



“BioClay™”: One Step toward the Sustainable and Novel Plant Protection Method

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Abstract

World peace depends on global food security with finite resources. In addition to these finite resources, biotic stress poses an additional threat to global food security. Plant breeding is an effective tool for combating biotic stresses. Scientists have developed various genetically modified crops tolerant to plant diseases and insects. However, Development of multiple biotic stress-tolerant cultivars through conventional breeding is a tedious and long-term process. However, the usage of pesticides is a rapid and short-term solution to fight against biotic stresses, but it will result in pesticide residues, which threaten the agricultural product's export to foreign markets and human health. To meet the UN Sustainable Development Goal 2030, sustainable solutions are needed to stay strong against these biotic stresses. In recent times, scientists have been approaching biotechnology and nanotechnology to counter biotic stresses. This study is solely based on the breakthrough innovation of “BioClay™” or dsRNA-LDH complex as a sustainable and eco-friendly potential for plant protection. “BioClay™” or dsRNA-LDH complex is used to induce RNAi and sustain delivery of dsRNA, which is a sustainable alternative to chemical usage. This technology is found to be prominent against various biotic stresses in plants. This information will be helpful to scientists for further research to fill the gap between previous research. This study also focuses on the future direction of “BioClay™” or dsRNA-LDH complex technology.

Keywords: BioClay™, LDH, RNAi, gene silencing, SIGS, nanotechnology

1. Introduction

Access, availability, and affordability of food are the major tools for world peace. The world population is projected to be 9.6 billion by 2050, while India will reach up to 1.53 billion population by 2050 (Coleman, 2004), food demand will increase by 60 percent by 2050, and arable land will not expand. (Frona et al., 2019). Global food erosion is up to 30 percent owing to biotic stress, excluding abiotic stress (Rizzo et al., 2021). Biotic stress includes the attack of insects- pests, diseases, fungal infections, and viral infections. (Ramakrishna and Kumari, 2017)

Currently, agricultural, and horticultural crops rely mostly on pesticide spraying for high yield. The pesticide has an immense harmful effect on human health (Rani et al., 2021) and the environment (Tudi et al., 2021), because Only 10% of agrochemicals were absorbed from the target site, the rest of the 90% was lost by volatilization, binds with other organic molecules in the soil, and leach out into the water bodies

(Mogul et al., 1996).

Biotechnology will provide sustainable plant protection solutions in the nightmare of pesticide residue's detrimental impacts. During 1980 to 1990 scientists first time observed RNAi (RNA interference) in plants (Napoli et al., 1990). In 1998 scientists implemented this technology to get protection against viruses in tobacco (Anandalakshmi et al., 1998). Transgenic delivery of dsRNA is among the earliest methods of dsRNA delivery in the living cell. However, as a non-transgenic alternative (Koch et al., 2016) performed a topical spray of naked dsRNA (Double-stranded RNA) to induce RNAi in plants. But major problem associated with the topical spray of naked dsRNA is inadequate absorption of dsRNA and the longevity of protection. Nanotechnology has provided novel and sustainable solutions to all these biotechnological problems. Nano-pesticides have the least effects on human health and the environment (Gomollon-Bel, 2019). The nanoparticle has prominent efficacy in delivering agrochemicals because of high



penetrations inside the organism (Giraldo, et al., 2014), large surface area (Su et al., 2020), and maximum absorptions of agrochemicals from the target site (Li et al., 2021).

Scientist at the University of Queensland, Australia has conducted a pioneering experiment of the usage of Layered Double Hydroxide (LDH) nano clay particle to deliver dsRNA into the plant cell, and this complex of dsRNA-LDH is popularly known as a “BioClay™” (Mitter et al., 2017). The LDH sheet was composed of Mg, Al, and hydroxide ions. Clay nanosheet will hold the dsRNA molecules and prevent the dsRNA’ wash-off by water and dsRNA’ degradation by nuclease enzyme, in contrast to naked dsRNA spraying. Degradation of the clay nanosheet in presence of water and CO₂ will eventually release dsRNA into the cell and induce gene silencing. This “BioClay™” extends plant protection up to 25–30 days as compared to naked dsRNA spraying. In other words, vaccination of the plant is also known as “BioClay™.”

