CHAPTER 1

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

Recent developments in the techniques and applications of in vitro culture and molecular biology of plants have opened new ways to manipulate plant genomes. The foreseen role of biotechnology in agriculture is based not on the actual production of any genetically superior plants, but on elegant demonstrations in model experimental systems. New hybrids, mutants and genetically engineered plants might be obtained by these methods if the same procedures can be further adapted for crop species.

1-A IN VITRO CULTURE OF PLANT TISSUES, CELLS AND PROTOPLASTS AS AN IMPORTANT TOOL OF PLANT BIOTECHNOLOGY

The use of synthetic media supporting the indefinite growth of plant tissues in vitro was first reported in 1939

(Gautheret, 1939; Nobecourt, 1939; White, 1939). Cultured tissues have been used as research tools in studying specific problems of plant cell physiology and biochemistry and in genetics and molecular biology. The potentials for applying microbial selection techniques to obtain mutant lines of higher plant cells were recognized (Blackley and Steward, 1964b; Street, 1973; Challef and Carlson, 1974; Maliga, 1978; Parke and Carlson, 1979; Flick, 1983; Widholm, 1983 and Gonzales and Widholm, 1985; Kucherenko, 1985). Selection of cell lines with novel phenotypes has been recognized as being important in obtaining cultivars useful to agriculture, horticulture and in the elucidation of basic problems.

Mutant cell lines usually are obtained from protoplasts or from cell suspension cultures. The use of protoplasts has merits, however, cell cultures are easier to handle in practise. In some instances, e.g., Zea mays, plant regeneration is possible from suspension cultures but not from protoplasts. Many variant cell lines (about 188) have been isolated and characterized biochemically by these methods (Flick, 1983).

Cell lines with the accumulation of high levels of various coloured compounds were selected in early years by visual selection. Thus, selections were made from callus with higher accumulation of β-carotene in Daucus carota (Eichenberger, 1951), anthocyanin in Happlopapus gracilis

(Blackeley and Steward, 1964a), red betalain in Beta vulgaris (Constable, 1967). Four clones with variable β-carotene and lycopene levels were isolated from mutagenized plated cells of Daucus carota (Nishi et al., 1974). Zenk et al (1977) used more sensitive techniques such as spectrophotometry or radioimmunoassay to identify strains overproducing high levels of serpentine and ajmalicine in Catharanthus roseus cell cultures.

Biosynthesis of certain amino acids in higher plants is regulated by the feedback control mechanism. The amino acid produced at the end of pathway controls the activity of the enzyme occuring early in the biosynthesis by allosteric control (Widholm, 1972b).

Genetic variants, overproducing end-products of metabolic pathways (i.e., proline, methionine, threonine, and lysine) have been obtained through selection experiments directed using analogues of the end products (Ohyama, 1974; Widholm, 1977a, b, 1978; Das and Widholm, 1983; Gonzales et al., 1984; Ahmed et al., 1986; Miah, 1987; Quang, 1987). The best studied system is the selection of cell lines resistant to tryptophan analogue, 5-methyl tryptophan (Widholm 1977a; Ranch and Widholm, 1983). The end product tryptophan inhibits the first enzyme, anthranilate synthetase. Widholm (1972a, b) selected tobacco cells resistant to 5-methyl-tryptophan. Once selected, the resistance was stable even in

in absence of selection pressure. The resultant mutant cell lines produced 27 times more tryptophan than the wild type cells. The acquired resistance to 5-methyl tryptophan was due to production of a variant form of anthranilate synthetase that had relaxed feedback control. In Catharanthus roseus a 1.5 fold increase in anthranilate synthetase activity was detected in lines with resistance to 5 methyl-tryptophan (Scott et al., 1979). However, anthranilate synthetase isolated from the regenerated plant was as sensitive to tryptophan as the wild type enzyme (Widholm, 1974).

