

**Citrus breeding 2.0**  
A novel approach integrating deciphered parentage and genomics-assisted selection

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Logos: NARO, N+G, M.A.F.P., 内閣府 (Cabinet Office, Government of Japan), 東京大学 (THE UNIVERSITY OF TOKYO)

### Mission of citrus breeding in NIFTS

- Development of diverse varieties to satisfy commercial needs
- Improvement of overall fruit quality for premium fruit
- Minimize the period to release a new citrus variety

Timeline of citrus varieties released:

- 1979: Kiyomi
- 1995: Amakusa
- 1999: Shiranuhi, Harumi
- 1999: Setoka
- 2002: Seinannohikari, Harehime
- 2004: Reiko
- 2005: Tamami
- 2006: Benibae
- 2009: Haruhi
- 2012: Asumi
- 2012: Mihaya
- 2014: Rinoka
- 2017: Asuki

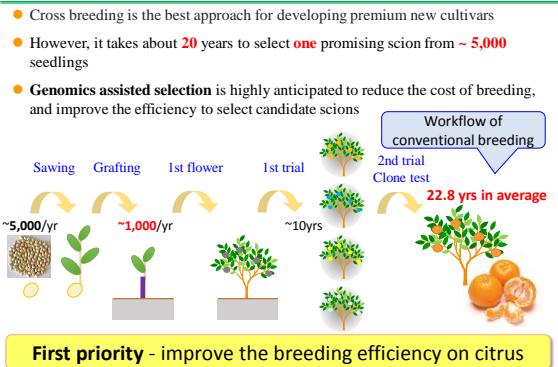
Logos: NARO

### Citrus breeding – three conventional approaches

- **Mutation breeding**
  - Mutant selection of a sport or from nucellar seedlings
  - Mutation induction by irradiation or chemicals
  - Less efforts to discover a mutant
  - Limited change of trait
- **Cross breeding**
  - Hybrid selection from a single cross of diploid
  - Valid to obtain unique variety
  - Longer breeding period than mutation breeding
- **Polyploid breeding**
  - Triploid or tetraploid selection
  - Benefit of seedless variety development
  - Large efforts to obtain parental lines



### Workflow of citrus breeding in NIFTS

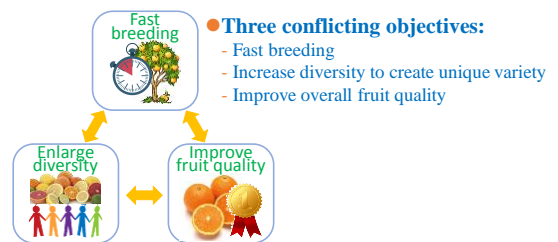


### Overview of citrus breeding in NIFTS

- Hybrid selection from various diploid crosses
- Released citrus varieties : **54** (49 crossbreds)
- Germplasm collections: **>1,200**
- Seeds obtained every year: **>12,000**
- Seedlings under evaluation: **~10,000**



### Trilemma in citrus breeding



It is difficult to accomplish these three objectives together by conventional breeding



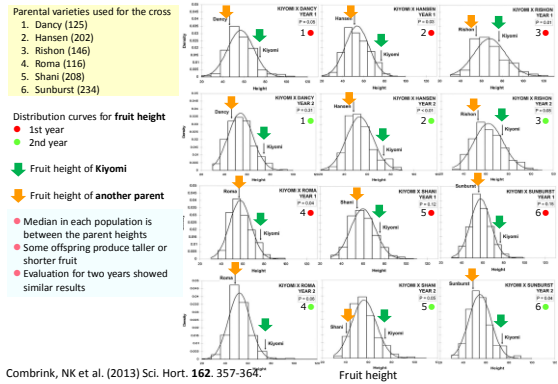
### Diversity observed among wide citrus varieties



- Size
- Shape
- Rind thickness
- Rind color
- Flesh color
- Seed number
- Acidity
- Brix
- Color break
- Aroma
- Taste
- Peelability
- Peel puffing
- Disease resistance
- Fertility
- Parthenocarp
- ...

