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TOBACCO: Bacterial and Viral Diseases

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Summary

Tobacco is a high value industrial crop, playing an important role in India's agricultural economy. Tobacco is cultivated in fifteen states of India producing 760 M kg of cured leaf from an area of 0.42 M ha. Several bacterial and viral diseases adversely impact tobacco production and quality. The tropical and subtropical conditions in the country besides imminent climate change effects are congenial for further escalation of their incidence and resultant losses. The major bacterial diseases observed in India are bacterial leaf spot, angular leaf spot, bacterial wilt and hollow stalk. They are not a severe menace in all the tobacco growing regions and are endemic to certain locations. Incidence of viruses and damage accrued to them are increasing of late. They are significant threats to quality tobacco production and are of great economic importance particularly tobacco mosaic virus, leaf curl virus, cucumber mosaic virus, etc. Tobacco mosaic virus (TMV) has been studied for almost a century and has wide applications in medicine, pharmaceutics, nanotechnology etc., but the development of resistant varieties is the only solution to manage it on tobacco. The spread of its vector can be managed by utilization of either natural resistance or engineered virus-resistant plants and efficient management of insect vectors. Engineered plants, expressing RNA-silencing nucleotides, are becoming increasingly useful and are likely to provide more effective protection in future Precise rapid detection kits may aid in understanding the specific pathogen and developing management methods. In this chapter, all these diseases and the causative agents were discussed in detail. The host range, biology, etiology and management aspects are presented in the chapter.

Keywords: Angular leaf spot, bacterial leaf spot, bacterial wilt, hollow stalk, tobacco mosaic virus, leaf curl virus and cucumber mosaic virus.

Introduction

Tobacco, the golden leaf is a high value commercial crop for India contributing significantly to the national economy. In India, it is cultivated in an area of about 4.20 lakh hectares with an annual production of 760 M kg cured tobacco leaf. It is the life line for 6 million farmers and supports 40 million workers. India exports about 244 M kg of tobacco and related products to about 100 countries, earning foreign exchange to the tune of ₹ 6000 crores besides an internal excise revenue generation of ₹ 28000 crores. Among the major tobacco

producers, India ranks second in area after China, third in production after China and Brazil, and fourth in productivity after China, Brazil and USA. A unique feature of tobacco production in India is that both flue-cured Virginia (FCV) and non-FCV types are cultivated under varied agro-ecological conditions. The different types of tobacco grown in the country are FCV, burley, chewing, cigar-wrapper, cheroot, oriental, HDBRG, *bidi*, *hookah*, *lanka*, *pikka*, *natu* and the first two are the major exportable types. They are grown in as many as 15 states, predominantly in Andhra Pradesh, Karnataka, Gujarat, and to a limited extent in Tamil Nadu, Uttar Pradesh, West Bengal, Bihar and Odisha. The states of Gujarat, A.P. and Karnataka occupy 1st, 2nd and 3rd positions respectively in both area and production accounting to lion share (> 80%) of Indian tobacco. During the past three decades (1998-2017) the production has increased from 543 M kg to 760 M kg despite the fact that area under tobacco cultivation remained more or less constant. This could be achieved through introduction of high yielding varieties, improved production practices, large scale use of chemical fertilizers and pesticides. Intensive cultivation practices with excessive dependence on chemical fertilizers, changes in varietal profile with wide spread cultivation of few high yielding varieties resulting in narrow genetic base and apparent changes in climatic conditions especially erratic rainfall pattern and temperature have tremendously influenced the intensity and geographical distribution of different diseases. Many diseases hitherto considered as minor, have become serious in many tobacco growing areas.

Cultivated tobaccos, *Nicotiana tabacum* L. and *N. rustica* L. are susceptible to several viral and bacterial diseases, apart from fungal diseases. These diseases not only reduce the yield of tobacco but also adversely impact quality of the cured leaf. Since tobacco is grown as a monoculture, year after year in the same traditional belt, ideal conditions are created for continuous perpetuation of the disease inoculums. They spread the disease in a severe form when weather conditions were favourable during their active growth period. Tobacco leaf being the economic product, any resultant blemish reduces the market value. Considerable research on some of these diseases, in basic and applied aspects has been carried out since last 70 years at the Central Tobacco Research Institute, Rajahmundry, with special emphasis on evolving resistant varieties and suitable management practices to combat them.

