Chapter 9
Genomic Designing for Biotic Stress Resistance in Sugarcane

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Abstract Sugarcane (*Saccharum* spp hybrid) is grown across the continents, principally for white sugar and bioethanol. It is a C4 plant, generates highest amount of biomass among the cultivated crops, and meets nearly 80% of the global white sugar requirement. The modern cultivated sugarcane is a derivative of *Saccharum officinarum* (noble canes) and the wild relative, *S. spontaneum*. Worldwide, breeding

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strategies have improved sugarcane yield till 1970s and later cane yield remained static across the countries. Many biotic constraints seriously affect productivity of the crop which is specific to cane growing countries. Among the diseases, smut, ratoon stunting, yellow leaf and mosaic are the major constraints in most of the countries. The diseases like red rot and wilt seriously affect cane production in South and South East Asian countries with many historic red rot epiphytotics causing huge crop losses in India. Similarly, the phytoplasma diseases, grassy shoot and white leaf are serious constraints in Asian region. Recently, the diseases like rusts, pokkah boeng, red stripe etc. emerged as major diseases in different countries. Among the insect pests, stalk borers are ubiquitous in nature with serious economic losses and each country or region has unique group of borer pests. Apart from the borer pests, many sucking pests and root grub are also of serious concern to sugarcane cultivation. Among the management strategies, host resistance is successfully exploited against various diseases and healthy seed, heat treatment, and chemicals are the other management strategies adopted in tandem. In case of insect pests, an integrated management is followed with more emphasis on biological control and chemicals depending on the pests and the location. Though remarkable gains were achieved through breeding strategies, complex polyploidy hinders genetic advancements for various traits in sugarcane. Recently, various genomic tools, especially transcriptomics were applied to understand gene functions and molecular markers are partially successful. Although, genetic transformation was successful in developing many transgenic lines against various biotic constraints, application of genome editing is in nascent stage due to multiple alleles. Overall, the various biotic constraints are managed through host resistance and other strategies in an integrated approach. Genomic applications have helped to understand genomes of the crop and pathogens/insects and, host resistance and genetic engineering supports trait improvement.

Keywords Sugarcane · Diseases · Insects · Stalk borers · Complex polyploidy · Genomic applications · Transgenics · Molecular markers

9.1 Introduction

9.1.1 Economic Importance of Sugarcane

Sugarcane (*Saccharum* spp. hybrid, Poaceae) a C4 tall perennial grass, is commercially cultivated in tropical and subtropical areas around the globe (Yadav et al. 2020). Though sugarcane cultivation dates back to 5000 BC in the Indian subcontinent, its cultivation expanded after it became an industrial crop during the last 100–120 years in the continents of Asia, Americas, Australia and Africa. Amongst C4 plants, the crop is highly efficient in converting solar energy and accumulates maximum yield in biomass (Henry 2010). Currently, sugarcane contributes >70% of total global sugar production, mostly consumed as refined sugar and to some extent as *khandsari*, *gur*

or other sweeteners in the Asian countries. Of late, the crop has received much attention as a bioenergy crop to produce bioethanol, which is the major renewable energy source to meet the increasing requirement for energy by decreasing greenhouse-gas releases, hence, it has stimulated a widespread attention on this crop (Souza et al. 2014). Globally, it also generates a high biomass of about 279 million tons annually of lignocellulosic biomass of leaves in the field and bagasse in the industry (Chandel et al. 2012). Apart from bioethanol, sugarcane supports electricity production in the sugar mills by burning bagasse, the fibrous part of stalk after juice extraction in different countries. Paper and pulp industries use bagasse as a raw material in to produce paper and newsprints. In addition, green leaves and tops of sugarcane are also used as animal feed and filter-cake (pressmud) from sugar industries is fortified as manure in different countries.

If we consider biomass production, sugarcane stands number one among the cultivated crops; positions amongst the top 10 commonly cultivated crops globally. In 2020, ~1.9 billion tons of sugarcane was produced worldwide from an area of 26.5 million ha grown in ~100 countries. Brazil occupies the numner one position in terms of production of cane and sugar. India follows Brazil and both the counties contribute to nearly 64.0% of the global production. China, Thailand, Mexico, Pakistan, the United States, Colombia, Australia, Cuba, and the Philippines are the other major sugarcane producing countries [http://www.fao.org/faostat (accessed on 24 December 2021)].

In the past five decades, global sugarcane production increased nearly threefold, largely due to the growing demand for sugar and bioethanol. Genetic advances in new sugarcane cultivars that suited to specific situations were attributed to the enhanced production. At the same time, improvements in agronomical measures also played a role in increasing cane productivity (De Morais et al. 2015). However, overall growth in cane output is mainly contributed by a drastic rise in cultivation area of the crop. For e.g. from 1973 to 2013 sugarcane cultivation in Brazil, Thailand, China and India witnessed increase by approximately 500, 286, 237 and 94%, respectively, whereas improved cane harvest per ha in the respective countries were only modest viz. 60, 11, 59 and 38% during the same period (Zhao and Li 2015a, b). Many countries are facing yield plateaus and incidence of pests and diseases, declining soil fertility and climatic conditions are attributed to the observed stagnation in cane yield (Yadav et al. 2020). Genetic enhancement of recent varieties is the continuous process to improve sugarcane productivity. In addition, there is a need to improve management practices of various biotic agents viz. diseases, insect pests, nematodes etc. to prevent crop losses. To address stagnant yield scenarios in sugarcane, strong breeding strategies are need to be combined with protection and production strategies. Although there are numerous issues intrinsic to the crop constrain breeding efforts, new avenues in biotechnology and molecular biology can complement realization of genetic improvement through breeding. Many biotic constraints affecting production and productivity of sugarcane can be resolved through a holistic approach of integrating conventional and modern scientific advancements. This chapter addresses major biotic constraints affecting sugarcane crop across the globe, strength of classical breeding to address them through host resistance, integrated management of

biotic constraints, newer applications in genetic engineering and genome editing to address the constraints and way forward to a sustainable sugarcane cultivation by effective management of all the major biotic constraints.

9.1.2 Reduction in Yield and Quality Due to Biotic Stresses

9.1.2.1 Fungal Diseases

In India, severe red rot epiphytotics occurred in almost all the preceding decades and due to breakdown of resistance several elite cultivars such as Co 213, Co 1148, Co 6304, CoC 671, CoJ 64, CoSe 95422, etc. were removed from cultivation. Presently, the popular cv Co 0238 is affected by a very severe epiphytotics in the subtropical India due to sudden failure in ~0.5 M ha in the region. The present crop losses were estimated to be 1.0–1.414 billion US$ and is considered as the largest crop losses recorded in sugarcane (Viswanathan et al. 2022a). Impact of red rot to sugarcane is also recorded in Pakistan, Bangladesh, Myanmar, Thailand, Nigeria, South Africa, Malaysia, Guatemala, Nicaragua and other countries (Viswanathan 2021a). Over a century, red rot epiphytotics followed 'boom' and 'bust' cycles regularly after adopting a particular cultivar over an extensive area in India and the recent red rot epiphytotics on Co 0238 became catastrophic due to adoption of the variety in more than 70% cane area in subtropical states (Ram and Hemaprabha 2020; Viswanathan et al. 2021a) and this has been found to mimic 'Vertifolia Effect" where a selection pressure for the pathogen has occurred for emergence of a highly virulent pathotype due to uniformity in the host variety under field conditions (Viswanathan et al. 2022a). For commercial release of a variety, red rot resistance along with high yield and quality is prescribed in India. Varietal breakdown in sugarcane posed by the new variants of the pathogen *Colletotrichum falcatum* is huge as we are unable to harness the benefit of elite varieties in the field for a long time (Viswanathan 2021b). This puts extra efforts on breeding group to come out with matching clones regularly.

C. falcatum infection causes rotting of stalk tissues and in most cases entire stalk rots and dries, becomes unfit for juice extraction. Further invertases produced by the pathogen cause inversion of sucrose into glucose and fructose and this biochemical changes results in poor sugar recovery. In general, diseased canes exhibit a significant loss in cane weight (29–83%) and juice extraction (24–90%) or total losses (Viswanathan 2010). Further, inversion of sucrose due to mixing of juice from infected and healthy canes during milling process affects sugar recovery. The disease affects the crop from germination stage onwards, till harvest. Most prominent symptoms are pronounced after cane formation as drying of canes in patches or throughout the field. During severe outbreak, the disease causes 100% crop losses in plant and ratoon crops (Viswanathan 2021a; Viswanathan et al. 2018a, 2022a). Hence the disease is of foremost importance for sugarcane cultivation in many Asian countries, most pertinent to Indian subcontinent.

Smut is another important fungal disease which occurs globally and impacts sugarcane significantly. The disease becomes more serious under favorable conditions and often complete crop failures occur in ratoon crops (Viswanathan 2012a, b). Besides direct loss in cane yield, *S. scitamineum* infection can cause a significant reduction in sucrose content, purity and other juice quality parameters (Kumar et al. 1989). Varied losses were reported in different varieties and climatic conditions viz. 10–30% cane yield and 3–20% sugar losses, 68–80% cane yield, 32% in juice quality and 62% in cane yield from Australia and India (Goyal et al. 1982; Solomon et al. 2000; Magarey et al. 2010a, b).

In India, wilt is another major disease affecting sugarcane production due to extensive drying of stalks, like red rot; hence huge economic losses were recorded (Viswanathan 2020). In 1970s, loss to cane yield of as high as 65% was estimated with severe disease incidences in ratoons (Sarma 1976). Further, wilt causes deterioration in juice quality and is primarily due to conversion of sucrose into reducing sugars and other biochemical changes (Singh and Waraitch 1981). Reductions of 14.6–25.8% and 3–20% in juice extraction and sugar recovery due to wilt were reported respectively (Gupta and Gupta 1976). Under field conditions, wilt affected canes recorded poor juice quality of 1.5–2.0 Brix as against 13–19.5 in the healthy canes (Viswanathan 2020). Reduced juice quality in the wilt-affected canes usually hampers sugar processing in the mills. It was estimated that wilt causes a loss of 3–6 tons of canes per ha and annually it is estimated to about 12.7–25.4 MT in various seasons, by which wilt caused losses of several million dollars in India. Apart from direct losses to the growers, the sugar mills encounter loss in terms of unrecoverable sugar every year (Viswanathan et al. 2006). Combined infections of red rot and wilt pathogens are very common in epidemic areas and such infections cause more severe crop losses than their separate infections (Viswanathan 2010, 2013a, b). Further, losses caused by wilt are largely ignored due to its recognition during the stages of crop maturity. In Bangladesh, wilt occurs throughout the country and causes significant losses to cane production (Hossain et al. 2017).

Earlier importance of pokkah boeng (PB), a fungal disease was ignored since it was a minor disease; however different states in India recently recorded severe outbreaks of the disease (Viswanathan 2018). PB affects cane yield to a tune of 40–60% in the susceptible varieties (Goswami et al. 2014). PB affected canes recorded a considerable decline in sugarcane production and sugar yield parameters (Dohare et al. 2003; Singh et al. 2006). The disease severity with 1–90% disease incidences on most of the commercial varieties were recorded during 2007–2013 in Uttar Pradesh state, which cultivates more than 50% of sugarcane in India (Vishwakarma et al. 2013). Further, the disease drastically reduces internodal elongation in the stalks (Viswanathan et al. 2014a). The disease severity forced the farmers to take up fungicidal sprays in different parts of Tamil Nadu state in India. In China, correlation analysis of disease severity with plant height, cane girth, single cane weight, yield, and Brix showed significant negative correlation (Wang et al. 2017a, b).

Orange rust was not a serious constraint before 2000 in Australia; however, later appearance of a new virulent race caused severe outbreaks of the disease. Breakdown of rust resistance severely affected the popular cv Q124 which was gown in 45% of

cane area in the country (Magarey et al. 2001a). The epidemics caused about $200 M losses to cane industry in Australia (Magarey et al. 2001b). Here, the affected crop suffered a substantial drop in sugar content. In Florida, USA, almost all the varieties under cultivation were found susceptible to brown or orange rusts during 2015–16 crop season (Raid et al. 2015). During the same time in Brazil, the popular cv RB 72454 was grown in 22.1% of the sugarcane area, however by 2010 the varietal area was reduced to 4.7%. Orange rust susceptibility was considered as one of the reasons for loss in area. After noticing orange rust in 2009, severe outbreaks of the disease were recorded in new areas within two seasons in the country (Sao Paulo State) and susceptible varieties incurred a loss of 15–30% in cane production (Barbasso et al. 2010; Klosowski et al. 2013; Daros et al. 2015; Gazaffi et al. 2016).

Brown rust severity reduced 33 and 31% in cane and sugar tonnage per hectare, respectively in Australia (Taylor et al. 1986). After a severe brown rust epidemic in a popular variety, which occupied 60% crop area in Cuba, a new policy of restricting cultivation of a variety below 20% was implemented in the region, to reduce the impact caused by rust outbreaks (La et al. 2018). In USA, yield losses to a tune of 10–50% were reported in many popular varieties due to brown rust. Breakdown of resistance to rust due to new virulent strains of the brown rust pathogen in many popular cvs CP 74-2005, CP 78-1628 and CP 72-1210 led to their withdrawal from cultivation in Florida. In addition, a sudden outbreak of brown rust in south Florida state during 1988 caused destruction of more than 50% visible dewlap leaves in the canopy causing ~40% losses in the cv CP 78-1247 and 20–25% on another popular cv CP 72-1210, which occupied 60% sugarcane area, causig a monetary loss of $40 million (Raid 1988; Comstock et al. 1992; Raid and Comstock 2006).

9.1.2.2 Bacterial Diseases

Ratoon stunting disease (RSD) caused severe yield losses in Australia, USA, India, Argentina, South Africa, China and other countries (Putra and Damayanti 2012; Taher-Khani et al. 2013; Li et al. 2014; Viswanathan 2001a, 2016; Magarey et al. 2021). RSD incidence increased with the ratoon number and sugarcane in dryland areas were more severely affected than those in waterlogged areas. Further, RSD has a significant impact on sugarcane yield, usually reducing sugarcane production by 12%–37% however during drought stress, the yield reduction increases to 60% (Wei et al. 2019). RSD causes ~10–15% losses in cane yield, however, losses in cane harvest can go up to 50% in disease-susceptible cultivars, under drought conditions (Benda and Ricaud 1977). Magarey et al. (2021) made an impact analysis on RSD to Australian sugar industry and suggested $25 M loss in the 2019 crop.

The leaf scald disease (LSD) bacterium may cause severe losses in susceptible varieties by death of entire stools and impaired juice quality (Viswanathan 2012a, b). Red stripe caused by *Acidovorax avenae* subsp *avenae* (Aaa) was considered as a minor disease earlier. However, increased severity of the disease was recorded in different countries. In Louisiana, Aaa caused significant effects on sugarcane yields and studies suggested careful management strategies to prevent losses (Johnson et al.

2016). The following factors like changes in climatic conditions, promoting susceptible cultivars in a large area and development of new Aaa stains with high virulence were found associated with the disease outbreaks (Fontana et al. 2013; Grisham and Johnson 2014; Ovalle and Viswanathan 2020; Viswanathan 2012a, b).

9.1.2.3 Virus and Phytoplasma Diseases

All the viruses systemically infect sugarcane and virus titre increases over the vegetative generations, hence severe expression of the disease occur in the ratoons and where healthy seed nursery programs are not adopted. In Florida, Brazil, India and Reunion Island, the major sugarcane growing countries recorded severe occurrences of yellow leaf disease (YLD) up to 100% incidences (Comstock et al. 2001; Rassaby et al. 2004; Vega et al. 1997; Viswanathan 2002). The virus infection adversely affects various growth parameters in various sugarcane cultivars. Viswanathan et al. (2014b) estimated losses of 44–57% in photosynthetic rate, 47–48% in stomatal conductance, 36–47% in transpiration rate, 30–34% in chlorophyll concentration and 31–33% in leaf area index. By this, photosynthate movement from source to sink is hampered in sugarcane (Yan et al. 2009). Further, all the symptomatic leaves recorded increased sucrose content due to prevention of photosynthates in virus-infected canes (Izaguirre-Mayoral et al. 2002). Such physiological malfunction leads to reduced cane growth in YLD-affected crop (Lehrer and Komor 2008). In Thailand, 30% cane yield reductions were recorded (Lehrer et al. 2008). In India, YLD-symptomatic plants of the susceptible cvs Co 86032, CoC 671 and CoPant 84211 recorded a loss in the range of 38.9–42.3% in cane yield; similarly, ~34.15% loss in juice yield due to the disease was recorded (Viswanathan et al. 2014b). Similarly, drastic reductions in cane yield and cane juice quality in YLD affected crops were recorded in China and Brazil (Vega et al. 1997; Yan et al. 2009).

Studies conducted during 1970s in Brazil revealed that tolerant varieties with 100% mosaic showed 18% losses whereas, up to 75% losses were recorded with only 25% mosaic in the susceptible varieties (Matsuoka and Costa 1974). Impacts of the disease on crop growth and growth parameters were estimated on popular varieties cultivated in tropical and subtropical regions like CoC 671, Co 740, CoS 767, CoLk 8102, CoPant 90223. The study evidently revealed significant reductions in CO_2 assimilation rate, number of millable canes, sugarcane growth traits like stalk thickness, number of nodes and cane yield and cane quality traits and sucrose and reducing sugars metabolism (Bhargava et al. 1971; Singh et al. 2003; Viswanathan and Balamuralikrishnan 2005). Recently, Putra et al. (2014) observed mosaic in ~30% of surveyed sugarcane fields in Java, Indonesia, indicating widespread occurrence of the disease in the country.

In mosaic affected sugarcane plants, due to destruction of chlorophyll and weakening of photosynthesis growth is significantly repressed (Bagyalakshmi et al. 2019a) and this causes in shorter internodes, lesser millable canes, poor root growth, and a considerably lower sett germination and lower cane yield (Singh et al. 1997, 2003). Sugarcane mosaic has become ubiquitous in its occurrence in many countries like

Argentina, Australia, Brazil, Cuba, China, India, USA, Indonesia, Thailand, Puerto Rico, etc causing huge economic losses (Lu et al. 2021; Wu et al. 2012). Unfortunately, the impact caused by the mosaic viruses is not realized by the sugarcane farmers and sugar industries. Although Sugarcane bacilliform virus (SCBV) symptoms were clearly described in different countries, its impact to cane growth is not reported except a few. SCBV infection caused reductions in cane weight, juice recovery and sucrose level in juice in China (Li et al. 2010). In India also, SCBV infected clones exhibited severe stunting and poor growth in germplasm whereas the hybrid varieties shown extensive discolouration followed by drying of leaf lamina under field conditions (Viswanathan and Premachandran 1998; Viswanathan et al. 2019a).

Sugarcane white leaf (SCWL) disease is highly destructive in Thailand, Vietnam, Taiwan, Sri Lanka and Iran and severe yield losses were reported. In India, sugarcane grassy shoot (SCGS) phytoplasma caused 5–70% and complete crop losses in plant and ratoon crops, respectively, in popular cultivars in different states (Nasare et al. 2007; Tiwari et al. 2012; Viswanathan et al. 2011b). Impact caused by SCWL to sugarcane in Thailand revealed a loss of over 30 million US dollars to Thai sugarcane industry each year. Such severe economic losses due to SCWL were reported from Taiwan, Vietnam and Sri Lanka (Kumarasinghe and Jones 2001; Hoat et al. 2012; Wongkaew 2012).

9.1.2.4 Other Diseases

In Australia, Magarey et al. (2013) reported pachymetra root rot infection in 50% or more farms in nine of the 12 surveyed areas; however some areas had more than 80% affected farms. About $50m per annum economic losses were attributed to the root rot disease in Australia. Root-lesion nematode, *Pratylenchus zeae* was reported on a higher proportion in Australia and all parasitic nematodes are estimated to cause an economic loss of ~$80m annually (Blair and Stirling 2007).

9.1.2.5 Insect Pests

Worldwide the yield loss in sugarcane due to insect damage accounts for more than 10% (Ricaud et al. 1989). The crop protection cost in sugarcane amounted to AUD 111 million in 1996 in Australia of which AUD 14 million and 97.4 million were accounted towards the production loss and management costs for the pests and diseases respectively (McLeod et al. 1999). In Brazil, losses due to *Diatraea saccharalis* differed between seasons. For each per cent of bored internode the sugar yield losses were estimated to be 8.83 and 19.8% in the first and second season respectively with significant differences in the quality of sugar (Rossato et al. 2013). In Louisiana, losses and management costs due to *D. saccharalis* is more than USD 8 million (Wilson 2021). The major borer pests of sugarcane cause yield losses of nearly 25–30% (Kalunke et al. 2009).

In South Africa, the stalk borer *Eldana saccharina* and thrips *Fulmekiola serrata* seriously affect the sugarcane yields (Keeping et al. 2014). The major borer pest in Mauritius is *Chilo sacchariphagus* with 40–60% infestation (Soma and Ganeshan 1998) and the top borer *Scirpophaga excerptalis* in India and Indonesia (Mukunthan 1989; Koerniati et al. 2020) cause enormous losses to farmers and sugar industry.

A loss of 0.25% sugar yield was observed for every one percent increase in the infestation levels of *D. saccharalis* (Gallo et al. 2002). In Panama, infestation of the stalk borer *Diatraeata bernelle* led to losses in fiber, cane weight and sugar recovery. In comparison with canes with no damage (level 0), canes with damage (level 3) yielded 2.56t lesser sugar per hectare. There was a positive correlation between internodes bored and loss of sugar ranged from 12.9 to 26.47% (Valdespino et al. 2016). Significant financial losses in major sugarcane areas of China had been incurred due to a host of factors such as continued increase in the borer population, stalk damage as well as dead hearts in maturity phase of crop and resultant reduction in sugar and cane yields (Xie et al. 2012; Li et al. 2013a, b, c). In China, 45% in cane yield and 6% sucrose were observed due to combined infestation of *Chilo infuscatellus* and *Tetramoera schistaceana* (Li et al. 2017a, b).

In Indonesia, cane height and other cane traits were negatively affected by moth borers among which *S. excerptalis* and stem borer caused a loss of 40.8 and 15% in stalk mass (Goebel et al. 2014). In India, *C. infuscatellus* causes 55–60% reduction in mother shoots by killing of meristems and 43–76% reduction in tillers and eventually 16–43% cane yield is reduced (Thirumurugan et al. 2006; Geetha et al. 2018).

In Ethiopia, combined infestation of stalk borer pests, *Scirpophaga calamistis, Eldana saccharina* and *Chilo partellus* resulted in significant losses on stalk length (10.24%), cane yield (24.86%), and sugar recovery (34.34%). The overall potential loss in yield was 27.3% and the damage was the highest in the grand growth phase of the sugarcane (Michael et al. 2018). Since its introduction in Reunion, Mauritius and Madagascar during the nineteenth century, *C. sacchariphagus* is a serious pest on sugarcane. Yield loss during heavy infestations was found to be 30% in many commercial varieties in comparison to the resistant variety (R570). Several field trials over multiple crop seasons established that the variety R579 was relatively more susceptible to *C. sacchariphagus* than R570 (Rochat et al. 2001).

In the Belize, heavy incidence of the frog hopper (*Aneolamia varia*) resulted in 10% loss of cane yield during 2006–2007 in the northern region (Thomas and Bautista 2020). White grubs are a serious constraint in sugarcane production in all countries cultivating sugarcane (Allsopp et al. 1991; Goble 2012) causing 25–80% loss in cane yield in India (Prasad and Thakur 1959; Tippannavar 2013; Lamani et al. 2017), 39% of yield reduction in Australia (Sosa 1984) and a yield loss ranging from 23 to 55 tonnes per hectare (McArthur and Leslie 2004) in South Africa.

9.1.3 Growing Importance in the Face of Climate Change and Increasing Population

Impact of climate change is witnessed across the continents and most of the crops under cultivation and animals face this threat. Across the countries climate change is expected to significantly affect sugarcane agriculture, specifically in the developing nations probably due to low capacity to adaptive strategies, highly prone to natural calamities and inadequate research infrastructure and management strategies (Zhao and Li 2015a, b). Climate change induced frequency and intensity of extreme environments may negatively affect sugarcane production and probably continue to be affected. Further, geographic location and mitigation strategies will decide the degree of impact caused by climate change on sugarcane. The key factors such as weather and CO_2 in the atmosphere, temperature, rainfall etc. influence the crop production, especially in developing countries. Cane and sugar production have fluctuated with climate extremities in different countries, especially drought and precipitation.