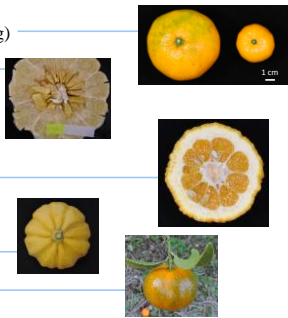
Most varieties are not suitable for the breeding. Why?

### Variation in fruit size and shape in Kiyomi tanger families



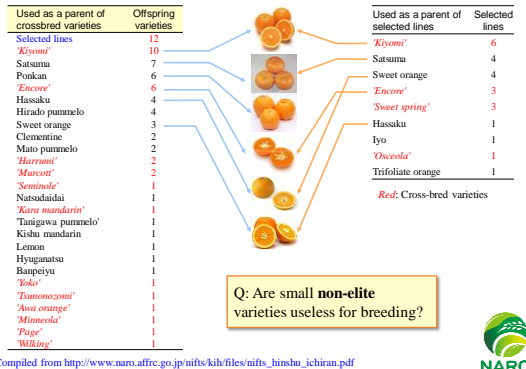
### Top 8 reasons to discard candidate seedlings

1. Small fruit (less than 100 g)
2. Seedy
3. Less Brix content
4. Acidic or less acidic
5. Hard to peel
6. Peculiar aroma
7. Appearance
8. Nonuniform coloring

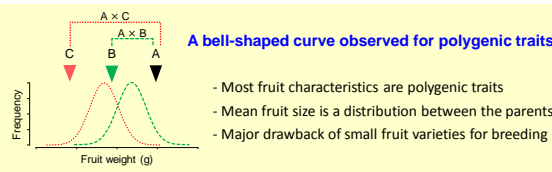


Many non-elite varieties have been considered useless as breeding parents

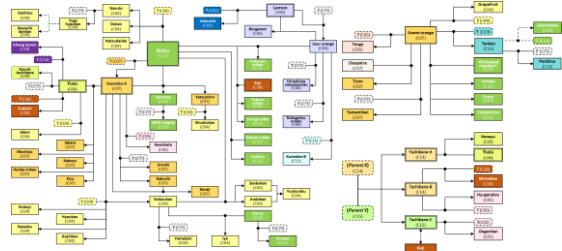
### A few elite varieties are commonly used in breeding programs



### Small fruit varieties yield small fruit offspring



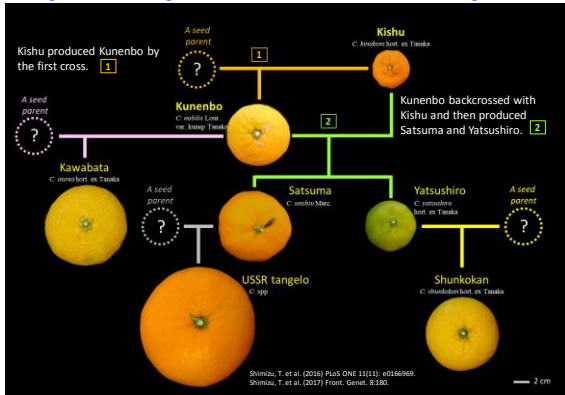
### Pedigrees of 67 citrus varieties were determined



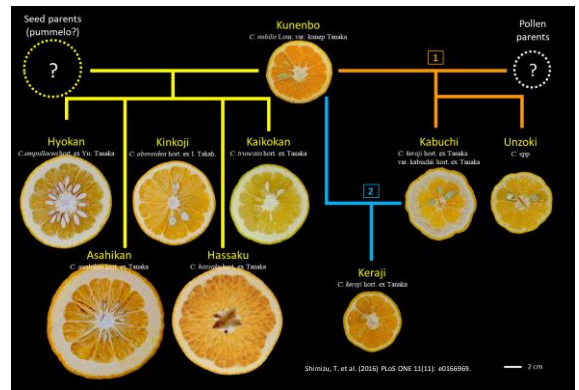
The pedigrees revealed demonstrated the key roles of non-elite small fruit varieties in expanding citrus diversity

Shimizu, T. et al. (2016) Plus ONE 11(11): e0166969.

