Review Article

Application of Tissue Culture in Breeding for Biotic stress Resistance

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Abstract

One of the problems facing agriculture today is related to the growing world population wich requires increase in food production while agricultural production is being challenged by biotic and a biotic stresses. Biotic stress includes various plant pathogens such as bacteria, fungi, viruses, nematodes, insects, and others whose infection arises from them frequently results in changes in plant physiology, the loss of biomass, early flowering, the decreased seed set, the accumulation of protective metabolites, and many other changes. One way to increase the quantity and quality of food is by reducing losses caused by insects, diseases and weeds by developing resistant crops or use of chemicals/ pesticides. Using chemical spray may have adverse effects on human health and the environment, including beneficial organisms and may lead to the development of chemical-resistant insects and weeds. Therefore, development of plants that continuously defend them-selves against attacks from bacteria, viruses, fungi, invertebrates, and even other plants is found to be important. The long-term goal of plant breeders is to improve crops for biotic stress tolerance. This can be achieved by the using modern breeding technique. Among them the use of Plant genetic engineering and tissue culture has made possible.

Key words*: Cell culture; invitro selection; protoplast fusion; somatic embryogenesis*

1. INTRODUCTION

Agriculture is the backbone of the economy of many of the developing nations. Also they have an important role in the food and food processing areas of life. In order to satisfy the growing populations and their food needs, many new revolutions have been made in the Agricultural industry to increase the yield of crop plants. Plant disease control is a major challenge to agriculture worldwide due to significant yield losses in crops caused by plant diseases. The concept of "integrated pest management", or IPM, has led to the development of useful pest management measures. Pest control measures throughout the world cost billions of dollars each year. However, use of pesticides is becoming more problematic due to development of resistance and to environmental concerns. Conventional plant breeding has made significant impact by improving the resistance of many crops to important diseases, but the time-consuming processes of making crosses, back crosses, and progeny selection makes it difficult to react quickly to the evolution of new virulent pathogen races. Moreover, plant breeding techniques are not a solution to many major diseases because there are no natural sources of resistance available to the breeder (Dasgupta, 1992;Melchers and Stuiver, 2000). Plant tissue culture technology is being widely used for large scale plant multiplication.

Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis (Akin-Idowu, *et al,*. 2009). Endangered, threatened and rare species have successfully been grown and conserved by micro propagation because of high coefficient of multiplication and small demands on number of initial plants and space. In addition, plant tissue culture is considered to be the most efficient technology for crop improvement by the production of somaclonal and gametoclonal variants. The micropropagation technology has a vast potential to produce plants of superior quality,

isolation of useful variants in well-adapted high yielding genotypes with better disease resistance and stress tolerance capacities (Brown and Thorpe 1995).

2. APPLICATION OF TISSUE CULTURE FOR BIOTIC STRESS RESISTANCE

Plant tissue culture has allowed a mass production of plants, increasing the capacity of plant multiplication with specific agronomical traits. It has also been a major tool for basic and practical investigation in agriculture. These new developments include clonal propagation, cell suspension culture, anther/pollen culture, embryo

culture, protoplast isolation and regeneration, and the development of new interspecific plantlets in vitro Are important techniques in devlopin stess resistant crop varieties.

2.1. DEVELOPMENT OF BIOTIC STRESS RESISTANT PLANTS THROUGH IN VITRO SELECTION

The yields of many commercially important crops remain relatively low due to susceptibility to various fungal, bacterial and viral pathogens. Chemical control of these pathogens is often difficult, costly and labor and resource-intensive (Bezier *et al*., 2002). In addition, some chemically synthesized fungicides can cause environmental pollution, being non-biodegradable they can build up heavy concentrations in soil reducing its productivity and in the water table posing health hazards to flora and fauna. Hence, studies on development of disease-tolerant plants through existing or novel methodologies have become increasingly important. Invitro selection is an attractive alternative approach for development of stress tolerant lines (Jayashankar *et al*., 2000; Ganesan and Jayabalan, 2006). In vitro selection through enhanced expression of pathogenesis-related (PR) proteins, antifungal peptides or biosynthesis of phytoalexins is an important tool for desirable plant selection (Ganesan and Jayabalan, 2006; Kumar et al., 2008a). This technology is easy and cost effective compared to the transgenic approach for the improved disease tolerance (Jayashankar *et al*., 2000). In vitro selection for resistance to a pathogen can be carried out using organogenic or embryogenic calli, shoots, somatic embryos or cell suspensions by exposing them to toxins produced by the pathogen, pathogen culture filtrate or to the pathogen itself (Kumar *et al.,* 2008a). The possibility of in vitro selection for disease resistance was first reported by Carlson (1973) in tobacco for Pseudomonas syringae. Since then, lines resistant to fungal, bacterial and viral pathogens have been isolated in many species (Gengenbach *et al*., 1977; Behnke, 1979; Brettell *et al*., 1980; Hartman *et al*., 1984; Chawla and Wenzel, 1987; Hammerschlag, 1988; Vidhyasekaran *et al*., 1990; Jayasankar and Litz, 1998; Jayashankar *et al*., 2000; Fuime and Fuime, 2003; Krause *et al*., 2003; Gayatri *et al.,* 2005; Ganesan and Jayabalan, 2006; Kumar *et al*., 2008a). In recent years, pathogen culture filtrate and phytotoxins are most commonly used for in vitro selection and regeneration of disease resistant plants in many crops (Kumar *et al*., 2008a). In vitro selection by adding host-specific phytotoxin such as fusaric acid and pathogen produced nonspecific phytotoxins i.e. deoxynivalenol (DON), crude pathogen culture filtrate or sometimes the pathogen itself to the growth media has been reported to increase the frequency of resistant plants, as compared with those obtained from tissue culture without selection (Gayatri *et al*., 2005).

