Chapter 3
Genomic Designing for Biotic Stress Resistance in Coconut

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Abstract Coconut (*Cocos nucifera* L.) is an economically important plantation crop grown widely in tropical and sub-tropical regions and coastal ecosystems worldwide. The impact of global warming on agriculture, in general, and perennials such as plantation crops, in particular, warrants the application of novel genomics-based approaches to safeguard the crops against abiotic and biotic stressors. Unlike the seasonal or annual crops, the damage of pests and diseases in coconut plantations is a serious threat to the coconut-based economy owing to the perennial nature of the crop. Against this backdrop, adopting genomic approaches for designing biotic stress tolerant coconut genotypes is inevitable. Coconut molecular breeding has witnessed the application of DNA markers in genetic diversity analysis and mapping of quantitative trait loci (QTLs). Further advancements in genome sequencing and transcriptome profiling have opened enormous avenues for utilising coconut-derived 'big data' in developing biotic stress-tolerant cultivars. This chapter discusses the important diseases and pests of coconut, genetic resources of coconut, approaches in

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conventional breeding to develop resistant genotypes, molecular mapping of resistance genes, QTLs and marker-assisted breeding, association mapping, glimpses of genome assemblies, and RNA-Seq approaches to develop disease and pest resistant genotypes.

Keywords Biotic stressors · *R*-genes · Molecular tree breeding · Phytoplasmal diseases · Coconut genetic sources

3.1 Introduction

Coconut (*Cocos nucifera* L.) is an economically important plantation crop grown widely in the humid tropical regions of the world. Coconut is cultivated around the globe, in not less than 90 countries, chiefly belonging to island and coastal ecosystems. Coconut production has been estimated to be 68,833 million nuts from an area of little over 12 million ha (ICC 2019). The coconut palm is fittingly called 'tree of life' (*Kalpavriksha*) because of its multitude of utilities ranging from nutrient-dense food and oil, therapeutically important virgin coconut oil (VCO), energy drink, inflorescence sap-based mineral-rich sugar, to other ancillary uses of the fiber, shell, timber for industrial purposes etc. The recent advocacy for nutrient diversity and nutritional security of the masses has opened up multiple avenues for the coconut as an invaluable health food with immense therapeutic potential.

Botanically, the coconut palm belongs to the family *Arecaceae*, sub-family *Coccoidea* and is a monotypic genus. Genetically, the crop is a diploid harboring 32 chromosomes ($2n = 2x = 32$). The genetic resources of coconut have been a critical component all these years, widely exploited in the process of conventional breeding to develop varieties with enhanced yield, productivity potential along with other agronomic features of abiotic and biotic stress tolerance (Niral et al. 2019). In general, breeding approaches of mass selection, hybridization, and elite palm selection to impart novel traits have been accomplished. The inherent traits of the palm, such as its perennial nature, heterozygosity and greatly extended juvenile phase and a requirement for suitable mass propagation techniques, have seriously hindered the varietal development programmes, especially to counter the biotic stresses and related exigencies (Arunachalam and Rajesh 2008, 2017).

Diseases and pests of coconut are major production constraints in addition to the climate-change-induced vagaries. Therefore, breeding for disease and pest resistance in coconut has been a main focus area of research in breeding programs across the globe. Among the diseases, phytoplasma causing lethal yellowing and root (wilt) diseases and fungal diseases such as bud rot, basal stem rot, stem bleeding, etc., are major stressors with serious implications on coconut productivity. Coconut breeding approaches worldwide have taken up the development of phytoplasma resistant varieties as a major thrust area followed by introgression of resistance against fungal diseases (Thomas et al. 2018). Further, the palm is infested by several pests of economic importance, namely rhinoceros beetle, red palm weevil, eriophyid mite, black-headed caterpillar and emerging or invasive pests like rugose spiralling whitefly. Efforts have been made to develop resistant varieties or identify resistant genetic sources of coconut to withstand pest pressure (Josephrajkumar et al. 2018a, b, c, d; Nampoothiri and Parthasarathy 2018).

Applications of DNA-based molecular markers have made rapid strides in the annual crops, and varieties have been developed to shield them against pests and diseases. The advent of these markers has supported genetic diversity analysis and mapping QTLs of economic and agronomic importance in coconut (reviewed by Rajesh et al. 2018, 2021a). Further, technological advancements in genome sequencing have greatly aided in generating three good quality genome assemblies of coconut depicting genetically diverse genotypes (Xiao et al. 2017; Lantican et al. 2019; Rajesh et al. 2020). In this context, this chapter provides glimpses of major diseases and pests of coconut, breeding efforts in developing varieties to withstand disease and pest attacks and application of genomics-assisted breeding in designing biotic stress tolerance in coconut.

3.2 Diseases of Coconut

A coconut-based cropping system warrants maximization of the returns by incorporating multiple crops and other components in a diversified manner. The concept of integrated disease management (IDM) requires adopting economically viable, ecologically safe and agronomically feasible approaches to ward off the diseases. Extensive research approaches are being evaluated in the research farms and on-field trials to ensure the safety and sustainability of integrated approaches to manage coconut diseases. The various economically important diseases of coconut are as follows:

3.2.1 Leaf Rot

Leaf rot, caused by pathogenic fungi, generally occurs along with root (wilt) diseased palms (Srinivasan 1991). The annual economic loss was estimated to be around Rs. 5.6 million (Menon and Nair 1948) due to the loss of 461 million nuts (Joseph and Rawther 1991). Extensive rotting of leaf tissue is preceded by the formation of water-soaked lesions that enlarge and coalesce. The rotten distal regions of the leaflets fuse to give a ‘fish bone-like appearance’, and these regions drop off after drying. This disease causes a severe reduction in the photosynthetic efficiency of the palms, thereby reducing the yield and attracting insect pests. It is caused by the fungi *Colletotrichum gloeosporioides* (Penzig) Penzig and Sacc., *Fusarium* spp. and *Exserohilum rostratum* (Drechsler) Leonard and Suggs (Srinivasan and Gunasekaran 1996). Disease management warrants that spindle leaf alone requires protection due to its increased susceptibility. Further, application of fungicide hexaconazole 5 EC on leaf rot affected palms and applying neem cake-sand mixture in leaf axils to control rhinoceros beetle are found to be effective in managing the disease. Moreover, various economic and environmentally feasible methodologies and integrated approaches have been adopted to manage the disease and the insect pests (Koshy 2000a; Koshy et al. 2002). Though palms in the early stages of infection recover, disease in the advanced stage may take over three years to recover following the recommended IDM practices completely.

3.2.2 Bud Rot

Bud rot of coconut is a disease of sporadic nature; however, epidemic scale outbreaks have also been recorded. Though the disease occurs in palms of all the growth stages, young palms are more susceptible and, therefore, severely affected. The disease was first reported in Grand Cayman in 1834. The disease has since been reported in many countries, including Cuba, India, The Philippines, etc. (Menon and Pandalai 1958; Child 1974; Renard and Darwis 1993). In India, bud rot occurs in coastal regions (Menon and Pandalai 1958; Radha and Joseph 1974; Sharadraj and Chandramohanan 2013; Chandran et al. 2017). In Côte d'Ivoire, infections have killed even up to 50% of the palms depending on the prevailing climatic conditions and the nature of the planting materials (Quillec and Renard 1984). Characteristic symptoms of the disease include spindle withering into pale color, or brown color, bending over of the spindle, rotting of internal tissues which emit a foul odor, slowly affecting the inner leaves and leaving only the matured leaves of the crown. Consequently, the palm succumbs (Menon and Pandalai 1958; Lingaraj 1972). Bud rot is caused by fungal pathogens, *Phytophthora palmivora* Butl., *P. faberi* Maubl. and *P. heveae* (Butler 1906; Shaw and Sundararaman 1914; Joseph and Radha 1975). The primary dry rot caused by *P. palmivora* is colonized by secondary invaders viz., *Fusarium* sp., *Xanthomonas, Pseudomonas* and *Erwinia,* causing wet rotting (Radha and Joseph 1974). Besides, *P. katsurae* was also identified as causal agent of bud rot of coconut (Thomas et al. 1947; Uchida et al. 1992; Chowdappa et al. 2003). Further, a significant inter and intra-specific variability among the *Phytophthora* spp. infecting coconut in India was recorded (Sharadraj and Chandramohanan 2016).

The disease is controlled by adopting IDM practices which involve improving the drainage, effective weed control, increasing the plant spacing, immediate removal of disease affected palms, removal of infected spindle leaf during the early stage, treatment of wound with chlorothalonil 75% WP. Prophylactic measures such as application of Bordeaux mixture around the base of the spindle and placing perforated sachets containing chlorothalonil 75WP (2 sachets palm^{-1}; 3 gm chlorothalonil sachet^{-1}) are also recommended. Also, antagonistic fungi such as *Trichoderma* sp., *Myrothecium roridum* and *M. verrucaria* have been effectively utilized to arrest the growth of *P. palmivora* and *P. katsurae.* Placement of *Trichoderma* coir pith cake in the inner whorls of coconut leaves is also an effective strategy (Chandramohanan et al. 2013). Besides nutrient management, appropriate irrigation scheduling and replanting of affected palms are necessary to ward off the disease. Growing of tolerant genotypes (Rennel Island Tall and local Indonesian talls, hybrids of Malaysian Yellow Dwarf × Rennel Island Tall or Local Tall, Red Dwarf × Tall coconut hybrids) is an alternate strategy (Mangindaan and Novarianto 1999).