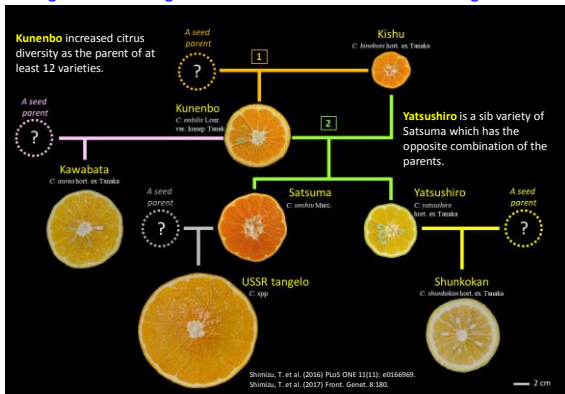
Introgression of the genome of **Kishu** to wide varieties through **Kunenbo**



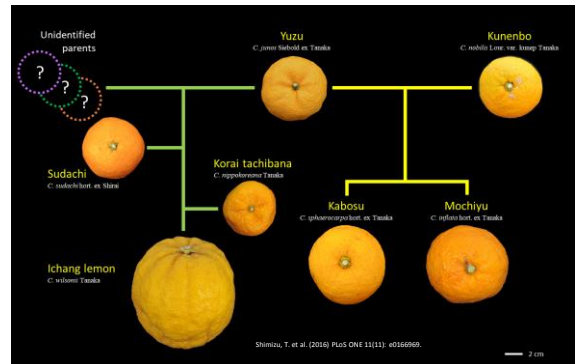
Diversity of fruit trait observed in half sibs of **Kunenbo**



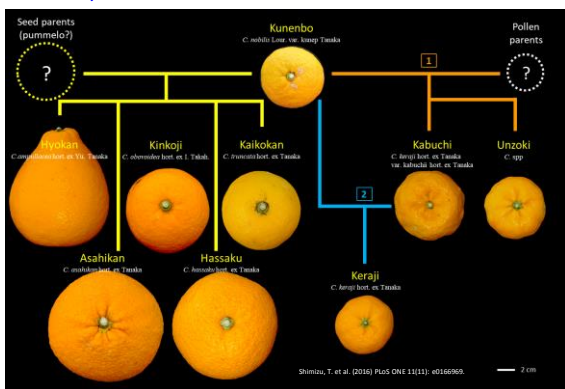
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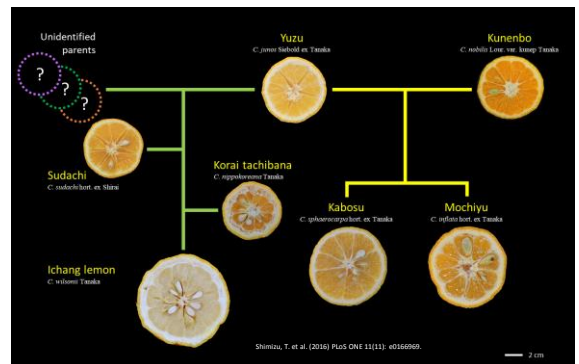
Diversity of fruit trait on acid citrus varieties observed in half sibs of **Yuzu**



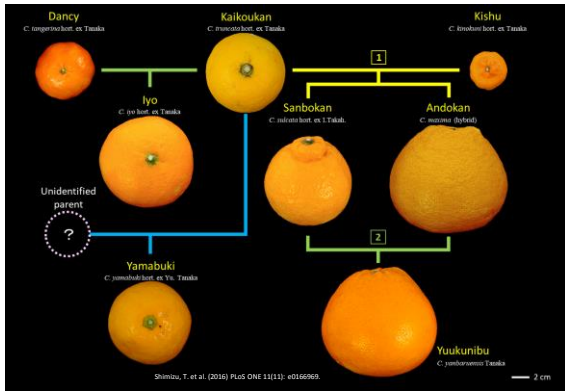
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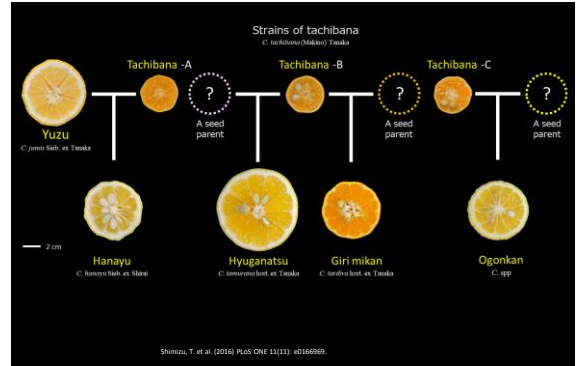
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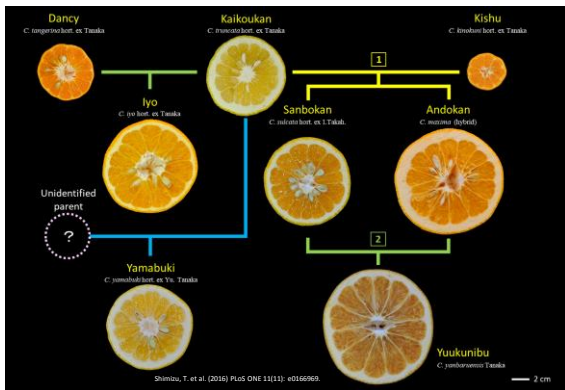
Wide diversity observed among the half sibs of **Kaikoukan**