Plant response to drought, heat, cold, salinity, high CO_2 concentrations, weeds, disease and pests in the changing climate are the best studied abiotic and biotic stresses (Pandey et al. 2017; Suzuki et al. 2014). Severe weather conditions have caused more incidences of diseases and overwintering pests with the corresponding input cost for control them. Changes in the precipitation and high diurnal temperature majorly influence the prevalence of insect pests (Hussain et al. 2018). Deviations from the regular patterns of temperatures may probably lead to changes in pest and disease incidences and this can impact crop production (Rosenzweig et al. 2014). Baez-Gonzalez et al. (2018) have suggested such associations with the infestations of sugarcane pests.

Since sugarcane crop is in the field for over 10 months, day and night temperature, rainfall pattern, and distribution and duration of light may have a key influence on growth of the crop. Further, they influence distribution of different pest and diseases in the crop during various growth phases and seasons. Deressa et al. (2005) observed a temperature increase by 2 °C and rainfall by 7% (doubling of CO_2) has negative impacts on sugarcane production in all sugarcane-growing regions of South Africa. Nevertheless, there are reports on positive side on raised CO_2 in controlled conditions enhanced water use efficiency, photosynthesis and biomass resulting high yield and productivity in sugarcane (de Souza et al. 2008). The enhanced temperature may change the incubation period of the pathogen in the host, may shorten the life cycle of the pathogen, may increase the spore numbers and more number of generations in crop cycle. Warm winters with high night temperatures enhances the survival of pathogens, life cycle of insect vectors, higher sporulating capacity and secondary aerial infection (Harvell et al. 2002).

Many pathogens spread their spore with help of wind and rain for a long distance. The wind direction may introduce the pathogen to the new areas where the crop is being grown and if the environment is favourable for infection and disease development, there is a chance for introduction of new diseases. Brown rust severity in

sugarcane has occurred in different countries or disease was introduced to new territories. Also rust resistant varieties quickly became susceptible due to faster gain of virulence by the new pathogenic races. Occurrence of orange rust was confirmed in Florida, Costa Rica, Guatemala, Nicaragua and Panama in 2007. Concerns were expressed over the sudden appearance of the disease in the American continent, probably due to climate changes (Viswanathan and Selvakumar 2021).

Smut outbreak was noticed on the east coast of Australia for the first time during 2006. Although it is due to climate change or not, it became a serious challenge to Australian sugar industry by initiating smut resistance programme (Croft et al. 2008a, b). Usually dry weather favoured the shedding the spread of smut spores in the field whereas a wet weather and rain negatively affects the spread. Since smut is distributed throughout sugarcane growing countries, it may emerge as a major constraint to cane cultivation in warmer environments. Pokkah boeng was earlier regarded as a minor constraint in India, however, its serious epidemics across the country in India is suspected due to favourable climatic factors for the disease development (Viswanathan 2018, 2020).

It is well established that abiotic and biotic factors influence disease development in sugarcane. Hence it is speculated that any impact to crop growth due to climate change would aggravate the crop to YLD seriously. In addition, climate changes on the vector i.e. sugarcane aphid *Melanaphis sacchari* in sugarcane ecosystem will also cause changes in disease epidemiology and disease build-up. Under field conditions in Guadeloupe, aphid population and YL disease progress had shown a correlation between them. In this study, precipitation during the first weeks of sugarcane growth showed a negative correlation to *M. sacchari* dispersal in the field and suggested that lack of rain or poor rain in initial crop phases favors severe YLD in a susceptible sugarcane variety (Daugrois et al. 2011). Similarly, late spring and early summer had the first *M. sacchari* incursion and aphid flow in Louisiana and this coincided with a high sequential increase of YLD (McAllister et al. 2008). Studies conducted at Coimbatore for four seasons revealed that precipitation pattern has a temporal fluctuation in aphid population (Viswanathan et al. 2022b).

Pest dynamics is synchronous with the vagaries of climate whether the changes are transient weather changes or seasonal or long term. As drought stress increases sugarcane vulnerability to pests (Showler 2012) and thus, developing multi-stress resistant varieties are vital (Dlamini 2021). For instance, in sugarcane, the borer *E. loftini* infestation increased during drought conditions. Crops irrigated adequately with well water had 82.8–90.2% lesser *E. loftini* eggs than those raised under drought situations (Showler and Castro 2010), as the leaves of drought stressed plants released oviposition cues. Similarly, during drought overproduction of reactive oxidative species (ROS) occurs, which escalates different pests including nematodes infections (Tsaniklidis et al. 2021).

Some of cultural practices as stalk burning before harvest or trash burning after harvest, mainly following during manual operations, impacts the climate severely, causing enormous heat and pollution disrupting the environmental balance. Self-detrashing varieties to minimize the drudgery of manual harvest and using the trash for mulching could be the options to refrain from trash or stalk-burning. Change

in pest status due to the variation in climate has been reported. Of the borers *D. saccharalis* and *D. flavipennella*, the dominant species changed from the former to the latter within a decade and the main reason suggested was intensive irregular rains favouring the latter (de Freitas et al. 2007).

9.1.4 Limitations of Traditional Breeding and Rational of Genome Designing

The major aim of any crop improvement activity would be to introgress one or a few favorable genes from donor into highly adopted variety, and to recover most of the recipient parental genome as rapidly as possible. Breeding for biotic and abiotic stress requires identification of stress tolerant genotypes mostly from the germplasm and accumulating their genes in current commercial cultivars. During the last 50 years, a remarkable accomplishment was made in plant breeding program by developing new improved sugarcane cultivars. Major emphasis was laid on sourcing genes contributing to better productivity and adaptability from related species and wild relatives through genetic manipulation at cultivar, interspecific or intergeneric level. Breeding for stress resistance through conventional means is challenging due to lack of knowledge on inheritance of disease resistance, transfer of undesirable genes from the wild accessions along with desirable traits and the presence of reproductive barriers especially in interspecific and intergeneric crosses.

Plant breeding has seen a major transition in the past decade as advances in biological sciences helped in evolving tools that can be applied to commonly accepted field techniques. Molecular markers have become a handy tool to accelerate plant breeding process by selecting desirable genotypes by following the genes or chromosomal segments in the crosses using markers that are closely linked to them. This is particularly important in the case of genes governing biotic and abiotic stresses where traditional methods of screening for the trait are laborious and time consuming. Sugarcane suffers from damages caused by various insect pests either by direct feeding of plant parts or by transmitting important viral diseases. Insecticides are used as a major control strategy to combat different insect pests. However, it was established that continuous use of insecticides results in development of resistance to the chemicals among the target insects and unintended harmful effects occur to beneficial insect population of pollinators, parasitoids and predators in the ecosystem. Hence, the best approach is to evolve plant varieties that are resistant to insects. For several years, breeding varieties for disease and pest resistance has been taken up. The inherent difficulties in the conventional screening and the misleading results in screening efforts, probably due to the polygenic control of resistance makes marker assisted selection (MAS) for pest and disease resistance a viable alternative. In marker-assisted selection, the selection is not on the elusive trait of interest but on the reliable molecular markers closely associated with the trait. Being environmentally independent and scorable even at very early stage of development; molecular markers ensure quicker

and clear-cut analysis at lower cost than phenotypic testing. Screening with molecular markers would be helpful especially when the trait is under polygenic control, most commonly seen in the case of pest and disease resistance. Biotechnological interventions play an important role to assist and improve classical plant breeding by integrating genomic tools that renders plant breeding programs more focussed, precision and less time consuming.

9.2 Description on Different Biotic Stresses

Throughout the world, negative impact of pests and diseases in sugarcane is reported and every sugarcane growing country suffers from insects and pathogens, although the type of causative organism varies. Nearly 125 diseases of fungal, bacterial, viral, phytoplasmal and nematode pathogens were reported from different continents (Rott et al. 2000). Although efforts are being made for the last 100 years to develop resistant varieties to various biotic constraints, the crop succumbs to many pests and diseases. The disease incidences and spread to new areas increased in different countries during the past decades. As per the report of International Society of Sugar Cane Technologists (ISSCT), each year several millions of dollars are lost due to diseases in sugarcane. Due to different diseases, each nation lose about 10–15% of their sugar production. Amongst, red rot, smut, and wilt are the major stalk diseases caused by fungal pathogens occur widespread across the sugarcane growing countries. Among the bacterial diseases, leaf scald (LSD) and ratoon stunting (RSD) caused by *Xanthomonas albilineans* and *Leifsonia xyli* subsp *xyli*, respectively occur in almost all the countries. Gumming disease and red stripe, the other bacterial diseases are known to inflict crop losses in some countries. Mosaic and yellow leaf (YLD) are the major viral diseases occur in all the sugarcane growing countries and affect sugarcane production considerably (ElSayed et al. 2015; Holkar et al. 2020; Lu et al. 2021). Besides these, phytoplasma diseases such as sugarcane grassy shoot (SCGS) and sugarcane white leaf (SCWL) seriously affect cane production in several countries in Asia. Foliar diseases such as rusts, eye spot, pokkah boeng, yellow spot, brown spot, ring spot, brown stripe, etc. occur throughout the world and their severity depends on the prevailing environmental conditions. Apart from these diseases, Sugarcane bacilliform virus causing leaf fleck has emerged as a serious constraint in different countries (Viswanathan et al. 2019a). Besides, Fiji disease confined to Australia and neighbouring countries and Pachymetra root rot limited to Australia are of regional importance.

9.2.1 Fungal Diseases

9.2.1.1 Red Rot (*Colletotrichum Falcatum* Went)

It seriously affects crop production in the countries like Bangladesh, India, Indonesia, Myanmar, Nepal, Pakistan, Thailand, Vietnam, and other Asian countries and is considered as a major stalk disease in USA, Brazil, Austrasia, Cuba, South Africa etc. Overall, the disease was reported from 77 countries in almost all the continents (Singh and Lal 2000). The fungal pathogen *C. falcatum* with perfect stage *Glomerella tucumanensis* [Speg.] Arx & Muller is associated with red rot. In Louisiana, the pathogen deteriorates the planted stalks or stubbles of sugarcane and this leads to failures in crop establishment (Hossain et al. 2020; Viswanathan 2021a). Sudden discolouration and drying of foliage, lesions of the rind and death of the affected stools are the field symptoms of red rot in a standing crop (Fig. 9.1a). The disease has been a serious menace from 1900 onwards in almost all the sugarcane growing countries when the disease was carried through seed canes from South East Asia. Over the decades, the disease menace has been reduced in many countries except South and South East Asia, where still epidemic occurrences of the disease destroy several thousands of ha. The pathogen causes extensive rotting of internal tissues and affected tissue turns red, hence it is called as 'red rot'. Typically, affected canes exhibit rotting of internal tissues with varying shades of red with characteristic white spots, perpendicular to the long axis of the cane (Fig. 9.1d).

The historic failures of elite sugarcane varieties in the past due to *C. falcatum* epiphytotics have started from the cv Co 205, the first man made hybrid sugarcane variety to the recent epiphytotics on Co 0238 were attributed to origin of new *C. falcatum* pathotypes. The new variants have gradually adapted to the new varieties which were hitherto resistant to the pathogen (Viswanathan et al. 2003a, 2022a; Viswanathan 2017, 2021a, b). Earlier, the new variants caused varietal breakdown or failures were designated as dark and light isolates based on the cultural morphology and usually light types were reported as virulent. In 1990s, a systematic study was conducted with a set of *Saccharum* spp and sugarcane hybrid varieties as differentials to characterize and designate the pathogenic variants in India (Padmanaban et al. 1996) and so far 13 pathotypes of *C. falcatum* were designated from different states (Table 9.1). This system of characterizing *C. falcatum* pathotypes ensures uniformity of using same pathotype to screen a common set of sugarcane varieties in advanced varietal trials in a region by different research centres (Viswanathan 2018).

C. falcatum exhibits enormous variation for pathogenicity, and also dynamic changes in virulence (Viswanathan et al. 2017a). Earlier studies of Malathi et al. (2006) revealed adaptation of *C. falcatum* to host varieties. In this, a resistant interaction becomes susceptible after repeated inoculations of the less virulent isolate on a resistant variety. Subsequent biochemical and molecular studies revealed pathogenicity factors that aid in pathogenicity of *C. falcatum* (Malathi and Viswanathan 2012a, b). Recently, detailed studies on red rot development from soil borne inoculum and plug method of inoculation on a set of varieties were conducted

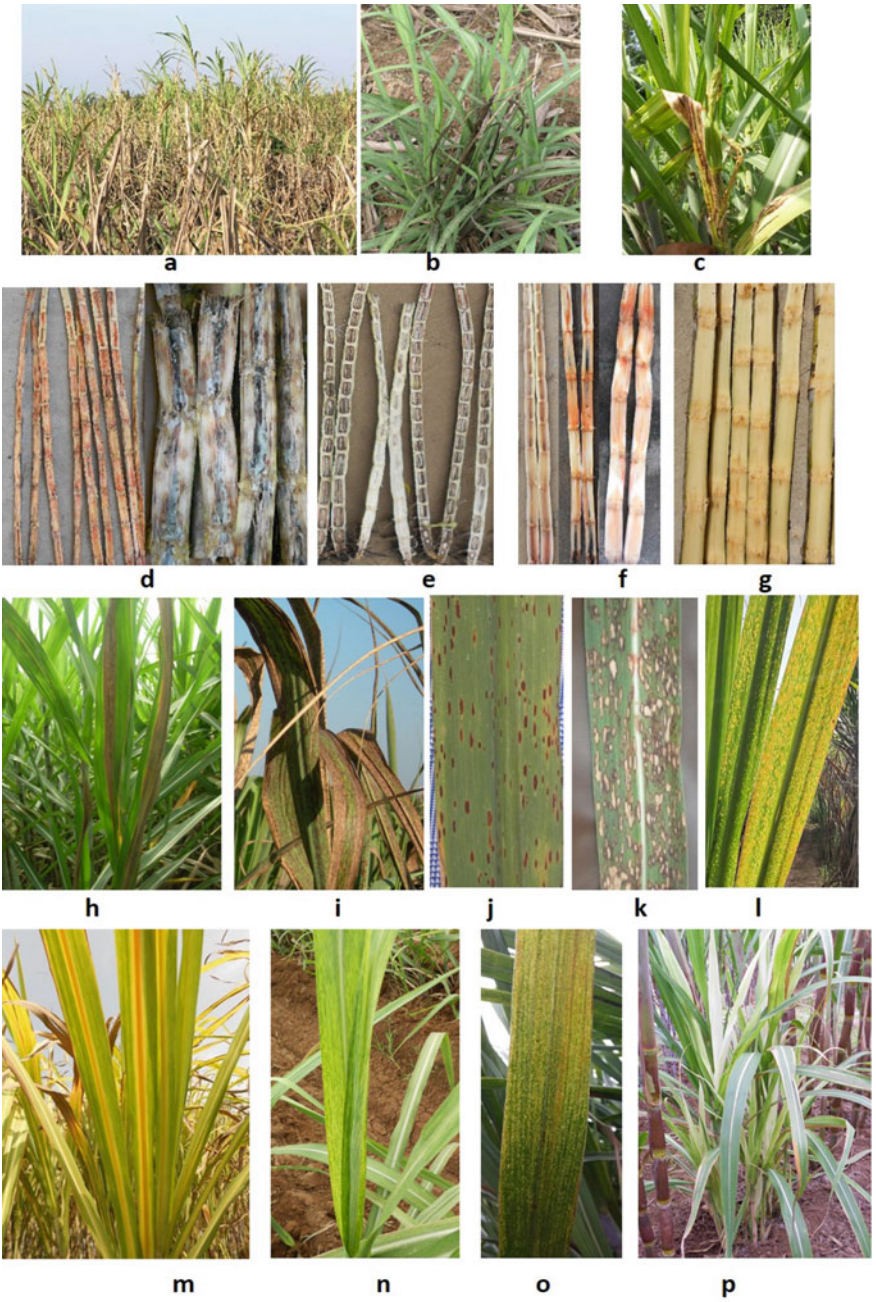

Fig. 9.1 Characteristic symptoms of major diseases of sugarcane. **a** Red rot-field symptoms, **b** smut, **c** pokkah boeng, **d** red rot-internal symptoms, **e** wilt internal symptoms, **f** pineapple disease, **g** ratoon stunting disease-internal symptoms, **h** leaf scald, **i** brown rust, **j** brown spot, **k** ring spot, **l** yellow spot, **m** yellow leaf disease, **n** mosaic, **o** leaf fleck, **p** grassy shoot disease

Table 9.1 Designated pathotypes of *Colletotrichum falcatum* in India

Pathotype	Host variety	Year of collection	Region
CF01	Co 1148	1997	Subtropical
CF02	Co 7717	1997	Subtropical
CF03	CoJ 64	1997	Subtropical
CF04	Co 419	1997	Tropical
CF05	Co 997	1997	Tropical
CF06	CoC 671	1997	Tropical
CF07	CoJ 64	2006	Subtropical
CF08	CoJ 84/CoJ 64	2006	Subtropical
CF09	CoS 767	2006	Subtropical
CF10	85A261	2006	Tropical
CF11	CoJ 64	2006	Subtropical
CF12	Co 94012	2009	Tropical
Cf13	Co 0238	2018	Subtropical

under field conditions. Pathogenicity of *C. falcatum* pathotypes from these assays clearly revealed that a pathotype exhibits a host adaptation to cause the disease in sugarcane (Viswanathan et al. 2020a, b). Further, the inoculum surviving in the soil makes repeated attempts to infect the host, finally succeeds to cause the disease in the field. By this, host resistance in a variety is compromised and 'resistance breakdown' or 'varietal breakdown' occurs (Viswanathan and Selvakumar 2020).

9.2.1.2 Smut

The whip smut caused by *Sporisorium scitamineum* (Phylum: Basidiomycota, Order: Ustilaginales) is a widespread disease of sugarcane across the continents, affecting both cane yield and sucrose content, hence substantial economic losses occur during severe cases (Bhuiyan et al. 2021; Rajput et al. 2021; Sundar et al. 2012). Emergence of long culmicolous smut whip (sorus) in growing point is the characteristic symptom of the disease or such whips on the axial buds and secondary tillers. The smut whips may be up to 1.5 m in length and contain millions of black teliospores (Fig. 9.1b). Severity of the disease is influenced by prevailing pathogenic races, environmental conditions, number of ratoons and varieties grown. Globally, efforts were made to identify race profile of *S. scitamineum* by assessing whip development in 14 locations across 10 countries on a set of standard host differentials. Although this study revealed existence of variability among *S. scitamineum* populations, a high level of pathogen diversity was found only in Taiwan (Grisham 2001).

Molecular studies with *S. scitamineum* isolates from 15 cane growing countries against 17 microsatellites revealed existence of a very low level diversity among African and American population as compared to the Asian population, which seemed as the major source of diversity in smut pathogen (Raboin et al.

2007). Although, molecular variation in smut pathogen has an association with their geographic origin, evidence for co-evolution between the host and the pathogen is lacking in China (Que et al. 2012). However, the studies from India suggested that *S. scitamineum* isolates originated from main sugarcane producing states exhibited a significant genetic and pathogenic variation. Further, prevailing environmental conditions and the varieties grown in the region are found to govern such pathogenic variation (Barnabas et al. 2018).

9.2.1.3 Wilt

Fusarium sacchari (E.J. Butler & H. Khan) W. Gams, (1971) (Nectriaceae, Hypocreales, Sordariomycetes, Ascomycota) is associated with the disease. Wilt is an important stalk disease, seriously affect production and productivity of sugarcane in different countries. Currently, it occurs in Bangladesh, India, Iran, Malaysia, Myanmar, Nepal, Pakistan and Thailand (Hossain et al. 2017; Rao and Agnihotri 2000; Viswanathan 2013a, 2018). Characteristic symptoms of wilt include stunted growth, drying of canes and internally, pith cavities and discolouration of the stalk tissues (Fig. 9.1e). In India, the disease occurs throughout the cane growing areas however; Indo-Gangetic plains of subtropical region, Gujarat and East Coastal deltaic regions witness disease severity to very high levels (Viswanathan 2018; Viswanathan et al. 2006). Only in the recent years, cause of wilt by *F. sacchari* has been established based on detailed pathogenicity and molecular analyses in India (Viswanathan et al. 2011a). The pathogen exhibits enormous variability for cultural and morphological characters (Poongothai et al. 2014a, b) and among the molecular markers, ISSR is more efficient followed by RAPD and rDNA IGS-RFLP to group the isolates (Poongothai et al. 2015).

9.2.1.4 Pokkah Boeng

Pokkah boeng (PB) is Javanese term meaning distorted or malformed spindle in sugarcane (Fig. 9.1c), earlier considered as a minor disease but now it occurs in many countries, devastating sugarcane productivity. Several *Fusarium* spp cause the disease and the following species *F. sacchari, F. andiyazi, F. verticillioides, F. proliferatum,* and *F. subglutinans* are reported from various continents/regions (Martin et al. 1989; McFarlane and Rutherford 2005; Govender et al. 2010; Mohammadi et al. 2012; Khani et al. 2013; Nordahliawate et al. 2008; Viswanathan 2020). *F. verticillioides, F. sacchari, F. proliferatum,* and *F. oxysporum* were reported as the casual organism in China, however, *F. verticillioides* is the dominant species associated with PB (Lin et al. 2014a; Bao et al. 2016; Meng et al. 2020). In the country, the disease occurs throughout year during both wet and dry seasons (Lin et al. 2014a). Further, *F. verticillioides* and *F. proliferatum* are reported as the cause of the disease in sugarcane and among the two, the former accounts for more than 90% of the records in the country. To confirm identity of the two species infecting sugarcane, a

species-specific PCR assay was developed (Lin et al. 2014a). In India, *F. sacchari* and *F. proliferatum* were isolated from the affected sugarcane; however, the former is frequently isolated from the infected samples (Viswanathan et al. 2017b). Majority of 55% *Fusarium* spp associated PB with knife cut symptoms in Iran was found to be as *F. verticillioides* and *F. subglutinans, F. proliferatum*, and *F. semitectum* are the other species associated with the disease. Almost all the isolates were pathogenic except *F. semitectum* isolates and among pathogenic species, *F. verticillioides* and *F. subglutinans* isolates were more pathogenic than isolates of *F. proliferatum* (Taher Khani et al. 2013). *F. verticillioides* and *F. proliferatum* were reported as the PB-associated pathogen in Mexico (Rosas-Guevara et al. 2014). Morphological features and molecular phylogenetic analyses grouped PB associated Fusaria and this broadly grouped them into two species *F. verticillioides* and *F. proliferatum* closely related to *F. sacchari* and *F. fujikuroi,* respectively (Leslie and Summerell 2006).

9.2.1.5 Pineapple Disease (Sett Rot)

In sugarcane, pineapple disease is a serious constraint and it causes rotting of the seed cane setts and rotting of standing canes. *Ceratocystis paradoxa* is the causative organism (anamorph: *Thielaviopsis paradoxa*). The disease is referred as pineapple disease because of sweet smell coming out of the diseased sugarcane was similar to ripened pineapple fruit. The disease is reported in more than 50 countries of both tropical and temperate regions. The disease causes 15–20% losses in sett germination, post germination death of seed cane sprouts and 10–15 tonne losses per hectare in cane yield (Girard and Rott 2000; Viswanathan 2012a, b). The pathogen affects standing canes particularly after damages caused by animal bites, lodging, water logging and red rot or wilt (Fig. 9.1f).

9.2.1.6 Rusts

Worldwide, two rusts, brown and orange rusts are regularly recorded on sugarcane (Rott et al. 2000). The former is caused by *Puccinia melanocephala* (Syd. & P. Syd) and the latter is caused by *P. kuehnii* (W. Kruger) E.J. Butler. During 2008, tawny rust, a new sugarcane rust, also referred as African sugarcane rust, was recorded in South Africa for the first time (Martin et al. 2017). Brown rust, also referred as common rust was recorded in ~29 sugarcane growing countries during 1980s, whereas currently it is reported from more than 40 countries (Egan 1980; EPPO 2019a). Severe outbreaks of brown rusts in Southern Karnataka on the cvs CoVc 03165, Co 0323 and other varieties like Co 94008, Co 98005, CoC 671, Co 94012 and VSI 434 with severe losses to crop production were recorded in the past (Fig. 9.1i) (Viswanathan 2012a; Selvakumar and Viswanathan 2019). Although orange rust was reported from ~18 sugarcane growing countries before 1980, the recent reports suggest that the rust occurs in nearly 45 countries, in the continents of Asia, Oceania, Africa and America (Egan 1980; Martin et al. 2017; Saumtally et al. 2011; EPPO 2019b). It was first

recorded during 2007 on the variety CP 80-1743 in Florida and subsequently it was recorded in other countries in America (Chavarría et al. 2009; Flores et al. 2009; Ovalle et al. 2008; Barbasso et al. 2010; Comstock et al. 2010).

