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In Vitro Studies of Ficus carica and its Application in Crop Improvement

Brij Mohan Singh[1], Chandra Mohan Rajoriya [2], Irshad Ahmad Wani[3], Rajveer Singh Rawat[4], Dr. Bhanwar Lal Jat[5*]
[1]*Department of Botany, SPC Govt PG College Ajmer, Rajasthan, India*
[2]*Research Scholar, Department of Geography, MDS University, Ajmer, Rajasthan, India*
[3]*Department of Botany, Bhagwant University Ajmer, Rajasthan, India*
[4]*RV Book Company, Ajmer, Rajasthan, India*
[5]*Department of Agriculture Biotechnology, Bhagwant University Ajmer, Rajasthan, India (Corresponding Author)*

Abstract: ***Recent advances in cell culture and molecular biology of higher plants, which are key components of plant biotechnology, have demonstrated the considerable power and potential of these technologies in the genetic modification and improvement of plants that cannot be accomplished by conventional genetic methods. This has stimulated a great deal of interest and activity in university as well as corporate research laboratories. Nevertheless, the fact remains that most of the success achieved so far has been with model plant species and the transfer of these new technologies to major crop species that are the principal targets of biotechnology has either been slow and difficult, or is non-existent. In order to have any meaningful impact on agriculture the developing biotechnology must be equally and readily applicable to important crop species. The cereals and grasses, which constitute the most important group of crop plants, have until recently been found to be very recalcitrant to cell culture techniques. It highlights the success achieved in establishing totipotent callus and cell suspension cultures, and reports the development of protoplast culture systems yielding somatic embryos and plants and the recent recovery of somatic hybrid cell lines and genetically transformed cell lines. The importance of the age and physiological state of the explant and the relative genetic stability of embryogenic cultures and regenerated plants is discussed. Proliferation of the cultured shoot apex started through the first two weeks of incubation in the culture room. Single shoots developed from the cultured apex reached 3- 4 cm tall during four weeks of incubation. No abnormalities were observed in the newly initiated shoots, and no callus was associated with their development. The initiation stage was thus terminated, and the new shoots were transferred to the multiplication stage. The shoots were divided into small nodal segments and cultured on fresh medium supplemented with various concentrations of BAP or kinetin. Enhanced Axillary shoot proliferation was observed in the shoot segments after 4-6 weeks in the media supplemented with BAP; however the number of new shoots varied according to BAP concentration. The number of branches per explant was highly influenced by the addition of BAP to the nutrient medium. The highest number of branches was achieved from the addition of 3.0 mg/1 BAP.***

Keywords: - Totipotency, Ficus carica, Proliferation, Shoot tip, Meristem.

I. INTRODUCTION

Plant tissue culture is not a separate branch of plant science like taxonomy, cytology, plant physiology etc. Rather it is a collection of experimental methods of growing large number of isolated cells or tissues under sterile and controlled conditions. The cells or tissues are obtained from any part of a plant like stem, root, leaf etc. This definition also extends to the culture of excised embryos and protoplast culture. Which are encouraged to produce more and more clones in culture and to express their totipotency? By this we mean that a non embryonic cell has the potential to dedifferentiate into an embryonic cell and then to develop into a complete new plant, if the environment is suitable. A root parenchyma cell, for example, may begin to divide and produce an adventitious bud and finally a mature, flowering shoot. All the genes for the production of the whole plant must exist in such dedifferentiated root cells .Totipotency is also illustrated by development of cultured callus tissue into new plants, and partial totipotency occurs when adventitious roots develop from stem cells and when xylem and phloem are regenerated from wounded cortex cells .In fact, totipotency might be advantageous to plants mainly because it provides them with a mechanism for healing wounds and reproducing vegetatively by cloning. In each of these examples of totipotency , several cells cooperate to form primordia from which the whole plant arises .Experiments in which the plants develop from single cells were pioneered by Frederick C. Steward and coworkers at Cornell University in the 1950s and are associated with his work on cytokinins .Steward found that single cells broke away from

pieces of callus derived from carrot root phloem .When conditions were changed ,single cells in the cell suspensions would occasionally divide to form multicellular embryoids .From these, new plants capable of producing seeds were formed. Cloning from single cells had been achieved. Even after Stewards experiments, there were some questions about whether single cells were totipotent because Stewards embryoids had always develop in the presence of many cells in suspension, although each plant apparently came from a single cell .Vasil and Hildebrandt (1956) answered this question by producing entire plants from isolated single cells. Nevertheless, the fact that some cells are totipotent does not prove that all cells have this property; in any tissue culture there are many cells that do not become embryos. Using this special characteristic of plants for large scale seedling production is known as plant tissue culture technology. Therefore to carry out the experiments using tissue culture techniques, a well equipped laboratory is first required. Cells or tissues are grown in different types of glass vitals containing a medium with mineral nutrients, vitamins, and phytohormones. Plant tissue culture is one of the most rapidly growing area of biotechnology because of its high potential to develop improved crops and ornamental plants .With the advances made in the tissue culture technology, it is now possible to regenerate species of any kind in the laboratory .To achieve the target of creating a new plant or a plant with desired characteristics, tissue culture is often coupled with recombinant DNA technology. The technique of plant tissue culture has already helped in the green revolution by improving the crop yield and quality. Many kinds of containers and container's lids were developed to improve ventilation and other growth conditions for plant tissue culture. Seedlings which have better growth capacity and higher survival rate in less production costs are obtained through all these improvements. The field finds a wide range of applications starting from mass clonal propagation to plant improvement, molecular biology, bio-processing as well as a basic research tool. It has advanced the production in forestry and agriculture to many folds. With the improvement in cultivation and management, the quantity and quality of anthurium plants has been enhanced. Ficus carica, the well known commercial fig, a member of Moraceae family is an important tree in many rural areas, it is native to Western Asia but distributed by man throughout the Mediterranean region (Morton, 1987), was one of the first plants ever to be cultivated by humans, it is a woody plant with highly varied forms, bushes, shrubs, small trees, very tall and large trees. Plants are usually monoecious with small flowers which they are all female and need no pollination in common fig, without petals and nectarines (Wang and Charles, 1991). The fruit color varies from yellowish-green to coppery, bronze, or dark-purple. The fruit is well known for its nutritive value and is consumed either fresh or dry worldwide and for their mild laxative activity and high alkalinity. Moreover, active ingredients derived from fig are used in various drug preparations. (Kirtikar and Basu, 1986). Fig trees are propagated via cutting of mature wood or grafts as a result of seeds are nonviable, this kind of vegetative propagation insures uniformity, relatively low multiplication rates because those materials can be obtained only from upright branches which results in poor rooting (Kumar et al., 1998). Therefore, multiplication by tissue culture techniques could be advantageous due to the production of high quality disease free, true-to-type plants independent of seasonal and other environmental conditions in a comparatively smaller space (Debergh and Zimmerman, 1991). The most important technique of micropropagation reported by various researchers using cultures of apical meristems and axillary buds to regenerate multiple shoots (Fraguas et al. 2004; Moon Kim et al. 2007; Al khaybari 2008; Flores- Mora et a/. 2009; Soliman et a/. 2010). Other important technique of Fig studies shown in callus induction and biochemical assessment of active compounds in fig calli induction from different parts in the plant (Nassar and Newbury 1987; Cormier et al., 1989). On the other hand, shoot was successfully regenerated from the axillary bud of mature trees (Deshpande et al. 1998), from apical bud (Kumar et al., 1998) and from the calli of stem segment (Jaiswal and Narayan 1985). The main objective in this study is to establish an in vitro shoot proliferation protocol for Ficus carica which is an important fruit in Kurdistan Region of Iraq. The result may lead to the develops a specific protocol for rapid shoot proliferation needed to be used for sustaining fig plantation in Iraq from genetically uniform and healthy plants that can be used for different objectives, in addition to identify the best medium for callus induction from the leaf segments. However, Ficus carica are prone to Ficus carica mosaic, an endemic disease that is widely distributed in most varieties and countries where Ficus carica are cultivated. Ficus carica mosaic can cause symptoms in both leaves and fruits. Infected leaves consist of various degrees of mosaic accompanied by yellow-green chlorotic lesions and deformation. Infrequently, similar chlorotic lesions also appear on immature fruits. As the disease progresses, fruits begin to drop prematurely and, in some cases, affected trees eventually die. To introduce Ficus carica cultivars that are susceptible to Ficus carica mosaic to establish new orchards, there is a need for certified healthy propagation material. A viable system for Ficus carica propagation through tissue culture has already been reported. Meristem culture has been used for production of virus-free plant material in many species, but in woody species, especially in Ficus carica, its use is rather limited. Sometimes, meristem culture fails to result in virus-free plants, so it should be combined with thermotherapy as an antiviral treatment. Thermotherapy has

