PLANT TISSUE CULTURE

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. Abstract

Plant tissue culture techniques are the most frequently used Biotechnological tools for basic and applied purposes ranging from Investigation of plant developmental processes, functional gene studies, commercial Plant micropropagation , generation of transgenic plants with specific industrial and agronomical traits,plants breeding and crop improvement, virus elimination From infected material to render high quality healthy plant material, presevation and conservation of germplasm of vegetative propagated plant crops, and rescue of threatened or endangered plant species. The significant effect of some factors such as medium components, phytoharmoes , explant type, and light on the regeneration ability of explant , recent reports evidence the involvement of Molecular singnals in organogenesis and embryogenesis responses to explant wounding , induced plant cell death, and phytoharmoes interaction. The cultured cell and tissue can take several pathways. The pathways that leads to the production of true-to-type plants in large number are the preferred ones of commercial multiplication. The process of micropropagation is usually divided into several stages., propagation , initiation of explants , subculture of explants for proliferation, shooting and rooting, adhardening. This stage are universally applicable in large-scale multiplication of plants. The delivery of hardned small micropropagated plants to growsers and market also requires extra care.

• **Key-words :-** Large scale propagations; metabolic engineering; plant cell culture; micropropagation; embryogenesis; mutliplication of cells; pathways.

• **Introduction**

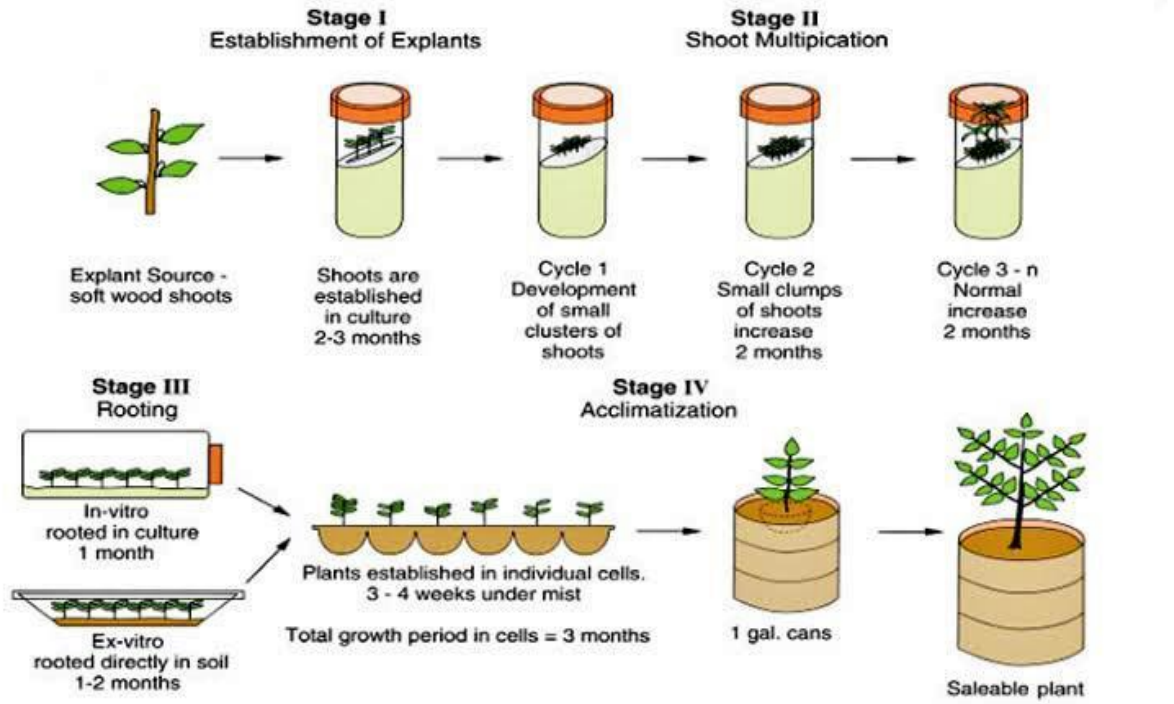
Plant tissue culture is a broad term that refers to the culture of any part of the plant (cell, tissue or organs) in artificial media, in aseptic conditions, and under controlled environment. The initiation of in vitro studies of plant cell and tissue culture dates back to 1902, when Gottlieb Haberland presented a “totipotency” hypothesis of each cell has all the genetic information needed to produce a perfect plants. The set of techniques emerged as an experimental approach to demonstrate the cell theory, which establishes that all living organisms are constituted of cells, the basic units of structure and reproduction, and also the totipotency concept, which is defined as the genetic potential of cell to generate an entire multicellular or organism. Several reports have shown the totipotent ability of plant cells through which the plant can be regenerated, which in turn Is widely used in several basic studies such as an micropropagation, Germplasm of conservation, and formation of genetically modified plants. Micropropagation commercially world wide, although the capability of plant regeneration Varies significantly varies in different genotypes.

