

Chapter 7

Genomics for Biotic Stress Tolerance in Jute



Pratik Satya, Soham Ray, B. S. Gotyal, Kunal Mandal, and Suman Roy

Abstract Jute (*Corchorus capsularis* and *C. olitorius*), valued for its industrial applications and environmental benefits, is attacked by selected pests and diseases that hamper its growth and development, reduce fibre yield up to 40% and negatively affect the quality of the fibre. As the fibre crop is predominantly grown in the Indian subcontinent, the changing subtropical climate would invariably modulate the pest and disease dynamics as well as the interaction of jute and the biotic stress causing organisms. To develop effective biotic stress mitigation strategies in jute, it is essential to understand both the nature of resistance in jute and interaction with the pest/pathogen at genetic, physiological and molecular level. This chapter first provides an outline of the major pest and disease of jute and their management strategies, genetics of resistance and traditional breeding approaches to combat the pests and disease of jute. Thence, standing on the backbone of traditional genetics and breeding, we scanned the recent developments in molecular genetics and genomics researches in jute that have helped to identify and exploit resistance genes, including their use in evolution, phylogeny and population structure analysis, molecular mapping of resistance loci and identification of QTLs. We then mined the genomic resources to identify the genes involved in host–pathogen interaction, particularly against *Macrophomina phaseolina*, the most dreadful pathogen of jute. Transgenic development for resistance is also gaining momentum in jute in recent years, although recalcitrance of jute is a challenging issue in developing stable transgenic system. Unraveling the resistance mechanisms in jute, a crop not preferred by many pests and pathogens, can help to devise novel resistance breeding strategies in other crops.

Keywords Biotic stress · Genomics · *Corchorus* · QTL · Resistance · Transgenics

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7.1 Introduction

7.1.1 Economic Importance

Jute is the most important industrial crop for production of non-textile fiber products. Jute fiber is used to make sacks, hessian, yarn, carpet backings and other jute diversified products. The fiber is obtained from two Malvaceous species, *Corchorus olitorius* L. and *C. capsularis* L., of which *C. olitorius* or ‘tossa jute’ is presently the dominant cultivated species (Satya et al. 2014). India and Bangladesh produce about 95% of the jute fibre, followed by China. During the twenty-first century, the area under jute has dwindled around 1.4 million ha, while the production was around 32–34 million tons of fiber per year (Fig. 7.1). While the production of jute in India has gradually declined from 67 to 51% during 2001–2019 particularly due to introduction of competing crops like maize, banana and sesame, the contribution of Bangladesh has increased from 30 to 48% during the same period. However, jute production in China has declined during this period from 2.1 to 0.8%.

As an industrial crop, the production of finished products (bags, sacks, hessian and diversified products) is equally important indicator of the jute crop economy. During 2018–19, India imported jute and jute products valued at about 160 million USD and exported jute products valued at 325 million USD (Source: National Jute Board, India; <https://www.jute.com/>). While the primary use of jute is as non-textile fiber, jute has a number of diversified end uses, which include both diversified use of fiber, the primary economic product as well as other non-fiber parts and use of whole biomass. Jute fibers are used to produce jute diversified decorative products, geotextiles, fiber-reinforced composites and can be used as source of cellulose and lignin. As health-promoting vegetable, jute leaf is popular in many Asian and African countries. The young seedlings of jute are consumed as vegetable in many African, Asian and Latin American countries for high nutritive values (Zeghichi et al. 2003; Ndlovu and Afolayan 2008). The cultivated and wild *Corchorus* species also have high ethnomedicinal value for treatment of cystitis, diarrhea, fever, and cold (Oboh et al. 2009).

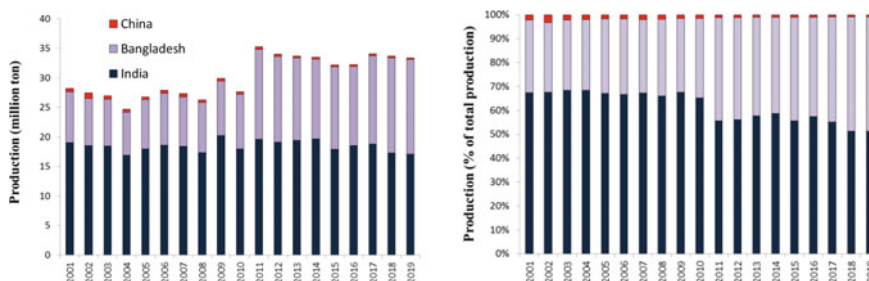


Fig. 7.1 Comparative jute production status in India, Bangladesh and China. *Data source* FAOstat (<http://www.fao.org/faostat/>). Accessed on 30.07.2021

7.1.2 *Reduction in Yield and Quality Due to Biotic Stresses*

Biotic stresses are one of the major reasons for reduction in yield potential of jute. Jute is grown in a hot and humid subtropical environment that also favors development of insect-pests and diseases. Consequently, the crop is attacked by many biological organisms including virus, bacteria, fungi, nematode, insects and non-insect pests, leading to a loss of about 15–20% in fiber yield. Pest problem is one of the major constraints responsible for low productivity of jute because the crop is prone to damage from seeding stage to harvest stage. For example, Yellow mite (*Polyphagotarsonemus latus* Banks), a major pest of jute, causes severe damage during early plant growth. As these mites are microscopic, their damage on jute often goes unnoticed in the initial phase. Fiber yield loss to the tune of 60% has been reported from the attack of yellow mite in jute (Kamruzzaman et al. 2013). The jute semilooper, a major lepidopteran pest of jute, causes 20–25% crop damage (Babu et al. 2020). Among the diseases, stem rot (c.o. *Macrophomina phaseolina*) causes the most serious damage to stem, reducing fiber recovery as well as fiber quality (Islam et al. 2012). It can attack any part of the plant at any growth stage and accounts for average yield loss of about 10% which, in severe conditions, may go up to 35–40% (Roy et al. 2008). Although fungicide application is an effective measure of controlling this disease, host resistance is much more preferable for the resource poor jute farming community as the pathogen can survive on several alternate hosts making it almost impossible to eradicate by chemical means (Islam et al. 2012).

7.1.3 *Growing Importance in the Face of Climate Change*

The service of jute crop to the restoration of the climate is extraordinary. When a jute crop is grown in an area of one hectare for four months, it produces over 50 tons of green biomass, thereby purifying more than 15 tons of CO₂ from atmosphere (Palit and Meshram 2008). The jute fiber is being grown primarily in the Gangetic delta of the Indian subcontinent over the past 250 years. Till the last phase of the twentieth century, jute was a monopoly crop of the Indian subcontinent, which contributed 97% of the total jute production of the World. Attempts to grow jute as fiber crop in other parts of the World, except China, did not meet with much success due to the unique climatic conditions required for jute. The rising global warming and environmental concern of synthetic fibers have opened up new scope for industrial and environmental applications of jute in the recent decades.

Climate change has a profound effect on the dynamics of insect-pests and diseases of crops. Although the responses of the insect-pests to global climate change are complex, it is predicted that for 41% of the insect-pests, response to climate change would lead to more damage to the crop (Lehmann et al. 2020). However, studies on the effect of climate change on the insect-pests and diseases of jute are limited. Species distribution modeling of these pests under predictable climates would not

only help reduce the pest problem but also would help in devising pest/disease specific breeding programs. For example, the optimum temperature for development of the yellow mite is estimated as 30 °C (Luypaert et al. 2014). Therefore a temperature range around 28–32 °C with high humidity would favor the growth of the mite. An increase in the temperature and intermittent rainfall during May–June (active growth stage of jute) would be more conducive to growth and development of the mite causing more damage to jute crop. Temperature and rainfall also plays key role in incidence of the jute semilooper. Among the major pathogens, *M. phaseolina*, which can withstand a higher temperature (up to 40 °C) by forming microsclerotia (Pandey and Basandrai 2021), is expected to remain a major threat for not only jute but for other crops like legumes, vegetables and oilseed crops.

7.1.4 Limitations of Traditional Breeding and Rationale of Genome Designing

The principal source of genetic diversity in most of the crops is natural selection that act upon variation created through mutation and recombination. Jute is a sexually reproduced self-pollinated species, though cross pollination may go up to 17% in *C. olitorius* (Satya et al. 2014). But the unique nature of jute cultivation does not allow the plant to enter into reproductive phase, as the whole plant is harvested during active vegetative growth. Consequently, natural mutations that accumulate during the vegetative period are not transmitted to the progeny and any chance of recombination is not possible. The only variability permitted in jute lies in the wild and weedy plants surviving in the nature. Moreover, the two species show high sexual incompatibility with each other and with other *Corchorus* species, further limiting the chance of introgression of genes from wild relatives. Consequently, genetic diversity in jute is extremely low, which is evident from low molecular marker polymorphism and diversity indices (Benor et al. 2012; Satya et al. 2014).

Although *C. olitorius* jute originated in Africa, the fiber type jute was possibly domesticated in Central and Northern India (Sarkar et al. 2019). While jute is being grown in the Indian subcontinents sporadically for about 2000 years, large-scale cultivation started only about 250 years ago. Traditional breeding started in jute with selection of local types by Finlow and Burkill during early twentieth century, resulting in release of two cultivars, D-154 of *C. capsularis* and Chinsurah Green of *C. olitorius*, which dominated the Indian subcontinent for about 50 years, gradually replacing the local landraces. During the latter half of the twentieth century these long duration cultivars were considered unfit for jute-rice cropping system that became very popular in the Indo–Gangetic plain after the spread of the photo-insensitive rice cultivars. This had to two major impacts, improvement in quality of the seed and loss of genetic diversity. Earlier, jute farmers saved their own seed by allowing a few lines to maturity for seed harvest, as they did not grow rice in the same field; rather they cultivated winter crops like potato, mustard or vegetables. As a consequence of

jute-rice cropping system, a seed production industry flourished that cultivated jute only for seed at Central and Southern India and supplied the seed for fiber crop in the Indo-Gangetic plain in India as well as Bangladesh. Replacement of farmers' saved seed ensured seed quality and varietal replacement, but the local genotypes were completely lost within a period of 10–20 years from both the countries, further limiting the existing gene pool of jute. The main variability in jute is thus limited to the germplasm collections of a few countries, breeding populations and some wild jute sporadically distributed in nature (Nasim et al. 2017). Many of the germplasm collections may have duplicate entries, as most of the germplasm collection was taken up by the International Jute Organization and was distributed to different jute growing countries. Since traditional breeding is principally dependent on variability available in the species, low genetic variability is the primary bottleneck of jute breeding. The novel genomic and genetic engineering tools that can transfer genes beyond the sexual compatibility barrier, and create new variations through targeted mutations can have tremendous impact on generating new variability in jute. However, research on genetic transformation, genomics and genome editing are still limited in jute, halting the progress of jute breeding.

