Understanding Biotic Stress and Hormone Signalling in Cassava (Manihot esculenta): Potential for Using Hyphenated Analytical Techniques

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Abstract: Biotic stresses often constitute major factors limiting global crop yields. A better understanding of plant responses to these stresses will facilitate efforts to improve stress tolerance and yields, especially in a climatically changing world. Numerous attempts have been made to confer tolerance/resistance to biotic stresses using both traditional and modern breeding methods. Mechanisms of biotic stress tolerance controlled by signalling networks and the analysis of genes controlling the yield and biotic stress tolerance are discussed. This review presents a report on the hormonal response of cassava to biotic stresses and the potential use of hyphenated analytical techniques to understand biotic stress hormonal responses. Hyphenated analytical techniques are reliable tools for understanding the response of cassava to biotic stresses, thereby accelerating the process of the development of biotic stress-tolerant/resistant genotypes for breeding purposes.

Keywords: cassava; biotic stress; hormone signalling; hyphenated analytical techniques

1. Introduction

Cassava (Manihot esculenta Crantz) is one of the most important crops grown by smallholder farmers globally, mainly for human consumption and as a cash crop [1,2]. The crop is consumed by around 800 million people globally, especially in Africa. Cassava has become an attractive crop for resource poor farmers due to its ability to withstand harsh growing conditions compared to other crops like maize, rice, wheat and sorghum. Thus, it is an ideal food security and industrial crop. Biotic stresses have a negative effect on crop production and yields globally, as reflected by a plethora of reports in various journal articles. Molecular methods to unravel the response of host plants to these stresses have played a major role in understanding the plant–biotic stressor interactions.

To develop control strategies for cassava, molecular mechanisms regulating the host plants’ responses to biotic stress is crucial. The literature has provided information about cassava’s response to insects and pathogens [3–6]; however, a lot more work to unravel a myriad of various cassava defence mechanisms against many of these stressors is still required. Genes and transcription factors that are regulated by salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signalling are either induced or repressed in response to biotic stress. These studies indicated that the expression of genes and transcription factors related to defence and hormone signalling were altered by biotic stressors.

Recently, hyphenated techniques have become some of the utilised methods to unravel plant–biotic stressor interactions [7–11]. These stresses include plant–insect pests and diseases, among others. The use of some of these hyphenated techniques/methods may be costly; however, understanding the interactions between the host plant and biotic stressor has shown progress. The findings from several studies have assisted significantly in solving major diseases of economic importance globally.
especially of staple crops grown for human consumption [3,10,11]. Due to its ability to withstand
growth under poor environmental conditions, cassava is one of the promoted staple crops globally
after maize, rice, wheat and sorghum.

Understanding the interactions of cassava and its biotic stressors is important in resolving
responses to the host plant and biotic stress. Although there are many reports in the literature on
cassava responses to biotic constraints [3–6,12,13], limited research has been carried out on hormone
signalling in response to biotic stress. The hormone signalling response of two or more stress
combinations still cannot be fully explained.

However, the crops’ productivity and yield are hampered by biotic stresses such as insect pests
and diseases. These insect pests and diseases reduce cassava yields, especially in Africa, where cassava
is an important food crop [14,15]. Major cassava diseases include cassava mosaic disease (CMD),
cassava bacterial blight (CBB) and cassava brown streak disease (CBSD) [3–5,16], while the major pests
are whiteflies, cassava green mites (CGM) and cassava mealybugs (CMB) [6,17,18].

1.1. Cassava Mosaic Disease

Cassava mosaic disease caused by the whitefly-transmitted Geminivirus species is one of the
important biotic constraints of cassava [16,19–22]. It is widespread in Africa and species that cause this
disease include African cassava mosaic virus, East African cassava mosaic virus and the South African
cassava mosaic virus. The disease was first identified in the Democratic Republic of Congo (DRC) and
virus transmission was done by a whitefly of the genus Bemisia [23,24]. Since it was first reported,
it has become a threat to cassava production in Africa. Whiteflies use their stylets to pierce the plant
tissues and inject toxic saliva during sucking of the phloem elements into the plant. Symptoms of
infection by CMD on cassava plants include chlorotic mosaic symptoms, leaf deformation and stunting.
Infection with CMD can cause between 20% and 60% of storage yield reduction and sometimes total
crop failure. Farmers use insecticides to control the spread of whiteflies from transmitting the virus
to other plants [19]. However, due to their ability to resist infection by the virus, the use of resistant
varieties is the control measure that is being given more attention. Any diseased plants that are found
in the field are also removed as a control measure.