3.2.3 Stem Bleeding

Stem bleeding of coconut is a disease of tropics reported in Sri Lanka (Petch 1906), India (Sundararaman 1922) and other countries like The Philippines, Malaysia, Trinidad, Papua New Guinea, Fiji, Ghana and Indonesia (Menon and Pandalai 1958; Renard et al. 1984). The disease has been reported recently in Brazil and China (Warwick and Passos 2009; Yu et al. 2012). Even though yield loss during the early stages of the disease is very minimal, enormous yield loss and even death of palms occur during later stages (Nambiar and Sastry 1988; Chandran et al. 2017). The characteristic symptoms of the disease are the appearance of dark colored patches at the base of the tree trunk, which progresses upwards, leading to longitudinal cracks on the bark exuding dark liquid. Eventually, these exudates dry up to form black encrustations having brownish-orange margins and tissue underneath and beyond the external encrustations starts to decay. The disease severity in young palms is high, causing considerable yield reduction and death of palms (Ohler 1984; Nambiar and Sastry 1988). Crown symptoms, characterised by premature yellowing of the outer whorl of leaves, are also common during summer and eventually dry up. Nut fall was found to be pronounced in palms that are exposed to drought-like situations. Reduction in crown size is also observed as the main trunk tapers towards the apex. The disease is caused by the fungus *Thielaviopsis paradoxa* (de Seynes) von Hohnel. It's perithecial stage [*Ceratocystis paradoxa* (Dade) Moreau] was also found associated with the disease (Menon and Pandalai 1958; Ohler 1984; Nambiar et al. 1986a, b). At times, the infection is further aggravated due to the infestation with *Diocalandra* weevil causing deterioration of the health of palms. The disease could be effectively controlled following IDM practices, viz., application of paste of talc-based formulation of *T. harzianum* CPTD 28 on bleeding patches, soil application of *Trichoderma harzianum* CPTD 28 (100 g) mixed with neem cake (5 kg), FYM (50 kg), and NPK fertilizer (500; 320; 1200 gm palm^{-1} year^{-1}). Summer irrigation, and avoiding any injuries to palm trunk, are important measures to control the disease. Genotypes such as Banawali Green Round Tall, Banawali Brown Round Tall and Malayan Orange Dwarf were found to be less susceptible to the disease. In contrast, Malayan Green Dwarf, Chowghat Orange Dwarf and Philippines Ordinary Tall were found to be more susceptible.

3.2.4 Ganoderma Wilt/Basal Stem Rot

Ganoderma wilt or basal stem rot or *Thanjavur* wilt is one of the most destructive diseases in coconut, first reported in the Indian state of Tamil Nadu in 1952 (Vijayan and Natarajan 1972). The fungal pathogen *Ganoderma lucidum* was associated with the disease in other parts of the country (Venkatarayan 1936; Nambiar and Rethinam 1986; Wilson et al. 1987). *G. boninense* Pat. was known to be linked with the disease in Sri Lanka (Peries 1974). In general, palms of 10–30 years old are more susceptible to the disease (43%) than younger ones (Vijayan and Natarajan 1972). The disease progresses with typical symptoms spread over five distinct stages: leaflet wilting, leaflet yellowing, decay and death of fine roots. In the next stage, bleeding starts from the base of the stem, with the lesions spreading upwards, concomitant with extensive root decay and declined or nil bunch production, button shedding and development of barren nuts. Progression of stem bleeding further causes the drooping and drying of leaves of outer whorls, and ultimately all the leaves dry up and stem shrivels. Further, the infection process is aggravated due to the infestation by scolytid beetle, *Xyleborus perforans* and the weevil, *Diocalandra stigmaticollis,* ultimately causing the death of the palm (Anonymous 1976; Rethinam 1984; Bhaskaran 1986). Occurrence of the disease could be detected before the expression of typical symptoms utilizing several methodologies such as colorimetric detection (Natarajan et al. 1986), analysis of physiological parameters like transpiration rate and stomatal conductance or resistance (Vijayaraghavan et al. 1987), and PCR-based detection (Karthikeyan et al. 2006; Rajendran et al. 2014) of *Ganoderma lucidum.*

To control the disease, integrated approaches are followed, including cultural practices that avoid pathogen establishment. Some of the common practices adopted are avoiding hardpan formation in the sub-soil region, avoiding water stagnation during monsoon, and applying summer irrigation (Rao and Rao 1966; Anonymous 1976; Ramasami et al. 1977; Satyanarayana et al. 1985). Growing indicator plants such as *Leucaena leucocephala* and *Gliricidia maculata* is an effective prevention strategy as these species are affected well before the palms show infection (Anonymous 1989). Further, the EDTA test also aids in the early detection of the disease (Natarajan et al. 1986; Vijayaraghavan et al. 1987). The IDM practices standardized by Coconut Research Station, Veppankulam, Tamil Nadu and Agricultural Research Station, Ambajipet, Andhra Pradesh (Anonymous 1990) suggest multiple approaches such as clean crop management practices, regular basin irrigation during summer months, avoiding flooding, application of organic manures, neem cake (5 kg palm^{-1} year^{-1}) fortified with *Trichoderma harzianum* (CPTD 28) talc formulation (50 g palm^{-1} year^{-1}), growing banana as intercrop and soil drenching of Bordeaux mixture thrice annually (1%, 40 L). Root feeding with 1% Hexaconazole 5EC and soil application of *Trichoderma harzianum* (CPTD 28) enriched neem cake @ 5 kg palm^{-1} at quarterly intervals, or soil application of *T. harzianum* (CPTD 28) enriched neem cake @ 5 kg palm^{-1} at three months interval followed by maintenance of moisture and mulching around palm basin were found very effective in the management of disease (Prathibha et al. 2020).

3.2.5 Immature Nut Fall

Immature nut fall is one of the common diseases observed in coconut plantations. The disease has been ascribed to various factors such as poor mother palm selection, extreme soil reactions (high acidity or high alkalinity), poor water management leading to drought or waterlogging conditions, imbalance in plant nutrition and poor pollination (Smith 1969; Ohler 1984; Prasada Rao 1988). Also, infestation due to eriophyid mite (*Aceria guerreronis* Keifer) is one of the prime reasons for nut fall besides encouraging secondary infections due to the fungus causing rot (ChandraMohanan and Baby 2004). Environmental factors like relative humidity and minimum temperature are also attributed to the disease (Venugopal and ChandraMohanan 2010). Nut fall or fruit rot is caused by *P. palmivora* (Butl.) and *Lasiodiploida theobromae* Pat. *P. meadii* Mc Rae has also been found to cause the immature nut fall for the first time in the Kodagu district of Karnataka State, India (Chowdappa et al. 2002). The disease can be managed by removing all the infected nuts from the palm and spraying with Bordeaux mixture 1% to the bunches two sprays at 30 days interval depending on the severity of the disease.

3.2.6 Grey Leaf Spot

Grey leaf spot is prevalent in all coconut growing regions, affecting young (nursery) seedlings and adult palms. In the latter, the yield reduction due to disease has been reported to be around 10–24% (Karthikeyan 1996). Further, delayed flowering is also observed when the disease infects the palms (Abad 1975). Minute yellow spots with gray margins appear on the outer whorl of old leaves, which later coalesce to provide a burnt appearance. Under severe conditions, drying and shrivelling of leaves occur, causing a 'blight' appearance. The pathogenic fungi *Pestalotiopsis palmarum* (Cooke) Steyaert has been identified as the causal agent of blight disease. Later, the application of molecular techniques enabled Maharachchikumbura et al. (2012) to describe several species of this pathogen. Incidence of the disease suggests the poor nutritional status of the palms either due to deficiency of potash or excess nitrogen. As a control measure, integrated nutrient management (INM), application of Bordeaux mixture or any copper fungicides or carbamates is suggested. Further, KCl application also significantly reduces disease incidence.

3.2.7 Lasiodiplodia *Leaf Blight of Coconut*

Lasiodiplodia leaf blight of coconut has been reported from almost all the major coconut growing countries such as Trinidad, Brazil, Malaysia, Sri Lanka and India (Ram 1993; Bhaskaran et al. 2007; Monteiro et al. 2013). The disease incidence severely weakens and causes the death of the palms growing in soils lacking drainage or under water stress and imbalance of nutrition. The fungus also infects seed coconuts (Raju 1984). Leaves and nuts are affected where the former appears as charred or burnt due to drying followed by apical necrosis of lower leaves, giving an inverted "v" shape and reminiscing the effects of water-deficit stress. The fungus causes systemic invasion and induces internal necrosis (Souza-Filho et al. 1979). A small black sunken region appears near the perianth of immature nuts. The nuts attacked by eriophyid mite are infected by the pathogen and cause rotting and shedding of immature nuts (Venugopal and Chadramohanan 2006). The fungus *Lasiodiplodia theobromoae* (Pat.) Griffon and Maubl causes the disease. Though 20 species have been identified based on conidial and paraphysis morphology, it was observed that the causal fungus is a complex of cryptic species (Alves et al. 2008). The disease could be effectively controlled by following phytosanitary measures such as (i) removal and burning of severely affected leaves to avoid further spread of inoculum; (ii) application of *Pseudomonas fluorescens* (200 g) along with FYM (50 kg) + neem cake (@5 kg palm^{-1} year^{-1}); (iii) spraying of Hexaconazole 5EC or copper oxychloride (0.25%) two times at 45 days interval during summer months; (iv) root feeding with Hexaconazole 5EC @ 2 ml in 100 ml of water at 3 month intervals.

