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Genetic Resistance to the Reniform Nematode in Cotton

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Abstract

Among major nematode pests of Upland, cotton production is the reniform nematode, which is a serious threat in various cotton-producing regions. The availability of germplasm lines with tolerance or resistance to this menacing pest is a valued asset. To date, various laboratories and research institutions have collaborated to transfer the reniform nematode resistance from wild gene pools of cotton into widely cultivated Upland cotton, which have led to positive results. This chapter focuses on the current status of these introgressions and resistance mechanisms in cotton. In this overview, four major themes are being pursued: (1) tolerance mechanisms in cotton to the reniform nematode, (2) genotype evaluations, (3) introgression of reniform resistance into Upland cotton, and (4) functional analysis of reniform infection in Upland cotton. Genetic resistance in Upland cotton to the reniform nematode is the only practical solution because conventional control measures are the most cost-effective and environmentally sustainable and therefore have been and will be actively pursued. Resistance genes, if successfully introgressed into crop plants from wild relatives, should complement management of the reniform nematode with traditional methods.

Keywords: functional analysis, introgression, reniform nematode, resistance mechanisms, tolerance

1. Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops of the world. However, in selected regions, yields are being reduced drastically by the reniform nematode, *Rotylenchulus reniformis*. This pest was reported in 1940 [1] and to date has become a significant pest

of cotton in the southeastern and south-central United States [2, 3]. The estimated cotton production loss that occurred in the United States due to reniform nematode in 2005 was 115×10^6 kg (526,000 bales), conservatively worth US\$1 kg⁻¹ [4, 5].

The reniform nematode is a semi-endoparasite and derives its name from having a kidney-shaped body, usually of the adult female, and the males are, however, vermiform. The reniform life cycle is in four stages beginning from the egg. The first juvenile stage (J1) molts to become the second juvenile (J2) which also occurs within the egg. The J2 then hatches 1–2 weeks after eggs are laid especially when conditions are favorable [5]. Cuticles may sometimes overlay the other and are seen usually in the third- and fourth-stage juveniles, J3 and J4 [6]. Generally, after molting, there is a reduction in the body size of the reniform nematode [5]. The young adult reniform nematode females move towards the germinating host seedlings and to certain organic substances secreted by the roots [7]. Penetration by the nematode is in the elongation zone of the host plant root, proximal to the meristem. The epidermis and cortex are pierced by the vermiform young female intracellularly with the pericycle being the permanent feeding site [8, 9]. Half to two-thirds of the nematodes' body usually remains outside the root, and the nematode swells with kidney-like shape morphology.

Among the cellular changes observed after penetration of the cells by the reniform nematode include the formation of dense granular cytoplasm, hardening of cell walls through accumulation of polysaccharides, disintegration of cell walls, and enlargement of cells [10–12]. The adult female reniform nematode oviposits into a gelatinous matrix, produced by the vaginal glands, and the number of eggs within an egg mass varies, from 60 to 200 by a single adult female nematode [13]. The reniform nematode has the capability to undergo anhydrobiosis, a survival mechanism in the absence of water, making it thrive for at least 2 years in the absence of a host in dry soil [14]. The state of anhydrobiosis for the reniform nematode can last for more than 20 years in the absence of host plants [15].

Root-knot nematode (*Meloidogyne* spp.), another damaging nematode of cotton, are sedentary endoparasites that feed within the roots of host plants, resulting in drastic yield losses [16, 17]. The major observable signs on plants are the root knots that indicate colonization of roots of infected plants by these organisms. Symptoms include low yields, stunted growth, wilting, and predisposition to other pathogens. The most damaging stage is the second-stage juveniles (J2), which penetrate and invade their hosts near root tips and then migrate intercellularly toward the vascular cylinder. These nematodes puncture the cell wall continuously, and there is secretion release from the stylet into the cytoplasm, and the cytoplasm contents are ingested. The cells enlarged to form giant cells [18], and these have multiple nuclei and are very active metabolically in their functions [19]. The life cycle of *Meloidogyne* lasts for about 3 weeks, and eggs are then released into a gelatinous matrix by the adult female. During this period of feeding within the plant roots, physiological changes occur which alter gene expression mechanisms of the host [20].