7.2 Description on Different Biotic Stresses

Pest problem is one of the major constraints responsible for low productivity of jute because the crop is inflicting damage by more than 40 species of pests including insects and mites from seedling stage to harvest of the crop. Bihar hairy caterpillar (*Spilarctia obliqua* Walker), jute semilooper (*Anomis sabulifera* Guenée), yellow mite (*Polyphagotarsonemus latus* Banks), stem girdler (*Nupsersha bicolor* Thoms.), indigo caterpillar (*Spodoptera exigua* Hübner), red mite (*Oligonychus coffeae* Nietner), stem weevil (*Apion corchori* Marshall) and gray weevil (*Mylloceris discolor* Boheman) are considered as the major pests of jute (Rahman and Khan 2012a). *Meloidogyne incognita* Chitwood is an important nematode pest of jute. Besides, gram caterpillar (*Helicoverpa armigera* Hübner), safflower caterpillar, *Condica capensis* Guenée, green semilooper, *Amyna octa* Guenée, leaf webber, *Homona* sp. Walker and leaf miner, *Trachys pacifica* Kerr are also emerging as insect-pests of jute in the recent past (Selvaraj et al. 2016; Gotyal et al. 2019). The list of major insect-pests, damage stage, nature of damage and distribution is provided in Table 7.1. For a detailed discussion on the insect-pests of jute, please refer to Selvaraj et al. (2016).

Table 7.1 List of insect and mite pests' scenario in jute crop

| Name of the insect-pests | Damage stage | Nature of damage | Distribution |
|--|--------------|--|---|
| Major pests | | | |
| Indigo caterpillar, <i>Spodoptera exigua</i> | Larva | The leaves are skeletonized; the older caterpillars often devour the entire lamina and causes defoliation. Typical damage is noticed in young seedlings, which are cut on the ground surface by the larvae causing reduction in plant stand | China, Indonesia, India, Japan, and Malaysia |
| Jute stem weevil, <i>Apion corchori</i> | Grub, adult | The female makes one or more punctures at the top nodes, where knot is formed and there are corresponding numbers of grubs seen inside the plant. Damage of apical meristem checks the vertical growth and makes multiple branching. The tissues damaged by the grub that binds the fiber together; which breaks at these points during fiber extraction and results in 'knotty fiber' | Jute tracts of India and Bangladesh |
| Jute semilooper, <i>Anomis sabulifera</i> | Larva | Approximately 95% of the damage is restricted to nine fully opened top leaves of the crop. The edges of the tender leaves are eaten, serrated, diagonal cuts seen in apical leaves In seed crop, scooping of terminal stem causes drooping of the plants and larvae damage seed pods by making holes that affects the quality of seed inside | Jute tracts of India, Bangladesh, Myanmar, Sri Lanka and in parts of Africa |

(continued)

Table 7.1 (continued)

| Name of the insect-pests | Damage stage | Nature of damage | Distribution |
|---|--------------|---|---|
| Yellow mite, <i>Polyphagotarsonemus latus</i> | Nymph, adult | Both nymphs and adults suck the sap from the ventral surface of young leaves. The infested leaves turn deep green with coppery-brown shades with typical inverted boat like shape and drop prematurely. The vertical vegetative growth of the crop is arrested, and significant yield loss occurs | Australia, Asia, Africa, North America, South America and the Pacific Islands |
| Bihar hairy caterpillar, <i>Spilancistris obliqua</i> | Larva | Young larvae feed gregariously and scrap the chlorophyll content and completely skeletonize the plant. The damaged leaves of the plant gives an appearance of net or web and under severe condition complete defoliation may occur. Damage seen during June and continued till mid-September coinciding with 60–100 day old crop | India, South-eastern Afghanistan, northern Pakistan, Bhutan, Bangladesh and Myanmar |
| Mealybug, <i>Phenacoccus solenopsis</i> | Nymph, adult | The damage is mostly caused by the immature stages of mealybug which suck the sap. Infested plants exhibits symptoms of distorted and bushy shoots, crinkled and/or twisted bunchy leaves, and plants become stunted and dry completely in severe cases. The vertical growth reduces and gives bushy appearance. Repeated attacks on the stem cause the development of crust due to which fiber bundles resist separation at the time of retting, resulting in the formation of 'barky fiber' | India, Indonesia, Japan, Malaysia, Philippines, Sri Lanka, Taiwan, Australasia, and Pacific islands |

7.2.1 Major Insect-Pests of Jute and Their Management

7.2.1.1 Indigo Caterpillar, *Spodoptera Exigua* (Noctuidae: Lepidoptera)

Once considered as minor pest, indigo caterpillar has recently becoming important for its regular occurrence in *C. olerarius*. Although average yield loss is estimated around 20%, its infestation in the early stage of the crop may cause complete crop failure requiring re-sowing of the crop. It mostly infests the seedling stage of the early sown crop (Fig. 7.2). During day time, the caterpillars defoliate the plants and hide in the bottom of the plant in the cracks and crevices. It is a highly polyphagous pest sporadically assumes destructive nature in the early sown jute crop. The young larvae after hatching feed on tender leaves in groups. The feeding activity of grown up larva is generally confined to a few hours early in the morning and late evening. March to April month is the peak period of infestation.

Integrated management

- Early infestation can be spotted by monitoring of the insect underside the clods and the base of the plants prior to the damage and initiation of spray.
- Destruction of egg masses/gregarious larvae by inspecting the field in the early hours when they are active on plant parts can reduce the damage to great extent.
- The early instar larvae can be controlled by spraying neem seed kernel extract 5% along with suitable sticker.
- In case of severe infestation, application of chlorpyrifos 20 EC @ 2.5 ml/L or synthetic pyrethroids such as cypermethrin 25 EC @ 0.5 ml/L, or lambda cyhalothrin 2.5 EC @ 1 ml/L should be done.



Indigo caterpillar feeding on jute leaf



Damage symptom of jute yellow mite



Gregarious larvae of hairy caterpillar



Jute semilooper larva feeding on leaf



Jute stem infested by mealy bug



Jute leaf folder caterpillar

Fig. 7.2 Major insect-pests of jute and their damage symptoms

7.2.1.2 Stem Weevil, *Apion Corchori* (Curculionodae: Coleoptera)

It's an internal feeder causing damage in all the jute growing tracts of India. The infestation by the weevil adversely affects the quality and yield of fiber. The grub tunnels and feeds inside the stem restricting the vertical growth and encouraging multiple branching. In the affected nodes mucilaginous substances accumulates, hardens which produces 'knotty fibers'. It attacks *C. capsularis* more than *C. olitorius*. The early season crop is more susceptible to weevil infestation. Yield loss in white jute is estimated to the extent of 18% (Datt 1958).

Integrated management

- Removal and destruction of stubbles and self-sown plants avoid the carry of the pest and reduce the infestation.
- Sowing of *tossa* and *white* jute during end of April considerably reduces its incidence while delayed sowing in late March to early April increases the risk of weevil infestation.
- Balanced application of nitrogenous, phosphatic and potassic fertilizers reduces the pest attack.
- In endemic areas preventive soil application of carbofuran (1 kg ai/ha) is effective in reducing the pest pressure. Need based foliar spray of cypermethrin 25 EC @ 0.5 ml/lit in early hours can control the damage caused by stem weevil.

7.2.1.3 Jute Semilooper, *Anomis Sabulifera* (Noctuidae: Lepidoptera)

Semilooper is one of the most important foliage feeding insects of jute, which occurs regularly in all the jute growing areas of the country (Rahman and Khan 2012a). Slender, light green semiloopers initiate the damage by feeding the young unopened leaves, later it spreads to fully opened leaves (Fig. 7.2). They remain in clusters up to 3rd instar, mainly feeding on the lower epidermis of leaves, hence often are difficult to find. From the 4th instar the larvae disperse in different plants, chewing the leaves leaving only ribs, which is a characteristic damage sign of this insect. In majority of the cases the 7–9 leaves of upper part of the standing crop are damaged (Datt 1958). Upon repeated infestation, crop growth reduces drastically and profuse branching is observed resulting in loss of fiber yield. It is a cosmopolitan pest, being distributed in wide geographical area and can damage other crops like pulses, groundnut, soybean and many vegetables.

Integrated management

- Balanced use of fertilizers is the key to reduce semilooper infestation. Plough the infested fields after harvest to expose and kill the pupae.
- *Bacillus thuringiensis* is an effective biocontrol agent against jute semilooper. Foliar spray of *Bt* formulation may be recommended @ 1 kg/ha.
- Individual economic injury level (EIL) for semilooper is 10% plant damage at 55 DAS. Whenever the damage by semilooper reaches 15% then any contact

insecticide such as profenophos 50 EC @ 2 ml/lit, fenvalerate 20 EC @ 2.5 ml/lit or cypermethrin 25 EC @ 0.5 ml/lit may be applied.

- The insecticidal sprays need to be targeted towards the apical portion of the plant rather than covering the whole plant from top to bottom as the infestation of the pest is confined to the top leaves.

7.2.1.4 Yellow Mite, *Polyphagotarsonemus Latus* (Tarsonemidae: Acari)

Yellow mite is the most destructive sucking pest of jute. The mite affected leaves curl down, become coppery-brown, dry and fall off (Fig. 7.2). The yield loss varied from 20 to 50% depending on the level of infestation and stage of the plant (Keka et al. 2008). High humidity and morning temperature enhances the rate of multiplication and damage by mite. *Tossa* jute is more susceptible to yellow mite than the white jute.