Cellular selection procedures have been used for selection of auxotrophic mutants (Savage et al., 1979; King and Khanna, 1980; King et al., 1980; Gebhart et al., 1981; Sidorov et al., 1981; Strauss et al., 1981), chlorate resistance (Muller and Graffe, 1978), antibiotic resistance (Maliga et al., 1973; Umiel, 1979; Umiel and Goldner, 1976), nucleic acid base analogue resistance (Littlefield, 1964; Bright and Northcote, 1974, 1975; Ohyama, 1974; Marton et al., 1978), and saline resistance (Nabors et al., 1980; Pandey and Ganapathy, 1984; Labrun et al., 1985; Salgado Garcigua et al., 1985; Warren et al., 1985).

1-B APPLICATION OF IN VITRO CULTURES OF PLANT TISSUE, CELL AND PROTOPLASTS IN DISEASE RESISTANCE

Plant tissue culture has been a useful tool in the field of plant pathology. The eradication of virus infections by

meristem tip culture and subsequent micro-propagation of the material is a good example (Walkey, 1968, 1978, 1980; Walkey et al., 1974; Mori, 1977; Quak, 1977).

During the last few years in vitro culture technique has been extensively applied to develop disease resistance in plants (reviews: Brettell and Ingram, 1979; Bajaj, 1981; Wenzel, 1985; Daub, 1986; Evans and Sharp, 1986). Species for which such an approach has proven successful are listed in Table 1. Specific examples will be noted in subsequent paragraphs.

Some isolates of bean callus cultures developed tolerance to the filtrate of the host specific haloblight bacterium Pseudomonas phaseolicola (Burkh.P Dows. (Bajaj and Saettler, 1970). This work was extended to another bacterial disease, the wild fire of tobacco caused by Pseudomonas tabaci (Wolf & Foster) Stev. (Carlson, 1973a). The plants were regenerated from protoplasts selected for resistance to methionine sulfoximine, a compound related to the toxin of P. tabaci. Potato calli resistant to the culture filtrate (CF) of fungus Phytophthora infestans (Mont.) de By. were regenerated into plants. Leaves from these plants also exhibited greater resistance to the filtrate than the parents (Behnke, 1980a). Thus selections for disease resistance were accomplished by challanging cultures with toxin filtrates from various pathogens (Gengenbach and Green 1975; Behnke and Lonnendecker, 1977; Gengenbach et al.,1977;

Table 1-1 : Disease resistant selections using in vitro cultures

Host	Pathogen	Selection pressure	Explant	Response	References
Phaseolus vulgaris cv. Monilov. Red (Kidney bean)	Pseudomonas phasolicola (Halo-blight)	Halo toxin	Excised roots, stem callus & cell suspension	Differential growth inhibition upto 77%; increase in abnormal cells; 55 fold increase in ornithine in resistant callus	Bajaj & Saettler, 1970.
Nicotiana tabacum L. (Tobacco)	Phytophthora parasitica var. nicotianae (Black-shank)	Fungus	Pith callus	In vitro resistance	Helgeson et al.1972, 1976
-- do --	Pseudomonas tabaci (Wild fire)	Methionine sulfoximine - analogue of methionine	Haploid protoplast	Plants resistant to the analogue	Carlson, 1973a.
Zea mays L. (Maize)	Helminthosporium maydis race T toxin (Southern corn leaf blight)	T-toxin	Embryo callus	Toxin-resistant cells and plants	Gengenbach & Green, 1975 Gengenbach et al.,1977
-- do --	-- do --	- do -	Leaf protoplast	Resistant protoplasts	Pelcher et al.,1978

contd...

Table 1-1 (contd.)

Saccharum officinarum (Sugarcane)	H. sacchari (eye spot)	Crude extrate	Cell suspension	Disease resistant clones	Heinz et al., 1977
Solanum tuberosum cv. Russet. Burbank (Potato)	Alternaria solani (Early-blight)	Semipurified toxin (lipid like) from CF	Leaf protoplasts	Partial resistance	Matern et al., 1978
S. tuberosum cv. Kennebec	Phytophthora infestans (Late-blight)	Cell wall extract (elicitor)	Leaf protoplasts	Rapid agglutination and death of protoplasts	Peters et al., 1978 (cf. Bajaj, 1981)
Brassica napus (Rape)	Plasmodio-phora brassicae (club-rot)	Spores	Stem embryo cultures of haploid	Resistant plants from the resistant callus portion	Sacristan & Hoffman, 1979.
S. tuberosum	Phytophthora infestans (Late-blight)	Culture filtrate (CF)	Callus cells	Resistant plants	Behnke, 1979, 1980a.
-- do --	Fusarium oxysperum (Wilt)	-- do --	-- do --	--do--	Behnke, 1980b.
Pennisetum americanum cv. PHB 10,12,14 (Pearl millet)	Claviceps fusiformis (Ergot)	Sclerotia extract	Embryos, excised, roots, mesocotyl and callus	Differential growth inhibition; tolerant tissues	Bajaj et al., 1980

