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RNAi INDUCED BY EXOGENOUS dsRNAs IN PLANTS AND ITS APPLICATIONS

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ABSTRACT

RNA interference (RNAi) mechanism has several several potential applications in agriculture, including disease and pest management and plant development. RNAi technology is applied either as transgenics or - host-induced gene silencing. Considering the public concerns about GMOs on human health and the environment, the application of RNAi-based transgenic plants has been a real challenge faced by researchers worldwide. Hence there emerged recent topical/exogenous delivery of double-stranded RNAs (dsRNAs), which are more acceptable than transgenics. The exogenously applied dsRNAs targeting specific genes to induce systemic gene silencing in plants is an alternative to genetic transformation.

TOPICALLY APPLIED RNA MOLECULES TO TARGET ENDOGENES

Topically applied exogenous RNA can effectively silence an endogenous gene. The polynucleotides being absorbed inside plant cells initiate systemic silencing of endogenous genes. The RNA will hybridize inside the plant cell to RNA transcribed from a endogenous target gene, thereby, affecting the regulation of the target gene by silencing. This was demonstrated in a 2011 Monsanto patent. An *in vitro* transcribed dsRNA was sprayed on *Nicotiana benthamiana* plants to target the endogenous phytoene desaturase gene (Sammons *et al*., 2011). In a study where the transient RNA interference (RNAi) approach was used for analyzing plant gene functions, dsRNA fused with polycation carrier peptide sequence was used to target the endogenous *chalcone synthase* gene. The dsRNA–peptide complex formed was

100–300 nm in diameter and positively charged. The fusion peptide used was a copolymer of histidine and lysine (Chen *et al.*, 2000). This complex was infiltrated into intact leaf cells of *Arabidopsis thaliana*. This resulted in the silencing of endogenous genes, demonstrating a quick method of gene silencing in specific tissues in plants (Numata *et al.*, 2014). Non-viral carriers consisting of nanoparticles and cationic polymers exhibit high gene delivery efficiency, and Jiang *et al.* 2014 reported gene silencing by a non-viral gene nanocarrier in which dsRNA applied to *Arabidopsis* root efficiently knocked down the expression of two critical developmental genes. The two genes were SHOOT MERISTEMLESS (STM) and WEREWOLF (WER). STM, Class I knotted-like homeodomain protein, is specifically expressed in the shoot apical meristem (SAM) and is required for SAM formation and maintenance throughout the plant life cycle.

TOPICALLY APPLIED RNA MOLECULES TO TARGET VIRAL PATHOGENS

A number of viruses affect economically important crop plants; hence, effective management of these viruses by different biotechnological interventions is a challenge. RNAi in plants has evolved as an antiviral defense mechanism which has been exploited in a large scale targeting different traits. There are many reports where transgenic plants expressing dsRNAs against viral proteins have been well documented (Khalid *et al.*, 2017; Pooggin, 2017). Viral sequence-derived dsRNA can be directly delivered to leaves either by inoculation by mechanical methods or via an Agrobacterium-mediated transient-expression assay (Tenllado *et al* 2001). In both the above said methods the viral RNA is targeted for degradation. In a study to control viral diseases in plants virus derived dsRNAs were produced in bacteria using an *in vivo* expression system and sprayed on plant surfaces demonstrating its effectiveness in controlling viral infections (Tenllado *et al* 2003). Maize dwarf mosaic disease caused by sugarcane mosaic virus (SCMV) is a serious threat for maize production in China. Bacterially synthesized dsRNA derived from plant viral sequences can interfere with virus infection when delivered into plants by homology-based gene silencing. In a study on maize, it was shown that spraying crude dsRNA-containing extracts produced *in vivo* in *E. coli* by two inverted-repeat expression vectors with the upstream CP1 fragment or downstream CP2 fragment from the SCMV coat protein gene. The SCMV CP1and SCMV CP2 dsRNA products synthesized in *E. coli* and were shown to be effective in inhibiting SCMV infection when applied to maize plants exogenously (Gan *et al*.,2010). A non-transgenic strategy to induce RNAi in *Nicotiana tabacum*

