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Review Article

GA_3 mediated mechanism of HLB mitigation in citrus

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A B S T R A C T

Huanglongbing (HLB), also known as citrus greening, poses a significant threat to the global citrus industry. The disease, spread by the Asian citrus psyllid (ACP), causes severe symptoms in citrus plants, leading to substantial yield losses and tree mortality.Gibberellic acid (GA3) has emerged as a promising tool for mitigating HLB-induced stress in citrus plants. In HLB-infected trees, GA3 plays a crucial role in countering both biotic stresses. GA3 modulates systemic acquired resistance (SAR) by upregulating genes involved in plant immune responses. It also enhances cuticle formation and integrity, promoting better growth and reduced disease severity. By degrading DELLA proteins, GA3 shifts the plant's balance towards growth over defence, thus improving the overall health and productivity of citrus plants affected by HLB. These findings suggest that GA3 not only aids in stress tolerance but also acts as an immunoregulator, highlighting its potential in sustainable citrus management against HLB.

Keywords: Greening, Immunoregulator, Oxidative Stress, Phloem Regeneration, Sustainability

Introduction

The production of citrus fruits worldwide includes products such as oranges, limes, lemons, grapefruits, and tangerines. Globally, citrus production has increased over the past 30 years, reaching more than 158.5 million tonnes,[1] with china being the largest producer followed by Brazil and India. India produced about 14.28 million tonnes of citrus across 1.09 million hectares.[2] The production is facing an unprecedented crisis due to Huanglongbing (HLB, also known as citrus greening), which is the most devastating disease in recent decades. HLB has caused a huge financial loss in the citrus industry. With the deepening of globalization, HLB has spread from Asia to Africa and the America.[3] It has affected major citrus-producing areas and severely hindered the development of the citrus industry. Due to HLB, 7.4 million trees were lost in Guangxi, China alone in 2020, and more than 10 million diseased trees were destroyed all over China each year.[4] To date, HLB has been confirmed in 58 of the 140 citrus-producing countries. The major citrus-producing regions without HLB, such as the Mediterranean region and Australia, are at great risk for ACP establishment and HLB spread.[5] HLB was first reported in Asia a century ago. The earliest description of HLB-like symptoms was from central India in the 1700s and was referred to as dieback. Perhaps the best early description of the symptoms was by Husain &Nath[6] who described a decline and death of citrus in the Punjab. They attributed the decline to psyllid feeding damage, but it was most likely HLB, especially considering their description of "insipid fruit," which is consistent with our modern interpretation of a bitter, acidic flavor of fruit from HLB-infected trees. This was also the first report of an insect, the Asian citrus psyllid (ACP), Diaphorinacitri, being associated with the problem, which

we now recognize as the major insect vector of the disease. Several reports of a similar malady subsequently emerged from southern China where Lin[7,8] in the Chaoshan district of Guangdong Province, eventually described the disease as a transmissible agent and gave it the name huanglongbing, which describes the diagnostic shoots of yellow, chlorotic, mottled foliage expressed in the spring and fall. Thus, it is likely that HLB may have become established in India before spreading to China.

It is challenging for biologists to fully understand the pathogenesis of citrus HLB. The challenges mainly result from the following aspects: First, CLas cannot be cultured by any in vitro methods so far. Consequently, traditional molecular and genetic analyses have limited usefulness in CLas studies. Second, CLas resides in the phloem, which is a specialized living environment that is difficult to manipulate. To accomplish the goal of HLB management, it is paramount to have a thorough understanding of the HLB pathosystem and come up with efficient and practical solutions to break down the HLB disease infection cycle.[9]

Symptoms

All species and hybrids of citrus are susceptible to greening disease, regardless of their rootstock. However, the severity of symptoms varies among cultivars. The most severe symptoms are observed in sweet orange (*Citrus sinensis*), mandarin (*Citrus reticulata*), tangelo (*Citrus tangelo*), and grapefruit (*Citrus grandis*). In contrast, less severe symptoms occur in lemon, rough lemon, and sour orange. While no citrus species are known to be resistant to greening disease, some cultivars demonstrate greater tolerance. For example, grapefruit exhibits more tolerance than sweet orange. Initially, pomelo (*Citrus maxima*) and kumquat (*Fortunella margarita*) cultivars were considered tolerant, but they eventually became infected and started showing mottling symptoms. In mature trees affected by HLB, the initial symptoms often include chlorosis in the terminal growth of shoots, which can resemble nutrient deficiencies (Figure 1). A distinctive and diagnostic symptom is characteristic asymmetrical blotchy mottle on leaves, vein corking, stunted growth, hardened leaves, small and upright leaves, similar leaves showing zinc or manganese deficiency. As the disease advances, twig dieback that results decreased canopy density, off-season, prolonged flowering period, root decay. The infected fruits are lobsided have aborted seeds, with a bitter taste and color inversion. With increasing severity, there is a rise in preharvest fruit drop, resulting in substantial yield loss[10] and sooty mould. The trees showing severe dieback symptom of HLB in kinnow, acid lime and sweet orange (Figure 2).