Integrated management

- *C. olitorius* jute varieties, JRO-204 and JROG-1 are comparatively more tolerant to mite.
- Early sown jute crop suffers more from mite infestation. Instead of March, the crop sown in April escapes the damage of mite to greater extent. Foliar spray of mineral oil @ 3 ml/lit + neem oil @ 3 ml/lit twice at 35 and 50 days after sowing (DAS) may be applied for management of yellow mite.
- Two sprays of spiromesifen 240 SC @ 0.7 ml/L, at 36 and 46 DAS may be applied for protecting the jute crop from yellow mite. Need based spray of abamectin 1.8 EC @ 0.8 ml/L or fenazaquin 10 EC @ 1.5 ml/L, alternatively at fortnightly interval is quite effective for mite management.

7.2.1.5 Bihar Hairy Caterpillar, *Spilarctia Obliqua* (Arctiidae: Lepidoptera)

Bihar hairy caterpillar (Fig. 7.2) has become a serious pest of jute in West Bengal, Bihar and some parts of Assam in India. In field, the initial damage can be spotted by seeing whitish jute leaves. High humidity, rains with intermittent sunny days with high temperature is the congenial condition for hairy caterpillar infestation.

Integrated management

- Regular monitoring to spot early oviposition and egg masses in the early stage, when the caterpillars remain gregarious on leaf, it is easy to destroy them after plucking such infested leaves and then dipping them in insecticidal solution.
- When caterpillars disperse, their control is achieved by insecticidal spraying of lambda cyhalothrin 2.5 EC @ 1.0 ml/L or indoxacarb 14.5SC @ 1.0 ml/L to reduce the pest population to a greater extent.

- Early instar larvae are more vulnerable to the larval parasitoid, *Protapanteles obliquae*. In case of greater activity of parasitoids, insecticidal interference may be avoided.

7.2.1.6 Mealybug, *Phenacoccus Solenopsis* (Pseudococcidae: Hemiptera)

Cotton mealybug, *P. solenopsis* (Fig. 7.2) has been reported to be a new pest of jute crop in 2012 in South Bengal. (Satpathy et al. 2016). It is highly polyphagous and occurs in many economically important crops. Earlier, three species of mealybug i.e. *Maconellicoccus hirsutus*, *Ferrisia virgata* and *Pseudococcus filamentosus* had been reported to infest jute. High temperature and stretches of dry period and less number of rainy days favor its infestation.

Integrated management

- Preventive seed treatment with thiamethoxam (70 WS @ 5 g/kg seed) or clothianidin (50 WDG @ 3 g/kg seed) is very effective against mealybug.
- The systemic insecticides are more effective against mealybug crawlers. Foliar spray of profenophos 50 EC @ 2 ml/lit or imidacloprid 17.8 SL @ 100-125 ml/ha or thiamethoxam 25 SG @ 200 g/ha is recommended for management of mealybug.
- The control of ants which help the mealybug colonies to grow and spread by soil application of chlorpyrifos 20 EC @ 2 ml/litre or malathion dust 5% @ 25 kg/ha restricts the mealybug crawlers to spread to non-infested plants.

7.2.1.7 Root Knot Nematode, *Meloidogyne Incognita* and *M. Javanica*

The nematodes colonize in the root zone and produce small knotty galls, which interfere with nutrient uptake. The infected plants show stunted growth, wilt and finally die. The population of nematode can increase rapidly in the soil. Rahman and Khan (2012a) observed >460% rise in the population of nematode in soil during 120 days crop growth period, resulting in about 15% plant loss.

Integrated management

- Soil amendment with lime, potash, sulphur, mustard oil cake and jute seed powder can reduce root knot nematode infestation.
- Cultural practices followed by crop rotation are effective with rice and wheat for two years reduced the *M. incognita* and *M. javanica* population in jute.
- Sunnhemp is a suitable trap crop for controlling nematode population in jute.

7.2.2 Diseases of Jute

The most severe pathogen that attacks jute is *Macrophomina phaseolina*, causing stem and root rot. Other serious diseases are anthracnose (*Colletotrichum corchori* and *C. gloeosporioides*), black band (*Lasiodiplodia theobromae*), soft rot (*Sclerotium rolfsii*), jute mosaic (Begomovirus) and Hooghly wilt (Table 7.2).

7.2.2.1 Stem Rot

M. phaseolina is a necrotrophic fungus that infects more than 500 plant species and causes several diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot, basal stem rot and root rot in many plant species including major agricultural crops. It is affecting both *C. capsularis* and *C. olitorius* species. It is prevalent in all jute growing regions. Its incidence makes significant reduction in yield and quality of fiber (Fig. 7.3). The fiber yield loss is generally about 10%, but can be as high as 40% in severe infestation (Roy et al. 2008). Attack at early stage even leads to the death of the plant resulting total crop failure, while infection at the latter stage damages the quality of the bast fiber. The sclerotia can survive in soil and crop residue over four years, starting a new cycle of infection under favorable condition by invading

Table 7.2 List of important diseases in jute crop

| Name of the disease | Causal organism | Damage symptom | Distribution in India |
|---------------------|---|---|--|
| Stem rot | <i>Macrophomina phaseolina</i> | Damping off, seedling blight, leaf blight, collar rot | Assam, West Bengal, Bihar and Odisha of India, Bangladesh |
| Anthracnose | <i>Colletotrichum corchori</i> and <i>C. gloeosporioides</i> | Small lesions with round, gray margins on leaves; sunken spots on stem | Assam, Bihar, UP and North Bengal of India, Bangladesh, southeastern China |
| Black band | <i>Lasiodiplodia theobromae</i> | Small dark brown to black lesions, enlarges to girdle the stem | Assam, West Bengal, Bihar and Odisha of India, Bangladesh |
| Soft rot | <i>Sclerotium rolfsii</i> | Blackish brown lesions or depressions on the stem | Sporadic in India and Bangladesh |
| Jute mosaic | Begomovirus | Yellow flecks on leaf lamina Yellow mosaic appearance | Sporadic in India and Bangladesh |
| Hooghly wilt | <i>Ralstonia solanacearum</i> , <i>M. phaseolina</i> and <i>Fusarium solani</i> | Plants droops, hang down, turn brown, and ultimately dies within a day or two | Hooghly, Howrah, North-24 Parganas and Nadia of India, Bangladesh |

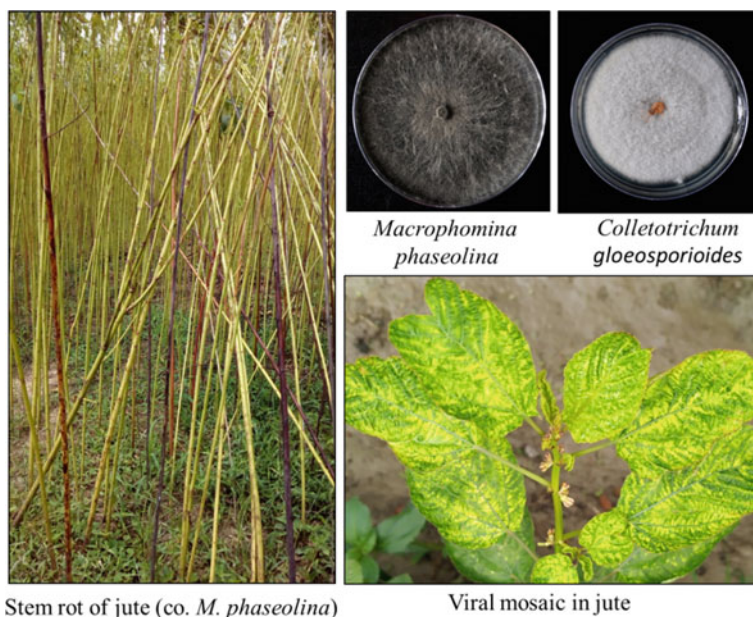


Fig. 7.3 Symptoms and pathogens of major diseases of jute

the plant cell wall mechanically or by releasing cell wall degrading enzymes (Islam et al. 2012).

Integrated management

- Medium to high land with well drained sandy loam soil is very good for jute crop.
- In field experiment with different dates of sowing of jute variety JRO-8432, early sown crop suffered from more stem rot than late sown crops.
- Sowing in the month of April reduce the incidence of stem rot than March sowing (De 2013). Stem rot disease showed declining trend in later sown crop.
- The infected seeds are the major source of the infection; seeds must be treated before sowing with carbendazim 50 WP @ 2 g/kg. When the disease incidence is 2% or more spraying of carbendazim 50 WP @ 2 g/L or copper oxychloride 50 WP @ 4 g/L or tebuconazole 25.9 EC @ 1.5 ml/L is recommended. If the infection is high, 3–4 sprays at 15–20 days interval may be used.

7.2.2.2 Anthracnose

It is a regular disease of capsularis belt of India. The disease entered India during 1930s along with jute germplasm from South-East Asia, particularly from Taiwan. Continuous rain, high humidity and temperature around 35 °C are congenial for anthracnose. It is widespread in southeastern China and is considered to be the most damaging disease of capsularis jute in China (Niu et al. 2016). It reduces both fibre

yield and quality, resulting in development of knotty fibre. It is a seed borne fungi, thus transmission from infected seed is a silent threat to the jute growers.

Integrated management

- Seed treatment with carbendazim 50 WP @ 2 g/kg or captan @ 5 g/kg and spraying of carbendazim 50 WP @ 2 g/L or captan @ 5 g/L or mancozeb @ 5 g/L check the disease spread.
- Seeds having 15% or more infection should not be used even after treatment. Removal of affected plants and clean cultivation reduce the disease (Sarkar and Gawande 2016).

7.2.2.3 Black Band

Once it was considered as minor disease but gradually it is spreading. Its incidence is seen both in *C. capsularis* and *C. olitorius* and causes serious damage to the older crop from July onwards, from which neither fiber nor seeds can be obtained. It first appears as small blackish brown lesions which gradually enlarge and encircles stem resulting in withering of apical shoot. On rubbing the stem surface, unlike stem rot profuse black sooty mass of spores adhere to the fingers. Crops grown from infected seeds show seedling blight.