1.2. Cassava Bacterial Blight Disease

Cassava bacterial blight disease is the most economically important bacterial disease of cassava
in cassava growing regions [25,26]. This disease, which is caused by Xanthomonas axonopodis pv.
manihotis [27,28], was introduced to Africa in the 1970s. Dissemination of the disease is mainly by
infected plant material [29] and symptoms include wilting and defoliation of infected leaves, and disease
severity can lead to death of the plants, especially of cultivars which lack resistance. During severe
epidemics which result from prolonged favourable conditions for the spread of the pathogen, complete
yield losses can also be experienced. Control measures include the use of disease-free planting material
and planting of resistant genotypes [30].

1.3. Cassava Brown Streak Disease

Cassava brown streak disease is a cassava disease caused by the cassava brown streak virus of the
genus Ipomovirus [31,32], which is transmitted by whiteflies [33]. The disease was first described in
Tanzania [34], and, since then, it has been found in all cassava growing regions, while it became endemic
to East African coastal regions. The disease can be disseminated by infected planting material [19].
Infected plants with CBSD show stem and root necrosis, which could lead to 70% loss of root weight
in susceptible cultivars [35]. Natural measures to prevent the spread among plants are still lacking.
Sources of resistance to CBSD are also assessed [36], together with in vitro techniques for elimination
of the disease.
1.4. Cassava Green Mites

Cassava green mites are important cassava pests that mostly occur during the dry season. They were first found infesting cassava in late 1971 in Uganda, East Africa [37]. Since the pest’s introduction in Africa, it has spread to the entire cassava belt, causing serious damage to cassava production [38,39]. Reduction in leaf size, defoliation and candlestick-like appearance of young shoots are observed in CGM infested plants. Different methods can be used for control of CGM. Due to the unsuccessful use of pesticides to control the pest, researchers at the International Institute of Tropical Agriculture (IITA) then established biological control of CGM. CGM’s true identity was established and its behaviour within the cassava ecosystem and damage to the cassava plants was studied. IITA researchers then introduced and established predator mites *Typhlodromalus aripo* and *Typhlodromalus manihoti*, and later *Neozygites tanajoae*, for biological control of CGM. The mites have been used since the 1990s as control measures in Africa [40,41].

1.5. Cassava Mealybug

Mealybugs are major insect pests infesting a wide range of crops and ornamental plants worldwide [42–44]. These phloem-sap sucking insects have been studied intensively for decades because of the economic losses they cause to agriculture, through direct damage to crops, virus transmission and decreases in yield and quality [45–49]. Cassava mealybug (*P. manihoti* and *P. herreni*) are polyphagous insects feeding on cassava leaves and stems [50]. They multiply explosively under dry favourable conditions and can quickly devastate cassava crops. Upon introduction into Africa, the mealybug spread quickly throughout all cassava producing regions, threatening the food security of over 500 million people [51,52].

Overall, the best strategy to offset the destructive effects of major insect pests and diseases is by breeding for resistance [53–56]. To achieve this, cassava germplasm resistant to diseases and pests was established in the 1970s by IITA in Mozambique [17]. The IITA and the Consortium of International Agricultural Research Centers (CGIAR) research teams introduced the germplasm through subsequent breeding programmes that have since generated high yielding, disease resistant genotypes. Most of the improved cultivars incorporating resistance to CMD and CBB are available at the IITA, Nigeria [17]. Several clones that combine resistance to CGM, CMB and other diseases and pests have also been developed; however, the challenges still persist. Hence, the interaction between plants and insect/pathogens should be better understood.

2. Plant–Insect/Pathogen Interactions

From over 350 million years ago, plants and insect pests/pathogens have continued to battle against each other [57–59]. Insect pests and pathogens have advanced to being able to recognise and detect their host plant for attack using natural signals during their coevolution. As immobile organisms, plants encounter several species of herbivorous insects sucking up their cell content and feeding on their vascular sap [60–62], whilst the pathogens apply necrotrophic, biotrophic and hemibiotrophic strategies to attack the plant [63,64]. An abundance of direct [65–67] and indirect defence [68–72] mechanisms have evolved to protect plants from pathogens and herbivorous insects [73]. These mechanisms can be constitutively present or induced by herbivory or pathogen stimuli. Accumulation of constitutive defences depends on the type of species infesting the plant, whilst with induced defences, characteristics are shared between the plants [57].

