Factors influencing morphogenesis

Morphogenesis in culture proceeds along a number of pathways. Of them, two are major pathways - organogenesis and somatic embryogenesis. Organogenesis includes direct genesis of adventitious shoots or roots and indirectly *via* callusing. Embryogenesis also possesses two pathways where the outcome differs in the form "bipolar somatic embryos" which in later stage form individual plantlets. Several factors influence the phenomenon of morphogenesis considerably during culture. They are: genotypes, explant, growth regulators, nutrients, other additives and physical environment.

Genotype

In the plant kingdom, certain plant groups appeared to respond more readily in culture than others. Members of carrot family (*Umbelliferae*) are considered to be a group that can readily form somatic embryos in culture. However, differences in response were observed among the different species of a genus and different cultivars in a species. It is now well accepted that genetic factors contribute to the response of plant tissues in culture. Though there are reports of recalcitrance among plant species to culture, this problem can be successfully overcome by manipulation of explants, culture medium or culture environment.

Explant

Although all cells in a plant are considered totipotent, there are striking differences from cell to cell and from organ to organ within a plant to regenerate plants. In general, embryonic, meristematic and reproductive tissues appear to have greater potential for growth and morphogenesis in culture. For woody species, it is possible to regenerate some types of organs only when embryos or young inflorescences are cultured. The inoculum must comprise actively dividing cells or juvenile cells. It is a well known fact that physiological stage of the mother plant, its nutritional and environmental conditions would also affect the explant for morphogenesis. So the mother plant should be grown in a well controlled environment to get reproducible results even though some changes in endogenous rhythm are not avoidable.

Growth regulators

It is known that the control of morphogenesis in the majority of the cultures is largely a function of the exogenous auxin/cytokinin ratio. High concentrations of kinetin cause

shoot initiation, whereas high levels of auxin favour rooting. In somatic embryogenesis, auxin is required for induction of embryonic cells and maintenance of proliferative growth. Embryo formation can be induced by transferring the callus to less auxin medium or a medium lacking auxin. Plant growth regulators other than auxins and cytokinins have been shown to play an important role in the induction and control of morphogenesis. Gibberellic acid has been used most successfully to obtain rapid growth of shoot apices and somatic embryos into plants.

Nutrient medium

Components of nutrient medium play critical roles in controlling morphogenesis in culture. Effects of many inorganic and organic nutrients have been studied extensively. One of the most important components of the medium in effecting morphogenesis is the source and concentration of nitrogen. Supply of high levels of reduced nitrogen appears suitable to shoot formation and essential to somatic embryogenesis. This is supplied in the form of ammonium nitrate and sometimes substituted with amino acids such as glutamine, glycine and alanine and their amides. Presence of potassium in the medium enhances embryogenesis.

Other additives

Supplementation of medium with casein hydrolysate and coconut milk also favour the morphogenesis *in vitro*. Coconut milk has been employed extensively as a medium component for somatic embryogenesis.

Culture environment

Temperature, photoperiod, light intensity and osmotic concentration are other factors that may have determining role in organogenesis and embryogenesis. The optimum temperature for culture is 24 ± 2^{o}C. Low temperature treatment of explants prior to culture favours their regenerative ability. Light also exerts a strong morphogenetic effect on plants in culture. Usually cultures produce shoots but the period of lighting should be maintained according to the photoperiodism of normal environment. The blue region of the spectrum promotes shoot formation and red light favours rooting. In the light, the somatic embryos of carrot formed plants; in the absence of light etiolation occurred. Overall osmotic concentration of a medium can also exert a profound effect on

morphogenesis. Increased osmotic levels in medium enhance shoot and somatic embryo formation. The osmotic level can be increased by adding additional sucrose.

Loss of morphogenetic ability

Cultures *in vitro* capable of morphogenetic potential initially lose the ability if they are subcultured repeatedly. Such subcultures may bring the changes at genetic, epigenetic and physiological levels. Variation in ploidy level of cells cultured is the usual change occurring at genetical level. Such variations may be either polyploidy or aneuoploidy. Sometimes gene mutations also occur in the cultured cells.

The epigenetic level changes occurring in culture are partially stable but reversible. Habituation to a partial particular component may produce morphogenetic loss in *in vitro* culture. For example, the embryogenic cultures grown in auxin plus medium would produce somatic embryos when the cultures are transferred to auxin free medium. The continuous culturing of callus or suspensions would lose the morphogenetic potential. This may be due to higher concentration of endogenous auxin. But these cultures can be made to produce embryos by depleting endogenous auxin level. For this the medium should have activated charcoal which has the potential to absorb certain amount of auxin.

Reduced growth rate less friability and senescence of cultures are the changes that occur at physiological level. These changes are temporary and unstable. By providing optimum chemical and physical environment, such morphogenetic losses can be overcome. Thus there are many reasons for the loss of morphogenetic ability by cultures, but there are indications of number of techniques that will help to reduce, if not eliminate, the problem.

Culture vessel to soil

The cellular totipotency is exploited in basic and applied aspects of plant science. This potential is not blocked with mere demonstration of organogenesis or somatic embryogenesis, but effectively utilized in propagating and producing entire plantlets, similar to mother plant and new genotypes respectively. The success of this technique depends on the method followed to establish plantlets in the soil, which have been cultured in an entirely new environment. The method requires details on rate of

multiplication of a particular explant and the rate of establishment of regenerated plantlets in soil. Adequate knowledge on manipulation of media, explant and culture environment to maintain the rate of multiplication at maximum is available. Having obtained a large number of regenerated plantlets, it is customary and necessary to transfer them to natural conditions. This is a critical period since the plantlets removed from the controlled environment of test tube or flask is going to face the real world. Under *in vitro* conditions, the plantlets have a carefully controlled supply of nutrient, humidity, temperature and photoperiod. The high humidity prevailing under culture conditions induces rapid shoot growth and proliferation. During this time, cuticle coverings of leaves and root hairs are poorly developed. If such plants are transferred to natural conditions, there would be substantial loss of water and desiccation due to cuticular and stomatal transpiration. So care must be taken during transfer of plantlets from *in vitro* condition to natural condition. Important points to be considered during transfer of plantlets to soil are:-

- Plantlets should be allowed to develop a good root system. The cultures with shoots may be transferred to a medium containing a weaker auxin for the better rooting.
- If the plantlets have been grown on agar-solidified medium, the agar may be removed by gentle washing with warm water.
- Damage to the root system should be avoided.
- After washing, the plantlets may be kept under higher intensity of light than the intensity of culture room for five to six days.
- The plantlets are then carefully planted in small plastic cups and the young roots surrounded with fine sand. It is better to sterilize the peat soil mixture in an autoclave to eliminate microbial pathogens.
- The small potted plantlets should be transferred to a controlled environment chamber, where control of light, temperature and humidity are possible.
- Then plantlets may be kept in mist chamber for increasing periods of light and temperature. During this hardening period, the plants will develop normal cuticular system with good rooting.

The above mentioned steps make regenerants to grow under natural conditions is collectively called as **hardening** and this process enhances the plant survival after transplanting.