2. From dsRNA Delivery Up To The Successful Gene Silencing

This LDH-mediated dsRNA delivery technology operates on the foundation of RNA interference (RNAi) mediated gene silencing or Post-transcriptional gene silencing (PTGS). RNAi-based crop protection is a satisfying way to counter an insect pest (Fletcher et al., 2020). Spray-induced gene silencing (SIGS) is an effective approach to induce RNAi (Qiao et al., 2021). Advanced studies using SIGS have shown that SIGS can effectively control viruses (Tenllado and Diaz-Ruiz, 2001), fungal pathogens (Wang et al., 2016), and coleopteran insects (San Miguel and Scott, 2016). Gene silencing is a way to suppress the expression of genes by degradation of the mRNA, which eventually stops the translation of a specific protein that leads to infection.

In this SIGS technique (Figure 1), topical application of dsRNA was performed to induce RNAi. LDH-loaded dsRNA were topically applied to plants. LDH-nanocarrier material breaks down after reacting with the atmospheric CO₂ and moisture, releasing loaded dsRNA (Xu et al., 2006). After absorption of site-specific dsRNA, dsRNA are transformed into the siRNA (small interference RNA) with the aid of the Dicer enzyme (Meister and Tuschl, 2004) (Tomari et al., 2007). siRNA was engaged with RNA-induced silencing complex (RISC) which contains Argonaute 2 protein (Matranga et al., 2005) (Ketting, 2011). Target mRNA bind to a complementary sequence of siRNA on RISC site and RNase H enzyme of the RISC cleaved the targeted mRNA and prevents translation of mRNA to protein. Eventually, protects plants from infections (Agrawal et al., 2003; Huvenne and Smaghe, 2010).

3. Potential of BioClay™ as a Plant Protectant Against Plant Virus

Scientists approach “BioClay™” for getting protection against the Cucumber Mosaic Virus (CMV). A significant reduction in local lesions of CMV infection was observed after topical spraying of CMV2b-dsRNA and “BioClay™” (CMV2b-dsRNA-

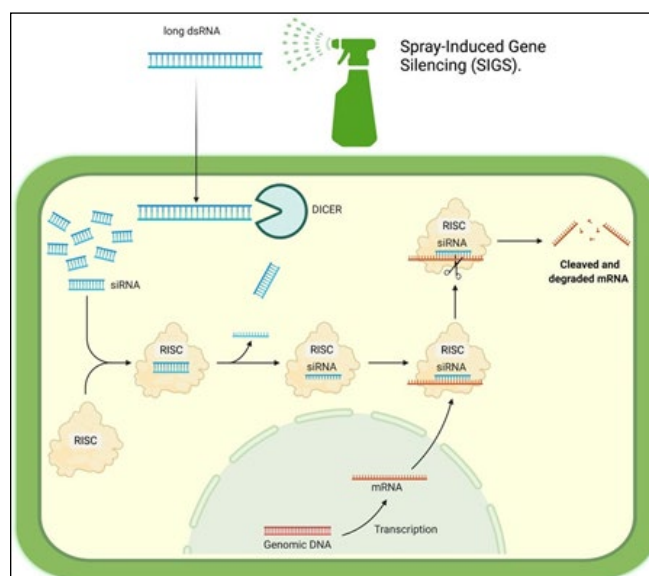


Figure 1: Spray-induced gene silencing mechanism. [Figure courtesy: (Hernandez-Soto and Chacon-Cerdas, 2021)]

LDH) in Cowpea leaves compared with water and LDH. Not only in local lesions, but scientists also observed that CMV2b-BioClay™ provided 93.75% systemic protection on unsprayed newly grown leaves after 20 days post spray against CMV virus, in contrast to only 12.5% systemic protection from the water (Mitter et al., 2017). Researchers found that LDH-mediated Tomato yellow leaf curl virus (TYLCV) specific dsRNA delivery protects the *Nicotiana benthamiana* L. plant against the TYLCV (Hernandez, 2021).