2. Tolerance and resistance mechanisms of cotton to reniform nematode

Studies have shown changes in host gene expression during infection of roots by nematodes especially within the syncytium [21–23]. During the formation of syncytium, a number of changes occur within plant cells, among these are changes in cell cycle mechanisms, hormone regulation events, and cell wall architecture [24, 25]. Establishment of nematode feeding cells is through specific processes which are controlled and directed by encoded products of nematode parasitism genes found within the esophageal glands. These products are then delivered into the feeding cell through the nematode stylet [26]. Processes and events occurring at the nematode feeding sites play roles related to various degrees of susceptibility of cotton plants to the reniform nematode. Among these events are early degradation of syncytia [10], formation of wall deposits, and absence of hypertrophy in pericycle cells. These are some known mechanisms proposed for cotton resistance to reniform nematode.

Various Upland cotton varieties were planted in United States (US) in 2015 [27]. Among the varieties grown in United States, the largest and least acreages of cultivation were 'Deltapine' (31.2%) and 'Seed Source Genetics' (0.02%), respectively. 'Deltapine' cultivar covered about 50.1% of the acreage planted to Upland cotton in the southeastern states (Alabama, Florida, Georgia, North Carolina, South Carolina, and Virginia). Bayer CropScience ('FiberMax' and 'Stoneville') also covered significantly higher acreages (21.61 and 16.93%), respectively. An early study was conducted on susceptible (DP50-HR: 'Deltapine 50' with higher reproduction) and resistant (DP50-LR: 'Deltapine 50' with lower reproduction) genotypes, which were inoculated with 3000 reniform nematodes [28]. Roots collected 3, 6, 9, 12, and 15 days after inoculation (dai) revealed reduced reproduction in susceptible genotypes with degeneration of the syncytial cells and absence of hypertrophy within the pericycle cells.

Reniform nematodes show significant variations in populations [29–34]. Cotton genotypes will, therefore, have varying reproductive and pathogenicity responses to reniform nematode variants if present in populations. The 'LONREN' genotypes ('LONREN-1' and 'LONREN-2' both of which are resistant to the reniform nematode) were used in association with susceptible genotypes ('FiberMax 966' and 'Deltapine 555BR') to six levels of inocula (0, 500, 1000, 5000, 10,000, or 50,000), consisting of eggs and vermiform life stages of reniform nematodes [35]. High inoculum levels (10,000 and 50,000) significantly reduced the root dry mass of 'LONREN' genotypes; however, higher levels in the susceptible genotypes rather stimulated root mass. Thus, the 'LONREN' genotypes may be involved in hypersensitive responses to nematode parasitism.

Another study was conducted on five cotton genotypes with various resistance/tolerance levels to reniform nematode isolates obtained from cotton field in Louisiana [36]. High reproduction of isolates was observed from Evan (33,793 juveniles/250 g soil) and Avoyelles (27,800 juveniles/250 g soil) genotypes. Data revealed that the *G. arboreum* ('A2-190') and 'LONREN-2' were the most resistant genotypes among the nematode isolates. However, 'TX-110' and 'BARBREN-713' both had high levels of resistance to the reniform nematodes in terms of pathogenicity. The 'LONREN-1', 'LONREN-2', and 'A2-190' genotypes showed hypersensitivity to reniform nematode invasion of roots as seen in stunted plant growth. This study therefore confirms the

presence of variation in both pathogenicity and reproduction of reniform nematode isolates in cotton fields. Isolates obtained from various infested cotton fields may have various adaptive mechanisms in the soils in which they occur [37, 38]. Further, genetic variation within specific genes, for example, rRNA and ITS1 and microsatellite loci of the reniform nematode [29–31, 34, 39, 40] might influence pathogenicity and reproductive ability of nematodes. Simple sequence repeat (SSR) markers have been used to reveal the polymorphism in reniform nematode sampled from infested fields in Mississippi [40].