3. Direct Defences

Direct defences may prevent the insects from feeding (trichomes, thorns, thick leaves) or produce toxic chemicals that include primary and secondary metabolites that destroy or impede the development of the insect [65,67,74]. The presence of secondary metabolites has been demonstrated to discourage feeding by herbivores in tomato [75] and in tobacco [76]. *Medicago truncatula* plants’ secondary
metabolites deter aphids from settling and feeding on the plants within six hours of release [77]. In cassava, both the grasshopper (Zonocerus variegatus) and burrowing bug (Cyrtopterus berigi), appear to be deterred from feeding by cyanogen in leaves and roots [78,79]. Furthermore, artificial infestation in a wild species of cassava, Jatropha gossypifolia [80], with cassava mealybug (Phenacoccus herreni) ended in 100% mealybug mortality within 48 h, due to the release of the cyanogen in the leaves.

One of the most significant elements of insect and pathogen defence in plants is a directly induced defence response [81]. These defences are insect/pathogen-specific and they have the advantage of reducing the metabolic cost to the host plant [66,72]. The cross-talk between the signal pathways results in the release of volatiles that further activate the expression of defence-related genes. To date, activation of plant defence signals by pierce-sucking, herbivore feeding insects has been reported to be similar to those in response to pathogen infection [82–87]. Examples of these studies include the pathogen Pseudomonas syringae pv. tomato infection in Arabidopsis thaliana [85]; green peach aphid (Myzus persicae) feeding on Arabidopsis thaliana [85,88]; Russian wheat aphid (Diuraphis noxia) feeding on wheat and barley [86,89]; potato aphid (Macrosiphum euphorbiae) feeding on tomato [90]; silverleaf whitefly (B. tabaci type B) feeding on A. thaliana [91]; greenbug (Schizaphis graminum) feeding on sorghum [92]; the grapevine mealybug (Planococcus ficus) feeding on grapevines [93]; cotton mealybug, (Phenacoccus solenopsis) feeding on cotton [94], and the mealybug (Phenacoccus manihoti) feeding on cassava [18]. The production of signal molecules such as the phytohormones JA, SA and ET is involved in biotic stress response (Figure 1), and abscisic acid production is induced upon encounter with abiotic stress [95]. These hormones have also been associated with plant–insect/pathogen interactions, which are triggered upon attack by herbivorous insects and pathogens [6,16,83,85,96–99].

Figure 1. A schematic representation of signalling hormones, salicylic acid, jasmonic acid and ethylene, involved in biotic stress response. The dynamism in the presence of biotic stress induces various cross-talk responses between signalling hormones but the resultant action (in the presence of stress) is defence gene expression and defence response. Upon exposure to the stress, SA regulates nonexpressor pathogenesis-related genes 1 (NPR1) and TGA transcription factors, JA activates transcription factor MYC2 and its closely related paralogs, while ET regulates the ethylene response transcription factors. An arrow end indicates a positive regulation; a blunt end indicates a negative regulation. Only a part of the regulatory relationships between the hormones are represented. The illustration of the cross-talk was sourced from Carvalho et al. [100] and Dolgikh et al. [101] and modified.

3.1. Jasmonic Acid (JA)

Jasmonic acid signalling is involved in diverse processes such as root growth and tuber formation, as well as in systemic acquired resistance (SAR) [102–104]. It plays a major role in inducing direct plant defence against insects and pathogens [105–107]. This phytohormone is reported to be synthesised by the oxylipin pathway and can interact with other phytohormones to facilitate transcriptional and
metabolic realignments in plants after insect/pathogen attack [108]. Halitschke et al. [109] indicated that the accumulation of JA appears to be induced by the oral secretions of a specialist herbivore Manduca sexta in Nicotiana attenuata. Moreover, the expression of SA and JA-responsive genes was induced by the green peach aphid (Myzus persicae) feeding in Arabidopsis [88], as well as a combination of pests’ and pathogens’ attack in Arabidopsis [85,96]. Similarly to other pests and pathogens, JA signal molecules were induced in cotton in response to whiteflies and aphids [110]. With regard to cassava, JA-responsive genes were either induced or suppressed in response to insect infestations or pathogen infections [111–114], depending on the response by the host.

