

How We Use Molecular Tools in the Strawberry Breeding Program

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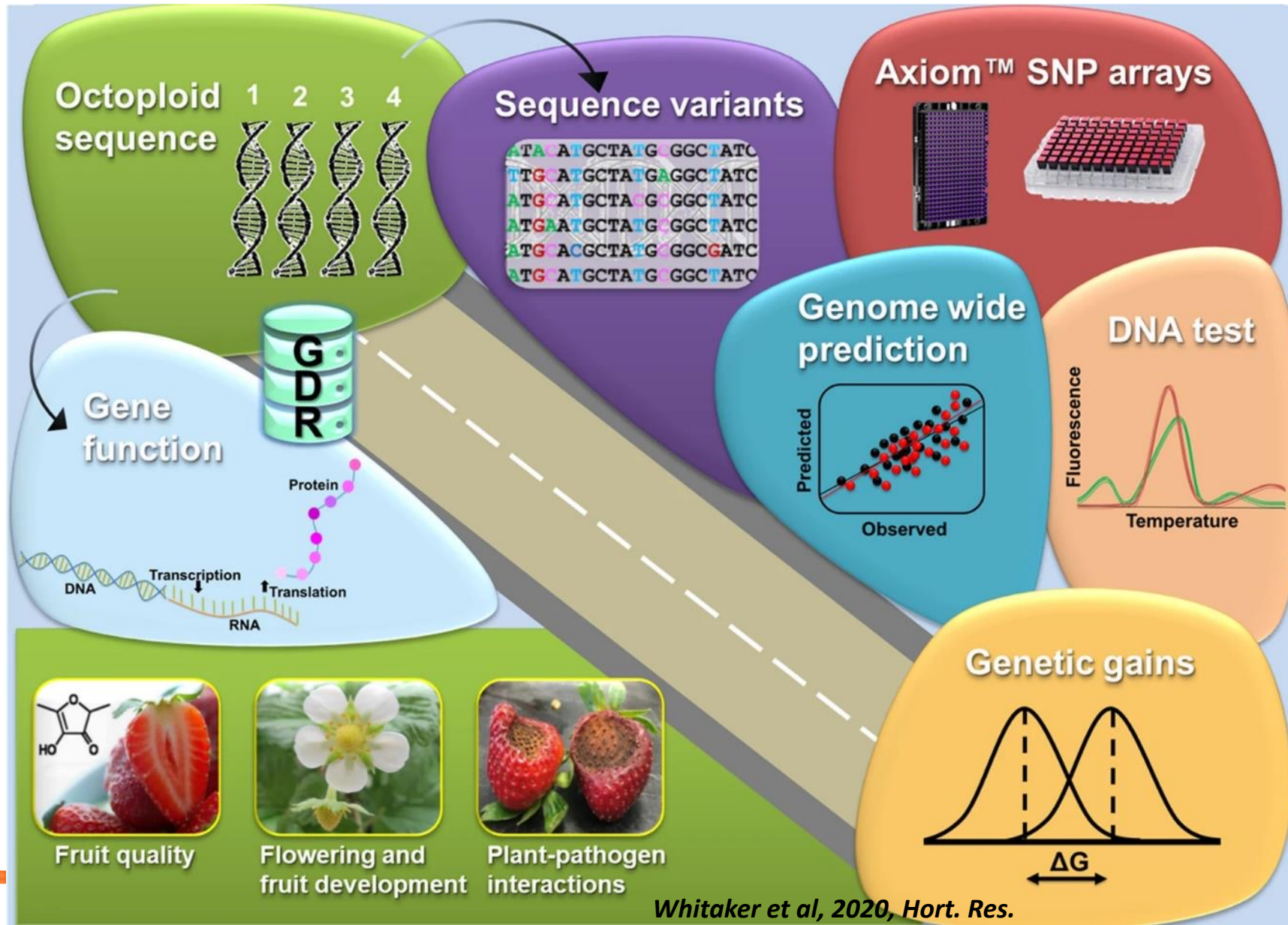
41ST ANNUAL AGRITECH



Outlines

- 1. DNA Markers and Marker-Assisted Seedling Selection for Improving Florida Strawberry Varieties**
- 2. Enhancing Disease Resistance Through Somaclonal Variation**
- 3. CRISPR Gene Editing for Accelerating Improvement of Strawberry Varieties**