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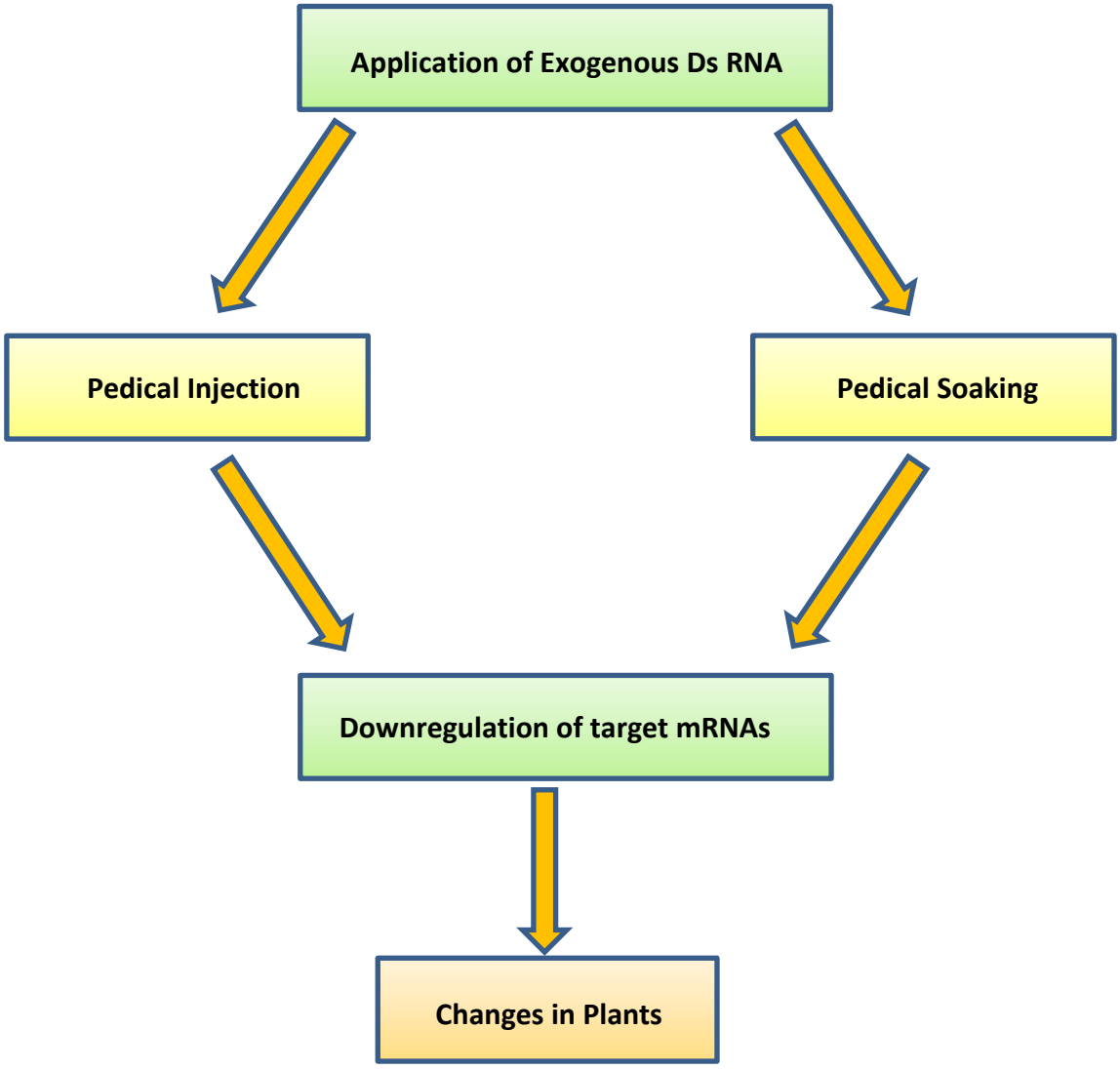
plants against Tobacco Mosaic Virus was demonstrated by *in vitro* production of dsRNA molecules for p126 (TMV silencing suppressor) and coat protein (CP) genes. This study demonstrated that the *in vitro*-produced dsRNAs, targeting two regions of the TMV genome when exogenously applied, induced RNAi against TMV resulting in resistance against virus infection in tobacco (Konakalla *et al.*,2016).



