pollen grains into the medium

 Initially isolated pollen grains were cultured either in hanging drops or on a filter paper raft placed on cultured anthers. Subsequently Nitsch and Co Workers first replaced the nurse tissue by an extract of cultured anthers and finally devised a completely synthetic medium for pollen culture the crucial ingredients of which were glutamine, L-Serine, and inositol

Slit technique

 Cutting the anther wall to release the microspore calluses/embryos rather than relying on natural dehiscence but this is a time consuming process eg. Tobacco, pennisetum

Advantages of haploids / anther culture / pollen culture

1. Haploid are useful in cytogenetic studies
2. Production of homozygous inbreds / isogenic diploids within a year as compared to the long inbreeding method which takes 4-6 years
3. To trace the parents of the hybrids
4. Haploids are valuable for mutation studies
5. By comparing the heterozygous diploid with haploid or homozygous diploid population recessive phenotypic characters can be identified
6. Development of pure lines and 100% male plants
7. Recovery of sexual inter specific hybrids between wild and domestic species (tomato)
8. For understanding the phylogenetic relationship between species the study of meiotic behaviour of haploid provide clues for chromosome duplication within a species
9. To study the inheritance pattern
10. Use of haploids in the production of monosomics, nullisomics, and other anueploids
11. Double haploid, that are homozygous and fertile are readily obtained enabling the selection of desirable gene combination

How to double the chromosomes in haploid?

Spontaneous duplication

 Homozygous diploid callus or embryoids may be formed by the spontaneous fusion of two similar nuclei of cultured pollen after first division. Haploid cells are unstable in culture and have a tendency to undergo endomitosis.

Induced duplication

 Colchicines treatment:- the young plantlets regenerated through anther culture are treated with 0.5% colchicines solution for 24 – 48 hours. Treated plantlets are replanted in the medium after through washing. In case of mature haploid plantlets , 4% colchicines paste may be applied to the axil of the leaves