The physiological state of the plant does have an influence on its response to tissue culture. The mother plant must be healthy and free from obvious signs of disease or pest. The shoot tip explants being juvenile contain a higher proportion of actively dividing cells. It is important to use quality mother plant stock to initiate cultures. The cultural conditions required to initiate and sustain plant cells in culture, or to regenerate intact plants from cultured cells, are different for each plant species. Each variety or clone of a species often have a particular set of cultural requirements. Nontraditional inducers such as some amino acids; light intensity and quality. weak electric current; and some antibiotics, for example, cefotaxime, avee also been reported to affect in vitro plant regeneration. Rathore

and Goldsworthy passed vera weak electric current 1 microamp between the tissue and the culture medium and noticed dramatic increases in tobacco callus growth. Azmi et al. Reported the beneficial effects of a mixed light color of LED (red and blue) on in vitro plant regeneration of Rosa kordesii. This review covers novel findings of how plants adjust regeneration in terms of the cellular, molecular and physiological aspects and discuss influence of developmental and environmental factors on plant regeneration efficiency.

1. Micropropagation :-

Micropropagation or in vitro clonal propagation is one of the most current extended commercial applications of tissue culture. Plant tissue culture is an excellent tool for the asexual multiplication of those species that are naturally reproduced asexually, but it is also used to overcome some problems of germination of seeds in different plant species; for example, recalcitrant species are particularly characterized for their short-seed viability (recalcitrant seeds), and therefore, asexual multiplication is a good alternative. Although tissue culture can be applied for the micropropagation of almost any plant species, it is recommended only for those that are economically profitable. Among the plant species that are currently micropropagated at the commercial level, the ornamentals occupy the first place.