3.2. Ethylene (ET)

Ethylene is a gaseous hormone that plays a role in regulating growth and development in plants [115–118], including leaf senescence and abscission, programmed cell death and response to stress and pathogen and herbivore attack [119,120]. Both abiotic and biotic stresses regulate ethylene signalling and establishment of SAR [121]. The first observation of ethylene burst due to insect attack was reported by Williamson [122], and, since then, more studies have demonstrated ethylene burst in response to both insect and pathogen attack. In tobacco (Nicotiana attenuata), an attack by the specialist herbivore Manduca sexta resulted in a dramatic release of ethylene [123]. The release of ethylene was also activated in cotton plants in response to infestation by the cotton bollworm (Helicoverpa armigera) in a study by Huang et al. [124]. During insect and pathogen attack and wounding, ethylene biosynthesis is regulated by kinases such as mitogen-activated protein kinases (MAPKs) and ethylene response factors. Maffei et al. [125] indicated that MAPKs play a role in signalling of abiotic and biotic stresses, pathogens and plant hormones in plants. For instance, in a study by Seo et al. [126], transcripts in tobacco encoding MAPKs began to accumulate only a few minutes after mechanical wounding, representing the presence of ethylene. Furthermore, upon infestation by the diamondback moth (Plutella xylostella) in Arabidopsis thaliana, transcripts encoding MAPKs were also activated [127], as well as in cassava in response to Colletotrichum gloeosporioides infection [99], supporting the role of ethylene signalling in response to insect and pathogen attack and wounding.

3.3. Salicylic Acid (SA)

Salicylic acid signalling plays an important role in regulating plant responses against biotrophic pathogens as well as phloem-feeding insects [128,129]. It also plays a role in the establishment of SAR, as shown through genetic studies [102–104,130]. SA signalling involves an important regulatory component, nonexpresser of pathogenesis-related gene 1. These components interact with TGA transcription factors and are involved in the activation of SA-responsive pathogenesis-related (PR) genes. In a study by Zhang et al. [131], feeding by the cotton mealybug Phenacoccus solenopsis induced the expression of PR genes (PR-1a and Glu A) in tomato. In addition, PR genes were differentially expressed during inoculation with Alternaria solani in tomato [132]. On the other hand, PR genes associated with SA-mediated signalling were induced by aphid feeding in sorghum [92] and tomato [133].

Studies on cassava revealed genes encoding the PR family which are involved in SA-induced pathogen/insect defence response [6,12,13,18,112,134]. For example, upregulation of PR genes in response to CBSV and UCBSV during early and late infections in cassava was reported by Anjanappa et al. [128,129]. In addition, Amuge et al. [12] and Rauwane et al. [18] reported similar PR gene family members in response to UCBSV and cassava mealybugs at different time intervals. Irigoyen et al. [112] further analysed the response of cassava PR genes after whitefly infestation. Expression of these PR genes responsive to SA signalling is therefore used as marker for SA-dependent SAR.

3.4. JA, ET and SA Interactions

Jasmonic acid, ET and SA are involved in plant defence by regulating plant defence responses against biotic and abiotic stresses [111,128,135,136]. In some studies [129,137,138], synergistic
interactions between JA, ET and SA have been reported. For instance, Spoel et al. [138] indicated that NPR1 plays an important role in interactions between SA and JA in Arabidopsis plants. Transcription factors MYC2 and ERF1 appeared to function as important nodes that integrate signals from JA and ET pathways upon herbivory by Pieris rapae and the bacterium Pseudomonas syringae in Arabidopsis thaliana [85,96]. Furthermore, an interaction between JA and ET-mediated defence signalling was regulated by transcription factor MYC2 in Arabidopsis thaliana in response to biotic stress [139]. Petersen et al. [140] reported the MAPK4 transcription factors as a negative regulator of SA signalling and a positive regulator of JA signalling in Arabidopsis. Moreover, in a study by Li et al. [141], the expression of WRKY70 in Arabidopsis thaliana caused enhanced expression of SA-responsive PR genes and concomitantly suppressed methyl-jasmonate-induced expression of JA-responsive marker gene plant defensin 1.2 (PDF1.2). Some of these regulatory proteins are also known to play a role in shaping the outcome of the Arabidopsis defence response.