Disease resistance has been identified following in vitro selection in a wide range of plant species including cereals, vegetables, fruits and other commercially important plant species. In vitro selection of disease resistant lines by using culture filtrate of pathogens have been effectively carried out in herbaceous plants including maize, potato, alfalfa, barley and rice. Some successful reports of in vitro selection for disease resistance in woody species involve peach, lemon, mango and grapes. Hammerschlag (1988) regenerated disease resistant plant of peach by screening embryogenic callus obtained from zygotic embryos against culture filtrate produced by a pathogenic bacterium Xanthomonas campestris cv. pruni. The nucellar embryogenic cultures of two polyembryonic cultivars of mango selected against the culture filtrate of Colletotrichum gloeosporioides exhibited resistance to the fungus in-vitro (Jayasankar and Litz, 1998). On similar lines, Jayashankar *et al*. (2000) screened proembryogenic mass of grapes against culture filtrate produced by Elsinoe ampelina, the causal agent of anthracnose disease and reported that regenerated plants showed enhanced resistance to the pathogen. Such studies have also been shown to be useful assays in testing for resistance in wheat (Yang *et al*., 1998), tomato (Fuime and Fuime, 2003), flax (Krause *et al*., 2003), turmeric (Gayatri *et al.,* 2005), cotton (Ganesan and Jayabalan, 2006), safflower (Kumar *et al.,* 2008a), sugarcane (Sengar *et al*., 2009), etc. In vitro selection by using phytotoxin has also been carried out by several workers. Cell suspension cultures of 'Peter Pears', a cultivar of Gladiolus×grandiflorus (Hort.), susceptible to the fungus Fusarium oxysporum f. sp. gladioli (Mass.), have been selected against fusaric acid, one of the toxins produced by this pathogen (Remotti *et al.,* 1997). Similarly, the calli of two genotypes of barley were used for selection of resistance using fusaric acid (Chawla and Wenzel, 1987). Gentile *et al*. (1992, 1993) regenerated 'mal secco' resistant lemon by screening embryogenic cultures of nucellar origin against a partially purified phytotoxin produced by Phoma tracheifila. Fusarium graminearum tolerant planlets of Triticum aestivum L. were successfully screened using deoxynivalenol as a selection agent in vitro (Yang *et al*., 1998). Toyoda *et al*. (1989) selected tobacco mosaic virus resistant tobacco in vitro using callus lines infected with tobacco mosaic viruses itself.

2.2. SELECTION FOR IN VITRO DISEASE RESISTANCE IN PLANTS

One of the earliest examples of selection for disease resistance exploited the structural similarities between an amino-acid analogue methionine sulphoximine and a toxin produced by Pseudomonas tabaci. (Carlson 1973) was the first to produce tobacco plants from the callus cultures which were resistant to Methionine sulphoximine, an analogue of tobaxin, produced by P. tabaci and enhanced resistance to the pathogen was

observed. Initially, (Bajaj and Saettler 1970) had studied the effect of Halotoxin containing filtrates of Pseudomonas phaseoiicola on the growth of bean callus tissue. (Krishnamurthi and Thaskal 1974) reported Fiji disease resistant sugarcane subclones {Saccharum officinarum Var. Pinder) from tissue culture.

successfully selected soybean (Glycine) plants from the suspension cultured soybean cells to the elicitor isolated from the fungal pathogen Phytophtora megasperma (var. Sojae. Gengenbach, *et al*. 1977) reported maize plants resistant to Helminthosporium maydis. (Sacristan & Hoffman 1979) studied direct infection of embryogenic tissue cultures of haploid Brassica napus with resting spores of Plasmodiophora brassicae. (Earle, *et al*., 19820) studied the effect of Helminthosporium phytotoxins on cereal leaf protoplasts. (Chaleff 1983) isolated sugarcane plants resistant to eyespot disease from callus and suspension culture, subjected to toxin of Helminthosporium sacchari, about 15-20% regenerated plants proved resistant. (Behnke 1979, 1980) selected potato plants resistant to un-fractioned liquid fungal growth medium containing exotoxins of Phyophthora infestans. (Bajaj, *et al*., 1981) studied differentiated tolerance of tissue cultures of pearl millet to Ergot extract. (Larkin and Scowcroft 1981) reported regeneration of sugarcane clones resistant to Helminthosporium sacchari from single cell population of susceptible clone treated with Heiminthosporoside. (Gengenbach, *et al*, 1981) studied mitochondrial DNA variation in maize plants regenerated during tissue culture selection. (Sacristan 1982) selected Phoma lingum resistant plants of Brasslca napus from cell and embryonic cultures. (Thanutong, *et al*. 1983) reported resistant tobacco plants from protoplast derived callus selected for their resistance to Pseudomonas & Alternaria toxins. (Solodkaya & Mezentsev 1983) regenerated clover plants from acell suspension cultivated in a medium containing the culture liquid of Sclerotinia trifollium Erikss. fungus. (Larkin and Scowcroft 1983) studied somaclonal variation and eye spot toxin tolerance in sugarcane.

Bhagyalakshmi, et al, (1984) obtained downy mildew resistant pearl millet plants from infected tissues cultured in vitro(Brettel, *et al*., 1997) reported regeneration from callus treated with toxic substances produced by Leptosphaeria nodonum Mutler, which cause Glume blotch disease of wheat. (Maribona, *et al*, 1984) isolated sugarcane callus culture resistant to Helminthosporium sacchari toxin.(Scala, *et al*., 1984) analysed the Tomato – Fusarium oxysporum system for the selection of disease resistance. The effect of selective toxin of Helminthosporium victoriae on oat tissues and protoplasts was studied by (Briggs, *et al*., 1984) and they were able to select Helminthosporium resistant plants from oat callus. Similarly, (Rines & Luke 1985) successfully selected oat plants insensitive to Helminthosporium victoriae toxin. The toxic effect of Fusaric acid on mesophyll cells of Asparagus officinalis in cell cultures was studied by (Julian in 1985). (Ling, *et al*., 1985) reported rice plants resistant to Helminthosporium oryzae through tissue culture.