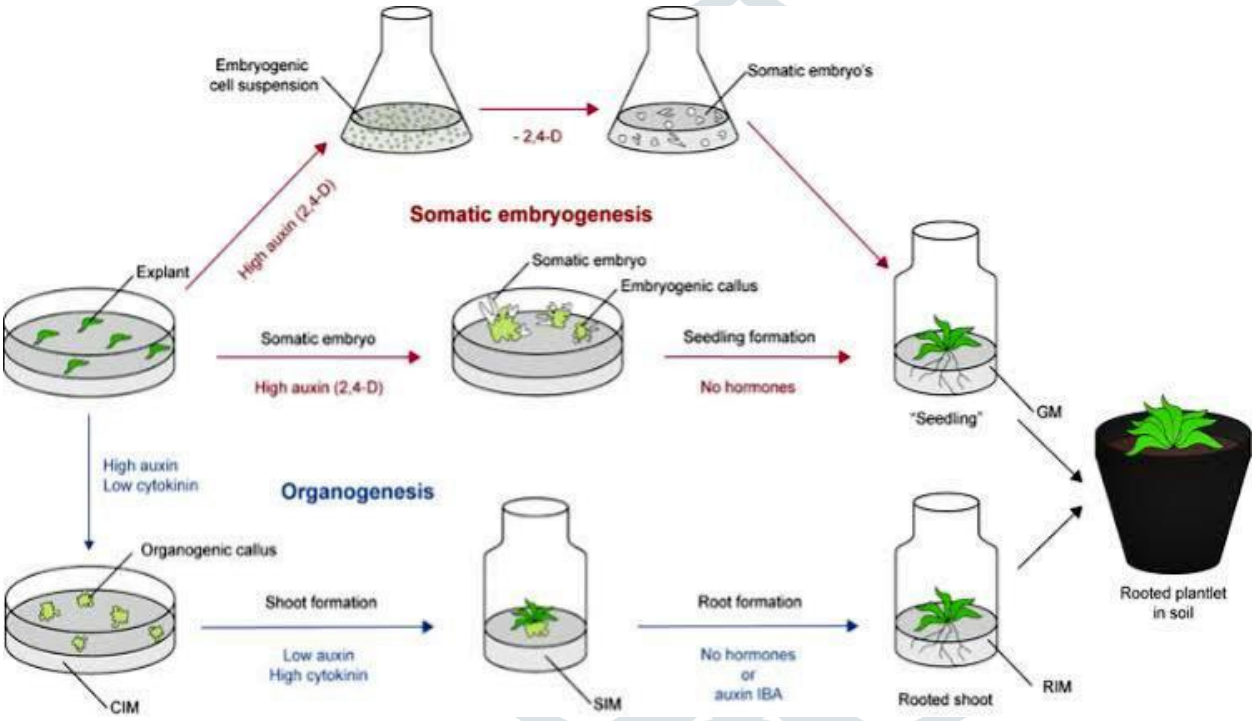
***2.* Organogenesis** :-

Plant shoots and roots are able to retain their apical meristem functions even after a part of their meristems is removed. However, when the whole meristems are excised, plant cells of differentiated tissues or organs have the ability to produce new shoots and lateral roots via organogenesis. In vitro plant regeneration by organogenesis is the result of organ formation through dedifferentiation of differentiated cells and reorganization of cell division to create particular organ primordia and meristems after the vascular connection between the explant and the newly regenerating organ.