Aerial spread of rust spores is of great concern since it will spread rapidly to the new areas in sugarcane growing countries. New variants of rust pathogens cause breakdown of resistance hence, many outstanding varieties under cultivation turn to be susceptible or resistant varieties have a tendency to pick up the disease slowly in the field (Braithwaite et al. 2009).

9.2.1.7 Other Foliar Diseases

Brown spot (*Cercospora longipes* E. J. Butler [1906]) is economically important in countries and regions where high relative humidity and mild temperatures of ~20–22 °C prevail (Saumtally and Sullivan 2000) (Fig. 9.1j). The disease is severely affecting productivity in the susceptible varieties like CoM 0265 in the tropical India and brown spot epidemics curtailed the spread of the variety in North Karnataka and South Maharashtra (Viswanathan and Ashwin 2020). Brown stripe caused by fungal pathogen *Bipolaris stenospilus* (teleomorph: *Cochliobolus stenospila*) is reported from various countries with severe damages to sugarcane cultivation in Cuba, Louisiana, Australia, Caribbean islands, Taiwan, India etc. The disease is favoured by factors such as drought or nutrient deficiencies resulting in huge losses to the crop (Martin and Egan 1989). Downy mildew caused by *Peronosclerospora sacchari* is characterized by leaf stripes of creamy white that become red upon aging with stunting of affected clumps. The disease is reported from Pacific, South Asia and South East Asian regions (Suma and Magarey 2000). Serious outbreaks have occurred in Australia, Fiji, Philippines and Taiwan and heavy yield loss is reported on susceptible varieties from 38 to 58% in Philippines. The yield losses can range from 20 to 90% with severe losses. Eye spot is another foliar disease caused by *Biploaris sacchari* is recognized by small "eye shape like" spots on laminar tissues and long streaks, several feet in length and sometimes 1/3 of an inch in width on susceptible varieties. The disease is considered as a minor disease and reported worldwide in the tropics and sub-tropics covering Africa, Asia, Americas, the Caribbean, Europe and Oceania (Comstock 2000). In India a severe disease outbreak occurred on the popular variety Co 419 in Karnataka (Kumaraswamy and Rabindra 1978). Cool moist weather favors the disease development. Ring spot, another minor disease caused by *Leptosphaeria sacchari*, generally infects the senescing leaves in tropical, high rainfall areas with humid conditions and is reported in more than 80 countries. Except the terminal leaves, the disease affects the entire foliage, hence it shows a burnt appearance from a distance. The disease may become a serious one in susceptible varieties if the preventive measures are not taken under disease favourable conditions (Fig. 9.1k) (Croft 2000). Similarly, yellow spot (*Mycovellosiella koepkei*) is prevalent in high relative humidity and heavy rainfall areas of sugarcane growing countries (Fig. 9.1l). The disease is of seasonal importance in sugarcane, reported worldwide from mild to severe form in India, East Asia, Central and South Pacific Islands, and also occurs

in Australia and Africa, Guyana, Trinidad, Barbados, Jamaica, Central and South America and North America. High yield loss is reported when 35–50% areas of the top 8–10 young leaves are affected due to the damage to the photosynthetic tissues. Sucrose content was affected in early maturing varieties and yield loss was reported in late maturing varieties under epidemic conditions (Ricaud and Autrey 1989).

9.2.1.8 Other Fungal/Oomycete Diseases

The other fungal diseases affecting sugarcane, reported worldwide in different countries with or without economic damages (Rott et al. 2000) are listed below:

Australian basal stem, root and sheath rot—unidentified basidiomycete fungus,

Banded sclerotial disease—*Thantephorous sasakii/T. cucumeris*,

Black leaf spot (tar spot)—*Phyllachora sacchari*,

Covered smut—*Sporisorium cruentum, Spacelotheca erianthi* and *Sporisoium schweinfurthiana*,

Dry top rot—*Ligniera vasculorum* (a plasmodiophoromycete fungus),

Ergot—*Claviceps purpurea*,

Leaf blight—*Leptosphaeria taiwanensis*,

Leaf scorch—*Stagnospora sacchari* and *Leptosphaeria bicolor*,

Marasmius basal stem, root and sheath rot—*Marasmius sacchari*,

Pachymetra root rot—*Pachymetra chaunorhiza*,

Pythium root rot—*Pythium arrhenomanes*,

Ramu orange leaf—unidentified Exobasidiales,

Red leaf spot (purple spot)—*Dimeriella sacchari*,

Red rot of leaf sheath—*Corticium rolfsii*,

Red spot of leaf sheath—*Mycovellosiella vaginae*,

Rind disease and sour rot—*Phaeocytostroma sacchari*,

Root and basal stem rot—*Xylaria* cf. *warburgii/X. arbuscular*,

Sclerophthora disease—*Sclerophthora macrospora*,

Sheath rot—*Cytospora sacchari*,

Veneer blotch—*Deightoniella papuana*,

White speck—*Elsinoe sacchari*,

Zonate leaf spot—*Gloeocercospora sorghi.*

9.2.2 Bacterial Diseases

9.2.2.1 Ratoon Stunting Disease (RSD)

Leifsonia xyli subsp. *xyli* (Lxx), the xylem-limiting bacterium is an unique bacterium causing RSD recorded almost in all the countries and is considered as a major disease constraint among the various sugarcane diseases (Viswanathan 2001a, 2016; Putra and Damayanti 2012; Taher-Khani et al. 2013; Li et al. 2014; Magarey et al. 2021). The disease is characterized by a stunted cane growth, which indicates reduced cane thickness, internode numbers and tillers. Also the internodes exhibit irregular shapes and ratoon crops express more pronounced disease symptoms. Usually RSD affected crops show a pale canopy due to loss of vigour and when it occurs with viral diseases of mosaic and YLD, a severe degeneration in the crop is noticed. Other than growth reduction, the disease is not recognized except orange-red nodal discolouration (Fig. 9.1g); hence the disease presence is largely ignored in many countries. It primarily affects cane yield, whereas other key economic parameter like sugar content show marginal impact. The disease expresses more severity in ratoons as well as in rainfed crops. RSD incidences varied from 48.9 to 100% depending on the sugarcane variety in China and it is the most significant disease constraint in the country, found widespread among the principal sugarcane diseases (Wei et al. 2019). In India, Lxx along with other viruses causing mosaic and YLD seriously affect cane productivity by means of varietal degeneration (Viswanathan 2004, 2016). Genome of Lxx is 2.6 Mb in length with 2,044 predicted open reading frames and genome analysis identified putative pathogenicity genes such as pectinase, lysozyme, wilt-inducing protein, desaturase and cellulase (Monteiro-Vitorello et al. 2004).

9.2.2.2 Leaf Scald

Xanthomonas albilineans, the gram −ve bacterium causing leaf scald disease (LSD) occurs in about 60 countries growing sugarcane, including Argentina, Australia, China, Brazil, India, Mauritius, Cuba, Reunion islands, Thailand, USA, etc. Like RSD, it is also a major disease of sugarcane and occurs worldwide (Rott and Davis 2000a; Lin et al. 2018). Typical manifestation of LSD vary from a narrow, single, white, sharp stripes or longitudinal blights to total wilting and necrosis of affected lamina, resulting in death of entire clumps (Fig. 9.1h) (Ricaud and Ryan 1989; Rott and Davis 2000a). Genetic relatedness of 218 *X. albilineans* isolates representing 31 regions worldwide revealed divergent populations of the bacterial pathogen. Worldwide, a narrow dispersal of the pathogenic variants was found (Davis et al. 1997). Recently *X. albilineans* complete genome was sequenced. The genome comprises a 3724 kb circular chromosome with a 31,536 bp plasmid. Whole genome analysis revealed an intra-species variability of *X. albilineans* and it further provided resources to explore its pathogenic potential and virulence (Zhang et al. 2020).

9.2.2.3 Red Stripe

Red stripe caused by the bacterial pathogen *Acidovorax avenae* subsp *avenae* (Aaa) in sugarcane occurs throughout the sugarcane growing countries, however its severity varies depending on the varieties under cultivation and prevailing environment. The disease manifests its symptoms in two phases viz. leaf stripe and top rot. Of the two, the latter is deleterious and causes severe crop losses since top rot phase causes death of the growing meristem or stunted cane growth (Martin and Wismer 1989; Rott and Davis 2000b). In molecular analyses, strains of Aaa in Argentina and other countries exhibited high degree of genetic variation (Fontana et al. 2013, 2019; Li et al. 2017a, b). The draft genome of Aaa is sequenced to ~5646 kb and it has a GC content of 68.6% (Fontana et al. 2016).

9.2.2.4 Other Bacterial Diseases

Other than these bacterial diseases, occurrences of the following bacterial diseases were reported in different countries, mostly as minor or seasonal diseases from different countries (Rott et al. 2000).

Bacterial mottle (*Pectobacterium chrysanthemi*),

False red stripe (*Xanthomonas* sp.),

Gumming (*Xanthomonas axonopodis* pv. *vasculorum*),

Mottled stripe (*Herbaspirillum rubrisubalbicans*),

Red streak (*Pseudomonas syringae* pv. *syringae*),

Spindle rot (*Acidovorax avenae* subsp. *avenae*).

9.2.3 Viruses

9.2.3.1 Yellow Leaf (YL) Disease

It was first reported during 1989 in Hawaii and later from other countries. Currently it occurs throughout cane growing countries and attained status of a major production constraint in India (El-Sayed et al. 2015; Holkar et al. 2020; Viswanathan 2021c). Sugarcane yellow leaf virus (ScYLV), a *Polerovirus*, is associated with YLD worldwide and the virus systemically infects phloem cells. The disease is characterized by mid rib yellowing, bunching of leaves in the spindle, drying of discoloured midrib and leaf tissues (Fig. 9.1m). Variation in the virus genome has been studied in detail based on complete genomes. Currently, 10 ScYLV genotypes occurring worldwide viz. from Brazil (BRA), China (CHN1-3), Colombia (COL), Cuba (CUB), Hawaii (HAW), India (IND), Peru (PER) and Reunion Island (REU) were characterized

(Moonan and Mirkov 2002; Abu Ahmad et al. 2006a, b; Chinnaraja et al. 2013; ElSayed et al. 2011; Gao et al. 2012; Lin et al. 2014a, b; Viswanathan et al. 2008a; Wang and Zhou 2010; Wang et al. 2012). The BRA genotype occurs in most of the countries but others are confined to few nations (ElSayed et al. 2015). Khalil et al. (2018) grouped 498 ScYLV isolates reported all over the world into 10 genotypes according to geographic origins.

9.2.3.2 Sugarcane Mosaic

Sugarcane mosaic virus (SCMV) subgroup of *Potyviridae* and Sugarcane streak mosaic virus (SCSMV) are associated with mosaic in sugarcane and the disease prevails worldwide (Viswanathan et al. 2018b). The viruses infect sugarcane, maize, sorghum, and many other grasses and cause yield losses. The disease symptoms are characterized by moderate to prominent forms of mosaic on leaves and in severe cases, entire leaf turn pale or yellow and causing yield decline (Fig. 9.1n). SCMV and SCSMV together or separately cause the disease in Asian countries, whereas Americas have infections of SCMV and/or Sorghum mosaic virus (SrMV). SCMV is predominantly reported from Africa and Australia however, recently infections of SCSMV have been reported from Ivory Coast (Koike and Gillaspie 1989; Grisham 2000; Gemechu et al. 2004; Chatenet et al. 2005; Xu et al. 2008; Viswanathan and Karuppaiah 2010; Gonçalves et al. 2012; Wu et al. 2012; Putra et al. 2014; Viswanathan et al. 2018b; Daugrois et al. 2020). Complete genomes SCMV were characterized from many countries with reports on prevalence of new strains, variation in genomes and recombinant isolates (Viswanathan et al. 2009, 2018b; Moradi et al. 2016; Xie et al. 2016; Bagyalakshmi et al. 2019b; Lu et al. 2021). SCSMV reported earlier as an unassigned member of *Potyviridae* (Hema et al. 1999); later it was characterized to a new genus 'Susmovirus' of *Potyviridae* (Viswanathan et al. 2008b) and it was subsequently rechristened as '*Poacevirus*'. The genome of SCSMV is characterized based on whole genome basis. Many whole genomes of SCSMV from Myanmar, China, Pakistan, India, Indonesia, Japan, and Thailand were reported (Fellers et al. 2009; Xu et al. 2010; Parameswari et al. 2013; Liang et al. 2016).

9.2.3.3 Leaf Fleck

Leaf fleck caused by Sugarcane bacilliform virus (SCBV) is a *Badnavirus* (*Caulimoviridae*). It was initially detected from Cuba in 1985 and subsequently from Morocco in 1986 (Lockhart and Autrey 1988). In India, the virus was initially reported from *Saccharum officinarum* and other germplasm clones (Viswanathan et al. 1996, 1999; Viswanathan and Premachandran 1998). However, recently prevalence of leaf fleck in severe form was recorded under field conditions on most of the popular cultivars under cultivation (Viswanathan et al. 2019a). Besides its occurrence worldwide, the virus is regularly detected in quarantine (Viswanathan et al. 2018b). Typically the disease symptom start as minute chlorotic specks, expand in size,

turn to yellowish and red and gradually the symptoms spread to entire leaf lamina. The severe expression of the disease on the older leaves and in severe cases entire foliage dries (Fig. 9.1o). SCBV exhibits enormous genomic variation (Rao et al. 2014). Initially two SCBV species Sugarcane bacilliform Morocco virus (SCBMV) and Sugarcane bacilliform Ireng Maleng virus (SCBIMV) were designated from Morocco and Australia, respectively (Bouhida et al. 1993; Geijskes et al. 2002). Later, complete genomes of SCBV from China, Guadeloupe and India were reported (Muller et al. 2011; Karuppaiah et al. 2013; Sun et al. 2016).

9.2.3.4 Other Viruses

Among the 23 virus species reported to infect sugarcane, SCMV, SCMV, ScYLV and SCBV are common in most of sugarcane growing countries (Boukari et al. 2020). In Australia, Fiji leaf gall caused by Fiji disease virus (FDV) was a major constraint to cane production (Smith 2000). Sugarcane mild mosaic virus (SCMMV), a *Closterovirus* has been reported as mixed infections with SCBV in germplasm from few countries (Lockhart and Autrey 2000). Peanut clump virus (PCV), a *Pecluvirus*, associated with red leaf mottle has been reported mostly reported from African countries (Rott and Chatenet 2000). Sugarcane striate mosaic associated virus (SCSMaV) associated with sugarcane striate mosaic disease, taxonomically intermediate between the genera *Carlavirus* and *Foveavirus* was reported from central Queensland, Australia (Thompson and Randles 2001). Ramu stunt virus with sequence homology to *Tenuivirus* genus causes Ramu stunt a serious constraint of sugarcane and is confined to Papua New Guinea (Braithwaite et al. 2019). There are reports of eight *Geminiviridae* members of the genus *Mastrevirus* species viz. Maize streak virus, Saccharum streak virus, Sugarcane streak virus, Sugarcane chlorotic streak virus, Sugarcane streak Egypt virus, Sugarcane white streak virus, Sugarcane streak Reunion virus, and Sugarcane striate virus (Bock et al. 1974; Peterschmitt et al. 1991; Hughes et al. 1993; Bigarre et al. 1999; van Antwerpen et al. 2008; Lawry et al. 2009; Candresse et al. 2014; Boukari et al. 2017, 2020; Yahaya et al. 2017). Two probable new viruses in *Umbravirus* and *Chrysovirus* genera were also reported after metagenomics studies (Filloux et al. 2018).

9.2.4 Phytoplasma Diseases

Sugarcane grassy shoot (SCGS) and sugarcane white leaf (SCWL) are the major diseases caused by phytoplasma and are confined to Asian countries and were not reported outside the continent. The countries, Bangladesh, China, Malaysia, Myanmar, Nepal, India, Thailand, Pakistan, Sri Lanka, Sudan, and Vietnam, and reported varying intensities of these diseases (Rishi and Chen 1989; Chen and Kusalwong 2000; Viswanathan 2000; Nithya et al. 2020). Both the diseases are characterized by excessive tillering, sprouting of axillary buds, leaves become short,

leathery and chlorotic and affected stools fail to produce millable (harvestable) canes (Fig. 9.1p). In the field, the diseases mainly spread through disease affected seed canes. The leafhoppers *Matsumuratettix hiroglyphicus* and *Yamatotettix flavovittatus* are the two known reported vectors for secondary spread SCWL disease in the field (Wongkaew and Fletcher 2004; Hanboonsong et al. 2006). However, role of insect vectors in spreading SCGS disease is not reported under field conditions. Usually the ratoon crops suffer severely and low cane productivity especially in the ratoons in these countries is attributed to severe outbreak of SCGS and SCWL diseases. SCWL phytoplasma and SCGS phytoplasma have close relations and come under the 16SrXI group (Wongkaew et al. 1997; Sdoodee et al. 1999). Detailed studies to characterize the phytoplasma associated with SCGS revealed occurrence of 16SrXI-B and 16SrXI-F strains in India however, there was no relation between phenotypic symptoms on sugarcane and the associated strains of phytoplasma (Nasare et al. 2007; Viswanathan et al. 2011b; Rao et al. 2017; Yadav et al. 2017). Recently, 0.505 Mb draft sequence of SCGS-phytoplasma genome from India was revealed with GC content of 19.86%, along with a putative plasmid of 2.9 kb (Kirdat et al. 2020).

9.2.5 Nematodes

Plant-parasitic nematodes occur worldwide in sugarcane. Species the following genera *Circonemella, Criconemella, Helicotylenchus*, *Hemicycliophora*, *Hemicriconemoides*, *Hoplolaimus*, *Pratylenchus*, *Rotylenchulus*, *Scutellonema*, *Meloidogyne, Ogma* and *Tylenchorhynchus* infecting sugarcane in 24 countries including Australia, Brazil, India, Kenya, Mauritius, Pakistan and South Africa (Stirling and Blair 2000; Ramouthar and Bhuiyan 2018). Symptoms of swellings and galls or lesions of varying dimensions are observed due to nematode infections. Such damages to root by the nematodes reduce plant growth which causes reduced tiller production and poor canopy coverage. Australian reports show 5–20% losses caused by nematodes every year and this loss is estimated to be more than $80 million in cane productivity. In the country, *Pratylenchus zeae* (lesion nematode) and *Meloidogyne* spp., especially *M. javanica* (root-knot nematode) are the major nematodes reported to impact sugarcane cultivation (Blair and Stirling 2007). For root-lesion nematodes, none of the commercial varieties evaluated in Australia are resistant. Recently, *Saccharum spontaneum*, was identified as a source of resistance to *Pratylenchus zeae* and *M. javanica* in Australia (Bhuiyan et al. 2019).

9.2.6 Occurrence and Distribution of Important Insect Pests

9.2.6.1 Major Borer Pests in Sugarcane

Most of the major pests of sugarcane are crambids *Chilo* spp. and *Diatraea* spp. of Lepidoptera, the former distributed throughout Africa and Asia and the latter confined to the new world (Bleszynski 1969). In tropics, *Sesamia* spp. and *Scirpophaga* spp. occur in large scale. The common sugarcane borers with the alternate crop hosts, occurring across the continents (Table 9.2) and in Asia are listed (Table 9.3).

Among the sugarcane pests, lepidopteran stalk borers are important pests (OECD 2016) causing enormous damage (Li et al. 2017a, b) leading to loss of quality (Sallam et al. 2010; Souza et al. 2013) and yield (Mengistu and Selvaraj 2013; Sattar et al. 2016) worldwide. They include the sugarcane giant borer *Telchin licus* (Drury) (Triana et al. 2020; Dinardo-Miranda and Fracasso 2013) in the Central and South America; the sugarcane stem borer *Diatraea saccharalis* (F.) in the Americas and the Caribbean region (Francischini et al. 2019), the *Eoreuma loftini* (Dyar), the Mexican borer in South Texas (Showler and Reagan 2017), in Mexico (Rodríguez-del-Bosque and Reyes-Méndez 2013), *Eldana saccharina* Walker, the African stem borer in South Africa (Keeping et al. 2014), the spotted borer *Chilo sacchariphagus* (Bojer) in China, South Africa, Swaziland and Mauritius, Réunion, Madagascar and Mozambique (Bezuidenhout et al. 2008), *Chilo sacchariphagus indicus* (Kapur), the internode borer in India (Geetha et al. 2010), *Proceras venosatus* Wlk (Weng et al. 2006), *Chilo infuscatellus* (Snellen), *C. sacchariphagus, Tetramoera schistaceana, S. inferens* and *Scirpophaga intacta* (Snellen) in China (Zhang et al. 2019).

Across the world atleast fifty crambid and noctuid borers of *Chilo, Sesamia* and *Diatraea* genera infest sugarcane (Wijayanti et al. 2021) while 36 species of them were recorded by Sallam (2006) in Asia and islands in the Indian ocean. In the old world, *Chilo* and *Sesamia* occur but *Diatraea* is a pest in the new world. The constantly occurring important stalk borers belong to the *Chilo* genus that are extensive and intensively distributed in Indian Ocean Islands (Williams 1983) and Mozambique, Africa (Youdeowi 1989; Kfir et al. 2002), China (Rossato et al. 2013) causing severe loss and easily spread by vegetative propagation (Rossato et al. 2013). In Indonesia, *C. sacchariphagus, C. auricilius* and *Scirpophaga excerptalis* are the most important sugarcane borers (Goebel et al. 2014) and *C. infuscatellus, T. schistaceana*, *S. inferens* and *Phragmataecia castenea* are minor pests (Achadian et al. 2011).

The noctuid sugarcane pink borer *S. inferens* has extended distribution in the East Asia (China, Japan) and many of the Asian countries including Philippines, Bhutan, Malaysia, Bangladesh, Brunei, Taiwan, Korea, Nepal, and Srilanka, (Jeevanandham et al. 2020) and infests various graminaceous hosts like finger millet, maize, sorghum wheat, rice, and citronella grass besides sugarcane (Fletcher 1920). This polyphagy enables persistent occurrence throughout the year in the ecosystem allowing the pest to multiply rapidly in the most favorable host before transferring on to sugarcane. Vast distribution of the stem borers *Sesamia calamistis* Hampson and *Chilo partellus*

Table 9.2 Major borer pests of sugarcane in the world

Pest species	Local name[a]	Alternative crop hosts
Argyroploce (*Tetramoera schistaceana* [Sn.])	Grey borer, sugarcane shoot borer	Nil
Chilo sacchariphagus (Bojr.)	Spotted borer	Rice, sorghum, maize
Chilo auricilius Dudgeon	Stalk borer, gold-fringed rice borer	Sugarcane, rice, maize and sorghum
Chilo infuscatellus (Snellen)	Striped stem borer, early shoot borer, yellow top borer	Rice, oat, maize, barley, sorghum, *Andropogon* sorghum
Chilo agamemnon Bleszynski	Purple lined borer, lesser sugar cane borer	Maize, rice, sugarcane, sorghum
Diatraea saccharalis (Fabricius)	Sugarcane stalk borer	Maize, rice, sorghum
Chilo terrenellus (Pagenstecher)	Sugarcane borer, sugarcane internode borer	Nil
Diatraea flavipennella (Box)	*Broca pequena da cana-de-acucar*	Nil
Diatraea indigenella (D. & H.)	Stem borer	Maize, sorghum
Diatraea considerate (Heinrich)	Stalk borer	Maize, sorghum
Diatraea rosa (Heinrich)	Stem borer	Nil
Diatraea grandiosella (Dyar)	South western corn borer	Maize
Diatraea tabernella Dyar	Stalk borer	Nil
Eldana saccharina (Walker)	Sugarcane stalk borer, African sugarcane stem borer, Eldana borer	Maize, sorghum, cassava
Eoreuma loftini (Dyar)	Mexican rice borer	Rice, maize, sorghum
Scirpohaga excerptalis (Walker)	The white top borer or sugarcane top borer	Rice, wheat
Sesamia inferens (Walker)	Purple borer	Wheat, maize, sorghum, ragi, rice
Sesamia grisescens (Walker)	Ramu shoot borer, the pink sugarcane borer, shoot borer, sugarcane borer, pink stalk borer,	Napier grass *Pennisetum purpureum*
Sesamia cretica (Lederer)	Sorghum borer, durra stem borer, sorghum stem borer, purple stem borer, the corn stem borer, sugarcane pink borer, pink corn borer, maize borer, large corn borer, greater sugarcane borer	Maize, sorghum

(continued)

Table 9.2 (continued)

Pest species	Local name[a]	Alternative crop hosts
Tryporyzanivella intacta (Sn)	Sugarcane top moth borer	Nil
Telchin licus licus	Sugarcane giant borer, banana stem borer	Banana
Coleoptera *Metamasius hemipterus* (L.)	West Indian cane weevil	Banana, pineapple, palms, maize
Rhabdoscelus obscurus (Boisd)	New Guinea cane weevil borer, beetle borer, cane weevil borer, New Guinea sugarcane weevil, Hawaiian sugarcane borer, sugarcane weevil borer	Palms, banana, maize

[a]Local names are those by which the pest is addressed in literature or by farmers

(Swinhoe) in main land Africa, and spread of *Sesamia cretica* Ledere upto Southern Europe has been reported by Sallam (2006). In South East Asia, *Chilo auricilius* is a major sugarcane borer and a serious stalk borer in northern India (Neupane 1990).