Figure 1.Various symptoms of HLB (Greening)

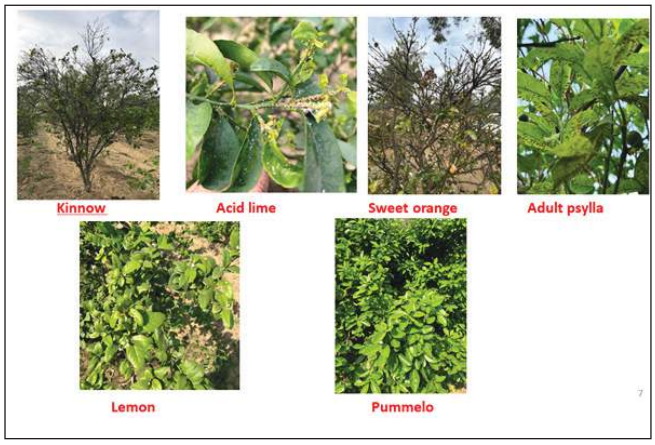

Figure 2.Susceptibility to HLB (Greening)

Description of the pathogen

At present, it is commonly believed that the HLB pathogen belongs to *Candidatusliberibacter* spp. of the α-proteobacteria, mainly divided into *Candidatusliberibacterasiaticus* (CLas), *CandidatusLiberibacterafricanus* (CLaf), and *Candidatusliberibacteramericanus* (CLam) according to regionality, heat sensitivity, and 16S rDNA.[11] Among them, CLas is the most pathogenic and widely distributed species. With the maturity of microscope technology, the peptidoglycan layer was observed under the electron microscope between the outer membrane and inner membrane of the HLB pathogen, which proved that it belongs to Gram-negative bacteria.[12]

Candidatusliberibacterasiaticus can infect almost all parts of the plant, but its distribution varies in different tissues.[13] The bacterial titter of leaves and stems is higher than in other parts of the plant. The Asian citrus psyllid (ACP) feeds on the phloem sap of citrus trees. CLas enters into the body of ACP and multiplies in the insect gut by this way. Then, they spread from the gut to the salivary gland and gonad by blood circulation. Eventually, CLas is transmitted to new hosts during ACP sucks sap from

healthy plants.[14,15] Bacteria deliver effector proteins into host cells through many kinds of secretion systems. CLas only encodes genes for type I secretion system (T1SS) and Sec-dependent secretion system, whereas the genes of other secretion systems are lacking.[16,17] CLas and CLam are vectored by *DiaphorinacitriKuwayama* (ACP), whereas CLaf is transmitted by *Trioza erytreae*.

Huanglongbing: The Indian Context

Citrus huanglongbing is attributed to one of the major causes of citrus decline in India. The presence of greening disease in India was first suspected in the 1960s.[18] Thereafter, it was reported from different citrus growing regions of India. A major survey and disease diagnosis project was initiated at the beginning of this decade at the National Research Centre for Citrus, Nagpur. Extensive surveys were conducted during 2002- 2007 in some of the major citrus belts of the country (Vidarbha and Marathwada regions of Maharashtra, Abohar and Hosiarpur regions of Punjab, Chettalli, Gudur and Periyakulum regions of Southern India, and different parts of North-East India) to record the incidence, distribution and documented the disease's incidence upto 47% (Table 1) (figure 3). Commercially important citrus cultivars like sweet orange (Mosambi, Sathgudi, Jaffa, Malta), mandarin (Nagpur, Kinnow, Coorg, Darjeeling), acid lime (Kaghzi, Vikram, Pramalini, Jayadevi) and lemon (Assam) were surveyed.[19]

- Recently, a 30–40% crop loss due to citrus greening has been recorded in the Nagpur mandarin production belts of Central India.[21]
- The citrus nursery at ICAR-CCRI, Nagpur has been rated as a '5 STAR' (*****) nursery in the country with an indexing facility for six important citrus pathogens namely Tristeza, Citrus Mosaic, Ringspot, Exocortis, citrus greening, and Phytoplasma.

Table 1.Extensive surveys by CCRI, Nagpur during 2002-2007 [20]

Crop	Incidence %
Mosambi sweet orange	8-43%
Malta sweet orange	30-40%
Sathgudi sweet orange	9-46%
Coorg mandarin	15-47%
Nagpur mandarin	10-16%
Sikkim mandarin	16-30%
Darjeeling mandarin	10-20%
Kinnow mandarin	15-20%
Acid lime	2-13%

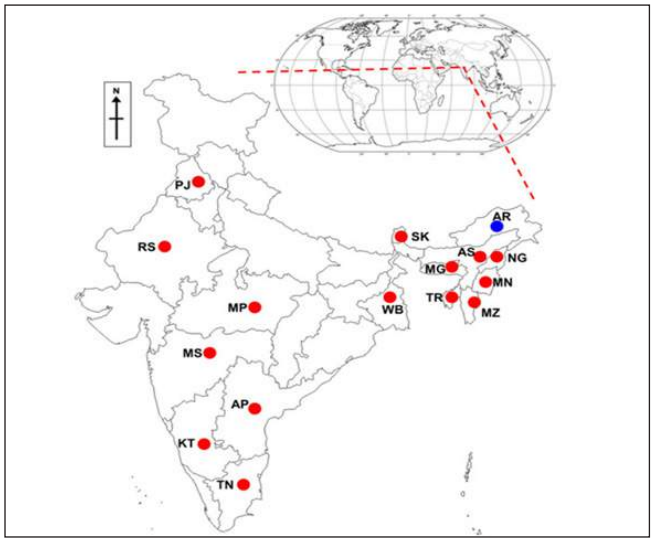

Figure 3.The surveyed citrus growing states of India[19]

Mitigation Strategies

To mitigate the effects of HLB, it is essential to develop integrated management strategies. Currently, no effective chemical control measures are available for managing HLB, making it increasingly challenging to contain the disease in new citrus-producing areas. An integrated disease management approach targeting three levels—pathogen, vector, and host—either separately or in combination, offers the most promising strategy to combat HLB. Present management options include controlling the psyllid population through chemical or biological means, removing CLas-infected trees, and planting disease-free nursery trees.

Physical Methods

Heat therapy: In the 1960s, heat therapy has been utilized to treat citrus HLB by Kongxiang Lin.[22] Among the three pathogenic bacteria, CLas is able to tolerate temperatures above 35◦C. CLaf is heat and dry weather-sensitive, found at elevations greater than 700 m and thriving at temperatures between 20 and 25 °C, whilst CLam is found to be heat tolerant and grow at comparatively higher temperatures. Infections of CLas and CLam are more severe than CLaf leading to tree death. Therefore, a temperature above 40◦C is usually selected as a heat treatment condition in the greenhouse. Limited by human and material resources, heat therapy is currently difficult to be applied in orchards.