Exploring of host resistance to nematode parasitism is the most effective and environmentally friendly method of nematode management. Resistant cultivars pose an incompatible interaction with nematodes. A hypersensitive reaction is produced in a response to the feeding or invading nematode leading to lignification of the cells in close proximity to the reniform nematode's head, or the cells either collapse or become necrotic [41, 42]. However, in a compatible interaction, there is a formation of active syncytia with cells becoming hypertrophic, dense cytoplasm, enlarged nuclei, in most instances a partial disintegration of cell walls [29]. Application of nematicides to the resistant genotypes of cotton, for example, 'LONREN' have a positive impact on plant response and yield [43]. This hypersensitivity which is produced can be reduced in seedlings after nematicide applications. Four-resistant breeding lines from ('LONREN-1' × 'FM966') cross, a susceptible line from the 'LONREN' × 'FM966' cross, 'LONREN-1', 'BARBREN-713', and the 'DP393' (susceptible cultivar) were explored in a nematicide trial [43]. There was an increase in plant height and yield among plants to which nematicides were applied in greenhouse experiments. Furthermore, the number of reniform nematodes isolated was about 50% lower in resistant genotypes 45 days after planting (DAP). Differences were also observed among 'BARBREN-713' and 'LONREN'-derived lines in relation to reniform nematode egg number counts, with lower counts noted on 'BARBREN-713'. There was also a reduction in stunting of genotypes that received aldicarb treatment.

3. Evaluation of *G. hirsutum* genotypes to reniform nematode

Within the *Gossypium* genus, about 50 diploid and allotetraploid species are known. Two widely cultivated diploids include *Gossypium arboreum* L. and *G. herbaceum* L., and the two cultivated allotetraploids are *G. hirsutum* L. and *G. barbadense* L. [44]. There are eight diploid genome groups (A, B, C, D, E, F, G, and K) with the same chromosome number ($2n = 26$) [45]. Among the wild diploid species, three geographical groups are known (Australian, American, and Afro-Arabian).

Towards the end of the 1980s, some germplasm lines ('La. RN 4-4', 'La. RN 909', 'La. RN 910', and 'La. RN 1032') were developed in Louisiana State University in Baton Rouge with low-to-moderate levels of resistance to the reniform nematode [46]. These lines were from Upland cotton and were the first with some levels of resistance to the reniform nematode. Towards the latter part of 1997, the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) scientists in Texas released four germplasm lines ('N220-1-91', 'N222-1-91', 'N320-2-91', and 'N419-1-91') with slightly higher levels resistance to the reniform nematode compared to earlier released lines [47].

Further screening of *G. hirsutum* genotypes has been conducted [46–49]. However, most of these lines have varying tolerance levels to the reniform nematode [47]. Several genotypes (*G. hirsutum* (110), *G. herbaceum* (7), *G. arboreum* (14), *G. barbadense* (6), wild *Gossypium* spp. (33), *Hibiscus* spp. (22), and other Malvaceae (7) were evaluated for their resistance against the reniform nematode, through their ability to reproduce on roots [50]. The genotypes (*G. stocksii*, *G. somalense*, and *G. barbadense* and 'Texas110' possessed high resistance to the reniform nematode. *Gossypium hirsutum* 893, *G. herbaceum* PI 408775, *G. arboreum* PI 41895, PI 417891, and CB 3839 also have low nematode egg productions (20%).

Resistance has also been explored in wild accessions of *G. hirsutum* and *G. barbadense* to *M. incognita* race 3 and reniform nematodes [51]. The *G. barbadense* accessions ('TX-1347' and 'TX-1348') had lower reproduction on them compared to the *G. hirsutum* accessions although were susceptible to *M. incognita* race 3. A greenhouse study was conducted on 52 cultivars of cotton to identify those with some resistance to the reniform nematode; however, all the cultivars were susceptible to this organism [48]. Among the various cultivars, those with the lowest reproductive factors (RF) were 'SG 105', 'DP 543 BGII'/'RR', 'DP 445BG'/'RR', and 'FM 989 R'. Other *G. hirsutum* lines ('MT2468 Ren1', 'MT2468 Ren2', and 'MT2468 Ren3') with resistance to the reniform nematode were released [52]. This effort was from Scientists at the USDA ARS and the Mississippi Agricultural and Forestry Experiment Station.

4. Introgression of reniform nematode resistance into Upland cotton

Researchers over the years have screened genotypes and believed to have resistance to the reniform nematode. They have proved that these genotypes are not 100% resistant to the reniform nematode [48–50]. This, therefore, has resulted in multi-institutional collaborations on the introgression of resistance into Upland cotton from close and distant relatives of *Gossypium* [53, 54]. Monosomic addition lines were developed with the aim of transferring resistance into Upland cotton from *G. longicalyx* [55]. However, after screening 12 lines that were segregating, resistance to this pest was minimal. However, there have been successful introgressions into the reniform nematode into *G. hirsutum* from *G. arboreum* [56], *G. aridum* [57], *G. barbadense* [58–60] making the introgressed lines resistant to the reniform nematode.