Management

- Destruction and removal of diseased plants is recommended. Before sowing treat seed with carbendazim 50 WP @ 2 g/kg. Foliar application of carbendazim 50 WP @ 2 g/L, mancozeb @ 4–5 g/L or copper oxychloride @ 4 g/L water is recommended.

7.2.2.4 Hooghly Wilt

This disease is mostly prevalent where *tossa* jute is followed by potato or other Solanaceous crop. During 1970s and 1980s, causing 30–34% crop loss in Hooghly district of India, particularly when the preceding crop was potato (Ghosh 1983). During late eighties and early nineties, 5–37% disease was recorded in Hooghly district and 2–20% in some areas of Nadia and North 24 Paraganas (Mandal and Mishra 2001). Presently this disease is not a serious concern. The disease is caused by infection of a microbial complex involving *Ralstonia solanacearum*, *M. phaseolina* and *Fusarium solani* (Ghosh 1983).

Management

- Solanaceous crops such as potato and tomato should not be grown before jute in the same field.
- Seed treatment with carbendazim 50WP @ 2 g/kg and spraying the same fungicide @ 2 g/L of water reduce root rot incidence which favors the incidence of wilt.

7.2.2.5 Jute Mosaic

The disease is caused by a begomovirus and is transmitted by whitefly (Ghosh et al. 2007). It is also called as leaf mosaic, yellow mosaic or jute golden mosaic, as small yellow spots appear on leaf that spread and coalesce to form patches. It is reported in capsularis jute from different jute growing belts of India and Bangladesh. In India, the disease is reported in capsularis jute from West Bengal (Ghosh et al. 2007) and from Assam. The incidence of the disease has increased from 20 to 40% (Ghosh et al. 2007).

Management

- Use of seeds from mosaic-free plants, rouging of diseased plant and field sanitation are recommended.
- Spraying of imidacloprid 17.8 SL @ 100–125 ml/ha could prevent the spread of the disease through vector control.

7.3 Traditional Breeding Methods

7.3.1 *Intraspecific Hybridization*

7.3.1.1 Pedigree Breeding

Pedigree breeding involving two diverse parents, often advanced breeding lines is the major approach in new cultivar development in most crops including jute. Screening for insect-pest and disease resistance is often performed at late generations at F₇-F₈ during station trials. Following the station trials the materials are tested in national evaluation trials, where extensive screening for field resistance to various pests and diseases are performed. Most of the jute varieties released in India are having field tolerance to major insect-pests and diseases of jute, which provides considerable resistance to the field level damages caused by various insect-pests and diseases. For example, the jute variety JRO 204 exhibits considerable resistance to stem rot with a disease incidence of 4.2% (Mandal et al. 2021). However, no resistant variety has yet been developed for viral diseases of jute. Although the damage caused by the viral diseases in jute is negligible, in the changing climate, viruses may be a major threat to jute production as observed in case of mesta, where yellow mosaic virus is becoming a potential threat in recent years.

7.3.1.2 Backcross Breeding

Only a few reports are available on breeding efforts made for incorporating resistance to diseases in jute using backcross breeding. A resistant parent OIN 154 and

popular high yielding cultivar JRO 204 were crossed in a backcross breeding program at ICAR-Central Research Institute for Jute and Allied Fibres at Kolkata, India (Satpathy et al. 2019). The F_1 was backcrossed to the recurrent parent JRO 204 and selections were made in BC_1F_4 . A total of 200 recombinant backcross inbred lines (BC-RILs) were advanced to BC_1F_4 generations and screened using artificial stem inoculation. A total of 20 resistant lines were identified and utilized in subsequent resistant breeding programs.

7.3.2 *Interspecific Hybridization*

Wild crop relatives are major source of plant defense related traits. In jute, Palve et al. (2006) evaluated 84 accessions against stem rot disease and stem weevil insect-pest. Out of them, 66 were from six wild *Corchorus* species, and 18 belonged to the two cultivated *Corchorus* species. They observed that the wild *Corchorus* species *C. fascicularis*, *C. pseudo-olitorius* and *C. tridens* were resistant, but some accessions of *C. aestuans* and *C. trilocularis* were susceptible to stem rot infection. On the other hand, *C. fascicularis*, *C. pseudo-olitorius*, *C. urticifolius* and *C. tridens* exhibited high resistance to stem weevil infestation. Recently, wild species like *C. aestuans* have been found to be an important source of resistance to *M. phaseolina* in jute. Through interspecific hybridization between *C. aestuans* and *C. olitorius*, a resistant genotype RS-6 has been developed. This genotype showed considerable resistance to stem rot infection under sick plot (2.6% infection), stem inoculation (lesion length 5.9 cm) and screening in growth chamber (15.3% infection) (Mandal et al. 2021).

7.3.3 *Limitations of Classical Genetics and Breeding in Developing Resistant Cultivars*

Only a few sources of resistance have been found in the existing gene pool of jute (see Sect. 7.4). Combined with low genetic diversity, absence of genetic polymorphism for the resistance to the biotic stresses in the parental lines has hindered the resistance breeding efforts in jute. Till date, sporadic attempts have been made to decipher the genetics of insect-pest and disease resistance in jute. As genetic analysis requires distinct resistant and susceptible lines, clearly defined artificial screening systems and scoring methodologies for distinguishing the resistant lines from the susceptible lines are necessary. Various screening techniques have been used for identification of stem rot resistance including evaluation under sick plot and stem inoculation (Mandal et al. 2021), but a standard screening system for evaluating stem rot resistance is not yet available. For example, Kamruzzaman et al. (2013) observed that at a later growth stages, the lignified stem tissues are not preferred by the yellow mite, thus the mite damage is more severe at early crop growth stage. Considering this, they suggested to

screen for tolerance to yellow mite at early crop growth stage (<90 days). Standard evaluation systems (SESs) for insect-pest/disease resistance have been rigorously developed only in few major crops, such as rice and maize. Resistance breeding and molecular analysis of resistance in many crops will remain elusive until and unless SES for these traits are well-established. Non-availability of good screening system has limited the power of classical genetic analysis as well as identification of linked molecular markers and high-effect quantitative trait loci (QTLs) for marker assisted resistance breeding (MARB) in several crops, including jute.

7.4 Genetic Resources of Resistance Genes

Development of cultivars exhibiting heritable resistance to biotic stresses is a safer long-term solution over chemical control. Often, such resistance sources are found in weedy and wild landraces, germplasm collection or wild crop relatives. Unfortunately for jute, no wild relative can be found in its primary gene pool, as none of the wild *Corchorus* species readily hybridizes with cultivated jute. Even the two cultivated species *C. olitorius* and *C. capsularis* are not easily crossable. Therefore the primary gene pool of jute is principally within-species. The unique nature of the cultivation of jute as fiber crop, where the plants are harvested long before induction of flowering, ensures non-survival of any natural mutant. Untapped genetic potential can be harvested from African countries like Ethiopia and Sudan, the center of origin and diversity of many *Corchorus* species. It is noteworthy to mention that jute is consumed as minor vegetable in many African and South-East Asian countries, and often used as an ethnomedicinal plant for treating of various ailments by African *shamans*. While a good number of African collections have been evaluated for genetic variability (Benor et al. 2012), very few landraces have been screened for resistance to insect-pests and diseases. Mir et al. (2011) evaluated 12 *C. capsularis* genotypes under field condition and identified a resistant line CIM-036 showing 6% disease incidence. In contrast, the popular cultivar JRC 321 exhibited 19.4% disease incidence, while another susceptible genotype JRC 412 exhibited 22% disease incidence. Meena et al. (2015) evaluated 13 *C. olitorius* landraces at two locations in India for two consecutive years and identified six accessions (OIN-125, OIN-154, OIN-467, OIN-651, OIN-853 and OEX-27) to exhibit moderate resistance against stem rot. Another 40 germplasm accessions were screened by Nasim et al. (2017) against various diseases including stem rot, die back, soft rot, root rot, black band, anthracnose, leaf mosaic, leaf curl and root knot at Bangladesh and reported that Acc. Numbers 1045, 1050, 1060, 1062, 1065, 1143, 1261, 1338, 3711, 3724, 4178, 5009 and variety O-72, were resistant to majority of the diseases. Conversely, Sharmin et al. (2012) reported that the cultivar O-72 was susceptible to stem rot disease. At ICAR-Central Research Institute for Jute and Allied Fibers, India, over 500 lines have been evaluated for resistance to major pests and diseases in natural conditions during the past decades. These studies identified a number of indigenous and exotic lines of jute as donors for resistance to insect-pests and diseases, particularly

under hot-spot conditions. For example, lines OIN-07, OIN-27, OIN-121, OIN-125 and CIM-07 exhibited lower stem rot incidence than the susceptible check variety JRC-412 (AINPJAF Annual Report 2020). Similarly, OIJ-08 recorded about 50% lower infestation than cultivar JRO-2407. Another landrace OIN-154 collected from Madhya Pradesh, India exhibited good resistance to root knot nematode infestation as well as to stem rot, and has been utilized in resistance breeding programme to develop elite breeding lines. The wild relatives of jute are good sources of resistance to major biotic stresses. However, a *C. trilocularis* accession was noted to be resistant to stem rot disease by Sharmin et al. (2012). They identified two xyloglucan endotransglycosylase/hydrolase (XTH) genes (*CoXTH1* from *C. olerorius* and *CtXTH1* from *C. trilocularis*) that expressed differentially upon challenged inoculation with *M. phaseolina*. The expression of *CtXTH1* gradually amplified over time while *CoXTH1* was found to be downregulated. Since XTH is a key player in cell wall development, enhanced expression of *XTH1* in the wild species is indicative of XTH polymerization that may provide resistance to stem rot disease. De and Mandal (2012) identified eight accessions, OIN-107, OIN-125, OIN-154, OIN-157, OIN-221, OIN-651, OIN-853 and OIJ-084 as moderately resistant to stem rot. Gotyal et al. (2014) screened jute germplasm against stem rot for two years and identified OIN-431 as a resistant germplasm. They reported that the *C. capsularis* germplasm exhibited more susceptibility than the *C. olerorius* germplasm. A list of important germplasm accessions showing good resistance to various biotic stresses are presented in Table 7.3.