3.2.8 Phytoplasmal Diseases of Coconut

Phytoplasma refers to small cell wall-less bacteria but enveloped by a single membrane and are known to cause various diseases in palms that are known by their characteristic symptoms. The advent of molecular detection of plant pathogens has greatly aided in the taxonomic characterization of many phytoplasmas associated with diseases of coconut.

3.2.8.1 Root (Wilt) Disease

Root (wilt) disease is an economically important, non-lethal yet debilitating disease of coconut. The economic losses due to husk damage and the decline in copra yield have been estimated to be around Rs. 3000 million. The disease was first reported in 1882 in the Kottayam district of the Indian State of Kerala. Later, several researchers have documented root (wilt) disease in Kerala (Butler 1908; Pillai 1911; Menon and Pandalai 1958; Koshy 1999). The spread of the disease received wide attention as it infects an area of 0.41 million ha in a contiguous manner in Kerala and in certain regions of Tamil Nadu, Goa and Karnataka (Solomon et al. 1999a, b; Koshy 2000a, b; Koshy et al. 2002). Further, the disease intensity survey had revealed that severity ranges from 2.1% (in Thiruvananthapuram district) to 48.0% (Alappuzha district) (Solomon et al. 1999a, b). Though earlier reports by Mathew et al. (1993) recorded a decline in yield of 45–60%, in West Coast Tall variety and D × T hybrids, respectively yield reduction of nuts to the extent of 80% is not uncommon when the disease is in advanced stage (Radha et al. 1962; Ramadasan et al. 1971). The characteristic foliar symptoms of the disease include flaccidity (in 67–97% palms), yellow discoloration (38–67% palms) and marginal necrosis (28–48% palms) of the leaflets (Varghese 1934; Menon and Nair 1952; Menon and Pandalai 1958). Further, the expression of disease symptoms varies with the age, variety, nutritional status of the palm, and crop management practices. In addition, inflorescence necrosis characterized by the lack of female flowers and sterile pollen and immature nut shedding are some of the symptoms (Varghese 1934; Varkey and Davis 1960). As the name suggests, root rotting or decay is another important symptom observed in more than 50% of the main roots (Butler 1908). Root decay may vary from 12 to 95% depending on the disease intensity (Michael 1964). Besides reducing the number of roots, degeneration of root anatomy, physiological aberrations and impaired water uptake are observed (Davis 1964; Michael 1964; Ramadasan 1964; Indira and Ramadasan 1968; Govindankutty and Vellaichami 1983). Multiple investigations based on electron microscopy, vector transmission studies have established phytoplasma as the causal agent of the disease (Solomon and Govindankutty 1991a, b). *Stephanitis typica* and *Proutista moesta* were reported as the insect vectors of the disease (Mathen et al. 1990; Anonymous 1997). Also, tetracycline treated trees exhibited remission of symptoms corroborating the phytoplasmal etiology of the disease. Manimekalai et al. (2010) reported that 16Sr XI group phytoplasma is associated with diseased palms.

Management of root (wilt) disease is cumbersome due to factors such as the perennial nature of the palms, pathogen persistence, and easy transmission due to vectors. Being a debilitating disease, crop management practices have attained immense importance; hence diverse strategies have been formulated for heavily and mildly infected areas (Anonymous 1986; Muralidharan et al. 1991). In the heavily infected areas, management of leaf rot, application of appropriate fertilizer dose, inclusion of organic manures, summer irrigation, intercropping with cassava, elephant foot yam and greater yam (Menon and Nayar 1978) and mixed farming approaches are suggested. In the mildly affected regions, removal of all the diseased palms by following systematic surveillance, adoption of appropriate disease detection tests including DAC ELISA (Sasikala et al. 2001, 2004) before the appearance of visual symptoms are recommended. It is followed by replanting with disease-free seedlings.

3.2.8.2 Lethal Yellowing

Lethal yellowing (LY) is an important disease that threatens the cultivation of coconut worldwide. The disease was initially documented in Grand Cayman Island in 1834 and Jamaica in 1884. However, at present, LY severely constrains the production potential of palms in the Southern United States, Central America and Caribbean region and east Africa (Harrison et al. 2014). The disease was known differently in diverse geographic regions: Cape St. Paul Wilt in Ghana, Kribi disease in Cameroon, Kaincope disease in Togo, Awka disease in Nigeria, lethal decline in east African countries (Brown et al. 2007). It affects coconut palms of all ages, and palms succumb within six months of the onset of symptoms (McCoy et al. 1983; Been 1995).

The characteristic symptoms of the disease include premature nut fall. The second stage is characterized by inflorescence necrosis followed by yellowing of fronds in the third stage. In this process, the death of the bud happens, and the emerging spear leaf will collapse. In the last or fourth stage of the disease, complete defoliation of the palm causes its decapitation (Brown et al. 2008; Harrison et al. 2014). Initially, electron microscopy and PCR-based detection identified phytoplasma as a causative agent (Heinze et al. 1972; Plavsic-Blanjac et al. 1972). Advancements in the PCR-based assays and serological analysis have helped to characterise the coconut LY group of phytoplasmas as belonging to the members of group 16SrIV having four subclades (16SrIV-A, 16SrIV-B, 16SrIV-C and 16SrIVD). Since phytoplasma is phloem limited, the cixiid, *Haplaxius* (*Myndus) crudus* was known to act as a vector that spreads disease in Florida (Howard et al. 1983). However, in Jamaica, the role of cixiid *Haplaxius* (*Myndus) crudus*, in the disease transmission could not be confirmed (Schuiling et al. 1976; Eden-Green 1978; Eden-Green and Schuiling 1978; Dabek 1974). Even though PCR detection of LY DNA in embryos was proven, seed transmission of this pathogen has not been proven unequivocally (Cordova et al. 2003). As a disease management strategy, clean cultivation practices, crop sanitation and prevention of the spread of insect vectors, and removal of weed hosts are critical (Lee et al. 2000). The application of oxy-tetracycline-HCL also suppresses LY symptoms (McCoy et al. 1976). Cultivating resistant cultivars MayPan hybrid (Malayan Dwarf × Panama Tall) in Jamaica has been an effective strategy; however, resistance breakdown reported in these genotypes is a concern (Wallace 2002).

3.2.8.3 Coconut Yellow Decline (CYD)

CYD is a debilitating disease first reported in Malaysia by Sharples in 1928, which considerably reduces the productivity of coconut in Malaysia. Nejat et al. (2009a) reported classic phytoplasmal symptoms such as yellowing and drying of fronds in Malayan dwarf ecotypes found in Selangor State in Malaysia. Therefore, the disease was called ‘coconut yellow decline’ (CYD). Leaves of the outer whorls show yellowing which gradually becomes light brown. Later the younger leaves become symptomatic, and the spear leaf also shows chlorotic symptoms. Further, premature nut fall and necrosis of inflorescence is observed. As the disease progresses, fronds collapse and rotting of the growing tip of palms occur, ultimately causing the death of palms (Nejat et al. 2009a). CYD in Malaysia was found to be caused by Bermuda grass white leaf group (16SrXIV, ‘*Candidatus* Phytoplasma cynodontis’ group) (in Malyan Red Dwarf and Malayan Tall palms), and *Candidatus* Phytoplasma malaysianum (16Sr XXXII-B) (in Malayan Yellow Dwarf) (Nejat et al. 2009a, b, 2013). A real-time PCR assay was developed for quantitative and rapid detection of the 16Sr XXXII-B (Nejat et al. 2010).

3.2.8.4 Tatipaka Disease

Tatipaka disease is a non-fatal but debilitating disease of coconut palms endemic in the east and west Godavari, Srikakulam, Nellore, Krishna and Guntur districts of Southern India (Rethinam et al. 1989; Rajamannar et al. 1993). Relatively young palms (below 20 years) are not generally affected, and also the spread of disease is sporadic (Solomon and Geetha 2004). The typical symptoms include a considerable reduction in the number and size of leaves, presence of chlorotic water-soaked spots on the leaves, abnormal bending of fronds and a marked reduction in crown size. The fasciated appearance of leaves is also a characteristic symptom. The bunches comprise both the normal and abnormal nuts, and the atrophied nuts are generally barren and at times ooze gummy exudates (Ramapandu and Rajamannar 1981). Sap transmission studies and electrophoretic analysis of DNA ruled out the possibility of virus or viroid infection (Rajamannar et al. 1984; Randles and Hatta 1980). Electron microscopy coupled with Dienes stain and fluorescence microscopy analysis of roots, meristem, petioles of developing leaves and rachilla of the tender inflorescence of diseased palms revealed the presence of phytoplasma in the sieve tubes (Rajamannar et al. 1993). There are no prophylactic or curative measures for the disease; hence diseased palms are to be removed immediately to arrest the spread of the disease.