Similarly, a WRKY70 transcription factor known to be a positive regulator of SA-mediated defences while repressing JA signalling was downregulated in a susceptible T200 cassava genotype in response to SACMV [16]. The authors suggested that the repression of this TF may contribute to suppression of the SA pathway, to subvert an induced resistance response in T200. In addition, the expression of JA/ET markers was constantly expressed in a resistant genotype compared to the susceptible genotype after cassava anthracnose disease (CAD) infection in cassava [99], suggesting the hormone signal interactions’ role in resistance to CAD. Likewise, the plant hormone signal transduction network between JA, ET and SA transduction pathways was reported in cassava in response to the mite Tetranychus urticae infestation [6]. The role of plant hormones in mitigating stress is studied using, among others, hyphenated techniques.

4. What Are Hyphenated Techniques?

Hyphenated techniques are so called because they are developed by coupling a chromatographic technique for the separation of compounds with a mass spectrometry system for mass analysis. Chromatography separates individual compounds in complexes such as plant extracts and the mass spectrometry analyses the masses of the individual compounds. With the aid of a database for searching data on retention time in the chromatographic column, mass data and data on the fragmentation pattern of the compound, the compounds are identified. These techniques are used to generate both qualitative and quantitative data on metabolites. They have wide-ranging applications in pharmaceutics, natural products and drug discovery and in studying responses of plants to various stimuli. When the techniques are represented, the hyphen is placed to separate one application, e.g., chromatography/separation, from the other, e.g., mass spectrometry, as is the case with LC-MS—the hyphen is placed between the LC and the MS. Examples of these hyphenated techniques are liquid chromatography-mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), liquid chromatography–nuclear magnetic resonance–mass spectrometry (LC-NMR-MS) and others (Figure 2). The coupling of the techniques is necessary to expand the scope of analysis as well as for better precision in detection, identification and understanding of complex analytes.

Liquid chromatography–mass spectrometry is developed by coupling liquid chromatography and mass spectrometry, and sensitivity, efficiency and resolving power can distinguish one LC component from another (Figure 2). However, the later developed ultra-performance liquid chromatography (UPLC) system offers shorter run time. Separation of compounds in the LC is determined by the physico-chemical properties of the stationary and the mobile phases. The stationary phase is the column through which the analyte moves during separation and the mobile phase is the solvent in which the analyte is dissolved. Separated compounds enter into the MS, where their masses are determined through searching the database. When mass information is not sufficient to identify a compound, a tandem MS, or an MS-MS, is useful to generate fragments and the fragmentation data supplement the mass data for accurate identification of the compounds. Further resolution of compounds is obtained by the use of various interfaces like the time of flight (TOF) and the quadrupole.
Figure 2. A workflow representation of the use of hyphenated techniques for understanding plant biotic stress.

A quadrupole mass analyser consists of four conduction rods arranged in parallel. In the middle of these rods is a space which acts as an ion channel. Separation of ions is based on the stability of their flight trajectories through an oscillating electric field generated with a radio frequency voltage. Only ions with a certain mass-to-charge (m/z) ratio are stable enough to pass through the channel, hence the selection of ions which fall within that particular m/z range. Ions outside this range are filtered out and do not reach the detector. In a triple quadrupole, three sets of the quadrupole are arranged, with the first and the third sets performing the mass filtering function described above. The middle quadrupole performs a collision function. The collision function generates fragments and a fragmentation pattern which, when combined with mass information, helps in compound identification.

The TOF interface in mass spectrometry enables the m/z ratio of ions to be detected through determining the time it takes for them to fly in the flight tube. The flight is enabled by ionization prior to the entry of the flight tube. The lighter ions travel faster and reach the detector before the heavier ions. From the detection, the m/z of the ions is determined. Ionization takes place in various forms. The various forms are electron impact ionization, fast atom bombardment, electrospray ionization, atmospheric pressure chemical ionization and matrix-assisted laser desorption ionization. The GC-MS platform works similarly to the LC-MS platform. However, the GC-MS targets small and volatile compounds. The sample to be analysed is vaporised and then pushed through a column by a carrier gas and then detected at the detection end the instrument. The LC-NMR is the coupling of LC with NMR. NMR lacks high sensitivity but remains useful in chemical structure elucidation. The NMR works by determining the nuclear properties of compounds exposed to an external magnetic field. A spectrum corresponding to unique emitted frequencies of compounds in the analyte is generated and its unique features are used for compound identification. These techniques have been used extensively in various plant science fields including the understanding of plant defence and signalling molecules, and the data generated supplement molecular data like gene expression profiling. The internal environment of the plant is composed of housekeeping compounds and compounds for adaptation to stresses. The production of plant hormones and the monitoring of hormone levels within the plant are conveniently done using hyphenated techniques. A multitude of studies have proven the usefulness of hyphenated analytical techniques in various plants including cassava.
5. How Have Hyphenated Techniques Been Used to Study Cassava? Biotic Stress Response and Hormonal Response?

