Haploid culture

Haploid plants bear the gametic chromosome number of a species and are generally derived from gametophytic tissue that develops during the reproductive phase of plants. Gametophytes develop after meiosis both in anthers (microsporogenesis) and in ovules (megasporogenesis). Haploid microspores develop into pollen grains (male gametophytes), whereas haploid megaspores generate an eight-celled embryo sac (female gametophyte) bearing the egg cell. The union of a haploid sperm cell from the male gametophyte with a haploid egg cell from the female gametophyte upon fertilization produces a zygote that develops into an embryo, restoring the somatic chromosome number of a species. An interruption of normal sexual development, either natural or induced, causes the microspore or megaspore to undergo mitotic divisions without fertilization, eventually resulting in a haploid plant. Such extraordinary development of these gametophytic tissues can be induced in plant tissue culture, resulting in androgenesis or gynogenesis of higher plants.

Anther culture: Guha and Maheshwari first demonstrated the possibility of androgenesis by anther culture of *Datura innoxia*. Although anther culture has been the most successful means to obtain haploid plants, microspore (pollen) culture and ovule culture have also been successful, especially for plants where anther culture has failed. Anther culture (called **androgenesis**) is a technique by which the developing anthers from unopened flower bud are cultured on a nutrient medium where the microspores within the cultured anther develop into callus tissue or

embryoids that give rise to haploid plantlets (**androgenic haploid**) either through organogenesis or embryogenesis. Haploid plants develop from anther culture either directly or indirectly through a callus phase. Direct androgenesis mimics zygotic embryogenesis; however, neither a suspensor nor an endosperm is present. At the globular stage of development, most of the embryos are released from the pollen cell wall (exine). They continue to develop, and after 4 to 8 weeks, the cotyledons unfold and plantlets emerge from the anthers. During indirect androgenesis, the early cell division pattern is similar to that found in the zygotic embryogenic and direct androgenic pathways. After the globular stage, irregular and asynchronous divisions occur and callus is formed. This callus must then undergo organogenesis for haploid plants to be recovered.

Application:
- Production of haploid plants.
- Production of homozygous diploid lines through chromosome doubling, thus reducing the breeding cycle.
- Production of useful gametoclonal variations.

Ovule culture: An ovule is a megasporangium covered by integument. Ovule culture (**gynogenesis**) involves the development of haploid from unfertilized cells of embryo sac present in ovule. As with androgenesis, gynogenic haploids may develop directly or indirectly via regeneration from callus. Direct gynogenesis usually involves the egg cell, synergids, or antipodal cells with organized cell divisions leading first to the formation of proembryos and then to well-differentiated embryos. In indirect gynogenesis, callus may be formed directly from the egg cell, synergids, polar nuclei, or antipodal cells, or may develop from proembryos. Plants regenerated from callus may be haploid, diploid or mixoploid.

The relative scarcity of haploid cells within an ovule compared to the thousands generally found within anthers has made ovule culture, or gynogenesis, less attractive alternative to anther or microspore culture for developing haploid plants. However, for a few species including onion (*Allium sativum*), ovule culture has been successful. San Noeum first reported successful ovule culture of barley.

Application:
- Production of haploid plants.
- Recovery of hybrid embryos overcoming embryo abortion at very early stages of development of zygote due to incompatibility barriers.
- Achievement of *in vitro* fertilization.