contd...

Table 1-1 (contd.)

S. tuberosum cv. Superior	*Alternaria solani* (Early blight)	toxin preparation from CF	Cell cultures from tuber	Resistant cells	Handa et al., 1982
S. khasianum	*F. oxysporum* (Wilt)	CF	Anther derived calli	Resistant calli	Ramnath et al.,1983
Musa L. spp. (Banana)	*F. oxysporum* (Wilt-'Panama')	Fusaric acid(FA)	in vitro clones	Resistant plants	Epp et al., 1984
Medicago sativa (Alfalfa)	*F. oxysporum* (Wilt)	CF	Callus from cotyledon and overy tissue and cells	-do-	Hartman et al., 1984 a,b.
Humulus lupulus (Hop)	*Verticillium alboatrum* (Wilt)	CF	Cell suspension	Resistant cells	Connell, 1985
Oryza sativa (Rice)	*Helminthosporium oryzae* (Brown spot)	Toxin	Cell suspension	Resistant plants	Ling et al., 1985
Glycine max (Soybean)	*Phialophora gregata*	CF	Calli	Resistant calli	Guan et al., 1986
Prunus persica (Peach)	*Xanthomonas campestris* (Black-rot)	Toxin	-do-	Resistant plants	Hammerschlag, 1986
Lycopersicon esculentum L. (Tomato)	*Phytophthora infestans* (Late-blight)	CF	-do-	Resistant calli	Illag & Dallacqua, 1986
Populus spp.	*Septoria musiva* (stem cankers)	-do-	Leaf disks	Clones increase in resistance	Ostryn et al., 1986

contd...

Table 1-1 (contd.)

Avena sativa (Oats)	Helminthosporium victoriae	Toxin	Calli	Resistant plants	Rines, 1986
L. esculentum	F. oxysporum f. sp. lycopersici race 2 (Wilt)	FA	Protoplasts	-do-	Shahin and Spevey, 1986
-do-	Alternaria solani (early blight)	CF	Calli	-do-	Shepherd & Sohndal, 1986

Mattern et al., 1978; Behnke, 1979; Bajaj et al., 1980; Epp et al., 1984; Hartman, 1984b, Connell, 1985; Ling et al., 1985). Genetic mosaics, occurring during the course of plant tissue cultures, also can be exploited to obtain disease resistant mutants (D'Amato, 1977). This strategy was used to develop resistance to Fiji disease-virus (Krishnamurthy and Tlaskal, 1974).

The isolation of disease resistant mutants through in vitro culture method can be achieved by one of the two methods: i) by in vitro selection of lines resistant to toxins of the pathogen followed by regeneration of plants from resistant cells (Behnke, 1980; Epp et al., 1984; Hartman, 1984 a,b; Connell, 1985; Shahin and Spivey, 1986) or by ii) Screening regenerated plants from unselected cell cultures and identifying resistant somaclonal variants (Heinz et al., 1977; Larkin and Scowcroft, 1981a, b; Ramnath et al., 1983). Both the approaches have been found equally effective in developing resistant plants. The selection of a particular one is an individual's choice. If one is working with a disease that has been well characterized, in which toxins have been identified and in which other in vitro selection schemes have been devised, then one may utilise in vitro selection. Otherwise it is better to screen unselected regenerants.

Tobacco is an important cash crop of our state. Crop yields are limited by wilt inducing fungus, Fusarium oxysporum f. sp. nicotianae (J.Johnson) Snyd. & Hans. (Valleau, 1952). Conventional breeding for wilt disease resistance has not been successful. Hence, we decided to address this problem using in vitro culture technology. At this stage, it would be useful to discuss the present status of research on wilt disease in plants.

