Green molecules in plant health management

Abstract

Green molecules, includes salicylic acid, jasmonic acid, saponins, chitosan, etc offers many promising avenues for plant health management. They offer a natural and sustainable approach for crop protection, with a lower environmental impact than synthetic pesticides and fertilizers. Furthermore, green molecules can enhance plant growth; improve abiotic stress tolerance such as drought, salinity, and heavy metal toxicity, improving crop performance under adverse environmental conditions which ultimately result in better yield. Green molecules have been shown to be effective against a range of plant pathogens, including bacteria, fungi and viruses. In the future, the use of green molecules in plant health management is likely to continue to grow. As consumers increasingly demand sustainable and environmentally friendly products, the demand for green molecules in agriculture is likely to increase. Furthermore, as research into the mechanisms of action of green molecules continues, new and more effective green molecules are likely to be developed, further expanding their potential applications in plant health management. Nevertheless, there are also some challenges associated with the use of green molecules in the field. For example, the effectiveness of green molecules can vary depending on environmental conditions, such as temperature and humidity. Furthermore, the use of green molecules can require higher application rates and more frequent applications than synthetic pesticides, which can increase costs and labor requirements.

Keywords: Green molecules, Salicylic Acid, Jasmonic Acid, Saponins, Chitosan, Plant health management

Introduction

Scientists have long been interested in understanding the natural mechanisms that plants use to defend themselves against pests and diseases, and this has led to the discovery of many biologically active compounds, popularly known as Green Molecules. Green molecules are organic compounds derived from natural sources that have been shown to have beneficial effects on plant health. These molecules can play a key role in plant health management by improving plant growth, increasing resistance to pests and diseases, and enhancing overall plant performance. Examples of a green molecule are salicylic acid, jasmonic acid, chitosan, saponins, etc. These molecules are synthesized by the plants in response to stress and help in mitigating them. The question comes, if these molecules are artificially synthesized and applied on plants, is these can provide the resistance against the different biotic and abiotic stress? Different green molecules and their application is discussed hereunder.

Salicylic acid

Salicylic acid (SA) is one of the common name in today's scientific era. It was first time used by Sumerians, who discovered the analgesic property of the willow plant. The analgesic and antipyretic effects of the willow's leave was well-known among the Assyrians and the Egyptians too. "In 1763, the Reverend Edward Stone wrote a letter to the Earl of Macclesfield wherein he described the use of powder derived from willow bark for treating ague (malarial fever) in 50 patients. Stone's work is generally regarded as the first modern scientific description of the medicinal use of willow bark" (Stone, 1963). SA was first reported to serve as an inducer of plant disease resistance by White in 1979. SA is a naturally occurring compound in plants that plays a critical role in plant defense against pathogens. SA is involved in systemic acquired resistance (SAR), a mechanism that allows plants to develop resistance against a broad range of pathogens after being exposed to a pathogen attack. "In higher plants, SA is synthesized from chorismate through two distinct metabolic pathways, each with multiple steps: the isochorismate synthase (ICS) and phenyl alanine ammonia-lyase (PAL) derived pathways" (Dempsey and Klessig, 2017).

Salicylic acid (SA), a plant hormone plays an important role in induction of plant defense against a variety of biotic and abiotic stresses through morphological, physiological and biochemical mechanisms. A series of experiments were performed to evaluate the biochemical response of the chickpea (*Cicer arietinum* L.) plants to a range of SA concentrations (1, 1.5 and 2 mM) by War *et al.* (2011). They suggested that SA at this 1.5 mM concentration could be utilized for the induction of plant defensive system that will enable the plant to withstand many biotic and abiotic stresses.

"SA also interacts with other hormones involved in the regulation of cell division and expansion, such as auxin, GA, and ethylene (ET), to modulate plant and organ growth" (Emamverdian *et al.*, 2020; Mazzoni-Putman *et al.*, 2021; Pokotylo *et al.*, 2021).

"Salicylic acid increases the plant's response to tolerance and resistance to various diseases affecting plants as it is found that increasing its internal concentration activates the protective role of pathogenic pathogens" (Kumar, 2014).