BACTERIAL DISEASES

Many bacterial diseases were reported on tobacco worldwide, namely angular leaf spot (*Pseudomonas syringae* pv. *angulata*), wild fire (*P. syringae* pv. *tabaci*), Granville wilt (*P. solanacearum*), bacterial leaf blight (*Pectobacterium carotovorum*), hairy root (*Agrobacterium rhizogenes*), leafy gall disease (*Rhodococcus fascians*) etc. Phyllody (Stolbur-*Candidatus* Phytoplasma spp.) is a phytoplasma disease reported to cause considerable damage in France. In India, angular leaf spot, bacterial leaf spot (*Dickeya dadantii*), Granville wilt and hollow stalk (*Erwinia caratovora* subsp. *caratovora*) are the major bacterial diseases infecting tobacco, particularly in Hunsur and Dinhata (ICTC 1960; Shenoi and Abdul Wajid 1987; Elias et al. 1987).

1. Angular leaf spot

The disease is caused by *P. syringae* pv. *angulata*. The bacterium is non-capsulate, non-spore forming, non-acid fast, Gram-negative, rod shaped bacteria (0.5 × 2.5 μ) with 1-6 flagella (Fromme and Murray 1919). Its prevalence is very little but causes considerable damage during wet season in Karnataka.

Symptoms

Angular dark brown to black coloured spots appear surrounded by yellow halo. Lesions are restricted between veins and leaves appear puckered and tears easily (Fig 1). It was regarded as a mutant of *P. syringae* pv. tabaci, which does not produce tobtoxinine. Morphologically they both seem to be similar (Braun, 1955). *P. syringae* pv. *tabaci* causes a severe type of angular leaf spot well known as wild fire. It was reported in Tamil Nadu (Gnanamanickam et al. 1977).

Fig. 1: Angular leaf spot in tobacco caused by *Pseudomonas syringae* pv. *angulata*.

Etiology

Cloudiness, stormy wind, high humidity, rainwater splashing, excess soil moisture, excess nitrogen and low potassium nutrition are the favourable factors for infection and rapid spread of the disease (Gopalachari 1984).

Management

- Discarding infected seedlings from the nursery
- Removal of diseased plants, their debris and host weeds
- Phytosanitary measures
- Spray of Bordeaux mixture in nurseries
- Application of Streptocycline @ 200 ppm in the planted crop.

2. Bacterial leaf spot

Bacterial leaf spot is caused by *Dickeya dadantii* (*Erwinia chrysanthemi*) in bidi tobacco and late planted FCV tobacco in Karnataka.

Symptoms

Plant develops circular water soaked yellow lesions with a minute brown centre, which expand with a translucent border and wide chlorotic halo (Wolf and Foster 1917). Later plants undergo vascular discoloration of the stems, wilting and stunting (Johnson 1923; Komatsu et al. 2002)

Etiology

The bacterium lies in the soil, and spreads through rainwater splashes to healthy plants. It occurs in epiphytotic form following heavy rains after the crop has been topped, and is more severe in thicker leaves. Low toppings with 8 leaves can considerably thicken the leaves and also enhance bacterial spot disease if it coincided with rains (Lucas 1975; Elias et al. 1987). High humidity and soil moisture, low K supply, subdued light influence the water-soaking of leaves (Johnson et al. 1943).

Management

- High topping at 14 leaf stage reduces the damage (Shenoi and Abdul Wajid 1987).
- Discarding infected seedlings from the nursery
- Removal of diseased plants, their debris and host weeds
- Phytosanitary measures
- Streptomycin sulphate + chlorotetracycline, streptomycin sulphate and streptomycin sulphate + oxytetracycline were effective against the leafspot (Elias et al. 1987).

3. Bacterial wilt

Bacterial wilt is an important disease of both 'Jati' (*N. tabacum*) and 'Motihari' (*N. rustica*) tobacco in *terai* region of West Bengal and has not been reported from other FCV or non-FCV tobacco growing regions of India. Bacterial wilt of tobacco is known to be caused by *Ralstonia solanacearum* and was coined 'Granville wilt' as it was first recognized in FCV tobacco in Granville County during 1880.

Symptoms

The disease initiates right from nursery stage and also seen in planted crop. In the field, the first symptom of the disease is drooping of 1-2 leaves during day which may recover during evening. One half of the affected leaves become flaccid, a characteristic symptom of bacterial wilt of tobacco. On slow progression of the disease, the affected leaves turn light green and may gradually turn yellow, midribs and veins get flaccid and large leaves may droop in an umbrella like fashion (Fig. 2).

Fig. 2: Bacterial Wilt in tobacco caused by *Ralstonia solanacearum*.