To understand biotic stresses and hormone signalling in plants, the use of hyphenated analytical studies plays a major role. These techniques/methods involve the use of a combination of analytical techniques to understand specific responses of a plant to different treatments from the same set of data. These studies can be from understanding the physiology of the plant, co-expression relationships using metabolome data [82,84,142] biochemical diversity of crops using metabolite profiling [8] and metabolite profiling in response to pests/pathogen attack [9–11,143] among others.

Specifically, for the detection, quantification and understanding of hormonal signalling in plants, hyphenated techniques have been broadly used [10,11,144,145]. Ghosh et al. [145] reported the accumulation of aromatic and aliphatic amino acids along with phenylpropanoid intermediates using GC-MS based metabolite profiling, which suggests the induction of secondary metabolism during pathogenesis. Alterations in carbon metabolism along with perturbation of hormonal signalling were highlighted. In addition, Aliferis et al. [144] highlighted the mobilization of carbohydrates, disturbance of the amino acid pool and activation of isoflavonoid, a-linolenate and phenylpropanoid biosynthetic pathways in soybean in response to Rhizoctonia solani infection. Components of the pathways included phytoalexins, coumarins, flavonoids, signalling molecules and hormones, many of which exhibit antioxidant properties and bioactivity, helping the plant to counterattack the pathogen’s invasion. Other studies that reported hormone signalling in plants in response to insect pests and pathogens include tomato–Botrytis cinerea interaction [7], rice and R. solani interaction [146] and cassava–whitefly feeding [11] among others.

Cassava production is currently receiving attention in terms of research, since it was labelled an orphan crop due to the slow progress in research for decades. Because cassava can be consumed fresh as well as processed, its improvement as a crop is of global relevance. Breeding cassava genotypes for resistance/tolerance to biotic stresses has been ongoing for decades. Multidisciplinary approaches such as the use of hyphenated techniques are fundamental to understand and identify plant responses to these stresses within a given condition. Among the most recently used technologies and techniques, the use of hyphenated analytical techniques plays a role in the breeding progress. Several studies have investigated the plant biotic stress interactions in cassava using these techniques [8,9,11].

In 2012, the cassava (Manihot esculenta Crantz) genome was sequenced, assembled and annotated into scaffolds [147]. The genome was further improved and re-annotated into 40,044 contigs, 18 chromosomes and 30,033 loci [148]. The sequencing and assembly of the cassava genome is invaluable in the application of molecular technologies towards cassava improvement, and this includes the use of techniques such as transcriptome, metabolome and SNP profiling, metagenomics and proteomics studies in response to herbivorous pests and pathogens. The genome information serves as a useful reference point for metabolome studies using hyphenated techniques because metabolites occupy the end of the spectrum of the central dogma of molecular biology.

