CHAPTER 1
GENERAL INTRODUCTION

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- Plant biotechnology and its impact on Agriculture

- Somatic cell genetical approach - general

- Somatic cell genetical approach - Disease resistance

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PLANT BIOTECHNOLOGY AND ITS IMPACT ON AGRICULTURE

Current world population of 5 billions is expected to increase to 10 billions in the mid half of 20th Century. Almost 90% of the global population increase is expected to take place in the third world countries of which millions of people are exposed to miserable hunger. To meet the demands of food needs of the future the immediate challenges of the plant biotechnologists are to develop high yielding plant varieties with greater resistance to diseases, pests and environmental stress like drought and salanity.

The need of such improved crop varieties is so immediate that the conventional time consuming breeding techniques will not be able to solve the problem in time. Plant Biotechnology based on new techniques involving the cell, protoplast, tissue and organ cultures as well as the genetic engineering techniques is useful to propagate interesting new varieties very rapidly and to create new ones respectively (Mantell et al, 1985; Perrik, 1987). Further impetus of plant biotechnology has come through their commercialization by multinationals like Unilever, Monsanto, Rhone Pulac, Shell, etc. According to a forecast made by an

international seed and plant-science consultancy agency the world food production will rise by 10 to 15% in the next 25 years as a result of application of biotechnologies alone (Withers and Alderson, 1986). Table 1 recapitulates some of the cultivated plants along with the probable marketing dates as a result of the progress made in their biotechnology and genetic engineering.

Table 1:

PROBABLE DATE FOR MARKETING NEW PLANT VARIETIES AS A RESULT OF PLANT BIOTECHNOLOGY (SELECTED EXAMPLES FROM L. WILLIAM TEWELES CO'S REPORT, 1983)

Crop Species	Dates of genetic engineering work	Probable period of release of transformed plants	Probable period of commercialization of transformed plants
Maize	Now	Early 1990's	Mid 1990's
Wheat	1981-1987	Early 1990's	Mid 1990's
Rice	1985-1987	Late 1980's	Early 1990's
Soybeans	Now	Early 1990's	Mid 1990's
Tomato	1983-1985	1984-1986	1986-1988
Sugarcane	1987-1989	Early 1990's	Mid 1990's
Cotton	1985-1985	Early 1990's	Mid 1990's

In Seventies of this century, the world has witnessed a remarkable progress in the field of agriculture by introduction of high yielding select varieties of rice and wheat which is hailed as green revolution. Almost all this progress is attributable to this conventional breeding technologies and development of selective and powerful insecticides and pesticides. The nintees and the first decade of 21st century will witness the fruitful products of biotechnology. This will be the second green revolution or gene revolution.

The space doesn't permit me to describe the newer and numerous such advances of biotechnology which are on horizon. The following description therefore outlines only one of such advancements called plant somatic cell genetics as the present research has relevance to it.

THE SCIENCE OF SOMATIC CELL GENETICS :

In vitro cultured cells, tissues and organs have been used as research tools in studying specific problems of plant cell physiology, biochemistry, genetics and molecular biology. (Blackley and Steward, 1964; Street, 1973; Chaleff and Carlson, 1974; Maliga, 1978; Parke and Carlson, 1979; Flick, 1983; Widholm, 1983; Gonzales and

Widholm, 1985; Kuchenrko, 1985). Problems of genetics like mutational breeding, hybridization and development of homozygous lines can be tackled by the novel science of somatic cell genetics where somatic cells are used in contrast to germ cells (pollen and egg) as used in the traditional genetics. The superiority of somatic cell genetics over the traditional genetics is its rapidity, simplicity, easyness, availability of large experimental units in selectively very small space and precision. On the otherhand it has disadvantage of uncontrolled genetical variation called Somaclonal variation which limits its widespread usage in agriculture and horticulture.