1-C PATHOGEN INDUCED WILT DISEASE

Wilt is an important plant disease characterised by loss of turgor followed by drying of the entire plant. It may result from injury to the root system, partial plugging of water conducting vessels, or toxic substances secreted by the pathogen. In angiosperms the propagules of the pathogen are easily carried in the vessels, but in the gymnosperms the discontinuity and resistance offered by short and narrow tracheids prevents such intercellular transport (Smith, 1967).

1-C.1 Causal agents :

The most common plant pathogens causing wilt are bacteria and fungi (Mace et al., 1981). Important plants such as potato, tomato, eggplant, groundnuts and banana are affected by the Pseudomonas solanacearum. E.F.Sm. Buddenhagen and Kelman (1964) delimited this species into 3 races. Race 1

has a wide host range, while Race 2 and 3 are restricted to a few plants. Xanthomonas, Corynebacterium and Erwinia are among other pathogenic bacteria inducing wilt (Harris, 1940; Main, 1964).

Among the fungi, Fusarium and Verticillium are the most common vascular pathogens (Mace et al., 1981). Production of the highly prized banana variety 'Gras Michel' was abandoned in many parts of the Western hemisphere because of the 'Panama disease' caused by Fusarium oxysporum f. sp. cubense (E.F.Sm.) Snyd. & Hans. (Green, 1981). The genus Fusarium is a persistent 'soil inhabitant' and once established, persists for several years, rendering the soil unfit for profitable crop production. It is more prevalent in warmer and tropical countries. Fusarium is a taxonomically complex genus. Snyder and Hansen (1940) grouped all forms into one species, F. oxysporum, comprising of several formae specialis named on the basis of their specialized hosts. Thus, F. oxysporum f. sp. vasinfectum (Atk.) Snyd. & Hans. infects cotton; F. oxysporum f.sp. lycopersici (Sacc.) Snyd. & Hans. infects tomato; F. oxysporum f. sp. cubense (E.F.Sm.) Snyd. & Hans. infects banana; F. oxysporum f.sp. lini (Bolley.) Snyd. & Hans. infects flax; and F. oxysporum f.sp. nicotianae (J.Johnson) Snyd. & Hans. infects tobacco.

The Fusarium wilt disease is reported in diverse crops such as bananas (Vakili, 1965), peas (Linford, 1928), tomato (Wellman, 1941; Foster, 1946), tobacco (Valleau, 1952), cotton (Kappelman, 1975; Smith and Snyder, 1975), chickpea (Kanniyan et al., 1984), sugarbeet (McDonald et al., 1976), flax (Wilhelm, 1981) and numerous others. The genus Verticillium was established in 1916 by Nees von Esenbeck (cf. Green, 1981) based on the characteristic verticillate conidiophore. V. albo-atrum Reinke & Berth. and V. dahliae Kleb. are considered to be the most important, causing wilt disease in a wide range of crop and ornamental plant species mainly in the temperate and cold regions of the world (Issac, 1967). Other fungi responsible for wilt diseases are Ceratocystis ulmi (Buism.) Moreau the agent causing Dutch elm disease in elm trees (Mace et al., 1981). Cephalosporium diospyri Crandall - persimmon wilt of oak (Crandall, 1945) and elm (van Arsdel, 1972) and Dothiorella (Cephalosporium) ulmi (Buism.) Moreau - wilt of American elm (Verrall and May, 1937).