3.2.8.5 Weligama Wilt Disease

Weligama wilt disease was first recorded in the Weligama region in the Matara district of Sri Lanka (Wijesekara et al. 2008; Perera et al. 2010). However, currently, the disease is prevalent in other districts of Sri Lanka (Perera et al. 2012). Flattening and bending of leaflets leading to flaccid appearance are the earliest symptoms. Palm crowns appear dark green, especially in the younger leaves, and it becomes prominent when the leaves are completely opened. Further, intense yellowing of lower whorls of leaves is also a characteristic feature of this disease. Drying up of fronds followed by leaf falling or ragged appearance of the crown occurs during later stages. Due to the reduced number of fronds, the palm crown becomes smaller, and trunk tapering happens. With the progression of the disease, female flower production declines and the productivity of the palm is severely affected (Wijesekara et al. 2008; Perera et al. 2010, 2012). Phytoplasma belonging to 16SrXI *Candidatus* Phytoplasma *oryzae* group has been reported as the causal agent of the Weligama wilt in Sri Lanka (Perera et al. 2012). Molecular detection of the infected palms is possible, and hence the removal of the diseased palms is advocated as a containment strategy.

3.2.8.6 Lethal Wilt Disease

Lethal wilt disease (LWD) of coconut, reported recently from East Coast of Tamil Nadu State of India, is another concern to the coconut farmers. The primary symptom of the disease is abnormal shedding of nuts, which is followed by inflorescence necrosis and yellowing of outer whorls of leaves. The foliar yellowing progresses to inner whorls and subsequently chlorotic leaves turn brown and necrotic. As the disease advances, necrosis and rotting of spear leaves and bud region occur. Affected palms die within 3–5 months leaving a bare trunk. The phytoplasma associated with LWD has been identified as '*Ca.* P. asteris'-related strain belonging to subgroup 16SrI-B (Babu et al. 2021). Since the disease is confined to a limited area, periodic surveillance and eradication of diseased palms form the primary management strategy.

3.2.9 Diseases Caused by Viruses and Viroids

3.2.9.1 Coconut Foliar Decay or Vanuatu Wilt

Coconut foliar decay (alternatively foliar decay *Mindus taffini* or New Hebrides coconut disease) is a disease known to occur in the introduced palms of the Malayan Yellow Dwarf in Vanuatu. The name *Mindus taffini* is derived from the plant hopper that transmits the disease.The local cultivars Vanuatu tall and Vanuatu Dwarf, though carriers, remain asymptomatic (Randles et al. 1992; Hanold and Randles 2003). Yellowing of leaves of positions 7–11 from spear leaf is the initial symptom, and as the yellowing spreads, the fronds break near the base and hang down. These symptoms happen in younger leaves too when they reach the position anywhere from 7 to 11. With the progression of the disease, the trunks tapers towards the top, and palms die in 1 or 2 years. The disease is caused by coconut foliar decay virus (CFDV) belonging to the family *Nanoviridae* (Randles et al. 1986) and transmitted by a planthopper *Myndus taffini* Bonfils (Cixiidae). Interestingly the virus and vector remain confined to the Vanuatu archipelago. Growing of tolerant cultivars (Vanuatu tall or the hybrid, Vanuatu Tall × Vanuatu Red Dwarf) is suggested as a disease management strategy. Further, FAO/IBPGR *Technical Guidelines for the Safe Movement of Coconut Germplasm* warrants the movement of coconut embryos in a sterile medium. The parts of the mother plant must be screened for the presence of virus or viroid before the transport of material.

3.2.9.2 Viroid Diseases

Coconut cadang-cadang and Tinangaja are two viroid diseases documented in the coconuts grown in The Philippines and Guam, respectively. Coconut Cadang-cadang is a lethal disease-causing severe economic losses reported from southern Luzon in The Philippines (Randles et al. 1987). In general, palms that have attained the flowering stage are affected, and infection of young palms is a rarity. During the early stage of the disease (2–4 years), the young nuts become more rounded, and equatorial scarifications are observed. Inflorescence shows a stunted appearance, and chlorotic spots characterize leaves. In the mid-stage of the disease (about two years), production of spathe, inflorescence and nut production decline but leaf spots become more prominent. In the late stage (5 years), leaf spots coalesce to result in chlorosis, the number and size of fronds decline, crown size is markedly reduced, and palm dies. The etiological agent of the disease has been identified as coconut cadang-cadang viroid or CCCVd (Hanold and Randles 1991). Even though no insect vector has been identified with the spread of the disease, viroid transmission in a small percent of progeny palms was observed when pollen from infected palms was used. The mode of natural transmission in the field is not known. CCCVd was also successfully transmitted to palms through contaminated harvesting scythes. Currently, there are no control measures to eradicate the disease; however, growing resistant palms, strict quarantine, and clean cultivation habits are suggested. The major diseases infecting coconut are described in Table 3.1.

3.3 Pests of Coconut

Coconut is infested by a wide array of pests, including 830 insect and mite species and 78 nematode species causing a serious decline in productivity (CPCRI 1979). Further, insect, mite and vertebrate pests in coconut result in a crop loss to the tune of 30% in the palm (Gitau et al. 2009). Damage due to the pest complex in Kerala State, India, has been estimated to be 618.50 million nuts annum^{-1}, suggesting the severity and extent of infestation (Abraham 1994). The pests of coconut could be classified as borers, defoliators, sap feeders, subterranean pests and emerging pests. The major pests infesting coconut are described in Table 3.2.

Besides the above-stated pests, other pests of importance are *Darna* (*Macroplectra*) *nararia* Moore (Limacodidae: Lepidoptera), *Parasa lepida* (Cramer); spiralling whitefly *Aleurodicus dispersus* Russell (Aleyrodidae: Hemiptera); and scale insects. Also, burrowing nematode (*Radopholus similis*), lesion nematode (*Pratylenchus coffeae*) and red ring nematode (*Rhadinaphelenchus cocophilus)* causing red ring disease and root-knot nematode (*Meloidogyne* spp.) are found to be pests of importance in the coconut ecosystem (Koshy 1986a, b).

Table 3.1 Major diseases infecting coconut and their causative pathogens

Sl. No.	Diseases	Causative agent(s)	Occurrence	References
I. Major fungal diseases				
1	Bud rot disease	*Phytophthora palmivora, P. heveae, P. katsurae P. nicotianae, Fusarium moniliforme, F. solani, Graphium* sp.	India, Côte d'Ivoire, Indonesia, Jamaica, Puerto Rico, Africa, Peninsular Malaysia and The Philippines	Butler (1906), Menon and Pandalai (1958), Quillec et al. (1984), Uchida et al. (1992)
2	Basal stem rot	*Ganoderma lucidum, G. applanatum, G. zonatum, G. boninense*	India, Florida, South America, Java, tropical Africa, Australia, Japan, Indonesia, Malaysia, The Philippines, Samoa, Sri Lanka and Tasmania	Peries (1974a), Satyanarayana et al. (1985)
3	Stem bleeding	*Thielaviopsis paradoxa/Chalara paradoxa*	Sri lanka, India, Indonesia, Malaysia, The Philippines, Fiji, Ghana, Trinidad	Petch (1906), Sundararaman (1922)
4	Leaf rot	*Exserohilum rostratum/Colletotrichum gloeosporioides/Fusarium solani* and *Fusarium moniliforme*	India	Varghese (1934), Menon and Pandalai (1958), Radha and Lal (1968), Srinivasan and Gunasekaran (1999)
5	Grey leaf blight	*Pestalotiopsis palmarum*	Guyana, India, Malaysia, New Hebrides, Sri Lanka, Trinidad, Nigeria	Copeland (1931), Cook (1971), Holliday (1980)
6	Leaf blight	*Lasiodiplodia theobromae*	India	Johnson et al. 2014
II. Phytoplasmal diseases				
1	Lethal yellowing	16Sr IV group Phytoplasma	Western Jamaica, Cuba, southern USA (Florida and Texas), southern Mexico	Nutman and Roberts (1955), Ashburner et al. (1996), Harrison et al. (1994, 2002), Myrie et al. (2007)

(continued)

Table 3.1 (continued)

Sl. No.	Diseases	Causative agent(s)	Occurrence	References
2	Root (wilt) disease	16Sr XI group Phytoplasma	India	Solomon et al. (1983), Manimekalai et al. (2010)
3	Coconut lethal disease	16Sr IV group Phytoplasma	Tanzania, Kenya, Mozambique	Schuiling et al. (1992), Mpunami et al. (1999)
4	Tatipaka disease	Phytoplasma	India	Rethinam et al. (1989)
5	Weligama wilt	16Sr XI group Phytoplasma	Sri Lanka	Perera et al. (2010)
6	Coconut yellow decline	16Sr XIV and 16Sr XXXII-B group Phytoplasma	Malaysia	Nejat et al. (2009a, b)
III. Virus diseases				
1	Coconut foliar decay or Vanuatu wilt	*Coconut Foliar decay virus* (CFDV)	Vanuatu	Calvez et al. (1980), Randles et al. (1986)
IV. Viroid diseases				
1	Coconut Cadang-cadang disease	*Coconut cadang-cadang viroid* (CCCVd)	Philippines	Randles (1975)
2	Coconut Tinangaja disease	*Coconut tinangaja viroid* (CtiVd)	Guam on Marianas Island	Boccardo (1985)
V. Protozoan diseases				
1	Fatal wilt or Heart Rot or Hartrot	*Phytomonas stahellii*	Central America (Costa Rica, Honduras and Nicaragua), South America (Brazil, Colombia, Ecuador, Guyana, Peru, Surinam and Venezuela) and the West Indies (Grenada, Trinidad and Tobago)	Waters (1978), Dollet (1984)

Table 3.2 Major pests infesting coconut, the damage symptoms and their control measures