3. Somatic embryogenesis :-

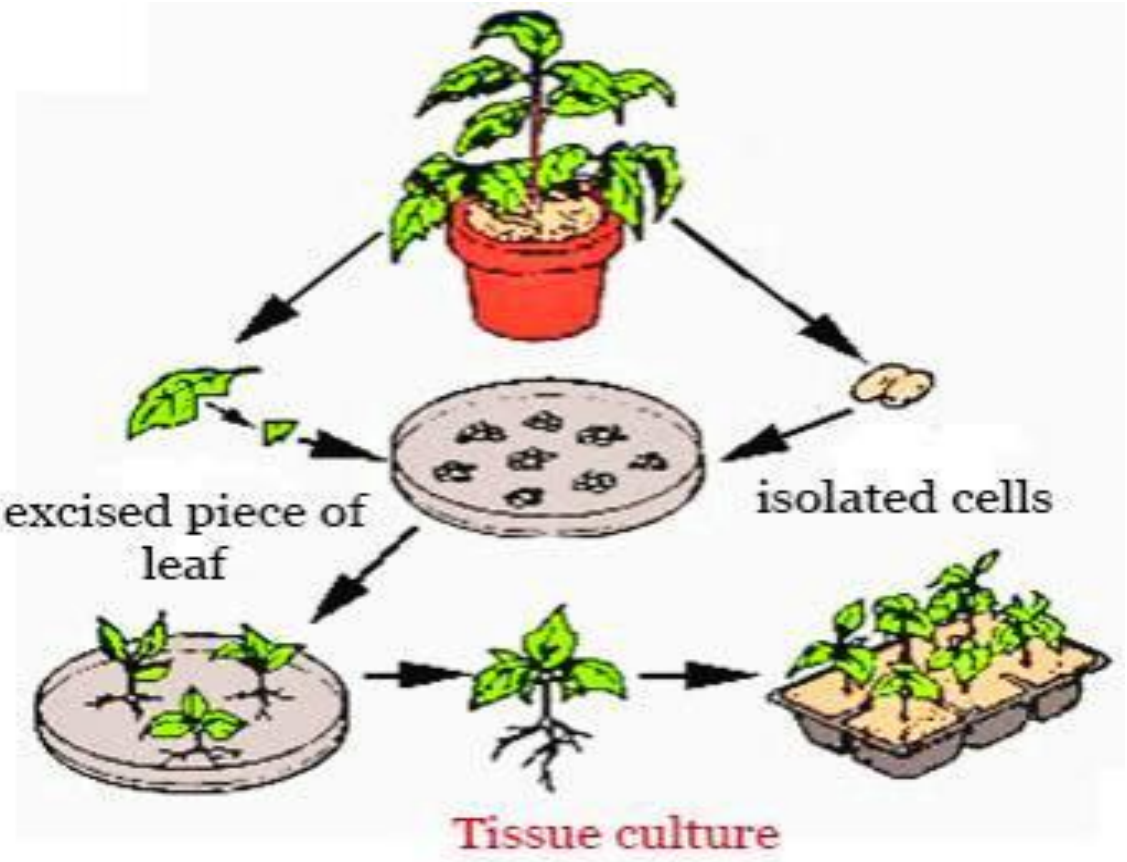
Somatic embryogenesis is one of the biotechnological techniques for multiplication of important economic cultivars. This process is a type of plant cell totipotency in which embryos arise from somatic or vegetative cells if no fertilization takes place. Several factors such as the origin of the explant, culture medium, and in vitro environmental conditions affect the success or failure of the somatic embryogenesis response. Somatic cells undergo embryogenesis stages by developing structures similar to zygotic embryos without merging of gametes.

