Breeding kiwifruit after the *Pseudomonas syringae actinidiae* incursion

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Abstract
Kiwifruit species (*Actinidia* spp.) are long-lived perennial vines that originate in central and southwestern China. Cultivated in New Zealand since the early 1900s (Ferguson, 2004), the kiwifruit grew to become our largest horticultural fresh-fruit export industry. Kiwifruit breeders in New Zealand have been selecting the best genotypes for five to seven generations utilising a conventional approach of reciprocal recurrent selection for general combining ability. Since the 2010 incursion of *Pseudomonas syringae* pv. *actinidiae* (Psa) a suite of approaches has been used and integrated into the traditional operations to accelerate and facilitate the development of new cultivars, and to ensure the sustainable development of populations available for long-term breeding.

Keywords: kiwifruit breeding, breeding strategies, pseudomonas syringaea, actinidia

Introduction

Kiwifruit breeding is complex. Three main species of *Actinidia* are the core of the current breeding effort. This species cluster is complemented with interspecific crosses and enriched with within and between ploidy groups. Kiwifruit species comprise a polyploid series with a basic chromosome number of x=29. The prevalent dioecious nature of kiwifruit adds another factor to the complex picture of breeding and population management (Fraser *et al* 2009). Since the early 2000s all new importations of germplasm have been limited to a small number of genotypes per year, and a large number of individuals are playing no role in the overall breeding path due to their high susceptibility to Psa. While at first sight, this raises flags about the long-term sustainability of the program, increasing the total genetic variance is possible through recombination, mutation, CRISPR-Cas9 and breeding.

Actinidia deliciosa (Actinida chinensis var. *deliciosa)*

With the largest source of available germplasm, *Actinida chinensis* var. *deliciosa* breeding is focused on providing a robust successor to the long standing king of the kiwifruit, the 'Hayward' cultivar. Until recently, its hexaploid nature (174 chromosomes) has meant that it has been passed over in molecular studies in favour of its diploid cousin. Factorial crossing designs have been the outstanding pillar for the development of progeny tested parents. This species naturally requires winter chilling to fruit and flower profusely as its centre of origin in central China tends to have colder winters. There are sufficient sources of variation to increase yield and a current study by Popowski (pers. comm.) is focusing on untangling the genetic basis of yield by aligning phenotypic yield observations with SNPs. Its apparent Psa tolerance is an advantage and it may be a source of resistance genes.
Hermaphroditism is a new addition to the *Actinidia chinensis* var. *deliciosa* breeding programme (Mcneilage, 1991) potentially benefiting the breeding cycle by reducing the

amount of progeny testing; an organised structure of the breeding lines is required to avoid potential associations with other commercial traits.
The quantitative nature of the most common breeding traits (e.g. fruit size, dry matter content) has been exposed through breeding. A stabilising selection model will reduce the chances of generating candidate cultivars with traits well beyond consumer needs.
With three years from planting to first year flowering, the life cycle of *A. chinensis* var. *deliciosa* is quite long.

Actinidia chinensis var. *chinensis*

This species was largely ignored until the early 2000 when Zespri commercialised the first gold fleshed cultivar. Two main ploidy groups (diploid and tetraploid) and two flesh colours (gold and red) are the focus of the breeding efforts through a reciprocal recurrent selection program similar to that described for *A. chinensis* var. *deliciosa*.
Many of the diploid *A. chinensis* var. *chinensis* genotypes were among the first to show extreme susceptibility to Psa (Vanneste, 2017, Da Silva *et al*, 2014). The apparently more tolerant tetraploid parents were the source of one of the most successful releases of a cultivar worldwide (Gold3; fruit marketed as Zespri® SunGold Kiwifruit).
The exploits of unreduced gametes as a new source of variance is exciting and potentially game changing for the breeding strategy (Seal *et al*, 2013). Psa reduced the amount of available germplasm; the risks associated with a strong reduction of population size are not clearly understood (as fruit traits do not seem to show a clear pattern of inbreeding depression). With only two years from planting to the first year of flowering, breeding with *A. chinensis var. chinensis* produces results faster than with *A. chinensis* var. *deliciosa*.
An extensive collection of expressed sequenced tag (EST) sequences is proving a valuable resource for studying a wide variety of traits and facilitating the identification of candidate genes for key traits (sex, vitamin C, flesh colour). A tremendous amount of genomic and genetic data has been accumulated for kiwifruit, and with the implementation of a series of modern data science technologies (Docker, Conda, Git, Jupyter Notebooks and Shiny) outputs from our parental trials can be quickly implemented in the breeding programme.

Actinidia arguta

A lesser known *Actinidia* species, this polyploid (2x, 4x, 8x) originates from colder areas in China, Siberia, Japan and Korea. New Zealand's commercialised *A. arguta* cultivars are sold as "Baby Kiwis" or "Kiwiberries". It is a short-storage berry with an edible skin and many kinds of skin colour. Psa tolerance is widespread among populations and *A. arguta* could prove to be a source of pyramiding genes for projects with *A. chinensis* var. *chinensis*.
With two to three years to first fruiting, accelerating flowering is a priority for kiwiberries (and for many of the other species) for speeding up the delivery of new cultivars.
Reciprocal recurrent selection has been implemented and the population divided in two with genotypes on both islands; germplasm sourcing is limited to existing genotypes on each island, potentially recreating Namkoong's multiple population strategy. In subdivided populations there is potential for rapid evolution through recurrent directional migration. If properly structured and with small population sizes, factorial crosses provide an opportunity to create populations with similar fitness values and potentially greater variability in allele frequencies.

Actinidia hybrids

Interspecific hybrids can result in completely new and unexpected products. However, with few barriers to hybridisation in *Actinidia*, a 'crossing frenzy' program can ruin the opportunity to focus and develop guided outcomes.
Recently commercialised hybrids include a 'Sweet Green' kiwifruit resulting from crossing *A. chinensis* var. *deliciosa* (6x) with *A. chinensis* var. *chinensis* (2x).
The extent of changes to chromosomal and mitochondrial DNA from interspecific hybrids is not well understood. The models for developing a robust strategy for specific hybrid combining ability might not be applicable if specific combining ability is more important than general combining ability. Interspecific hybridisation fuels genomic and transcriptional alterations and thus hybrids can be adept at responding to environmental changes, with the caveat that those changes may also drive evolution of pathogenicity that, in the light of Psa, will be important to keep at bay.

Other *Actinidia* species

Other important *Actinidia* species such as *A. eriantha*, *A. rufa* and other subspecies of *Actinidia* are also present in the germplasm collection and are subject to studies associated with particular traits.
Flowering synchronicity and ploidy issues can be overcome and the establishment of new inter- and intraspecific hybrids may increase the opportunity for selecting new cultivars with traits highly associated with consumer and industry demands.

Concluding remarks

During the first 50 years of *Actinidia* breeding, a small number of robust cultivars have been developed using traditional breeding approaches. The robustness and plasticity of early cultivars allowed the development of a resilient industry and were the key to the success of the program.
In the coming years, the pathway and structure of breeding populations will remain, but modern technologies are starting to add value and breeders are realising the potential of genomics to develop new cultivars faster and with more accurate predictions.
Environmental challenges will put pressure on the longevity of kiwifruit cultivars; kiwifruit breeders need to be ready for them.

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