Sl. No.	Pests (common name)	Biological name	Crop loss, symptoms and damage	Control measures	References
I. Borers					
1	Asiatic rhinoceros beetle	*Oryctes rhinoceros* Linn. (Coleoptera: Scarabaeidae)	• Central crown damage levels of 40–60%, 60–80% and 80–100% cause 12, 17 and 23% nut losses, respectively • Adults bore unopened spear leaves, spathes and tender nuts. When injured spindles open up, the green leaves present a geometric 'V'-shaped cut pattern • In juvenile palms, stunted growth and delayed flowering • In freshly transplanted coconut seedlings, infestation causes the wilting and improper establishment • Beetle attack provides egg-laying sites for red palm weevil and entry of fungal pathogens	• Regularly monitor and identify any damage on the spear leaf of the juvenile palm or at the collar region of seedlings • Hook out the beetle if any chewed up fibres are noticed from the leaf axils • In juvenile palms, place naphthalene balls (4 g/axil) and fill with sand or place chlorantraniliprole (0. 4% GR) or fipronil (0.3% GR) (3 g in perforated sachet/ axil) on top most 3–4 leaf axils • In adult palms, fill the top most 3–4 leaf axils with 250 g neem cake/maroti cake/Pongamia cake mixed with an equal volume of sand • Incorporate *Clerodendrum infortunatum*, a weed, into the manure pits to induce larval-pupal abnormalities in feeding grubs • Apply entomopathogenic green muscardine fungus (*Metarhizium majus*) into the breeding pits @ 5×10^{11} spores/m^3 • Release 10–12 virosed (*Oryctes rhinoceros* nudivirus) beetles for auto-transmission of nudivirus	Ghosh (1911), Pillai (1919), Nirula (1955b), Howard et al. (2001); Rajan et al. (2009), Josephrajkumar et al. (2015), Mohan et al. (2018), Josephrajkumar et al. (2016, 2019c)

(continued)

Table 3.2 (continued)

Sl. No.	Pests (common name)	Biological name	Crop loss, symptoms and damage	Control measures	References
2	Red palm weevil (RPW)	*Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae)	• RPW attack juvenile palms of age less than 20 years • All life-stages are confined within the infested palms with an action threshold determined as 1% • Internal feeder and hence difficulties in early diagnosis • Yellowing and wilting of the inner and middle whorl of leaves. Holes and tunnels trunk, ooze out a brown viscous fluid, emanating fermented odour	• Early diagnosis through acoustics-based RPW detector • Periodic monitoring and regular crown cleaning • Destroy crown toppled palms in a garden to avoid the lateral spread of the pest • Avoid causing any physical injury to the palms and cut the petioles at least 1 m away from the trunk • Spot application of imidacloprid 17.8 SL @1 mL/L of water or spinosad 45% SC @5 mL/L through the bore holes • Stimulo-deterrence for the disorientation of weevils through crop pluralism approach • Follow all regular management practices against rhinoceros beetle and leaf rot disease	Ghosh (1911), Menon and Pandalai (1960), Abraham and Kurian (1975), Abraham et al. (1998), Josephrajkumar et al. (2014a), Rajan et al. (2009), Josephrajkumar et al. (2017, 2018c, 2019c, 2021)
II. Defoliators					
1	Coconut black-headed caterpillar	*Opisina arenosella* Walker (Lepidoptera: Oecophoridae)	• Caterpillars produce silken webs reinforced with excreta and scrapes of leaf bits • Severe pest damage cause complete drying of the middle to the inner whorl of fronds • Annual loss due to infestation has been estimated as INR 7280 ha^{-1}	• In case of very severe infestation, remove and destroy fully dried 2–3 outer whorl of leaves • Augmentative release of stage-specific parasitoids viz., • larval parasitoids *Goniozus nephantidis* (Bethylidae) @ 20 parasitoids/palm, *Bracon brevicornis* (Braconidae) @ 30 parasitoids/palm • pre-pupal parasitoid, *Elasmus nephantidis* (Elasmidae) @ 49 /100 pre-pupae • pupal parasitoid *Brachymeria nosatoi* (Chalcididae) @32/100 pupae at the appropriate time • Ensure balanced nutrition and timely irrigation for recovering palm health	Rajagopal and Arulraj (2003), Mohan and Sujatha (2006) Mohan et al. (2010) Rajan et al. (2009), Josephrajkumar et al. (2018a, 2019c)

(continued)

Table 3.2 (continued)

Sl. No.	Pests (common name)	Biological name	Crop loss, symptoms and damage	Control measures	References
III. Sap Feeders					
1	Coconut eriophyid mite	*Aceria guerreronis* Keifer (Acarina: Eriophyidae)	• Infestation occurs in the developing young buttons after pollination and is seen in the floral bracts (tepals) and the soft meristematic portions beneath the perianth • Dispersal of the pest through wind, honeybees and other insects • Yellow halo around the perianth in the initial stage turns into brown and show necrotic patches, and finally, the damage is manifested as warts and longitudinal fissures on the nut	• Crown cleaning should be taken up routinely • Spray 2% neem oil-garlic emulsion or 0.004% azadirachtin (10,000 ppm ai) @ (4 mL/L) on bunches after pollination or root feeding with neem formulations containing azadirachtin (50,000 ppm ai) at 7.5 mL or azadirachtin 10,000 ppm at 10 mL with an equal volume of water three times during March–April, October–November and December–January • Spray 20% palm oil-sulphur (0.5%) emulsion on bunches after pollination • Application of talc-based preparation of *Hirsutella thompsonii* @ 20 g/l/palm containing 1.6×10^8 cfu per gram with a frequency of three sprayings per year during March–April, October–November and December–January • Adopt integrated nutritional management practices such as applying NPK fertilizer as recommended, recycling biomass or raising green manure crops in coconut basins and in situ incorporation during flowering, timely summer irrigation, and adopting appropriate moisture conservation measures	Moore and Howard (1996), Sathiamma et al. (1998), Haq (2001), Nair (2002), Rajan et al. (2009), Josephrajkumar et al. (2016, 2019c)
IV. Subterranean pests					
1	Coconut white grub	*Leucopholis coneophora* Burm. (Coleoptera: Scarabaeidae)	• Seedling damage occurs due to the feeding of roots, and death happens • In adult palms, root damage causes impaired water and nutrient conduction, yellowing of fronds, and yield loss	• Mechanical collection and destruction of adult beetles emerging during June • Repeated ploughing to expose the grubs to predators/digging and removal of grubs during October–December • Targeting early-instar grubs during July–August using Imidacloprid 17.8 SL @ 0.7 ml/l of water, 3–4 L of spray solution/m^2 area or Bifenthrin 10 EC @ 3 mL/L, 3–4 L of spray solution/m^2 area or Chlorpyriphos 20 EC @ 3 mL/L @ 3–4 L spray solution/m^2 area • Soil application of aqua formulation of entomopathogenic nematode, *Steinernema carpocapsae* @ 1.5 billion IJ/ha during September–October	Nirula et al. (1952), Rajan et al. (2009), Prathibha et al. (2013, 2018), Rajkumar et al. (2019), Josephrajkumar et al. (2019c)

(continued)

Table 3.2 (continued)

Sl. No.	Pests (common name)	Biological name	Crop loss, symptoms and damage	Control measures	References
V. Emerging pests					
1	Rugose spiralling whitefly	*Aleurodicus rugioperculatus* (Aleyrodidae: Hemiptera)	• It is an invasive pest of coconut • Desapping by whitefly could induce stress on the palms due to removal of water and nutrients • The economic loss is restricted mainly to the loss of photosynthetic efficiency due to the formation of sooty mold fungus (*Leptoxyphium* sp.)	• Pesticide holiday approach (i.e., no insecticides are recommended) to conserve habitat niche of natural enemies (*Encarsia guadeloupae, Apertochrysa* sp., lady beetles) and sooty mould scavenger beetle (*Leiochrinus nilgirianus*) by conservation biological control • Installation of yellow sticky traps on the palm trunk and interspaces for trapping adult whiteflies • In severe cases, water or neem oil (0.5%) spray is recommended on the lower surface of palm leaflets • Encourage good palm health by soil-test-based application of nutrients, organic recycling of residual biomass, irrigation, crop pluralism by growing various inter/mixed crops and ecological intensification to infuse biodiversity • Avoid the movement of infested seedlings (coconut, banana, ornamental palms) from endemic regions	Mohan et al. (2017), Josephrajkumar et al. (2018a, b, 2019b, c, 2020a, b)
2	Coreid bug	*Paradasynus rostratus* Dist. (Coreidae: Hemiptera)	• Loss is due to the shedding of developing buttons and immature nuts ranging from 18.2 to 66.4% • Deep furrows, crinkles and gummosis on the nut surface are typical symptoms	• Crown cleaning to destroy eggs and immature stages of the pest • Spray azadirachtin 300 ppm (Nimbecidene) @ 0.0004% (13 mL/L) two times during May–June and September–October • Spray thiamethoxam 25% WG (0.2 g/L) preferably in September–October on bunches after pollination during afternoon hours to safeguard pollinators • Encourage the predatory weaver ant, *Oecophylla smaragdina,* in the palm system	Kurian et al. (1972), Rajan et al. (2009), Josephrajkumar et al. (2019c)
3	Nesting whiteflies	*Paraleyrodes bondari* Peracchi and *Paraleyrodes minei* Iaccarino (Aleyrodidae: Hemiptera)	• Exotic whitefly smaller than RSW and desapping from under the surface of palm leaflets • Exudates honey dew and sooty mould developed on the upper surface of leaflets interfering with photosynthetic efficiency	• In juvenile palms, spraying water with jet speed could dislodge the whitefly and reduce the feeding and breeding potential of the pest • Ensure good nutrition and adequate watering to improve the health of juvenile and adult palms • Effective nitidulid predators belonging to *Cybocephalus* sp. and lady beetles were observed on the palm system and pesticide holiday is advised for conservation biological control	Mohan et al. (2019), Josephrajkumar et al. (2019a, 2020a)