The literature provides several examples of studies that investigate cassava–insect/pathogen interactions using hyphenated techniques. For example, natural variation in resistance of cassava to whitefly Aleurotrachelus socialis was characterised using complementary metabolite profiling approaches [11]. A range of secondary metabolites dominated by the presence of phenylpropanoids and flavonoids were characterised using LC-MS, whilst the analysis of polar extracts facilitated the annotation of compounds and components of the intermediary and primary metabolism using GC-MS. In addition, secondary metabolites were identified and quantified in different parts of cassava plants infested and non-infested by mealybug Phenacoccus manihoti using HPLC technology [9]. Extracts from the apical and basal leaves of the different cassava cultivars contained caffeic acid, p-coumaric acid, ferulic acid, rutin and trace amounts of gallic acid. Caffeic acid was mainly detected in the basal leaf extracts for all evaluated cultivars. Furthermore, rutin concentrations were higher in apical leaves than in basal leaves for all cultivars, regardless of whether the plants were infested by P. manihoti.
Given the successes of hyphenated techniques in delineating other biochemical processes of cassava which has been demonstrated by these studies, studies of hormonal responses of cassava to stress should surely yield results. Other studies have also been reported, including capturing biochemical diversity in cassava through the application of metabolite profiling [8]; metabolic profiles of six African cultivars of cassava (*Manihot esculenta* Crantz) to highlight bottlenecks of root yield [149]; photosynthesis, metabolites and soluble carbohydrates in cassava, focusing on potassium and storage root development [150]; elucidation of starch and carotenoid composition of thirteen Nigerian cassava landraces using gene expression and metabolite profiling techniques [151]. These studies support the existing reports that cassava responses to biotic and other stresses in general trigger a common cohort of responsive metabolites, providing insight into the general activation of metabolic pathways in response to stress. However, studies on the cassava hormonal defence machinery need broadening and intensification to reveal all underlying mechanisms. Other response mechanisms, besides hormonal responses, need to be studied for an in-depth understanding of the responses of cassava to biotic stresses.

6. Future Prospects

Elucidation of all the defence compounds in cassava is ongoing and, as in all plants, may take time to complete. The search for these compounds requires the persistent and painstaking exercise of exposing plants to different stresses and then extraction of compounds for analysis. The production of different defence compounds requires exposure to different stresses and combination of stresses under a controlled environment. Large-scale projects to conduct this work require the use of automated instruments to rapidly determine the compounds in the plant extract. This work can supplement other efforts such as next generation sequencing to understand the genome, transcriptomics and proteomics to improve breeding strategies for more efficient breeding programmes which rely on biomarker discovery and the linking of biomarkers with superior plant traits. Moreover, work needs to be done to discover molecular signatures of the already existing cultivars to determine cultivar differences and identify cultivars for fitness improvement. Large-scale elucidation of defence compounds requires the use of various application platforms including those reviewed in this manuscript, namely LC-MS, GC-MS and LC-NMR-MS. This will assist in achieving the goal of complete elucidation of cassava defence compounds. As the stress compounds are discovered, a catalogue for the newly discovered compounds must be created and efforts must be made to enrich the existing databases of cassava. Work to correlate data generated using various “omics” tools and other non-omics data such as physiological data, agronomic data, etc. can add the much-needed value to understand the dynamics of the plant as it copes with stress. This kind of work is becoming more important considering the looming threat of climate change and its effects.

7. Conclusions

This review article is an account of biotic stresses, specifically diseases and insects of cassava, and the role of hormones JA, ET and SA in plant biotic stress responses. The interaction between the cassava plant and the pests/pathogens is intricate and is therefore delineated using hyphenated analytical techniques, among other applications used to study and understand the response of cassava to diseases. We reviewed the techniques used to study the response of cassava to its major stresses. The focus was on five major biotic stresses, namely CMD, CBB, CBSD, CGM and CMB. The reviewed hyphenated techniques used to study cassava were LC-MS, GC-MS and LC-NMR-MS. Although these techniques have been used to study cassava responses to biotic stresses, the authors feel the need for the broadening and intensification of these studies to understand the hormonal mechanisms underlying the response, as well as other responses which, by no means, are linked to hormonal responses. The molecular responses of plants to biotic stresses involve an orchestrated regulatory network which has been explored in higher plants and in important crop species. The regulatory network involves genes, proteins and metabolites, and the understanding of this machinery is important for crop improvement. Progress in the research of the plant defence metabolome differs from plant to plant.
Progress regarding model plant species like *Arabidopsis* has advanced more than in many crops which are not broadly utilised. Similarly, potato, rice, wheat, maize and other leading crops have been broadly studied. Unfortunately, the metabolic responses of cassava, including hormonal responses, have not been studied as broadly as is the case with the leading crops, thus leaving a vast opportunity for efforts to study the metabolomic responses of cassava to biotic stresses. From these study efforts, understanding of the cassava biotic stress machinery can be better understood and the generated knowledge can be used for breeding and cassava improvement. The use of hyphenated analytical techniques can fast-track progress. Hyphenated techniques have throughput power and therefore can generate multitudes of data with just a few runs, thus creating vast knowledge.

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