CHAPTER 2
TOBACCO AND SUGARCANE

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- Commercial importance
- Diseases and damages
- Somatic cell genetical approach for disease resistance
- Fusarium wilt disease of tobacco
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TOBACCO

Commercial Importance :

Tobacco is one of the most remunerative cash crops of India. It is grown for its leaves in 87 countries and in 7 continents. India is the third in world tobacco production. Two species *N. tabaccum* and *N. rustica* of tobacco are grown in India for the commercial purpose in around 4 - 4.5 hundred thousand hectares.

Tobacco was exported from India equivalent to foreign value of about Rs. 230 crores in the year 1984. Tobacco occupied the second place among excise commodity and revenue realised by the Government through excise duty of tobacco and tobacco products was of the order of over 850 crores in the year 1984. In terms of employment about 5.5 million people are employed in tobacco industry.

Among the tobacco growing states of India viz., Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu, West Bengal, Orissa, Bihar, Uttar Pradesh and Maharashtra, Gujarat ranks first from productivity view point (Anonymous, 1981-82).

Tobacco - Diseases :

However, loss of tobacco in India due to various diseases like powdery mildew, black shank, mosaic, and nematodes may be around 5 - 10% in a normal year and 50 to 70% in an epiphytotic year. This is equivalent to 34 million Kg or 170 to 230 million Kg loss of tobacco as on data accumulated for the year 1976-77. The major diseases of tobacco are listed in the table3. .

Table 3:

TOBACCO - DISEASES

Sl.No.	DISEASE	CASUAL ORGANISM	AREAS WHERE IT IS PREVALENT
I.	FUNGAL		
01.	Damping off	Predominantly by *Pythium aphanidermatum*	Andhra Pradesh, Gujarat
02.	Black Shank	*Phytophthora parasitica* var, Nicotianae	Karnataka, A.P.,Gujarat
03.	Anthracnose	*Colletotrichum tabaccum*	In all tobacco growing tracks of India.
04.	Powdery Mildew	*Erysiphe cichoracearum* var Nicotianae	A.P., Karnataka
05.	CHITRI (Root Rot Complex)	Complex of *Fusarium oxysporum* f.sp. nicotianae & *F. solani* f.sp. nicotianae	Gujarat
06.	Frog-eye spot	*Cercospora nicotianae*	Andhra Pradesh, etc.
07.	Brown Spot	*Alternaria longipes*	In Tobacco grown in monsoon season.
08.	Sore Shin	*Rhizoctonia solani*	Considered as minor diesease. Occurs both in Nursery as well as in Plantation field.
09.	Leaf Blight	*Helminthosporium speciferum*	Hariyana
II.	BACTERIAL		
01.	Angular Leaf spot	*Pseudomonas tabacii*	Karnataka, Tamil Nadu
02.	Hallow Stalk	*Erwinia carotovora*	Karnataka, A.P.
III.	VIRAL		
01.	Mosaic	Nicotianae Virus I or Marmor tabacii	Widely distributed
02.	Leaf Curl	Nicotianae Virus 10	Widely distributed

Fusarium wilt

In Gujarat State bidi tobacco (N. tabaccum) is cultivated in about 99,400 ha. The principal diseases attacking the young plants of bidi tobacco in nursery are damping-off caused by Pythium aphanidermatum (Edson) Fitz Patrick., wilt caused by Fusarium oxysporum (Schelecht) f. sp. nicotianae, black shank by Phytophthora parasitica f.sp. nicotianae (Breda de Haan) Tucker., brown spot by Alternaria alternata (Fries) Keissler, anthracnose (Colletotrichum dematium (Pers. ex Fr.) Grove), leaf curl mosaic and root-knot nematodes (Meloidogyne incognita (Trenb) Chitwood.

Among these, the most disastrous disease of bidi tobacco is wilt disease locally known as 'Chitri' disease (Prasad et al. 1957). Due to considerable loss caused by this disease, it has become a menance to tobacco cultivation in this area. Actually Chitri is a complex of wilt and root-rot (Fig. 1). Local name 'Chitri' given to the disease in which groups of plants are affected as patches (= Chitri) which extend irregularly but slowly. Usually the disease appears in months of October and November. It is considered that the disease complex involves F. oxysporum f.sp. nicotianae and F. solani f.sp. nicotianae (Prasad and

FIG.1 . Fusarium wilt :

A. yellowing and wilting of leaves on one side of the plant.

B. wilting of entire plant (right)

A

B

Patel, 1952). However wilting of the plants is due to F. oxysporum (Akehurst, 1981).