"In plants, the positive correlation between endogenous levels of SA and resistance responses against biotrophic and hemibiotrophic pathogens are well established" (Glazebrook, 2005). "In addition, the exogenous SA application induces local and systemic acquired resistance in different plant species against various types of pathogens, including *Fusarium oxysporum*, *Alternaria alternata*, *Magnaporthe grisea*, *Colletotrichum gloeosporides*, *Xanthomonas* spp., different kinds of viruses and etc". (Jendoubi *et al.*, 2017, Le Thanh *et al.*, 2017, Wang and Liu, 2012, Kundu *et al.*, 2011, Daw *et al.*, 2008, Esmailzadeh *et al.*, 2008, and Radwan *et al.*, 2007).

“Notably, exogenous application of 1 mM SA almost completely suppressed powdery mildew disease development in cucumber plants. However, SA’s roles in plant defense against necrotrophic pathogens are not fully understood yet, due to its complexity” (Koo *et al.*, 2020).

“Exogenous SA treatment boosts the defense system of the host” (Zhang and Li, 2019). When plants are exposed to a pathogen attack, SA levels increase in the plant tissues, and this triggers a series of biochemical and physiological changes that enable the plant to defend itself against the pathogen. SA induces the expression of defense genes and activates the production of pathogenesis-related (PR) proteins, which are known to have antimicrobial properties.

Xi *et al.*, 2021 studied “the disease severity of a clubroot-sensitive cultivar of pakchoi, Xinxiaqing, and its reduction with 0.6 mM exogenous SA after the infection of *Plasmodiophora brassicae*. To investigate the mechanism of SA-reduced disease severity against clubroot, then they analyzed the plant growth, alteration of antioxidant enzyme system, and related gene expression of Xinxiaqing. Results showed that the clubroot incidence rate and disease index were decreased after being treated with 0.6 mM exogenous SA. Furthermore, plant growth, reactive oxygen species (ROS) contents, and membrane lipid peroxidation were changed. The activities of antioxidant enzymes, including superoxide dismutase (SOD), ascorbic acid-peroxidase (APX), catalase (CAT), and glutathione reductase (GR), were increased. Additionally, the production rates of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and superoxide anion (O_2^-) were inhibited. The expression levels of genes, encoding SOD, APX, CAT, and GR, were increased”.

“It is evident that SA also stimulates the production of reactive oxygen species (ROS), which play a key role in the plant's defense against pathogens by damaging the pathogen's cells. In addition, The phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), Cinnamate 4-hydroxylase (C4H), p-Coumaric acid-3-hydroxylase (C3H) activities was higher in resistant genotypes at interval of 24 h.a.i for PAL, 48 h.a.i. for PPO and C4H while 0 h.a.i for C3H. The presence of one constitutive isoform and an induced isoform was noted for PPO. Isoform PPO-2 was absent in infected and non-infected susceptible genotypes at 48 h.a.i. Thus, overall results indicated the involvement of several of the phenolics and quinones in the processes of resistance” (Jadhav *et al.*, 2013).

“Continuous cropping and climate change threaten plant production *via* multiple abiotic stresses induced by heavy metals, salinity, ozone, ultraviolet, temperature and drought” (Connor, 2002). “Intriguingly, SA is not only regulating the resistance to biotic stresses, but also the tolerance to various abiotic stresses” (Khan *et al.*, 2015 and Horvath *et al.*, 2007).

The underlying mechanisms of SA-induced abiotic stress tolerance include that SA-mediated (a) accumulation of osmolytes, such as glycinebetaine, proline, soluble sugars and amines, which can help maintain osmotic homeostasis, (b) regulation of mineral nutrition uptake, (c) enhanced reactive oxygen species scavenging activity, (d) enhanced secondary metabolite production, such as terpenes, phenolics, and compounds with nitrogen (alkaloids, cyanogenic glucosides, non-protein amino acids) and sulfur (glutathione, glucosinolates, phytoalexins, thionins, defensins, and allinin), and (e) regulation of other hormone pathways.

SA can modulate the expression of plant hormones such as jasmonic acid and ethylene, which are involved in plant defense against different types of pathogens.

SA has been shown to be effective in controlling a wide range of plant diseases caused by bacteria, fungi, and viruses. For example, SA has been used to control powdery mildew in cucumbers, tomato spotted wilt virus in tomatoes, and bacterial wilt in tobacco.

However, it is important to note that the efficacy of SA in disease management depends on several factors, such as the concentration and timing of the application, the type of pathogen, and the plant species. Therefore, it is important to use SA in combination with other disease management strategies such as cultural practices, biological control agents, and chemical fungicides for effective disease control.