Wide diversity observed among the sibs of small fruit variety **Tachibana**



Wide diversity observed among the half sibs of **Kaikoukan**

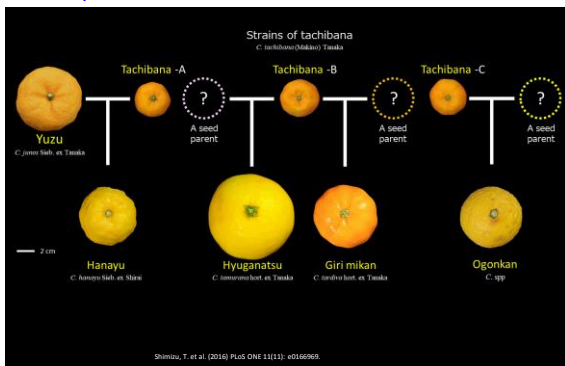


Traits changed during single generation



- ✓ Single cross is enough to increase diversity and select promising scions
- ✓ Multiple generation is not mandatory to exclude undesirable traits
- ✓ Non-elite varieties show potential as breeding parents

Wide diversity observed among the sibs of small fruit variety **Tachibana**



Using **non-elite varieties for breeding will**

- Contribute to increasing diversity of fruit traits
- Decrease the overall fruit quality by introgression of bad trait
- Extend total breeding period for continuous cross to segregate out unwanted traits

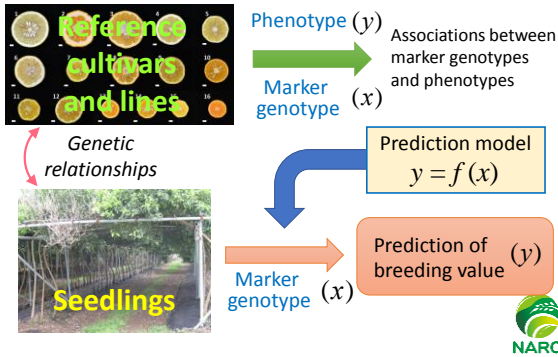
**Conventional citrus breeding evaluates more than 20 traits:**

- Selecting a promising candidate exceeding the thresholds of most of the traits is mandatory
- Many fruit traits are complex polygenic quantitative traits
- Simple MAS for a specific trait is insufficient
- Most fruit characteristics are combination-dependent

Two genomics-assisted selection methods will increase both **diversity** and **fast-breeding**

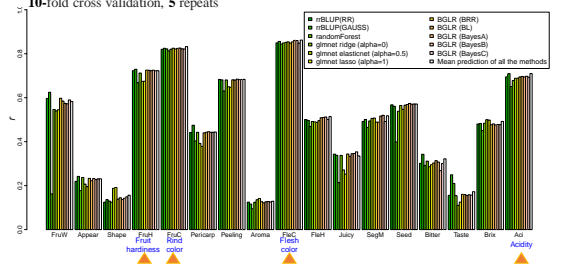


## Genomic selection (GS)



## Prediction accuracy for 17 fruit traits showed variation

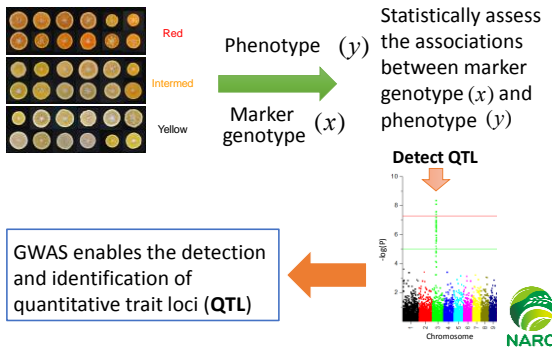
Prediction accuracy was evaluated in total families. Training population: Parental and breeding population 10-fold cross validation, 5 repeats