Etiology

Plants aged 35-60 days were found to be highly susceptible to bacterial wilt infection in the field following high temperature (25-30ºC) and rainfall. Water logged plots were predisposed to rapid build-up and spread of the disease. In spite of latent infection in plants, they do not express the disease due to tolerating ability in full grown adult plants. The survival of the pathogen in soil and weed hosts is difficult (CTRI 2005). Increase in temperature from 20-30 °C favored wilt development, whereas temperature below 20 °C did not produce symptoms and remained latent. High soil moisture, 20-30 °C temperature and high relative humidity (> 80 %) in atmosphere are the highly favorable predisposing factors for rapid build-up and spread of the disease (CTRI 2006).

Management

Cultural management of the disease includes uprooting diseased plants in the field, burning or composting. The recommended cultural practice from Central Tobacco Research Institute against bacterial wilt of tobacco is application of lime @ 1 t/ha followed by ploughing and a special operation called laddering and kept fallow for 20-30 days. Laddering is the procedure undertaken after the application of lime; the soil is ploughed and the overturned clods are pounded with the help of ladders tied to bullocks. Incorporation of dhaincha as green manure has also found to be beneficial (CTRI 2011). Potato, brinjal, chilies, tomato, jute and mesta are the wilt susceptible crops grown in *terai* region of West Bengal for over 2 decades led to the progressive build-up and spread of the pathogen in soil and crop debris. It is hence an endemic disease of economic importance in that region (Roy et al. 2008).

Fig. 3: Hollow stalk in tobacco caused by *Erwinia carotovora* sub sp. *carotovora*.

4. Hollow stalk

Hollow stalk caused by *Erwinia carotovora* sub sp. *carotovora* (syn. *Pectobacterium carotovorum* sub sp. *carotovorum*) has been reported from U.S.A, Canada and China affecting primarily the stem (black leg) and cured leaves in FCV tobacco. From India hollow stalk has been reported from Motihari tobacco (*N. rustica*) only and to a lesser extent in Jati tobacco (*N.*

tabacum) from *terai* region of West Bengal, where it is endemic (CTRI 2005; 2006; Roy et al. 2008).

Symptoms

Pith undergoes rapid browning and hollowing due to soft rot and eventual collapse of the tissue (Fig. 3). The top leaves wilt and the infection spread is downwards (Lucas 1975). Black leg phase of the disease is characterized by the formation of blacks stripes or bands girdling the stalk and cured leaves have been reported from USA, Canada (Lucas 1975) and China (Xia and Mo 2007) but not from India.

***Etiology*:** Hollow stalk in the field usually appears first at topping and suckering time. The disease may appear at any time due to stem injury but it is commonly observed 35-40 days after topping operations. Losses on an average caused to the crop range from 5-30%. In case of higher latent infection in plants, the entire crop can be wiped out in the event of high rainfall and water logging (CTRI 2005; 2006).

Management

Utmost care should be exercised during field operations; unsterilized sickles and knifes should not be allowed lest the disease spreads to healthy plants. Field operations like desuckering and topping should be avoided during damp and cloudy weather. Effective prophylactic management of the disease has been recommended spot application of Bordeaux mixture or copper oxychloride as slurry/paste on topped and de-suckered points of stalks (CTRI 2006). After the onset of disease, the most effective management strategy lies in providing deep incision with sterilized knife at the stem base or desuckered points of leaf followed by application of Bordeaux mixture or copper oxychloride. Manasi is a tolerant variety of chewing tobacco released by ICAR-CTRI in 2004. Out of a total of 185, *N. rustica* germplasm accessions screened for resistance to hollow stalk disease, only two accessions *viz*. White Pathar and Bengthuli exhibited resistant disease reaction (less than 2 cm linear soft rot of pith) on artificial inoculation (CTRI 2010).

VIRAL DISEASES

Many plant viruses were observed affecting global tobacco production, namely tobacco mosaic virus (TMV), tobacco leaf curl virus (TLCV), tobacco distorting virus (TDV), cucumber mosaic virus (CMV), potato virus Y (PVY), tomato spotted wilt virus (TSWV), tobacco ring spot virus (TRSV), tobacco etch virus (TEV), tobacco necrosis virus (TNV), tobacco bushy top virus (TBTV), tobacco rattle virus (TRV) etc. The latter is vectored by the stubby root nematodes, *Trichodorus* and *Paratrichodorus* sp.