been successfully used in virus eradication in many plant species. In our study, an attempt was made to obtain mosaic virus-free Ficus carica plant material through thermotherapy. This study included *in vitro* culture of shoot tips developed during the treatments, rooting and root formation. The plantlets obtained by this method were transplanted into vials and stored under high humidity conditions. *Ficus carica* leaves have also been a long standing remedy against hemorrhoids, and it is also known that phytosterols and some polyunsaturated fatty acids, such as linoleic acid also found in Ficus carica, are known for their anti-carcinogenic properties, another reason to believe that *Ficus carica* may exert anti-cancer effects. Recent studies reveal that *Ficus carica* latex may not only be good on the treatment of warts and other traditional herbal remedies but also exert certain anti¯inflammatory and anti-oxidant activity. *Ficus carica* latex was •used in some preparations to treat painful or swollen piles, insect sting and bites, but it was never thought that additionally this herb could also exert anti-inflammatory and anti-oxidant activity. The anti-inflammatory and the anti-oxidant activity of *Ficus carica* is probably exerted due to the presence of steroids and flavonoids for its free radical scavenging activity.

II. MATERIALS AND METHODS

A. *Laboratory Requirements for Tissue Culture*

1) *General Organization:* Localize each portion of the tissue culture procedure in a specified place in the laboratory. An assembly-line arrangement of work areas such as, media preparation, glassware washing, sterilization, microscopy, and aseptic transfers) facilitates all operations and enhances cleanliness. Media (tissue culture and nutrient agar) should be available. Laminar flow hoods should also be available.
2) *Glassware:* Use glassware that has only been used for tissue culture and not other experiments. Toxic metal ions absorbed on glassware can be especially troublesome. Wash glassware with laboratory detergent, then rinse several times with tap water and, finally, rinse with purified water.
3) *High-purity Water:* Use only high-purity water in tissue culture procedures. Double glass distilled Water or deionized water from an ion-exchanger are acceptable. Water should not be stored, but used immediately. Regular maintenance and monitoring of water purification equipment are necessary. Purified water for tissue culture can also be purchased.
4) *Plant Material:* Plants used in tissue culture need to be healthy and actively growing. Stressed plants, particularly water-stressed plants, usually do not grow as tissue cultures. Insect and disease-free greenhouse plants are rendered aseptic more readily, so contamination rate is lower when these plants are used in tissue culture procedures. Seeds that can be easily surface sterilized usually produce contamination-free plants that can be grown under clean greenhouse conditions for later experimental use.
5) *Aseptic Technique:* The essence of aseptic technique is the exclusion of invading microorganisms during experimental procedures. If sterile tissues are available, then the exclusion of microorganisms is accomplished by using sterile instruments and culture media concurrently with standard bacteriological transfer procedures to avoid extraneous contamination. Media and apparatus are rendered sterile by autoclaving at 15 lbs/inch2 (121°C) for 15 minutes. The use of disposable sterile plastic ware reduces the need for some autoclaving. Alternative sterilization techniques such as filter sterilization must be employed for heat-labile substances like cytokinins. Aseptic transfers can be made on the laboratory bench top by using standard bacteriological techniques (i.e., flaming instruments prior to use and flaming the opening of receiving vessels prior to transfer). Aseptic transfers are more easily performed in a transfer chamber such as a laminar flow hood, which is also preferably equipped with a Bunsen burner. If experimental tissues are not aseptic, then surface sterilization procedures specific to the tissues are employed. Common sterilants are ethyl alcohol or chlorox with an added surfactant. Concentration of sterilants and exposure time are determined empiric ally.
6) *A plant tissue culture laboratory:* The culture laboratory ideal organization will allow a separate room for each of the following functions:-Media preparation, aseptic procedures, incubation of cultures, and general laboratory operations. If one has the opportunity to plan an in vitro laboratory in advance, the component facilities should be arranged as a production line. The area involved with washing and storage of glassware should lead to the facilities for oven sterilization and media preparation. Materials should then move from autoclave sterilization to the aseptic transfer facility. After the aseptic operations, the cultures are transferred to incubators or controlled-environment chambers. The cultures should be in close proximity to the laboratory containing microscopes and facilities for evaluation of the results. Discarded and contaminated cultures are transferred back to the washing area. It is of the utmost importance to give careful consideration to the

arrangement of the aseptic procedures. Because some laboratories do not have a separate sterile room, some type of laminar flow cabinet or bacteriological glove box is required. This facility must be located in an area free from drafts and with a minimum of traffic. In addition, it should not be located in the vicinity of scientists from other research groups working with airborne microorganisms. Discarded cultures, as well as contaminated ones, are autoclaved briefly in order to liquefy the agar and to kill any contaminants. The culture glassware is easier to wash after the spent medium has been liquefied and removed. After scrubbing with a brush in a hot detergent bath, the glassware is rinsed repeatedly with tap water, and then given two or three rinses in distilled water. If an automatic dishwashing machine is used, a final rinse with distilled or demineralized water should be used to remove any possible traces of detergent. After washing, the glassware is oven dried prior to storage. Certain cell cultures require scrupulously clean culture vessels; therefore, a routine dishwashing program is inadequate. New glassware may release chemicals that are toxic to the cultured tissues. Although media preparation requires a balance sensitive to milligram quantities for weighing hormones and vitamins, a less sensitive scale may be used for weighing agar and carbohydrates. The media reagents should be shelved near the balance for convenience. A refrigerator in the media room is necessary for sowing stock solutions and chemicals that degrade at room temperature. A combination hot plate and magnetic stirrer is a time saver for dissolving inorganic reagents. Either a pH meter or pH indicator paper is required for adjusting the final pH of the medium. Relatively large quantities of single-and double distilled water must be available in the media room. Sterilization equipment is an integral part of media preparation. An electric stove is the most economical type of oven sterilization. Wet-heat sterilization involves either an autoclave or a pressure cooker. Some hormones and vitamins are sterilized by ultra-filtration. After sterilization of the culture vessels by dry heat and autoclaving the medium, the culture tubes are poured in the transfer chamber.