Disease symptoms :

Clear symptoms of Chitri disease are observed 6 to 8 weeks after planting, though some plants may show the symptoms even 3 to 4 weeks after transplanting. Affected plants show the wilting of leaves. The fungus enters the host through wounds. Preliminary 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 and distinguished symptoms of this disease appear from 2 to 4 weeks after infection. Yellowing and wilting of the older leaves on one side of maturing plants slowly spreads either upward along 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 discoloration is due to deposition of melanin pigments formed by oxidation and polymerization of phenols of the

host in the cell walls and lumens of the middle lamella due to macerative action of pectic enzymes of the fungus. Tyloses are formed by the extensions of plasmalemma 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 the infected plants (Dimond, 1970).

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 notable among them are by Brian (1958), Dimond (1955, 1970), Gaumann (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; Diamond 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, 1985) and increased rate of transpiration in infected plants (Dimond and Waggoner, 1953 b; Beckman et al., 1962).

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

Tobacco cell and Tissue culture - Disease resistance (Table 4):

Tobacco is the model species where tissue culture is concerned, particularly with respect to plant

regeneration ever since totipotency of cells was demonstrated in it (Vasil and Hildebrandt, 1965). Production of haploids from anthers (Bourgin and Nitsch, 1967). Whole plant regeneration from the isolated protoplasts (Nagata, and Takabe, 1971) and the first somatic cell hybrids between Nicotianae glauca and N. langsdorfii (Carlson, Smith and Dearing, 1972) have been achieved in tobacco. Thus tobacco is an ideal system for in vitro manipulations to develop disease resistant plants.

Table 4:

IN VITRO SELECTIONS FOR RESISTANCE TO DISEASES IN TOBACCO

DISEASE	PATHOGEN	SELECTION PRESSURE	INHERITANCE OF RESISTANCE	REFERENCES
FUNGAL DISEASES				
Black Shank	Phytophthora parasitica var. Nicotianae	Fungal mycelium	In vitro resistance	Helgeson et al 1976,
Brown Spot	Alternania alternata	Toxin fraction from culture filtrate (CF)	Transmitted to progeny	Thanutong et al, 1983.
Wilt	Fusarium oxysporium	Culture filtrate (CF)	Co-dominant pattern of inheritance	Selvapandiyai et al, 1987.
BACTERIAL DISEASE				
Wild fire	Pseudomonas tabacii	Methionine sulfoximine (analogue of methionine)	Transmitted to progeny	Carlson 1973
Wild fire	Pseudomonas Syringe c.v. tabacii	Partially purified tabtoxin	Transmitted to progeny	Thanutong et al, 1983

It was Carlson (1973) who demonstrated first time that somatic cell genetical approach can be successfully employed for disease resistant breeding. Carlson used Tobacco-Pseudomonas tabacii as the experimental system. This bacterial pathogen causes wild fire disease in tobacco.

The pathogen produces a toxin methionine sulfoximine (= MSO) which is an analogue of naturally occuring protein amino acid glutamine. Application of methionine sulfoximine to tobacco leaves induced several chlorotic lesions seen typically during pathogenesis. Population of mesophyll protoplasts derived from haploid tobacco plants were challenged with toxic concentration of MSO. Surviving protoplasts were resistant to the toxin. Regenerated plants derived from these resistant protoplasts were also resistant to the attack of pathogen Pseudomonas tabacii. Resistance was transmitted to the progeny. In this case, however, resistance to the toxin and to the pathogen were not the same because the bacterial multiplication was not inhibited in the toxin resistant plants.

Similarly protoplast-derived calli of N. tabaccum cv. 'Samsum' were selected for their resistance to toxins from Phytophthora syringeae pv tabacii which also causes

wild fire disease and from Alternaria alternata that causes brown spot disease (Thanutong, Furusawa and Yamamoto, 1983). Plants were regenerated from each of the toxin-challenged population of calli. Resistance was traced all the way to R2 generation. Resistance to wild fire disease, however seems to be unrelated to resistance to brown-spot disease.

Helgeson et al (1976) envisaged screening of tobacco callus clones with zoospores of Phytophthora parasitica var nicotianae. They reported that the callus tissues derived from resistant plants were all resistant to zoospores attack in vitro. Hence, the resistance gene that is expressed in intact tobacco plants is also expressed in in vitro. Other examples in which expression of disease resistance in tissue cultures has been investigated were tobacco/Pseudomonas tabacii, P. pisi and P. fluorescens.