2.3. CELL CULTURE AND SELECTION

Plant tissue culture represents the simplest of the biotechnologies available to plant scientists today. The realization that certain in vitro conditions could induce heritable changes, called somaclonal variations, in the genomes of plant cells opened an avenue for the selection of various desirable traits from in vitro cultures, including herbicide resistance (Maliga, 1984). Using cell culture procedures, BASF Inc. produced a corn hybrid (DK404SR) that is resistant to the sulfonylurea herbicide, sethoxidim. In their method, a mutant cell line (named S2) was identified following continuous culture of corn embryo tissues under high sethoxidim selective pressure. Plants regenerated from this somaclonal mutant line were found to contain a form of the enzyme, acetolactate synthase (ALS, target of sulfonylureas), which was insensitive to the herbicide. This resistance was subsequently transferred to the commercial hybrid (DK404SR) by backcrossing the S2 line with both of its parental lines. Further investigations showed that the sethoxidim tolerance was inherited as a single partially dominant allele. Zambrano et al. (2003) selected a glyphosate-tolerant sugar cane cell line in liquid medium containing 0.8 mM glyphosate and regenerated plants that could tolerate up to 5-fold the concentration of glyphosate that killed plants from unselected cells. Analysis of these plants by random amplified polymorphic DNA (RAPD) markers revealed a 564-bp band that was unique to the plants derived from the selected cell line, indicating a possible selection of a pre-existing variability among the cells. A similar method was used to select glyphosate-resistant cells of chicory that subsequently produced glyphosateresistant plants (Sellin *et al*., 1992) Cell culture under lethal concentrations of certain herbicides also results in gene amplification in surviving cells that leads to resistance through the overproduction of enzymes targeted by herbicides.

A petunia cell line with resistance to glyphosate was selected in this manner and plants regenerated from it survived lethal levels of glyphosate (Steinrucken *et al*., 1986). This resistance was found to be due to amplification of the gene encoding 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that caused its overproduction in the cells (Steinrucken *et al*., 1986). Similarly Caretto et al. (1994) selected carrot cells and subsequently regenerated plants that were resistant to the sulfonylurea herbicide, chlorsulfuron. Resistance in these plants was due to amplification of the acetolactate synthase gene. In vitro development of phosphinothricin (PPT) resistant rice has also been reported by inducing plantlet regeneration in explants

collected from 7-day old seedlings on medium supplemented with sublethal doses of PPT (Toldi *et al.*, 2000). Other in vitro cell selection studies have developed resistance to paraquat in tomato cells (Thomas and Pratt, 1982), resistance to glyphosate in carrot and groundnut cells (Murata *et al.*, 1998; Jain *et al.*, 1999) and resistance to a Protoporphyrinogen oxidase (PPO) inhibitor in soybean cells (Warabi *et al*., 2001); however, no viable plant regeneration was reported in these studies.

2.4. SOMATIC EMBRYOGENESIS

Somatic embryogenesis is not only a process of regenerating the plants for mass propagation but also regarded as a valuable tool for genetic manipulation. The process can also be used to develop the plants that are resistant to various kinds of stresses (Bouquet A, Terregrosa L 2003) and to introduce the genes by genetic transformation (Maynard *et al*. 1998). A successful protocol has been developed for regeneration of cotton cultivars with resistance to Fusarium and Verticillium wilts (Han *et al*.2009).

2.5. PROTOPLAST FUSION

Disease resistance in breeding program may come either from closely related species or from more distantly related species. Problems are generally encountered if an effort is made in crossing distantly related species. Protoplast fusion is one of the methods that can be used to circumvent problems in introgression genes for resistance. By this method, factors that contribute to crossing barriers between species can be avoided and viable hybrids (Cybrids) have been recovered even between distantly related species (Harms, 1985). Examples of disease resistant plants, produced from protoplast fusion are shown in

Table 1: Some of disease Resistant Plants Produced from Protoplast Fusion

Species used for fusion	Diseases	Reference
Lactusa sativa	Downy mildew(Bremia lactucae)	Maloy, (2005)
Brassila oleracea and Raphanus sativus	Clubroot (Plasmodiophora brassical)	Maloy, (2005)
Brassica napus and Brassica nigra	Black leg (Phoma lingum) club root	Maloy, (2005)
Solanum brevidens and Solanum tuberosum	Bacterial soft rot (Erwinia spp)	Maloy, (2005)

3. VIRUS RESISTANCE

3.1 GENERATION OF VIRUS FREE PLANT MATERIAL

Another most interesting application for which plant tissue culture Therefore, meristematic tissues are sometimes free of is uniquely suited is in the obtaining, maintaining and pathogens; it is possible to recover non-infected plants mass propagating of specific pathogen-free plants by by in vitro meristem culture techniques and to grow them meristem culture technique. This technique was first into healthy plants. In addition, thermotherapy, developed for virus eradication by Morel and Martin (Morel and Martin 1952) chemotherapy and surface sterilization applied to the on Dahlia and leads to pathogen-free plants. Recently, whole plant as well as explants followed by meristem meristem culture has been used successfully in the culture has been successfully used for elimination of removal of viruses from many plants (potato, sugarcane, many viruses in plants (Lizarraga *et al*,. 1986). strawberry) and is now used routinely for the eradication of many viral diseases from plant materials. Infected plants are sometimes free of viruses. In certain diseases, insects, antibiotics, salts etc have been isolated cases, such as potato virus X (PVX) and tobacco rattle from haploids derived from anther culture and (ii) because virus in potatoes, only the apical dome and the first dihaploids which is derived from haploids, can be used young primordial leaf are free of viruses.