In India, of the more than 200 pests recorded on sugarcane only a few borers and sucking pests severely affect the cane yield and quality of the produce (Figs. 9.2 and 9.3). The loss due to insect pests in sugarcane production is 20–25% (Kumar et al. 2019).

9.2.6.2 Genetic Divergence of Insect Populations

Very few records of biotypes of insects on sugarcane are available. Genetic divergence of the borer species *D. saccharalis* between the populations of southern United States, Mexico and Brazil was observed (Pashley et al. 1990). Different biotypes of the sugarcane moth borers that belong to the genera of *Diatraea, Chilo, Eoreuma, Sesamia* and *Bathytricha* within collection localities and across their distribution could be identified through molecular characterization of COII and 16S sequences (Lange et al. 2004). Though (Joyce et al. 2016), the widely distributed *D. saccharalis* is still assumed to be a single species Joyce et al. (2014) demonstrated the existence of two different genotypes in United States.

Divergence analyses often has the ability envisage the expansion and invasion of a pest. Francischini et al. (2019) analyzed the genetic diversity of *D. saccharalis* in America through molecular markers targeting entire genome and comparing the mitochondrial gene sequences. The clustering analyses indicated three distinct groups, which showed the distribution pattern of genetic diversity in the Americas suggested possible extensive spread through human-mediated movement. In India, the host based genetic divergence in the populations of *S. inferens* was established through SSRs (simple sequence repeats) analysis (Reetha and Mohan 2018).

Table 9.3 Major stemborers in Asia with their host plants and distribution (Adapted from Hittori 1971)

Species	Host plant					Distribution												
	Rice	Sugarcane	Maize	Wheat	Others	China	Japan	Formosa	Philippines	Indonesia	Malaysia	Vietnam	Thailand	Burma	Pakistan	India	Srilanka	Others
C. infuscatellus		✓	✓		✓			✓	✓	✓				✓		✓	✓	Afghanistan, Central Asia, Timor, Vulcan
C. sacchariphagus		✓			✓				✓	✓	✓							Madagascar, Mauritius, Reunion
C. s. indicus	✓	✓			✓													
Chilo tumidicostalis		✓														✓		Nepal
Chilo auricilius	✓	✓	✓		✓			✓	✓	✓			✓	✓		✓	✓	Bhutan, Nepal, Sikkim, Sangir, Molucca
Chilo partellus	✓	✓	✓		✓										✓	✓	✓	Afghanistan, Africa
Chilo polychrysus	✓	✓	✓		✓	✓			✓	✓	✓		✓			✓		Assam
C.s. straminellus		✓			✓	✓	✓	✓										
Acigona steniellus		✓										✓				✓		
Tryporyza nivella		✓				✓	✓	✓	✓	✓				✓		✓	✓	New Zealand, Africa
T.n. intacta		✓			✓					✓								
Sesamia inferens	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Korea

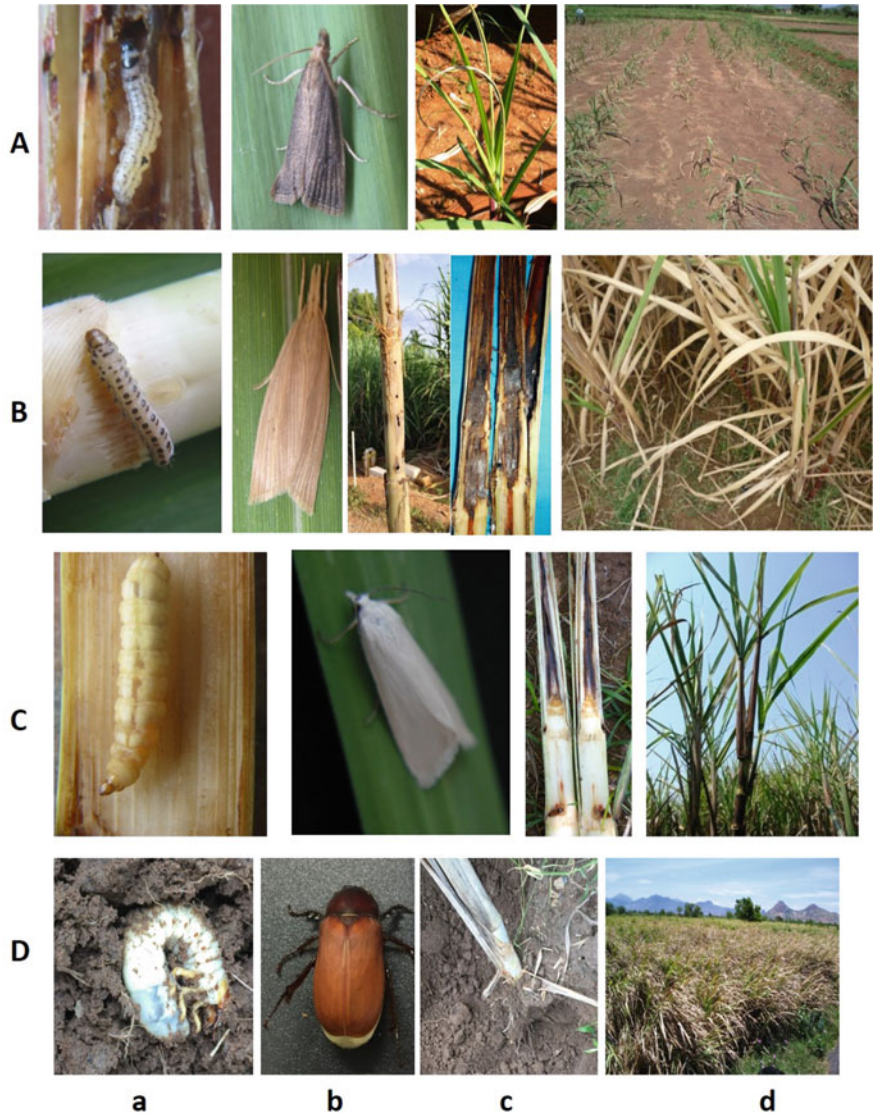

Fig. 9.2 Major insect pests of sugarcane; **A** shoot borer: *Chilo infuscatellus*, **B** internode borer: *Chilo sacchariphagus indicus*, **C** top borer: *Scirphophaga excerptalis*, **D** white grub: *Holotrichia serrata*; **a** larva (grub), **b** adult, **c** damaged plant, **d** affected field

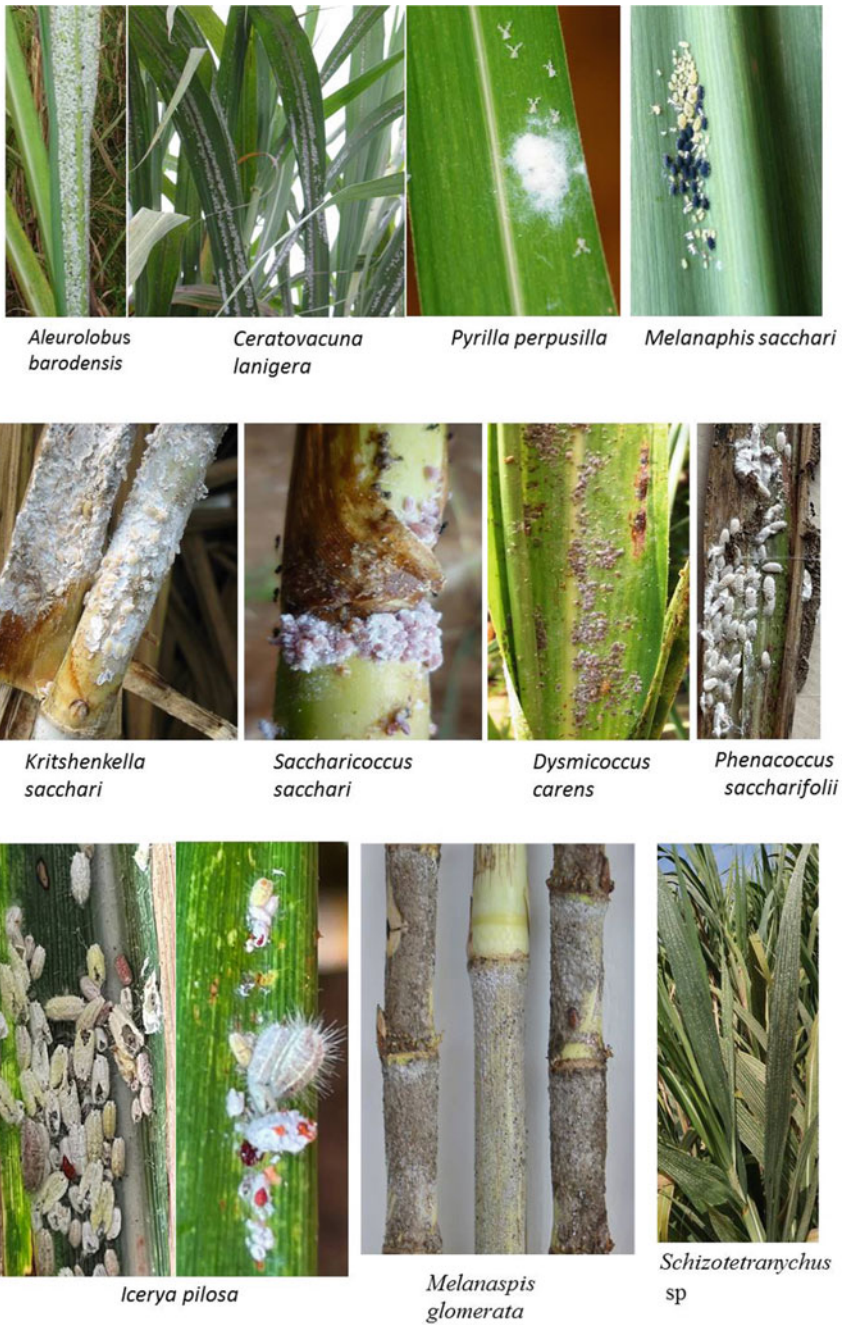

Fig. 9.3 Major sucking pests of sugarcane

9.2.7 Stages and Extent of Damage

9.2.7.1 Diseases

In sugarcane, vegetative propagation through seed canes (setts) is commonly practiced across the countries. The crop is harvested during 12–18 months after planting. Rarely, the crop is harvested 24 months after planting in places like Hawaii. After harvest of the plant crop, the second crop referred as ratoon is raised from the stubbles for many seasons. Number of ratoons again varies from country to country. In India, two ratoons are common, of course there are also 20–25 ratoons successfully grown in certain isolated pockets. Overall, sugarcane crop is grown as a plantation crop in large estates in different countries especially in the continents Americas, Australia and Africa, however, the farmers grow sugarcane in small holdings, in most of the Asian countries. Hence type of cultivation has a major influence on pest and disease outbreaks and management strategies to be adopted. Major fungal, bacterial, viral and phytoplasmal diseases are transmitted mainly through infected seed canes (setts). Hence the infected setts introduce diseases in the field and its manifestation may occur during 0–60 days in germination phase, active tillering during 60–150 days, grand growth during 150–270 days and maturity phase from 270 days to till harvest, depending on the initial pathogen load, additional inoculum in the soil, inoculum carried through secondary sources (air, water, vectors etc.) and prevailing environment. Similarly, inoculum left in the stubbles of plant or ratoon crops serves as primary inoculum for the succeeding ratoon crops.

Since many stalk diseases like red rot and wilt cause death of entire stalks, such canes become unfit for sugar extraction and left in the field. Farmers bear the loss in cane yield due to death of canes (Fig. 9.1a, b, d, e). During the milling process, infected canes either partial or full crushed with healthy canes, spoil juice quality thereby reduces sugar yield. When canes are purchased based on tonnage as practiced in many Asian countries, the millers bear the loss in sugar yield. Practically, diseased canes are also taken together with healthy canes to the mills. Wherever sorting system followed in cane yards of the mills, all the dead canes due to red rot, wilt or sometimes pineapple disease are removed and only healthy canes are taken for milling. The first author recorded heaps of rejected wilt affected canes in the yards and this scenario portrays supply of healthy canes to milling and also prevalence of severe wilt in the east costal region in India (Viswanathan 2012a, b, 2020). Such sorting processes are now discontinued in many mills due to labour scarcity and industries suffer due to poor juice quality in the milling process, especially in deltaic regions and areas prone for waterlogging. Scale insect infestation is a serious issue in sugarcane, because the insects cannot be removed from the canes (Fig. 9.3). Crushing of the insect affected canes affect the juice quality due to direct loss in sugar by the insect feeding and chemical compounds from crushed insects also impair juice quality. Apart from red rot and wilt, smut causes severe yield losses in terms of number canes produced in a stool or area. Foliar diseases affect cane growth and yield and ultimately sugar yield, depending on the severity of infection during tillering and grand growth

phase. Infection of these diseases during maturity phase does not affect cane yield significantly since the crop does not grow much during this phase or flowering takes place. However, severe infection during this phase affects sucrose accumulation in the canes.

Overall, severity of the stalk diseases and varietal degeneration caused by non-fungal diseases is more in ratoon crops. Poor ratoon productivity in canes in many countries is attributed to degeneration of canes and also due to increased availability of fungal inoculum to cause severe diseases in cases of red rot, wilt and smut. Crop losses to a tune of 100% are recorded on several occasions in India after severe outbreaks of these three major fungal diseases, mostly in ratoons (Viswanathan 2018, 2020, 2021a). Severe disease outbreaks or varietal degeneration restricts number of ratoon crops and this affects profitability to farmers and sugar mills.

9.2.7.2 Factors Affecting Insect Pest Infestation

Climate, cultivars, cultivation practices and disturbance in the ecosystem determines a pest status in any given location or in a crop. Climate, cultivar and crop type majorly impacted the infestation and damage of sugarcane stalk borers in different locations (White et al. 2001; Mengistu and Selvaraj 2013). For example, population and thus the intensity of *D. saccharalis* infestation is affected by the adopted cultivars, varying temperature and moisture conditions throughout the year and population of natural enemies among others. Multiple overlapping generations throughout the year and the occluded larval and pupal stages of the pest inside the plant make it difficult to estimate populations and control the pest. The response of a variety to borers varied according to the different climatic conditions. Humidity was the most important factors limiting the borer infestations. The unirrigated fields were found to be more infested than irrigated ones. Plant canes tend to be more susceptible than ratoons (Williams 1983). Rajabalee et al. (1990) showed a positive correlation between the percent internodes damage and sugar loss.

When new areas are brought under cultivation of existing crop or in case of introduction of new crop into a location, the pest status gets altered. In Brazil, extended area under sugarcane and application of vinasse had been suspected to boost the population buildup of the root pest *Sphenophorus levis* that often kills the host plants (Martins et al. 2020). Geographic distribution of the insect pests on sugarcane is extremely narrow except for a few that are cosmopolitan (Mengistu and Selvaraj 2013). In Indonesia, species predomination among different borer species is impacted by several ecological components such as climate, cropping system varieties and edaphic factors resulting in changes in the individual pest behavior biology and population dynamics in borer composition and distribution (Wijanaythi et al. 2021). Soil moisture and soil clumping might interfere with underground movement of borer and thus suppress the population. Changes in species predominance have been observed due to the change in weather patterns and varieties. In China, increasing temperature during winter and introduction of newer varieties has altered the species

dominance in the borer complex. Reversal in the status, damage potential and distribution of the important borers *Tetramoera schistaceana* and *Chilo sacchariphagus* (Leul and Thangavel 2013; Li et al. 2013a) was observed.

Biochemical composition of the plant, i.e., the internal plant environment of the cane influences the build-up of the pest. Higher infestation of root knot nematodes in sugarcane, *Meloidogyne* spp. was linked to lower concentrations of free amino acids in plants (Heppner et al. 2008). Sugarcane plants stressed by drought had higher levels of dry leaf tissue and elevated concentrations of amino acids in the cane stalks, such plants were preferred for oviposition by *E. loftini* (Showler and Castro 2010). In Bihar, India, *C. auricilus* became a major pest due to the excessive usage of nitrogen fertilizers, extension of area under soft and high sugar varieties (Kumar et al. 1987).

9.2.8 Conventional Methods of Control

9.2.8.1 Diseases Management

Disease-Free Planting Materials

Multiplication of sugarcane through setts favors carry over of most of the pathogens except foliar pathogens through seed canes (setts) and hence planting of disease-free seed canes is emphasized to prevent disease introduction in the field. Disease-free canes are raised in designated nursery plots. In case of non-fungal diseases, the disease can be managed effectively only through healthy seed nursery programme. Nowadays, tissue (meristem) culture is recommended to produce viral and phytoplasma-free planting materials; however, molecular assays for the designated pathogens are done to ensure a total freedom of the pathogens in the seedlings. After YLD assumed a major menace to sugarcane production in India, sugarcane varieties were multiplied in large-scale through meristem culture. To support this venture, molecular diagnosis was made compulsory to produce ScYLV-free plantlets (Viswanathan 2012a, b). Additionally, the plantlets were also indexed for SCMV, SCSMV and SCGS phytoplasma to address varietal degeneration in India (Viswanathan 2016, 2021c; Viswanathan et al. 2018c). Efficacy of meristem culture in virus elimination has been validated in RT-qPCR assays by comparing virus titre in virus-free seedlings and asymptomatic plants in the field (Chinnaraja et al. 2014).

Elimination of SCGS phytoplasma through tissue (meristem) culture combined with PCR assays in healthy seed programme has been found as a major approach to control the disease under field conditions. Further, multiplication of disease-free plants through single bud settling nurseries ensures very rapid multiplication of healthy seed for the popular varieties in India (Viswanathan 2016, 2018; Viswanathan et al. 2018c). Although, heat treatment of seed canes either by aerated steam or moist hot air practiced in India, it is partially effective in inactivating the phytoplasma (Viswanathan 2016, 2018). By tissue (meristem tip) culture SCWL-plants are produced extensively in Thailand (Wongkaew and Fletcher 2004). Change of

cane planting time from autumn to spring season reduces the SCWL disease incidence due to reduced population of *M. hiroglyphicus* during low temperature during winter.

Cultural

Combining agronomical measures along with the use of disease free seed canes have completely controlled WLD in Taiwan (Leu and Kusalwong 2000). However, in tropical country like Thailand, sanitation, planting WLD-free planting materials and use of green manure plants in crop rotation are the most appropriate strategies to contain the disease (Wongkaew 2012). Since the vector insects movement is low in the field, the insecticides can be effectively used against them. Hence an integrated WLD-free seed cane production methodology is recommended in Thailand involving tissue culture, multiplying seed canes in large, isolated areas, and applying insecticides to control vectors (Hanboonsong et al. 2021).

Fiji leaf gall was effectively managed through an integrated approach of involving virus-free or certified seed, resistant cultivars and effective quarantine in Australia. In addition, to manage Fiji leaf gall, sugarcane varieties with certain level resistance is desired since during the favourable conditions for plant hopper vectors, susceptible varieties succumb to the disease and severe outbreaks occur. Further, to reduce inoculum availability in the field, virus-free seed is necessary to manage this disease (Smith 2000).

Disease Resistance

Disease resistance in the varieties is an important component in red rot management strategy in sugarcane and the new varieties with high sugar and cane yield are recommended for cultivation in India only if they possess red rot resistance. By this strategy, the disease epidemics were overcome inspite of the ravages caused by each of the red rot epidemics occurred during the past 120 years (Viswanathan 2021b). Much progress has been achieved in identifying resistant parents, inheritance of disease resistance and resistant clones in the germplasm, parents and inter-specific and -generic hybrids (Ram et al. 2005; Babu et al. 2010; Nair et al. 2017; Viswanathan et al. 2017c, 2018d, 2021b). Further, new screening methods, rapid screening of large number of clones under controlled conditions, a method to assess field tolerance and a new method to assess nodal resistance to *C. falcatum* by cotton swab inoculation were developed and being used (Mohanraj et al. 2012; Viswanathan et al. 2018a). Comparison of red rot ratings in the controlled condition testing method with the standard plug method revealed that this rapid screening method possesses adequate precision and matching disease reactions with plug method (Viswanathan et al. 2021c). Recent studies conducted at ICAR-SBI demonstrated *C. falcatum* soil borne inoculum as a source to induce disease development in sugarcane varieties and established field tolerance potential of the varieties to different pathotypes (Viswanathan et al. 2020a).

C. falcatum gradually adapts to the newly released varieties and comes out with new variants capable of breaking host resistance; hence the pathotypes are characterized regularly and resistance to the new pathotypes in the varieties, parental clones and germplasm is updated regularly (Malathi et al. 2006; Viswanathan and Selvakumar 2020; Viswanathan et al. 2022a).

Before 1998, ~70% of the varieties under cultivation in Australia were susceptible to smut (Sundar et al. 2012) hence a systematic breeding for sugarcane smut resistance has been initiated. This has brought a significant increase from 0.4 to 52% in smut-resistant crosses in Australian breeding programs from 2000 to 2007 (Croft et al. 2008a, b) and by the end of 2011, smut-resistant clones nearly doubled (Bhuiyan et al. 2013a, b). Similarly, many elite smut-resistant sugarcane cultivars were developed in different countries through their breeding programs.

As like red rot and smut, managing wilt through host resistance in sugarcane is a viable strategy to reduce crop losses in epidemic regions. Hence the pre-release varieties are artificially inoculated on the standing canes of the varieties following the standard plug method to assess wilt resistance in India. Alternatively, the pathogen is applied to the root zone to allow the pathogen to infect the canes from root and stalk. Recently a 0–9 scale was developed to rate sugarcane varieties for wilt resistance from R to HS (Viswanathan et al. 2022c). Further resistance to wilt and PB in sugarcane was identified among the parental clones numbering 700 maintained at National Hybridization Garden (NHG), the Indian national facility being used by ~24 sugarcane research centres to develop new sugarcane varieties. Sudden outbreak of these Fusarium diseases benefitted to identify sources of resistance among the parents that are frequently used in sugarcane breeding (Viswanathan et al. 2014a, 2019b). Overall it was found that parental clones of subtropical states exhibited relatively more resistance to these diseases as compared to tropical clones.