Selvapandiyan et al (1987) regenerated large number of tobacco plants of wilt susceptible A_{ex} variety from the cell clones that were resistant to the LD50 concentration of culture filtrate of the pathogen F. oxysporum. When these regenerated plants were inoculated in the field with the pathogen, they showed

1.5 to 2 times greater tolerance to the pathogen than the seed-derived plants as well as the control regenerated plants i.e. plants regenerated in the non-selective medium.

Disease protection by Genetic Engineering :

The possibility of engineered plant protection against the pathogens has arisen with the development of methods to insert foreign genes into the plant genome and to express these gene product which confers in its turn protection to donor plants against disease.

Abel et al (1986) used A. tumefaciens to introduce the coat protein gene of tobacco mosaic virus in the tobacco using the leaf disk technique of Horsch et al (1985). Inoculated leaves of the transgenic regenerated plants show fewer chlorotic or necrotic lesions compared with control plants, when challenged by TMV. Further, the rate of systemic spread of the virus was also reduced.

Similarly Cuozzo et al (1988) constructed a Chimeric Coat Protein (CP) gene of another virus called Cauliflower mosaic virus (= CMV) and introduced it into tobacco plants by Agrobacterium-tumefaciens. The gene was introduced in both sense and antisense (i.e. 3' -> 5' and 5' -> 3') orientations. Symptom development and

virus accumulation reduced or absent in the plants having anti sense orientation of CP gene, independent of the strength of the inoculum. Antisense-CP plants were protected only at low inoculum concentrations. Moreover the conferred resistance is stably inherited through successive tobacco generations tested.

A bacterial chitinase gene from Serratia marcescens (Chi A) that encodes a protein which has been shown to possess in vitro anti-fungal activity, was also introduced successfully into tobacco plants through Agrobacterium. Ti plasmid based plant cell transformation in plants that express the Chi A protein at high levels were regenerated (Jones, et al., 1988). Those transformants showed high accumulation of Chi A protein i.e. 25% of total soluble leaf protein and showed higher chitinase enzyme activity.

SUGARCANE

Commercial Importance:

Sugarcane is well known and widely used source of sugar that goes into a wide variety of food and beverage products including speciality sugars, molasses and rum as well as other fermentation products.

Sugarcane is grown in more than a hundred different countries located between latitudes of about 40 degrees North and South of equator. The major producers of Sugarcane includes Brazil, India, Cuba, China, Mexico, the Philippines, South Africa, Australia and the U.S.A. as well as many smaller nations in the Caribbean, Latin America, Africa, the Far East and the Pacific islands. Major sugarcane growing states in our country are UP, Bihar, Haryana, West Bengal, Andhra Pradesh, Tamil Nadu, Karnataka, Maharastra, Gujarat, etc.

Diseases of Sugarcane :

Diseases are among the major constrains to optimum economic returns from sugarcane per unit area, time and inputs in India. As a result cost of sugar tend to rise and make international sugar market tougher. Declining returns from sugarcane prompt farmers as well as

industrialists to pay more attention to other crops. Research data indicate that about 10% increase in yields of sugarcane can be obtained by managing diseases in particular and plant protection in general.

TABLE 5:

COMMON SUGARCANE DISEASES AND THEIR DISTRIBUTION IN INDIA

Name of the diseases	Causal organism	Areas where disease prevalent
Blight of seedlings	Alternaria alternata	Common in sugarcane growing areas
Red-rot of sugarcane	Colletotricum falcatum	Bihar, MP, Rajasthan, WB, UP, AP, Punjab, Haryana, TN, Maharashtra.
Wilt & dry rot of cane	Fusarium moniliformae	South Arcod, and Nellikuppam in Tamilnadu, Nizamsagar in AP.
Smut of Cane	Ustilago Scitaminea	Common; Bihar, MP, UP, and Haryana.
Downy Mildew	Sclerospora sabbhari	Pusa, Bihar
Grassay shoot disease	GSD virus	----
Ratoon stunning and varietal detrioration	RSD bacteria	----

In 1966, the losses due to diseases were roughly estimated to be between Rs. 30-40 crores a year in India alone. Sugar Industry Commission (1973) put it at Rs. 130 crores annually in India. This figure would be much higher if indirect losses too were taken into account.

Major diseases and their causal agents that affect the sugarcane yield are listed in the table 5. Of the listed diseases, two major diseases namely red-rot and smut are responsible for the drastic decline of sugarcane production in India. These two diseases are wide-spread in the major sugarcane growing part of India.