In India, the following viral diseases were found to be infective in artificial inoculation studies: Cabbage ring spot virus (Azad and Sehgal 1959); Chilli mosaic virus (Raychaudhuri and Mishra 1962); Cowpea mosaic virus (Nariani and Kandaswamy 1961); Cucumber mosaic virus (Nariani and Nyako 1963); Potato virus X (Sharma and Raychaudhuri 1962); Sunnhemp mosaic virus (Das and Raychaudhuri 1963); Tobacco yellow net virus (Dhingra and Nariani1961); Turnip crinkle virus (Verma and Verma 1961); Vegetable marrow mosaic virus (Ramakrishnan and Narayanaswamy 1965) . Among the tobacco viruses, TMV, TLCV, CMV, PVY, TEV, TDV were reported to infest the crop naturally in

India, with stray incidences of tobacco ringspot virus (TRSV) and tobacco bushy top virus (TBTV).

1. Tobacco Mosaic Virus (TMV)

TMV is a single-stranded, positive-sense, rod shaped RNA virus in the genus *Tobamovirus* in the family Virgaviridae. It is the first pathogen to be identified as a virus. In 1879, Adolf Mayer observed a peculiar disease of tobacco at Wageningen, The Netherlands, which was not attributed to any of the known pathogen types. In 1886, he first named this syndrome `tobacco mosaic' and reported that it could be transferred between plants, similar to bacteria. In 1892, Dmitri Ivanovsky produced concrete evidence on the existence of a non-bacterial infectious agent, that the infected sap was infectious even after filtering through the finest Chamberland filters (Bos 1999). Beijerinck (1898) found that the filterable agent of tobacco mosaic disease was neither bacterium nor corpuscular body, but rather that was '*contagium vivum fluidum*' referring to a contagious living fluid. In 1955 Heinz Fraenkel-Conrat and Robley Williams showed that a functional virus could be created out of purified RNA and a protein coat. Hence, TMV was the first virus to be discovered over a century ago and was the first virus ever purified (Stanley, 1935; Bawden et al. 1936) first to be detected in centrifuge and in electrophoresis apparatus (Eriksson- Quensel and Svedberg 1936) and to be visualized in an electron microscope (Kausche et al. 1939; Zaitlin 1998). The TMV coat protein (CP) was the first virus protein to be sequenced (Anderer et al. 1960; Tsugita et al. 1960) and TMV's particle structure was the first to be elucidated in atomic detail (Bloomer et al. 1978; Namba et al. 1989). TMV is a preferred didactic and symbolic model to illuminate the essential features that define virus. It is a tool to study host–pathogen interactions, cellular trafficking, and also to produce pharmaceutical proteins in tobacco (Scholthof 2004).

Because of the adverse effects on the crop it is an economically important disease. TMV is worldwide in distribution and reduces cured tobacco yield, quality and price. Tobacco mosaic virus has a wide host range, including 199 species from 30 families. However, other solanaceous hosts are important sources of inoculum for tobacco (Shew and Lucas 1991).

Symptoms

Characteristic symptoms include irregular mosaic pattern of dark and light green areas on leaf, leaf malformation and stunted plant growth. This mosaic pattern is the result of intermingled yellow-green mottling on the foliage of the tobacco plant. Young leaves of infected plants are often malformed and may show puckering or wrinkling of the leaf tissue. Nearly mature leaves that are infected may show "mosaic burn" (Fig. 4) with large, irregular, burned or necrotic areas on the foliage that can cause extensive damage to the crop (Lucas 1975).

Fig. 4: Tobacco Mosaic Virus.

Etiology

Tobacco mosaic virus is sap transmissible and is one of the most infectious plant viruses and survives for more than fifty years in soil, debris and compost. Primary sources of infection may include perennial weeds and infected crop debris in the soil; usually only responsible for a small proportion of disease spread. Gooding (1969) reported that only about 10% of TMV infection in North Carolina is the result of primary infection by crop debris in the soil. Therefore, secondary spread of the virus accounts for most TMV infections. Secondary infections through contact may occur when a worker's hands, clothing, or equipment, previously in contact with an infected plant, comes in contact with a healthy plant. Cultivation practices such as hoeing, topping, spraying pesticides and insecticides, and other field operations can also spread the disease (Lucas 1975).