1-C.2 Disease syndrome :

After germination of the spores in the host exudate, the fungi (Fusarium and Verticillium) enter the host through wounds or via the tissue. Formative symptoms such as vein clearing (Foster, 1946; Kalyanasundaram, 1954; Raade and Wilhelm 1958), leaf yellowing and epinasty (Hall, 1952; Threlfall, 1959) and development of adventitious roots may begin as early as 48 hr.

after root infection (Dimond, 1955). However, the conspicuous symptoms appear from 2 to 4 weeks after entry into the host. Yellowing and wilting of the older leaves on one side of maturing plants slowly spreads either upward along the elongated stem or inward in rosette type plants (Talboys, 1958). Dwarfing and stunting are observed as the late symptoms before the death of the plant (Engelhard and Bragonier, 1957; Selman, and Pegg 1957; Talboys, 1958, 1970). Brown discolouration of the cortical region and sloughing off of the epidermis of the root arise from rotting of the outer parenchymatous tissues. Discolouration extends also to the lower portion through the whole length of the stem including petioles and leaf veins. The discolouration is due to deposition of melanin pigments, formed by oxidation and polymerization of phenols of the host, in the walls and lumens of the middle lamella due to macerative action of pectic enzymes. Tyloses are formed by the extensions of xylem parenchyma into the vessels (Pennypacker and Nelson, 1972; Emberger and Nelson, 1981; Stuchling and Nelson, 1981; Harling and Taylor, 1985). Other responses of the xylem parenchyma to infection are hyperplasia and hypertrophy which have been attributed to increased auxin levels in infected plants (Dimond, 1970).

The rapid gelation response, together with the hyperplasia, were considered to be responsible for the physical containment of the fungus in the resistant cultivars and are

therefore, an important part of defense mechanism in the host against the pathogen (Harling and Taylor, 1984). The vascular plugging formed sometime during pathogenesis is one of the major factors responsible for the wilt.

1-C.3 Mechanism of Wilting :

It is agreed that water shortage is the cause of wilting, but the precise factors leading to water deficits have not been established. Many theories have been proposed by Brian (1958), Dimond (1955, 1970), Gäumann (1957), Sadasivan (1961), Saraswathi Devi (1964) and Talboys (1970) to explain the mechanism of wilting. The two possible ideas proposed are the 'plugging' and 'toxin' theories.

The support for the plugging or occlusion theory is based on the following observations: i) reduced rate of water flow in vessels (Melhus et al., 1924; Harris, 1940; Beckman et al., 1953; Dimond and Waggoner, 1953b), ii) presence of obstructions in the vessels: these are the pathogens themselves / or their metabolites, products of host tissue degradation such as gels and gums, and finally pathogen-induced host responses (tyloses, gums) (Harling and Taylor, 1984) and iii) reduced rate of transpiration in infected plants (Dimond and Waggoner, 1953b; Beckman et al., 1962).

In contrast to the plugging theory, Gäumann (1951) advanced the theory that wilting is due to production of toxins by the pathogen. It is envisaged that low molecular weight substances

(Dimond and Waggoner, 1953a) produced by wilt pathogens in roots, are translocated to the leaves. There they adversely affect membrane permeability. Due to breakdown of osmoregulation, excessive loss of water occurs. Hence, a heavy loss of ions, especially potassium, causes an 'ionic imbalance'. There is some evidence that the ionic derangements and water loss are brought about by low molecular weight toxins (Misaghi, 1982).

The first chemically defined wilt toxins were lycomarasmin (Plattner and Clauson-Kaas, 1945) and fusaric acid (Gäumann, 1957) isolated from F. oxysporum f. sp. lycopersici. Lycomarasmin, chemically defined as N-(hydroxypropionic acid) - glycylasparagine (Wooley, 1948), is transported in the tomato plant more rapidly and is more, toxic as an iron chelate (Braun and Pringle, 1967). Fusaric acid (5-butlypicolinic acid) increases transpiration and causes a furrowing over the petiole vascular bundles and leaf necrosis. Kuo and Scheffer (1964) evaluated fusaric acid as a factor in the development of Fusarium wilt and concluded that the toxin was not responsible for wilt symptoms but could play a secondary role in disease development. Ethylene is also considered as a wilt toxin, known to be produced by injured plants (Hall, 1951, 1952)*

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* Some other low molecular weight toxins are listed in Chapter 4.

Fusarium produces an abundance of pectolytic enzymes such as pectin methyl esterase and deplopolymerases (Pierson et al., 1955) and their many isozymes (Madhosingh, 1980). These enzymes are considered to cause reduction transpiration, chlorosis, vascular browning followed by wilting in the host (Pegg, 1981). Secretion of pectinases that dissolves pectic substances in the middle lamella of the xylem parenchyma is also noticed (Pierson et al., 1955).