Interrupting the pathway of transmission: The two major transmission pathways of HLB are grafting diseased branches and spreading by ACP The "three-step method" has been proven to be effective in reducing the spread of HLB.

- Cultivation of pathogen-free seedlings.
- The uprooting of the diseased trees.
- Controlling of the Asian Citrus Psyllid.

Chemical Methods

Antibiotics: Tetracycline has been widely used to treat HLB in various countries as early as the 1970s. Today, hygromycin and streptomycin have been allowed to be used commercially.[23]

Antibacterial peptides: Antimicrobial peptides are small-molecule proteins with extensive antimicrobial activity secreted by the host and have a regulatory effect on immune response. Huang et al.[24] have identified a short antimicrobial peptide (SAMP) that was only present in HLB-tolerant citrus by analysis of small RNA and mRNA. Compared with antibiotics, the greatest advantages of SAMP were its thermal stability and high efficiency, which made it more suitable for practical application.

Chemical repellents and antifeedants: Chemical repellents, such as neonicotinoids, flonicamid, and imidacloprid, along with antifeedants, have been suggested to reduce the psyllid population in citrus orchards. These chemicals can affect the fertility of major predators of the Asian citrus psyllid (ACP), contributing to controlling the spread of the disease

Nanomaterial: Field experiments showed that ZnO–nCuSi had strong antibacterial activity to control citrus canker effectively. Gosh et al.[25] Found that nano-ZnO-2S albumin protein could significantly inhibit the growth of CLas.

Chemical immune inducers: SA induce the expression of pathogen-associated proteins (PRs), which can improve the disease resistance of plants. However, genome studies have revealed that CLas, CLaf, and CLam have genes encoding SA hydroxylase, which degrades SA to hydroxyl SA, a form that cannot induce the expression of PRs. To counter this, exogenous application of immune activators like imidacloprid, β-aminobutyric acid, and 2,3-benzothiadiazole, which are more stable than SA and not easily degraded by bacterial SA hydroxylase, can be effective against HLB. Methods such as surface spraying or trunk injection of these immune inducers can enhance plant resistance for some time by inducing PR expression.[26]

Natural hormones: brassinosteroid (HBR) treatment in 2-year-old diseased citrus has shown significant improvement in symptoms and even a reduction of bacterial titer to an undetectable level.[27] Melatonin and GA3 are another important plant regulator.

Biological control

Entomopathogenic fungal therapy involves fungi like *Isariafumosorosea* and *Hirsutellacitriformi*s, which are [28] insects such as *Tamarixiaradiata* and *Psyllaephagus*, which target ACP populations effectively. Insect viruses also present a promising approach, with certain viruses associated with ACP being potential vectors for delivering RNA interference (RNAi) directly to ACP.[29] This method offers an alternative to using the *Citrus tristeza virus* (CTV) vector system. Natural enemy predation is another approach where predators such as ladybugs, spiders, and syrphid flies help control ACP populations. Additionally, repellents like guava leaves and their volatiles, along with essential oils from lavender, rose, tea tree, and other natural organic compounds, have been found to repel ACP. Intercropping citrus with crops that are averse to ACP is also an effective method to reduce Huanglongbing (HLB) transmission. Certain volatile organic compounds, such as β-caryophyllene, have also been noted to repel ACP.

Nutrient management

Sieve tube occlusion caused by *Candidatusliberibacterasiaticus* limits nutrient transport, but additional nutrient supply can alleviate symptoms and extend plant life.[30] Studies show higher N, Mn, Zn, and SA in nutrient-supplied trees compared to those without and Zn-containing additives can alter microbial abundance.[31]

Biotechnology

Overexpression of genes like *AtNPR1* enhances disease resistance in crops, including citrus, against HLB by lowering bacterial titer and improving growth under stress. Other transgenic approaches, such as overexpressing thionein or synthetic peptides like D4E1, also increase HLB tolerance.

However, the selection of scions and rootstocks tolerant/resistant to HLB is the main component for sustainable strategies aiming to tackle citrus HLB.

Coevolution of the pathogen and host – A Continuous process A hypothetical model was explained by Jones and Dangal[33] about the coevolution of pathogen and host. When a pathogen invades a plant, the plant's immune system initially recognizes pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs), triggering Pattern Triggered Immunity (PTI). This response forms the first line of defence, activating various defence mechanisms to restrict pathogen growth. However, pathogens evolve effector proteins (Avr proteins) that can suppress PTI and facilitate infection, leading to a second layer of immune response called Effector Triggered Immunity (ETI). ETI is typically stronger and more specific, as plants evolve resistance (R) proteins that recognize these effectors directly or indirectly, often resulting in a hypersensitive response (HR) and programmed cell death (PCD) (Figure 4). This on-going evolutionary arms race, where plants and pathogens continuously adapt to each other's strategies, shapes the complex and sophisticated plant immune system. The overall level of disease resistance or susceptibility is determined by the balance between PTI, ETS, and ETI.

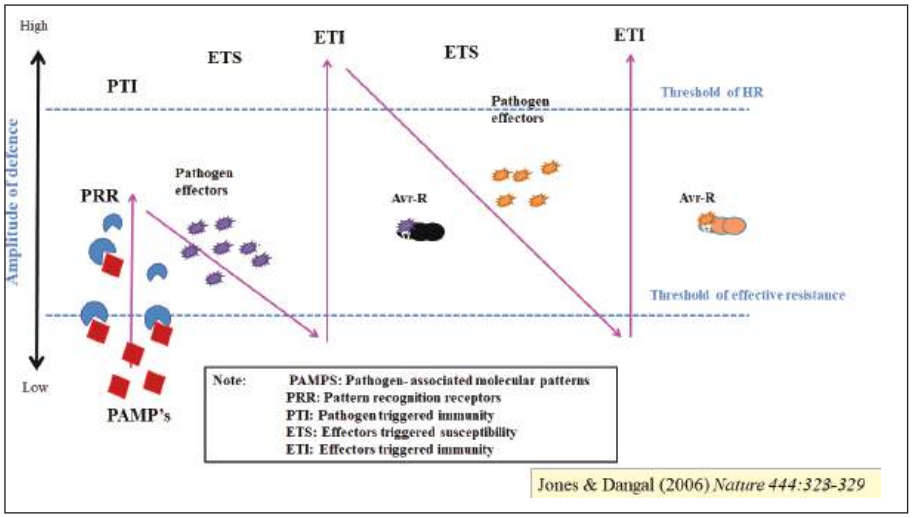

Figure 4.A zigzag model illustrates the quantitative output of the plant immune system