Breeding Toolbox: Traditional Breeding + DNA Technology



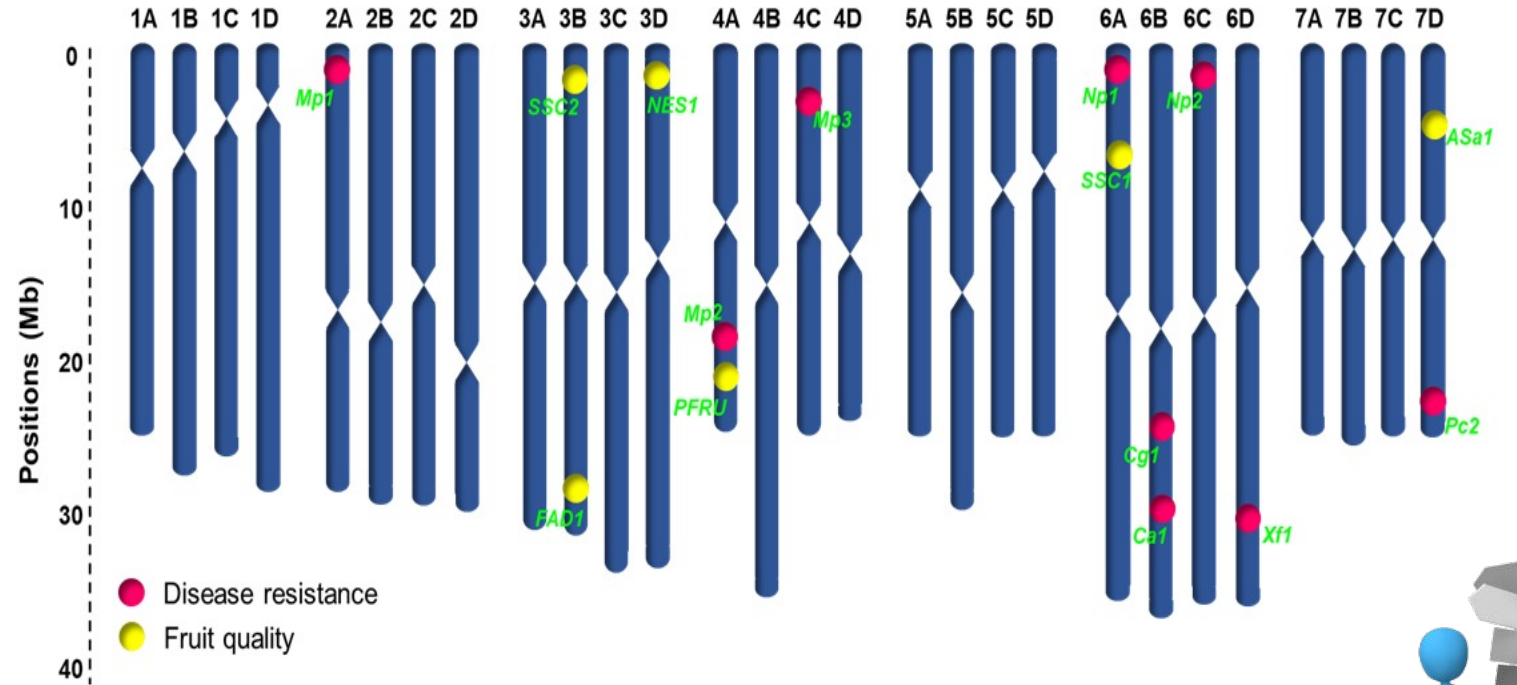
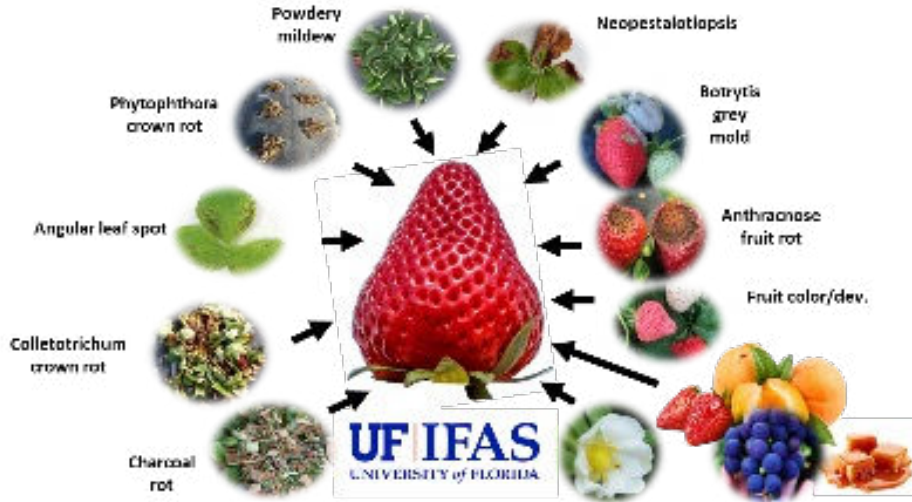
**Tools Available
For Genomics-
Enabled
Breeding In
Octoploid
Strawberry:
2015 - 2022**