Jasmonic acid

Oxylipins are oxygenated fatty acids that participate in plant development and defense against pathogen infection, insects, and wounding. Their production begins with the oxygenation of polyunsaturated fatty acids by lipoxygenases (LOXs; EC 1.13.11.12) to form 9- or 13-hydroperoxides. These are substrates for several enzymes involved in the synthesis of final oxylipins, which can act as signal molecules and/or direct antimicrobials. Jasmonates (JAs) are the best-studied group of plant oxylipins. Jasmonic acid (JA) is a natural plant hormone that plays a critical role in plant growth, development, and response to stress. Crosstalk of JA with various other plant hormones regulates the balance between plant growth and defense. JA is synthesized from the amino acid linolenic acid and is involved in many physiological processes, including root growth, flowering, and fruit ripening. JA and its derivatives, including its methyl ester (MeJA) d its isoleucine conjugate (JA-Ile), are collectively called jasmonates (JAs).

One of the most well-known functions of JA is its role in plant defense against herbivorous insects and some pathogens. When a plant is attacked by an insect or pathogen, JA levels increase, triggering a series of biochemical and physiological changes that help the plant defend itself. For example, JA can stimulate the production of secondary metabolites such as alkaloids and terpenes, which can repel or deter insects and pathogens. JA can also activate the expression of genes involved in plant defense, such as those encoding for pathogenesis-related (PR) proteins.

Completion of the castor bean genome sequence now permits genome-wide analysis of the LOX gene family in castor as well as comparison with LOX in Arabidopsis. Mhaske *et al.*, in 2012 identified “12 candidate LOX genes in the castor bean genome. Phylogenetic analysis indicated that these LOX members cluster into two groups, designated types 1 and 2, as expected from previous studies. Out of which six LOX gene specific primers were designed to amplify castor LOX genes i.e. LOX1, LOX2, LOX3, LOX4, LOX5 and Dox. Sequence analysis showed conserved five iron binding sites in the LOX domain of all the Rc-LOX, however, only LOX5 contained consensus (positions 547, 556, and 715) histidines residue. Expression analysis of

LOX2, 3, 4, 5 and DOX genes in resistant and susceptible genotypes of castor at 0 days after infection (DAI), 5 DAI and 10 DAI (30 days after sowing) was carried out using quantitative real time (RT)-PCR during castor bean - *Fusarium oxysporium* f. sp. *ricini* interaction. Results suggest that 2 (LOX2 and LOX5) of 6 Rc-LOX genes were detectable. Resistant genotypes (48-1 and SKP-84) exhibited appreciably higher expression of LOX5 during castor bean–F. oxysporium interaction, which further suggest the participation of Rc-LOX5, a type-1 LOX predicted to be 9-LOX, in wilt resistance".

Patt *et al.*, 2018 observed that "foliar applications of methyl jasmonate (MJ), induced systemic resistance pathway. It elicits the emission of defense-related volatiles in citrus foliage, and what effect this might have on the host-plant searching behavior of *Diaphorina citri*. Comparisons were made of volatiles emitted from growing shoots of uninfected and Las-infected 'Valencia' sweet orange (*Citrus sinensis*) trees over two consecutive sampling days. A settling behavioral assay was used to compare psyllid attraction to MJ-treated vs. Tween-treated citrus sprigs. All three main effects, Las infection status, plant signaler application, and sampling day, influenced the proportions of individual volatile compounds emitted in different treatment groups. MJ- and SA-treated trees had higher emission rates than Tween-treated trees. Methyl salicylate (MeSA) and β-caryophyllene were present in higher proportions in the volatiles collected from Las-infected + trees. On the other hand, Las-infected + MJ-treated trees emitted lower proportions of MeSA than did Las-infected + Tween-treated trees. Because MeSA is a key *D. citri* attractant, this result suggests that MJ application could suppress MeSA emission from Las-infected trees, an approach that could be used to discourage psyllid colonization during shoot growth. MJ application enhanced emission of E-β-ocimene, indole, volatiles attractive to many of the psyllid's natural enemies, indicating that MJ application could be used in an 'attract and reward' conservation biological control strategy. Volatile emissions in SA-treated trees were dominated by MeSA. MJ application elicited aggregation behavior in *D. citri*. Similar numbers of psyllids settled on MJ-treated versus Tween-treated sprigs, but a significantly greater percentage of the MJ-treated sprigs had aggregations of nine or more psyllids on them. Taken together, the results of this study indicate that exogenous applications of MJ or SA could be used to influence Asian citrus psyllid settling behavior and attract its natural enemies".