Defence response of susceptible genotypes against CLas

Figure 5: Defence response of susceptible genotypes against CLas. In phase 1 of this model, citrus plant receptors detect the CLas PAMPs. In phase 2, a PAMP-triggered immunity (PTI) response is initiated, resulting in the production of gibberellic acid (GA), salicylic acid (SA) and the SA-dependent gene expression activation (in blue). In phase 3, CLas deliver effectors leading to effector-triggered susceptibility (ETS). In phase 4, effectors are recognized by plant proteins, activating effector-triggered immunity (ETI). In phase 5, ETI triggers a series of genetic events (in red), including the induction of callose synthases and pp2 expression. This exaggerated response could be considered as hypersensitive cell death (HR), since the attempt to isolate spatially the CLas leads to callose and PP2 accumulation that cause phloem dysfunctions. The phase 6 represents the starch accumulation in the mesophyll chloroplasts.[34]

Curtoloet al.[34] conducted a transcriptomic analysis on citrus infected with HLB and derived a hypothetical model explaining the response of susceptible genotypes to *Candidatus liberibacter asiaticus* (Figure 5). When the infection happens Citrus receptors detect CLas PAMPs, triggering a PTI response. This leads to the production of GA and SA and the activation of downstream genes, keeping the plant asymptomatic. Pathogen Progression: CLas delivers effectors that interfere with PTI resulting in effector-triggered susceptibility (ETS). Advanced Response Effectors activate an ETI, amplifying PTI. This induces callose synthase and PP2 gene expression, causing callose and PP2 accumulation. These changes lead to hypersensitive cell death (HR).

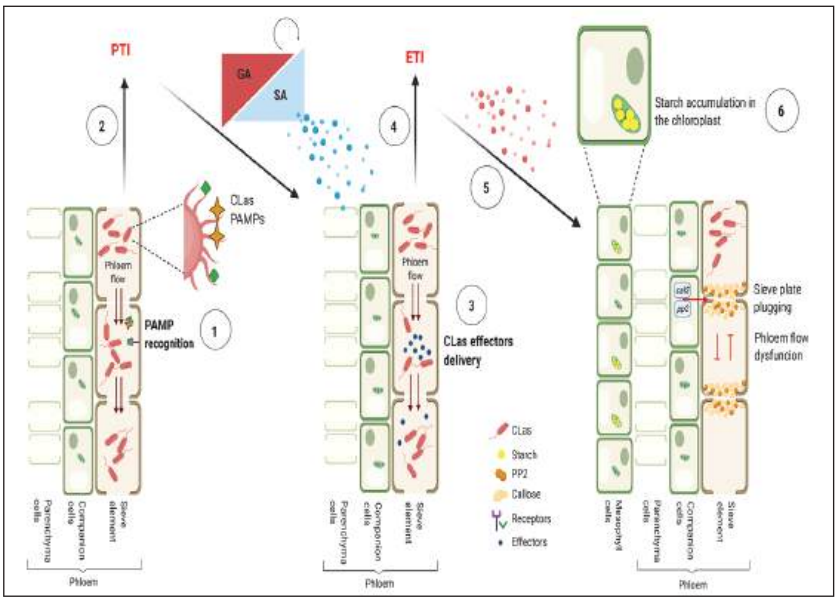

Figure 5.Defence response of susceptible genotypes against CLas

Gibberellins (GAs) in plants

Gibberellins are tetracyclic and diterpenoid acids (Figure 6). These hormones are highly concentrated in rapidly growing plant regions such as shoot apices, young leaves, developing seeds, and fruits. First observed in Japan as causing "foolish seedling" disease in rice, GAs were linked to the fungal pathogen *Fusarium*. Gibberellins were isolated from the fungus *Gibberellafujikuroi* and later from plants, leading to the identification of multiple GA types (e.g., GA1, GA2, GA3). Today, 136 gibberellins have been identified, named GA1 to GA136 based on their discovery order.[35] Gibberellins (GAs) are transported throughout the plant via both the phloem and xylem. In the phloem, they move along with carbohydrates and other substances, while in the xylem, GA translocation occurs due to lateral movement between vascular bundles. Unlike the polar transport of auxins, GA movement is generally non-polar.

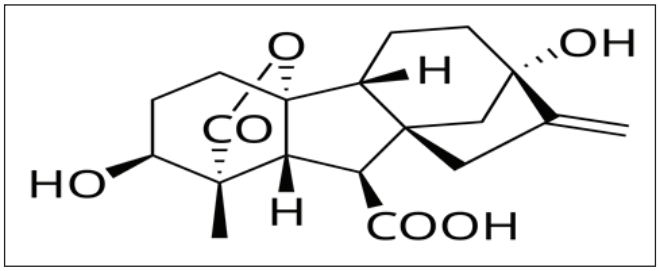

Figure 6.Chemical structure of gibberellin (GA3)

Physiological Roles of Gibberellins[36]

- **Stem Elongation:** GAs promote stem and leaf sheath elongation by inducing cell division and elongation; lack of GAs causes dwarfism.
- **Reversal of Dwarfism:** GAs can restore normal growth in genetically dwarf plants like corn and peas by promoting internode elongation.
- **Bolting and Flowering:** GAs induce rapid stem elongation (bolting) in rosette plants before flowering, even without specific photoperiod or cold requirements.
- **Parthenocarpy:** GAs induce the formation of seedless fruits (e.g., tomatoes, grapes), enhancing fruit size and yield commercially.
- **Breaking Dormancy:** GAs break seed and bud dormancy, promoting germination in conditions where seeds usually need cold, light, or specific photoperiods.
- **Sex Expression:** GAs shift flower sex expression towards maleness, promoting male flowers in genetically female or monoecious plants.
- **De Novo Synthesis of Hydrolytic Enzymes:** GAs stimulate the production of enzymes like amylases during seed germination, aiding in the breakdown of stored starch.
- **Prevention of Senescence:** GAs delay leaf and fruit ageing (senescence), maintaining quality and preventing disorders in stored fruits.