7) *Incubation of the cultures:* The freshly prepared cultures are grown under carefully regulated environmental conditions, i.e., temperature, light, and humidity. This is accomplished with an incubator, plant growth chamber, or controlled environment room. If cell suspensions are cultured, some type of shaker or aeration equipment will be necessary. Several engineering aspects should be c safety and convenience of the electrical onside red in designing a culture room: s temperature regulation; arrangement of the Y stem; air flow for uniform era shelving; and elimination of airborne contaminants.
8) *Shoot tip and Meristem Culture of Ficus carica:* The tips of shoots (which contain the shoot apical meristem) can be cultured *in vitro*, producing clumps of shoots from either axillary or adventitious buds. This method can be used for clonal propagation .shoot meristem cultures are potential alternatives to the more commonly used methods for cereal regeneration as they are less genotype-dependent and more efficient (seedlings can be used as donor material) The tips of shoots (which contain the shoot apical meristem) can be cultured *in vitro*, producing clumps of shoots from either axillary or adventitious buds. This method can be used for clonal propagation .shoot meristem cultures are potential alternatives to the more commonly used methods for cereal regeneration as they are less genotype-dependent and more efficient (seedlings can be used as donor material) .Healthy growing shoots of Ficus carica collected from, Rajori district of Jammu city in Jammu and Kashmir, in April, 2015. The shoots were thoroughly washed using tap water to remove dirty and dusts, followed by surface disinfestations by immersing in a mixture of 5% NaC1 solution containing few drops of Tween-20 surfactant. A gentile vacuum was applied for 15 min. to dislodge the air bubbles possibly mass captured within the tissues. Under aseptic conditions, the disinfectant was discarded and the shoots were rinsed 3 times' autoclaved deionized water. The shoot apex, consisting of apical Meristem, leaf primordial were excised and transferred to culture vessel containing (25ml) nutrient medium. Murashige and Skoog (1962) was the base medium used Cultivation of axillary or apical shoot meristems, particularly of shoot apical meristem, is known as meristem culture. Meristem culture involves the development of an already existing shoot meristem and subsequently, the regeneration of adventitious roots from the developed shoots. It usually does not involve the regeneration of a new shoot meristem. Shoot apical meristem lies in the shoot tip beyond the youngest leaf or the first leaf primordium.
9) *Explant:* The *Ficus carica* fruit with large fruit size 75-80g, black fruit flesh and a short neck, dense fruit flesh good for transportation, easy peeling, and a small ostiolum. The fruit does not show any cracking, has a pH of 4.55, contains 0.19% total acidity, 17.52% reduced sugars, and needs 32.000 growing de re to ripen .It measures upto about 100 in diameter and 250 gm in length. Thus a shoot tip of 100- 500 pm would contain 1-3 leaf primordia in additicm to the apical meristem. In practical, the shoot tips of Ficus carica tip to 1 nun are used, jot should be free from virus .Shoot tip culture is widely used for rapid clonal propagation for which much larger, e.g. 5-10 mm, explants are used. Therefore, most cases of meristem culture are essentially

shoot tip cultures. Nodal explants of various. Sizes are also commonly employed for rapid clonal propagation. When the objective is vegetative propagation, the size of shoot tip used for culture is not important. But when the explant is to free from a virus, it is essential that the apical meristem should be excised along-with a minimum of the surrounding tissue. The shoot tip of Ficus carica is cut into fine pieces to obtain more than one plantlet from each shoot-tip. In some species like cauliflower, pieces of curd (the inflorescence) are used, while in plants having underground stems shoot tips or tissue pieces bearing buds of such stems may be used. Generally, explants taken from actively growing plants at the beginning of growing season are the most suitable. These small pieces of shoot apices are sterilized by incubating them in a sodium hydroxide solution for ten minutes. Then the explant is thoroughly rinsed four times in sterilized distilled water. After that we transfer each explant to a sterilized Petri dish. We remove the outer leaves from each shoot apices of Ficus carica with a Pair of forceps. This lessens the possibility of cutting into the softer underlying tissue. Due to the removal of outer leaves of Ficus carica, the apex is exposed. We cut the apex with the help of scalpel and transfer only those less than l mm in length to the surface of the medium.

10) *Culture Medium:* In general, MS medium has been found satisfactory for most plant species. But In general, MS medium has been for some species a much lower salt concentration may be adequate or even necessary since the high salt concentration of MS medium may be deleterious or even toxic. Agar, gelled medium is the most widely used mainly for convenience. But in some s pieces, use of liquid medium is either necessary .The GR requirement depends on the stage of culture process, viz., (i) culture initiation, (ii) shoot multiplication, (iii) rooting of shoots and (iv) Transfer of plantlets to soil. Culture initiation consists of surface sterilization of explants and establishing them in vitro. The main feature of this stage is the detection and elimination/control of contamination; growth of explants may or may not occur. In cases of heavy contamination or endophytic contamination (bacteria/fungi present inside explant), a suitable antibiotic, e.g., trimithoprim, and/or fungicide, e.g. Bavistin, may be added to the culture medium. The Ficus carica medium consists of a combination of distinct concentrations of activated charcoal with benzyladdenine (BA), kinetin and gibberllic acid (GA_3) and wood with kinetin. The regular strength of woody plant y plant medium w illediumin combination with 0.5mg per lit kinetin was the best condition re k for shoot proliferation of Ficus carica .In addition the activated charcoal in the of illediumin completely inhibit shoot The inclusion proliferation. p benzyladdenine (BA) in the medium induces excessive callus formation as well as small and vertified shoots, while gibber . Acid (GA_3) induces excessive elongation associated with vertification, chlorosis and tip burned shoots.MS medium supplemented with vitamins, 100mg per liter myo-insitol, 30g per liter sucrose, 0.5mg per liter benzyladdenine (BA), 0.5mg per liter 2ip, and 2.5mg per liter phytogel in the presence of 100mg per liter ascorbic acid and 150mg per liter citric acid. After 2-3 weeks, the cultures are transferred to a shoot multiplication medium designed to promote axillary branching. This medium generally contains a cytokinin (usually, 1-2 mg/1, but up to 30 mg/1 has been used) either alone or in combination with an auxin (commonly 0.1-1.2 mg/1) chiefly depending on the plant species. BAP is the most commonly used cytokinin, but with some species. Among auxins, NAA, IBA and IAA are generally employed; 2, 4-D is not used as it promotes callusing. Higher concentrations (>2 mg/1 BAP) of cytokinin induce adventitious shoot buds, and retard shoot growth; the latter may necessitate a culture of shoots on basal/low cytokinin/GA_3 medium for shoot elongation before they can be rooted. Therefore, a GR combination should be determined to obtain optimum shoot multiplication rates with the minimum risk of adventitious shoot buds and, if Possible without the need of shoot elongation step (to save time, labour and cost).

11) *Environment during Culture:* During culture initiation and shoot multiplication phases of Ficus carica, the culture are generally kept at a constant temperature of 27°C and are illuminated with about 1,500 lux White' from fluorescent tube.' during rooting, higher light intensities, e.g., 3,000-10,000 lux, are commonly used since have a beneficial effect on rooting and •on plant survival on transfer to the soil. The minimum of sixteen hours of light is required.

12) *Browning of Medium:* In *Ficus carica* species, phenolics leach into the medium from the cut surfaces of explants. These phenolics turn dark brown on oxidation and are detrimental to the cultures. This problem is very common in case of all the woody species, particularly when explants are taken from mature trees. The problem of phenolics can be overcome, in Ficus carica species, in one of the following ways, but in some species like mango control of phenolics is the chief problem since the entire explant turns black and dies. (i) Frequent (every 3-7 days) subculture of *Ficus carica* explants on agar medium may be sufficient to overcome this problem in many species. (iii) A brief period (usually 3-10 days) of culture in liquid medium is effective in this species, viz *Ficus carica*. This may remove from the explants not only polyphenols, but other possibly inhibitory substances in order to check this oxidation of polyphenols an antioxidant like ascorbic acid (50-100 mg/1), cysteine-HCl (100 mg/1) or citric acid (150 mg/1) may be used. (iv) Adsorbents like activated charcoal (0.5-2 g/1) or PVP

(polyvinylpyrrolidon) may also be used to absorb the polyphenols secreted into the medium. (v) Culture in dark may be helpful since light enhances poly phenol oxidation.

13) *Rooting of Shoots:* As soon as the growing single leaf shoot obtained from the shoot tip of *Ficus carica* starts developing roots, we transfer them to hormone free medium. In general, the rooting medium has low salt, e.g., 1/2 or even 1/4 salts of the MS medium, and reduced sugar levels (usually 1 g/1), reduced salts being essential for rooting in some species of Ficus carica. In some species, e.g. Narcissus, strawberry, etc., rooting occurs on GR-free medium. But in most species, 0.1-1 mg/1 NAA or IBA is required for rooting. In plants like Citrus, however, a pulse treatment with an auxin (10 min with 100 mg/1 NAA or IBA) gives optimum rooting. Shoots are usually rooted in an agar medium, but the recent trend is to root them directly in vermiculite or potting mix. The cut ends of shoots are treated with a suitable auxin solution or rooting powder mix, transplanted in pots and kept under high relative humidity and low light intensity. This saves cost as rooting and soil transfer stages are combined and e rooting medium is eliminated. Rooting takes about 10-15 days, depending mainly on species. Plantlets with 0.5 to 1 cm roots are usually transplanted into pots since longer roots tend to get damaged during the transfer. For *Ficus carica*, different BA and GA_3 applications did not significantly affect the shoot formation however, the highest shoot development (63.1%) was obtained on medium with 0.2 mg/1 GA_3 + 2 mg/1 BA had a greater effect than GA_3 on shoot development. Long-term thermotherapy treated meristems showed the highest shoot formation. For root formation, there were no significant differences between the applications. However, the highest root formations, 55.6 %without BA or IBA Meristem culture on medium without thermotherapy treatment showed better root formation than treatments with thermotherapy.