In addition to its role in plant defense, JA also plays a role in response to abiotic stress, such as drought, salinity, and extreme temperatures. JA can modulate the expression of genes involved in stress response, such as those encoding for heat shock proteins and antioxidant enzymes, helping the plant cope with adverse environmental conditions.

Furthermore, JA has been shown to enhance plant growth and yield, especially under stress conditions. JA can promote root growth, increase photosynthesis, and improve nutrient uptake, leading to improved plant performance and productivity.

Saponins

Saponins are a group of natural compounds that are widely distributed in the plant kingdom and have diverse biological activities. In plants, saponins serve as protective agents against herbivores and pathogens, and they can also act as growth promoters, elicitors of plant defense responses, and biopesticides. Being natural plant antimicrobials, saponins have potential for use as biopesticides. Nevertheless, their activity in plant–pathogen interaction is poorly understood.

Saponins have been shown to have a range of beneficial effects on plant health, including improving nutrient uptake and enhancing plant growth. Saponins can act as natural surfactants, which can improve the solubility and bioavailability of nutrients such as phosphorus and nitrogen, making them more accessible to plants. This can lead to improved plant growth, development, and yield.

Saponins can also enhance plant defense against pests and diseases by stimulating the production of phytoalexins, which are natural compounds that are toxic to pathogens. Saponins can also activate the plant's defense system by inducing the expression of genes involved in plant defense, such as those encoding for PR proteins.

Tida *et al.*, 2019 performed "a comparative study of saponins' antifungal activities on important crop pathogens based on their effective dose (EC50) values. Among those saponins tested, aescin showed itself to be the strongest antifungal agent. The antifungal effect of aescin could be reversed by ergosterol, thus suggesting that aescin interferes with fungal sterols. They tested the effect of aescin on plant–pathogen interaction in two different pathosystems: *Brassica napus* versus (fungus) *Leptosphaeria maculans* and *Arabidopsis thaliana* versus (bacterium) *Pseudomonas syringae* pv *tomato* DC3000 LOX1, LOX2, LOX3, LOX4, LOX5 and Dox (Pst DC3000). They analyzed resistance assays, defense gene transcription, phytohormonal production, and reactive oxygen species production. Aescin activated *B. napus* defense through induction of the salicylic acid pathway and oxidative burst. This defense response led finally to highly efficient plant protection against *L. maculans* that was comparable to the effect of fungicides. Aescin also inhibited colonization of *A. thaliana* by Pst DC3000, the effect being based on active elicitation of salicylic acid (SA)-dependent immune mechanisms and without any direct antibacterial effect detected. Therefore, this study brings the first report on the ability of saponins to trigger plant immune responses. Taken together, aescin in addition to its antifungal properties activates plant immunity in two different plant species and provides SA-dependent resistance against both fungal and bacterial pathogens".

Furthermore, saponins have been shown to have allelopathic effects, meaning they can inhibit the growth and development of competing plants, leading to improved crop yields.

Chitosan

Chitosan, which is a natural and eco-friendly linear polysaccharide made from chitin by a chemical process involving deproteinization, demineralization, decolouration and deacetylation, has received considerable attention because of its properties. Due to its fungicidal effects and elicitation of defense mechanisms in plant tissue, chitosan has become useful and highly appreciated as a natural biodegradable high molecular polymer compound that is a nontoxic and bioactive agent.

Chitosan is a natural and biodegradable biopolymer derived from chitin, a component of the exoskeletons of crustaceans such as crabs and shrimp. Chitosan has been shown to have many beneficial effects on plant health and is used widely in agriculture for plant growth promotion, disease control, and stress tolerance.

“In sustainable agriculture, chitosan can be used alone or in combination with other compounds in plant nutrition with the aim of withstanding abiotic stress, stimulating plant defense systems, combating plant diseases, promoting plant seed germination and seedling growth, and increasing crop production and quality” (Ahmed *et al.*, 2020). In addition, chitosan can purify soil and agricultural wastewater from heavy metals such as mercury, copper, uranium, and lead and thus can be reused for irrigation.