Gibberellin signalling in plants

The signalling of GA in plants involves a homeostatic balance between the gene expression involved in the GA biosynthesis, GA receptor, and enzyme concentration (Figure 7). When gibberellin binds to GID1, it induces a conformational change that allows GID1 to interact with the DELLA repressor protein, forming the GID1-GA-DELLA complex. This complex is recognized by the SCFGID2/SLY1 E3 ubiquitin ligase, which promotes the polyubiquitylation and subsequent degradation of the DELLA protein. This releases transcription factors like PIF3, PIF4, and bHLH, which then activate GA-regulated genes.[37]

In the context of HLB, GA3 has been studied for its potential to mitigate the symptoms of the disease and improve the health and productivity of infected citrus trees.

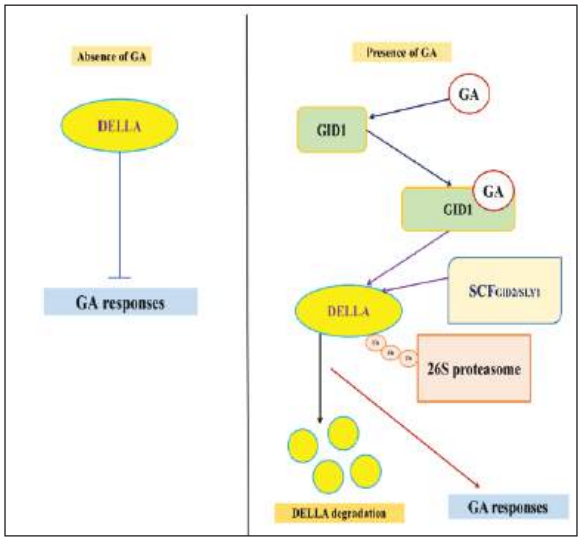

Figure 7.Gibberellin signalling in plants[37]

Role of GA3 in amelioration of stresses

HLB induces 2 types of stress in plants.

- Biotic resulting from the infection of Clas.
- Abiotic because of physiochemical changes associated with attack (like reduction in nutrient mobilisation, and water stress as a result of phloem blockage).

Shah *et al.* (37), explain that exogenous application of GA_3 helps plants counter these negative effects (Figure 8). GA_3 improves plant growth by enhancing physio-biochemical processes in chloroplasts and reducing oxidative stress through the scavenging of ROS. By promoting the degradation of DELLA proteins and facilitating the expression of defensive genes in the nucleus. These genes help produce antioxidants and osmolytes, boosting the plant's stress tolerance. Consequently, GA_3-treated plants exhibit improved root growth, delayed senescence of leaves, and overall increased resilience to abiotic stresses.

Tang et al.[38] studied the "Effects of exogenous gibberellic acid (GA3) on Huanglongbing-affected 'Valencia' sweet orange trees in Florida"

Based on the concept that:

- Only <1% of the so turn into harvestable fruits.
- A significant amount of resources used for flower formation is lost during production season due to flower and fruit abscission.
- The flower number is inversely proportional to the fruit number or fruit size.
- GA increases leafy inflorescence and leafy inflorescence set large apical flowers and fruits.

They applied GA3 monthly from September to January (Figure 9). In 2018, untreated trees had 234 flower buds per frame in spring, while GA3-treated trees had 62 to 71 buds per frame. Ga3 trees had one-third fewer flowers compared to the control. In untreated trees, 39% of buds emerged in February. GA3 treatments reduced bud emergence to less than 2% in February, delaying major sprouting until early March (Figure 10). Therefore, exogenous GA3inhibits flowering by promoting leaf development of SAM. When applied before buds become determined.