Little information is available for resistance to insect-pests, which are more difficult to screen as unlike fungi, insects move around to cause damage in different parts of the plant. In addition, plants have different mechanisms like antibiosis, antixenosis or tolerance, which are difficult to score. For example, yellow mite, one of the most serious insect-pests of jute can damage the plant at any crop growth stage, move from one leaf to another and can spread from one plant to another plant. Since artificial screening against yellow mite is troublesome, natural field reaction is considered the most appropriate approach for identifying tolerant lines. It was observed that the *C. capsularis* cultivars are less preferred than the *C. olerorius* cultivars by yellow mite

Table 7.3 Sources of insect-pest resistance in the primary and secondary gene pool

| Common name of the pest/disease | Resistant source | References |
|---------------------------------|--|------------------------------|
| Hairy caterpillar | <i>C. aestuans</i> (WCIN-179) | Gotyal et al. (2014) |
| Jute semilooper | OIN-87, OIN-88, OIN-89, OIN-92, OIN-94 | AINPJAF Annual Report (2017) |
| Jute stem weevil | OIN-95, OIN-114, OIN-121, OIN-100, OIN-110 | |
| Yellow mite | OIN-91, OIN-96, OIN-97, OIN-98, OIN-103 | |
| Stem rot | <i>C. aestuans</i> WCIN-136-1 | Germplasm reg. no. INGR21036 |

(Rahman and Khan 2012b). However, significant variation was observed among *C. capsularis* genotypes under field screening. Kamruzzaman et al. (2013) observed that infestation of female yellow mite was lower ($2.2/\text{cm}^2$) in moderately tolerant cultivars BJC-83 and CVL-1, but damage of yellow mite caused yield loss of 54%. In contrast, BJC-7370 with higher female mite infestation ($3/\text{cm}^2$) exhibited 60% yield loss. In addition to cultivars the incidence of yellow mite is dependent on environmental conditions including temperature, relative humidity, plant canopy structure and the age of the plant (Islam et al. 2020). The population of yellow mite was found to concentrate more on the five apical leaves, while the lower leaves have less population. Moreover, the population is more at noon than morning and afternoon. A standard screening system for yellow mite, thus, has to give proper weightage to these factors for identifying effective resistant lines.

7.5 Resistance Gene-Based Marker Development and Utilization

Resistance gene analogs (RGA) are genomic sequences sharing conserved regions of plant resistance genes. The RGAs are often part of R genes, or may be tightly linked with R genes. RGA markers are a group of functional DNA markers that reveal variability in these RGAs, which is used for genetic diversity analysis, evolutionary studies and plant genetic resource characterization (Satya and Chakraborty 2015). Molecular markers based on RGAs are functionally related to plant defense response, thus these have unique advantages for studying genetic diversity of isolated populations adapted under different environmental conditions (Satya and Chakraborty 2015). Genetic polymorphism of RGA sequences or resistance gene analog polymorphism (RGAP) is a unique functional marker system, which has been used in many crop species for genetic mapping of R genes, genetic polymorphism analysis of population, evolution of plant resistance, population ecology and plant genetic resource characterization. Such information is desirable not only for devising strategies for genetic improvement in a breeding program or to transfer genes from sexually compatible species, but also to gain insight in the evolutionary processes of plant defense related genes (Michelmore and Meyers 1998). The evolution of RGAs is influenced by several factors such as host–pathogen co-evolution, geographical isolation, adaptation of a crop to a particular ecology, and extent of cultivation as a crop. Currently, two models are suggested for the origin of multiple R genes. One model considers that recombination and unequal crossing overs at intergenic regions have led to divergence of a single gene into large multi-gene families (Richter and Ronald 2000). The other hypothesizes that the plant R genes have evolved through a birth and death process involving recombination and gene conversion that alter the structures of the functional domains followed by divergent selection (Michelmore and Meyers 1998). The first process results in a concerted evolution with fixation of highly homogeneous populations with high inter-population diversity. In contrast,

the birth and death based divergent selection would allow more diversity within each population and more possibilities for inter-population genetic exchange.

7.5.1 Utilization of Resistance Gene-Based Genic Markers for Domestication and Population Genetic Analyses in Jute

As a dominant marker system, RGAs have certain advantages for genome mapping and phylogenetic analysis. RGAs are dispersed throughout the genome, making these markers suitable for genetic diversity studies at genome level. RGAs are principally associated with functional regions of genome, being distributed in tandem array of repeated gene families (Poczai et al. 2013). Many of these genes may be functionally inactive at certain reference points (pseudogenes), but may express under different disease scenario or environmental conditions. RGA markers thus may be more suitable than neutral markers for study of geographically isolated populations domesticated under different agro-ecological conditions. Genetic diversity of 80 accessions of the cultivated and the wild *Corchorus* species when evaluated using RGA markers, amplified a total of 182 fragments in the Indian *C. olitorius* group, of which 76.4% were polymorphic. In the African group 164 RGA fragments were amplified exhibiting 84.8% polymorphism (unpublished data, P. Satya).

7.5.2 Use in Phylogenetic Analysis

Markers associated with stress evolve with adaptation, which makes them useful for identification of geographically isolated groups (Satya et al. 2014). RGA-based phylogenetic analysis of 80 *Corchorus* accessions following weighted neighbor-joining method identified four major genetic groups (Fig. 4a). The first group was a mixture of African wild jute and Indian fiber type cultivars along with mutants that were mostly derived from these cultivars. The second group consisted of only Indian jute landraces. The third group was heterogeneous (25 accessions), comprising of African, Indian jute accessions and wild relatives. Similar results were also observed using principal coordinate analysis (Fig. 4b). As the African *C. olitorius* genotypes have broader genetic base than the Indian *C. olitorius* genotypes for RGA loci, a differential adaptation for RGA loci in African and Indian jute can be envisaged. Of particular interest was *C. aestuans*, which was distantly placed from other *Corchorus* species, indicating it might harbor different resistant genes. Indeed, *C. aestuans* is being used in resistance breeding to develop stem rot resistant genotypes (Mandal et al. 2021).

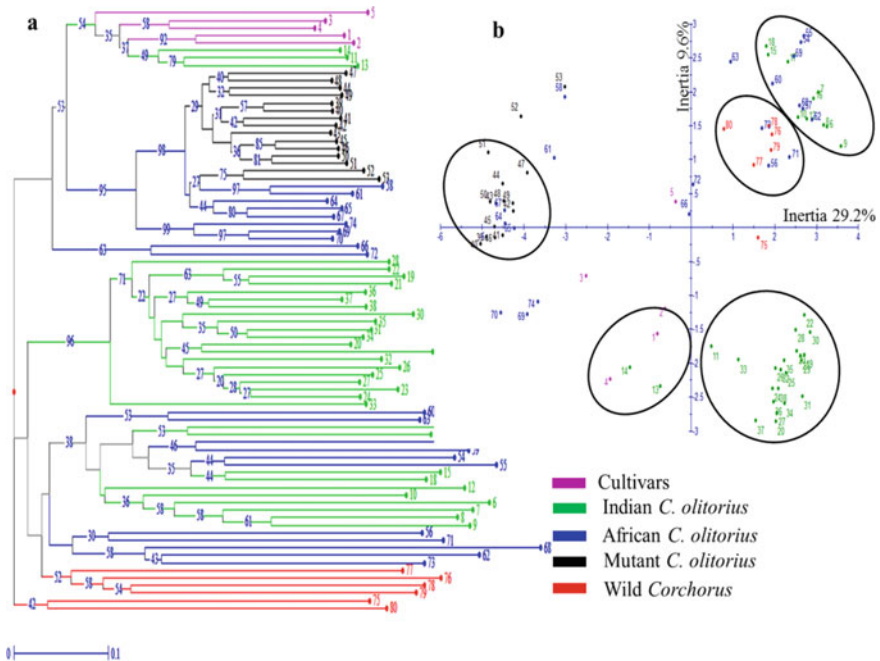


Fig. 7.4 Phylogenetic analysis of jute germplasm using RGA markers. **a** Nearest-neighbor phylogenetic tree of 80 *Corchorus* accessions (color-coded). **b** Principal coordinate analysis of 80 *Corchorus* accession

7.5.3 Use of RGA in Population Structure Analysis

RGA-based population structure analysis following Bayesian approach resulted in clear differentiation of genetic structures (unpublished data, P. Satya). At $K = 2$, the proportion of two genetic clusters under non-admixture model were 61.82% and 38.18% (Fig. 7.5). The first cluster comprised all the wild species, 43.5% Indian *C. olitorius* accessions, 60% of African *C. olitorius* accessions and 72% of *C. olitorius* accessions from other countries. All the fiber type cultivars and mutant genotypes of *C. olitorius* and fiber type cultivars of *C. capsularis* from India were classified under cluster II. At $K = 3$, cluster I remained largely unchanged (60.9%), while cluster II was subdivided into two clusters, having 15.45% (cluster II) and 23.64% (cluster III) accessions, respectively. Cluster III comprised the 15 mutant genotypes of *C. olitorius*, three accessions from Kenya, two accessions from Nigeria, three accessions from Russia, two accessions from Myanmar and one accession from Thailand. The results of admixture model closely followed the outputs from non-admixture model (Fig. 7.5). The African and India *C. olitorius* also shared high genetic relatedness for RGA loci, which indicates tossa jute might have migrated during early civilization periods in India, most possibly through land as an herbal medicinal or vegetable plant. In another study, Satya et al. (2014) observed clear

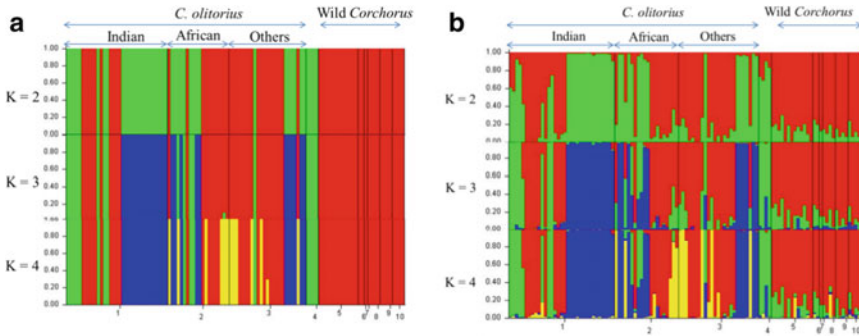


Fig. 7.5 Optimal population structure of *Corchorus* based on RGAP under non-admixture (a) and admixture (b) models. Each vertical line represents one population, each color represents one cluster. The length of the colored segment represents estimated proportion of membership of each cluster in the population

population structure difference between Indian and African jute genotypes using neutral simple sequence repeat (SSR) and functional peroxidase gene-based markers, which also indicate early domestication and introduction of jute in India.