The introgression of the reniform nematode resistance trait of *G. longicalyx* into Upland cotton was achieved through the development of two 52-chromosome trispecies hybrids [(*G. hirsutum* × *G. longicalyx*) chromosome-doubled × *G. armourianum*] (abbreviated as HLA) and [(*G. hirsutum* × *G. herbaceum*) chromosome-doubled × *G. longicalyx*] (abbreviated as HHL) [61]. The *G. longicalyx* (F₁) had the resistance trait. In this approach, there was a need to include a genotype that could reduce ploidy characteristics existing between the 'donor' and 'recipient' genotypes. Therefore, either *G. armourianum* Kearney (D₂₋₁) or *G. herbaceum* L. (A1) were used in genetic crosses. A number of recurrent backcrossings were undertaken that produced 28 lines with reniform nematode resistance, and segregation (1:1) was observed in all the backcrosses for resistant and susceptible traits [61, 62]. This demonstrated that the trait responsible for reniform nematode resistance is single gene controlled and heritable. This effort

led to the release of two *G. hirsutum* germplasm lines, 'LONREN-1' (Registration # GP-977, PI 669509) and 'LONREN-2' (Registration # GP-978, PI 669510). The release was by both USDA and Cotton Incorporated to enable cotton farmers' access to the reniform nematode-resistant germplasm with improved yields. The introgressed chromosome segments in both lines differ slightly in terms of size, with that of 'LONREN-2' much smaller than that of 'LONREN-1'. Experimental assays revealed a 95% reduction in nematode populations in growth chambers and 50–90% reductions in field trials. A disadvantage with these lines, however, is the reduced plant growth and stunting in fields with very high populations of reniform nematodes, reflecting in poor yields [63, 64]. This gene conferring high resistance to the reniform nematode has also been introgressed from the African species *G. longicalyx* (Hutch. & Lee, $2n = 2x = 26; 2F_1$) [65]. The allele conferring this resistance (Ren^{lon}) was linked to chromosome 11. In that study [65], two trispecies hybrids made up of *G. hirsutum* (AD)₁, the recipient species, *G. longicalyx* (F_1) the donor parent, and two wild diploids, *G. armourianum* (D_{2-1}) and *G. herbaceum* ($A1$), were used as 'bridges'.

Various screens were performed on *G. hirsutum*, *G. longicalyx*, *G. armourianum*, and HLA trispecies hybrids to identify markers that could be linked to resistance in the reniform nematode [65]. Sixty-two simple sequence repeat (SSR) markers were used in amplifying pooled DNA from *G. hirsutum* cultivars ['Auburn (Aub)-623', 'AcalaNemX', and 'Deltapine (DP)-458'] for the identification of polymorphisms on the A-sub-genome. Some primer pairs revealed polymorphisms among various groups of resistant (F_1 and HLA) and susceptible (AD)₁ and (D)₂₋₁ genotypes. These primer pairs were selected and used for screening genotypes in BC2F1 populations to identify markers associated with the reniform resistance locus *Ren*. A phenotypic marker in *G. longicalyx* could be linked with green seed fuzz (*Fzglon*) which was closely associated with the reniform nematode. This association was confirmed by screening 984 resistant and susceptible genotypes in various backcrosses.

Transfer of reniform nematode resistance into Upland cotton has also been successfully achieved through crossing the resistant *G. arboreum* (A-genome) with a D-genome species [66]. Backcrossing with Upland cotton (AD)₁, and screenings of the BC2F1 and the BC2F2 for resistance to reniform nematode showed plants with resistance to this pest. Another successful reniform nematode resistance introgression into *G. hirsutum* was from *G. aridum* [57]. This study involved mapping of traits from various genotypes that have been backcrossed. This included a trispecies hybrid, [*G. arboreum* × (*G. hirsutum* × *G. aridum*)], crossed with 'MD51ne' (*G. hirsutum*). One hundred and four simple sequence repeat markers were then used to identify markers linked with resistance in the reniform nematode in 50 and 26 resistant and susceptible progenies, respectively, obtained from the above crosses. Among the various markers utilized, 25 of them were resistance specific to the reniform nematode and were all localized to chromosome 21 of cotton. The locus of resistance was Ren^{ari} and two of these markers identified were BNL3279_132 and BNL2662_090.