Papaya (*Carica papaya* L.), cultivation in many countries is affected by a number of viral diseases, among which papaya ring spot disease (PRSD), caused by papaya ring spot virus (PRSV), is economically the most important. A study that demonstrated the dsRNA mediated protection against PRSV by topical application of dsRNA in papaya has shown that, *in vitro* produced dsRNA molecules from both the coat protein (CP) and the helper component-proteinase (HCPro) genes of Papaya Ring Spot Virus conferred resistance. Systemic papaya leaves of the dsRNA-treated plants were virus-free at 14 days post-inoculation, confirming the

robustness of this non-transgenic virus control strategy (Vadlamudi *et al.*,2020). In most of these studies, the efficacy of exogenously applied dsRNAs against viral pathogens have largely been done under controlled experimental conditions, which is considered a major drawback. More studies to test under field conditions have to be executed.

TOPICALLY APPLIED RNA MOLECULES TO TARGET FUNGAL PATHOGENS

Foliar applications of dsRNAs significantly decreased white stem rot caused by *S.sclerotiorum* in canola (*Brassica napus*). The target genes associated with fungal pathogenicity for RNAi were identified by RNA sequencing. A comparison of global gene expression of *S. sclerotiorum* grown on susceptible and tolerant leaves of *B. napus* to those grown *in vitro* was performed. Exogenous application of dsRNA resulted in knock down of target genes. The target genes involved toxin biosynthesis, ROS response, and cell cycle regulation. Targeting RNAi transcripts of the above said genes resulted in significant reductions in lesion size by 85%, 71%, and 45%, respectively (McLoughlin *et al*.,2018).

The emerging evidence on cross-kingdom RNAi has expanded our knowledge of host-pathogen interactions and potential disease management approaches (Wang *et al.*,2017). Host-induced gene silencing has shown great potential for controlling pests and diseases in crop plants. The fungal pathogen *Botrytis cinerea* which is the causal agent of gray mold disease is a serious threat to almost all vegetables, fruits and flowers. It delivers small RNAs (Bc-sRNAs) or the pathogen effectors into host plant cells and utilizes host RNAi machinery to suppress host immunity genes (Weiberg, 2013). Based on the above listed research findings, it is evident that dsRNAs could be exploited in pathogenic fungal infestation in plants.

TOPICALLY APPLIED RNA MOLECULES TO TARGET INSECT PESTS

RNA interference (RNAi) technology has greatly impacted the insect pest control strategies in crop plants. In host-delivered RNAi management dsRNA has to be taken up by plants and also has to retain it for long to enable target insects to ingest it through feeding (Huvenne and Smagghe 2010). A first report of exogenous dsRNA application and stability of dsRNA treatments in field conditions for insect pest management was in Citrus and grapevine (Hunter *et al*.,2012). dsRNA targeting Arg kinase of two psyllids (*Diaphorina citri* and *Bactericera cockerelli*) and the sharpshooter *Homalodisca vitripennis* were injected and could

detect the presence of dsRNA even after 7 weeks. In rice, root absorption of dsRNA targeting the brown plant hopper *Nilaparvata lugens* and in Maize dsRNA targeting Asian corn borer reduced the pest infestation (Li *et al*., 2015).

CONCLUSION

Exogenous/topical dsRNA introduction into plant cells results in targeted gene silencing by specific degradation of target mRNA by post transcriptional gene silencing (PTGS). There are a number of factors that affect the efficiency of exogenously applied dsRNA. The size and concentration of dsRNAs, the method of application used, and also the stability under environmental conditions. All these factors together determine the uptake of dsRNAs by plant cells to initiate RNAi. The main drawback of exogenous applications of naked-dsRNAs is their stability.

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