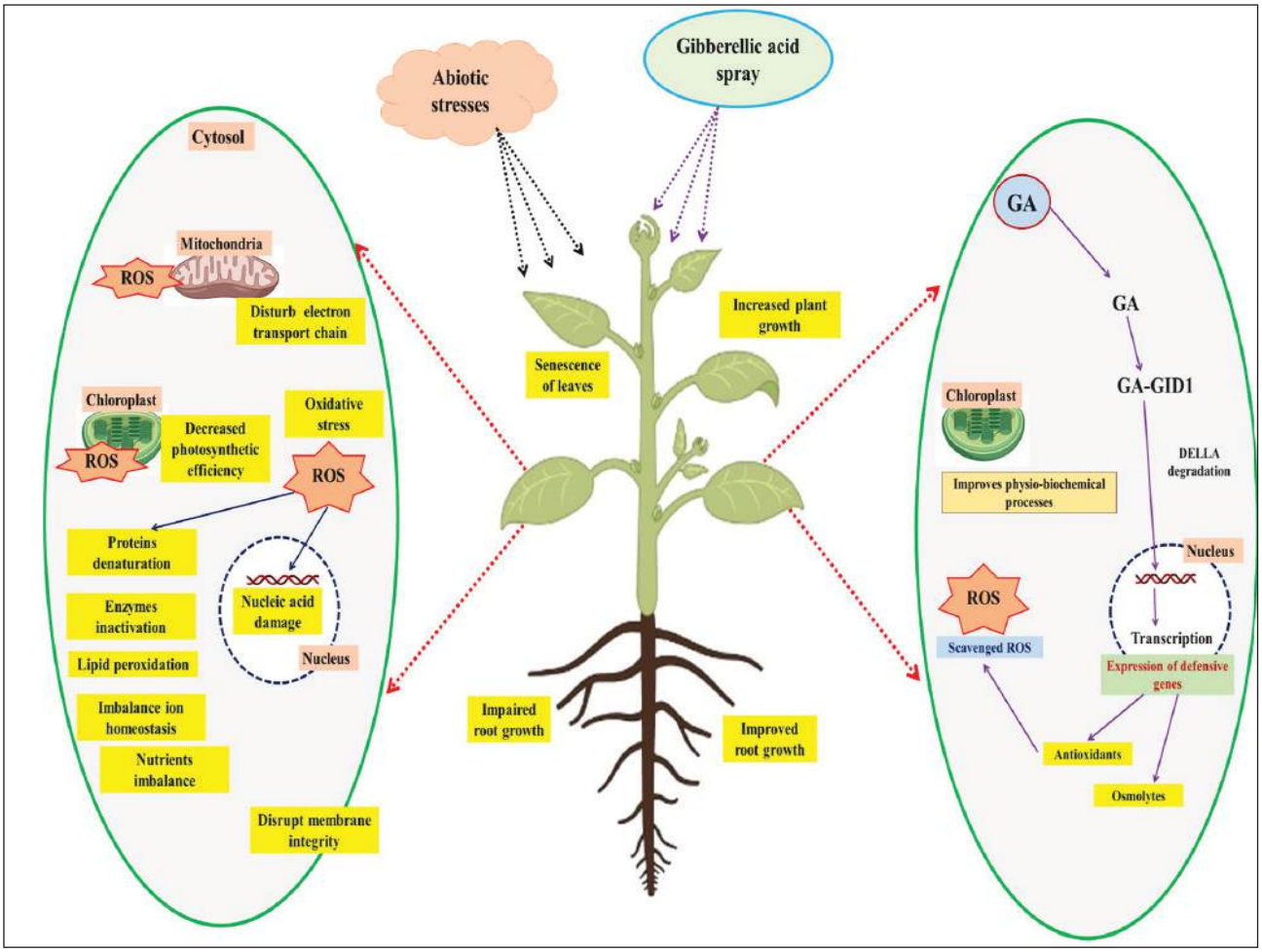

Figure 8.Role of exogenously applied GA3 in alleviating the negative effects of abiotic stresses by improving physio-biochemical processes and defence system of plants[37]

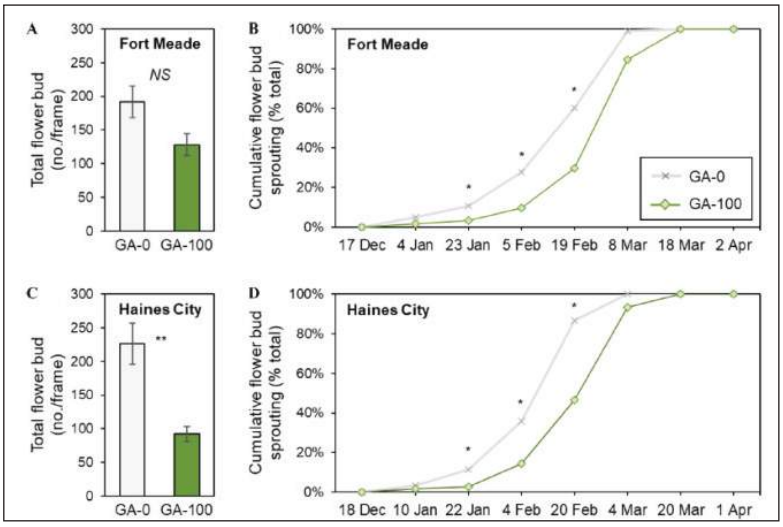

Figure 9.Number of total elongated flower buds per frame produced in spring and cumulative rate of flower buds on individual survey days in untreated control 'Navel' sweet orange trees and trees applied with gibberellic acid monthly from September to January at the rate of 49 g/ha

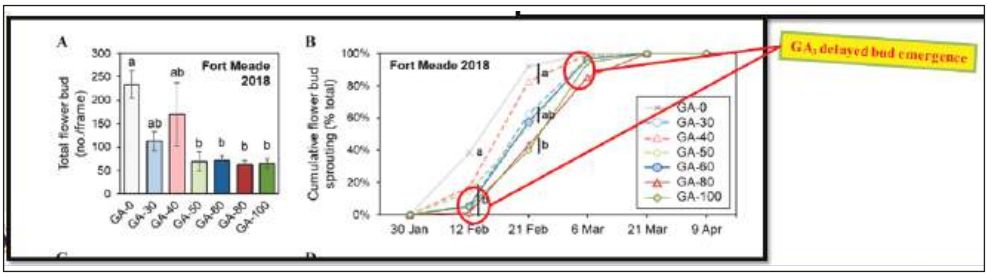

Figure 10.Number of total elongated flower buds per frame produced in spring and the cumulative rate of flower buds on individual survey days in untreated control 'Valencia' sweet orange trees and trees applied with gibberellic acid monthly from September to November, September to December, and September to January at the rate of 25 and 49 g/ha.