No large differences were observed among the 11 prediction methods for 17 traits

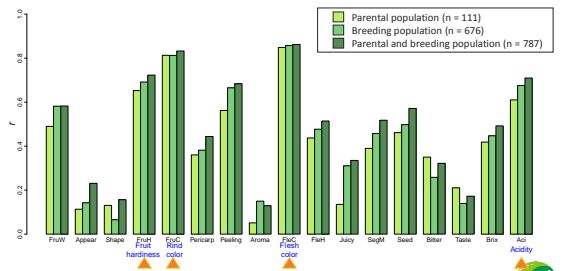
Minamikawa, M.F. et al., 2017. *Scientific Reports*, 7(1), p.4721.

## Genome-wide association study (GWAS)



## Integrating population data improved the prediction accuracy of GS

Prediction accuracies were improved by integrating additional population data



Minamikawa, M.F. et al., 2017. *Scientific Reports*, 7(1), p.4721.

## GS and GWAS in citrus

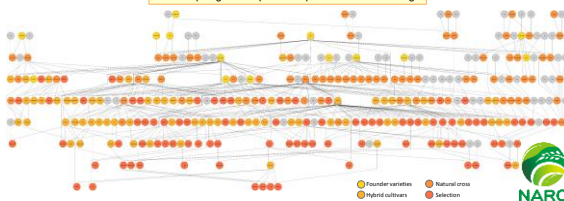
### Plant samples for modeling:

- 111 parental varieties
- 35 breeding populations, total 676 individuals

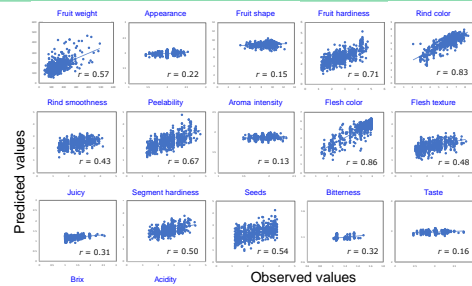
### Genotype data:

- Verified 1,841 SNPs (Illumina GoldenGate Assays)
- Designed from resequencing data of 15 citrus varieties

Part of pedigrees of plant samples used for modeling



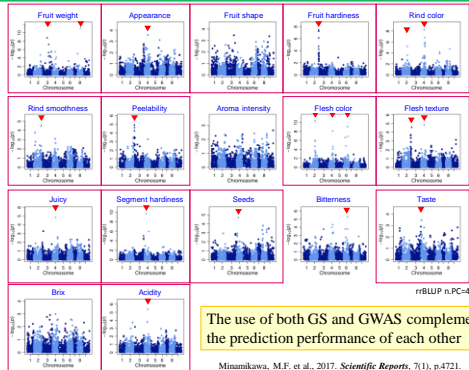
## GS is valid for predicting a part of fruit traits



Heritability and mode of inheritance of trait, accuracy of phenotyping, and populations used for modeling affect prediction accuracy

Minamikawa, M.F. et al., 2017. *Scientific Reports*, 7(1), p.4721.

## GWAS is valid for identifying QTL of fruit traits



## Citrus breeding 2.0

### Citrus breeding 2.0 will

- **Contribute to increase trait diversity** by referring to known pedigrees
- **Improve overall fruit quality** by selecting high-quality seedlings by using GS and GWAS
- **Achieve fast-breeding** by selecting a promising scion from single cross and avoid the trilemma in citrus breeding

### In progress:

- Increase the number of plant samples and DNA marker genotypes for remodeling
- Integration of this strategy for the breeding system to increase diversity
- Application in the rebreeding of Satsuma and others

Shimizu, T. *in press*.

## Acknowledgements



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