3.2 ELIMINATION OF VIRUSES

The elimination of viruses can be achieved by a combination of apical meristem culture and thermotherapy. Meristem culture is the most commonly used method to free plants from virus diseases. Virus-free plants have been obtained by thermo-therapy and meristem culture in several species (Morel and Martin, 1952; Belkengren and Miller, 1962; Mullin *et al.,* 1974; Boxus, 1976). The plant meristem is a zone of cells with intense divisions, situated in the growing tip of stems and roots. The virus travels through the plant vascular system, which is absent in the meristem. Moreover the cell-to-cell movement of the viruses through plasmodesmata cannot keep up with the growth and elongation of the apical-tip. The high metabolic activity of meristematic cells, usually accompanied by elevated endogenous auxin content in shoot apices may also inhibit virus replication. Thus, the meristem is highly protected from infection (Limasset *et al.,* 1949). Based on this finding, meristem culture has been extensively used to eliminate viruses, bacteria and fungi from plants. The culture of meristems or alternatively small shoot tips, in combination with enhanced cell division in vitro and/or thermal pre-treatment allows the elimination of viruses in plants propagated from vegetative parts. If explants are too big, they are likely to contain virus particles in the associated vascular tissue. For thermotherapy, the plants are first are grown at high temperature (ca. 38-420 C) for 4-6 weeks. Under tropical or subtropical conditions, this can be accomplished simply by installing a small compartment of a glasshouse equipped with a roof vent on one end and an exhaust fan on the opposite end, both temperature-regulated. This approach removes the excess heat and provides a constant high temperature daytime treatment. In climates with temperate conditions, the same effect can be achieved by placing fluorescent lights including ballasts and/or heat producing incandescent lamps in the necessary minimum distance to avoid damage over the plants to be treated in a dark box just large enough to include the plants. Such a system has been used for virus elimination in sweet potato.

After thermotherapy, 0.2 –0.4 mm explants are preferentially cultured singly in test tubes. If the explant is too big, it likely has a vascular system that may contain microbial contaminants including viruses. The plants thus obtained are multiplied, and re-indexed. A better strategy is to culture 2-5mm long explants for 4-5 weeks, maintain the in vitro grown plant at high temperature for 4-5 weeks, and excise 0.2-0.4mm or even longer explants to initiate subcultures. This procedure avoids in vivo contamination problems and gives highsurvival and multiplication rate. In potato, in vitro cultures were established from several millimeter long shoot tips and axillary buds after in vitro thermotherapy to free potato from all relevant virus diseases. By using this method, it was possible to eradicate viruses A, Y and X from potato varieties in one single step. A method called multiple lateral shoot technique of in vitro elimination of three common viruses, X, Y and S of potato, has been also reported (Zapata et al., 1995). In this method, a stem with at least five nodes is treated with ribavirin in liquid medium, and cultured for 5 days at room temperature. The stems are subjected to thermotherapy at 32-350 C for 25 days, apical buds are taken from the lateral shoots cultured on solid medium, and checked for virus infection with ELISA. The survival of explants in relation to the applied temperature stress and their size is always inversely proportional to the pathogen elimination success. It is known from molecular and traditional microbiological surveys that effectively all in vitro plants contain certain microorganisms, mostly bacteria but also mycoplasmas, viroids, and fungi, many of which cannot be cultured without the host. Such microorganisms may not show symptoms, and in some cases may even have a positive effect on the growth performance of the host plant (Bell *et al.,* 1995, Bensalim *et al.,* 1998, Boddey *et al.,* 1995, Cassells, 1997). Shoot-tip culture is used for the multiplication of plants that are already freed from known diseases. It should be emphasized that it does not free the plant from viruses. In fact, it favors propagation of viruses and increases virus concentration in the daughter plants. In many ornamentals, variegation is due to the presence of certain viruses or mycoplasms (Lee *et al.,* 1997). The removal of such viruses is therefore not desirable. In such ornamental plants, multiplication from shoot-tip and axillary bud culture is the better choice, because meristem culture may remove the virus, and the ornamental variegation is lost (Cassells *et al.,* 1980).

In certain varieties with variegations as in Petunia and Pelargonium meristem culture may dissolve the existing chimera, thus producing material without the desired character. Viruses may also produce certain phenotypes that are characteristic of a plant variety. For example, in sugarcane cv. ‘Co 740’, the yellow irregular leaf streaks were initially considered as a diagnostic character till they were proven to be disease symptoms. The removal of the virus by meristem-tip culture led to the disappearance of the yellow streaks, which reappeared on re-infection (Hendre *et al.,* 1975). Erroneously, the yellow streaks are still recognized as a varietal diagnostic trait of ‘Co 740’. Recently, endogenous para-retroviruses have been reported that integrate in the genome of the plant material (Harper *et al.,* 1999). Under in vitro stress, sequences of such viruses have been detected in the micro propagated banana plants.

4. WEED AND INSECT RESISTANCE

4.1. HERBICIDE TOLERANCE

Farmers must control weeds that compete with their crops for water, nutrients and sunlight. Herbicide tolerance is an important agronomic trait that has been used to control weeds very efficiently for several decades. A faster approach is the use of biotechnology techniques such as in vitro cell culture.