For plant crops that are difficult to breed or have a poor genetic basis, somaclonal variation can be a very useful option for breeders as a new option. Indirect plant regeneration is carried out by organogenesis or embryogenesis in two steps.In the first step, callus is induced, followed by the second stage, in which the shoot meristems or somatic embryos are initiated from the callus tissues, resulting in an organ formation. Choosing the right explant, medium, phytohormones, genotype, carbohydrate, and gelling agent, as well as some other agents such as light regime, temperature, and humidity, noticeably affects organogenesis and embryogenesiss processes. Shoot clumps can be regenerated from shoot tips or bud stems that have only one bud, various mature somatic tissues, pollen, andprotoplast. Protoplasts possess the ability to develop new cell wall and to regenerate complete plants when grown in an appropriate culture medium. Crop improvement could be facilitated by genome editing in regeneration from protoplastss . By genome editing, it is possible to modify genome sequences as well as modify the arrangement of gene expression patterns in a pre-specified area of an organism. Genome editing covers wide spectra of techniques applying either a site-specific recombinase (SSR) or site-specific nuclease (SSN) system. Genome editing is speedy with a very low hazard of unforeseen effects, and can be employed with any crop, even those that have complex genomes and are difficult to breed.



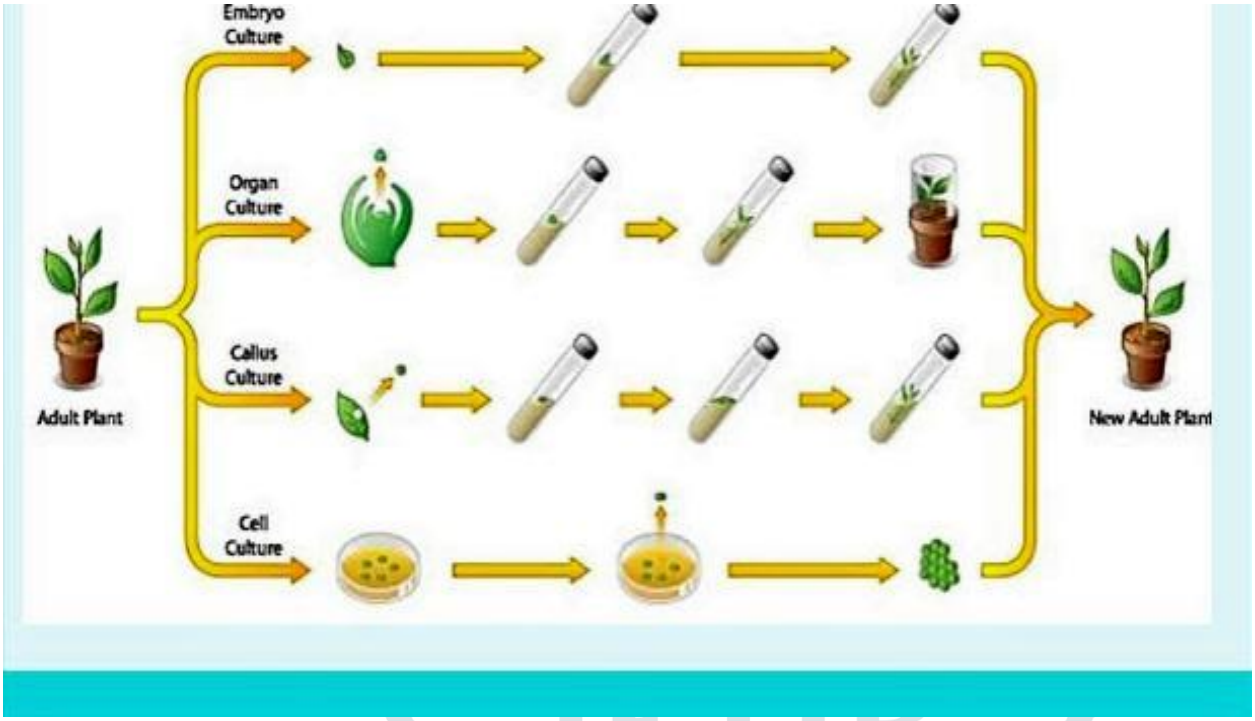
4. Genetic engineering :-

Plant genetic engineering is possible thanks to the use of plant tissue culture systems combined with recombinant molecular biology techniques. The goal of plant genetic engineering is to manipulate genetic material from different organisms in such a way to have specific sequences coding for specific genes that confer particular characteristics when they are introduced and integrated into a plant genome. Once a gene of interest is isolated, a construct is prepared in an appropriate vector to carry out the genetic transformation using either biological (Agrobacterium tumefaciens-mediated infection) or physical methods (usually microparticle bombardment). Genetic transformation has been achieved with important crops such as corn, wheat, cotton, rice and soybean, among others, and millions of hectares are currently planted with transgenic crops resistant to pests or herbicide.



5. Plant Tissue Culture :-

Genomicss (the study of gene structure, function and regulation, and related techniques), transcriptomics (the study of the transcriptome or the set of genes that are transcribed in an organism), proteomics (the study of the set of proteins translated in an organism), and metabolomics (the study of all metabolites present in an organism) have become essential for the study of biological processes in plants. The knowledge on plant genomes, transcriptomes, proteomes, and metabolomes has impacted favorably in the comprehension of complex developmental processes, such as in vitro organogenesis, embryogenesis, or dedifferentiation, and the genetic changes induced during in vitro conditions. Additionally, metabolomics can be very useful to investigate secondary metabolism not only during morphogenetic processes but mainly in cell, tissue, and organ cultures of plant species producing secondary metabolites of industrial and pharmaceutical interest.



6. Cellular Origins and Plant Regeneration :-

The cellular behaviour studies are very important in plants to differentiate between embryogenic and nonembryogenic calli. Taha and Wafa investigated cellular behaviour to detect the somaclonal variations in vitro. However, cellular behaviour in regenerates and intact plants needs to be evaluated to determine the occurrence of somaclonal variation in the plant regeneration process.

6.1. Changes in Cellular Behaviour during In Vitro Plant Regeneration

Plants possess a greater cellular plasticity than those observed in the other organisms, which dramatically guarantees the cell's ability to regenerate. Recent findings on plant tissue and organ regeneration indicate that a cell may commence follow four regeneration process including cell death, division, dedifferentiation, and trans-differentiation. These studies have outlined comprehensive perspectives of regeneration at the cellular level and help a lot to know the regenerative capacity of plant cells.