Reniform nematode resistance introgression into *G. hirsutum* from *G. arboreum* accession ('A2-190') crossed with a hexaploid ($AD1$) × $D4$ bridging line (G 371) is another success story [56]. The progenies were tetraploid triple-species hybrids. They were progressively backcrossed into *G. hirsutum* producing 277 BC₁ new genotypes. Growth chamber experiments

revealed that the 'G371', a hexaploid bridging line, poses resistance to the reniform nematode. Therefore, resistance to the reniform nematode was controlled by dominant genes.

Trispecies hybrids with *G. thurberi* have been utilized in transferring resistant traits into *G. hirsutum* [67]. This hybrid [(*G. hirsutum* × *G. thurberi*) a hexaploid [2n = 6× = 78, (AADD)₁ × D₁] × *G. longicalyx* (F)] was used in cytogenetic analysis. Fifteen simple sequence repeat (SSR) markers were also utilized in accessing the introgressions in the various hybrid plants through specific bands, which confirmed successful introgressions. Cytogenetic analysis revealed a chromosome configuration 2n = 52 = 14.13 I + 15.10 II + 1.03III + 0.9 IV + 0.03 V + 0.13 VI (I, II, III, IV, V, and VI refer to univalents, bivalents, trivalents, tetravalents, pentavalents, and hexavalents, respectively). Mitotic chromosome analysis provided evidence on the number of chromosome within the genomes of the species and hybrids. The chromosomes varied in number for *G. hirsutum* (52), *G. thurberi* (26), *G. longicalyx* (26), and (*G. hirsutum* × *G. thurberi*) (78), and three-species hybrid [(*G. hirsutum* × *G. herbaceum*) × *G. longicalyx*] (HTL) (52).

Large germplasm lines ('1866' and '907') of *G. hirsutum* and *G. barbadense*, respectively, have been effectively screened in efforts to identify resistance to the reniform nematode [68]. Introgressions from *G. barbadense* into *G. hirsutum* against the reniform nematode, and markers linked to resistance in this pest have been a success [58–60, 68, 69].

Greenhouse assays also complement field screening of germplasm lines for nematode resistance in these lines [70]. In one of these assays, a single test plant was screened against six susceptible and resistant plants of *G. hirsutum*, 'Deltaple-16' and moderately resistant *G. barbadense* 'TX-1348', respectively [51]. Screening assays revealed about 5 and 12% of *G. hirsutum* and *G. barbadense* accessions having lower numbers of reniform nematodes compared to as susceptible check 'TX-1348', respectively. Moderate resistance was observed in the *G. barbadense* cultivar 'TX-110' (PI 163608) and *G. barbadense* accession 'GB 713' (PI 608139). Among the various *G. barbadense* accessions screened with moderate resistance to *M. incognita*, none of these was resistant to the reniform nematode.

Texas AgriLife Research released two breeding lines 'TAM RKRNR-9' (Reg. No. GP-941; PI 662039) and 'TAM RKRNR-12' (Reg. No. CP-942; PI 662040) of Upland cotton in 2010 [58]. Both of these lines poses resistance to *M. incognita* and the reniform nematode. These germplasm lines were developed through the crosses between 'M-315 RNR', an *M. incognita* resistant *G. hirsutum* line, and a reniform nematode-resistant *G. barbadense* line 'TX-110'. Both germplasm lines are commercially available and can be utilized in cotton breeding research. In other collaborative efforts, Research Scientists at the USDA and Mississippi Agricultural and Forestry Experiment Station released three germplasm lines, 'M713 Ren1' (Reg. No. GP-958, PI 665928), 'M713 Ren2' (Reg. No. GP-959, PI 665929), and 'M713 Ren5' (Reg. No. GP-960, PI 665930) in 2012 [59]. The reniform nematode resistance within these lines was from the *G. barbadense* accession 'GB 713'.