4.2. CELL CULTURE AND SELECTION

Herbicides that interfere with basic metabolic activities are expected to inhibit growth of cultured cells as well as of the whole plant. In such instances, herbicide tolerant mutants can be selected by culturing cells in the presence of a herbicide concentration that is toxic to normal cells, favoring subsequent identification of the herbicide-tolerant target enzyme. Using cell culture techniques, BASF Inc. produced a maize hybrid that is resistant to the sulfonylurea herbicide, sethoxidim. In their analysis, a mutant cell line (named S2) was identified following continuous culture of maize embryo tissues under high sethoxidim selection pressure. Plants regenerated from this soma clonal mutant line were found to contain a form of the enzyme, acetolactate synthase (ALS, target of sulfonylureas/imidazolinones), which was insensitive to the herbicide. This resistance was subsequently transferred to the commercial hybrid (DK404SR) by backcrossing the S2 line with both of its parental lines. Further investigations showed that the sethoxidim tolerance was inherited as a single partially dominant allele. Similarly, Zambrano *et al.,* (2003) selected a glyphosate-tolerant sugar cane cell line in liquid medium containing 0.8 m M glyphosate and regenerated plants that could tolerate up to fi ve-fold the concentration of glyphosate that killed plants from unselected cells.

Cell culture under lethal concentrations of certain herbicides also results in gene amplification in surviving cells that leads to resistance through the overproduction of enzymes targeted by herbicides. For example, a petunia cell line with resistance to glyphosate was selected in this manner and plants regenerated from it survived lethal levels of glyphosate (Steinrucken and Amrhein 1986). This resistance was found to be due to ampli fi cation of the gene encoding 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase that caused its overproduction in the cells. Similarly, (Caretto *et al.,* 1994) selected carrot cells and subsequently regenerated plants that were resistant to the sulfonylurea herbicide, chlorsulfuron. Resistance in these plants was due to ampli fi cation of the ALS gene. In vitro development of phosphinothricin (PPT) resistant rice has also been reported by inducing plantlet regeneration in explants collected from 7-day old seedlings on medium supplemented with sublethal doses of PPT (Toldi *et al.,* 2000) . Other in vitro cell selection studies have developed resistance to paraquat in tomato cells (Thomas and Pratt 1982) , resistance to glyphosate in carrot and groundnut cells(Jain *et al.,* 1999) and resistance to a protoporphyrinogen oxidase (PPO) inhibitor in soybean cells (Warabi *et al.,* 2001) ; however, no viable plant regeneration was reported in these studies.

Even though there are many advantages to creating the resistance of plants to insects the process that has its own disadvantages also. The use of synthetic toxic insecticides has created a greatest threat to the environment and health in their persistence and negative effects to non target organisms (Ishaaya, *et al,* 2007). There are problems linked with the resources of air, water and soil pollution, directly. There are also problems that are counter parts of the soil which include loss of crop, wild plant and animal genetic resources, elimination of natural enemies of pests, Pest resurgence and genetic resistance top pesticides, chemical contamination and complete destruction of natural control mechanisms (Conway *et al.,* 1991).

One of the important pesticide included problem is the development of resistance by the insect pests. The use of these insect resistance plants has led to the development of cross resistance in insect pests. Thus once the resistance is developed by the pests, they become resistance to all other crops in future. Globally about 504 insects and mites, 150 plant pathogens are known to have developed resistance. Resurgence is yet another problem being faced. They occur in two ways firstly rapid resurgence of pests populations exposed to the pesticide, nextly, minor pests or unimportant pests or target species developing into major pests as a result of decreased competition for food and shelter. Also it has led to social and health problems, such as birth defects, nerve damage, cancer and other effects that might occur over a period of time (Peshin *et al.,* 2009).

The process of breeding to produce insect resistance to plants using tissue culture is effective as they include meristerm tip propagation, callus and single cell culture, haploid plant production and protoplasm isolation, culture, transformation, fusion and regeneration to whole plants. The potential of these techniques is further augmented by molecular technologies. Tissue culture of insect resistance plant is useful on plants like strawberries, apple, bananas, sugarcane and potatoes. The plants generated from the culture are isolated and are tested for the insect resistance. These cultures were processed for the production of resistant dihaploids from

Haploid plants by addition of chemicals like Colchicine. The insect resistance of the plants can be increased by protoplast fusion. This results in the production of Cybrid cells containing the nucleus of one cell and the cytoplasm of the other which are produced by the fusion of hybrid cells containing the nuclei and cytoplasm of both protoplasts. These cells produced are genetically transformed into the plant cells. There are various methods available for the same. This includes direct DNA uptake, microinjection of DNA, liposome mediated delivery of DNA, use of plant viral vectors and by the use of natural gene vector system of A. Tumefaciens. When DNA are introduced into plant cells, DNA gets integrated into the plan chromosomal DNA. When this DNA carries appropriate regulatory genes recognized by the plant cells, the DNA is expressed. So far only micro projectile bombardment and Agrobacterium system have been used successfully to introduce into plants specific new genes that were then expressed by the plant (Agrios, 2005).

5. SUMMARY

Plant tissue culture is now a well-established technology. Like many other technologies, it has gone through different stages of evolution; scientific curiosity, research tool, novel applications and mass exploitation. Initially, plant tissue culture was exploited as a research tool and focused on attempts to culture and study the development of small, isolated segments of plant tissues or isolated cells. Around the mid twentieth century, the notion that plants could be regenerated or multiplied from either callus or organ culture was widely accepted and practical application in the plant propagation industry ensued. Today plant tissue culture applications encompass much more than clonal propagation and micro propagation. The range of routine technologies has expanded to include somatic embryogenesis, somatic hybridization, virus elimination as well as the application of bioreactors to mass propagation. The list includes:- Clonal propagation, Axillary shoot multiplication, Direct (adventitious) organogenesis, Callus to organogenesis, Somatic embryogenesis, Virus elimination, In vitro grafting,In vitro gene banks, stock plant banks, cell culture, protoplast culture,etc are used to develop resistant crop varieties to biotic and abiotic stress.

6. REFERENCE

Akin-Idowu PE, Ibitoye DO, Ademoyegun OT (2009) Tissue culture as a plant production technique for horticultural crops. Afr. J. Biotechnol. 8(16): 3782-3788.