6.2. Programmed Cell Death in Plants :-

Programmed cell death (PCD) in plants often occurs as a result of DNA damage, showing

autolytic features, and has a noticeable role in the induction of tissue and organ regeneration. However, the underlying mechanisms responsible for these mechanisms remain largely unknown. Induction of PCD takes place by some plant-specific transcription factors such as *SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1)* and *ETHYLENE RESPONSE FACTOR115 (ERF115)-PHYTOCHROME A SIGNAL* TRANSDUCTION1.Thee induced plant cell death accelerates regeneration responses, which in turn changes the expression of genes involved in cell division process, resultingg in enhanced cell division. Although it is not clear yet how regenerative cells are induced in response to the cell death, mechanical disarray caused by cell death, affecting orientation in cell division of appending cells, reinforces the possibility of mechanical regulation in regeneration process. Any cellular modifications to reduce specialization are called dedifferentiation, whereas transdifferentiation is defined as the jump from one type of specialized cell to another type. Nguyen and McCurdy asserted that dedifferentiation could be part of transdifferentiation. Because of the property of callus ss proliferating mass of dedifferentiated cells, dedifferentiation is strongly associated with callus formation.

6.3Cell Fate Reprogramming and Pluripotency Acquisition :-

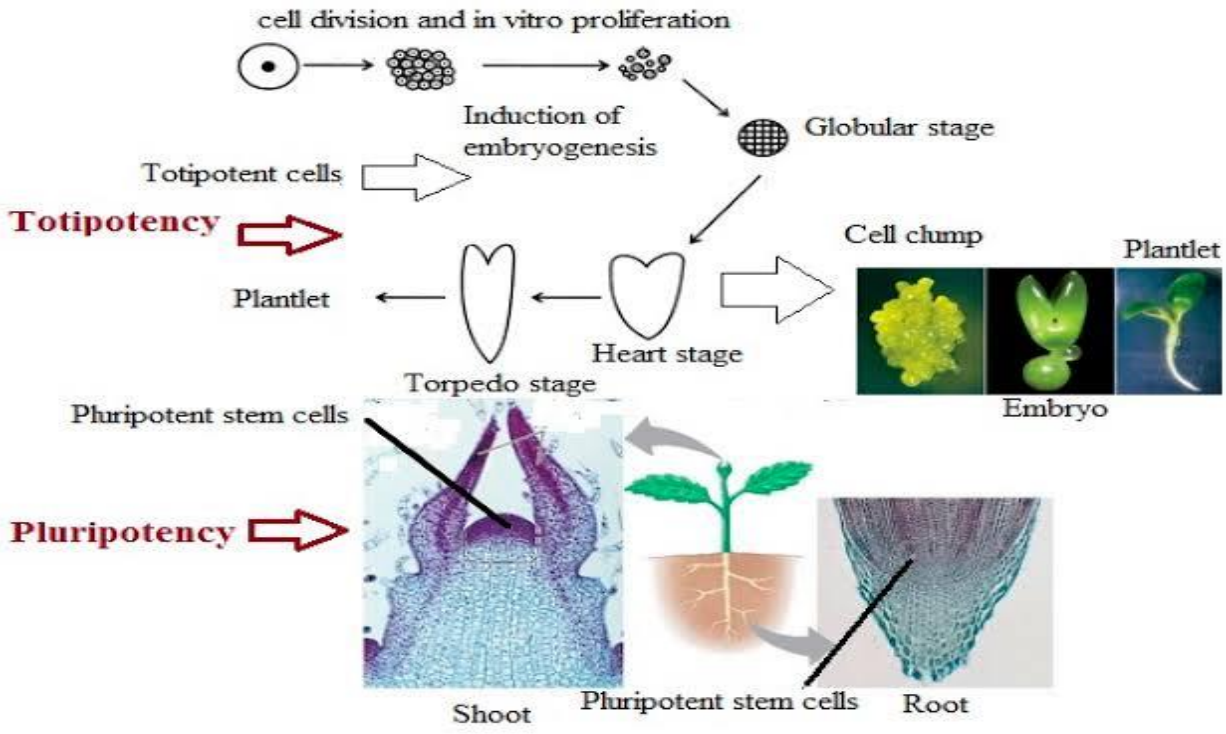


Figure . Totipotency and pluripotency in plant regeneration

Pluripotency is defined as the ability of unique cells in the plant's meristems to become an adult organism in response to environmental agents. Pluripotent cells are present in the root and shoot apices, where they create cells and tissues, but do not have the capability to create an embryo. Vice versa, under different circumstances, a somatic plant cell can dedifferentiate to generate a totipotent embryogenic cell that has the capability to produce an embryo. According to Ikeuchi et al., plants' regeneration process is performed throughout two distinct cellular strategies. One is by reactivating cells that are not sufficiently differentiated, and the other is by reprogramming them into somatic cells. In both cases, regeneration relies on the phenomenon of cellular flexibility, which can be widely specified as the capability to redefine cell fate. Recent findings have demonstrated that finally differentiated cells can be reprogrammed into pluripotent cells, which corroborate the reversibility of cell differentiation. Therefore, modulation of signaling pathways may enhance somatic cell reprogramming. However, the mechanisms by which somatic cells dedifferentiate into pluripotency are still unknown and need to be

6.4. Wound Responses and Signaling during Plant Regeneration :-

Wounding in the explant is the first incident in plant regeneration. Wound signals suchas electric current, hydraulic pressure, Ca2+, reactive oxygen species (ROS), oligopeptide system, oligosaccharides, jasmonic acid, salicylic acid, ethylene, abscisic acid, and changes in various metabolic processes of plant metabolism play a very important role in the regeneration process.The results of analysis of the genes downstream of wound signaling indicated that wounding significantly affects plant regeneration. Wounding possesses intricate biological impact and has multiple tasks in plant regeneration, but how the wound re-activates cell proliferation and accelerates cellular reprogramming is not very clear yet and needs to be addressed more than ever to clarify all aspects of these process.