3.4 Genetic Resources of Resistance/Tolerance Genes

The first systematic account of the classification of coconut genetic resources was performed by Narayana and John (1949). Later multiple variants for a specific phenotypic feature of the coconut accessions were recognized. For instance, habit is characterized with dwarf; intermediate forms; and talls, the stem has branching; polyembryony; and suckering variants, vegetative parts of the palm exhibit variants ranging from albinism; chimaera; rosette-type seedlings; the fusion of leaflets ('plicata'); and forking of leaves portions. In inflorescence too, twin spadix; multiple spathes; incomplete spike suppression; secondary; splitting of spikes; spikes unbranched ('spicata'); secondary spikelets; viviparous germination; bulbils; midgets; persistent stem inflorescences are the variants observed. Fruit variants are numerous such as jelly-like; fragrant, and sweet forms, to name a few. Against this backdrop, International Coconut Genetic Resources Network (COGENT) was set up in 1991 to conserve and utilize the coconut genetic resources to achieve sustainable productivity goals. It had led to the establishment of five multi-site International Coconut Gene banks (ICGs), viz., (1) Indonesia—Southeast and East Asia; (2) India—South Asia and the Middle East; (3) Papua New Guinea—South Pacific; (4) Côte d'Ivoire—Africa and the Indian Ocean; (5) Brazil—Latin America and the Caribbean which constitute a network of ex-situ conservation and collection of coconut accessions. The International Coconut Genetic Resources Database (CGRD) under the aegis of COGENT reveals that even though over 1416 coconut accessions were being conserved, the country-specific crop improvement programs utilize less than 5% of the germplasm holdings (Batugal 2005). Nonetheless, the development of catalogues of coconut genetic resources providing descriptors have greatly enhanced the utility of these genotypes in national breeding objectives (Hamelin et al. 2005). At present, 24 gene banks spread worldwide have 1837 accessions for use in varietal development programs considering the local and national requirements (Nampoothiri and Parthasarathy 2018). Accordingly, South American and African countries aim for evolving disease tolerant genotypes to counter lethal yellowing disease (LYD), whereas Vanuatu focuses on coconut varieties that could withstand coconut foliar decay. In India, the objective is to develop root (wilt) disease tolerant lines and accessions in the management of invasive spiralling whitefly, among others.

Efforts in the characterization of coconut genetic resources have discovered trait-specific germplasm, and several of them have been registered with the ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) in India. A robust evaluation and breeding by selection methodology at Indian Council of Agricultural Research-Central Plantation Crops Research Institute, Kasaragod, Kerala, along with the many coordinating Research Centres under the All India Coordinated Research Project on Palms (AICRP on Palms) and State Agricultural Universities (SAUs) resulted in the release of over 30 improved coconut cultivars (Niral et al. 2009). In addition, the multi-pronged approach of screening of accessions for tolerance to biotic/abiotic stressors, laying special emphasis on the root (wilt) disease resistance, drought tolerance, and climate resilience, have yielded an appreciable collection of germplasm

(Rajagopal et al. 1990; Nair et al. 2004; Kasturi Bai et al. 2006; Hebbar et al. 2013, 2018).

3.5 Classical Genetics and Traditional Breeding

Traditionally the main focus of coconut breeding has been the development of high yielding speciality varieties that could withstand the biotic and abiotic stresses (Jerard et al. 2016). Accordingly, the variability in yield and economic parameters were given importance. The traits such as fruit weight, percentage of the husk and the nut yield were considered of paramount importance to develop high yielding varieties (Niral et al. 2009). A defined breeding objective to evolve insect-resistant varieties in coconut does not exist in this context. Nevertheless, conventional breeding approaches have developed coconut varieties and hybrids resistant to some notable biotic stressors (Table 3.3). Preliminary screening of genotypes against leaf-eating caterpillar (*Nephantis serinopa* Meyr.) (Kapadia 1981) and rhinoceros beetle (*Oryctes rhinoceros* Linn.) (Sumangala Nambiar 1991) revealed considerable variability among the coconut genotypes. Screening of coconut accessions for Eriophyid mite (*Aceria guerreronis* Keifer) resistance suggests that multiple nut characteristic features including its color and shape, tightness of tepals around the nut, space between the rim of the fruit and tepal aestivation confer resistance to mite attack (Moore and Alexander 1990; Arunachalam et al. 2013). Hence, genotypes such as Kulasekharam Green Dwarf (KGD) and Chowghat Orange Dwarf (COD) and selection of KGD called Kalpa Haritha and few other accessions viz., Navasi Tall, Gangapani Tall, Jamaica Tall and East Coast Tall displayed lesser mite incidence (Niral et al. 2014; ICAR-CPCRI 2019).

Similarly, whenever COD was a pollen parent, rhinoceros beetle infestation was severe, and this phenomenon was equally observed whenever a dwarf accession constitutes a parent in a hybrid combination. Hence, West Coast Tall (WCT), East Coast Tall (ECT) and hybrid combinations of Laccadive Ordinary Tall × Cochin China Tall and Gangabondam Green Dwarf × East Coast Tall were found to have the least incidence of beetle infestation (Muthiah and Bhaskaran 2000). In a survey for red palm weevil infestation and search for resistant genotypes, CGD, COD, and Benaulium Tall have ovipositional preference implying their susceptible nature to the pest (Faleiro and Rangnekar 2001). Screening for coconut scale insect resistance divulged that the prevalence of leaf glandular trichomes conferred resistance to the insect attack. Hence, Coco Nino Dwarf (194.4 trichomes cm^{-2}) was resistant compared to the susceptible Laguna Tall (81.6 trichomes cm^{-2}) (Galvez et al. 2018).

In the field of disease resistance, root (wilt) disease (RWD) is a serious concern for coconut cultivation, and field screening suggests that the cultivar CGD exhibit > 90% tolerance in field conditions. Various cross combinations and extensive screening for resistance have resulted in the development of a variety Kalparaksha- a selection of a cultivar Malayan Green Dwarf (Nair et al. 2009). Further, CGD was found to be

Table 3.3 Improved coconut varieties and hybrids developed, and other genetic sources of resistance identified to tackle various biotic stresses

Sl. No.	Variety or hybrid or resistance source	Organization(s) involved/Reference	Major characteristic features
1	Kalpa Haritha	ICAR-CPCRI	Less eriophyid mite damage Dual-purpose variety suitable for tender nut and copra
2	Kalparaksha	ICAR-CPCRI	Yields high [in terms of nut/copra yield] in the root (wilt) disease (RWD) prevalent tracts Semi-tall statured variety suitable for tender nut purpose characterized with green fruits
3	Kalpasree	ICAR-CPCRI	Yields high in the root (wilt) disease (RWD) prevalent areas Dwarf variety with green fruits and yields good quality oil
4	Kalpa Sankara	ICAR-CPCRI	A hybrid derived from CGD X WCT cross Exhibits tolerance to root (wilt) disease (RWD), yields high
5	Malayan Dwarf × Panama Tall (Maypan hybrids)	Harries and Romney (1974)	Resistant to lethal yellowing disease (LYD)
6	Sri Lankan Dwarf, Indian Dwarf sand King Coconut	Been (1981)	Promising sources of resistance to Lethal yellowing disease
7	Donají hybrid (Malayo Enano Amarillo cv. Acapulco × Alto Pacífico cv. Escondido)	Experimental field of Oaxaca Coast of INIFAP (Serrano et al. 2011)	Resistant to LYD
8	PB 121 hybrid and progenies	Bourdeix et al. (1992)	Excellent level of tolerance to nut-fall caused by *Phytophthora katsurae*
9	Nias Yellow Dwarf × Palu Tall and Bali Tall	Brahmana et al. (1993)	Resistant to bud rot (*Phytophthora palmivora*) due to high polyphenol content
10	ECT × BSR-resistant ECT	Karthikeyan et al. (2005)	Higher survival percentage, higher nut yield against basal stem rot less disease incidence

(continued)

Table 3.3 (continued)

Sl. No.	Variety or hybrid or resistance source	Organization(s) involved/Reference	Major characteristic features
11	Kenthali Orange Dwarf and Chowghat Orange Dwarf	Ramaraju et al. (2000), Nair (2000)	Lower incidence of eriophyid mite infestation
12	Hybrids Java Giant Tall × East Coast Tall, Ayiramkachi Tall × West Coast Tall, Cochin China Tall × Philippines Ordinary Tall and West Coast Tall × Chowghat Orange Dwarf	Muthiah and Rajarathinam (2002)	Moderately tolerant to eriophyid mite
13	Varieties namely BSI, Chowghat Orange Dwarf, Philippines Ordinary Tall and Spicata Tall, and hybrids, viz. Philippines Ordinary Tall × San Blas Tall and Cochin China Tall × Philippines Ordinary Tall	Muthiah and Natarajan (2004)	Moderately resistant to eriophyid mite
14	Sri Lankan Yellow Dwarf and Gonthembili Tall	Perera (2006)	Tolerant cultivars to eriophyid mite
15	Java Giant Tall and Ceylon Green Tall	Raju et al. (2006)	Moderately resistant to eriophyid mite
16	Gangabondam Green Dwarf	Girisha and Nandihalli (2009)	Less mite incidence attributable to the tight attachment of perianth to nut surface
17	Laccadive Ordinary Tall and East Coast Tall × Godavari Ganga	Sujatha et al. (2010)	Lowest mite damage index
18	Jamaican Tall, BSI, Philippines Lono Tall, Guam Tall and Orange Dwarf	Badge et al. (2016)	Minimum infestation of eriophyid mite
19	Laccadive Ordinary Tall × Cochin China Tall and Gangabondam Green Dwarf × East Coast Tall	Muthiah and Bhaskaran (2000)	Minimum damage to rhinoceros beetle (*Oryctes rhinoceros*)
20	MAWA (Malayan Yellow Dwarf × West African Tall)	Capuno and de Pedro (1982)	Resistant source of Red Spider Mite (*Oligonychus velascoi*)

a promising donor of the RWD resistance gene, which led to the development of a variety ‘Kalpasree’ and a (D × T) hybrid, ‘Kalpa Sankara’ (Nair et al. 2006).