One of the most well-known functions of chitosan in plant health is its ability to induce systemic acquired resistance (SAR) in plants. SAR is a plant defense response that is activated in response to pathogen attack and can provide long-lasting protection against subsequent infections. Chitosan can stimulate the production of endogenous plant defense compounds such as phytoalexins, PR proteins, and reactive oxygen species, which can enhance plant resistance against various pathogens.

Chitosan can also promote plant growth and development by improving nutrient uptake and enhancing photosynthesis. Chitosan can act as a natural biostimulant by increasing root growth and branching, leading to improved nutrient and water absorption. Chitosan can also improve chlorophyll content and photosynthetic activity, leading to improved plant growth and yield.

Additionally, chitosan has been shown to enhance plant tolerance to abiotic stress such as drought, salinity, and heavy metal toxicity. Chitosan can activate the expression of stress-related genes and increase the activity of antioxidant enzymes, helping plants cope with adverse environmental conditions.

“Chitosan is efficient in inhibiting hyphal growth, mycelial elongation, spore formation, spore germination, spore viability, germinal tube, and fungal virulence factor production of phytopathogenic fungi” (Chakraborty *et al.*, 2020). “The ability of chitosan to penetrate the plasma membranes of phytopathogens depends on the degree of membrane fluidity. Chitosan-sensitive fungi possess polyunsaturated fatty acid-rich membranes such as linoleic acid (high fluidity membrane), while chitosan-resistant fungi possess saturated fatty acid-rich membranes such as palmitic acid or stearic acid (low fluidity membrane)” (Palma-Guerrero *et al.*, 2010).

A broad spectrum fungicidal activity of chitosan has been reported against many pathogenic fungi, for example, *Botrytis cinerea*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Sclerotium rolfsii*, *Pythium aphanidermatum*, and *Rhizopus stolonifer*.

N. A. Musmade and Lalit Mahatma (2021) observed that “chitosan and chitosan-mediated silver nanoparticles exhibited antifungal properties against soil-borne pathogens *viz.*, *Sclerotium rolfsii* and *Pythium aphanidermatum* at different concentrations. Chitosan also causes excessive mycelial branching; abnormal shapes, swelling and hyphae size reduction in *F. oxysporum* f. sp. *cubese*, *F. solani* f. sp. *glycines*, *Botrytis cinerea*, and *A. alternata*”.

Chitosan is also responsible for cytological alteration, protoplasm dissolution, and large vesicles of fungus. Chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of *B. cinerea* and *F. oxysporum* f. sp. *albedinis*.

The ability of chitosan to exhibit intracellular antimicrobial action depends on its molecular weight. Low molecular weight chitosan has the highest inhibitory effect on *Rhizopus stolonifer*, while high molecular weight chitosan shows better efficacy on *Fusarium oxysporum* f. sp. *vasinfectum*, and *Alternaria solani*. Fungi that have been exposed to chitosan make fewer spores than untreated fungi.

Despite substantial study on chitosan, the mechanism of action of chitosan in regulating plant immunity and reducing pathogens has not been thoroughly explained. Chitosan's mechanism of action is anticipated to be more complex, involving a number of overlapping elements that will need to be researched more in the future.

Conclusion

Green molecules in plant health management refer to environmentally friendly and sustainable chemical compounds or substances that are used to protect and promote the health of plants. These molecules are designed to minimize negative impacts on the environment, non-target organisms, and human health while effectively managing pests, diseases, and other factors that can affect plant growth. Salicylic acid (SA) and Jasmonic acid (JA) is a naturally occurring plant hormones and signaling molecules that plays a crucial role in plant health management, particularly in the plant's defense mechanisms against herbivores, pathogens, and abiotic stressors. The role of these plant hormones in plant health management is complex and can vary depending on the specific plant species, the type of pathogen or stressor, and environmental conditions. Saponins are naturally occurring chemical compounds found in various plants, and they have diverse functions, including acting as defense compounds against herbivores and pathogens, promoting plant growth, and contributing to soil health. The effectiveness of chitosan in plant health management can vary depending on factors such as the plant species, the type and concentration of chitosan used, and the specific pests or diseases being targeted. Additionally, chitosan is often used as part of integrated pest management (IPM) strategies, where multiple tactics are employed to optimize plant health and minimize the use of synthetic chemicals. The use of green molecules in plant health management aligns with sustainable agriculture practices, reducing the reliance on synthetic chemicals and promoting long-term ecosystem health. It also contributes to the production of healthier and safer food for consumers.

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