4. Potential of BioClay™ as a Plant Protectant Against Plant Insects

Whitefly is a highly destructive and polyphagous pest in agriculture (Lu et al., 2019). Whitefly caused huge monetary losses annually in to agriculture industry (Hasanuzzaman et al., 2016). To control the whitefly populations, the most important step is to control an egg, and nymph stage instead of the adult stage. In 2021, scientists evaluated the effect of concatemer dsRNA- LDH on egg and nymph of the whitefly in cotton plants in this study they found out that, “BioClay™” caused 23–17% more mortality of the egg and nymph of whitefly in the cotton plant than naked concatemer dsRNA (Jain et al., 2022). Among the vegetables tomato is the major host of the whitefly. In the same study, scientists discovered that the Suc+DNaseI-BioClay™ treated tomato plant caused 57% mortality of whitefly eggs and nymph, which is more than the naked dsRNA.

Pod/seed loss into legume crops extends up to 60–70% owing to the Bean Common Mosaic Virus (BCMV). BCMV was transmitted through the Aphid (*Myzus persicae* L.). Scientists gained 100% protection against BCMV through BCMVCP-BioClay™ in *Nicotiana benthamiana* L., in contrast to only 20% protection in untreated plants. With the same BioClay

molecules, scientists got 91.7% protection against BCMV in cowpea plants, in contrast to only 44% protection in untreated Cowpea plants (Worrall et al., 2019).

5. Potential of BioClay™ as a Plant Protectant Against Fungal Infections

LDH-mediated dsRNA delivery is not only limited to pest and viral protection, but scientists also found it is effective against fungal infection. Scientists discover that spraying of dsRNA via nanocarrier (LDH) is effective in silencing targeted genes like FoCYP51, FoChs1, and FoEF2 of *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis and Shomaker (FORL), it resulted into severe reduction in pathogenicity of fungi (Mosa and Youssef, 2021). These fungi cause a fusarium crown and root rot disease in tomatoes. Researchers from China found that RsCRZ1-dsRNA-LDH induced stronger RNAi than RsCRZ1-dsRNA to control the growth of *Rhizoctonia solani* (Chen et al., 2022).

6. Future Direction of “BioClay™”

In addition to an attack of sucking pests, lepidopteran and coleopteran pests also cause devastating damage to crops. Farmers use insecticides to control their attacks, but after continuous use of the same insecticides, an insect has developed an insecticide resistance mechanism (Ribeiro et al., 2003) (Van den Berg and du Plessis, 2022). In option to that, scientists started to work on biotechnological advancement to develop a Genetically modified (GM) crop. GM crops decrease pesticide usage by 37% (Klumper and Qaim, 2014). Despite this advantage of GM crops, GM crops lost popularity due to toxic effects on human health (Zhang et al., 2016), significant off-target effects, and ecological risk (Losey et al., 1999). After that, scientists started work on RNAi-mediated gene silencing in lepidopteran and coleopteran insects, as a result of a series of experiments scientists successfully implemented the RNAi mechanism in lepidopteran and coleopteran insects (Macedo et al., 2017) (Sharath Chandra et al., 2019), but a major limitation for scientists is to avoid degradation of the dsRNA in insect gut (Garbutt et al., 2013) (Prentice et al., 2017; Guan et al., 2018). Scientists are still finding solutions to prevent the degradation of the dsRNA. So, there is such a huge gap in the development of sustainable RNAi mechanisms for the lepidopteran and coleopteran insects and analysis of the efficiency of LDH tools in combination with dsRNA to control lepidopteran and coleopteran pests. In addition to that, scientists found that direct application of dsRNA was not stable in soil and did not protect plants from fungal infection (Qiao et al., 2021). So, future generation researchers have an opportunity to stabilize the dsRNA in soil with the help of LDH sheets to get protection from devastating fungal diseases. However, no studies have been conducted on the effect of LDH sheets on RNAi efficiency in nematodes. A major concern for the researcher would be, How insects will react in response to long-term application of RNAi technology? and What will be the next step for the scientists, if insects will develop a resistance mechanism against RNAi technology?

7. Conclusion

“BioClay™” is a non-GMO alternative to counter biotic stresses. Despite of promising lab development, it needs to be implemented in farmland. A major constraint in an adoption of this technology is high cost of plant protection, which is hurdle for marginal farmers, and not having easy access to this kind of sustainable measure of plant protection. Scientists must address several concerns like non-target effects, long-term hazards, and regulatory approval for a better future of this technology.

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