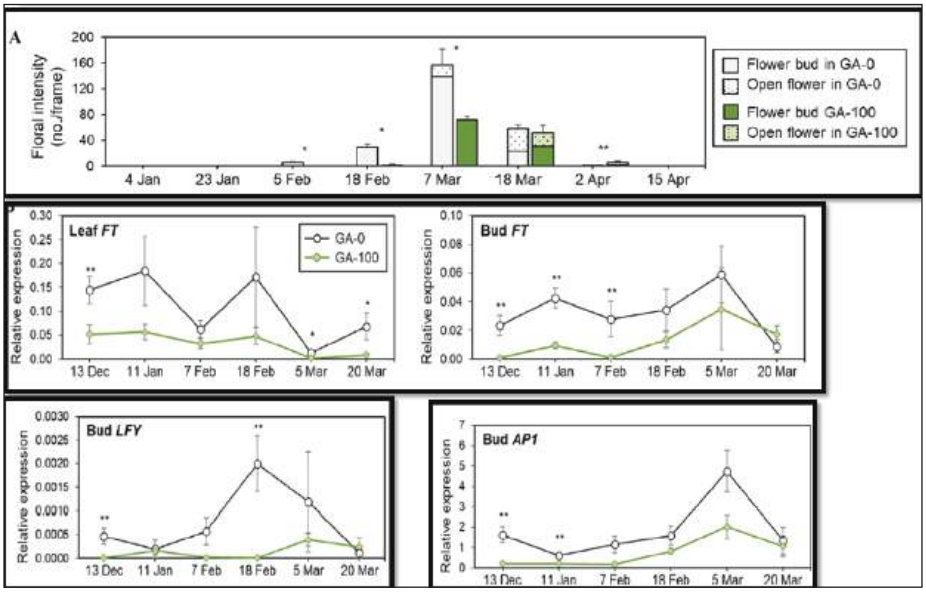

Figure 11.Floral intensity, expressed as the sum of elongated flower buds and open flowers per frame, and the relative expression of flowering locus t (FT), suppressor of overexpression of constans1 (SOC1), leafy (LFY), and apetala1 (AP1) in untreated control 'Valencia' sweet orange trees and trees applied with 49 g/ha gibberellic acid monthly from September to January

GA3-applied trees observed less floral intensity, on February 5, February 18, and March 7, due to lower levels of key floral genes FT, LFY, and AP1 (Figure 11). There are several other reporters who suggest that GA3 can down-regulate AGAMOUS key genes for flower differentiation in grapes. Thus, GA3 delayed bud emergence, compressed the bloom period, and reduced floral intensity by regulating flowering gene expression and improving the endogenous gibberellic acid levels in 'Valencia' and 'Navel' sweet orange trees.

Inference: GA_3 treatments on delaying bud emergence, compressing the bloom period, and reducing floral intensity by regulating the expression of flowering genes and endogenous gibberellic acid concentrations in 'Valencia' and 'Navel' sweet orange trees

A single application of gibberellic acid (GA) in December significantly increased yield and stabilized flower bud differentiation in HLB-affected citrus, suggesting it as a cost-effective alternative to multiple applications.[39] Gibberellic acid (GA3) treatment mitigates biotic (CLas-infection) and abiotic (osmotic) stresses in HLB-affected 'Valencia' sweet orange by enhancing vegetative growth, improving carbohydrate translocation, reducing stress-induced carbohydrate accumulation, and modulating phytohormone levels, thereby improving photosynthetic activity, gas exchange, fruit retention, and overall productivity.[40] Foliar application of gibberellic acid (GA3) in HLB-affected 'Valencia' sweet orange trees improves yield, increases mature fruit size, reduces fruit drop, maintains canopy density, and enhances stress mitigation and plant defense mechanisms, offering a potential strategy to sustain tree health and productivity despite the disease.[41]

Gibberellin Signalling in Plant Innate Immunity

According to vidhyashekaranet al.,[43] GA3 modulates plant defence responses by regulating SA–JA–ET signalling systems, which form the backbone of the immune system. The interaction between GA and SA (salicylic acid) signalling systems boosts the plant's resistance to *Pseudomonas syringaepv*. tomato.

Figure 12, Eui is an enzyme that breaks down active gibberellins (GAs). When eui is overproduced in genetically modified plants, these plants have low levels of GAs and reduced amounts of salicylic acid (SA). This shows there is a positive regulation between GA and SA.

GA induces systemic acquired resistance (SAR)

SAR enhances defence throughout the plant following a local infection which is mediated by SA. MeSA produced as a presuppose of systemic infection moves from infected leaves to pathogen-free parts, inducing resistance against biotrophic and hemibiotrophic pathogens as seen in figure 13. Bakar *et al*.[43] reported GA3 induces systemic acquired resistance (SAR) against *Pseudomonas syringae* in Arabidopsis by restoring cuticle formation by mutations in the GLABRA1 (GL1) gene that improves cuticle integrity. Several genome annotation studies showed that genes encoding SA hydrolase are found in CLas, CLam, CLaf (*Hu et al*., 2021). That can degrade SA and cannot induce the expression of PR proteins. In order to overcome this some of the exogenous chemical immune inducers, like gibberellin, can be used, and these are more stable and cannot be degraded by C. *liberobactor*.

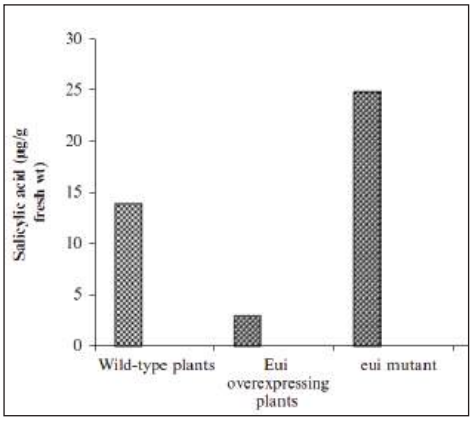

Figure 12.Eui, a P450 monooxygenase, deactivates active GAs. Overexpressing eui in transgenic plants leads to low GA levels and suppresses SA accumulation

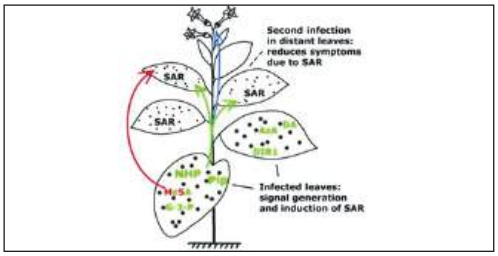

Figure 13.SAR (methyl salicylate (MeSA)) moves from infected leaves to pathogen-free parts, inducing resistance against biotrophic and hemibiotrophic pathogens