14) *Transfer of Plantlets to Soil:* Rooted shoots from the cultures were planted in a (50% peat / 50% Perlite) Mixture in plastic trays. Relative humidity was adjusted to 96-99% during the first week before being gradually reduced over the next 2 weeks. Later, the plants with external environment by gradually opening there acclimated to plashes. At last that we transfer these plantlets to tunnels that covered the trays. At last that we transfer these plantlets to soil is the most critical step in micro-propagation. The plantlets are maintained under highly protected conditions *in vitro*, i.e., high humidity, low irradiance, low CO_2 levels and high sugar content. These conditions lead to some or several of the following anatomical and physiological consequences: (i) Poor cuticle and cuticular wax development, (ii) A greater stomatal density, (iii) Larger and more spherical stomata, (iv) Improper stomatal function so that they remain open even under severe dehydrating conditions, (v) Poorly developed vascular bundles in leaves, (vi) Lower chlorophyll contents, (vii) Reduced levels of proteins involved in harvesting light, (viii) Reduced Rubisco activity, and (ix) Lower net photosynthesis. Of these effects, those concerned with photosynthesis are due mainly to the high sucrose content of the tissue culture media. As a result, tissue culture-raised plantlets have to become photoautotrophic from their earlier photo heterotrophic state, following their transfer ex vitro. In addition, they have to become adapted to a lower humidity level and higher irradiance, which impose on them transpiration-induced water stress. Therefore, these plantlets show poor survival when they are transferred ex vitro. In view of this, tissue culture-raised plantlets are subjected to specific culture regimes aimed at making them capable of surviving the uncontrolled and harsher ex vitro environments; this is called hardening or acclimatization. Hardening procedures may be based on (i) modified nutrient media and culture conditions, (ii) chemical additives in culture media, or (iii) co-culture with microorganisms.

15) *Modified Nutrient Media and Culture Conditions:* Several strategies have been tried to promote acclimatization of plantlets, e.g., increased aeration of culture vessels by using caps made of gas permeable materials, increased light intensity, enhanced level of CO_2 in culture vessels, lower sucrose level in culture medium, and reduced humidity in culture vessels. There is considerable evidence that low sucrose content of medium, higher CO_2 levels in and increased aeration of culture vessels, and higher light intensities promote photo autotrophy, improve stomatal structure and/or function, and hasten cuticle deposition on transfer ex vitro and, thereby, improve plantlet survival. Low sucrose content in the medium favors' photo autotrophy, and inhibits ethylene biosynthesis; as a result, plantlet survival on transfer ex vitro is improved. High irradiance promotes survival mainly due to improved photo autotrophy and an early formation of cuticle. Similarly, enhanced CO_2 concentration in culture vessels also promotes autotrophy, but a combination of high irradiance and CO_2 levels seems more desirable. Similarly increased CO_2concentration is more effective under autotrophic (low sucrose in medium) than mixotrophic (high sucrose in the medium) conditions. For example, culture of rose plantlets under high CO_2 level (2 m mol/l) and light intensity of 100 m mol/m2/sec irradiance doubled the photosynthetic activity in the plantlet. A gradual reduction in sucrose content of medium is reported to favor photo autotrophy.

16) *Contamination Control:* Endophytic bacterial contaminations are usually managed by antibiotic treatments. An ideal antibiotic should be soluble, stable, unaffected by pH and the media, without side effects, broadly active, bactericidal, nonresistance-inducing, inexpensive, active in combination with other antibiotics, and nontoxic to human health. Carbenicillin, cephalosporin, gentamicin, polymixin, rifampicin, streptomycin, and Timentin are commonly used. Knowledge of the effects of antibiotics, on both bacteria and plants, is crucial for the elimination of contaminants and recovery of healthy plants. Attention should be paid since antibiotics may be inactivated by heat, light, the tissue culture medium, pH, etc. The continued use of antibiotics in the medium or repeated treatments with a single antibiotic may lead to bacterial resistance. Therefore, combinations of antibiotics may be used, but it should be ensured that the antibiotics are not antagonistic in action. Some other steps that would minimize contamination are as follows, (i) The stock plants used as sources of "Plants should be grown in greenhouse/growth chamber, (ii) The stock plants should be irrigated with filtered water rather than tap water, (iii) Freshly prepared solutions of surface sterilants should be used. Finally, (iv) that laboratory cleanliness and air source must be controlled, and the (v) operators must be strictly trained in aseptic techniques.

17) *Crop improvement through tissue culture:* Tissue culture plants of Ficus carica are diseases free and pest free and become available round the year and ensure uniform growth, early maturing and high yields. They also produce non-cracking fruits of uniform size. Plant tissue culture also comprises a set of in vitro techniques, methods and strategies that are part of the group of technologies called plant biotechnology. Tissue culture has been exploited to create genetic variability from which crop plants can be improved, to improve the state of health of the planted material and to increase the number of desirable germplasm available to the plant breeder. Tissue-culture protocols are available for most crop species, although continued optimization is still required for many crops, especially cereals and woody plants. Tissue culture techniques, in combination with molecular techniques, have been successfully used to incorporate specific traits through gene transfer. *In vitro* techniques for the culture of protoplasts, anthers, microspores, ovules and embryos have been used to create new genetic variation in the breeding lines, often via haploid production. Cell culture has also produced somaclonal and gametoclonal variants with crop-improvement potential. The culture *of* single cells and meristems can be effectively used to eradicate pathogens *from* planting material and thereby dramatically improve the yield *of* established cultivars. Large-scale micropropagation laboratories are providing millions *of* plants *for* the commercial ornamental market and the agricultural, clonally-propagated crop market with selected laboratory material typically taking one or two decades to reach the commercial market through plant breeding, this technology can be expected to have an ever increasing impact on crop improvement as we approach the new millennium. Tissue-culture techniques are part of a large group *of* strategies and technologies, ranging through molecular genetics, recombinant DNA studies, genome characterization, gene-transfer techniques, and aseptic growth of cells, tissues, organs, and in vitro regeneration of plants that are considered to be plant biotechnologies. The use of the term biotechnology has become widespread recently but, in its most restricted sense, it refers to the molecular techniques used to modify the genetic composition of a host plant, i.e. genetic engineering. In its broadest sense, biotechnology can be described as the use of living organisms or biological processes to produce substances or processes useful to mankind and, in this sense, it is far from new. The products of plant breeding and the fermentation industries (e.g. cheese, wine and beer), for example, have been exploited for many centuries. We no longer have to rely on pollination and cross-fertilization as the only ways to genetically modify plants. That the newer molecular and cellular technologies have yet to make a broad based significant impact on crop production is not surprising since a plant-breeding process of 10 to 20 years duration is still required to refine a selected plant to the stage of cultivar release. The applications of various tissue-culture approaches to crop improvement, through breeding, wide hybridization, haploid, somaclonal variation and micropropagation, are the subjects of this review.. Historically, selection of plants was made by simply harvesting the seeds *from* those plants that performed best in the field. Controlled pollination of plants led to the realization that specific crosses could result in a new generation that performed better in the field than either of the parents or the progeny of subsequent generations, i.e. the expression of heterosis through hybrid vigour was observed. Because one of the two major activities in plant breeding is manipulating genetic variability, a key Prerequisite to successful plant breeding is the varn availability of genetic diversity. It is in this area, creating genetic diversity and manipulating genetic variability, that biotechnology (including tissue-culture techniques) is having its most significant impact. In spite of the general lack of integration of most plant-biotechnology and plant-breeding programmes, field trials of transgenic plants have recently become much more common. There are therefore

reasons to believe that we are on the verge of the revolution, in terms of the types and genetic makeup of our crops that has been predicted for more than a decade. In almost all cases, some type of tissue-culture technology has been used to recover the modified cells or tissues. In fact, tissue-culture techniques have played a major role in the development of plant genetic engineering. Tissue culture will continue to play a key role in the genetic-engineering process for the foreseeable future, especially in efficient gene transfer and transgenic plant recovery

18) *Embryo Culture:* The most common reason for post -zygotic failure of wide hybridization is embryo abortion due to poor endosperm development. Embryo "kier has been successful in overcoming this major barrier as well as Solving the problems of low seed set, seed dormancy, slow seed germination, inducing embryo growth in the absence of a symbiotic partner, and the production of monoploids of barley. The breeding cycle of Iris was shortened from 2 to 3 years to a few months by employing embryo rescue technology. A similar approach has worked with orchids and roses and is being applied to banana and Colocasia. Interspecific and intergeneric hybrids of a number of agriculturally important crops have been successfully produced, including cotton, barley, tomato, rice, jute, etc.