1-C.4 Mechanisms of plant resistance to wilt :

The plant growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D), naphthaleneacetic acid (NAA), indoleacetic acid (IAA), 2,3,5-triiodobenzoic acid (TIBA), β-naphthaleneacetic acid (β-NAA) induced resistance against Fusarium wilt in the susceptible tomato. This was reported first by Davis and Dimond in 1953. Thereafter many people used growth regulators for increasing resistance, for example, in Verticillium wilt of cotton (Buchenauer and Erwin, 1976; Erwin et al., 1976), Dutch elm wilt (Beckman, 1958; Smalley; 1962), oak wilt (Venn et al., 1968) etc. Greater levels of resistance induced by ethylene were found in Verticillium wilt of tomato by Pegg (1976). Russel (1975) found gibberellic acid apparently decreases resistance in tomato to Verticillium wilt.

Resistance to wilt disease may be decreased by injuries and infections. The inoculum densities of the nematodes and

the wilt fungus determines the degree of susceptibility. Conroy et al. (1972) observed that progressive increase in the Verticillium wilt of tomato occured when the density of either microsclerotia or nematode increased in mixed inoculations.

Cellulose amendments increase the severity of Fusarium wilt in peas (Guy and Baker, 1977). Similarly certain other chemicals can reduce the resistance, presumably because they increase the inoculum density in soil.

Resistance to wilt disease may be controlled by a single dominant gene, as in the case of melon, pea, tomato, cabbage, sunflower and upland cotton or by two or more dominant genes as in Egyptian cotton, bananas, sweet potatoes and elms (Bell and Mace, 1981). Resistance mechanisms might work in the host plant any time between spore germination on the plant surface to colony formation in the xylem vessels (Harling and Taylor, 1984). The resistance effective against one wilt fungus is also effective against others. Thus resistance of soybeans to Cephalosporium is also effective against Verticillium (Tachibana, 1971). Similarly resistance of cotton to Verticillium is also effective against Fusarium (Bell and Mace, 1981).

The resistance of the plant varies depending on the age of the plant. The juvenile resistance of elms to Ceratocystis is lost largely during the second growing season and mature trees

reach a peak of susceptibility (Ceroselli and Feldman, 1951; Schreiber, 1970; Townsend, 1971). With respect to individual leaves, there is a difference to wilt fungi even when whole plant is increasing in resistance (Busch and Edington, 1967; Busch and Schooley, 1970; Howell et al., 1976).

Sequential changes in host resistance vary considerably during growth and development (Bell and Mace, 1981). Trees show marked seasonal as well as annual change in resistance. Consequently, trees infected during the peak of susceptibility in late spring may recover as resistance increases during summer (Wilhelm and Taylor, 1965).

The genetic potential of fungal virulence also affects the resistance to wilt disease. Resistant cultivars may become proportionally more susceptible as inoculum concentrations exceed certain critical levels (Rauscher et al., 1974).

1-C.5 Disease complexes-nematodes and wilt fungi :

These are the interactions of two plant pathogens to cause synergistic increase in disease incidence or severity. While feeding on their host, the parasitic nematodes may cause wounds in the host tissues which may provide portals of entry for other pathogens. Fusarium and Verticillium are often involved in such complexes with parasitic nematodes (Green, 1981). As early as in 1902 (cf. Green, 1981) it was observed that

Fusarium wilt of cotton was more severe in the presence of root-knot nematode infection than when the nematode was absent. Similar results were reported by others with different complexes, e.g., nematode Radopholus similis - F. oxysporum in banana (Newhall, 1958) and nematode Meloidogyne javanica - F. oxysporum in tomato (Bergeson et al., 1970).

In contrast to these results, Fassuliotis and Rau (1969) found that resistance of cabbage to cabbage yellow caused by F. oxysporum f. sp. conglutinans was not altered by root-knot nematode Meloidogyne incognita. Johnson and Littrell (1969) also failed to demonstrate a change in resistance to Fusarium wilt in Chrysanthemum using three different species of root-knot nematodes.