Multiple screening for lethal yellow disease (LYD) resistance in Ghana (Vanuatu Tall and the Sri Lanka Green Dwarf), Jamaica (MYD) and Tanzania (dwarfs such as Cameroon Red Dwarf, Equatorial Guinean Dwarf and Brazilian Green Dwarf), Nigeria (dwarfs MGD, MYD and MOD) identified that the respective genotypes were relatively free from the disease. Hence, it was suggested to deploy a range of partially resistant genotypes, and the accessions of SE Asia and Mexico were found to be promising (Villarreal et al. 2002; Serrano et al. 2011; Odewale et al. 2013). In general, Malayan dwarfs or hybrids involving Malayan dwarfs and talls inherit resistance to LYD. Hence, Maypan hybrid (90%), Malayan Dwarf (96%), Panama Tall (Malayan Dwarf × Fiji Dwarf) and other dwarf cultivars of India and Sri Lanka showed field resistance (Been 1981).

Screening of hybrids for resistance against stem bleeding (caused by *Ceratocystis paradoxa*) in India identified a genotype (from the cross Cochin China Tall × Gangabondam Green Dwarf) as least infected (Radhakrishnan and Balakrishnan 1991). Though detached leaf petiole inoculation of the fungi to screen 26 coconut genotypes did not reveal any resistance to the disease, the lesion size was least in Banawali Green Round (Ramanujam et al. 1998). Among the three juvenile hybrids of Brazil screened for leaf spot (caused by *Bipolaris incurvata*) resistance, PB 121 was promising (Gomez-Navarro et al. 2009). In India, MYD and CGD displayed relatively low disease incidence (Govindan et al. 1991); Tiptur Tall was resistant (Ghose et al. 2006) (Table 3.3).

3.6 Association Mapping Studies

Association mapping is a promising way of resolving the genetic basis of complex traits and identifying trait-associated markers based on naturally existing collections with uncertain pedigree relationships. A concept of natural population-based genetic mapping is well suited for perennial crops like coconut due to its high resolution, allelic richness. Moreover, it does not necessitate a developed mapping population for tracing the QTLs. Thus, linkage disequilibrium or genome-wide association studies (GWAS) utilize the principle of linkage disequilibrium in a set of crop accessions to identify QTLs. Studies on association mapping of traits in coconut germplasm are very limited. To date, a few preliminary association studies have been reported in coconut. Geethanjali et al. (2018) and Zhou et al. (2020) employed GWAS strategy to study the population architecture and the fatty acid content traits, respectively. Analysis of genetic diversity of 79 coconut accessions revealed the presence of 2–7 alleles and two major clusters differentiating talls of Indo-Atlantic and South Asia from the accessions of Indo-Pacific and SE Asia region. Also, association analysis in a subset of 44 genotypes in the same study detected a single SSR locus, CnCir73, putatively associated with fruit yield component traits (Geethanjali et al. 2017). Zhou et al. (2020) performed linkage analysis in 80 accessions for fatty acid content resulting in the grouping of germplasm into sub groups comprising higher-fatty acid and lower-fatty acid groups. Further, SSR markers linked to fatty acid content in chromosome 11 and donor genotype (Aromatica Green Dwarf) carrying an allele CnFAtB3-359 with major positive effects were identified for use in coconut oil breeding.

The adoption of high-throughput sequencing technologies coupled with the developments in bioinformatics and statistical methodologies has greatly accelerated the genetic mapping of economically important crops (Elshire et al. 2011). The whole-genome sequence resources (Xiao et al. 2017; Lantican et al. 2018; Rajesh et al. 2020) have been effectively utilized by Yang et al. (2021) to develop a high-density linkage map of coconut by adopting a genotyping-by-sequencing (GBS) approach. This study had arranged the coconut genome sequence onto 16 pseudomolecules and placed over two-thirds of the coconut genome onto these 16 linkage groups. This chromosome-scale genome assembly of coconut would certainly facilitate the implementation of robust molecular breeding programmes (Yang et al. 2021). Also, recently, GBS technique was employed to study the genetic diversity of 16 coconut accessions originating from diverse regions of the globe and to discover novel SNPs (Rajesh et al. 2021b). GBS strategy yielded a total of 10,835 high-quality SNPs and around 80% of them exhibited polymorphism information content (PIC) values in the range of 0.3–0.4. Further phylogenetic and population structure analysis based on Bayesian model suggest that coconut genotypes clustered depending upon their morphoforms (talls *vs.* dwarfs) although clustering based on geographical origin was also observed. The pattern of Linkage disequilibrium (LD) in coconut reveals that it is reported to decay at a relatively short genetic distance of 9 Kb. This study has paved the way for application of forward genetic approaches such as GWAS and development of GS models in coconut (Rajesh et al. 2021b).

3.7 Molecular Mapping of Resistance Genes and QTLs and Marker-Assisted Breeding

A genetic linkage map is a linear map that shows the relative positions of genes along with a chromosome or linkage group. Genetic distances among them are established by linkage analysis, which determines the frequency at which two gene loci become separated through chromosomal recombination. Availability of a good quality genetic linkage plays a vital role in genetic analysis of a trait, helps in acceleration of breeding programmes, facilitates the identification of novel loci governing important traits. Hence, linkage mapping is considered an integral component of any marker-assisted breeding (MAB) programs. Though the physical maps could provide the order and distances of molecular markers, genetic maps are required to validate them. They would greatly assist in improving the de novo genome assemblies. Characterization and mapping of loci corresponding to quantitative traits refer to QTL mapping would help analyse the segregation pattern of QTLs and assist the genomics-based breeding in coconut. In coconut, the mapping strategies, (a) linkage mapping and (b) association mapping or linkage disequilibrium, are followed to identify QTLs, but the latter is minimally explored.

The first genetic map of coconut was made in the year 1991, with a population developed from cross EAT × LAG ('African Tall' × 'Laguna Tall') using inverse sequence-tagged repeat (ISTR) markers (Rohde et al. 1999). This work was further extended with a population generated using a cross Malayan Yellow Dwarf (MYD) × Laguna Tall (LAGT) to identify QTLs associated with early germination traits. This was the first opportunity developed for marker-assisted selection in coconut. After that, Ritter et al. (2000) identified QTLs for leaf production, girth using 52 F_1 progenies generated from the cross Laguna Tall (LAGT) and Malayan dwarf (MYD), the markers used for the study were RAPD, ISTR, AFLP. Another genome map has been constructed with half-sib families of CRD × RIT ('Cameroon Red Dwarf' × 'Rennell Island Tall') using 227 markers (AFLP and SSRs), detected QTLs for the yield-related traits like the number of bunches and number of nuts (Lebrun et al. 2001). With the addition of new markers to the same mapping population, i.e. CRD × RIT, 52 putative QTLs were identified for the 11 traits, 34 of them were probably correspond to the single pleiotropic genes, and the others had relatively large effects on the individual traits (Badouin et al. 2006); QTLs linked to fruit components such as weight, endosperm humidity and fruit production were identified at different locations of a genome. Studies were also taken up in coconut to identify QTLs governing major cuticular wax components of coconut, which are involved in the plant's defence against abiotic and biotic stresses. Around 46 QTLs related to biosynthetic pathways of five different wax components were identified by Riedel et al. (2009).

Application of molecular markers in coconut improvement has spanned wide areas including in analyzing the genetic differences and genetic diversity analysis among the genotypes (Lebrun et al. 1998; Ashburner and Been 1997; Perera et al. 1998; Rohde et al. 1992; Manimekalai and Nagarajan 2006; Rivera et al. 1999; Rajesh et al. 2014, 2015; Jerard et al. 2017; Preethi et al. 2020), habit detection (Rajesh et al. 2013, 2014), mite resistance (Shalini et al. 2007), LYD resistance (Konan et al. 2007) among others. As stated above, only a few studies have been conducted in coconut to identify genes or loci associated with biotic stress resistance. DNA-based molecular markers have enormous advantages over conventional phenotypic markers for applications in plant breeding, especially in perennials such as coconut. Identification of AFLP markers linked to root (wilt) disease has greatly aided the process of resistance breeding in coconut (Rajesh et al. 2002). Deciphering the population structure of apparently disease-free and susceptible palms from the disease endemic districts of Southern Kerala towards RWD utilizing microsatellite markers revealed two distinct populations of resistant WCT along with several sub-populations (Deva Kumar et al. 2011). It was suggested to use these populations for genomics-assisted disease resistance breeding. Building on the findings of Rajesh et al. (2015), which unravelled the transcriptomic response of CGD leaf samples against RWD, Rachana et al. (2016) amplified, sequenced and characterized putative resistant gene analogues (RGA) from the same cultivar. Further, the coconut RGAs exhibited a high degree of sequence similarity to monocot NBS-LRRs and were expressed highly in RWD resistant genotypes (Rachana et al. 2016). Whole-genome sequence of root (wilt) disease-resistant cultivar CGD and comparative transcriptomic approach identified a total of 112 NBS-LRR encoding loci of six different classes (Rajesh et al. 2020).