Although, YLD can be managed by healthy seed nursery programme, it is costly and seed replacements need recurring cost under field conditions. Hence host resistance to ScYLV has been studied in detail and resistant sources in the germplasm, varieties and parental clones in different countries. In Florida, *S. spontaneum* was identified as the most resistant group for ScYLV with 7% incidence whereas, *S. officinarum* was the most susceptible group with 76% in world collections of sugarcane and related grasses (Comstock et al. 2001). ScYLV infection showed a wide variation in the range of 0–100% in Colombia. In a cross of resistant male parent and a susceptible female parent resulted in progenies of YLD resistance (Victoria et al. 2005). Artificial virus inoculation procedures using aphids were developed in Louisiana, Hawaii, Brazil and other countries to develop YLD resistance in sugarcane varieties (Viswanathan 2021c). Studies of Fartek et al. (2014) found a positive phenotypic and genetic correlation among *Melanaphis sacchari* resistance in sugarcane varieties and disease incidence. They found a two-fold lower mean virus incidence in 22 resistant varieties than the 159 susceptible varieties. Previously, Zhu et al. (2010a, b) revealed that YLD-tolerant cultivars have limited ScYLV population than the disease susceptible ones. Recent studies of Burbano et al. (2021) in Brazil found a greater broad-sense heritability of 68% in qRT-PCR assays for ScYLV whereas it was 52.62% in

YLD-phenotypic expression to identify possible immune clones to the virus. ICAR-SBI, Coimbatore, India conserves the largest collections of sugarcane germplasm of nearly 4066 different *Saccharum* spp and hybrids at Kannur, Kerala, India. Detailed surveys were conducted for YLD incidence and severity in the germplasm clones, using the new 0–5 resistant grading system, identified about 463 and 773 among the hybrid genotypes and *Saccharum* spp, respectively as resistant sources (Viswanathan et al. 2016a).

By developing effective mechanical inoculation assays for SCMV, screening for virus resistance in sugarcane clones was successfully done, under greenhouse conditions (Chaves-Bedoya et al. 2011; Gemechu et al. 2004; Pinto et al. 2013; Srisink et al. 1994). Recently, da Silva et al. (2015) reported a combination of phenotypic evaluation of mosaic and SCMV diagnosis by ELISA assays to efficiently select mosaic-resistance sources. This has helped to detect the virus in asymptomatic genotypes and to identify 22 genotypes as resistant to SCMV Rib-1 strain. In China, mosaic phenotyping in sugarcane germplasm was done and identified resistant sources to mosaic from intergeneric hybrids of *S. officinarum* and *E. rockii* and *Erianthus arundinaceus* and *S. spontaneum* clones to SCSMV, SCMV and SrMV using severity grades of mosaic and RT-PCR (Li et al. 2013b, 2018, 2019). At ICAR-SBI, Coimbatore a 0–6 scale was developed to screen the *Saccharum* spp germplasm for mosaic resistance. This study clearly indicated that mosaic is widely prevalent in the germplasm maintained at Agali and about 97% of the genotypes/varieties have infections of SCMV and SCSMV, either alone or together. Indexing through RT-PCR assays revealed infections of SCSMV, SCMV and both in 77, 51 and 46 of the 88 *Saccharum* spp clones. Three *S. robustum* cones were identified as mosaic resistant from the study and probably these clones would be the donors for mosaic resistance in India. Further, the 0–6 scale adopted will be highly useful to phenotype mosaic resistance in other *Saccharum* spp clones and parental clones (Bagyalakshmi and Viswanathan 2021).

Work done at Australian researchers clearly demonstrated that Fiji leaf gall disease can be controlled though resistant varieties. The breeding strategy involves screening all the clones for resistance, avoiding crosses between susceptible parents and developing better methods of rating for resistance like breeding plant hoppers on virus-infected sugarcane in the glasshouse and subjecting new clones to a defined hopper numbers, rating clones based on percent of virus-infected plants and disease symptoms severity (Smith 2000).

Different methods and scales were developed for rust screening for resistance in various countries. Leaf whorl inoculation was optimized in Florida, where in uredospores @10^5 per ml were placed in whorls of spindles and rust development was observed after 30 days. Alternatively, wrapping of rust affected leaves with young shoots was found to be superior as compared to dusting or spraying uredospores. A 0–4 rust rating scale was adopted and graded them as resistant to susceptible; this criterion was followed to identify orange rust resistance in sugarcane clones under field conditions by adopting leaf whorl method of inoculation (Sood et al. 2009, 2013). In China, sugarcane germplasm was sprayed with uredospores suspension and screened for rust resistance using 0–9 scale (Wang et al. 2013). Under Indian conditions, leaf whorl inoculation method was found ideal to artificially induce severe

rust on susceptible clones and the method was recommended to screen sugarcane varieties for brown rust resistance (Viswanathan, unpublished). At Coimbatore in tropical India, 275 parental clones of sugarcane were screened under field conditions for rust resistance and among them ~60% of clones were rust-free and 20% were moderately resistant. In the remaining 20% susceptible group, about 13 and 7% behaved as moderately susceptible and susceptible, respectively (Selvakumar et al. 2018).

RSD resistance is assessed based on the pathogen population densities in sugarcane (Roach 1992; Davis et al. 1994; Miller et al. 1995). Limited colonization by the pathogen is found associated with LSD resistance in sugarcane varieties and wild relatives of *Saccharum* spp (Rott et al. 1997). Host resistance is the major strategy followed to manage all the leaf spot diseases, red stripe and pokkah boeng in different countries. At times, resistance breeding is focused on red rot, wilt, smut, mosaic or other diseases specific to their region hence foliar diseases are not given due weightage. In many countries susceptible varieties are rejected during varietal selection process; only disease resistant types are advanced and recommended for cultivation. Although this method does not give true resistant types, it is followed in places where resources and man power are limited.

Biocontrol Approaches

In sugarcane, effectiveness of native plant growth promoting rhizobacteria (PGPR) and fungal antagonist *Trichoderma* against *C. falcatum* was established under laboratory conditions and field situations (Viswanathan and Samiyappan 2002a, 2008; Malathi et al. 2008; Singh et al. 2008; Hassan et al. 2010, 2012; Joshi et al. 2019; Viswanathan and Malathi 2019). PGPR mediated mechanism of induced systemic resistance was governed by specific induction of oxidative enzymes and PR-proteins. Further, antagonistic activities and biocontrol potential of endophytic PGPR strains were established against *C. falcatum* (Viswanathan and Samiyappan 2002b; Viswanathan et al. 2003b, c; Jayakumar et al. 2021).

Chemical Control

As mentioned earlier, pathogenic inoculum from sett- and soil serve as primary sources for spread of different diseases in sugarcane, hence sett treatment with fungicides or bioagents will reduce fungal disease development from seed canes and soil. Practically no diagnosable disease symptoms exist on the setts, hence sett treatment with fungicides is recommended in disease prone areas or susceptible varieties are grown. Thiophanate methyl, a systemic fungicide was found effective against *C. falcatum* (Malathi et al. 2004). The same fungicide was found to be compatible with *Pseudomonas fluorescens* strains and the combination exhibited more efficacy against the pathogen inoculum survives in the soil (Malathi et al. 2002). Conventional immersion of setts in fungicide solution for a short duration is ineffective

against the deep-seated fungal propagule; to overcome this concern, mechanized fungicide treatment of setts with fungicide was developed and improved efficacy of fungicides and disease control was demonstrated under field conditions against smut and red rot diseases (Viswanathan et al. 2016b; Malathi et al. 2017). Recently Shailbala (2016) reported efficacy of the fungicide combination Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC against red rot, smut and rust diseases in sugarcane.

Currently both the sugarcane rusts are common in Florida. The farmers prefer to grow high yielding cultivars though they are brown rust susceptible by adopting fungicide sprays. A high yielding cv CP96-1252, but moderately susceptible to brown rust is preferred by the growers in Florida hence it occupied 29% of the sugarcane area during 2016 (Raid et al. 2018; Rott 2018; Van Weelden et al. 2017). Recently Chaulagain et al. (2019a) reported 40–42% less rust severity in the brown rust affected field after spraying fluxapyroxad + pyraclostrobin on the popular cv CP 96–1252 during three seasons and obtained an increase of 27–35% in mean stalk weight. For effective control of brown rust, they suggested a minimum of two fungicide sprays. They also found beneficial effects of fungicide sprays in orange rust susceptible varieties in Florida and recommended fungicide sprays with different modes of action such as two successive sprays of fluxapyroxad + pyraclostrobin and later one spray of pyraclostrobin, fluxapyroxad, metconazole or fluxapyroxad + pyraclostrobin during three succeeding months, to prevent emergence of fungicide resistant pathotypes (Chaulagain et al. 2019b).

Spraying of 60 g streptomycin + tetracycline in 500 l water per ha in the two month crops at 15-days intervals has been found effective to manage LSD severity in Tamil Nadu, India. Further, spraying of this antibiotic combination immediately after noticing the disease symptoms reduced LSD severity significantly (Viswanathan 2012a).

Heat Treatment

Across the countries, hot-water treatments are used to inactivate the pathogens in seed cane. Immersing setts in running water (ambient-temperature) for 40 h and then by hot water treatment for 3–4 h at 50 °C before planting is used to inactivate LSD bacterium. This treatment is reported to provide control efficacy of 95% (Rott and Davis 2000a). Treating RSD affected seed canes with hot water at 50 ± 0.5 °C improved bud germination, crop growth, higher cane yield in the range of 9.5–54.7% and sugar content in the range of 0.68–1.7% (Wei et al. 2019). Moist hot air treatment or aerated stream therapy is followed in different sugar mills to inactivate pathogens causing RSD and grassy shoot with partial success. There were claims on their efficacy against sett borne fungal inocula of red rot or smut, however only incomplete success was achieved. To inactivate grassy shoot phytoplasmas in the setts, information on varied thermal tolerance in the varieties and varying regimes of temperature and timing are required (Viswanathan 2001b).

9.2.8.2 Insect Pest Management

Host Plant Resistance—Borer Pests

Unlike other crops, efforts of resistance breeding in sugarcane are rather lagging probably because of its genetic complexity and polygenic inheritance (White et al. 2010). Mostly, field observations on damage help to identify the resistant varieties among the already popular varieties in a locale and conventional breeding for insect resistance is not in vogue in sugarcane. Between two major varieties cultivated in Reunion, R570 was more resistant than R579 (Nibouche and Tibére 2010). Differences in the varietal response to *D. saccharalis* were observed by White et al. (2008) in Louisiana.

Though voluminous work has been published on screening or evaluation of resistant varieties to borers of sugarcane globally, quantification of the benefits accrued due to prevention of pest or increase in yield has not been accomplished. For example, with regard to *C. sacchariphagus* in sugarcane, no information is available on the resistance status or the gain accrued by improved varieties in Reunion (Nibouche and Tibere 2009). Further, a constantly changing varietal scenario undermines the efforts of identifying resistant varieties, as the popular varieties gain area under cultivation only based on their performance with reference to major diseases, cane yield and quality.

Morphological characteristics are primary indicators of borer resistance in sugarcane. Several traits in sugarcane correlated to borer resistance were leaf width, color of the stalk, fiber composition, waxy rind, self-de-trashing leaves, elongated spindles, slim stalks, thin erect leaves, elevated fiber composition, content, wax, increased crop vigor, juice percentage, and attraction to ovipositing females (Reagan et al. 2008). However, through the years, the rind hardness and fiber content of the cane have been suggested, tested and found to be correlated with borer resistance (Reagan and Martin 1982; Bessinet al. 1990). Despite their importance in breeding for resistance against *D. saccharalis*, constant search for varieties with lower borer infestation would also lead to accretion of unfavorable characters like increased fiber content and lower sucrose thus reducing sugar recovery (White et al. 2006). Borer resistant varieties are currently cultivated on more than 60% of sugarcane area in Louisiana (Wilson 2021).

In an effort to identify sources of multiple pest resistance among the genotypes from *S. barberi, S. robustum, S. spontaneum* and *Erianthus* sp., a few genotypes had exhibited resistance to two to five major sugarcane pests (Mukunthan 1994) and it could be seen that *S. robustum* and *Erianthus* had more genotypes with multiple pest resistance compared to *S. barberi* while *S. sinense* and *S. spontaneum* did not possess any such genotype. Cane length as well as the length and girth of the vulnerable portion had a positive correlation with *C. sacchariphagus indicus* infestation on 20 genotypes tested. The sugar content, phenol, cellulose and tannin played a role in INB resistance in sugarcane (Asha et al. 2019). Antibiosis resulting in lower weight gain and prolonged larval duration was exhibited by the *D. saccharalis* resistant variety, HO 08-9003 (White et al. 2011; Salgado et al. 2021). Poor neonate establishment

due to rind hardness conferred resistance to *D. saccharalis* and nine fold differences of such response were observed among the cultivars (Salgado et al. 2021). Tomaz et al. (2018) Identified sugarcane varieties with resistance to leaf feeding as well as stalk entry and tunnelling, for use in breeding programs to achieve increased level of borer resistance.

In Louisiana, decades of sustained efforts have been made since 2001 to breed resistant cultivars for management of the stalk borers, *E. loftini* and *D. saccharalis* (Wilson 2011; Wilson et al. 2015; Reay-Jones et al. 2003; Reagan et al. 2008) through continuous rigorous screening and monitoring protocols and programs (Reagan and Martin 1982; Schexnayder et al. 2001; Posey et al. 2006; Reagan et al. 2008). Three resistant varieties, L 99-226, HOCP 04-838 and L 01-299 are currently recommended for resistance to stem borers in Louisiana. Similarly, five among the recommended commercial varieties have been declared to be susceptible to *D. saccharalis*. Among the currently popular sugarcane cultivars 5–10 fold differing levels of borer resistance has been observed (Wilson et al. 2015, 2021).

Among the South African sugarcane varieties, N21 is highly tolerant to drought (Kvedaraset al. 2009). The survival and developmental rates of insects increases during drought due to increased plant nutrient levels, lowered plant defense, and availability of improved temperature niches (Atkinson and Nuss 1989). Hence Reagan and Mulcahy (2019) suggested that drought tolerant varieties can be successfully used in managing the borer populations during the adverse climatic conditions. Borer resistance in sugarcane is polygenic, governing any resistant trait and several genotypes showing antixenosis and antibiosis against borers have been identified in Brazil (Pimentel et al. 2017). Complex genetic control of sugarcane borer resistance with high genetic variation among Brazilian sugarcane genotypes was demonstrated by Tomaz et al. (2018, 2019). Notable work on single clone and family selection for breeding resistance to *E. saccharina* had been done by several workers (Farrag et al. 2018; Zhou 2015, 2016; Zhou and Mokwele 2016).

Cultural Control

Routine or specific field operations, planting time, irrigation/fertilizer schedule, spacing, harvesting are the common aspects of crop husbandry which can incidentally or intentionally favor or suppress build-up of pests is understood as cultural control. This is an eco-friendly non-chemical method but can be expensive, time consuming and execution may be dependent on several factors in crop cultivation. Increased incidence of stalk borers in ratoon crops is a consequence of elimination of the natural enemies (Macedo and Araujo 2000), due to the stalk burning of the previous crop as compared to non-burned sugarcane (Pholan et al. 2005).

In Brazil, movement and utilization of infested sugarcane seedlings has been the major factor for incursion and increased infestation of billbug *S. levis*. Also, application of vinasse (a byproduct during the production of ethanol from molasses) through fertigation increases the pest population, as the volatiles from vanasse are

attractive to the beetles. The increased soil moisture levels due to vinasse applications enhance the survival of the billbug populations (Martins et al. 2020).

In South Africa, Keeping et al. (2014) observed increased survival of *E. saccharina* due to higher rates of nitrogen application while silica application reduced the infestation and this protection was greater on susceptible varieties. A loss of 30% sugar yield due to *E. saccharina* in susceptible varieties could be prevented through foliar application of silicon (Keeping and Meyer 2002). Further, susceptible varieties grown in soils under water-stress but rich in silicone reinforced the barrier effect of the stalk to the borer (Pene et al. 2018). Flooding has been suggested for the management of white grub such as *Tomarus subtropicus* (Buss 2003) but this method is inoperable in areas of poor water supply where the infestations with white grub are intense. De-trashing combined with spraying of imidacloprid reduced *A. barodensis* resulting in higher yield and sucrose (Rao et al. 2011). Repeated ploughing during May–June exposes the hibernating population of white grubs to natural enemies like birds, pigs and dogs for predation.

Chemical Control

The insecticides applications for borer management have not been adopted as they had not been economically viable (Showler and Reagan 2017) and hugely impractical due to the large canopy. It is further difficult to manage the internal feeders, which were inaccessible for the sprays. As the larvae of *E. saccharina* bored inside the sugarcane within 24 h after hatching, insecticidal sprays were rendered useless (Heathcote 1984). Similar is the case with many other borers such as *E. loftini* in South Africa, *C. sacchariphagus* in many Asian countries and *C. sacchariphagus indicus* in India.

Selection of chemicals and application strategies determine the efficacy against borers. In Louisiana, if systemic chemicals were applied as high volume spray, early in the season, sugarcane could be protected from *D. saccharalis* injury, as the concentrations remained effective even after eight weeks post application (Wilson et al. 2021). Synthetic neonicotinoids, imidacloprid and thiamethoxam are highly effective against the sugarcane whitefly species specifically *Aleurolobus barodensis* (Bhavani and Rao 2013; Chaudhary and Jaipal 2006; Vijayaraghavan and Regupathy 2006). Among the newer chemicals, thiamethoxam (Vijayaraghavan and Regupathy 2006) and dinotefuran (Koohzad-Mohammadi et al. 2017) brought down the populations of *A. barodensi* and *N. andropogonis*, respectively.

The most effective insecticide combination may be detrimental to conservation of biocontrol agents. A combination of thiacloprid with deltamethrin was effective against *N. andropogonii* but harmful to the two parasitoids. Hence use of pyriproxyfen (a juvenile hormone) was the safest insecticide with >50% parasitoids emergence could be more prudent (Behnam-Oskuyee et al. 2020). Integrated pest management (IPM) program had effectively used for whitefly management. In this regard, IPM involving stripping the affected leaves, spraying imidacloprid, azadirachtin, 2% urea reduced *A. barodensis* population resulting in higher yield of sugar and cane

(Bhavani and Rao 2013). Similarly, a combination of de-trashing, release of *Chrysoperla carnea*, yellow sticky traps, pesticide application reduced the population of *A. barodensis* to 0.48 per cm^2 of leaf (Bhatti et al. 2019).

Biocontrol

Since the scope of chemical control is restricted due to the dense crop canopy specifically during the grand growth phase, natural control of pests thrives in sugarcane ecosystem. However, the ecological differences among ecosystems may influence the success of biocontrol agents. Sugarcane cultivated yearlong or as a semiperennial offers a relatively stable habitat (Kfir et al. 2002). For establishment and success of natural enemies, habitat stability is crucial (Hall and Ehler 1979; Cameron et al. 1993). However, climate change can restrict the adaptability and success or may often result in the undesirable impact on non-target insects (Lu et al. 2015). Temperature essentially impacts both establishment and efficacy of a natural enemy (Lu et al. 2013). Biopesticides are eco-friendly. Since they aid in the the reduced application of toxic chemical insecticides, safer food and cleaner environment can be ascertained (Hall and Menn 1999).

The most prominent parasitoids that had been frequently used in classical biological control programs in sugarcane are *Cotesia flavipes* and *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae). Their successful establishment has been proven on several occasions. In Asia and Indian Ocean islands, *C. flavipes* has a broad host range occurring on $4/5^{th}$ of recorded species of the borers (Sallam 2006). Conlong and Goebel (2002) found that the introduced parasitoid *X. stemmator* remarkably brought down the INB infestation at all the released sites in Mozambique. Soil application of the fungus *Metarhizium anisopliae* at the time of earthing up would significantly effective in reducing white grub population and recorded higher yield than untreated control (Purwar 2013; Lamani et al. 2017; Thirumurugan et al. 2020).

9.3 Genetic Resources of Resistance Genes

9.3.1 Available Germplasm

The genus *Saccharum* consists of *S. officinarum*, *S. sinense, S. barberi*, *S. edule* (cultivated species) *S. spontaneum* and *S. robustum* (wild species) (D'Hont et al. 1998; Irvine 1999). Two duplicated world collections of sugarcane germplasm 'World collections of sugarcane germplasm' are preserved by ICAR-SBI, Coimbatore, Tamil Nadu at Kannur in Kerala, India and the 'National Germplasm Repository' in USA at Subtropical Horticulture Research Station, USDA-ARS, Miami, Florida. Constituents of Miami collections predominantly include *S. officinarum*, *S. spontaneum* and sugarcane hybrids. It also has other *Saccharum* spp. and species of

other genera such as *Imperata* spp., *Coix gigantea*, *Miscanthus floridulus*, *M. sinensis*, *Miscanthus* spp., *Miscanthus* hybrids, *Narenga porphyrocoma*, *Sorghum plumosum*, and *S. arundinaceum*. There were 1002 accessions maintained at Miami mostly the survivors of Andrew Hurricane occurred in 1992 and other fresh accessions (Spurthi et al. 2014). ICAR-SBI, Coimbatore, India regularly undertakes the collection, characterization, conservation in the respective repositories and documentation of genetic resources including *Saccharum* spp and allied genera. Besides, the institute maintains the basic species of clones and related genera of *Sachharum* complex. Further, ICAR-SBI also maintains Indian hybrids, Indo-American clones, improved clones of *S. barberi* (Population improved barberi-PIB), *S. robustum* (Population improved robustum-PIR), *S. officinarum* (Population improved officinarum-PIR), *S. spontaneum* (Population improved spontaneum-PIS), interspecific hybrids (ISH) and intergeneric hybrids (IGH). About 2680 'Co' selections (hybrids) developed by ICAR-SBI, since its inception in 1912 are maintained at Coimbatore, India and these are part of the longest sugarcane improvement activities across the globe (Amalraj and Balasundaram 2009) and that is active now also. The germplasm availability of predominantly used species clones of *Saccharum* complex and related genera at major breeding centres of the world are presented below.

S.No.	Species	India	USA	Brazil[a]	China#	Thailand[b]
1.	*S. officinarum*	764	238	103	32	4785[c]
2.	*S. barberi*	43	38	29	3	–
3.	*S. sinense*	29	22	17	25	–
4.	*S. edule*	16	–	3	–	–
5.	*S. robustum*	145	45	39	6	–
6.	*S. spontaneum*	978	319	211	690	991
7.	*Erianthus* spp.	202	–	40	404	957

[a]Sum of the collections maintained at Serra do Oura, Biovertis, Gran Bio and Devaneio (*Source* Cursi et al. 2022)
[b]Sum of collection maintained at Department of Agriculture (DOA), Khon Kaen University (KKU), Kasetsart University (KU), Office of the Cane and Sugar Board (OCSB) and MitrPhol (MitrPhol Innocation and Research Centre (MitrPhol) (*Source* Khumla et al. 2022)
[c]including *Saccharum* spp. hybrids
#*Source* Zhang and Govindaraju (2018a, b)

Besides above breeding materials, the Thailand breeding population has accessions of *Miscanthus* and *Sclerostachya*; *Narenga*, *Imperata* and *Pennisetum* in China. Brazil has *Miscanthus*, unknown species and hybrids in their germplasm collections. These genetic materials have substantial diversity for various traits and many useful genes for many phenotypic characters, cane and sugar yield, biomass and resistance to different stresses etc and serve as excellent breeding materials for sugarcane advancement.

9.3.2 Primary Gene Pool

This sugarcane gene pool comprises five different species of *Saccharum* and their hybrids, which hybridize easily among them. Major part of the species germplasm had been screened for red rot resistance. Very few clones of *Saccharum* spp. showed resistance to red rot (Alexander 1987; Malathi and Viswanathan 2012b), whereas, the number of red rot resistant clones in *S. barberi* was relatively more as compared to other cultivated species (Alexander and Rao 1976). Among the *S. officinarum* germplasm, seven clones were reported to be resistant to red rot and 15 clones were moderately resistant (Sreenivasan and Nair 1991). Baragaua, Seleri and Saipan G showed resistant consistently. About 237 clones were found to resistance or moderately resistance to smut. Alexander (1987) reported ~95 *S. officinarum* clones from the world germplasm collections as resistant to smut. Naidu and Sreenivasan (1987) reported that *S. officinarum* (97 out of 428 clones) and *S. spontaneum* (137 out of 324 clones) had the highest resistant sources among five species clones of *Saccharum* whereas *S. sinense* (15), *S. barberi* (9) and *S. robustum* (3) showed a lower level of resistance against smut pathogen. Unlike resistance to red rot, more number of *S. officinarum*, many *S. spontaneum* and *S. robustum* clones showed resistance to smut (Srinivasan and Alexander 1971; Alexander et al. 1983).