7.6 Genomics-Aided Breeding for Resistance Traits

7.6.1 Genomics to Decipher Plant-Pathogen Interaction Pathways in Jute

Plants manifest their defense mechanism against numerous biotic agents by expressing diverse genes associated with resistance. Several genes are involved in recognition of pathogen/pest and development of cascades of signal transduction pathways. A good number of genes are involved in the KEGG plant-pathogen interaction pathway (KO04626) that expressed in hypocotyl (earliest growth stage) even in absence of any biotic stress (Table 7.4) indicating that jute expresses an array of pathogen recognition and resistance genes.

Analysis of hypocotyl transcriptome (GCNR00000000) and bast transcriptome (GBSD00000000) of white jute (*C. capsularis*) cv. JRC-212 was performed in order to identify genes which can potentially be involved in stem rot resistance. Jute expresses an array of biotic stress resistance related genes both in hypocotyl and bast tissues (Fig. 7.6). Classification of these genes in different categories depicts enrichment of two different classes—(i) General biotic stress-related genes and (ii) Pathogenesis-related (PR) proteins (Fig. 7.6). General biotic stress-related genes are a mixed class of genes having diverse functionalities, but in general, are upregulated during all kind of biotic assault. On the other hand, PR-proteins are a group of toxic plant proteins which are also structurally diverse in nature. These are constitutively

Table 7.4 Jute genes expressing in KEGG pathway KO04626 (Plant-Pathogen interaction) at early developmental stage

| KEGG orthology | Gene name | Jute genes mapped in KEGG pathway |
|----------------|--|--|
| K00864 | Glycerol kinase | unigene_S3_18878, unigene_S3_22035, unigene_S3_29038 |
| K02183 | Calmodulin | unigene_S3_13054, unigene_S3_34974, unigene_S3_35082, unigene_S3_35105, unigene_S3_37730, unigene_S3_37911 |
| KO4079 | Molecular chaperone HtpG | unigene_S3_8998, unigene_S3_13558, unigene_S3_31587 |
| KO4368 | Mitogen-activated protein kinase kinase 1 | unigene_S3_19240 |
| KO5391 | Cyclic nucleotide-gated channel, plant | unigene_S3_5842, unigene_S3_5843, unigene_S3_8206, unigene_S3_8207, unigene_S3_8208, unigene_S3_8436, unigene_S3_31190, unigene_S3_31281, unigene_S3_31336, unigene_S3_31457, unigene_S3_31677, unigene_S3_31776, unigene_S3_42931, unigene_S3_42937 |
| K09487 | Heat shock protein 90 kDa beta | unigene_S3_16535, unigene_S3_31076, unigene_S3_32140 |
| K12795 | Suppressor of G2 allele of SKP1 | unigene_S3_24963, unigene_S3_30641, unigene_S3_44523 |
| K13412 | Calcium-dependent protein kinase | unigene_S3_6046, unigene_S3_6047, unigene_S3_8069, unigene_S3_9373, unigene_S3_10367, unigene_S3_10787, unigene_S3_11019, unigene_S3_11616, unigene_S3_13721, unigene_S3_21586, unigene_S3_30827, unigene_S3_31104, unigene_S3_31448, unigene_S3_32948, unigene_S3_33750, unigene_S3_33780 |
| K13413 | Mitogen-activated protein kinase kinase 4/5 | unigene_S3_11687 |
| K13414 | Mitogen-activated protein kinase kinase 1 | unigene_S3_31308, unigene_S3_31359 |
| K13416 | Brassinosteroid insensitive 1-associated receptor kinase 1 | unigene_S3_16444 |
| K13420 | Leucine rich repeat (LRR) receptor-like serine/threonine-protein kinase FLS2 | unigene_S3_17046, unigene_S3_44483 |
| K13424 | WRKY transcription factor 33 | unigene_S3_33360, unigene_S3_33982 |

(continued)

Table 7.4 (continued)

| KEGG orthology | Gene name | Jute genes mapped in KEGG pathway |
|----------------|---|--|
| K13425 | WRKY transcription factor 22 | uniGene_S3_39301 |
| K13427 | Nitric-oxide synthase | uniGene_S3_30358 |
| K13429 | Chitin elicitor receptor kinase 1 | uniGene_S3_2478, uniGene_S3_2479, uniGene_S3_6662, uniGene_S3_6663, uniGene_S3_6664, uniGene_S3_19454, uniGene_S3_40624 |
| K13430 | Serine/threonine-protein kinase PBS1 | uniGene_S3_12240, uniGene_S3_30782 |
| K13434 | Pathogenesis-related genes transcriptional activator PTI6 | uniGene_S3_11274 |
| K13436 | <i>pto</i> -interacting protein 1 | uniGene_S3_18586, uniGene_S3_29132, uniGene_S3_33171 |
| K13447 | Respiratory burst oxidase | uniGene_S3_6079, uniGene_S3_6615, uniGene_S3_25996, uniGene_S3_32011, uniGene_S3_39638 |
| K13448 | Calcium-binding protein CML | uniGene_S3_8981, uniGene_S3_12622, uniGene_S3_13244, uniGene_S3_13853, uniGene_S3_14800, uniGene_S3_19509, uniGene_S3_28107, uniGene_S3_29343, uniGene_S3_30322, uniGene_S3_30611, uniGene_S3_30637, uniGene_S3_33699, uniGene_S3_34923, uniGene_S3_34949, uniGene_S3_35760, uniGene_S3_35842, uniGene_S3_38062, uniGene_S3_38224, uniGene_S3_38517, uniGene_S3_39026, uniGene_S3_39356, uniGene_S3_40103 |
| K13456 | <i>RPM1</i> -interacting protein 4 | uniGene_S3_29227 |
| K13457 | Disease resistance protein RPM1 | uniGene_S3_11841, uniGene_S3_12078, uniGene_S3_12156, uniGene_S3_12201, uniGene_S3_31985 |
| K13458 | Disease resistance protein RAR1 | uniGene_S3_40708 |
| K13459 | Disease resistance protein RPS2 | uniGene_S3_16907, uniGene_S3_31867, uniGene_S3_32694 |
| K18834 | WRKY transcription factor 1 | uniGene_S3_6366 |
| K18835 | WRKY transcription factor 2 | uniGene_S3_10459, uniGene_S3_31188 |
| K18875 | Enhanced disease susceptibility 1 protein | uniGene_S3_33706 |

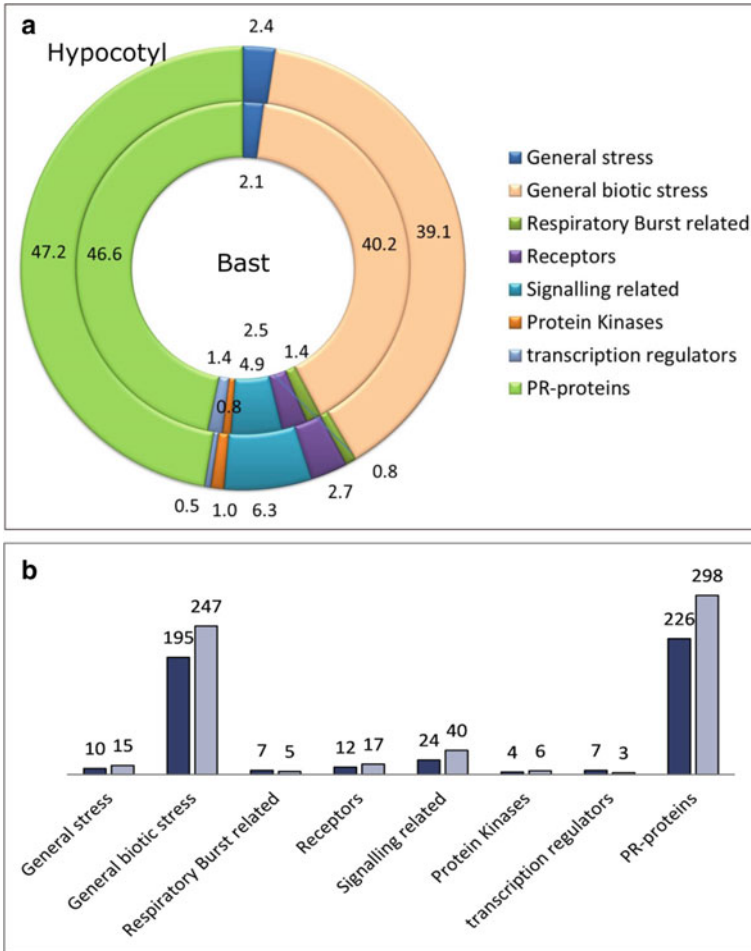


Fig. 7.6 Categorization of biotic stress-related genes obtained from hypocotyl and bast transcriptomes of jute. **a** Percentage contribution of each group of genes to the total biotic stress-related genes expressed, presented in doughnut charts. **b** Vertical bar chart representing the absolute number of gene expressed under each category. Each pair of bars indicates genes expressed in bast (first bar) and hypocotyl (second bar)

expressed at a low level in normal condition but are upregulated by several-folds in response to invading fungal pathogens (Van Loon 1997).

PR-proteins can get accumulated at intracellular and intercellular spaces and variation of the type- and content- of PR-proteins also depend upon the type of the tissue. Cell wall is one of the major sites of PR protein accumulation (Agrios 2005). Among the expressed PR-proteins of hypocotyl and bast, we could identify two specialized classes—non-expressor of pathogenicity related (NPR) proteins and proteinase inhibitors (PI) (Fig. 7.7a).