SSR markers associated with reniform nematode quantitative trait loci (QTLs) involved in resistance are known [69]. This was achieved through genotyping of 300 F₂ populations of 'GB713' × 'AcalaNem-X' crosses. QTLs were localized to chromosomes 21 and 18, respectively. The QTLs on chromosome 21 were on map positions 168 (LOD 28.0) and 182.7 (LOD 24.6),

with the specific SSR markers BNL 1551_162 and GH 132_199 on position 154.2 and 177.3 and BNL 4011_155 and BNL 3279_106 on positions 180.6 and 184.5 associated with these loci, respectively. However, the only single QTL on chromosome 18 was on the map position 39.6 (LOD 4.0) with the specific SSR markers BNL 1721_178 and BNL 569_131 on positions 27.6 and 42.9, respectively. The authors, therefore, suggested the following designations *Ren*^{barb1} and *Ren*^{barb2} for QTLs located on chromosome 21 and *Ren*^{barb3} for those on chromosome 18. Further experiments in controlled environments on 'GB-713' showed a reduction in reniform and *M. incognita* numbers by 90% [60].

5. Functional analysis of reniform nematode infection in cotton

5.1. Parasitism genes

Sequencing of the nematode parasitome usually involves gene products that the nematode secretes during its cycle; critical study of genes will enhance the understanding of the nature of damage caused by nematodes to host plants [22]. Majority of these genes encode cell wall-modifying proteins such as galactosidases, xylanases, pectinases, and expansins [71]. Isolation of the parasitome from plant-parasitic nematodes, for example, β -1-4-endoglucanases (cellulases) was from the subventral glands of *Heterodera glycines* and *Globodera rostochiensis* [72, 73]. These proteins were the first isolated molecules in plant-parasitic nematodes. Cellulases are needed by nematodes to degrade cell walls for easy penetration to the roots [74].

Cellulase genes have been identified within reniform nematode [75–77], *H. glycines* [78], *G. tabacum* [79], *Pratylenchus penetrans* [80], *M. incognita* [81], and *H. schachtii* [82]. Within the cyst nematode, cellulase activity has been observed in J2 juveniles, but rarely in J3 juveniles [78]. These cellulases are associated with the glycosyl hydrolase family 5, with two domain structures (i.e., a catalytic domain with and without a cellulose-binding domain). The cellulase gene (*RR-ENG-1*) of 1341-bp length has also been molecularly characterized in the reniform nematode. This gene had a 19-bp 5'-untranslated region (UTR), a 1245-bp open-reading frame (ORF), and an 80-bp 3'-UTR region [75]. Multiple sequence alignment of the cDNA and genomic sequences revealed seven introns and eight exons for *RR-ENG-1* gene.

Further BLAST analysis gave hits to *HG-ENG-6* mRNA in *H. glycines*. Semiquantitative RT-PCR used in studying gene expression revealed *RR-ENG-1* highly expressed in the second-stage juvenile (J2) and adult vermiform life stages. However, expression levels in the adult female were much lower. Recent characterization of the reniform nematode genome revealed hits to *HG-ENG-6* and *ENG-1* genes in *H. glycines* (Accession # AA025506) and *Radophilus similis* (Accession# ACB38289), respectively [77]. Similarly, transcriptome sequencing of the reniform nematode revealed hits to *ENG-2* (Accession # AAK21881.1 and AAK21883.2), *ENG-1* (Accession # AAD45868.1), and *ENG-7* (Accession # AAK21887.1) genes in *Meloidogyne* spp. [76].

Two cellulase cDNAs (*HG-ENG-1* and *GR-ENG-1*), that code for a secretion of signal peptide, cellulase catalytic domain, cellulose binding domain (CBD), and a small peptide link-