19) *Protoplast Fusion:* Protoplast fusion has often been suggested as a means of developing unique hybrid plants which cannot be produced by conventional sexual hybridization. Protoplasts can be produced from many plants, including most crop species. However, while any two plant protoplasts can be used by chemical or physical means, production of unique somatic hybrid plants is limited by the ability to regenerate the fused product and sterility in the interspecific hybrids rather than the production of pro le Perhaps the best example of the use of protoplasts to improve crop production is that of Nicotiana, where the somatic hybrid products of a chemical fusion of protoplasts have been used to modify. The alkaloid and disease-resistant traits of commercial fusing al tobacco cultivars. Somatic hybrids were produced by plasts, using a calcium-polyethylene glycol treatment, from a cell suspension of chlorophyll-deficient N. rusfica' an albino mutant of N. tabacum . The wild N. rusfica parent possessed- desrable traits of high alkaloid levels and resistance to black root rot. Fusion products were selected as bright green cell colonies, the colour being due to the genetic complementation for chlorophyll synthesis the hybrid cells. Plants recovered by shoot organogenesis showed a wide range of leaf alkaloid content but had a high level of sterility. However, after three backcross generations to the cultivated N. tabacum parent, plant fertility was restored in the hybrid lines, although their alkaloid content and resistance to blue mould and black root rot were highly variable. Interestingly, neither parent was known to possess significant resistance to blue mould. The hybrids resulting from this study were found to be resistant to root rot nematodes and spider mites, important agricultural traits. However, they were also completely sterile and could not be incorporated into an aubergine-breeding programme. Two possible way Selection of hybrids and use of protoplast fusion for hybridization in ti‘°1) Plants has been reported in Brassicas, citrus, rice, carrot, tomato, and the forage legumes and clove have recommended that production of novel hybrids through protoplast fusion should focus on four areas:- (i) agriculturally important traits; (ii) achieving combinations that can only be accomplished by protoplast fusion; (iii) somatic hybrids integrated into a conventional breeding programme; and (iv) the extension of protoplast regeneration to a wider range of crop species.

20) *In the case of the Haploids:* Haploid plants have the gametophytic (one-half of the normal) number of chromosomes. They are of interest to plant breeders because they allow the expression of simple recessive genetic traits or mutated recessive genes and because doubled haploids can be used immediately as homozygous breeding lines. The efficiency in producing homozygous breeding lines via doubled *in* vitro-produced haploids represents significant savings in both time and cost compared with other methods. Three *in vitro* methods have been used to generate haploids:- (i) culture of excised ovaries and ovules (ii) the *bulbosum* technique of embryo culture and (iii) culture of excised anthers and pollen. At least 171 plant Species have been used to produce haploid plants by pollen, microspore and anther culture. These include cereals (barley, maize, rice, rye, and wheat), forage crops (alfalfa and clover), fruits (grape and strawberry), medicinal plants (Digitalis and Hyosc Yamusl ornamentals (Gerbera and sunflower), oil seeds (canola and rape), plantation crops (cotton, sugar cane and tobacco), and vegetable crops (cabbage, carrot, pepper, potato, sugar beet, sweet potato, tomato and wing bean).

21) *Somaclonal Variation:* In addition to the variants/mutants (cell lines and plants) obtained as a result of the application of a selective agent in the presence or absence of a mutagen, many variants have been obtained through the tissue-culture cycle itself. These somaclonal variants, which are dependent on the natural variation in a population of cells, may be genetic or epigenetic, and are usually observed in the regenerated Plantlets. Somaclonal variation itself does not appear to be a simple Phenomenon, and may reflect pre-existing cellular genetic differences or tissue culture-induced variability. The variation may be generated through several types of nuclear chromosomal re-arrangements and losses, gene amplification or de-amplification

non-reciprocal mitotic recombination events, transposable element activation, apparent point mutations, or re-activation of silent genes in multi gene families, as well as alterations in maternally inherited characteristics. Many of the changes observed in plants regenerated in-vitro have potential agricultural and horticultural significance. These include alterations in plant pigmentation, seed yield, plant vigour and size, leaf and flower morphology, essential oils, fruit solids, and disease tolerance or resistance. Such variations have been observed in many crops, including wheat, triticale, rice, oats, maize, sugar cane, alfalfa, tobacco, tomato, potato, and oilseed. The same types of variation obtained from somatic cells and protoplasts can also be obtained from gametic tissue. One of the major potential benefits of somaclonal variation is the creation of additional genetic variability in co adapted, agronomically useful cultivars, without the need to resort to hybridization. This method could be valuable if selection is possible in vitro or if rapid plant-screening methods are available. It is believed that somaclonal variants can be enhanced for some characters during culture in vitro, including resistance to disease pathotoxins and herbicides and tolerance to environmental or chemical stress. However, at present few cultivars of any agronomically important crop have been produced through the exploitation of somaclonal variation.

22) *Wide Hybridization:* A critical requirement for crop improvement is the introduction of new genetic material into the cultivated lines of interest, whether via single genes, through genetic engineering, or multiple genes, through conventional hybridization or tissue-culture techniques. During fertilization in angiosperms, pollen grains must reach the stigma of the host plant, germinate and produce a pollen tube. The pollen tube must penetrate the stigma and style and reach the ovule. The discharge of sperm within the female gametophyte triggers syngamy and the two sperm nuclei must then fuse with their respective partners. The egg nucleus and fusion nucleus then form a developing embryo and the nutritional endosperm, respectively. This process can be blocked at any number of stages, resulting in a functional barrier to hybridization and the blockage of gene transfer between the two plants, such as the failure of pollen to germinate or poor pollen-tube growth may be overcome using in vitro fertilization. Post-zygotic barriers (occurring after fertilization), such as lack of endosperm development, may be overcome by embryo, ovule or pod culture. Where fertilization cannot be induced by in vitro treatments, protoplast fusion has been successful in producing the desired hybrids. In vitro Fertilization has been used to facilitate both interspecific and intergeneric crosses, to overcome physiological-based self incompatibility and to produce hybrids. A wide range of plant species has been recovered through *in vitro* fertilization via pollination of pistils and self- and cross-pollination of ovules. This range includes agricultural crops, such as tobacco, clover, corn, rice, canola, poppy and cotton. The use of delayed pollination, distant hybridization, pollination with abortive or irradiated pollen, and physical and chemical treatment of the host ovary have been used to induce haploidy.