Viswanathan et al. (2016a) reported that in case of *Saccharum* spp, 86% of *S. robustum* were resistant to YLD, followed by *S. sinense* (80%), *S. officinarum* (78%), and *S. barberi* (76%) in the germplasm collections maintained at Kannur, India. Similarly, occurrence of YLD in the germplasm collections of sugarcane at Miami, USA was highest in *S. officinarum* (75.8%) followed by *S. robustum* (62.5%), *S. sinense* (46.2%), *S. barberi* (13.6%), and *S. spontaneum* (7.0%). A cross involving a YLD-susceptible noble cane and a resistant wild relative gave a higher percentage of clones, which were free from the virus for more than 10 seasons. Similarly, the wild parent (IND-81-146) remained free from virus infections during the period indicating that *S. spontaneum* is highly tolerant to ScYLV whereas, *S. officinarum*, is highly susceptible. In sugarcane, resistance to YLD was found as a dominant trait (Comstock et al. 2001).

Chu et al. (1982) presumed that modern commercial varieties possess rust susceptible genes transmitted from some *S. officinarum* clones. Later, inheritance of rust resistance was studied with self-progeny of the sugarcane cv R570. Reaction of the progenies to rust was assessed under controlled greenhouse and field trials and rust phenotyping revealed a definite 3:1 segregation for resistant and susceptible reactions in the progenies. This study indicated that the brown rust resistance is probably governed by a dominant resistant gene and identified 'Bru1' gene in the cv R570. The Bru1 gene was found to check infections caused by different brown rust isolates from various geographic locations (Asnaghi et al. 2001; Daugrois et al. 1996). Later, the second major brown rust resistant gene 'Bru2' was identified and it prevented rust fungal sporulation (Raboin et al. 2006; Costet et al. 2012). Heritability for rust resistance was reported to be intermediate (Tai et al. 1981; Gonzales et al. 1987). Further, high narrow-sense and broad-sense heritability values of 0.84 and 0.73 respectively,

for rust resistance were reported (Comstock et al. 1992). In another study, Hogarth et al. (1993) reported 0.84 and 0.78 heritability values for rust. Costet et al. (2012) analysed 380 recent varieties and breeding clones from different breeding centers of more than 30 across the globe with 22 molecular markers. They identified 17 genotypes viz., B 41227, Co 214, MEX 73 523, MQ 76 53, N 53 216, NCo 334, R 84 693, Q 127, Q 136, R 570, R 572, R 573, R 575, R 577, H 72-8597, R 579, R 83 1592 genetically linked to Bru1 as the stable resistant source for rust.

9.3.3 Secondary Gene Pool

S. spontaneum and *S. robustum,* the wild species of sugarcane constitute the secondary gene pool of sugarcane. It is very interesting to note that sugarcane improvement started with utilisation of secondary gene pool sugarcane rather than primary gene pool and the source for red rot resistance is mainly contributed by *S. spontaneum* (Srinivasan and Alexander 1971; Natarajan et al. 2001). A large number of clones with red rot resistance are available in *S. spontaneum* germplasm collection maintained at ICAR-SBI (Alexander et al. 1983). Of the 170 *S. spontaenum* clones, 69 were resistant and 59 moderately resistant to red rot (Kandasami et al. 1983) and 91 of the *S. spontaneum* clones were resistant to smut. Five *S. spontaneum* clones were found to be resistant to rust and 91 clones resistant to ratoon stunting disease. Among the 30 Japanese wild sugarcane (*S. spontaneum*) accessions and five cultivars, JW 90, Iriomote 8, Iriomote 15, Iriomote 28, and T16 were found resistant and the cultivar Ni F8 was moderately resistant to the only one race of smut pathogen prevalent in Japan (Sakaigaichia et al. 2018). Interspecific hybrids (ISH) developed from the tall, thick and broad leaved *S. spontaneum* from Arunachal Pradesh produced progenies, of which 35% were resistant to red rot and only 7% were highly susceptible (Mohanraj and Nair 2012). Inter-specific crosses involving PIO, PIS and PIR clones and commercial varieties exhibited higher scope for developing red rot resistant progenies and gave about 35% red rot resistant progenies with heterotic vigour for other economic traits. Three F_1 progenies of improved *S. officinarum* x *S. spontaneum* cross combination viz., 96-38, 96-195, 95-108 (Alarmelu et al. 2010) produced more resistant progenies.

9.3.4 Tertiary Gene Pool

Genetic diversity tertiary gene pool of sugarcane which include the allied genera such *Eriatnus, Miscanthus*, *Narenga*, *Sclerostachya*, *Imperata* and *Pennisetum* are a treasure house of much value for the sugarcane breeding programme in future. Among them, *Erianthus* offers greater scope by being an important source for higher biomass production, pest and diseases resistance and tolerance to abiotic stresses. However, success of *Saccharum* x *Erianthus* hybridisation is low because of high rate of selfs

and resemblance of hybrids with *Saccharum* parent. After developing *Erianthus* specific markers, intergeneric hybrids (IGH) involving *S. officinarum*, *S. robustum*, *S. spontaneum* and commercials with *Erianthus* were developed. These IGHs showed superiority over the ISHs involving *S. spontaneum* for sucrose content (Mohanraj and Nair 2010). Sorghum x *S. officinarum* hybrids have been developed that are unique in having sorghum cytoplasm (Nair 1999). Amongst the six taxonomic groups of *Saccharum* spp. comprising five different species of *Saccharum* and *Erianthus* spp, the clones belonging to the *Erianthus* spp section *Ripidium* were found to be the most resistant clones whereas clones of *S. officinarum* and *S. robustum* are highly susceptible (HS) (Burner et al. 1993). A total of 79 backcross progenies (BC1 and BC2) of *E. arundinaceus* were assessed for smut resistance. In this study, seven BC1 and three BC2 derivatives of *E. arundinaceus* exhibited greater resistance against smut pathogen and these lines may serve as potential donors for smut resistance in sugarcane (Shen et al. 2014). Among the *Erianthus* germplasm, 10 clones were stated to be resistant to red rot (Sreenivasan et al. 2001). Mukunthan and Nirmala (2002) screened 285 accessions of *S. barberi*, *S. robustum*, *S. sinense*, *S. sponataneum* and *Erianthus* species for their response to white grubs and reported that 61 clones are tolerant. The majority of the tolerant clones were accessions of *Erianthus* collections. Pest reaction of 20 *Erianthus* clones had been reported with respect to 7 key pest of sugarcane (Sreenivasan et al. 2001).

9.4 Glimpses on Classical Genetics and Traditional Breeding

9.4.1 Classical Mapping Efforts

9.4.1.1 Morphological Characterization

S. officinarum (noble canes) is characterized by having showy colours with juicy stalks and broad leaves. These are known for their thick stalks and high sucrose content. Though single cane weight is high, its tillering ability is poor. Hence, noble canes were replaced by the improved inter-specific hybrid varieties involving *S. officinarum* and *S. spontaneum*; however, some noble canes are cultivated for festivals and for chewing purposes in many Asian countries. The clones of *S. barberi* are thin, hardy with narrow or medium leaves. In spite of having very high tillering ability, the yield is poor because of very low single cane weight. *S. sinense* known as Chinses canes have medium thick canes with good tillering ability and satisfactory level of yield and sucrose. *S. robustum,* a wild relative of sugarcane has medium thick canes and broad leaves. The other wild relative of sugarcane, *S. spontaneum* shows wide variation from grassy type to thin cane types (Ramana Rao et al. 1979). *S. barberi* clones were classified into four groups, viz., Mungo, Saretha, Sunnabile and Nargori

based on the morphology and *S. sinense* was placed under Pansahi group (Barber 1916, 1918).

9.4.1.2 Cytological Studies in *Saccharum* Complex

Jagathesan et al. (1970) observed the chromosome number in 585 *S. officinarum* clones from the World collections of sugarcane and identified typical $2n = 80$ and atypical $2n \neq 80$. Chromosome analyses of about 442 *S. spontaneum* clones from various locations in India established polyploid aneuploid nature of the species. The Indian accessions had $2n = 40$–80, 80–112 and 112–118 from central, eastern and western regions, respectively (Panje and Babu 1960). Among the number of cytotypes in *S. spontaneum* reported, $2n = 64$ is the most common cytotype and distributed in most parts of India (Nair and Praneetha 2006). Among the 30 *S. officinarum* and 20 *S. spontaneum* clones studied, *S. officinarum* clones had $2n = 80$ except NG 77-26 which had $2n = 70$ whereas in *S. spontaneum,* the number of chromosomes was in the range of $2n = 64$–72 (Sobhakumari 2009). Sobhakumari (2020) based on the chromosome analysis of 524 *S. spontaneum* accessions inferred that North-eastern region of India was found to have a higher evolutionary activity in *S. spontaneum* due to multiple cytotypes occurrence and sympatric growth with other species and genera. Crosses between Vellai an *S officinarum* with $2n = 80$ and Coimbatore local, an *S. spontaneum* with $2n = 64$ resulted in hybrids having $2n = 112$ having $2n + n$ chromosome transmission (Dutt and Rao 1933). This mechanism favoured the transmission of whole nuclear genome of noble canes to hybrids resulting in superiority of hybrids for most of economic traits. However when atypical *S. officinarum* clones were crossed with *S. spontaneum*, $n + n$ transmission was observed. Parthasarathy and Rao (1947) reported somatic chromosome number of five forms of *Sclerostachya fusca* collected from different locations as $2n = 30$. Chromosome number in *Erianthus munja*, *E. ravennae* and *E. arundinaceous* clones were determined (Rao and Raghavan 1951). *E. ravennae* had only one cytotype of $2n = 20$ while the other two species had $2n = 30$, 40, and 60 chromosomes. Later, a detailed survey on the chromosome number of the *S. spontaneum*, *S. officinarum* and *Erianthus* spp., *Narenga*, *Sclerostachya* and *Imperata* collected from North east India was made (Sreenivasan and Sreenivasan 1994). Modern sugarcane cultivars originate from hybrid derivatives obtained from the cross combinations involving noble canes with $x = 10$; $2n = 8x = 80$ (*S. officinarum*) and wild canes with $x = 8$; $2n = 5x\text{-}16x = 40$–128 (*S. spontaneum*). The progeny clones have 130–140 chromosomes, of which, *S officinarum* contribute 70–80% and *S. spontaneum* 10–20%. The residual 10% are of recombinants between these two *Saccharum* spp (D'Hont et al. 1996). Differential contribution of the male and female predecessor was revealed by a genomic study involving in situ- and fluorescent in situ-hybridization assays in the hybrid R 570 genome. Earlier, isozyme variation was used as potential biochemical markers in sugarcane genetics and breeding (Glaszmann et al. 1989). This work has given the way for the use of markers as an effective means of finding linkage groups in genome of sugarcane.

9.4.2 Breeding Objectives

Improving the sugarcane yield and sucrose content in the varieties are the most important breeding objectives. Under the worst scenario of climate change, where the minor diseases becoming major, the fast development of biotypes of insect pests and of pathotypes of pathogens and increased virulence of pathogens, development of pests and diseases resistant varieties become foremost important. Red rot was considered once as diseases of subtropical India and smut as diseases of tropical region. In subtropical breeding programme, red rot resistance was given major emphasis while smut resistance in the tropical India. Nowadays red rot prevails in most of the states in India and new varieties succumb to the disease before their potential realized (Viswanathan 2021a, b). Hence, without red rot resistance, no sugarcane variety gets released or notified for commercial cultivation in India. Ratoon stunting causing yield loss up-to 15–50% in South Africa (Bailey and Bechet 1986) and 29% in Fiji (Johnson and Tyagi 2010) is a major constraint in sugarcane across the nations. This made the sugarcane breeders to consider this disease during their selection process in these countries. Before 1998, majority of the sugarcane varieties in Australia were smut susceptible which caused yield loss upto 26%, hence, resistance breeding to smut has become one of the primary objectives in Australian sugarcane varietal development program (Sundar et al. 2012). The climate changes also necessitate developing varieties tolerant to water logging, drought, salinity, cold, frost and other climatic extremities. Winter ratooning ability is important breeding objective of developing varieties for sub-tropical region of India. Some of the other objectives in sugarcane breeding like short duration varieties, special varieties for jaggery production, high fibre, high biomass etc. are driven by the demand of sugar industries. However, recently sugarcane has also become a bio energy crop (Souza et al. 2014) for producing ethanol. Nowadays, technological advancement in other fields made possible to find more utility for the by-products of sugarcane viz., filter muds, molasses (for cane ethanol, other alcohols, acetic acid, citric acid, cattle feed and cooking fuel, baggase (fuel, fibreboard, paper, bioplastics, power generation, biogas, fertilizer) etc. (Moore et al. 2013). Accordingly, the breeding objectives in sugarcane improvement are dynamic according to the priorities and future requirement.

9.4.2.1 Positive and Negative Selection in Sugarcane

In sugarcane, most of yield and sucrose quality traits are selected in positive side. The objective of breeding varieties for the purpose of bio-ethanol production can be met by developing varieties with very high sucrose content. In case of fibre content, a negative selection is effected. However, this trait is selected positively when we breed for energy canes. In case of tropical regions of India like Karnataka and southern parts of Maharashtra, flowering is a negative character and hence this trait is selected negatively. Other trait where negative selection is practiced in sugarcane is crop maturity duration. Recently developed variety at ICAR-SBI, Coimbatore, Co 11015,

the high sucrose % at 8th month of crop maturity on par with that of ruling variety Co 86032 at 12th month was achieved (Durai et al. 2020). This variety was developed applying the negative selection for days to crop maturity.

9.4.3 Achievements in Classical Breeding

The idea of utilizing a wild species for improvement of cultivated crop was thought out and initiated in sugarcane breeding at Coimbatore during 1912 and now it is being practiced in most of the crop improvement programmes across the world. The first hybrid Co 205 developed from the cross between *S. officinarum* and its wild relative *S. spontaneum* recorded 50% more yield than the indigenous varieties in Punjab, India and well adopted to the climatic and soil conditions of the subtropical region because of the ancestry of *S. spontaneum*. There had been steady improvement in sucrose content in the varieties bred at Coimbatore from mean of 15.89% prior to 1960s to mean of 19.54% in the 2000s (Hemaprabha et al. 2012). During the last two decades, substantial improvement in cane yield was observed in the major sugarcane growing countries. A significant improvement in sugarcane yield of Colombia was achieved from 5 t of sugar/ha/year during the year 1950 to 12 t/ha/year in 2000 (Cock 2001). During the same period, sugarcane production in Brazil has increased from 64 to 70 t ha. In Florida, from 1968 to 2000, sucrose, cane yield and sugar yield of the commercial cultivars progressively increased by 26.0, 15.5 and 47.0%, respectively (Edmé et al. 2005). In China, during the past 60 years (1961–2013), a rapid increase in sugarcane production from 2.643 MT to 126.13 MT was observed. Tremendous improvement in cane productivity (24.0–67.4 t/ha) and the mean sucrose % (less than 13% to >14.5%), with some varieties now record an average over 16% sucrose was observed during October to April (Zhang and Govindaraju 2018a, b). Increase in sugarcane productivity of Australian sugarcane varieties attained at the end of 1999 (95 t/ha) could be credited chiefly to the genetic improvement of varieties (Ming et al. 2010). Apart from yield and quality improvement, all other traits for stress tolerance are expected from the sugarcane varieties. Red rot problem in India is majorly managed through deployment of resistance varieties. Sugarcane cultivation in problematic areas was possible because of development of varieties with tolerance mechanism in sugarcane for drought, salinity, water logging etc. Among the diseases that affect the sugarcane production in Louisiana, smut, brown rust, orange rust, pokkah boeng, leaf scald, red stripe and top rot, mosaic (both sorghum mosaic virus (SrMV) and SCMV), are primarily managed through host plant resistance (https://www.lsuagcenter.com/).

9.4.4 Limitations of Traditional Breeding and Rationale for Molecular Breeding

The main objective of any plant breeding programme is to introgress one or a few favourable genes from donor into highly adopted variety and to recover most of the recipient parental genome as rapidly as possible. Breeding for biotic and abiotic stress requires identification of stress tolerant genotypes mostly from the germplasm and accumulating them in current commercial cultivars. Remarkable achievement was achieved during the past five decades in evolving new improved crop cultivars through traditional breeding in sugarcane. Major emphasis was laid on sourcing genes contributing to better productivity and adaptability from related species and wild relatives through genetic manipulation at cultivar, interspecific or intergeneric level. Breeding for stress resistance through conventional means is challenged by a poor understanding on inheritance of disease resistance, transfer of undesirable genes from the wild accessions along with desirable traits and the presence of reproductive barriers especially in interspecific and intergeneric crosses.

Most of modern varieties of sugarcane are products of only few inter-specific crosses involving around 15–20 genotypes developed at Java and India (Roach 1989). Even now in classical breeding programme, old genetic materials are widely used in crosses thus restricting the few recombinations from the original founder parents leading to narrow genetic base. Among the plant species available on earth, genetics of sugarcane is found as one of the most complex. Nevertheless, gene mining of *Saccharum* spp complex by genomic research helps the breeders (Abberton et al. 2016) to incorporate the variety of alleles in the breeding materials. Sucrose and cane are primary products from sugarcane. However, there was no significant improvement in top sugarcane producing countries in the last two decades for cane yield (Yadav et al. 2020). Further, the increase in sugar yield in most of the varieties of Florida was because of increase in cane yield rather than sugar (Zhao and Li 2015a, b). There was not a marked difference with respect commercial cane sugar (CCS) % between the older and new varieties of Australia (Jackson 2005). These facts clearly indicate that further improvement in sugarcane is possible by understanding molecular mechanism of sugar accumulation and metabolism. It was demonstrated that knocking down the pyrophosphate: fructose 6-phosphate 1-phosphotransferase (PFP) activity enhances sucrose accumulation in immature internodes of canes (Groenewald and Botha 2008).

Plant breeding has seen a major transition in the past decade as advances in biological sciences helped in evolving tools that can be applied to commonly accepted field techniques. In the context of plant breeding, molecular markers became a handy tool in selecting desirable genotypes by following the genes or chromosomal segments in the crosses using markers that are closely linked to them. This is particularly important in the case of genes governing biotic and abiotic stresses where traditional methods of screening for the trait are laborious and time consuming. Sugarcane suffers from insect damage, either by directly damaging the crop tissues or by its role as vectors of plant viruses. Continuous use of chemicals to protect the crop plants against insects harms the environment seriously. Hence it is essential to evolve plant

varieties that are resistant to insects. For several years, breeding varieties for disease and pest resistance has been taken up. The inherent difficulties in the conventional screening and the misleading results in screening efforts, probably due to the polygenic control of resistance makes marker assisted selection (MAS) for resistant to biotic constraints a viable alternative. In MAS, selection is not on the elusive trait of interest but on the reliable molecular markers closely associated with the trait. Being environmentally independent and scorable even at very early stage of development, molecular markers ensure quicker and clear cut analysis at lower cost than phenotypic testing. Screening with molecular markers would be helpful especially when the trait is under polygenic control, most commonly seen in the case of pest and disease resistance.

In the age of climate change, transgenic technology in sugarcane is boon by developing transgenic events tolerant to various biotic and abiotic constraints. The very first transgenic variety having tolerant to drought was commercially released in Indonesia. This transgenic genotype has plant cells stabilizing compound called betaine a bacterial gene (http://www.thejakartapost.com/). Progress to develop varieties resistant to stalk borer, ScYLV and herbicide resistance was possible through transgenic approach (Arencibia et al. 1997; Gilbert et al. 2009; Enríquez-Obregón 1998). Further, to tap the potential of cellulose in the leaves of sugarcane and bagasse, lignin is to be modified into simpler form which can be easily degraded by modifying its chemical structure through genetic engineering and studies were initiated in Brazil (http://agencia.fapesp.br/en/167560) and Australia (Harrison et al. 2011). Presently sugarcane is considered as an ideal plant for producing medicinal and industrial values like therapeutic protein and natural precursors of biopolymers (Wang et al. 2005; Petrasovits et al. 2007). In order to fulfil these objectives of utilising the sugarcane in present and future, molecular approaches are essentially required along with conventional breeding.

9.5 Marker-Assisted Breeding for Resistance Traits

9.5.1 Germplasm Characterization and DUS

The concept of plant variety protection received the emphasis by Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPs). Being a member to TRIPs, it is mandatory to protect varieties of crop plant by patents, by an efficient *sui generis* system, or by both in India. The choice of *sui generis* method of plant crop varieties protection was selected by Indian Government and 'Protection of Plant Varieties and Farmers' Right Act in 2001 was enacted to encourage research, variety development, protection to varieties, ensures farmers rights and for the growth of seed industry. To claim the protection under this act, the variety must satisfy four criteria of novelty (variety should not be commercialized for more than one year before the grant of protection and in case of tress of vines earlier than six years),

distinctness (variety must be distinguishable from already available old varieties by one or more identifiable morphological, physiological and other characters), uniformity (in appearance), stability (expression of the essential traits remains unchanged over the successive generations of propagation) (DUS) (Anonymous 2006).

DUS testing is done to know the whether the new variety bred in particular species is distinct from already available old varieties and the character(s) of distinctness is expressed stably over the period. ICAR-SBI, Coimbatore is designated as a National DUS center by the Protection of Plant Varieties & Farmers' Rights Authority (PPVFRA) for sugarcane. The sugarcane varieties are subjected to the DUS testing at four centers viz., Coimbatore and Agali for tropical varieties and at Lucknow and Karnal for sub-tropical ones.

In case of sugarcane, seed quantity of 400 single buds taken from top portion of the 8–10 month old mother cane are taken as planting materials for DUS testing. Care is taken to select the setts for planting from healthy and vigorous material without any incidence of pest and diseases. Further, the seed material for DUS testing is expected to have high level genetic purity, uniformity and phyto-sanitary standards and it should not be taken from in vitro propagation and not subjected to any chemical or bio-physical treatment. The DUS test is conducted on payment of testing fee by the nominating centers.

In case sugarcane varieties developed from a sub-tropical region of the country, the DUS testing is done at two locations viz., ICAR-SBI, Regional Centre, Karnal, Haryana and ICAR-Indian Institute for Sugarcanes Research, Lucknow, Uttar Pradesh. ICAR-IISR maintains reference varieties of sugarcane numbering 153 that includes released and notified varieties from Central Varietal Release Committee (CVRC) and state governments and clones from advanced varietal trials (AVT) of All India coordinated research project on sugarcane (AICRP[S]) from different sugarcane research centers (Anonymous 2019). Similarly, a total of 167 reference varieties are maintained at ICAR-SBI Regional Centre, Karnal. A total 233 reference varieties are maintained clonally at ICAR-SBI at two of its test locations viz., ICAR-SBI, Coimbatore and its Research Centre in Agali clonally and separately for DUS testing of the varieties developed from tropical region of the country (Anonymous 2020). DUS testing guidelines are used to record DUS characters on the reference collections and the reference varieties were characterized and the database of the all the reference varieties are developed along with photographs of the major morphological features (Anonymous 2020). Three characters viz., growth habit, leaf blade, leaf sheath adherence are taken to group the reference varieties. A group of reference varieties to which candidate variety shows most similarity is selected to plant along with candidate variety for DUS testing. The DUS trial is conducted in plot size of four rows of 6 m length with row to spacing of 0.90 m. Observations specific to different stage of crop maturity stages as per the guidelines of PPV and FRA are recorded to see the distinctness and similarity of candidate varieties to the reference varieties.

Morphological characters on growth habit, leaf sheath hairiness, shape of ligule, shape of inner auricle, colour of dew lap, leaf blade curvature and leaf blade width are seen during the end of grand growth phase of the crop (240 days). During the

maturity phase (300 days), those adhering on leaf sheath, colour of inter-node both exposed and not exposed to sun, characteristic features of inter-node like, diameter, shape, alignment, growth crack (split), rind surface appearance and waxiness, bud characters like shape of bud, size of bud, bud groove, bud cushion, bud tip in relation to growth ring, prominence of growth ring and width of root band are observed. Cane characters like cane height, number of millable canes, pithiness and internode cross section are recorded during harvest (360 days) of the crop.