Red-rot disease :

Red-rot was first discovered in Java in 1893. It is caused by the pathogen Glomerella tucumanesis. This became a major cause for the decline of sugarcane Industry in USA, Australia, India and Hawaii. In 1939-42 a virulent strain of red-rot appeared in UP and Bihar, which threatened the very existence of Sugarcane Industry. Again in 1967-77, UP was ravaged by red-rot. Many excellent and promising varieties like CO 281, CO 290, CO 421, etc., became susceptible to this disease.

Red-rot appears to gain foot hold in peninsular India as

well, which has been free from this disease till recently. It has spread to many parts of AP and threatens adjoining Maharastra and Gujarat. This scales down the tonnage as well as the recovery thereby hitting the farmers as well as miller.

External symptoms of the Red rot disease are:

i) The uppermost leaves of a shoot looses colour and droop slightly,

ii) Mid rib shows dark reddish areas,

iii) The affected canes will show longitudinal reddened areas in internodes,

iv) Reddening is more conspicuous in the vascular bundles and progress towards pith.

It is emphasized that in sugarcane any injury leads to the reddening of the tissue. But the characteristic cross bars in reddened area will give an indication that disease is due to red-rot pathogen.

Mode of Infection :

The pathogen infects the host mainly through the leaf scars, thereafter enters the parenchyma and grows intracellularly. It forms intra cellular mycelium in the later stages.

The fungal hyphae penetrate the host cell wall during the progressive stage of disease by forming penetration peg, seen as constriction in the hyphae. The hydrolytic enzymes are not produced in the presence of sugar, but they are produced at a later stage when the tissues begin to die and the pathogen grows on the dead cells of the host.

Sugarcane cell and Tissue Culture for development of disease resistance :

High yielding, disease and pest resistant clones have been developed during past 50 years through conventional breeding and selection programmes which require 10-15 years to release a new variety.

However sugarcane breeding is complicated and has limitations due to 1) high degree of polyploidy, 2) odd cytogenetic behaviour, 3) low seed viability, and 4) limitations in flowering. Even if desired new variety is produced by overcoming all the above said hurdles, large scale multiplication of sufficient planting material of the new variety by normal conventional vegetative method is time consuming. Therefore crop improvement by tissue culture and subsequent clonal propagation of the improved variety is of immense

potential.

A considerable amount of work has been carried out on in vitro culture of both callus and suspension tissues of sugarcane, following the initial work of Nickell (1964). To date, the most promising use of sugarcane cell cultures for crop improvement is in the development of somaclones of commercial varieties possessing disease resistance. Heinz (1973) reported the development of eye spot-resistant somaclones from a susceptible parent clone. Krishnamurthy and Tlaskal (1974) have reported the development of resistant somaclones from Fiji and downy mildew - susceptible parent clones. (Table 6).

Eye spot disease of sugarcane is caused by Helminthosporium saccharii, which produces a toxin, helminthosporoside (Steiner and Strobel, 1971). Suspension cultures of susceptible clone CP 57-603 were used in an attempt to screen for resistance in vitro using helmenthosporoside as screening agent. Suspension cultures were treated with a chemical mutagen, methyl methane sulphonate or ionizing radiation or were left untreated and subsequently cultured in vitro in 0.1 or 5% helmenthosporoside (Crude extract). Plantlets were differentiated, planted in the field and screened for

Table 6:

DISEASE RESISTANCE THROUGH TISSUE CULTURE

DISEASE RESISTANT LINES OF SUGARCANE OBTAINED FROM UNSELECTED IN VITRO CULTURES

Pathogen	Tissue source	Inheritance of resistance	Reference
Fiji disease virus	callus	Transmitted to progeny	Krishnamurthy & Takshal (1974)
Sclerospora sacchari	callus	not tested	Reddi & Gluinadi (1970)
Helminthosprorium sacchari	callus	Transmitted to progeny	Heinz et al, 1977

Table 7:

DISEASE RESISTANT LINES OF SUGARCANE OBTAINED FROM SELECTED IN VITR CULTURES

Pathogen	Toxin	Toxin resistance		Inheritance of resistance	References
		Cell Culture	Regenerated plant		
Helminthosporium sacchari	partially purified toxin	+	+	Transmitted to progeny (asexual)	Heinz et al (1977)

resistance to Helminthosporium sacchari.

Similarly development of Fiji disease-resistant subclones from susceptible parent has been carried out in Fiji with the susceptible clone, pindar (Krishnamurthy and Tlaskal, 1974). The Fiji disease-resistant pindar somaclones were tested over a number of years in several locations and have maintained resistance.