GA in enhancing SAR against Candidatus liberibacter asiaticus

Martinelliet al.[44] studied molecular responses to small regulating molecules against HLB and reported that GA3 and BA treatmenthas been shown to enhance systemic acquired resistance (SAR) against *Candidatus liberibacter asiaticus*, SAR involves the activation of defence by inducing upregulation of WRKY transcription factors MYC2, and EDS1 involved in plant immune responses, Increased alpha-amylase expression for starch breakdown, Up-regulation of heat shock proteins (HSP82) and cell wall metabolism enzymes. Induced expression of salicylic acid-mediated defence genes, better growth compared to untreated trees. Lead decrease in the level of the HLB pathogen

GA3 is an immunoregulator

Ma et al.[45] studied Citrus Huanglongbing, a pathogen-triggered immune disease that can be mitigated with antioxidants and gibberellin. The study investigated the effects of gibberellin (GA3) and antioxidants (uric acid and rutin). GA3 was chosen for its known role in modulating plant immunity and growth. Figure 14, illustrates the role of GA3 in suppressingROS-mediated cell death. Foliar spray of C. *sinensiswith* GA3 @5ppm and 25ppm suppressed cell death, reduced tissue H2O2 levels, ion leakage, and differences in foliar appearance.

The expression profiling of ROS-related genes between GA and non-GA (nGA) treated *Citrus sinensis* protoplast cells in the presence of 1.8 mM H2O2. RNA-seq analysis revealed that GA treatment induced the expression of H2O2 scavenging enzymes (POD, GPX, SOD, CAT, GPX, APX) and inhibited RBOHD expression, reducing oxidative stress (Figure 15).

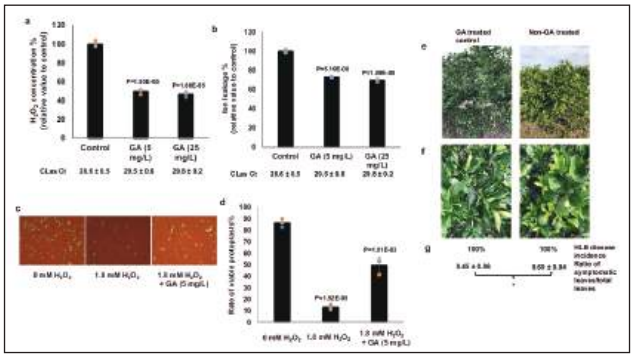

Figure 14.The immunoregulator gibberellin (GA) suppresses HLB development, a, b GA suppresses ROS-mediated cell death, c, d GA suppresses cell death of *C. sinensisprotoplast* cells, e–g Foliar spray of GA suppresses HLB symptoms

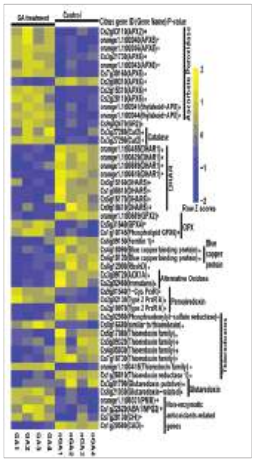

Figure 15.The expression profiling of ROS-related genes between GA3 and non-GA3 treated *Citrus sinensisprotoplast* cells in the presence of 1.8mM H2O2

Inference: The use of GA3 (gibberellic acid) shows potential in mitigating HLB symptoms by reducing oxidative stress in infected citrus plants. GA3 can help regulate the balance of reactive oxygen species (ROS), decreasing cell death in the phloem tissue. By promoting new growth and improving overall plant health, GA3 contributes to reducing the impact of HLB on citrus trees. This hormone-based approach, when combined with other horticultural practices like antioxidant treatments and nutrient management, offers a promising strategy for managing HLB in citrus production areas.

GA3 in HLB mitigation

- Enhancing physio-biochemical processes in chloroplasts and reducing oxidative stress through the scavenging of ROS
- Degradation of DELLA proteins activates growth-related genes this shifts the balance from defence towards growth over defence.
- Modulates plant defence responses by regulating SA–JA–ET signalling
- GA3 induces SAR by upregulating several genes that encoding proteins and enzymes like alpha-amylase and HSP82
- It is an immunoregulator by upregulating certain genes that encode ROS scavenging and downregulating NADPH oxidase genes.

Conclusion and Future Thrust

- In recent decades, Huanglongbing (HLB), also called citrus greening, has been the most devastating disease for citrus crops.
- Several physical and chemical approaches are available but have several limitations.
- Prevention of callose plugging and efficient phloem regeneration capacity are important mechanisms for tolerance to HLB in citrus.
- As there is no permanent solution for this, the development of tolerant/resistant scion hybrids is the most effective way to managing HLB. However, the choice of rootstocks can provide limited resilience to HLB in the citrus scions and it hold several limitations for the existing commercial orchards
- GA treatment leads to significant yield improvement in HLB-affected trees. It boost plant defence responses and reduce oxidative stress. It maintains canopy vigour and potentially improves the source-to-sink ratio. However it does not eradicate the HLB pathogen in citrus trees, it offers an alternative method for growers to manage production issues associated with HLB, such as:
- Yield decline
- Reduced fruit size
- Increased preharvest fruit drop
- Rapid decline in tree health

- Therefore, the recent discovery that GA3 (Gibberellic Acid) treatment can be a temporary solution for citrus growers to ameliorate the production issues until more permanent solutions are available and offers a sustainable solution to enhance citrus production, benefiting farmers.
- While the initial results are promising, the broader application and effectiveness of GA3 treatment across various conditions and citrus varieties need further validation.

Conflicts of Interest: None

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