9.5.2 Molecular Markers for Biotic Stresses

Conventional plant breeding has contributed immensely on improving yield in major crops and with the advancement of modern genomic tools a paradigm shift is being made in evolving better varieties. Breeding programs aim at introgressing one or a few favorable genes from donor into highly adopted variety while at the same time recovering most of the recipient parental genome. Breeding for biotic and abiotic stresses requires identification of tolerant genotypes mostly from the germplasm, and hence the knowledge on the genetics of resistance/tolerance is very valuable. Remarkable accomplishments were achieved during the last five decades in evolving new improved crop cultivars resistant to plant diseases and pests through plant breeding. Genomic tools are beginning to support plant breeding programs in a way as never before in evolving crop varieties, resilient to various biotic and abiotic stresses with improved productivity. Major emphasis was laid on sourcing genes contributing to better productivity and adaptability from related species and wild relatives through genetic manipulation at interspecific or intergeneric level. Breeding for stress tolerance through conventional means is challenging due to poor understanding on inheritance of resistance to diseases, transfer of undesirable genes from the wild accessions along with desirable traits and the presence of reproductive barriers especially in interspecific and intergeneric crosses and needless to say that the time taken in evolving such varieties is very long.

9.5.2.1 Red Rot

In sugarcane breeding programs especially in India, red rot resistance is a prerequisite in identification and commercial release of new varieties. Also, prevalence of many *C. falcatum* pathotypes complicates breeding for *C. falcatum* resistance (Viswanathan 2021b). Further, inheritance patterns to red rot resistance/susceptibility are not clear since the studies revealed existence of both race-specific and non-specific (vertical and horizontal) resistance (Babu et al. 2010). Hence, sugarcane genome complexity does not permit any genetic manipulation for *C. falcatum* resistance by conventional breeding gene introgression methods. The only way of getting resistant varieties is screening the highly variable F_1 progeny population and the subsequent clonal propagation for *C. falcatum* resistance. However, there is need to categorize

genomic regions imparting resistance against constantly evolving new variants of *C. falcatum*. In sugarcane, almost all the traits of interest are quantitative and multi-allelic (Selvi and Nair 2010) and mapping them even with the currently available genomic and bioinformatics resources and tools is a tedious process.

9.5.2.2 Rust

MAS has been highly successful in sugarcane breeding for rust resistance. Genetic basis of rust resistance among the selfed progenies of the resistant cv R570 established a 3:1 segregation ratio for resistant and susceptible. Resistant allele 'Bru1' was identified in the cv R570, which is dominant, single copy and monogenic (Daugrois et al. 1996). To locate the major gene on the cv R570, genetic map based on restriction fragment length polymorphism (RFLP) was used (Grivet et al. 1996). Resistance to brown rust was found to be very much transmissible and additive; hence breeding for disease resistance became fast and effective. Simple inheritance to brown rust in a complex polyploidy crop like sugarcane favored bacterial artificial chromosome (BAC) library construction and map based cloning of the cv R570. In the context of genomic complex of sugarcane Bru1 became the finely categorized Mendelian trait and Bru1 provided resistance against many rust races of the pathogen (Asnaghi et al. 2001). Even though the cv R570 has been cultivated intensively in Réunion Island, resistance breakdown to the gene has not been observed for nearly two decades.

9.5.2.3 Yellow Leaf Disease

Only a very few genetic studies were made to describe YLD resistance in sugarcane. Using a quantitative triat loci (QTL) strategy of involving progenies between a susceptible (S) variety and a resistant (R) clone, the first key quantitative trait allele (QTA) was tagged for ScLYV resistance and named as *Ryl*1 (Costet et al. 2012). Here, resistance in the 196 progenies of R570 (S) x MQ76-53 (R) was evaluated using tissue-blot immunoassay (TBIA) for 10 years. Genotyping was accomplished with different molecular markers (1299 amplified fragment length polymorphism (AFLP), 247 simple-sequence repeats (SSR), 115 RFLP) resulting in 2822 polymorphic markers. The major QTA *Ryl*1 contributed 32% for YLD resistance in the resistant cv MQ76-53.

9.5.2.4 Other Diseases

Another association mapping study by McIntyre et al. (2005) with 192 progenies made from a cross between the cv Q117 and a clone 74C42. In fact, this was the first association mapping study attempted in sugarcane. The Q1 progeny were evaluated for their disease resistance to pachymetra root rot (PRR), brown rust and genotyped using RFLP (7 RFLP and 31 resistance gene analogues), 31 AFLP and 30 SSR

markers. An elite clone set consisting of 154 clones representing diverse Australian breeding material was used to validate the identified markers. Linkage map and association analysis were carried out and 30 markers were identified for brown rust and PRR. The total phenotypic variations described by the specific markers were in the range of 4–16% for PRR and 4–18% for brown rust. QTL's identified from biparental cross were validated in the elite clone set. Three markers were found highly associated for PRR and one marker was significantly associated to brown rust. This study provided a foundation that association mapping can be successfully employed in sugarcane crop.

Gouy et al. (2015) screened 183 sugarcane accessions representing worldwide sugarcane germplasm with SSR, DArT, and AFLP (1406 AFLP, 1892 DArT and 29 SSR) markers, and the population was characterized for agro-morphological and disease resistance characters across five locations. R12H16_PCR marker located in the Bru1 gene was used as a diagnostic marker. Diagnostic markers are derived from the polymorphism that directly contributes to the trait or in strong linkage disequilibrium (LD) with allele.

A mapping panel consisting of 154 sugarcane clones representing parental materials and cultivated varieties were studied for markers associated to disease resistance PRR, Fiji leaf gall, leaf scald and smut. Genetic analysis with AFLP (1068) and SSR (141) markers indicated that the number of markers identified almost halved when population structure was considered for all the diseases except for leaf gall. The numbers of markers significantly associated at $P \leq 0.001$ within groups were 12 for smut, 5 for leaf scald, 4 for Fiji leaf gall and 5 for Pachymetra (Wei et al. 2006).

These genes which are often identified as candidate genes with several other gene sets in other biotic as well as abiotic studies not only in sugarcane but also in other crops, are being studied further and probably these would support directly to red rot resistance, and potentially apply MAS in sugarcane breeding. Although association between identified markers and phenotype is not well established in sugarcane for routine selection process, it could be a valuable means to understand the resistance potential of the genotypes used in the breeding programs.

9.5.2.5 Insect Pests

The genome of sugarcane, a complex polyploid with QTL for borer pests remains relatively unexplored. In sugarcane, differentially expressed cDNA fragments for sugarcane stalk borer Eldana were identified by Butterfield et al. (2004). Using an RFLP approach, genes involved in resistance mechanisms such as peroxidase, catalases and several receptor kinases were probed on a set of population of 78 sugarcane clones. They identified 69 polymorphisms exhibiting correlation with Eldana resistance followed by 59 to smut, and 35 to SCMV. Distinct markers with the largest effects accounted for 20.2% of the variation in case of Eldana and 15.9% of the phenotypic variation in smut score.

Randomly amplified polymorphic DNA (RAPD) and SSR markers were applied to assess genomic diversity amongst cane cultivars varying in resistance for top

borer and to identify their association with borer resistance and susceptibility. DNA from R, moderately resistant (MR) and HS clones were bulked and screened with polymorphic primers. Sixty-two of the 125 primers generated polymorphic profiles. Among them OPC201020, NKS7186, NKS8334, NKS61221 and NKS9615 showed a relation with top borer resistance/susceptibility in resistant varieties whereas two markers NKS5684 and OPV17917 showed such relation in the susceptible varieties. Finally, these markers were validated with a set of foreign hybrids showing resistance and identified three NKS7186, NKS61221 and OPV17917 that are useful for screening top borer resistance in sugarcane (Selvi et al. 2008).

Inter specific and intra specific breeding for resistance in sugarcane is viable due to the genetic compatibility and availability of resistance sources. A wild relative of sugarcane, *Erianthus arundinaceus* has tolerance against abiotic stresses (Shabbir et al. 2021) which may also have tolerance to different pests therefore they can be extensively used in current sugarcane improvement programs to develop varieties with insect pest resistance and high sucrose (Cai et al. 2012).

9.6 Genomics-Aided Breeding for Resistance Traits

9.6.1 Structural and Functional Genomic Resources Developed

With the recent advances in genome sequencing technologies, several relevant genes were identified/characterized and novel information on process and pathways are continually emerging. The major developments include the development of molecular markers, generation of expressed sequence tag (EST) databases, gene expression methodologies, development of microarray technologies, development of computational abilities and algorithms etc., sequencing of the several plant genomes and transcriptomes along with the advances in automated sequencing. These developments enabled the structural and functional characterization of several genes governing economically important traits and their further use in enhancing the breeding efficiency. The largest transcriptome resource for sugarcane (http://sucest.lbi.ic.unicamp.br/en/), containing about 238,000 ESTs sourced from 26 cDNA libraries of 12 cane varieties. The cDNA libraries represented different stages of crop development and environment and subjected to different biotic treatments (Arruda 2001). Brazilian modern cultivar's (SP80-3280) gene space assembly was created and that comprises 373,869 genes of the whole sequence with their upstream regions to identify regulatory promoter elements, BACs of R570 and SP80-3280 and CRISPOR, a CRISPR/Cas9 assisting tool. The SUCEST-FUN database also hosts functional genomics resources for insect and pathogen interaction with sugarcane.

Nuclear genomes of modern cultivars have two sub-genomes; the one from *S. officinarum* with basic monoploid genome size of about 1 Gb and the other from *S. spontaneum* with size of 750–843 mb (Zhang et al. 2012). Linkage mapping

in autopolyploid is difficult because of arbitrary combination of many homologous chromosomes, detection of many spots/bands by nucleic acid probe/primers and segregation of alleles with different dosage level (Ming et al. 2010). Recently, French sugarcane variety R 570 (2n = 115) was selected by the sugarcane genome sequencing initiative (SUGEST) and this variety is characterized intensively (Aitken et al. 2016). Other cultivars, used for gene sequencing are SP 80-3280, Q 165, LA 9 Purple and IJ 76-514 (*S. officinarum*), SES 208 and Mandalay (*S. spontaneum*). First linkage map was created from the progenies of a cross combination of *S. spontaneum* and its doubled haploid having 64 linkage groups from 276 RFLPs with 208 single dose randomly primed PCR loci of *Saccharum* complex (da Silva et al. 1995). Linkage groups in all the nine available linkages have partially represented less than 50% of the genome of the genotypes taken for study (Ming et al. 2010). IND 81-146 with about 58% of genome coverage had the fewest chromosome number (26–28), which is the best criteria for selecting the genotypes for a saturated genetic map.

The differential chromosome number (100–130), plenty of transposans/retrotransposons present through the genome, repetitive elements and differential ploidy levels for genes account for about 50% of genome in the crop making sugarcane monoploid genome 10 times bigger than the model plant species like *Arabidopsis*. The large and complex polyploidy nuclear genome and organeller genome of sugarcane are responsible for less advancement in sugarcane genomics.

A monoploid reference sequence of sugarcane hybrid cv R570, an allele defined genome of *S. spontaneum* and a long read reference transcriptome are some of the sugarcane genomics resources developed recently (Hoang et al. 2017, Garsmeur et al. 2018; Zhang et al. 2018). Further, advances in proteomic research resulted in expansion of a huge reference proteomes of around 20,382 as on January 2022, in the Uniprot database, consisting more than 8714 bacterial, 10,069 viral, and 1805 eukaryote proteomes. For structural genomics, the protein database PDB, hosts various information on crystal structure, electron microscopy, x-ray diffraction studies, and nuclear magnetic resonance (NMR) studies of proteins from plants, viruses, bacteria and fungi. For sugarcane to be specific, crystal structures of defensin (de Paula et al. 2011), canecystatin (Valadares et al. 2013), sugarcane serine/threonine protein kinase SAPK10 (Righetto et al. 2019; PDB Accession 5WAX), UDP-glucose pyrophosphorylase (Cotrim et al. 2018), an antifungal protein Sugarwin (Maia et al. 2021) are available in PDB. In addition structural information from model plants like Arabidopsis (1720) entries, maize (23), Tobacco (10), rice (28), sorghum (18, consisting of important genes like peroxidase, caffeoyl-CoA O-methyltransferase, Phenylalanine ammonia-lyase), structure of effector protein, chitin deacytylase, fungal alcohol oxidase etc. from *Colletotrichum* species (10), structure of proteins for fungal toxin, replication protein, kinesin etc. from *Ustilago* species are available for references.

The large volume of sequence data generated by next-generation sequencing (NGS) are simultaneously characterized functionally using high-throughput assays, DNA microarray, gene chips, serial analysis of gene expression (SAGE), oligoarrays, and single cell RNA sequencing etc. to identify candidate genes on a large scale. A huge DNA sequence information were generated from these projects and

the online databases such as http://www.ncbi.nlm.nih.go (NCBI-National Centre for Biotechnology Information), http://www.tigr.org (TIGR-The Institute of Genome Research), http://www.ebi.ac.uk (EBI-European Bioinformatics Institute) have all the sequence deposits. The availability of genome sequences for several crops and microbes CCBI Database as on January 2022, (Eukaryotes [20672 out of which 1754 are plant genomes https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/Plants]), Prokaryotes (372,288), Viruses (46,556), Plasmids (34,863) and Organelles (21,232). Recent progress in genome editing (GE) methods has made advances in breed for practically any given desired character. Improvements in GE tools like transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) have made it possible to manipulate accurately any gene of interest by the molecular biologists. By applying gene-editing approach, non-genetically modified (Non-GMO) crop plants with the desired gene of interest or trait will be achieved and this will contribute to enhanced yield by effectively managing various biotic and abiotic stresses.

The new genomic science allowed the scientists to investigate the plant genome by many approaches and on a dimension, which was earlier unthinkable. Shortly, plant breeders will have the choice of using many genes, to develop a desired plant of required genetic makeup with more efficiency than the past. Moreover, in the coming days interaction between different genes that work together in plants to give a desired crop features will be achieved through genomics. By this, a combination of desired genes could be assembled into cultivars by means of very accurate plant breeding procedures. Thus a combination of biotechnology and genomics programs is greatly aiding to confront the many challenges facing production, management, and sustainability in agriculture.

9.6.2 Genome-Wide Association Study (GWAS) and Genomic Selection (GS)

Genomic-selection method initiated by Meuwissen et al. (2001) is a new approach for selection of individuals in breeding experiments and is appropriate for the augmentation of complex-traits requiring long-term field experiments. Genomic-selection utilize the whole-marker information by concurrently calculating the outcome of each marker covering the whole genome to anticipate the genetic-value of individuals. Because of its complex genome (aneu-polyploidy), genetics and genomics research has not been very successful in sugarcane, unlike other crops. However, with the advances in genomics and decreasing costs of NGS tools, development of high-density markers enabled genetic maps is currently possible. During last two decades, GWAS were developed to find QTLs related to biotic resistance in sugarcane (Debibakas et al. 2014; Gouy et al. 2015; Singh et al. 2016; Gutierrez et al. 2018; Islam et al. 2018; Yang et al. 2018, 2019; You et al. 2019, 2020; Aono et al. 2020; Pimenta et al. 2021).

9.6.2.1 Red Rot

An association mapping approach used on a panel of 119 sugarcane genotypes fingerprinted for 945 single sequence repeats alleles was carried out to find markers associated with resistance to three pathotypes *C. falcatum* of viz., CF01, CF08 and CF09 (Singh et al. 2016). Mixed-linear models identified four markers that were able to describe ten to sixteen percentage of individual trait variation. General linear model (GLM) analysis identified three (IISR_90_360; IISR_298a_140; IISR_256_240), one (IISR_198_170) and five (IISR_148_200; IISR-137-240; IISR_46b_170; SCB10_410; ESTA69_400) markers linked with resistance to the pathotypes CF01, CF08 and CF09, respectively. MLM identified only four markers viz., IISR_256_240 & IISR_298a_140 for the pathotype CF01 and IISR_137_240, IISR_46b_170 for the pathotype CF09, which were able to elucidate 16.6, 10.7, 14.5, and 11.7%, respectively of the total phenotypic variation. Many genes involved in host-defense like Serine/threonine protein kinase, MAP Kinase-4, Transporter-1, Cytochrome-P450, Ring finger-domain protein, Glycerol-3-Phosphate, and others were confined to the region of these four markers. Similarly, from ICAR-SBI, Coimbatore, the populations of BO 91 × Co 775, Co canes, Co 86002 × BO 91 and CoM 0265 × Co 775 were scored for red rot resistance and identified more number of clones under MR category. Clonal selection was done in all the populations for sucrose, red rot reaction and other criteria and broad sense heritability was calculated for all the traits. The heritability % for the red rot trait from the populations ranged from 94 to 97%. All the genotyped clones subjected to develop genomic selection/prediction models. BayesA, BayesB, BL, genomic best linear unbiased prediction (GBLUP), Reproducing Kernel Hilbert space (RKHS) Single models showed significant Single nucleotide polymorphisms (SNPs) for the sucrose and red rot traits. The correlation between prediction models for the sucrose trait with training and testing population was high (>0.9) and the prediction accuracy was high for 100 testing population (>0.65 for the for the sucrose trait). The prediction models for red rot resistance showed the accuracy of 0.56 (Manimekalai et al. unpublished).

9.6.2.2 Smut

Recently hybrid-transcript based mapping assembly method was followed to decode genome-wide expression conversion at iso-form level and alternative splicing (AS) land-scapes regulation in a moderately resistant genotype following *Sporisorium scitamineum* inoculation (Bedre et al. 2019). Approximately 5000 (14%) sugarcane genes were identified using de novo and comparative genome-wide transcript mapping that undergo alternative splicing in reaction to *S. scitamineum* infection. A total of eight hundred and ninety-six events have been established that expressed differentially at various stages of infection with *S. scitamineum.* The open reading

frames (ORFs) of these genes deciphered changed proteins, which change and regulate cell wall, reactive oxygen species (ROS) homeostasis, transcription and defense-hormone signaling. Although AS approach has cleared the path up to some extent but still there are many folds that have to come out. In addition to the abovementioned methods, there are new opportunities to develop emerging GWAS method for sugarcane and *S. scitamineum,* which allows for the concurrent detection of genes interconnect between sugarcane and its pathogen.

9.6.2.3 Rust

Several works have been executed by different workers in sugarcane to know the resistance to *Puccinia kuehnii*. Yang et al. (2018) based on genome-wide association method detected 3 quantitative-trait loci (qORR4, qORR109 and qORR102) for sugarcane rust in a population of 173 progenies attained from a bi-parental crosses (CP88-1762 and CP95-1039). Resistance gene encoded maker-G1 was identified through PCR method. Probably the putative QTL and G1-marker identified in this work can be successfully used in breeding programmes to ease the selection process for orange rust disease management. Later, Yang et al. (2019) evaluated 308 accessions from the World germplasm collections maintained at Miami for resistance to *P. kuehnii* by genotyping and phenotyping studies. They also characterized many DNA sequence variants by NGS through target-enrichment sequencing. They detected 91 putative DNA-markers and eighty two candidate genes remarkably related to resistance to orange rust. These results throw lights on the genetic bases of the rust resistance in sugarcane. Although MAS is successful for rust resistance, all the related alleles are yet to be discovered and the accompanying regions vary between genotypes, thus regulating methodology generalization. Aono et al. (2020) used GBS method to find out the genomic regions resistant to brown rust in full sib progenies. They detected about 14,540 SNPs that guided to achieve 50% Mean Prediction Accuracy. By this method, they attained up to 95% accuracy with 131 SNPs dataset related to brown rust resistance.

9.6.2.4 Leaf Scald

Molecular markers resistant to leaf scald disease (Causal organism: *Xanthomonas albilineans*) through MAS were developed by Gutierrez et al. (2018) with progenies from a cross of resistant (LCP 85-384) and susceptible (L 99-226) parental cultivars. QTL analysis detected 8 genomic regions on 7 linkage groups controlling leaf scald response. QTLs qLSR77; qLSR37; qLSR262 and qLSR104 were accumulative for 30 percentage of the resistance response. They were able to locate one representative gene around three QTLs using syntenic information of sorghum reference genome and comparative genome analysis. Upon *X. albilineans* infection of meristematic or lamina tissues, a clear upregulation in their expression of the genes linked to major QTLs was found. c5_1527, the codominant marker tightly allied to the QTL can

be used along with other linked SNP markers as diagnostic markers in MAS for *X. albilienans* resistance in sugarcane.

9.6.2.5 Ratoon Stunting Disease

You et al. (2020) evaluated 146 individuals of selfed progenies of the cv CP80-1827 resistant to ratoon stunting disease through GWAS. Eighty-two Lxx resistant genes were recognized by exploring the twenty-three quantitative trait loci regions on their tune in with forty-four genes on Sorghum genome, twenty genes from the genome of sugarcane cv R570, and 18 genes from *Saccharum spontaneum* genome. They recognized quantitative trait loci administering ratoon stunting resistance along with the associated single nucleotide polymorphism markers will help in MAS to reduce the depletion of sugarcane yield due to this disease.

9.6.2.6 Yellow Leaf Disease

A GWAS was performed to identify QTL governing YLD resistance on a sugarcane varieties panel numbering 189 (Debibakas et al. 2014). About 3949 DArT and AFLP polymorphic markers were used to fingerprint the panel. During the study, the varieties were phenotyped for the virus infections in 2 trials for 2 crop seasons at Guadeloupe, which has conducive conditions for natural infections of ScYLV. For ScYLV resistance, 6 independent markers were identified in relation with phenotype, but 2 markers were identified frequently during the GWAS analysis. After detailed bioinformatic analyses it was found that many genes involved in interaction of the plant with the aphid vector or the virus are found in the marker regions. Later, two QTLs administering yellow leaf virus resistance were detected and cumulatively they reduced disease incidence by 31% (Islam et al. 2018). These earlier reports improved understanding of the molecular resistance mechanisms to ScYLV. But this study were carried out either on bi-parental populations or in cultivar panels and this hinders the chance of identifying the potential ScYLV resistant loci owing to contracted genetic basis in elite sugarcane cultivars. Hence, to avoid these limitations, high density markers were used and found ninety one putative-markers and eighty two significantly associated candidate genes for YLD resistance (Yang et al. 2019). Recently, an Axiom Sugarcane100K SNP array was constructed using more than three hundred *Saccharum* spp lines (TES method) from the sugarcane germplasm and this generated about 4 million single nucleotide polymorphisms. QTL analysis identified eighteen QTLs controlling ScYLV resistance segregating in the 2 mapping populations, harboring twenty-seven disease resistant genes (You et al. 2019). Progress made in identifying QTLs for ScYLV resistance has been very positive to identify YLD resistant parental lines or germplasm. We are hopeful that GWAS application will ably support breeding for YLD resistance in sugarcane.

9.7 Recent Concepts and Strategies Developed

9.7.1 CRISPR/Cas System Mediated Resistance to Sugarcane Diseases

Despite the tremendous advances made through traditional sugarcane initiatives, long breeding cycle of 12–14 years, slow breeding improvement, narrow genetic variability, complex polyploidy of the genome and poor fertility obligated to produce novel varieties make further genetic gain of superior sugarcane varieties difficult (Babu et al. 2021; Ram et al. 2021). Achievements made using genetic engineering technique for incorporating tolerance/resistance to biotic stress in sugarcane is given in Table 9.4. Due to complex nature of biotic stress resistance in sugarcane and the lack of genetic information posed a serious challenge. Advances in genomics, such as NGS strategies and the availability of the mosaic monoploid genome of sugarcane, facilitated identifying new genes linked to both biotic and abiotic traits, expanded understanding of the response of the crops to stress, and these developments are likely to speed up the development of sugarcane-based products (Babu et al. 2021).

New genomic modification tool using the cleavage mechanism of RNA-guided Cas9 with the target specificity allows precise control of the gene editing (Doudna and Charpentier 2014). Gene editing was successfully demonstrated in plant systems for various traits (Feng et al. 2013; Li et al. 2013c; Nekrasov et al. 2013; Shan et al. 2013; Xie and Yinong 2013). Genome-editing technologies include clustered regularly interspaced short palindromic repeats/CRISPR-associated systems (CRISPR/Cas9), zinc finger nucleases (ZFNs), sequence specific nucleases (SSNs), meganucleases (MNs), transcription activator like effector nucleases (TALENs), CRISPR/Cas12a (Cpf1, CRISPR from Prevotella and Francisella 1), and Cas9-derived DNA base editors. Though application of genome editing was successfully demonstrated in sugarcane for improvement of agronomically significant characters (Jung et al. 2012; Kannan et al. 2018; Zhao et al. 2021; Eid et al. 2021; Oz et al. 2021) (Table 9.4), gene editing for inducing resistance to biotic stresses is not carried out till date.