Bajaj, Y.P.S and A.W. Saettler, 1970. Effect of Halotoxin containing filtrates of Pseudomonas phaseolicoia on the growth of bean callus tissue. Phytopathology, 60: 1065-1067.

Bajaj, Y.P.S., P. Kumar, K.S. Labaua and M.M. Singh, 1981. Regeneration of plants from seedling explants and callus cultures of Arachis hypogea L Indian J. Exp. Biol., 19: 1026-1029.

Barthou, H., C. Briere, C. Caumont, M. Metitprez, J. Kallerhoff, C. Borin, A. Souvre and G. Alibet, 1997. Effect of atmospheric pressure on sunflower (*Helianthus annuus* L.) protoplast division. Plant Cell Rep., 16: 310-314.

Behnke, M., 1979. Selection of potato callus for resistance to culture filtrates of Phytophthora infestans and regeneration of resistant plants. Theor. Appl. Genet. 55, 69–71.

Behnke, M., 1980. General resistance to late blight on Solanum tuberosum plants regenerated from callus resistant to culture filtrates of Phytophthora infestans. Theor. Appl. Genet, 56: 151-152.

Behnkev, M., 1979. Selection of potato callus for resistance to culture filtrates of Phytophthora infestans and regeneration of resistant plants. Theor. Appl. Genet, 55: 69-71.

Belkengren, R.O. and Miller, P.W. 1962. Culture of apical meristems of Fragaria vesca strawberry plants as a method of excluding latent A virus. Plant Disease Rep. 46: 119-121.

Bell, C.R., Dickie, G.A, Harvey, W.L.G. and Chan, J.W.Y.F. 1995. Endophytic bacteria in grape vine. Can. J. Microbiol. 41: 46-53.

Bensalim, S., Nowak, J. and Asiedu, S.K. 1998. A plant growth promoting Rhizobacterium and temperature effects on performance of 18 clones of potato. Amer. J. Potato Res. 75: 145-152.

Bezier, A., Lambert, B., Baillieul, F., 2002. Study of defense related gene expression in grapevine leaves and berries infected with Botrytis cinerea. Eur. J. Plant Pathol108, 111–120.

Bhagyalakshmi Prasad, Satishchandra Prabhu and C. Shantamma, 1984. Regeneration of downy mildew resistant plants from infected tissues of pearl millet (Pennisetum americanum) cultured in vitro. Curr. Sci. t., 53(15): 816-817.

Boddey, R.M., Oliveira de, O.C., Urquiaga, S., Reis, V.M., Olivares de, F.L., Baldani, V.L.D. and Döbereiner, J. 1995. Biological nitrogen fixation associated with sugar cane and rice: Contributions and prospects for improvement. Plant and Soil 174: 195-209.

Bouquet A, Terregrosa L (2003) Micropropagation of grapevine (Vitus spp.). In: Jain SM, Ishii K, editors. Micropropagation of woody trees and fruits. The Netherlands. 75: 319-352.

Boxus, P. 1976. Rapid production of virus-free strawberry by in vitro culture. Acta Hotic.66: 35-38.

Brettell, R.I.S., Thomas, E., Ingram, D.S., 1980. Reversion of Texas male-sterile cytoplasm maize in culture to give fertile, T-toxin resistant plants. Theor. Appl. Genet. 58, 55–58.

Briggs, S.P., R.P. Scheffer, A.R. Haug, 1984. Selective toxin of Helminthosporium victoriae: Terminal relationship in effects on oat tissues and protoplasts. Phytopathology, 74: 768-774.

Brown DCW, Thorpe TA (1995) Crop improvement through tissue culture. World J. Microbiol & Biotechnol. 11: 409-415.

Caretto S, Giadina MC, Nicolodi C, Mariotti D (1994). Chlorsulfuron resistance in Daucus carota cell lines and plants: Involvement of gene amplification. TAG, 88: 520-524.

Carlson, P.S., 1973. Methionine sulfoximine resistant mutants of tobacco. Science, 1980:1366-1368.

Carlson, P.S., 1973. Methionine sulfoximine-resistant mutants of tobacco. Science 180, 1366–1368.

Cassells, A.C. 1997. Pathogen and Microbial Contamination Management in Micropropagation. Kluwer Acad. Pub. Dordrecht.

Cassells, A.C., Minas, G. and Long, R. 1980. Culture of Pelargonium hybrids from meristems and explants: Chimeral and beneficially-infected varieties. In: Tissue Culture Methods for Plant Pathologists. Ingram, D.S. and Helgeson, J.P. (Eds.) Blackwell Scientific Pub. Oxford. Pp.125-130.

Chaleff, R.S., 1983. Isolation of agronomically useful mutants from plant cell cultures. Science, 219: 676-82.

Chawla, H.S., Wenzel, G., 1987. In vitro selection for fusaric acid resistant barley plants. Plant Breeding 99, 159–163.

Dasgupta, M.K. 1992. Rice sheath blight: the challenge continues. pp130-157. Edited by Singh, U.S. Plant Diseases of International Importance. Volume I. Diseases of Cereals and Pulses. Prentice Hall, Englewood Cliffs, New Jersey.

Earle, E.D. and E.G. Vernon, 1982. Effects of Helminthosporium phytotoxins on cereal leaf protoplasts. Proc. 5th Intl. Cong. Plant Tissue and Cell culture 663. Ebel, J., Ayers, A.R., Albersheim, P. (1976). Host – pathogen interactions.

Eck, J.M.V. and Smith, N. (1996) Tissue culture. in : Environmentally Safe Approaches to crop disease control'',N.A.Rechcigl. CRC Press,BocaRaton, New York, pp. 317-331

Fuime, F., Fuime, G., 2003. Use of culture filtrates of Pyrenochaeta lycopersici in tests for selecting tolerant varieties of tomato. J. Plant Pathol. 85, 131–133.