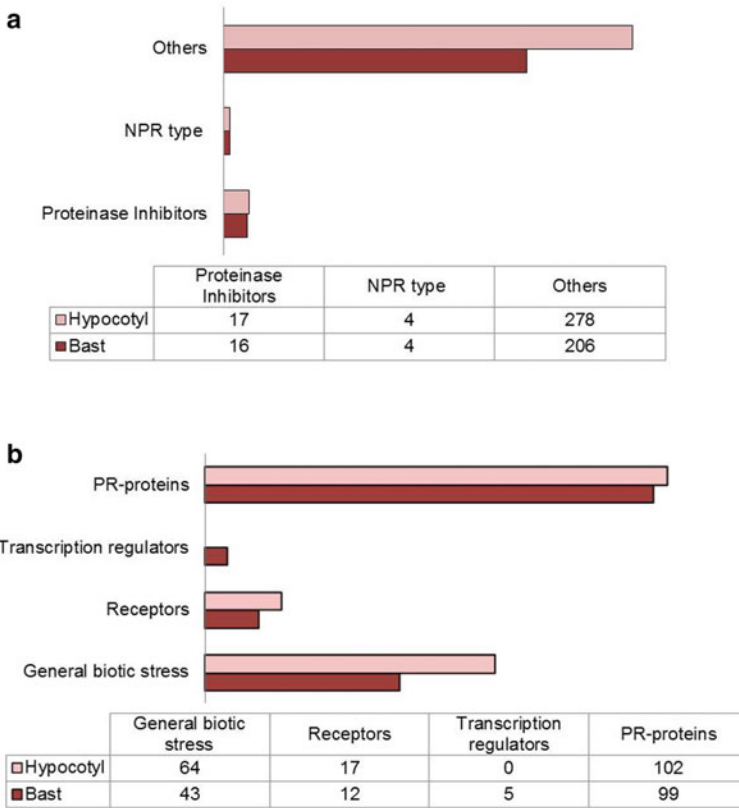


Fig. 7.7 Classification of PR-Proteins (a) and Leucine rich repeat (LRR)-motif containing proteins (b) into functional categories. The horizontal bar chart depicts relative abundance of each group and the table below each graph represents absolute number of expressed gene in two different tissue-types

7.6.2 Genomics for Identifying Genes Involved in Resistance to Stem Rot Disease

Among all the diseases of jute, stem rot caused by *M. phaseolina* is the most prominent and destructive one. There are reports of some well-known basic pathways which tend to be upregulated or activated during defense response in general, like cell wall biosynthesis, production of reactive oxygen species, programmed cell death, synthesis of gaseous phytohormones such as ethylene, jasmonic acid, (JA), salicylic acid (SA) and abscisic acid (ABA) and hypersensitive response (HR) (Biswas et al. 2014). They also identified 1715 contigs in disease inoculated plants. Of these, 158 were expressed in response to abiotic or biotic stress, about 22% being involved in biotic stress tolerance. Gene ontology analysis revealed that the majority of these

genes were hydrolases, transferases, protein binding factors and molecular transporters. These genes were involved in several gene ontology pathways, namely, “hydrolase”, “oxidoreductase”, “secondary metabolic process”, “cellulose and pectin containing cell wall” and “lyases”, being overexpressed in disease inoculated plants. The study also identified 22 miRNAs that could be master regulators of the systemic acquired resistance (SAR) pathways. A number of disease resistance genes from jute could be placed on the KEGG ‘plant-pathogen interaction pathway’ (Table 7.3). Additionally, we also classified the LRR containing proteins, a well-recognized class of proteins implicated in plant disease resistance (McHale et al. 2006, Lee and Yeom 2015), into different functional categories (Fig. 7.7b) and observed that most of the LRR proteins belong to PR-proteins. Kabir et al. (2021) identified 119 APETALA2/Ethylene-Responsive Factors (*AP2/ERF*) genes. They observed that a group of different transcription factors, namely *CoERF-01*, *CoERF-39*, *CoDREB-18*, *CoDREB-23* and *CoDREB-13* were downregulated in stem rot infected tissues, which indicate possible involvement of these genes in disease signal transduction pathway. Three genes, *CoDREB-01*, *CoDREB-28* and *CoDREB-30* were upregulated, which might be involved in response to pathogen induced stress.

7.6.3 Genomics for Deciphering Systemic Acquired Resistance

Up-regulation of a large number of PR-proteins in a cohort manner is a key characteristic feature of SAR, a well-established defense strategy of plants against necrotrophs, which does not involve HR mediated cell death (Durrant and Dong 2004). There is an intricate balance of plant defense system maintained by interplay of NPRs and PIs. Plants have two distinct defense mechanisms, one against biotrophs (pathogens that requires live host for nutrient acquisition) and the other against necrotrophs (pathogens that acquires nutrition from dead host). Defense against biotrophs is mediated by SA signaling where NPR1 functions as master regulator and the process is culminated by HR-mediated death of infected cell. While on the other hand, defense against necrotrophs is regulated by JA signaling which is culminated by up-regulation of PI-I and PI-II group of proteins, which do not induce cell death but owing to their antimicrobial activity make the cellular environment of the host inhospitable for the necrotrophic pathogen (Rahman et al. 2012). The intricate balance between two defense mechanisms is maintained by transcription factor *TGAI* which is, in turn, regulated by *NPR1* master regulator and suppresses JA dependent defense signaling during biotrophic infection (Rahman et al. 2012). While another transcription factor *SNCI*, which we found to be expressed specifically in bast tissue in our transcriptome data, is reported induce *TGAI* mediated, but *NPR1* independent, upregulation of defense signaling (Rahman et al. 2012) and hence, might be associated with defense against necrotrophs.

7.6.4 Deciphering Role of Chitinase in Resistance

Chitinases are well-annotated class of genes implicated in defense against fungal pathogens. These are the group of protein which disintegrates chitin—the prime polysaccharide component of fungal cell wall (60% w/w of the cell wall)—by enzymatic hydrolysis and limits the fungal infection (Pusztahelyi 2018). In fact, four (PR-3, PR-4, PR-8 and PR-11) out of 17 defined families of PR-proteins contain different types of chitinases (Moosa et al. 2018). The hypocotyl transcriptomes of jute cv. JRC 212 contains 12 chitinases. Evidence of up-regulation of plant chitinases in response to *M. phaseolina* infection has also been reported in several plants (Saima and Wu 2019).

7.7 Brief Account of Molecular Mapping of Resistance Genes and QTLs

Genetic mapping of resistance to fungal pathogens using molecular markers is a well-established approach to identify additive and dominance components of genetic effects and also to identify molecular markers linked to the trait in concern to aid MARB. Since *C. olitorius* is the principal cultivated jute species, development of resistant cultivar is crucial in *C. olitorius* to avert major economic loss from stem rot infection. The genetic basis of resistance to this disease is not well investigated, and only a few resistant genetic stocks have been identified till date (De and Mandal 2012). A study in *C. olitorius* reported quantitative inheritance exhibiting presence of both additive and dominant gene actions (De and Kaiser 1991). Quantitative inheritance of resistance to *Macrophomina* is observed in many crop species. This approach has been a major strategy to combat *Macrophomina* in several crop species including cowpea, sorghum and bean. Only a few reports of genetic mapping and quantitative trait loci (QTL) identification are available in jute. A few linkage maps have been created in *C. capsularis*, using randomly amplified polymorphic DNA (RAPD), sequence-related amplified polymorphisms (SRAP), inter-simple sequence repeats (Chen et al. 2014) and single nucleotide polymorphism markers (Biswas et al. 2015), but no QTL was placed on these maps. QTLs for fiber yield and associated characters on SSR and restriction-site-associated DNA (RAD) based linkage map have been identified in *C. olitorius* (Das et al. 2012; Topdar et al. 2013; Kundu et al. 2015).

Plants harbor a large array of defense related genes including pathogen specific active resistance (R) genes, RGAs as well as genes involved in HR including synthesis of pathogen inducible proteins (PR-proteins) and modulation of oxidative burst. Among these, two groups of genes are present in large families (superfamilies), the R gene families sharing common nucleotide binding site-Leucine rich repeat (NBS-LRR) motif encoding sequences and the peroxidase gene superfamily that comprise of a large number of plant peroxidase genes (POGs). These genes are often distributed

as gene clusters in particular genomic regions in plant genomes (Chen et al. 2015). Many of the candidate R genes and RGAs have been mapped and found to be linked with resistance phenotype against a number of pathogens (Marone et al. 2013; Chen et al. 2015).

The first linkage map for locating resistance to stem rot disease was developed by Mir et al. (2011) using 69 F₂ from a cross of CIM-036 (resistant) and JRC-412 (susceptible). They used RAPD and SCAR (sequence characterized amplified region) markers from SSR loci and identified two linkage groups LG1 (seven markers) and LG2 (two markers). In our study (Satya et al. 2016), *C. oltorius*, OIN 154 was used as donor for resistance by backcrossing to high yielding cultivar JRO 204 as a recurrent parent. A backcross mapping population was constituted comprising of 120 BC₁F₂ genotypes. Under a project funded by Department of Biotechnology, Government of India, a total of 88 SRAP primer combinations were screened for polymorphism among the parents. Likewise, 110 SSR, 20 RGA and 12 POG markers were also screened for polymorphism. A genetic map was constructed carrying 66 SRAP, 11 SSR, 6 RGA and 6 POG markers (Fig. 7.8). The number of markers in different linkage groups (LGs) ranged from 3 (LG7) to 21 (LG1). Chromosome-wide marker interval varied from 3.3 cM (LG2) to 19.2 cM (LG7), with a genome-wide marker interval of 8.3 cM. The distribution of SRAP markers was random over all the LGs, but LG4 comprised of only SRAP markers. The distribution of SSR markers was also random, though no SSR marker could be mapped on LG4. However, the distribution of RGA and POG markers was non-random, showing clustering of RGA and POG loci on certain LGs. The RGA markers were present on LG2, LG3 and LG5. Similarly, the POG markers were also distributed on LG2, LG3 and LG5, showing clustered distribution on LG5 (Satya et al. 2016).