Table 9.4 Application of genome editing in sugarcane for improvement of agronomic traits

Targeted genes	Technique	Improved traits	Repair pathway	References
COMT	TALEN	Reduction in lignin content for bioethanol production	NHEJ	Jung and Altpeter (2016), Kannan et al. (2018)
ALS	CRISPR	Herbicide tolerance	HDR	Oz et al. (2021)

9.7.2 Nanotechnology

Nanotechnology is the term related to resources and processes involving particles of 100 nm. Nanotechnology has the ability to create massive changes with ecological earnings in agricultural systems offering a chance to exercise a more proficient, safe and precise control of time and location of pesticide release (Kuzma and VerHage 2006). The use of nano particles in the new formulations of pesticides and insect repellants has been reviewed by El-Wakeil et al. (2017). Application of nanotechnology has led to better pest management among other benefits with least impact on environment (Hofmann et al. 2020). Thus subsequently mitigating consequences of climate change Nanoparticles could be used in the new formulations of pesticides preparation and insect repellants. Nanoparticle-mediated gene delivery in many plant species is more efficient than the traditional technologies due to the higher efficiency of genetic transformation (Ahmar et al. 2021).

Nano pesticides can replace the conventional pesticides as they deliver higher efficay at lower doses minimizing negative effects (Kah et al. 2018, 2019; Ahmar et al. 2021). Prolonged sustainable efficacy is ensured due to the accurate delivery and slow gradation of active components (Chhipa 2017). The pesticide nanoemulsions have certain advantages over other methods such as broad range of applicability, superior adherence on target sites with better perviousness (Feng et al. 2018). Globally, in the nano pesticide market, insecticides formed the highest share of 41% revenue in 2019 (Research Corridor 2020). Several reports and reviews have adequately emphasized the merits and risks associated with nanopesticides, and their fate in the environment (Sharma et al. 2017; Peixoto et al. 2021; Rajiv et al. 2020; Nguyen et al. 2012; Adak et al. 2012; Shukla et al. 2020; Kah et al. 2013; Mukherjee et al. 2016). Agricultural products may retain the nanopesticide residues (García et al. 2010) and thus enhanced persistence of pesticide molecules in the target organism or plant achieved through nanocapsules or nanoemulsions may pose greater risk (de Francisco and García-Estepa 2018).

9.8 Genetic Engineering for Resistance Traits

9.8.1 Establishment of Genetic Transformation in Sugarcane

The lack of resistant sources in *Saccharum* germplasm to many diseases or absence of viable management practices has opened new avenues especially genetic engineering and gene editing to circumvent the constraints and to improve production of sugarcane. Due to inaccessible crop canopy, insect pest management in sugarcane by chemical application is difficult. Moreover, borer larvae are impervious to chemical control as larvae are cryptic internal feeders. Although traditional insect host-plant resistance involves quantitative attributes at numerous loci, progress in developing a resistant cultivar has been limited. The availability of resistance gene

sources in the breeding pool as well as onerous screening procedures make traditional breeding for resistant types difficult. Chawdhary and Vasil (1992) successfully used particle bombardment and electroporation methods to transfer pBarGUS genes into sugarcane suspension cell cultures. Following that, tremendous progress was made in sugarcane genetic transformation and transgenic sugarcane development for a variety of traits. Among the several approaches used to introduce the desired gene in sugarcane protoplasts, cells or calli, *Agrobacterium* mediated transformation (Arencibia et al. 1998; Joyce et al. 2010; Kalunke et al. 2009; Manickavasagam et al. 2004; Mayavan et al. 2013) is popular. The other methods of gene transfers involved either chemicals (Chen et al. 1987) or devices (Franks and Birch 1991; Snyman et al. 2006; Babu and Nerkar 2012) or electrical perforations in the target tissue (Rathus and Birch 1992).

Genetic transformation in sugarcane has been extremely successful and transgenics for various biotic stresses developed (Babu et al. 2021) are listed in Table 9.5. These include resistance to diseases such as mosaic, yellow leaf, leaf scald, red rot etc. (Jain et al. 2007; Gilbert et al. 2009; Zhang et al. 1999; Kanchana 2007) and to pests like sugarcane borers (Kalunke et al. 2009; Gao et al. 2016), Sugarcane has also been modified genetically for the better economically important traits namely yield of sugar, quality of juice (Botha and Groenewald 2001; Vickers et al. 2005) and value-added unique sugar that is more beneficial to consumers (Wang et al. 2005).

9.8.2 Disease Resistance in Sugarcane

9.8.2.1 Viral Diseases

In order to induce resistance to viral diseases, SCMV-coat protein (SCMV-CP) gene of was transferred by genetic transformation in sugarcane. The transgenic lines of sugarcane plants carrying the coat protein gene was tested and found to be superior to that of non-transformed plants. Sugarcane hybrid CC84-75 was transformed through particle bombardment using ScYLV coat protein DNA fragment. Most of the PCR positive for ScYLV coat protein exhibited negative for ScYLV even after 10 months after infection (Rangel et al. 2005). Microprojectile transformation of sugarcane cv Q124 with FDV segment 9 ORF1 resulted in resistance to Fiji disease. Of the 47 transgenic lines investigated, some of the resistant lines showed no Fiji disease symptoms (McQualter et al. 2001).

Transgenic sugarcane lines conferring with mosaic resistance in high yielding and high sucrose varieties were developed in many countries through particle gun bombardment methods (Ingelbrecht et al. 1999; Yao et al. 2004; Gilbert et al. 2005; Guo et al. 2008). However, the transgenic lines had high copy numbers of target gene inserts (Arencibia et al. 1998) and transgenic lines obtained through gun bombardment methods had difficulties to prove the sites of insertion, and border sequences. Post-transcriptional gene silencing (PTGS) mediated transgene development was reported as the most widely adapted method to confer mosaic resistance in high

Table 9.5 Sugarcane transgenics developed for different biotic stress resistance/tolerance

Disease	Gene used	References
SCMV	Sugarcane mosaic virus coat protein (SCMV-CP)	Joyce et al. (1998) Apriasti et al. (2018)
Sugarcane leaf scald	albicidin detoxifying (albD)	Zhang et al. (1999)
SrMV	Sorghum mosaic virus coat protein (SrMV-CP)	Ingelbrecht et al. (1999)
ScYLV	Sugarcane yellow leaf virus coat protein (ScYLV-CP)	Rangel et al. (2005)
SCMV	Sugarcane mosaic virus coat protein (SCMV-CP)	Gilbert et al. 2005
Sugarcane rust (*Puccinia melanocephala*)	Glucanase, chitinase & aprotinin 24	Enriquez et al. (2000)
Fiji leaf gall	Fiji disease virus segment 9 ORF 1 (FDVS9 ORF 1)	McQualter et al. (2001)
Red rot (*Colletotrichum falcatum*)	Dm-Anti microbial peptide 1 (amp1) and chitinase	Kanchana (2007)
Red rot (*C. falcatum*)	Chitinase and 1,3-β-glucanse	Kanchana (2007)
Red rot (*C. falcatum*)	*Trichoderma* β-1,3-glucanase gene	Nayyar et al. (2017)
Red rot (*C. falcatum*)	barley chitinase class-II genes and *HarChit*and *HarCho*	Ijaz et al. (2018), Tariq et al. (2018)
Insect pests	Gene used	References
D. saccharalis	Crystal toxin gene (cry1Ab)	Braga et al. (2003)
C. infuscatellus	Crystal toxin gene (cry1Ab)	Arvinth et al. (2010)
Sugarcane borers	Crystal toxin gene (cry1Aa3)	Kalunke et al. (2009)
P. venosatus	*Bt*Crystal toxin gene (cry1Ac)-modified	Weng et al. (2006, 2011)
Cane grub	*Galanthus nivalis* L. (snowdrop) agglutinin (gna)	Legaspi and Mirkov (2000)
Cane grub	*G. nivalis* (snowdrop) agglutinin (gna)	Nutt et al. (1999)
E. loftini	*G. nivalis* (snowdrop) agglutinin (gna)	Setamou et al. (2002a, b)
E. loftini	*G. nivalis* (snowdrop) agglutinin (gna)	Tomov and Bernal. (2003)
Sugarcane stalk borer	*G. nivalis* (snowdrop) agglutinin (gna)	Irvine and Mirkov (1997)
E. loftini	*Galanthus nivalis* L. (snowdrop) agglutinin (gna)	Nutt et al. (1999)
Ceratoacuna lanigera	Snow drop lectin	Zhangsun et al. (2007), Romeis et al. (2003)

(continued)

Table 9.5 (continued)

Insect pests	Gene used	References
Top borer *S. excerptalis*	Aprotinin	Christy et al. (2009)
Early shoot borer (*Chilo infuscatellus* Snell)	Crystal toxin gene (cryIF)	Thorat et al. (2017)
Sugarcane weevil	Sugarcane cysteine peptidase inhibitor 1 (CaneCPI1)	Schneider et al. (2017)
D. saccharalis, Ceratovacuna lanigera	AVAc-SKTI	Deng et al. (2008)
Sphenophorus levis	*HIS Cane CPI-1*	Ribeiro et al. (2008)
E. loftini	*gna*	Setamou et al. (2002a)

yielding and high sucrose mosaic susceptible varieties. During 2005, the first SCMV resistant transgenic lines developed with untranslatable SCMV strain E-CP gene by following biolistic transformation methods in USA and these lines were evaluated for agronomic performance and field disease resistance. Around 100 transgenic lines derived from the cvs CP 84-1198 and CP 80-1827 when evaluated for resistance against the disease and agronomic traits in one plant crop and two ratoons, the transgenics developed from CP 84-1198 had recorded a significant improvement in cane yield and sucrose with reduced mosaic disease incidence (Gilbert et al. 2005).

In China, transgenic sugarcane lines resistant to mosaic were developed using Sorghum mosaic virus (SrMV) (SrMV) CP gene by following RNA interference (RNAi) approach. The RNAi vector pGII00-HACP contained hairpin interference sequence and herbicide-tolerant gene, *cp4-epsps* and the expression cassette was transferred to sugarcane cv ROC22 by following *Agrobacterium*-mediated transformation. The SrMV transgenic lines were confirmed by challenge inoculation and herbicide screening. The genetically modified cv ROC22 were reported with 87.5% SrMV resistance rate (Guo et al. 2015). Similarly, RNA silencing approach was followed to develop transgenic sugarcane against SCMV with the expression of a short hairpin RNAs (shRNA) directing SCMV-CP gene, in Punjab province in Pakistan. Based on SCMV conserved CP region, two independent shRNA transgenic lines expressing stem and loop sequences derivative of microRNA, sof-MIR168a—an active regulatory miRNA in sugarcane, siRNA 2 and siRNA4 were engineered as RNAi constructs with the polyubiquitin promoter control. Particle bombardment method was used to deliver the constructs into sugarcane cvs SPF-234 and NSG-311 as separate experimentations. Challenging the transgenic lines with SCMV by mechanical inoculation revealed that the degree of mosaic resistance is more in shRNA4 transgenic lines in both cultivars with 80–90% reduction of SCMV-CP gene expression (Aslam et al. 2018).

In India, efforts were made to develop mosaic resistant transgenic sugarcane plants with RNAi technology using the SCSMV suppressor protein genes SCSMV-P1 and HC-Pro. Both the gene constructs were evaluated in model plant *Nicotiana tabacum* under GFP tagged transient expression assay in that the P1 gene was identified as

playing a major role of RNA silencing suppressor (Bagyalakshmi and Viswanathan 2020). Recently, Hidayati et al. (2021) made a comparison of RNAi and pathogen-derived resistance (PDR) approaches toassess effectiveness of transgenic sugarcane plants with resistance to SCMV in Indonesia. Transgenic plants harbouring RNAi mediated resistance were reported with high level of SCMV resistance based on delayed symptom expression at 26 dpi with mosaic symptoms only 50% of the inoculated plants as compared to 77.8% in PDR transgenic plants and with less number of plants with 36.7 kDa SCMV-coat protein. With this RNAi mechanism generated SiRNA mediated control was reported as effective against the SCMV.

Agronomic evaluation of five independent sugarcane transgenic clones with SCMV resistance was done based on field performance, resistance to the virus, and stability of transgene in comparisons with Badila, the wild-type parental clone in China. All the transgenic lines were reported with higher tonnes of cane/ha, higher sucrose % along with low mosaic incidence than Badila. Among the five independent sugarcane transgenic lines, the line B48 showed very high resistant to the virus with only 3% or less incidence. Further, the resistant line recorded an average of yield of 102.72 t/ha, whereas the parental clone Badila recorded 67.2% lesser cane yield and the transgene expressed stably over many vegetative generations. With this study, the China has developed a transgenic Badila as a valuable SCMV resistant germplasm source for future development of mosaic resistant genotypes (Yao et al. 2017).

In Florida, USA Gilbert et al. (2009) developed two transgenic clones (6–1 and 6–2) resistant to ScYLV from the CP92-1666 cultivar by particle bombardment methods using two different transformation vectors under the same maize ubiquitin promoter with untranslatable ScYLV-CP gene construct in antisense orientation; and the other construct with modified antibacterial Cecropin B gene along with *npt*II selectable marker gene. The transgenic lines as well as tissue culture material had shown low yield potential compared to the parental cultivar in plant crop followed by two subsequent ratoons. But, transgenic lines had a high level of ScYLV resistance with very low infection rates of 0–5% compared to 98% in parent cultivar. This study revealed that transgenic lines cannot be acceptable for commercial cultivation as such due to poor yield potential but serve as donor parents to develop ScYLV resistance. Later, Glynn et al. (2010) reported that it could be overcome or reduced by transgene transfer to sexual progeny of sugarcane true seeds. In the same way, Zhu et al. (2010a, b) developed transgenic lines resistant to ScYLV from a susceptible cv H62-4671 in Hawaii using the particle bombardment method. Two different transformation constructs were used, one with untranslatable CP gene of ScYLV in a sense orientation driven by a maize ubiquitin promoter while the other with *npt*II selectable marker under a sugarcane ubiquitin promoter. Based on viral titer and symptom phenotype, the transgenic lines were evaluated. Of nine transgenic lines, six exhibited ScYLV resistance with atleast 10^3 fold lower virus titer than the wild susceptible parent.

9.8.2.2 Other Diseases

Nine putative transgenics harboring the chitinase gene was tested against *Colletotrichum falcatum* and eight plants showed susceptible reaction whereas a single transgenic G11-1 showed partial resistance. Increased resistance was observed in transgenics GM-8 and GM-9, which co-expressed with Dm-antimicrobial peptide (Amp1) and chitinase. On the other hand two sugarcane transgenics P-2 and P-4 harbouring the genes chitinase and β-1,3-glucanase, respectively showed moderate tolerance to red rot (Kanchana 2007). The transgenic sugarcane lines of the cv CoJ 83 expressing *Trichoderma* spp β-1,3-glucanase gene exhibited tolerance to *C. falcatum* CF08 and CF09 pathotypes in glass house environment. The expressed gene in parenchymatous tissues in stalks inhibited fungal growth by lysis. Further, the expressed protein of β-1,3-glucanase gene sliced β-1,3-glycosidic bonds that causes damage to mycelia of *C. falcatum* (Nayyar et al. 2017). Further, transgenic lines with expression of *HarChit* encoding Chitinase and *HarCho* encoding Chitosanase are found to show strong inhibition against *C. falcatum* (Ijaz et al. 2018; Tariq et al. 2018). These studies clearly showed potential of the barley chitinase class-II genes to inhibit red rot pathogen in sugarcane stalk tissues.

9.8.3 Insect Pests Resistance in Sugarcane

Genetic transformation in sugarcane has helped to fortify a superior variety that already excels in most agronomic features but is susceptible to pests. Introduction of insecticidal genes through transformation enhances pest resistance in sugarcane thus maximizing and sustaining the crop yields even though IPM approaches complement the previously existing tolerance (Allsopp and Manner 1997; Allsopp and Suasaard 2000). Several genes that confer insect resistance have been found from various sources and effectively used in commercial sugarcane genetic transformation for pest management (Table 9.5). The widely exploited insect resistance genes include protease inhibitors, crystal toxins, lectins, secondary plant metabolites, proteins that inactivate ribosomal activity, and viruses. These genes are used either singly or in combination to generate commercially valuable insect resistant transgenic plants. Sugarcane transformed with proteinase inhibitor genes were resistant to grubs (Atkinson et al. 1993; Falco and Silva-Filho 2003; Nutt et al. 2001). Significant growth inhibition was observed in stalk borers reared on sugarcane transformed with lectin genes (Legaspi and Mirkov 2000). *Bt*-transformed sugarcane was found to be resistant to *D. saccharalis* (Arencibia et al. 1997; Wu et al. 2009). ELISA studies of the integrated Cry 1Aa 3 gene showed ten-fold increase in the level of expression (Kalunke et al. 2009). Borer larvae fed with transformed sugarcane possessing a gene coding for aprotinin suffered significant weight loss (upto 99.8%) which could be due to the cumulative antibiosis effect (Christy et al. 2009). Transgenic sugarcane lines over expressing Cry1F showed resistance to *C. infuscatellus* (Thorat et al.

2017). Arvinth et al. (2009, 2010) developed transgenic sugarcane expressing cry 1 Ab gene for management of shoot borer.

Transgenic sugarcane plants expressing both Cry1Ab and EPSPS were resistant to *D. saccharalis* and tolerated the herbicide Glyphosate but were agronomically poorer than the native sugarcane plants. Also, variations in the copy number of the target fragment in the transformants and expression of both of the target genes in less than 70% of the resistant plantlets (Wang et al. 2017a, b), probably due to exogenous gene silencing were the other issues.

Success of transformation and its inheritance in progeny plants are determined by the integration and expression of the desired gene in the genomic DNA of the plant. *Agrobacterium* mediated gene transfers in sugarcane by Dessoky et al. (2020) resulted in less than 25% of transgenics with varying levels of integration and the expression of cry1Ac gene. Only two transgenic sugarcane lines showed highest toxicity against the borer *S. cretica* at lower concentration of toxin, which may be due to single copy of the gene integrated. Different concentrations of endotoxin produced by each sugarcane transgenic line possessing *Cry1Ab- Cry1Ac* (Koerniati et al. 2020) could affect the efficacy against the target pest *Scripophaga excerptalis.* No differences in the morphological traits in the transformant sugarcane plants with resistant gene targeting *D. saccharalis* and the aphid *C. lanigera* were observed though the growth was slower compared to the non-transformed plants (Deng et al. 2008).

Work on transgenics for cane grub resistance is scarce. In Australia, the transgenics caused severe antibiosis in cane grub *Antitrogus consanguineus.* Grubs reared on sugarcane incorporated with a proteinase inhibitor gene attained <5% weight of those raised on non-transformed sugarcane. In yet another instance, *Dermolepida albohirtum* larvae on sugarcane transgenics with lectin gene attained less than 21%larval weight in controls (Nutt et al. 2001).

Despite the proven successes in transgenic sugarcane development, it remains a strenuous process involving rigorous and complicated procedures of tissue culture and regeneration that have to be standardized for each sugarcane genotype. Therefore, it is a tedious procedural challenge to standardize and evolve effective transformation protocols for every new sugarcane variety. Besides these, molecular protocols required for commercial release. Even after the transgenic event is achieved, due to genetic complexity and absence of a completelyanalysed reference genome for sugarcane, it is extremely difficult to execute the molecular studies to ascertain the number of copies, expression levels, insertion site and create other data for commercial release by regulatory authorities (Budeguer et al. 2021).

9.8.4 Safety of Transgenic Sugarcane

In Brazil, the earliest transgenic sugarcane variety (Bt sugarcane CTC175) expressing the Cry1Ab protein to manage *D. saccharalis* has been approved in 2018 for commercial production and distribution (ISAAA 2018a; Gianotto et al. 2018, 2019). US FDA

issued approval for Bt sugarcane from Brazil by declaring the sugar from such canes was not different that obtained from non-transformed varieties (ISAAA 2018b). Two more sugarcane events expressing cry1Ac gene (CTC91087-6 and CTC93209-4) have also been released in Brazil, recently (ISAAA 2021).

Sugar from Bt sugarcane has been proven to be safe for consumption (Gianotto et al. 2018). Sugar derived from genetically modified sugarcane was not found the products of introduced genes and was not any different from that from non-transgenic canes thus cultivating genetically modified varieties should be continue to maintain the way sugar is used as food source (Joyce et al. 2013; Cullis et al. 2014; Gianotto et al. 2018, 2019; Lajoloi et al. 2021). In South Africa positive consumer acceptance of sugar from genetically modified cane has been reported (Vermeulen et al. 2020).

Safety and impact of transgenic, specifically Bt-crops on non-target organisms have been studied in many crops (Abbas 2018; Marques et al. 2018) though such studies are scare in sugarcane. The Bt-transformed sugarcane did not have any negative impact on the structure or diversity of microbes or enzymes in the rhizosphere (Zhou et al. 2016). However, the proteins of the transformants may be toxic to parasitoids and may interfere with their ability to locate their hosts (Schuler et al. 1999).

9.9 Future Perspectives

9.9.1 Potential for Expansion of Productivity

As discussed in Sect. 9.1, increased cane productivity and sugar/ethanol production in the past 50 years was attributed to expansion of sugarcane area. However, we cannot ignore the genetic gains achieved through conventional breeding. Although outbreaks of different diseases or insect pests occurred in different continents, they have been contained by varietal replacements. Further, as discussed in the introduction, many countries experience yield plateaus attributed by pests and diseases, declining soil fertility and climatic conditions (Yadav et al. 2020). In India, varietal degeneration as the cause for decline in sugarcane productivity in many popular varieties was established. By this, yield potential of a variety comes down after few years in the field due to systemic accumulation of non-fungal pathogens causing RSD, YLD and mosaic (Viswanathan 2001a, 2016; Viswanathan and Balamuralikrishnan 2005; Viswanathan et al. 2014b). In addition, phytoplasmas causing SCWL and SCGS diseases affect productivity in the ratoons in almost all the countries in South and South East Asia (Rishi and Chen 1989; Nithya et al. 2020). The major fungal diseases like smut, red rot and wilt are tackled by host resistance, whereas, we could not manage the non-fungal diseases owing to various reasons. Lack of resistant sources, ignorance of impact caused by these pathogens, complication of disease management by disease spreading insect vectors and non-adoption of healthy seed programmes contribute to perpetuation of non-fungal pathogens hence poor cane

yield under field conditions. Recently, many success stories have emerged from different countries to manage YLD, WLD, RSD and other non-fungal diseases by adopting an integrated approach of obtaining healthy seed after meristem culture, molecular indexing of the mother plants or seed canes and heat treatment of seed canes and disease surveillance and monitoring under field conditions (Hanboonsong et al. 2021; Viswanathan et al. 2018d; Wongkaew and Fletcher 2004; Wei et al. 2019). By adopting ScYLV-free nursery programme, potential yield of 250 tonnes/ha was achieved under tropical India in the popular cv Co 86032 and the disease epidemic was managed (Viswanathan et al. 2018d). Such successful disease management programmes will address varietal degeneration due to non-fungal diseases and there is a scope of getting additional cane production of 60–80 million tonnes of canes in India without increasing cane area (Viswanathan 2018). Hence, vertical expansion of cane growth is the only way to meet increased demand for sugarcane in most of the countries. However, regular deployment of resistant varieties to different biotic constraints with improved cane and sugar yield potential is needed to take advantage of genetic gain in them.

9.9.2 Potential for Expansion into Non-traditional Areas

Raising demand for sugar and ethanol accelerated expansion of sugar industry across the continents. Hence, global sugarcane production witnessed three-fold increase during the last five decades however, it is contributed by the drastic increase in area of crop cultivation in the major cane growing countries like Brazil, India, China, and Thailand (Zhao and Li 2015a, b). Sugarcane needs copious water for its growth that too throughout the year. Hence, irrigation water availability throughout the growing season is a key for cane cultivation in many of the Asian countries. At this situation, expanding sugarcane in nontraditional areas will be difficult in these countries. Whereas cane cultivation is expanding in many African countries during the last 20 years by clearing forest land or reserving land for sugarcane from other land categories. Scope of rainfed sugarcane cultivation or availability of irrigation water from perennial water sources like Nile or other rivers favored cane area expansion. Many sugar estates in the continent realized good yield from virgin soil with high organic matter. In contrast, scope of expanding cane acreage is limited in many Asian regions due to land requirement for other crops, especially food crops. Further, the present cane area is also impacted by rainfall in counties like India, China and Thailand.

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