Another source of infection is air-dried tobacco. Workers who smoke cigarettes, or who use chewing tobacco or snuff containing air-cured tobacco, may introduce the virus to the plants. This is especially common when the workers are performing plant bed operations. Symptoms do not appear on nursery beds but could easily be spread at transplanting time when the infected plants come in contact with workers and equipment. Age of the plant, amount of inoculum and growth conditions in general determine whether symptom expression is acute or chronic. Usually the time required for symptom appearance is shortened by increases in temperature and light intensity. Above 38°C infection is inhibited and there will not be any symptoms below 10^{o}C and above 27^{o}C.

Management

The most efficient way to control mosaic is to keep the crop TMV-free (Lucas 1975). Preventative measures include use of resistant varieties, adoption of phytosanitary measures, rouging of diseased plants early in the season; planning inter-culture operations in infected fields at the end, disinfection of implements before entering healthy fields; washing hands with soap water before and after entering infected fields, Prevention of smoking and use of other tobacco products. Prophylactic sprays of 0.5% skimmed milk on 30th, 40th and 50th DAP prevents spread of the disease (Hare and Lucas 1959). Foliar spray of

dodine and glyodin, was found to reduce the amount of damage caused by TMV (Chow and Rodgers 1973).

Host plant resistance is an effective means of controlling TMV. Apple et al. (1963) found that host resistance derived from *N. glutinosa* reduced losses due to TMV compared to milk treatment of seedlings. The first recorded resistance to TMV was from the Colombian variety 'Ambalema' (Nolla and Roque 1933). Holmes (1938) used inter-specific hybridization to incorporate resistance into Turkish variety Samsun from *Nicotiana glutinosa*. That 'N' gene was successfully incorporated into burley tobacco also. However, when the 'N' gene was incorporated into flue-cured tobacco, Chaplin et al. (1961) reported reductions in cured tobacco yield and value, and Chaplin and Mann (1978) concluded that the N factor may be inherently difficult to disassociate from the adverse yield and quality characteristics in flue-cured tobacco. Reddy and Nagarajan (1981) observed that among the 268 collections of *N. tabacum* and *N. rustica*, 8 FCV, 10 Burley, 10 Air cured, 3 *rustica*, 2 each of hookah and cigar and 1 each of natu and snuff types were found resistant, the resistance being *glutinosa* type, showing typical hypersensitive local lesion reaction. Sastri et al. (1981) found that three (*Nicotiana* 1) resistant flue-cured tobacco varieties *viz*., TMVRR-1, 2 and 3 were superior over their susceptible counter parts *viz*., Virginia gold, CTRI Spl. and Kanakaprabha under heavy infection conditions. Shenoi et al. (1992) screened 216 *Nicotiana* germplasm and found that 36 entries were resistant to TMV showing hypersensitive *glutinosa* reaction, and one as tolerant to TMV. Banket A1 was the resistant burley variety introduced by ICAR-CTRI in 1984. MRGTH 1 (2006), GT 9 (2001) were the bidi types developed with TMV resistance. S 5 (1977) was also a resistant cheroot variety. VT-1158 (1998), Jayasri-MR (1986), Godavari special (1981), CTRI special – MR (1980) were the thriving TMV resistant FCV varieties, released by ICAR-CTRI. An interspecific high yielding FCV variety Sulakshana with TMV resistance has been released in 2019 by ICAR-CTRI (Murthy et al. 2019). FCR-15 was identified for release.

Prophylactic sprays of plant extracts like *Basella* or *Bougainvillea* or neem @ 1% prevents spread of the TMV disease (Nagarajan and Murthy 1975). Nagarajan and Murthy (1977) screened 27 plants and found that *Acacia Arabica*, *Boerhaavia diffusa*, *Lawsonia inermis*, *Nerium odorum*, *Peltophorum ferrugenium*, *Pithecalobium dulce* and *Prosopis specigera* inhibited above 80 percent infection. Shamarao et al. (2011) found that application of Viroson 2% followed by Bougainvillea leaf extract 5% and neem @ 1500 ppm reduced the TMV incidence substantially. However, higher plant height, leaf length and leaf width were recorded in Viroson, neem 1500 ppm and cow urine application indicating their role in triggering host defense and plant growth promotion.

2. Tobacco Leaf Curl Virus (TLCV)

The disease is caused by a virus of the genus *begomovirus* belonging to Geminiviridae. Singh et al. (2011) found that the begomovirus of tobacco leaf curl in Pusa, Bihar were recombinants of extant begomoviruses and satellite DNAs spreading in the Indian sub-continent and South-East Asia. Begomoviruses are associated with subviral agents named alpha

and betasatellites (Masuta and Shimura 2017). The association enables the virus to infect hosts that were not infected naturally (Jose and Usha 2003).