1-C.6 Genesis of resistance :

Resistance mechanisms might inhibit any of the four stages of pathogenesis (Bell and Mace, 1981): i) germination of the propagules of fungus and its establishment on the plant surface, ii) fungal penetration through the outer root or stem tissues into xylem vessels, iii) fungal proliferation within xylem vessels and iv) growth of the fungus from xylem vessels into sorrounding stem or leaf tissues. In these stages, wilt pathogens encounter different types of cells and tissues that may exert different kinds of magnitudes of resistance.

1-C.7 Sources of Resistance to wilt disease :

Despite the difficulty in isolating host genes that would confirm absolute resistant to the pathogen, breeders have produced several tolerance varieties to Fusarium spp. by conventional breeding techniques. The first wilt resistant flax, 'N.D 52', 'N.D.73' and 'N.D.114' were selected by Bolley in 1907 (cf. Wilhelm, 1981) from common varieties by screening in a heavily infested field with Fusarium in North Dakota, USA. A long range breeding effort aimed at the improvement of cultural and brewing characters of hop in England (Keyworth, 1947, cf Wilhelm, 1981) provided agronomically promising hybrid clones for screening against Verticillium wilt. After several years of research, Chamberlain and Bernard (1968) found that in soybeans, out of the more than 2,000 varieties and collections, none was immune to vascular infection. However a high resistance was identified in P.I. 84946-2 (soybean variety) a line of unknown origin selected from a Korean seed accession. Put (1958, cf. Wilhelm, 1981) tested sunflower varieties and inbred lines under natural field conditions' in Manitoba and obtained disease reactions ranging from 100% susceptible to highly resistant upto 79%. Resistance to F. oxysporum f.sp. albedinis (Killian & Maire.) Malencon. has been identified in a number of date varieties (Pereau-Leroy, 1958). In 1982, Nene and Kannaiyan screened 11,000 entries of Cajanus cajan to F. udum and identified 33 resistant lines. However, only one line

ICP. 8863 was found to be resistant in both greenhouse and laboratory screening tests.

The advantages of planting F1 hybrid seed are well known for many crops (Wilhelm, 1981). In 1974, Davis demonstrated the applicability of this breeding system to the development of early-maturing, high yielding and high quality hybrid cultivars that are adaptable to local requirements. He produced a number of commercial Gossypium hirsutum L. X G. barbadense L.F1 hybrids with high wilt resistance.

1-C.8 Mutation Breeding for induced resistance :

Further success in obtaining wilt resistant mutants through irradiation was reported by Broertjes (1969) and Horner and Melouk (1976) as mentioned by Wilhelm (1981). 11,000 rhizome pieces of Scotch spearmint (Mentha cardiaca Gerard ex Baker) were irradiated with 8,000 to 12,000 radiation dosage units of Cobalt ^{60} gamma rays. Shoots growing from these rhizomes were excised and immersed in the suspension of Verticillium spp. Out of an initial 1,500 plants that were found resistant in soil, several showed high degree of durable resistance. But the same technique failed in soybean to select for Phialophora wilt (Chamberlain and Bernard, 1968).

1-D SCOPE OF STUDY

I am presenting in this thesis, our work on the production of novel wilt disease resistant tobacco plants using cellular breeding technique. Attempts have also been made towards

understanding the resistant behaviour of tobacco to the pathogen F. oxysporum and the nature of the toxin responsible for wilting.

Tobacco is the model species where tissue culture is concerned, particularly with respect to plant regeneration (Vasil and Hildebrandt, 1965) ever since totipotency of cells was demonstrated in it. Production of haploids (Bourgin and Nitsch, 1967), regeneration from the isolated protoplasts (Nagata and Takebe, 1971) and the first somatic cell hybrid between N. glauca and N. langsdorfii (Carlson et al., 1972) also were achieved. All these were easily accomplished with tobacco because it is the easiest material to manipulate in vitro. The effects of growth regulating auxins and cytokinins tested on it are quite specific and reproducible. These incited me to work on N. tabacum to apply the cellular selection approach for wilt resistant lines.