7. Future Perspectives :-

Plant cell, tissue, and organ cultures have been applied to a range of different purposes including micropropagation, which is the most extended and successful application at commercial level and surely will continue in the future, and genetic engineering of important crops to confer tolerance mainly to pests and herbicides enabling the increase in production and yield with less applications of toxic insecticides and herbicides in millions of hectares worldwide. A significant impact is predicted in the production of different transgenic crops resistant or tolerant to drought, salinity, or cold under these stress conditions in the near future. Additionally, genetic transformation will be certainly a strategic tool for facing the global warming and its consequences by generating transgenic plants resistant to abiotic factors.

Genetic engineering is still expected to contribute to the development of transgenic crops with increased nutritional or nutraceutical value or resistant to diseases caused by fungi, bacteria, or viruses. Plant metabolic engineering contribution to the development of more metabolically efficient crops or with modified biochemical pathway leading to the production of commercial secondary metabolites has been slow and modest, but it should have great promise to regulate the biosynthesis of target diverse secondary metabolites of industrial and pharmaceutical interest. Much more difficult is to evaluate quantitatively the impact that tissue culture has had or will have on plant breeding and crop improvement using embryo rescue, double-haploid generation, or somatic hybridization, but of course they will be contributing to get improved hybrid crops to increase productivity.

The development of high-throughput genome and transcriptome sequencing techniques, the application of protein separation and sequencing, and the improvement of extraction, separation, and identification of metabolites, as well as the availability of data in public databases, have helped to decipher genome organization, gene function and regulation, and prediction of protein function and to know the set of metabolites produced in different plant species. Omics have therefore become fundamental tools for the study of basic biological processes in plants. Integration of omics is desirable for a better understanding of whole biological phenomena. It is evident that omics will be of great benefit to investigate in vitro morphogenetic processes and should facilitate the establishment of more efficient in vitro plant regeneration protocols if master control genes of differentiation and development are identified and characterized.

On the other hand, the combination of different omics should enable the metabolic engineering of interesting biochemical pathways in order to manipulate specific characteristics for the optimization and production of secondary metabolites of industrial and pharmaceutical importance.

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