Ganesan, M., Jayabalan, N., 2006. Isolation of disease-tolerant cotton (Gossypium hirsutum L. cv. SVPR 2) plants by screening somatic embryos with fungal culture filtrate. Plant Cell Tissue Organ Cult. 87, 273–284.

Gayatri, M.C., Roopa Darshini, V., Kavyashree, R., 2005. Selection of turmeric callus for tolerant to culture filtrate of Pythium graminicolum and regeneration of plants. Plant Cell Tissue Organ Cult. 83, 33–40.

Gengenbach, B.G., G.E. Green, C.M. Donovan, 1977. Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Natl. Acad. Sci., USA, 74: 5113-17.

Gengenbach, B.G., Green, C.E., Donovan, C.M., 1977. Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. Proc. Natl. Acad. Sci. U.S.A. 74, 5113–5117.

Gengenbach, B.G., J.A. Connelly, D.R. Pring and M.F. Conde, 1981. Mitochondrial DNAvariation in Maize plants regenerated during Tissue culture Selection. Theor. Appl. Genet, 59: 161-167.

Gentile, A., Continella, G., Tribulato, E., Vardi, A., 1992. Differential responses of citrus calli and protoplasts to the culture filtrate and toxin of Phoma tracheiphila. Theor. Appl. Genet. 83, 759–764.

Gentile, A., Tribulato, E., Deng, Z.N., Galun, E., Fluh, R., Vardi, A., 1993. Nucellar callus of 'Femminello' lemon, selected for tolerance to Phoma tracheiphila toxin, shows enhanced release of chitinase and glucanase into the culture medium. Theor. Appl. Genet. 86, 527–532.

Hammerschlag, F.A., 1988. Selection of peach cells for insensitivity to culture filtrates of Xanthomonas campestris pv pruni and regeneration of resistant plants. Theor. Appl. Genet. 76, 865–869.

Han GY, Wang XF, Zhang GY, Ma ZY (2009) Somatic embryogenesis and plant regeneration of recalcitrant cottons (Gossypium hirsutum). Afr. J. Biotechnol. 8(3): 432-437.

Harper, G., Osuji, J.O., Heslop-Harrison, J.S. and Hull, R. 1999. Integration of banana streak badna virus into the Musa genome: molecular and cytogenetic evidence. Virology 255: 207-213.

Hartman, C.L.,McCoy, T.J., Knous, T.R., 1984. Selection of alfalfa (Medicago sativa) cell lines and regeneration of plants resistant to the toxin(s) produced by Fusarium oxysporum f. sp. medicaginis. Plant. Sci. Lett. 34, 183–194.

Hendre, R.R., Mascarenhas, A.F., Pathak, M., Nadgir, A.L. and Jagannathan, V. 1975. Growth of virus free sugarcane plants from apical meristem. Indian Phytopathology, 28:175-178.

Jain M, Bhatnagar RK, Sarin NB (1999). Isolation and biochemical diagnosis of cell lines of groundnut (Arachis hypogaea L.) selected on glyphosate. Pestic. Sci., 55: 843-849.

Jayasankar, S., Litz, R.E., 1998. Characterization of resistance in mango embryogenic cultures selected for resistance to Colletotrichum gloeosporioides culture filtrate and phytotoxin. Theor. Appl. Genet. 96, 823–831.

Jayashankar, S., Li, Z., Gray, D.J., 2000. In vitro selection of Vitis vinifera Chardonnay with Elsinoe ampelina culture filtrate is accompanied by fungal resistance and enhanced secretion of chitinase. Planta 211, 200–208.

Julian, 1985. Toxicity of fusaric acid on mesophyll cell cultures of Asparagus officinalis: Implications for selection to resistance. Proc. 6th Asparagus Symp Guleph., pp: 238-2445.

Krause, I.R., Mankowska, G., Lukaszewicz, M., Szopa, J., 2003. Regeneration of flax (Linum usitatissimum L.) plants from anther culture and somatic tissue with increased resistance to Fusarium oxysporum. Plant Cell Rep. 22, 110–116.

Krishnamurthi, M and J. Thaskal, 1974. Fiji disease resistant Saccharum officinarum Var. Pindar subclones from tissue cultures. Proc. Int. Soc. Sugarcane Technol., 15: 130-137.

Kumar, J.V., Ranjitha Kumari, B.D., Sujatha, G., Castano, E., 2008a. Production of plants resistant to Alternaria carthami via organogenesis and somatic embryogenesis of safflower cv. NARI-6 treated with fungal culture filtrates. Plant Cell Tissue Organ Cult. 93, 85–96.

Larkin, P.J. and W.R. Scowcroft, 1983. Somaclonal variation and eyespot toxin tolerance in sugarcane. Plant Cell Tissue Org. Cult, 2: 111-121.

Larkin, P.J. and W.R. Scwocroft, 1981. Somaclonal variation a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., 60: 197-214.

Lee, I.M., Klopmeyer, M., Bartoszyk, I.M., Gundersen-Rindal, D.E., Chou, T.S., Thomson K.L. and Eisenreich, R. 1997. Phytoplasma induced free branching in commercial poinsettia cultivars. Nature Bio/Technology 15:178-82.

Limasset, P., Cornuet, P. and Gendron, Y. 1949. Recherche du virus de la mosaïque du tabac (Marmor tabaci Holmes) dans les organes aériens de tabac infectés. C.R. Acad. Sci., Paris, 228: 1888-1896.

Ling, D.H., P. Vidhyasekharan, E.S. Borromeo, F.J. Zapata, T.W. Mew, 1985. In vitro screening of rice germplasm for resistance to brown spot disease using phytotoxin. Theor. Appl. Genet, 71: 133-135.

Lizarraga, R., P. Tovar, U. Jayasinghe and J. Dodds 1986. Tissue culture for elimination of pathogens. Specialized Technology Document 3. International Potato Center, Lima, Peru., pp: 22.