Based on multiple QTL mapping, a total of three QTLs were identified on LG3 and LG5. Of these, *qRM-3-1* was a major disease resistance QTL, explaining 29.4% of the phenotypic variance with a log of odd's ratio (LOD) peak of 11.04 on LG3. A second QTL, *qRM-3-2* was mapped on LG3 with LOD values of 2.0, explaining

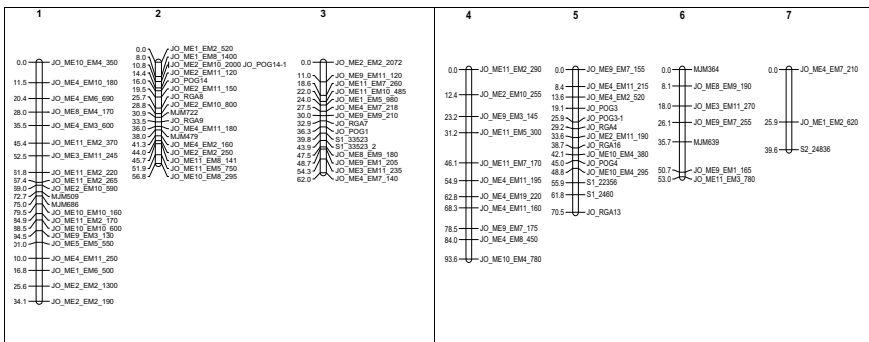


Fig. 7.8 Genetic linkage map of seven LGs of *C. oltorius* constructed with SRAP, RGA, POG and SSR markers. The genetic distances (cM) from top to bottom are indicated to the left of the LGs

4.4% of the phenotypic variance, respectively. A third QTL, *qRM-5-1* was identified on LG5, with a peak at 45.0 cM. Large effect QTLs for resistance to *Macrophomina* was also reported by Reddy et al. (2008) explaining up to 19.29% of the phenotypic variation.

While marker development for disease resistance in jute has made a moderate progress, little effort has been directed towards identification of markers lined with insect-pest resistance. Ghosh et al. (2010) developed a linkage map by crossing *C. olitorius* variety O-7/95, tolerant to yellow mite attack and var. O-72, sensitive to mite attack. They genotyped 150 F₂ plants using 88 SSR markers, phenotyped using a mite tolerability index (MTI) and constructed a genetic map comprising of 21 SSR markers distributed over five LGs and proposed three markers to be linked with MTI based on chi-square test. However, the trait was not mapped on any of the LGs and is consequently of little further use.

7.8 Brief on Genetic Engineering for Resistance Traits

Development of transgenic organisms has become a routine practice in the field of plant molecular biology for the purpose of gene functional validation. Apart from this, commercial transgenic crops have also shown promise worldwide in the direction of food and livelihood security. In India, although transgenic food crop has still not obtained approval for commercial cultivation, but a lone fiber crop—transgenic Bt-cotton—has shown its worth by propelling the country to become a major cotton producer in the world. But transgenics have not flourished much in case of jute—the second-most important commercial plant fiber grown in the world. The major obstacle for producing transgenic jute lies in its extreme recalcitrance to tissue culture mediated plant regeneration (Saha and Sen 1992; Sarker et al. 2008; Saha et al. 2014). Nevertheless, efforts have been strengthened in recent times to optimize transgenic protocol in jute and also transfer desirable traits. The first report of jute transformation dates back to the year 2008 when two different protocol of jute transformation were proposed by groups working at University of Dhaka, Bangladesh. One of these two studies reports *Agrobacterium tumefaciens*-mediated transformation of petiole-attached cotyledons and mature embryo explants obtained from two different varieties of white jute (Sarker et al. 2008). However, this study validated positive transformants only by GUS (*β-glucuronidase*) expression and polymerase chain reaction. Sajib et al. (2008) developed a tissue culture independent method of transforming *C. olitorius* utilizing *in planta* transformation technique. Juvenile jute plants were subjected to transformation by pricking with fine needle at the shoot apical meristem followed by agroinfiltration (Sajib et al. 2008). Production of transgenic jute harboring artificial microRNA (amiRNA) and hairpinRNA (hpRNA) targeting downregulation of monolignol biosynthetic genes were achieved using this protocol (Shafrin et al. 2015, 2017). Two different transformation protocols for *C. capsularis* were reported from Indian Institute of Technology, Kharagpur, India. Both of these protocols reported transformation of popular white jute variety JRC 321. One of these

two protocols reported production of a transgenic hairy root system carrying *gusA* reporter gene in jute through *A. rhizogenes*-mediated root infection, which can further be used as a continuous source of explant for obtaining transgenic plants (Chattopadhyay et al. 2011). While the other protocol reported successful stable transformation of jute with bialaphos resistance gene (*bar*) by using particle bombardment of apical meristematic tissues of one day-old germinated seedlings (Bhattacharyya et al. 2015). Stable transformation technique for *C. capsularis* var. JRC 321 through *A. tumefaciens*-mediated shoot tip transformation is also available (Saha et al. 2014). This protocol was used to introduce Cry1Ab/Ac δ -Endotoxin (Majumder et al. 2018a), rice *chitinase11* (*OsChi11*) and *Phosphinothricin N-acetyltransferase* (*bar*) genes in jute (Majumder et al. 2018b) for resistance to lepidopteron pests, stem rot disease and herbicide (Phosphinothricin), respectively. The major genetic transformation efforts for introducing biotic stress tolerance in jute ARE presented in Table 7.5.

An important trait which has been incorporated in transgenic jute is resistance to lepidopteran insect-pests. Transformation of white jute with synthetically fused *cry1Ab/Ac* gene of *Bacillus thuringiensis* (Bt) resulted in increased protection to jute- semilooper (*Anomissa bulifera* Guenee), hairy caterpillar (*Spilarctia obliqua* Walker) and indigo caterpillar (*Spodoptera exigua* Hubner). The transgenic lines expressed Cry1Ab/Ac endotoxin in the range of 0.16 to 0.35 ng/mg of leaf which resulted in high insect mortality (66–100% in case of semilooper and hairy caterpillar, while 87.50% in case of indigo caterpillar) in detached leaf assay (Majumder et al. 2018a). Apart from this, transgenic expression of rice chitinase (*Chi11*) and *Phosphinothricin N-acetyltransferase* (*bar*) genes in white jute has shown promising results in controlling *M. phaseolina* infection as well as imparting herbicide tolerance in jute. The transgenic plant harboring dual-gene construct of *Chi11* and *bar* not only demonstrated high chitinase induced antifungal activity against *M. phaseolina* in detached leaf assay but also successfully withstood 10 mg/L glufosinate ammonium in culture media as well as glufosinate herbicide (0.25%) (Majumder et al. 2018b). In this study, a 473 bp long cDNA of rice chitinase 11 (X54367) was cloned downstream of constitutive promoter CaMV35S and shoot tips of jute was transformed through *Agrobacterium* mediated transformation method. Crude protein extract obtained from T₂ transgenic plants were found to degrade chitin much more effectively than control non-transgenic plants both in gel diffusion assay and in in-solution assay. Results of whole plant antifungal bioassay carried out in transgenic and non-transgenic-plants at about 80–90 days after sowing also demonstrated much reduced lesion length (typical to stem rot) in transgenic plants compared to non-transgenic strategies. Finally, better quality of fiber was also observed after retting from transgenic plants compared to non-transgenic controls.

7.9 Future Perspectives

Despite presence of numerous pests and diseases causing economic damage of jute crop at various growth stages, progress in genomic research on the biotic stress

Table 7.5 Summary of the jute transformation techniques developed

| Transformed species | Variety used | Transformation technique | Explant | Gene transferred | References |
|----------------------|-----------------|--|---|--|-----------------------------|
| <i>C. capsularis</i> | CVL-1 and CVE-3 | <i>Agrobacterium tumefaciens</i> -mediated | Petiole-attached cotyledons and mature embryo | β -glucuronidase (<i>gusA</i>) | Sarker et al. (2008) |
| <i>C. olitorius</i> | O-72 | <i>Agroinfiltration</i> | Shoot apical meristematic region | β -glucuronidase (<i>gusA</i>) | Sajib et al. (2008) |
| <i>C. olitorius</i> | O-9897 | <i>Agroinfiltration</i> | Shoot apical meristematic region | 5H-amiRNA and C3H-amiRNA | Shafirin et al. (2015) |
| <i>C. olitorius</i> | O-9897 | <i>Agroinfiltration</i> | Shoot apical meristematic region | COMT-hpRNA and C4H-hpRNA | Shafirin et al. (2017) |
| <i>C. capsularis</i> | JRC 321 | <i>A. rhizogenes</i> mediated | Root tip | β -glucuronidase (<i>gusA</i>) | Chattopadhyay et al. (2011) |
| <i>C. capsularis</i> | JRC 321 | Particle bombardment | Apical meristematic tissues | Bialaphos resistance gene (<i>bar</i>) | Bhattacharyya et al. (2015) |
| <i>C. capsularis</i> | JRC 321 | <i>A. tumefaciens</i> -mediated | Shoot tip | β -glucuronidase (<i>gusA</i>) | Saha et al. (2014) |
| <i>C. capsularis</i> | JRC 321 | <i>A. tumefaciens</i> -mediated | Shoot tip | Cry1Ab/Ac δ -Endotoxin | Majumder et al. (2018a) |
| <i>C. capsularis</i> | JRC 321 | <i>A. tumefaciens</i> -mediated | Shoot tip | <i>OxChi11</i> and <i>bar</i> | Majumder et al. (2018b) |

tolerance in jute is limited. However, a number of genes associated with resistance to various biotic stresses have been identified recently in jute. Future research should focus on development of markers linked to the resistance genes and use of these markers in breeding for pest and disease resistance. However, as jute is not preferred by many insects and diseases and have inherent high-phenolics and flavonoid content, the molecular mechanism of non-preference of jute as a host by major pests and pathogens can be an interesting research area to identify novel R genes. For example, jute is not attacked by most of the mold fungi, and is little affected by leaf spot causing pathogens. Functional characterization of such unique R genes from jute can open up new avenues for genomics assisted improvement in biotic stress resistance in other crops. Furthermore, due to its unique geographical adaptation and restrictions in interspecific genetic exchange, jute can be an ideal system for studying host–pathogen coevolution in a relatively isolated environment.

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