The virus is transmitted by the insect vector, whitefly, *Bemisia tabaci* Gennadius. *B. tabaci* has about 900 host plants all around the world and transmits over a hundred virus species. It is currently recognized as a complex of cryptic species with two most important phylogenetic groups: MEAM1 (Middle East-Asia Minor 1; biotype B) and MED (Mediterranean; biotype Q). It vectors the leaf curl disease in many solanaceous crops and cause huge losses. In severe infection, losses vary from 60-70% as infected leaves do not cure well. In mild infections, the yield loss is very marginal. Valand and Muniyappa (1992) reported the highest incidence of disease in Andhra Pradesh (77%) followed by Gujarat (59%), Karnataka (17%), Bihar (11.6%) and West Bengal (5.4%).

Symptoms

Diseased leaves curl, become brittle, puckered, downward curling of margins with enations or leafy outgrowths on the under surface of leaves (Osaki and Inouye 1981). Leaves show vein clearing, abnormal vein thickening and twisting of petioles. Internodes get shortened resulting in dwarfing of the plant (Fig. 5). Late infected plants do not show the severe symptoms although the top leaves look small and tend to curl inwards (Nagarajan and Reddy 1984).

Etiology

It is neither seedborne nor transmitted mechanically. The vector of TLCV is the whitefly, *Bemisia tabaci* Gennadius, a member of the family Aleyrodidae. Intensity and spread of the disease depend on plant age at the time of infection and population of whiteflies. The virus remains persistent in the insect for about 6 days. Infective whiteflies insert the virus into phloem tissues by means of the stylets while feeding on the leaves. The incubation period varies from 12 to 33 days depending on temperature, plant age and vigor. The disease is seldom seen in the nursery, but usually appears 2 to 3 weeks after transplanting. However, whiteflies feed and transmit TLCV to the seedlings in the nursery also. Single viruliferous whiteflies were found to be efficient vectors for about one week (Pruthi and Samuel 1941). Whiteflies are more abundant and active in relatively dry season and as a result, leaf curl is more prevalent during dry weather (Lucas, 1975). Whereas high rainfall, more number of rainy days and higher relative humidity, accompanied by less bright sunshine hours favoured low leaf curl incidence (Monga and Tripathi 1988).

Fig. 5: Tobacco Leaf curl Virus.

Management

As TLCV is a virus, no direct method for control is available. The incidence of TLCV can be managed by vector control alone. The application of insecticides has been reported to be effective in the management of *Bemisia tabaci*. The use of insecticides to control the insect vectors of viral diseases will remain, at least in the short to medium term, an important strategy of IPM, to manage TLCV, allowing the farmer to continue to produce economically quality tobacco crop. Under these circumstances identification and use of selective insecticides in IPM programmes assume significance. Several new molecules *viz.,* imidacloprid 17.8 SL @ 0.005%, thiamethoxam 25 WG @ 0.005%, flonicamid 50 WG, pymetrozine 50 WG @ 0.02% have been evaluated and found effective against the TLCV vector, *B. tabaci* (Sreedhar 2011; Sreedhar 2020a). As these insecticides are used at very low a.i./ha the produce will be free of pesticide residues. Integrated management of sucking pests with sorghum as barrier crop and need based application of selective insecticides was found to be quite effective in management of *B. tabaci* and minimizing the leaf curl incidence in flue cured Virginia tobacco (Sreedhar and Krishnamurthy 2010; Sreedhar 2012; Sreedhar and Murthy 2014; Sreedhar 2015a and b; Sreedhar 2016; Sreedhar and Rao 2016; Sreedhar 2019).

The integrated module developed at ICAR-CTRI against TLCV vector is as follows:

- Avoidance of leaf curl infected seedlings while planting.
- Removal and destruction of diseased tobacco plants within one month of planting when infection is less than 2%.
- Alternate weed hosts for whitefly should be removed and destroyed, in and around tobacco fields.
- Tobacco grown in the areas where brinjal, chillies and sunflower are cultivated has high chances of TLCV incidence.
- Installation of yellow sticky traps (grease/castor oil coated) @ 12 per hectare to monitor the whitefly population.
- When whiteflies are observed on the sticky traps, or leaf curl is noticed in the field the following insecticides should be sprayed at an interval of 15 days in the nursery as well as planted crop. Imidacloprid 200 SL @ 2.5 ml or thiamethoxam @ 2 g or pymetrozine 50 WG @ 4g or flonicamid 50 SG @ 4 g/10 litres of water ten days before pulling of seedlings in the nursery and 10 days after transplanting in the field 2-3 times at an interval of 15 days.

