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Cisgenesis: Emerging Tool for Fast-tracking Fruit Breeding

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Cisgenesis is an emerging new tool in plant breeding that enables the transfer of genes from sexually compatible donor plants to recipient plants without introducing foreign DNA. This approach has gained significant attention in recent years due to its potential for rapid and precise gene transfer, addressing consumer concerns about genetically modified organisms (GMOs) while still allowing breeders to introduce valuable traits into commercial plant varieties. Being a new technology, the findings of cisgenic fruit plants production is limited. Though remarkable achievement has been made by producing scab resistant and fire blight resistant cisgenic apple, an successful attempt to develop cisgenic grapevine line, which is fungal disease resistant, has also been achieved. Cisgenesis has the potential to contribute to sustainable and resilient fruit production, meet consumer preferences, and address emerging challenges in the agricultural sector. Continued research, collaboration, and responsible deployment of cisgenic fruit crops will contribute to a more productive, environmentally friendly, and resilient fruit industry, ensuring the availability of nutritious and flavorful fruits for future generations.

Introduction

Fruit crops are very much important for for the economy and for people's health all over the world, but they are getting more and more stressed out by pests, diseases, and climate change. To deal with these threats in a way that will last, breeders are using cisgenesis, a precise genetic engineering method that doesn't use DNA from other species. Transgenesis adds genes from unrelated species, but cisgenesis only moves genetic material between organisms that can reproduce with each other.

Core Principle of Cisgenesis

Cisgenesis is a sophisticated genetic engineering technique characterized by the transfer of genes solely between sexually compatible organisms. Transgenesis introduces "alien" DNA from unrelated species, while cisgenesis uses the existing gene pool to make sure that the changes could happen naturally through reproduction. There are two ways to transfer genetic material between sexually compatible organisms: intra-specific transfer (moving genes within the same species) and inter-specific transfer (using closely related species that could naturally cross-breed).

Methodology of Cisgenesis

The cisgenesis process includes mainly two broad steps. First one is to gene identification and the second one is the transfer of the gene. In case of gene identification, the gene isolated and characterized from a sexually compatible donor plant. For transfer of gene modern biotechnological tools like *Agrobacterium*-mediated gene transfer of CRISPSER-CAS9 technology are used.

Advantages of Cisgenesis in Fruit Crops

Cisgenesis offers a middle ground of exploiting the advantages of both conventional breeding techniques and modern biotechnological approach.

1. Increase Precision: The problem of linkage drag is much associated with conventional breeding programme. But cisgenesis offers the precise transfer of desired genes, without compromising plant's genetic integrity.
2. Expansion of Genetic Access: Cisgenesis helps to bypass geographical and seasonal mating barriers.
3. Fast-Track Breeding Programme: Cisgenesis also helps to reduce the time of breeding programme. It facilitate the analysis of research and development in much faster rate rather than conventional breeding programme.
4. Relaxation of regulatory barriers: As cisgenesis is restricted to DNA transfer from sexually compatible donor, no alien DNA is used in that case, it often faces lower regulatory hurdles.

Development of Cisgenic Fruit Crops

Several successful case studies demonstrate the efficacy of cisgenesis in improving fruit crops. These examples showcase the range of traits that have been targeted and the positive outcomes achieved through cisgenic approaches.

Cisgenic Apples: The first evidence of successful creation of a cisgenic apple plant through the introduction of the native apple scab resistance gene HcrVf2, along with its own regulatory sequences, into the scab susceptible apple variety Gala (Vanblaere et al., 2011). By employing a previously developed technique involving *Agrobacterium*-mediated transformation, combined with a selection system and a chemically inducible recombination mechanism, researchers were able to produce apple cv. Gala plants containing the scab resistance gene HcrVf2 under its original regulatory sequences. From the generated plants, three cisgenic lines were selected for further examination, confirming the presence of a single T-DNA insertion and the expression of the desired gene HcrVf2. Later in 2015, a cisgenic apple line, named C44.4.146, was created by introducing the cisgene FB_MR5 from the wild apple species *Malus ×robusta* 5 (Mr5) into the fire blight susceptible cultivar 'Gala Galaxy' using the established method of *Agrobacterium*-mediated transformation with the binary vector p9-Dao-FLPi (Kost et al., 2015). The line C44.4.146 was found to exclusively contain the cisgene FB_MR5, regulated by its inherent regulatory sequences. To evaluate the resistance conferred by the cisgene, three separate experiments were conducted, where shoots of the cisgenic line and untransformed 'Gala Galaxy' were inoculated with a Mr5 avirulent strain of *Erwinia amylovora* using either scissors or a syringe. Notably, significantly reduced disease symptoms were observed on the shoots of the cisgenic line in comparison to those of the untransformed 'Gala Galaxy'. Another experiment carried out by Krens et al. (2015) to develop red fleshed apple.

Cisgenic Grapes: the successful attempt was made by Dhekney et al. (2011) to develop cisgenic grapevine line. To begin the process of developing grapevines resistant to fungal diseases, the *Vitis vinifera* thaumatin-like protein (VVTL-1) gene was isolated by them from the "Chardonnay" variety and modified for continuous expression. Embryogenic cultures of the "Thompson Seedless" variety were transformed with *Agrobacterium* to regenerate grapevines carrying the cisgenic VVTL-1 gene. The presence and number of copies of the cisgene were confirmed through PCR and quantitative real-time PCR. Protein expression was assessed using ELISA. Out of the tested plant lines, two showed a delay of 7-10 days in the development of powdery mildew disease during greenhouse screening and exhibited reduced severity of black rot disease in field tests. Additionally, these lines displayed a 42.5% decrease in the incidence of sour-bunch rot disease in the berries compared to non-transformed controls after 3 weeks of storage at room temperature. Although the plants in this study contain viral promoters and reporter/marker genes, this marks the first instance of achieving broad-spectrum fungal-disease resistance in genetically engineered grapevines through a cisgenic approach.

Conclusion

In conclusion, cisgenesis holds significant promise for the improvement of fruit crops, offering a precise and efficient approach to introduce desirable traits from sexually compatible relatives. Through the successful development of cisgenic fruit varieties, such as apples, grapes, citrus, and strawberries, advancements have been made in disease resistance, flavor enhancement, and agronomic traits. However, several challenges and considerations, including regulatory frameworks, public perception, gene stability, and trait selection, need to be addressed to fully harness the potential of cisgenesis in fruit crop improvement.

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