23) *Micro propagation:* During the last 30 years it has become possible to regenerate plantlets from explants and or callus from all types of plants. As a result, laboratory-scale micro propagation protocols are available for a wide range of species and at present micro propagation is the widest use of plant tissue-culture technology. Murashige (1990) reported that there were over 300 commercial operators World-wide in 1990. The role of micro propagation in crop improvement has been recently reviewed in considerable detail. Along with the impressive successes there are several limiting factors to its use. The cost of the labour needed to transfer tissue repeatedly between vessels. Problems of vertification, acclimatization and contamination can cause great losses in a tissue-culture laboratory. Genetic variations in cultured lines, such as polyploidy, aneuploidy and mutations, have been reported in several systems and resulted in the loss of desirable economic traits in the tissue-cultured products. There are three methods used for micropropagation:- (i) enhancing axillary-bud breaking (ii) production of adventitious buds and (iii) somatic embryogenesis. In the latter two methods, organized structures arise directly on the explant or indirectly from callus. Axillary-bud breaking produces the least number of plantlets, as the number of shoots produced is controlled by the number of axillary buds cultured, but remains the most widely used method in commercial micro propagation and produces the most true to-type plantlets. Adventitious budding has a greater potential for producing plantlets, as bud primordia may be formed on any part of the inoculum. Unfortunately, somatic embryogenesis, which has the potential of producing the largest number of plantlets, can only presently be induced in a few species. Nevertheless, the production of somatic embryos from cell cultures presents opportunities not available to plantlets regenerated by the organogenic routes, such as mechanization. One approach envisages the use of bioreactors for large-scale production of somatic embryos and their delivery in the form of seed tapes or artificial seeds. No commercial operation based on somatic embryogenesis exists but such embryogenesis is playing an important role in improving herbaceous dicots, herbaceous monocots and woody plants.

24) *Synthetic Seed:* A synthetic or artificial seed has been defined as a somatic embryo encapsulated inside a coating and is considered to be analogous to a zygotic seed. There are several different types of synthetic seed: somatic embryos encapsulated in a water gel; dried and coated somatic embryos; dried and uncoated somatic embryos; somatic embryos suspended in a fluid carrier; and shoot buds encapsulated in a water gel. No large-scale system for producing such seeds has yet been developed. Several applications for synthetic seeds have been reviewed. The use of synthetic seeds as an improvement on more traditional micropropagation protocols in vgetatively propagated crops may, in the long term, have Tissue *culture possess other applications in crop improvement also like a* cost saving, as the labour intensive step of transferring plants from in vitro to soil or field conditions may be overcome. Other applications include the maintenance of male sterile lines, the maintenance of parental lines for hybrid crop production, and the preservation and multiplication of elite genotypes of woody plants that have long juvenile developmental phases. However, before the widespread application of this technology, somaclonal variation will have to be minimized, large-scale production of high quality embryos must be perfected in the species of interest, and the protocols will have to be made cost-effective compared with existing seed or micro propagation technologies.

25) *Virus Eradication:-*Many Important plants contain systemic viruses which substantially reduce their potential yield and quality .It is, therefore important to produce virus free stocks which can be multiplied. Generally, highly meristematic tissue of a virus infected plant remains free from virus due to their fast mitotic activity. Therefore, shoot tip and meristems of a virus infected plant are the ideal explants to produce a virus free stock. This technique is also valuable for the maintenance of carefully defined stocks of specific varieties and cultivars in disease Free State. The size of the meristem explants is critical for virus eradication. Often so called meristem tip cultures have failed to eliminate virus infection because the explants contain shoot apices with vascular tissue instead of true meristem. This technique, combined with heat treatment (thermotherapy) or chemical treatment (chemotherapy) has proved to be very effective in virus eradication. Heat treatment is done by placing an actively growing plant in a thermotherapy chamber. Over a period of two weeks the temperature is increased to 38 degree inside the chamber and the plants are maintained at this temperature for two months. After that period, the apical meristem is excised, surface sterilized and transferred aseptically to agar medium. Using this technique 85% to 90% virus free plants have been obtained. Without heat treatment, shoot tips or meristems can be grown on chemotherapeutants added medium for virus eradication. Commonly used chemotherapeutants are 2,4-D,mela-chife green, thiouracil etc. Shoot tips or meristem culture have enormous horticulture value e.g in the production of plants for the cut flower industry when stock plants of registered lines must be maintained in as near-perfect condition as possible. Any infection by virus that affect the growth or physical characteristics of size and shape of flowers , is obviously very serious problem from commercial point of view .Meristem culture technique to clean up the stock could , therefore, avert a commercial disaster . Similarly, in the agricultural world, the production or yield of a crop can fall dramatically as a result of viral infection and render that particular variety no longer salable or commercial value. Meristem culture could be of value in restoring the original properties of the variety by removing the infection.

26) *Germplasm Preservation:-*One way of conserving germplasm, an alternative to seed banks and especially to field collections of clonally propagated crops, is in vitro storage under slow-growth conditions (at low temperature and or with growth-retarding compounds in the medium) or cryopreservation or as desiccated synthetic seed. The technologies are all directed towards reducing or stopping growth and metabolic activity. Techniques have been developed for a wide range of plants. The most serious limitations are a lack of a common method suitable for all species and genotypes, the high costs and the possibility of somaclonal variation and non-intentional cell-type selection in the stored material.

27) *Concluding Thoughts:-*Plant tissue-culture technology is playing an increasingly important role in basic and applied studies, including crop improvement. In modern agriculture, only about 150 plant species are extensively cultivated. Many of these are reaching the limits of their improvement by traditional methods. The application of tissue-culture technology, as a central tool or as an adjunct to other methods, including recombinant DNA techniques, is at the vanguard in plant modification and improvement for agriculture, horticulture and forestry. Although cultured roots are difficult to measure in situ during culture, an attempt will be made to approximate the linear growth rate. Daily measurements can be made on a single culture, within the flask, without exposure and risk of contamination. Place the flask on a sheet of graph paper, and align the root with the markings on the paper. Compare the growth rates of root cultures that have been stationary to the growth rates of other cultures that received agitation on a shaker. Effective utilization of cell and tissue culture methods in Zea mays research requires cultures capable of plant regeneration. These differentiated plants would provide a direct link with conventional genetic and

breeding procedures.

III. RESULTS AND DISCUSSION

In order to determine proper sterilization procedure, shoot tips were successfully sterilized by immersion in 3:1 chlorax: water coupled with slight vacuum, followed by washing the samples three times in deionized distilled water and surface sterilization was efficient in eliminating various pathogens such as bacteria and fungi, as well as prevention of browning of explants during fig tissue cultures by daily transfer to fresh media. Proliferation of the cultured shoot apex started through the first two weeks of incubation in the culture room. Single shoots developed from the cultured apex reached 3- 4 cm tall during four weeks of incubation. No abnormalities were observed in the newly initiated shoots, and no callus was associated with their development. The initiation stage was thus terminated, and the new shoots were transferred to the multiplication stage. The shoots were divided into small nodal segments and cultured on fresh medium supplemented with various concentrations of BAP or kinetin. Enhanced axillary shoot proliferation was observed in the shoot segments after 4-6 weeks in the media supplemented with BAP; however the number of new shoots varied according to BAP concentration. The number of branches per explant was highly influenced by the addition of BAP to the nutrient medium. The highest number of branches was achieved from the addition of 3.0 mg/1 BAP . While the lowest number of branches per explant was recorded in the control treatment. A reverse correlation can be found between the number of branches and the mean length of branches. This reduction in length may be attributable to the competition on nutrient media and space in cultures received high BAP concentration. The highest number of leaves per explant was recorded for the (3.0 mg/1 BAP) treatment. Which was significantly reduced .This has the ability to form callus. The data indicated that the highest survival percentage (100%) was obtained on MS medium supplemented induction with 10.0 mg/1 of each of BA and NAA, whereas the lowest one was found with 4 mg/1 2,4-D (66%). The highest callus formation percentage (86%) was obtained on MS medium supplemented with 0.2 mg/1 kinetin and 2.0 mg/1 2,4-D, which also increased callus weight . Callus was sub cultured monthly and maintained on the same medium. In this respect, MS medium containing 10 mg/1BAP and 10 mg/1 NAA formed callus from stem explants of *F. carica*. While, in case of Ficus carica, callus was obtained from leaf explant when cultured on MS medium containing 0.5 mg/1 2,4-D. Establishment of regeneration system. Regeneration via indirect somatic embryogenesis. The best concentrations of growth regulators to produce shoots from callus. Results show that the highest shoot formation percentage (83%) was obtained on MS medium supplemented with 30 mg/lit 2iP followed by 79% obtained on MS medium supplemented with 7 mg/1 TDZ in combination with 0.25 mg/1 NAA compared to the other treatments. Also, the best results of mean number of formed shoots per explant were produced on concentration of 30 mg/1 2iP and 7 mg/1 TDZ in combination with 0.25 mg/1 NAA This study aims to establish a regeneration and transformation system using both Agrobacterium tumefaciens-mediated and biolistic gun transformations for the local fig cultivar Sultani using the bar and the gus genes as selectable marker and reporter genes, respectively. Recent advances in cell culture and molecular biology of higher plants, which are key components of plant biotechnology, have demonstrated the considerable power and potential of these technologies in the genetic modification and improvement of plants that cannot be accomplished by conventional genetic methods. This has stimulated a great deal of interest and activity in university as well as corporate research laboratories. Nevertheless, the fact remains that most of the success achieved so far has been with model plant species and the transfer of these new technologies to major crop species that are the principal targets of biotechnology has either been slow and difficult, or is non-existent. In order to have any meaningful impact on agriculture the developing biotechnology must be equally and readily applicable to important crop species. The cereals and grasses, which constitute the most important group of crop plants, have until recently been found to be very recalcitrant to cell culture techniques. This article describes the advances made in cell culture of important members of this group of crop species. It highlights the success achieved in establishing totipotent callus and cell suspension cultures, and reports the development of protoplast culture systems yielding somatic embryos and plants and the recent recovery of somatic hybrid cell lines and genetically transformed cell lines. The importance of the age and physiological state of the explant and the relative genetic stability of embryogenic cultures and regenerated plants is discussed. Plant transformation is now a core research tool in plant biology and a practical tool for cultivar improvement. There are verified methods for stable introduction of novel genes into the nuclear genomes of over 120 diverse plant species. This review examines the criteria to verify plant transformation; the biological and practical requirements for transformation systems; the integration of tissue culture, gene transfer, selection, and transgene expression strategies to achieve transformation in recalcitrant species; and other constraints to plant transformation including regulatory environment, public perceptions, intellectual property, and economics. Because the costs of screening populations showing diverse genetic changes can far exceed the costs of transformation, it is important to distinguish absolute and useful transformation