er, are within the cyst nematode genome. Secretion of these cellulases was within the subventral esophageal gland cells, and their presence was confirmed through mRNA in situ hybridization and immunolocalization. The cellulase activity is usually enhanced by the presence of the CBD. In *G. rostochiensis*, characterization of its cellulase cDNA (*GR-ENG-2*) showed absence of a CBD, which inhibits synthesis of crystalline cellulose [72, 83]. Horizontal gene transfer from prokaryotic microbes such as bacteria to nematodes is known [84]. Cyst nematode cellulases are similar to those found in bacteria, and theories of horizontal gene transfer to ancestor cyst nematodes have been proposed [26, 85]. This phenomenon has also been observed in *Meloidogyne* species EST data and the existence of associations to parasitism [86]. Two *Meloidogyne* genes of rhizobial origin encode L-threonine aldolase (mi01644) and a protein of unknown function (mi00109). Sympatric organisms share the same soil ecological niche and both *Meloidogyne* and rhizobia fall into this class [87]. Another group of parasitism genes characterized in the reniform nematode genome are the C-type lectins (CTLs) [76, 77, 88]. The C-type lectins (CTLs) are a family of Ca²⁺-dependent carbohydrate-binding proteins with roles in innate immune response. A 5'- and 3'-RACE analysis was used in the identification of 11 reniform nematode CTL transcripts (*RR-CTL-1-RR-CTL-11*), and these ranged from 1083 to 1194 bp with 93–99% sequence identity with the other [88]. Multiple sequence alignment of cDNA and genomic sequences showed three intronic regions. Specific BLAST hits were to *Heligmosomoides polygyrus* and *H. glycines*. The genes *RR-CTL-1*, *RR-CTL-2*, and *RR-CTL-3* expressions were constant in the life cycle of the reniform nematode. The *Rr-ctl* transcripts were not constant in the various juvenile stages and were 839-fold higher in sedentary female nematodes compared to any other juvenile stage. A previous expressed sequence tag (EST) study [89] revealed C-type lectin domain peptides. These groups of peptides are carbohydrate-binding proteins, and therefore, calcium is a requirement for their effective functioning [90] and it is found in metazoans [91]. The CTL genes have been characterized in some animal-parasitic nematodes, *Heligmosomoides polygyrus*, *Toxocara canis*, and *Nippostrongylus brasiliensis* [92, 93]. These CTLs have also been identified in the subventral glands of *M. graminicola* [94]. Characterization of the reniform nematode genome and transcriptome through 454 sequencing showed hits to *BM1_02750* (Accession # XP_001892052) and *CLEC-180* (Accession #NP_501229.2) in *Brugia malayi* and *Caenorhabditis elegans*, respectively [77].

5.2. Parasitism proteins

Parasitism proteins released from the cell of nematodes induce changes in host cell physiology through specific signals in the nucleus within the host cells [95]. The regulation of the host's cells by nematodes occurs during transcription and direct interaction between nematode-secreted protein and plant-protein target has been observed. In the root knot nematode (RKN), a 13-aa secretory peptide 16D10 has interactions with two SCARECROW-like transcription factors [96].

The root knot and cyst nematodes both secrete chorismate mutase (CM) which affects the cellular shikimic acid pathway [71]. An overexpression of this protein in *M. javanica* in plant

roots influences indole acetic acid (IAA) secretion which may result in improper tissue development [97]. Other proteins (14-3-3, *sxp-ral-2*, and *ranbpm*-like family proteins) influence cell-cycle, calcium binding, and defense regulation mechanisms in plants [71]. The 14-3-3 proteins have also been identified in reniform ESTs and these had homologies to 14-3-3 sequences of *C. briggsae* (Accession # XP_002643936.1) and *C. elegans* (Accession# NP_509939.1) [76]. Within the reniform, nematode genomic sequence hits to 14-3-3 sequences were observed in *Bursaphelenchus xylophilus* (Accession# ACZ13351), *Ancylostoma caninum* (Accession # ACO59962), and *M. incognita* (Accession# AAR85527) [77].

Expansin-like proteins in the potato cyst nematode (*G. rostochiensis*) can imitate some of their host genes in their function. An example is the *hg-syv46* parasitism gene with a C-terminus having a similar function to *CLAVATA3/ESR* (CLE), a conserved domain in *Arabidopsis thaliana* [98, 99]. Three reniform nematode genes that code for putative CLE motifs (*rr-cle-1*, *rr-cle-2*, and *rr-cle-3*) have been isolated [100]. These peptides have an amino-terminal signal peptide with specific roles in secretion and pose a C-terminal CLE motif which can be associated with that of *Heterodera* spp. The parasitism gene (16D10) is a conserved gene found in root knot nematode species controlling signaling events in RKN and its host associations. A double-stranded RNA (dsRNA) within the genomes of nematodes produces RNA interference (RNAi) of the targeted transcript. The technique of RNAi was first studied in *C. elegans* [101] and has been applied in gene silencing of variety organisms [102]. There are *in vitro* assays that facilitate stimulation of parasitic J2 nematodes to intake dsRNA from solutions through the nematodes stylet for RNAi induction [96, 103]. Specific nematode parasitism genes that are targeted could be easily knocked out and their functions effectively studied at the molecular level through RNAi approach [103].