3. Cucumber Mosaic Virus (CMV)

The virus belongs to the genus cucumovirus of the family bromoviridae. CMV is known worldwide to infect over 200 plant species with the broadest known host range for any virus (Ohshime et al. 2016). CMV constitutes two subgroups I and II based on severity of symptoms and virulence (Blume et al. 2011). Symptoms are similar to that of TMV and often misdiagnosed.

Fig. 6: Cucumber Mosaic Virus.

Symptoms

Typical mottling and mosaic patterns appear, sometimes accompanied by stunting and narrowing and distortion of the leaves (Fig. 6). Severe strains may cause interveinal discoloration and the oak-leaf pattern of necrosis on the lower leaves. 'Mosaic-burn' or sun-scald frequently appears on the upper leaves of infected plants. Vein banding, inter venal yellowing, are accompanied with leaf blisters, shrivelled, chlorotic and necrotic lines causing filiform leaves. Mixed infections are quite frequent in combination with other viruses (Blancard et al. 1999). Mild strains cause only a faint mottling of the leaves. It also is more difficult than tobacco mosaic to transmit mechanically. Thus, cucumber mosaic tends to progress more slowly than tobacco mosaic in a field.

Etiology

The virus particles enters into the leaf through wounds, principally those made by aphid. The cucumber mosaic virus overwinters in perennial weeds and may be transmitted to healthy plants by aphid vectors or by mechanical means. Bradley (1968) showed that the mid veins and secondary veins were excellent sources of CMV and that aphids probing infected tissue for less than a minute became highly viruliferous. The cucumber mosaic virus cannot withstand drying or persist in the soil.

Management

Varietal resistance is the primary management strategy. Couzzo et al. (1988) demonstrated coat protein (CP) mediated protection in CMV. Quemada et al. (1991) engineered the coat protein (CP) gene from cucumber mosaic virus (CMV) strain C and transferred them into genome of tobacco (*N. tabacum* 'Xanthi'). They infected transgenic tobacco plants with CMV strain C of chi of sub group I and strain WL of subgroup II, transmitted mechanically or by aphids. They found significant degree of protection when challenged with CMV strains of either sub-groups. Rizos et al. (1996) reported that transgenic plants expressing coat protein (CP) of an Australian isolate of cucumber mosaic virus (CMV) were resistant to infection with the homologous and two heterologous CMV strains. The level of resistance observed in CP-expressing plants was related to the virulence of challenging CMV isolate and not to the similarity between the CP expressed by transgenic plant and CP of the challenging virus. A mutant of the cucumber mosaic virus subgroup I A strain Fny (Fny-CMV)

lacking the gene encoding the 2b protein (Fny- CMV delta2b) induced symptomless systemic infection in tobacco. Both the accumulation of Fny-CMV delta2b in inoculated tissue and systemic movement of the virus appear to proceed compared to wild type Fny-CMV (Soards et al. 2002).

Viral diseases cannot be controlled once the plant is infected. Therefore, every effort should be made to prevent introduction of viral diseases into the field. Infected plants should be removed immediately to prevent spread of the pathogens. Perennial weeds and ornamentals, which may serve as alternate hosts, should be controlled in and adjacent to the field. Several selective insecticides were identified for effective management of tobacco aphid *Myzus persicae nicotianae.* Imidacloprid and thiamethoxam are being used for the last two decades for management of tobacco aphid (Ramaprasad et al. 1998; Sreedhar et al. 2011; Sreedhar 2011). Recently flonicamid, pymetrozine, spirotetramet + imidacloprid, sulfoxaflor, flupyradifurone and afidopyropene were found to be quite effective which are alternative to neo nicotinoids, effective against the tobacco aphid and relatively safe to the natural enemies (Sreedhar and Rao 2017; Sreedhar 2018; Sreedhar 2020b). Barrier crop sorghum along with need based application of selective insecticides was found to be an effective strategy for management of aphid in flue cured Virginia tobacco (Sitaramaiah et al. 2005; Sreedhar and Krishnamurthy 2010; Sreedhar 2012; Sreedhar and Murthy 2014; Sreedhar 2015a and b; CTRI 2019)

4. Tobacco Distorting Virus

TDV is a member of the genus *Polerovirus* of the family Luteoviridae. It was noticed in the sun-cured *Natu* tobacco in Andhra Pradesh for the first time in 1968 at Karimnagar and Warangal (Chandwani and Reddy 1971). The virus causes abnormal suckering, stunting and the leaves get mottled, puckered, distorted with rat-tails. Only long midrib is seen without lamina at times. It produces necrotic local lesions on *Datura*, *Chenopodium amaranticolor* and *Colocasia esculenta.* Thermal inactivation point of the agent is as high as 97 °C. D.G. 3 (Desi Guntur) is an improved Natu tobacco variety which is resistant to TDV virus (Gopalachari 1984).