efficiencies. The major technical challenge facing plant transformation biology is the development of methods and constructs to produce a high proportion of plants showing predictable transgene expression without collateral genetic damage. This will require answers to a series of biological and technical questions, some of which are defined. Plant tissue culture involves the culture of all types of plant cells, tissues and organs under aseptic conditions. This definition also extends to the culture of excised embryos and to protoplast culture. An overview of tissue culture techniques and their applications in plant propagation and genetic improvement of plants is presented. The areas under review include: (1) embryo culture, (2) meristem culture, (3) micropropagation, (4) somatic embryogenesis, (5) somaclonal variation, (6) *in vitro* selection, (7) anther culture and (8) protoplast culture. Problems and limitations of each of the techniques are also discussed. Examples are given of work that has been undertaken or that is currently in progress on the application of these techniques to the improvement of Queensland's subtropical horticultural industries. Key examples are: (1) embryo culture to facilitate incorporation of genes conferring disease-resistance from wild *Cucurbita* species into cultivated varieties, (2) meristem culture for virus elimination in strawberries *(Fragaria x ananassa)* and sweet potato *(Ipomoea batatas)*, (3) micropropagation for rapid increase in new varieties of ginger *(Zingiber officinale)* and pineapple *(Ananas comosus)* to enable more rapid field evaluation and early release, (4) micropropagation of disease-free, genetically uniform planting material of superior female papaya *(Carica papaya)* selections and banana *(Musa* spp.) selections and (5) the use of somaclonal variation and gamma-irradiation for the genetic improvement of banana. Finally, future opportunities for the utilization of tissue culture in plant propagation and improvement in Queensland's horticultural industries are summarized.

A. Micropropagation Industry in India

Micropropagation is the application of tissue culture technique to the propagation of plants starting with very small parts grown aseptically in a test tube or other suitable containers. Micropropagation is one of the key tools of plant biotechnology that has been extensively exploited to meet the growing demands for elite planting material in the current century. There exists a large demand for disease free clones of superior quality plants in ornamental, horticultural, and floricultural.

B. Role of Government in Plant Tissue Culture Industry

To encourage the tissue culture industry, various central and state government departments have framed several schemes and have announced incentives.

1) *Ministry of Agriculture:* The Department of Agriculture and Cooperation under the Ministry of Agriculture, Government of India provides financial assistance up to Rs. 21 lakhs and Rs. 10 Lakhs for setting up tissue culture units in public and private sectors respectively, subject to a maximum of 20% of the project cost. Under integrated development of fruits scheme, financial assistance in the form of subsidy, up to 50% is provided for purchase of tissue culture banana plants by various state Governments. The Government of India has set up a national facility for virus diagnosis and quality control of tissue culture plants at New Delhi with 5 satellite centers catering to the needs of the tissue culture industries in various parts of the country.
2) *Agriculture and Processed Food Products Export Development Authority (APEDA):* Under the Ministry of Commerce and Industry, state-of-the-art airfreight trans-shipment centre has been set up for tissue culture plants (perishables) at New Delhi, Bombay and Bangalore airports. Airfreight subsidy up to 25% of the freight cost is provided to tissue culture plants. 50% subsidy is given for the development of infrastructure like refrigerated van, packing, export promotion, market development, consultancy services, feasibility studies, organization building and human resource development. Financial assistance is also given for strengthening quality control facilities and implementation of ISO 9000.
3) *National Horticulture Board (NHB):* For setting up tissue culture lab there is a provision for back-ended capital subsidy not exceeding 20% of the project cost with a maximum of Rs. 25 lakh per project. Such subsidies are also extended to build up greenhouse and climate controlled poly house/shade house.
4) *Small Farmers Agri-business Consortium (SFAC):* SFAC under the Ministry of Agriculture gives soft loans up to 50 lakhs for setting up small tissue culture labs by co-operative societies formed by small scale farmers.
5) *Department of Biotechnology (DBT):* DBT supports research and development projects across the country at various laboratories in the universities and research institutions for development and standardization of tissue culture protocols. The private tissue culture units are entitled for expansion of existing units as a Phase II activity under a scheme called Small

Business Innovation Research Initiative (SBIRI). To promote the adoption of tissue culture technology by the industry and the end user, the department has established two micropropagation technology parks (MTPs) which provide a large number of service packages and have an important mandate of training and generating skilled manpower. The MTPs have transferred about 10 technologies to the industry and have also provided consultancy and taken up turn-key projects for various end users and state departments. The department has also set up a national facility for virus diagnosis and quality control of tissue culture raised plants, which are located at 6 different centers in India to ensure supply of disease free plants to the end users.

6) *State level incentives:* The states of Karnataka, Gujarat, Maharashtra, and Andhra Pradesh are giving financial assistance for setting up tissue culture units under the new agro-industrial policy. Karnataka gives capital subsidy of 20% on investments. All the above schemes have encouraged the establishment of tissue culture industry.