5.3. Host-pathogen interactions

Plant host-pathogen interactions have fascinated plant pathologists over hundreds of years [104]. Contemporary studies on interactions began with seminal work on the gene-for-gene concept in plant host-pathogen interactions [105]. The gene-for-gene concept states that for every host R (resistance) gene, a corresponding Avr (avirulence) gene exists; thus, a successful host-defensive response requires a successful interaction [105]. The *ranbpm*-like family proteins and chorismate mutase (CM) interact with plant R genes, indicating nematode parasitism proteins act as avirulence genes [106, 107].

Proteins made by animal parasitic nematodes have been found in some plant parasitic nematode genomes and they are conserved [108]. These proteins function to challenge the host's immune system invoking specific responses [109]. The last two decades have advanced the global approach to studying gene expression. We now have advanced recombinant DNA technologies to study gene expression at the mRNA or total RNA (transcriptome) and protein (proteome) levels. Approaches employed in the study of plant response to nematode parasitism include differential display [110, 111], promoter-reporter gene fusions [112–114], RNA blotting, protein immunolocalization, *in situ* hybridization [79, 115], and differential library screening [116]. Identification of genes playing useful roles in parasitism have been achieved through development of cDNA libraries from

esophageal gland cells of the soybean cyst nematode [117, 118]. Other techniques used in the study of gene expression in plants at a single time point during early stages of infection of nematodes include oligonucleotide and cDNA microarrays [119, 120]; for example, in soybean cyst nematode, 1358 cDNAs from the esophageal glands were identified in expression analysis [121].

Recently, RNA-Seq analysis has been used in transcriptome sequencing of cotton (*G. hirsutum* L.) genotypes to measure comparative transcript abundance in reniform nematode susceptible ('DP90' & 'SG747'), resistant ('BARBREN-713'), and hypersensitive ('LONREN-1') genotypes of cotton (*G. hirsutum* L.) with and without reniform nematode infestation [122]. Several resistance genes that encode proteins known to be tightly linked to pathogen perception and resistance, for example, LRR-like and NBS-LRR domain-containing proteins were identified.

Most gene expression is a regulated process with genes being active in some situations and inactive in others. Gene expression relates to the physical signals from the environment and developmental cues of the organism in question. The rate of protein synthesis was once thought to be proportional to the concentration of mRNA; therefore, gene expression regulation depends on the regulation of the steady-state concentration of mRNAs [123]. However, mRNA levels and protein concentrations only partially correlate, a finding based on thoroughly composed reference datasets accounting for factors where ribosome occupancy and density and open-reading frame (ORF)-specific translation elongation rates were considered [124]. Therefore, many regulatory mechanisms involved with gene expression operate at many levels. The mechanisms influence on alterations in DNA structure, modification of transcription, stability, or translation of mRNA, or alterations in protein activity through post-translational modification.

Plants display varying levels of resistance to most pathogens in their environment, often being able to recognize pathogens through specifically distinct methods of detection [125]. A series of mechanisms of defense have been developed by plants, some of which can be constitutive or inducible. Resistance in plants is defined as the inability of a pathogen to propagate and spread on a host plant, usually involving a response referred to as the hypersensitive response (HR) [125]. The lines between a plant and its pathogens are truly battlegrounds where there are deployments of defense. The most current description of the action that takes place applies also to cotton-reniform interactions. Inducible defense responses in this type of interaction follow the 'zigzag' model [126].

According to the model suppression of immune-associated macroscopic programmed cell death (PCD) triggered by MAPK cascades or by the ETI cognate elicitors R3a/Avr3a occurs in susceptible hosts [126, 127]. The study showed that nematodes injected into the plant, thereby suppress PAMP-triggered immunity (PTI) or effector-triggered immunity (ETI) that are associated with the activation of PCD. Therefore, resistance is the ability of the host plant to evade the suppression of PCD. In other words, the host is resistant when its cells are able to undergo PCD and susceptible when the cells cannot undergo PCD.

6. Conclusion

There has been much progress and success made by researchers in introgression of reniform nematode resistance into Upland cotton from distant and close relatives. However, introgressed genotypes, solely developed to withstand reniform nematode parasitism, may have low crop yields in fields where mixed-populations of nematodes occur. The challenge now is to develop germplasm lines that may be able to withstand more than two nematode types in the same field. The rapid advances in genomics, transcriptomics, and proteomic analysis provide huge datasets, from which several resistance genes to the reniform nematode and other nematodes have been identified. These genes could be further explored and transferred into Upland cotton with various trials initiated in specific cotton-producing localities, for this overarching goal to be achieved.

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