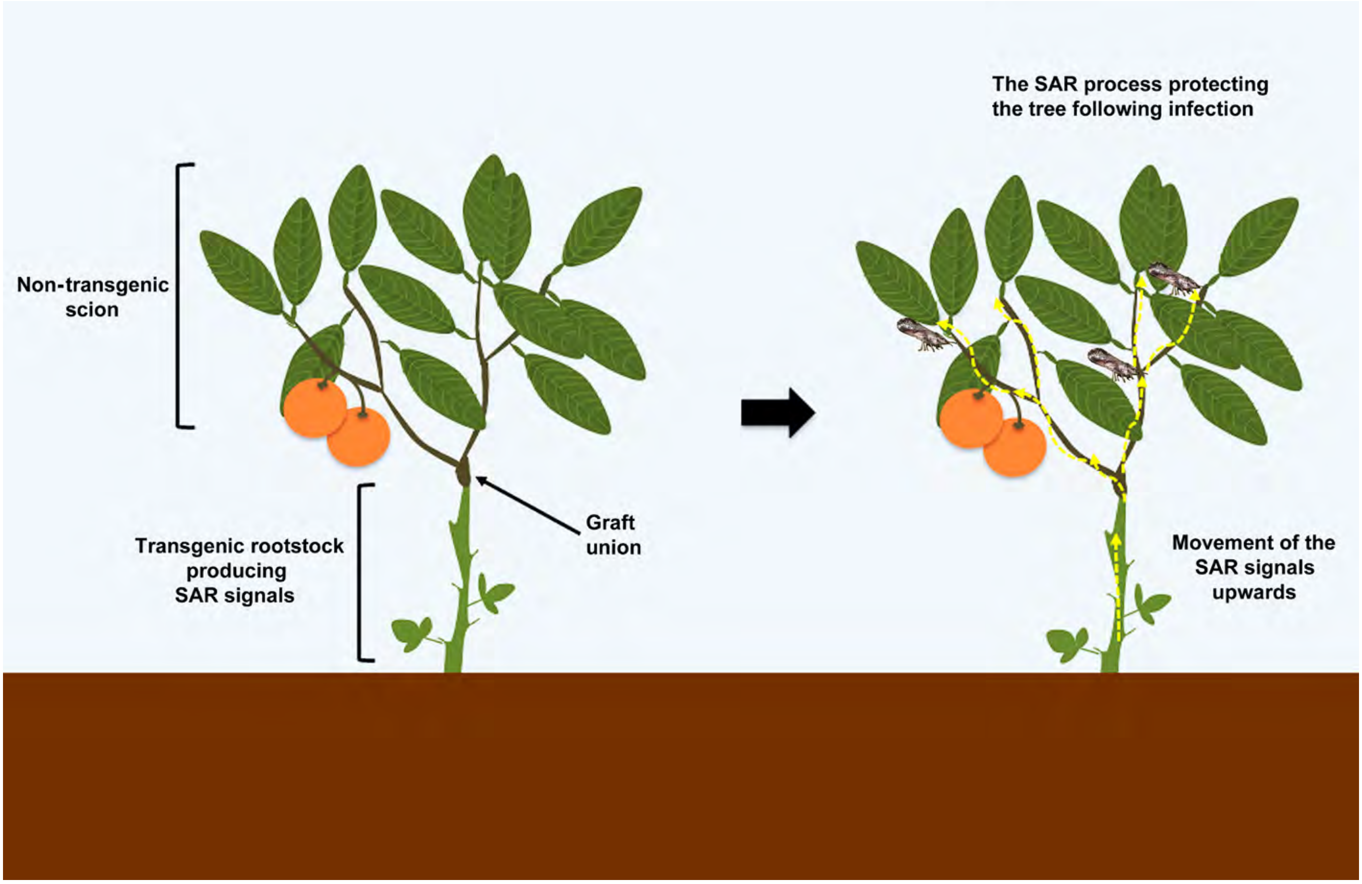

Figure 1. The systemic acquired resistance (SAR) process from a transgenic rootstock to a non-transgenic scion following infection.

Boosting citrus tree immunity to combat HLB

By Manjul Dutt, Juliana Soares and Jude Grosser

Land plants such as citrus are generally anchored to a specific location by their roots. Owing to their immobile nature and constant exposure to pathogenic microbes, plants are very vulnerable. However, you might be surprised by how much plants can protect themselves and survive against multiple adversities encountered during their lifetime. A reason for the survival ability of plants is their sophisticated and unique defense mechanisms, known as innate immunity, that were developed through evolution.

SYSTEMIC ACQUIRED RESISTANCE

Systemic acquired resistance (SAR) is a part of the innate immunity of plants, and it is activated upon pathogen attack, leading to the generation of warning signals that translocate throughout the vascular system to notify the entire plant of a potential danger. These signals activate the plant's defense responses, providing resistance at the whole plant level like vaccine-mediated immunization in humans.

Although SAR protection does not last as long as human immunization, it can last for a week, a month or even an entire season depending on the plant species. An advantage of SAR over human vaccines is that it protects plants against a broad spectrum of pathogens. However, a disadvantage is that once the effect of SAR disappears, the defense responses are reactivated only when there is another pathogen attack, resulting in a delay in the entire protection process.