USE OF SOMATIC CELL GENETICS IN DEVELOPING - DISEASE RESISTANCE PLANTS:

It was Carlson(1973) who demonstrated for the first time that somatic cell genetical approach can be successfully employed for breeding plants for disease resistance. During last few years in vitro culture technique has been extensively applied to develop disease resistance in plants (for review see Brettel and Ingram, 1979; Bajaj, 1981; Wenzel, 1985; Daub, 1986; Evans and Sharp,

Table 2:

SOME EXAMPLES OF SUCCESSFUL SELECTIONS OF DISEASE RESISTANT PLANTS FROM SOMATIC CELLS GROWN IN VITRO

(A) : Selection pressure used at Cellular level :

Pathogen/ Disease	Host	Selection Agent in cell culture	Pattern of Inheritance of disease resistance	Reference
Pseudomonas syringnae pv. nicotianae, Wild fire disease	Tobacco	Fungal Toxin (Methionine Sulfoximine)	Single codominant gene for disease resistance	Carlson (1973)
Helminthosporium maydis	Maize	Fungal toxin (HMT-Toxin)	Maternal inheritance	Gengenbach et. al. (1977)
Helminthosporium sacchari	Sugarcane	Partially purified HS toxin	Transmitted to progeny	Heinz et al (1977)
Fusarium oxysporum f.sp. nicotianae	Tobacco	Culture Filterate	Transmitted to progeny	Selvapandiyan (1987)
Fusarium oxysporum f.sp. lycopesici	Tomato	Fusaric acid	Single dominant gene for disease resistance	Shahin & Spivey (1986)

1986). Species for which such an approach has proven successful are listed in Table 2. Specific examples will be described in the subsequent pages.

The isolation of disease resistant mutants using Somatic cell can be achieved through one of the following methods:

1) By in vitro selection of cell or protoplasts resistant to the pathogen cells or its toxin followed by regeneration of plants from resistant cell/protoplast (Behnke, 1980a,b; Connell, 1985; Epp et al., 1984; Hartman et al., 1984a,b; Selvapandiyan, 1987; Shahin, and Spivey, 1986). This is a direct method and often being used.

2) Screening in vitro regenerants derived from population of unselected cells/protoplasts followed by identifying resistant somaclonal variants using conventional screening method (Heinz, et al. 1977; Larkin and Scowcroft, 1981 a,b; Ramnath, et al., 1983). This is an indirect and random method but is useful where fundamental information about in vitro selection agent(s) is lacking.

3) Production of transgenic plants in which foreign gene of viral coat protein or pathogen repellent toxic

protein gene is incorporated. which confers resistance to virus and pest respectively (Abel et al., 1986; Cuozzo et al., 1988; Hilder et al., 1987; Johnson et al., 1989; Vaeck et al., 1987).

4) Antisense method - in which whole genome or part of genome of virus has been inserted in reverse direction (5' -> 3' instead of 3' -> 5') in the transgenic susceptible plant. Because of reverse orientation, it interferes with the transcription of viral DNA when the plants are infected with the putative virus. This is a new molecular approach and shows a great promise to breed plants for resistance to viral diseases.

Thus development in the science of somatic cell genetics has opened new ways to manipulate plant genome for increased disease resistance. Further genetically fixed resistance to a disease is the cheapest and most prophylactic procedure of plant protection against a number of diseases as it promises cleaner environment.

OBJECTIVES AND THE SCOPE OF STUDY

We have selected Tobacco (*Nicotiane tabaccum*) and Sugarcane (*Sacharum spp*) as our experimental systems. Both these are cash crops. One is seed propagated, the

other is propagated vegetatively. Losses in both these crops due to various diseases are very high.

Earlier in our laboratory Selvapandiyan (1987) regenerated large number of tobacco plants of variety Anand-2 from a selected cell line which was resistant to the culture filtrate of a wilt disease causing fungi Fusarium oxysporum. This variety is very sensitive to this disease. Many of the regenerated plants when challenged by F. oxysporum exhibited resistance to the pathogen. However he could not establish the genetical basis of this resistance. I therefore undertook extensive genetical analysis of this wilt disease resistant tobacco plants upto three generations and the results of this work is presented herein. As an offsping of this work, it was also discovered for the first time that this pathogen produces a proteinaceous toxin in its culture filtrate. When applied to healthy tobacco plants this toxin could induce several disease symptoms typical of this wilt disease. Several tests were carried out which suggest that this toxin exhibits some kind of host specificity. In case of sugarcane, whole plant regeneration protocol was established from cell culture. This is a pre-requisite for carrying out experiments on developing disease resistant sugarcane.

Regenerated plantlets were screened for their total content of DNA and the Tm value of their DNA.