To identify genetic loci associated with lethal yellowing (LY) disease resistance, Cardena et al. (2003) analyzed three different coconut populations with contrasting characteristics [susceptible West African Tall (WAT), the resistant Malayan Yellow Dwarf (MYD), and a resistant population of Atlantic Tall (AT) plants]. Using bulk segregant analysis, RAPD markers were selected if their frequencies were high in MYD and AT and low in WAT. A total of 82 RAPDs could differentiate the DNA pools derived from the MYD and WAT. The 12 RAPDs selected during the analysis of MYD and WAT are invaluable markers for differentiating the genetic makeup of the coconut materials. Konan et al. (2007) utilized 12 microsatellite markers to analyze LYD resistance revealing a total of 58 alleles. This study also identified the 10 specific alleles (CnCir series of SSR loci) associated with LYD resistance by screening tolerant Vanuatu Tall (VTT), Sri Lankan green Dwarf (SGD), and susceptible West African Tall (WAT). Further, the F_{st} index suggests that around 60% of the total allelic variability could explain the differences among the three genotypes studied. And these marker types could be useful for identifying the resistance material for taking up breeding programmes. Later search for resistance-conferring genes in coconut by Puch-Hau et al. (2015) using degenerate primers resulted in amplifying nucleotide-binding site (NBS)-type DNA sequences from coconut genotypes that were either resistant or susceptible to LYD. Interestingly, all the resistant gene analogues derived from coconut clustered with a non-TIR-NBS-LRR subclass of NBS-LRR genes. Further, gene expression analysis suggests that these RGAs exhibited variability in their expression for external salicylic acid. This study has set the stage for the exploration of RGAs in coconut. Putative receptor-like kinase (RLK) genes from coconut genotypes under the threat of Cape St Paul wilt disease was characterized by Swarbrick et al. (2013). Further sequence analysis of intron sequences of these putative RLKs identified three potential single nucleotide polymorphisms (SNPs) that could significantly differentiate susceptible and resistant genotypes.

Efforts were also made to identify molecular markers linked to coconut eriophyid mite (*Aceria guerreronis* 'Keifer') resistance. In the process of identification, coconut genotypes and mite-resistant and -susceptible accessions were collected. Thirty-two simple sequence repeat (SSR) and seven RAPD primers were used to identify the association between resistant trait-associated loci. Based on single marker analysis, nine SSR and four RAPD markers associated with mite resistance were identified. Combinations of 5 markers (SSR and RAPD) associated with eriophyid mite resistance have been discerned based on combined step-wise multiple regression of both SSR and RAPD data (Shalini et al. 2007).

3.8 Genomics-Aided Breeding for Resistance Traits

3.8.1 Whole-Genome Sequence Assemblies

The de novo nuclear genome assembly of coconut (cv. Hainan Tall) unravelled that the genome harbours 28,039 protein-coding genes. In contrast, related palm genera such as *Elaeis guineensis* and *Phoenix dactylifera* have 34,802 and 28,889–41,660 protein-coding genes, respectively (Xiao et al. 2017). Molecular evolutionary analysis based on Bayesian genetics suggests that coconut had diverged from oil palm around 46 million years ago. Comparative genomics further divulge that gene families encoding plasma membrane transporters (especially those involved in K^+ and Ca^{2+}, and Na^+/H^+ antiporters) are prevalent in coconut genome suggesting its adaptability to saline environments (Xiao et al. 2017). It was followed by sequencing of dwarf cultivar 'Catigan Green Dwarf' (CATD) by Lantican et al. (2019) that aided in characterization of novel 7139 microsatellite markers, 58,503 SNP variants and 13 gene-linked SSRs following a comparative analysis of dwarf and tall coconut genomes. Also, SSRs linked to drought tolerance and other biotic stress tolerance identified were promising resources for molecular breeding in coconut. These efforts were further complemented by characterizing the nuclear and organellar genomes of indigenous coconut cultivar, Chowghat green dwarf (CGD), showing resistance against root (wilt) disease (RWD) (Rajesh et al. 2020). Among these three genome assemblies, the efforts of Rajesh et al. (2020) would greatly help in identifying genetic factors responsible for resistance to root (wilt) disease and to introgress those genetic elements in susceptible cultivars by resorting to genomics-assisted breeding.

3.8.2 Transcriptomic Approaches

Transcriptome analysis, using RNA-sequencing (RNA-Seq), has been performed in coconut to decipher the molecular response of diseases such as coconut yellow decline (Nejat et al. 2015) and root (wilt) disease (RWD) (Rajesh et al. 2015, 2018). However, prior to this, researchers have utilized a comparative genomics approach to analyze the expression dynamics of *R* genes of coconut using related palms, mainly date palm. Resistant gene analogs (RGA) derived from coconut were utilized to comprehend the expression dynamics of coconut *R*-genes expression in response to root (wilt) disease. Conserved domains of nucleotide-binding site-leucine-rich repeat (NBS-LRR) class genes of oil palm and date palm were used to design primers and study coconut RGAs (Rajesh et al. 2015). Three putative RGAs were isolated in coconut. Their relatively high expression status in the leaves of RWD resistant cultivar suggests their potential utility in genomics assisted resistance breeding in coconut (Rachana et al. 2016).

RNA sequencing of apparently healthy and diseased coconut cultivar Chowghat Green Dwarf (CGD) revealed the underlying host molecular response to the disease progression (Rajesh et al. 2018). Differential transcript expression analysis of healthy and diseased RNAs reveals that many transcripts (~2700) are differently regulated in the wake of the disease. Interestingly, a genetic regulatory network analysis based on the transcriptome data shows that RNA encoding calmodulin-like 41, WRKY DNA-binding proteins are upregulated. This transcriptome analysis also put forth a molecular model of coconut's response to RWD involving host protein kinases, calcium-binding proteins and a signaling cascade involving salicylic acid in concurrence with the dynamic expression of TFs such as WRKY and NAC-domain proteins. (Rajesh et al. 2018).

Similarly, comparative transcriptome analysis of healthy and diseased coconut yellow decline (CYD) diseased infected leaves have identified that genes involved in defence response and signal transduction pathways are highly upregulated (Nejat et al. 2015). In the phytoplasma infected tissues, genes coding for pathogenesis-related proteins (PRs) were highly expressed. This study proved that the active defence response of the host is stimulated during the phytoplasma invasion (Nejat et al. 2015).

3.9 Conclusion and Future Perspectives

In summary, it is evident that the application of genomics science in improving coconut has been lagging compared to many other crops. Also, a very handful of successful applications of MAS has been witnessed in coconut, especially in developing insect or disease-resistant cultivars. Further identification and validation of major QTLs linked to traits of agronomic importance are strategically required to unleash the potential of genomics-assisted breeding in coconut. Considering its perennial nature, applying concepts such as 'speed breeding' to reduce the timeline for developing novel varieties warrants progress and the use of robust in vitro propagation techniques. To increase the genetic gain, adopting techniques such as genomic selection (GS) models is imperative as it helps in the shortening of breeding cycles and improves the efficiency of selection procedures. Deployment of genomics assisted breeding has to be integrated with the current conventional breeding strategies for developing biotic stress tolerance in coconut. Applications of transgenics or genetic engineering technologies have long been overlooked in horticultural, perennial crops, unlike the annuals wherein successful deployment of GM crops has helped manage deadly pathogens and pests in the field conditions. Nonetheless, it is anticipated that break-through in coconut in vitro clonal propagation techniques along with developments in the field of gene prospecting would spur the development of GM coconut aimed at pests and disease tolerance. Availability of good quality genome assemblies and application of functional genomics has a great potential to decipher gene-function relationship in coconut. Further, genome-editing approaches utilizing CRISPR/Cas9 tools are imminent in coconut to develop biotic stress-tolerant genotypes by way of suppressing host susceptibility factors. Though applying this technology is challenging in perennials such as coconut, it is highly desirable in the context of ‘fast-forward breeding’ approaches envisioned. In addition, resequencing of a large number of coconut accessions possessing specific biotic stress tolerance traits (disease or pests) would provide a genetic blueprint for accelerating the genetic gain in the field of genomics assisted resistance breeding.

In addition, the role of bioinformatics in solving the bottlenecks in breeding for biotic stress tolerance in coconut is required now more than ever because large-scale data pertaining to the genome, transcriptome sequences are available in the public databases. Mining of these databases to develop robust genic markers, EST-based full-length gene sequences, reconstruction of transcriptome profiles, identification of novel functional genetic elements and other gene regulatory elements are fundamental to reap the benefits of big data-enabled molecular breeding in coconut.

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