////Title: Harnessing Genetics for Sustainable Fruit Production

////Stand-first: People are becoming increasingly aware about the health benefits of eating a diet that's rich in fruit and nuts. However, farmers are struggling to meet the increasing demand, due to crop damage caused by climate change and emerging plant diseases. As a solution, Dr Vladimir Orbovic, based at the University of Florida, develops and evaluates new methods to manipulate the genetic make-up of plants to rapidly create resilient crops for sustainable future.

////Body text:

In recent years, consumers around the world have become increasingly health conscious, which has driven up the demand for high-quality fruit and nuts.

For fruit and nut growers, keeping up with this increasing demand is a fine balance. While production has been growing steadily over the past few years, climate change, emerging diseases and the depletion of natural resources have been putting a strain on the yield and quality of many fruit and nut crops. In response to these challenges, farmers and breeders are keen to develop new crop varieties that are not only more resistant to environmental stress and disease, but that also exhibit higher yields.

Conventional selective breeding has been used for centuries to produce the crop varieties we have today. However, for most of these crops, progress moves at a snail's pace, because plants have a very long juvenile period before they start producing fruit. In the wild, this period ensures that the plant does not start producing fruit too young, but for farmers this means a long wait before harvest.

In a recent paper published in *Frontiers in Plant Science*, Dr Vladimir Orbovic of the University of Florida with his co-authors, Dr Song from Michigan State and Dr Prieto from INIA in Chile, discusses how methods to manipulate the genetic make-up of important crops have become an efficient and rapid alternative to selective breeding. These methods allow breeders to introduce specific genes into crops in a much shorter period of time. Recent success stories include plums that are resistant to plum pox and apples that don't go brown.

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Plant genetic engineering has become one of the most important tools in the modern crop breeding. This approach is particularly valuable if it is difficult or impossible to introduce a desirable trait through selective breeding methods. Over the past few decades, researchers have made significant progress in developing new and more efficient methods to manipulate the genetic make-up of plants.

Dr Orbovic explains how this all started back in 1985, with the first successful genetic transformation in tobacco plants. In this context, transformation refers to the insertion of a new gene into the tobacco plant's DNA using a modified bacterial vector called *Agrobacterium*. As a common plant pathogen, *Agrobacterium* already possesses the ability to transfer DNA between itself and plants. Motivated by the success achieved with tobacco plants, several research groups around the world developed new transformation protocols for a variety of fruit and nuts crops, including apple, pear, plum, cherry, grapes, walnuts, kiwifruits, citrus, chestnuts and blueberry.

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Depending on the selected gene to transform, the resulting plant can be called transgenic or intragenic. In broad terms, transgenesis involves introducing a gene from one species into another, in what is commonly known as genetic modification, or GM.

In a recent study published in *Scientia Horticulturae*, Dr Orbovic and his colleagues produced transgenic citrus crops by inserting a specific gene that can prevent or slow down cell death. This important study was undertaken in response to a worldwide drive to create transgenic citrus plants that are resistant or at least tolerant to citrus canker, 'greening' disease, and other emerging diseases.

The team found that adding one or more copies of the gene improved the plant's survival when under attack by a disease-causing virus or bacteria, and slowed down the process of stress-induced aging. The plant's resilience to infection was measured in terms of its resistance to transformation by the gene vector *Agrobacterium*. As the transgenic plants were more difficult to transform than normal plants, Dr Orbovic and his colleagues concluded that these plants would also be more resilient when under attack.

To test the impact of environmental stress on their new plants, the researchers cut small branches, placed them in water and observed them in time. The leaves and branches of the non-transgenic plants appeared dehydrated and discoloured after nine weeks, whereas the transgenic plants remained fresh and vibrant.

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Dr Orbovic and his colleagues hope that their new transgenic plants could become a solid foundation to develop stress tolerance and disease resistance in citrus plants. This method has been used in different plants by Dr Orbovic's team and other research groups around the world, but as many consumers are uncomfortable with the idea of eating genetically modified crops, progress has been severely hindered.

In search of a solution, researchers have now started to develop methods that consumers are more comfortable with. As mentioned earlier, one of the other options available is intragenesis. This method can be considered a 'softer' version of transgenesis, where the inserted genes come from the same species.

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Going back to Dr Orbovic's article in *Frontiers in Plant Science*, he also discussed another innovative approach of incorporating desirable traits into crops, called FastTrack Breeding. FastTrack breeding is like the conventional process of selective breeding, but in high speed.

The idea is to use crop varieties that have been transformed with genes that induce early fruit production, to speed up the process of selecting for the required traits. Once happy with the result, scientists can then select plants that don't have the genetically modified genes, but still have the desirable traits, and use those for large scale production. In this way, the method allows for the use of genetically modified plants during the breeding process, but the product that reaches the market is not considered to be genetically modified.

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Another promising approach is based on an agricultural practice that is over 3000 years old: grafting. Traditional grafting involves joining the upper part of one plant (the scion) onto a root and trunk system of another plant (the rootstock), so that they grow as a single plant.

In his paper, Dr Orbovic describes how researchers now use grafting with a twist. Developed in the 1990s, 'transgrafting' combines this old practice with new technology. In this case, the rootstock is genetically engineered to endow the whole plant with resistance to a particular disease, but the fruits produced are not actually genetically modified themselves. The key advantage of transgrafting is that the plant can transport nutrients across the graft, without ever changing DNA sequences in the fruits.

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For the past 5 years, many research groups including Dr Orbovic's team have started using a revolutionary gene editing technology called CRISPR, to manipulate the genetic make-up of plants with a precision never seen before. This technology can recognise and alter specific DNA sequences, without affecting the rest of the plant's genetic material. Importantly, CRISPR simply 'edits' the plant's DNA, and does not introduce genes from other organisms.

This novel approach is still in its early stages, but initial studies are pointing in the right direction. Positive results have been demonstrated in apple, grape, orange, grapefruit and kiwifruit, suggesting that CRISPR may one day occupy a special place in a breeder's arsenal when it comes to introducing disease resistance and climate resilience into crops.

Meet the Researcher

Dr Vladimir Orbovic
Core Citrus Transformation Facility
University of Florida
Gainesville, FL
USA

Contact
E: orbovic@ufl.edu
W: https://crec.ifas.ufl.edu/facilities/transformation-lab/orbovic/

This SciPod is a summary of the papers '*Agrobacterium*-Mediated Transformation of Tree Fruit Crops: Methods, Progress, and Challenges', from *Frontiers in Plant Science*, and 'Production and characterization of transgenic Citrus plants carrying p35 anti-apoptotic gene' from *Scientia Horticulturae*.