IV. CONCLUSION

Recent advances in cell culture and molecular biology of higher plants, which are key components of plant biotechnology, have demonstrated the considerable power and potential of these technologies in the genetic modification and improvement of plants that cannot be accomplished by conventional genetic methods. This has stimulated a great deal of interest and activity in university as well as corporate research laboratories. Nevertheless, the fact remains that most of the success achieved so far has been with model plant species and the transfer of these new technologies to major crop species that are the principal targets of biotechnology has either been slow and difficult, or is non-existent. In order to have any meaningful impact on agriculture the developing biotechnology must be equally and readily applicable to important crop species. The cereals and grasses, which constitute the most important group of crop plants, have until recently been found to be very recalcitrant to cell culture techniques. It highlights the success achieved in establishing totipotent callus and cell suspension cultures, and reports the development of protoplast culture systems yielding somatic embryos and plants and the recent recovery of somatic hybrid cell lines and genetically transformed cell lines.The importance of the age and physiological state of the explant, and the relative genetic stability of embryogenic cultures and regenerated plants is discussed. The field finds a wide range of applications starting from mass clonal propagation to plant improvement, molecular biology, bio-processing as well as a basic research tool. It has advanced the production in forestry and agriculture to many folds. Plant tissue culture has an important role to play in the manipulation of plants for improved agronomic performance. Plant tissue culture is an integral part of molecular approaches to plant improvement and acts as an intermediary whereby advances made by the molecular biologists in gene isolation and modification are transferred to plant cells. Some of the simpler techniques that are more approachable and have been found to be applied directly in plant propagation and genetic improvement of plants are:- (i) Micropropagation. (ii) Meristem culture. (iii) Somatic embryogenesis. (iv) Somaclonal variation. (v) Embryo culture. (vi) *In vitro* selection. (vii) Anther culture, and (viii) Protoplast culture. Most applied and well translated among these is the technique of micropropagation, which has revolutionized the modern agriculture industry. *Ficus carica* has been cultivated for a long time in various places worldwide for its edible fruit. It is supposed to originate from Western Asia and spread to the Mediterranean by humans. It is also an imperative crop today. The areas with typically mild winters and hot dry summers are the major producers of edible figs. Fruits can be eaten raw, dried, canned, or in other preserved forms. *Ficus carica* possibly originate from the Middle East, which is one of the early cultivated fruit species and currently is an important crop worldwide. Nowa days, the common fig still grows wild in y he Mediterranean basin. Morphological data propose that the fig is gynodioecious, whereas from a functional standing point, the fig is considered dioecios with two tree morphs Capri fig and edible fig. The fruit and reproduction systems of species in the genus ficus are exclusive, It can also be pollinated by their associated agaonid wasps (Hymenoptera: chalcoidea: agaonide), and inturn the wasps can only lay eggs within their associated fruit. For successful pollination and reproduction of species of ficus carica to occur, their associated species of ficus carica must be present. Phytochemical studies of ficus carica revealed the presence of numerous bioactive compounds such as phenolic compounds, polysterols, organic acids, anthocyanin composition, and volatile compound such as hydrocarbons, aliphatic alcohols, and few other classes of secondary metabolites from different parts of ficus carica. The leaf of ficus carica consists of various volatile compounds which are identified and distributed by distinct chemical classes, such as aldehydes: methyl-butanol, 2-methyle butanol, 2-pentanal, hexanal alcohols: butanol, heptanol, benzyle alcohol, ketone etc. Fifteen anthocyanin pigments were isolated from the fig fruit and bark of ficus carica. Most of them contain cyanidin as a glycone and some pelargonidin derivatives. The phenolic compounds , phenolic acid, chlorogenic acid, flavones and flavonols have been isolated from fresh and dried fig skins of *Ficus carica* and dried figs contained total higher

amounts of phenolics that the pulp of fresh fruits , owing to the contribution of the dry skin. Ficus carica has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anyi-inflammatory remedy. Leaves, fruits and roots of ficus carica are used in native medicinal system in different disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, cough and bronchial problems), inflammatory and cardiovascular disorders. Fruits of ficus carica can be eaten fresh or dried or used as jam. Figs are used as excellent source of minerals, vitamins, carbohydrates and dietary fiber because it is fat and cholesterol free and contain high amount of amino acids. The fruit's juice of ficus carica mixed with honey is used for hemorrhage. % Indian medicine, these fruits is used as a mild laxative, expectorant and diuretic. It is also used as aid in liver and spleen dl paste of ficus is applied to swellings, tumors and inflammation for reweaving pain. The methanol extract of ficus carica showed a strong antibacterial activity against oral bacteria. The combination effects of methanol extract with ampicillin orgentamicin were synergistic against oral bacteria that showed that figs could act as a natural antibacterial agent. Hexane, chloroform, ethyle acetate, and methanol extracts of *Ficus carica* latex were investigated for their antimicrobial activity. Many interesting biological activities of ficus carica have been carried out, which can be further explored to make use of them as a healing method for the future. For example, the leaves have shown irritant activity. Consequently they can be investigated against parasitic infection and ovicidal activity. The majority of the pharmocological studies which have been carried out on ficus carica were conducted with uncharacterized crude extracts: it is difficult to produce the gradesd of these Studies and identify the bioactive metabolites. Phytochemical research carried %it on ficus carica has led to the isolation of new classes of plant metabolites .Most of the Phytochemical works have been employed on leaves and fruits of *Ficus carica*., while there is little information on stem and root phenolic profiles. However the vast traditional uses and established pharmocological activities of ficus carica point out that an enormous scope still exists for its Phytochemical exploration using bioassay-guided isolation. The result of future research in the above mentioned area will afford a persuasive support for the future clinical use of ficus carica in contemporary remedy.

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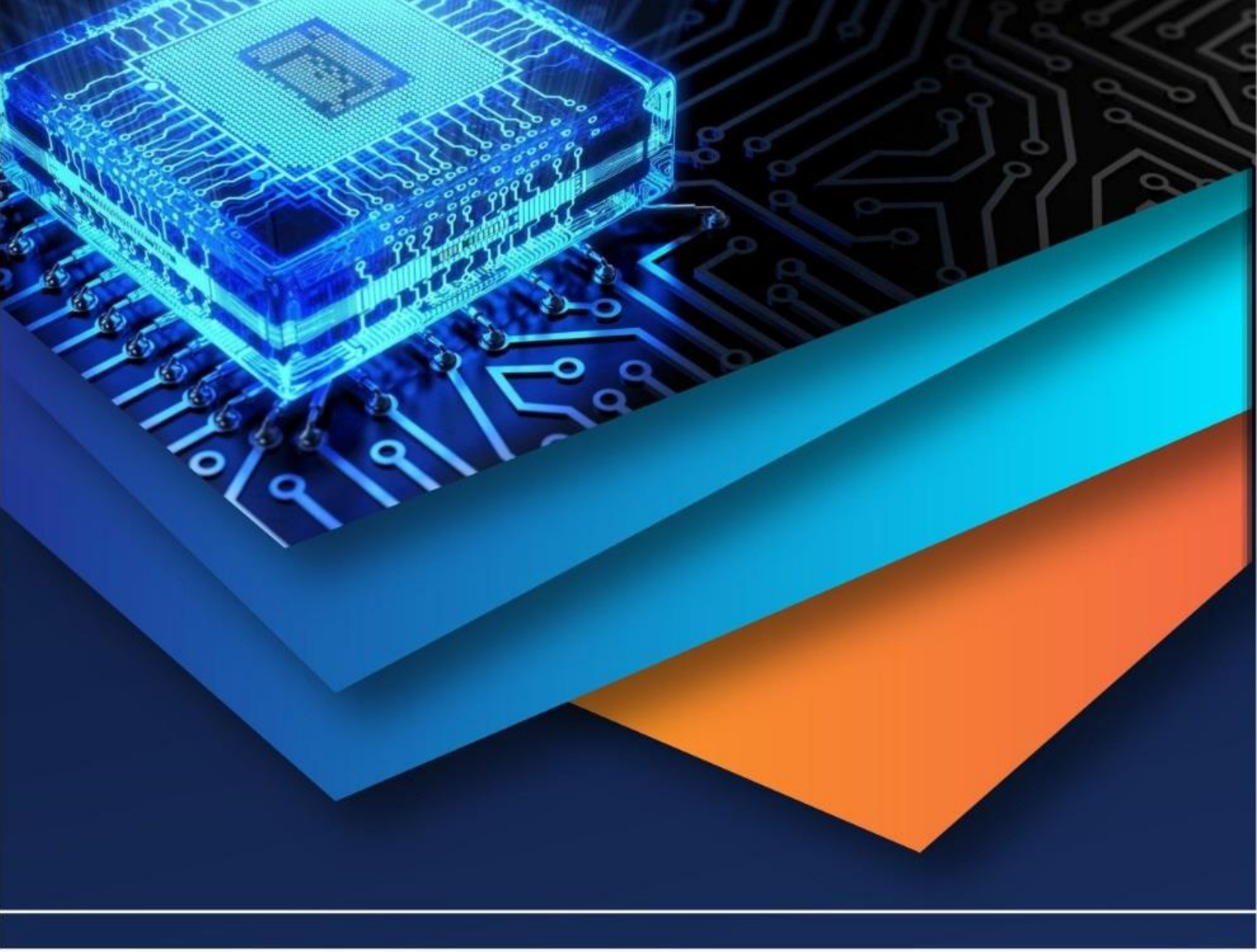

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