Maliga P (1978). Cell culture procedures for mutant selection and characterization in Nicotiana plumbaginifolia. In: Vasil IK (ed). Cell culture and somatic cell genetics of plants, Vol. 1. Academic Press, New York, pp 552-562.

Maloy, O.C. (2005) "Plant disease management. The plant instructor. D01:10 1094/PHL – 1 – 2005– 0202 – 01.

Maribona, R.H., M.R. Leal and S.B. Korneva, 1984. Resistance expression to Helminthosporium sacchari toxin in sugarcane tissue culture. PI Tissue Cell Cult. Appl. Crop Imp., pp: 359-360.

Maynard C, Xiang Z, Bickel S, Powell W (1998) Using genetic engineering to help save American chestnut: a progress report. J. Am Chestnut Found. 12: 40-56.

Melcher, L.S., and Stuiver, M.H. 2000. Novel genes for disease resistance breeding. Curr. Opin. Plant Biotech. 3:147-152.

Morel, G. and Martin, C. 1952. Virus-free Dahlia through meristem culture. C. R. Hebd. Seances Acad. Sci. Paris 235: 1324-1325.

Morel, G.M. and C. Martin, 1952. Guerison de dahlias atteintsd'unemaladleAvirus. Comptesrendushebdomaire des s~ances de l' Academie des Sciences, Paris., 235: 1324-1325.

Mullin, R.H., Smith, S.H., Frazier, N.W., Schlegel, D.E. and McCall, S.R. 1974. Meristem culture frees strawberries of mild yellow edge, pallidosis and mottle diseases. Phytopathalogy 64: 1425-1429.

Murata M, Ryu J-H, Caretto S, Rao D, Song H-S, Widholm JM (1998). Stability and culture limitations of gene amplification in glyphosate resistant carrot cell lines. J. Plant Physiol. 152: 112-117.

Remotti, P.C., Loffler, H.J.M., Vanvlotendoting, L., 1997. Selection of cell-lines and regeneration of plants resistant to fusaric acid from Gladiolus grandiflorus cv. Peter pears. Euphytica 96, 237–245.

Rines, H.W. and H.H. Luke, 1985. Selection and regeneration of toxin. insensitive plants from tissue cultures of oats {Avena sativa) susceptible to Helminthosporium victoriae. Theor. Appl. Genet, 71: 16-21.

Sacristan, M.D. and F. Hoffmann, 1979. DirectMnfection of embryogenic cultures of haploid Brassica napus with resting spores of Plasmodiophora brassicae. Theor. Appl. Genet, 54: 129-32.

Sacristan, Mf.D., 1982. Resistance responses to Phoma lingum of plants regenerated from selected cell and embryogenic cultures of haploid Brassica napus. Theor. Appl. Genet, 61: 193- 200.

Scala, A., P. Bettini, M. Baiatti, P. Gogani, G. Pellegrini and F. Tognoni, 1984. In vitro analysis of the tomato Fusarium oxysporum system and selection experiments. PL Tissue Cell Cult Appl. Crop Impr. Meet., pp: 361-62.

Sellin C, Forlani G, Dubois J, Nielsen E, Vasseur J (1992). Glyphosate tolerance in Chicorium intybus L. var. Magdebourg. Plant Sci., 85: 223-231.

Sengar, A.S., Thind, K.S., Kumar, B., Pallavi, M., Gosal, S.S., 2009. In vitro selection at cellular level for red rot resistance in sugarcane (Saccharum sp.). Plant Growth Regul. 58, 201–209.

Solodkaya, LA. and A.V. Menzentsev., 1983. In: the abstracts of the 2 All Union Conference on Genetics of somatic cells in Culture. Moscow. Zvenigorod, p. 37 (In Russian).

Thanutong, P., I. Furusawa and M. Yamamoto, 1983. Resistant tobacco plants from protoplast - derived calluses selected for their resistance to Pseudomonas and Alternaria toxins, Theor. Appl, Genet., 66: 209-215.

Thomas BR, Pratt D (1982). Isolation of paraquat-tolerant mutants from tomato cell cultures. TAG, 63: 169-176.

Toldi O, Toth S, Oreifig AS, Kiss E, Jenes B (2000). Production of phosphinothricin- tolerant rice (Oryza sativa L.) through the application of phosphinothricin as a growth regulator. Plant Cell Rep. 19: 1226-1231.

Toyoda, H., Chatani, K., Matsuda, Y., Ouchi, S., 1989. Multiplication of tobacco mosaic virus in tobacco callus tissues and in vitro selection for viral disease resistance. Plant Cell Rep. 8, 433–436.

Vidhyasekaran, P., Ling, D.H., Borromeo, E.S., Zapata, F.J., Mew, T.W., 1990. Selection of brown spot-resistant rice plants from Helminthosporium oryzae toxinresistant calluses. Ann. Appl. Biol. 117, 515–523.

Warabi E, Usui K, Tanaka Y, Matsumoto H (2001). Resistance of a soybean cell line to oxyfluorfen by overproduction of mitochondrial protoporphyrinogen oxidase. Pestic. Manag. Sci., 57: 743-748.

Yang, Z., Yang, X., Huang, D., 1998. Studies on somaclonal variants for resistance to scab in bread wheat (Triticum aestivum L.) through in vitro selection for tolerance to deoxynivalenol. Euphytica 101, 213–219.

Zambrano AY, Demey JR, Gonzalez V (2003). In vitro selection of a glyphosate-tolerant sugarcane cellular line. Plant Mol. Biol. Rep. 21: 365-373.

Zapata, C., Miller Jr., J.C. and Smith, R.H. 1995. In vitro eradication of viruses from potato. In vitro Cell Dev. Biol.-Plant. 31: 153-159.