5. Tobacco Etch Virus

TEV belongs to the genus *Potyvirus* of the family potyviridae. It has a monopartite strand of positive-sense, single-stranded RNA surrounded by a capsid. It is not a common pathogen, but infects flowering plants of tobacco, transmitted by sap and also by aphids in a non-persistent manner. It infests solanaceous plants, and causes rat-tailing in *Datura* sp. On lower leaves vein clearing is seen along with some necrotic lines or etching. Mottling is seen with chlorotic and necrotic spots. Its thermal death point was found to be 60-62 °C. Nagarajan and Reddy (1978) reported the virus on burley tobacco varieties - 'La Burley', 'Ky-58', 'Burley-21' and also on FCV varieties-'Virginia Gold' and 'CTRI special' in and around CTRI, Rajahmundry (Gopalachari 1984).

6. Potato Virus Y

PVY is one of the most important tobacco viruses and has a worldwide distribution. This virus is classified in the genus *Potyvirus*, family Potyviridae with a single stranded positive-sense genomic RNA. The virus is transmitted by aphids. Kanavaki et al. (2006) observed differences in probing behavior of

Myzus persicae nicotianae, and *Myzus persicae* (*sensu stricto*), where the latter transmitted the PVY^N virus less efficiently during the early phase of host selection in Greece. This virus causes tobacco veinal necrosis or vein banding i.e. appearance of dark green bands along the brown necrotic veins. It has a local lesion host *Chenopodium amaranticolor*. Necrosis extends to the vascular region and plants die out of pith necrosis (Gopalachari 1984). It can be managed by using insecticides late in the season, when aphids thrive on tobacco with those mentioned for CMV. Alternate host plants and weeds should be destroyed. Jowar can be raised as barrier crop around tobacco.

Conclusion

Bacterial and viral diseases cause significant loss of yield and quality of tobacco. Bacterial diseases were reported from some of the tobacco growing regions of the country and could be nearly managed with good agricultural practices. Viral diseases being ubiquitous are critical in tobacco and can cause significant losses if they occur in epidemics. Ever since viruses were identified as new pathogenic entities unknown to the world, they continue to be unmanageable problems, especially those that are sap transmissible like TMV. It can only be prevented by developing resistant varieties and following strict phytosanitary measures. For vector transmitted viruses, timely insect or the specific vector management can subside the spread to some extent. Due to lack of knowledge in the tobacco farmers about viral diseases, the long lasting solution against these diseases can only be through incorporation of host plant resistance.

Virus occurrence may be completely excluded if preventive strategies are established. Plant variants that possess increased natural resistance could substitute for susceptible cultivars. Although this "classical breeding" is a powerful method to produce the resistant plants, it is usually costly and time consuming. In addition, features such as crop quality and quantity may be compromised by breeding for resistance. In contrast, genetic manipulation is a relatively rapid method to introduce virus resistance. Transgenic plants expressing the RNA-silencing pathway have been shown to efficiently resist viral infection. This pathway perhaps represents the most specialized molecular strategy that plants use to combat viruses. Thus, RNA-silencing based approaches might be an effective way of reducing crop loss caused by viruses. More important are the methods that induce viral RNA-silencing without altering the plant genome since these methods overcome risks associated with transgenic plants. ICAR-CTRI, premier institute working on the model plant has been relentlessly pursuing and advocating the utilization of resistant varieties and prevention of viral disease spread by integrated vector management strategies. Pervasive *in vitro* studies on the virus biology, etiology, regulation of putative virulent genes and *in vivo* rapid detection tools can be the way forward. In the near future, knowledge of changes in the mRNA, protein and cellular metabolites after virus infestation will lead to a greater understanding of the plant virus interaction. This will in turn enhance the efficiency of the current approaches and allow the development of new strategies. Comprehensive thrust on plant virology, backed by national policies to allocate commensurate funds,

can create new avenues for concerted research on cell cultures, development of accurate diagnostic kits and efficient plant disease management.

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