To facilitate the achievement of quicker responses, researchers are investigating how specifically a plant's warning signals regulate resistance, the routes that are activated and the products being produced to induce defense responses. This information can be used to engineer plants to directly produce SAR-related products, thereby keeping the alert system always activated and avoiding any delay in resistance responses.

The citrus genetic improvement team at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Citrus Research and Education Center (CREC) in Lake Alfred is investigating methods to more quickly activate and increase the SAR process in citrus plants. The objective is to confer long-lasting protection, thus making plants tolerant to huanglongbing (HLB) disease.

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CONVENTIONAL BREEDING

Every year, several hundred hybrids between selected breeding parents with good potential toward HLB tolerance are produced in the CREC citrus genetic improvement program. These breeding parents may be derived from protoplast fusion (somatic hybridization) or prior conventional crosses.

Crosses are also performed between the inedible HLB-tolerant citrus relatives, such as *Citrus latipes* (Khasi papeda) or the edible *Citrus australasica* (Australian finger lime) with conventional citrus rootstocks and scions. In a recent study, several putative HLB-tolerant hybrids that resulted from these crosses were analyzed. Researchers observed enhanced expression of some genetic markers, indicating enhanced SAR activity within the plant. Thus, it is indeed possible that enhanced SAR activity could play a significant role in the development of conventionally bred HLB-tolerant rootstocks and scions in the future.

GENETIC ENGINEERING

The NPR1 protein, a key regulator of SAR, was discovered in Arabidopsis, a small flowering plant related to cabbage, cauliflower, broccoli and mustard. This gene has shown great potential in inducing resistance against pathogens in several crops, including citrus, rice, wheat, soybean, grape, carrot, tomato and strawberry.

The citrus improvement team developed genetically engineered sweet orange expressing Arabidopsis NPR1. Trees planted in high HLB pressure fields showed tolerance to *Candidatus* Liberibacter asiaticus. Many of these trees have survived in a healthy state for many consecutive years. Each tree that had enhanced tolerance to HLB also had enhanced expression of the same genetic markers that were observed in the conventionally bred population described above.

These genetically engineered trees are still being monitored every year to evaluate the durability of the tolerance effect mediated by the Arabidopsis NPR1 gene. Based on current results, this gene seems to be a good candidate for engineering durable resistance to HLB. The citrus improvement team has also identified other SAR-inducing genes from other crop plants that enhance tolerance to HLB. Engineered plants with these gene(s) are also being evaluated in a high disease pressure field site and have shown promising results.

In citrus, there is already evidence that plant signals can move from rootstock to scion. This was achieved when a transgenic rootstock expressing the FT early flowering gene induced earlier flowering in grafted juvenile non-transgenic scions. SAR signals are known for their ability to promptly move throughout the plant. Therefore, the citrus improvement team hypothesizes that these molecules are also going to easily move from the engineered rootstocks, eliciting a rapid response to HLB development in the scion and allowing graft transmissible movement of SAR-enhancing signaling molecules (Figure 1, page 14).

In this scenario, an engineered

scion would not be required. The fruit or juice from the tree might not be considered a genetically modified organism (GMO) at all if GMO proteins are not detectable. Time-tested rootstocks (genetically modified) and scions can be used in this approach, and this would have a positive impact on consumer acceptance. The citrus improvement team is currently exploring this mechanism to provide durable tolerance to regular sweet orange scions. However, studies of this nature require time because field testing is a slow process, and it will be a few years before conclusive evidence is obtained.

Interestingly, citrus also has its own NPR1 gene. The question thus arises: Why does a foreign DNA need to be inserted into citrus when this gene is already present in citrus? The simple answer is that there are several genes that actively interfere with the expression of citrus NPR1. These genes are known as negative regulators or genetic suppressors. Turning off these negative regulators could potentially allow the endogenous citrus NPR1 gene to become functional, negating the need for the introduction of a foreign DNA. To resolve this issue, a powerful technique called CRISPR-Cas9, which can target specific negative regulators and silence them, is being utilized. More details on the CRISPR-Cas9 process will be discussed in a future issue of this magazine.

CONVENTIONALLY BRED VS. GENETICALLY MODIFIED CITRUS

Both conventional breeding as well as genetic engineering strategies have risks and benefits. Conventionally developed citrus has been around for a long time, and all cultivated citrus plants originate from either sexual crosses or mutations.

Cisgenesis is another technique gaining traction in recent years. Cisgenesis is a genetic modification system in which a natural gene from a crossable/sexually compatible cultivar (that could also be transferred using traditional breeding techniques) is inserted into the recipient plant to include a value-added trait. This technique could be used to potentially transfer one or more tolerance gene(s) from an HLB-tolerant citrus relative, preserving cultivar integrity without adding the negative traits that are commonly observed following conventional breeding.

The genetic modification of citrus rootstocks using SAR-related genes is also an interesting approach that can be included in citrus improvement programs. The defense signals generated using this approach are mostly mobile and can potentially translocate from a genetically modified rootstock to a non-transgenic scion. Therefore, as explained above, this process can be used in engineering rootstocks that can potentially induce resistance in non-transgenic scions. This can result in the generation of citrus fruits free of any transgene.

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