Dr. Verma,
Sujet

DNA Markers In UF Strawberry Breeding



Strawberry DNA Markers and MASS

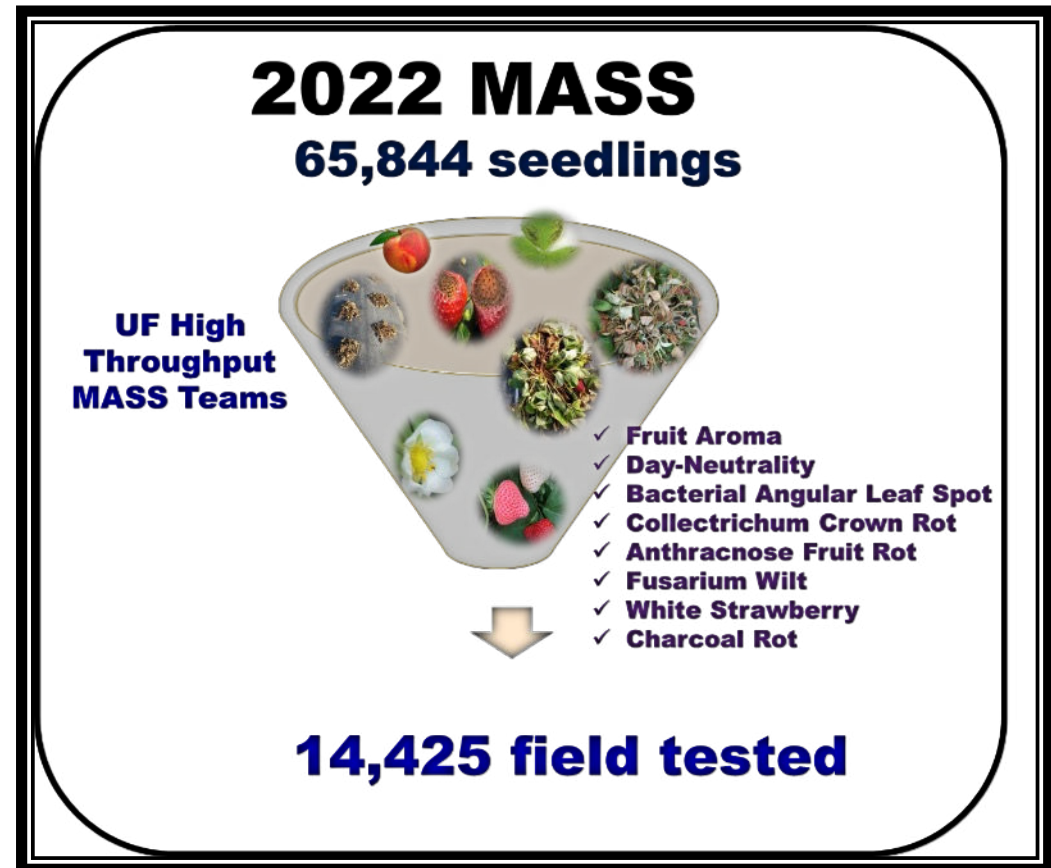
Traits

Disease resistance

- Phytophthora crown rot resistance
- Colletotrichum crown rot resistance
- Anthracnose fruit rot resistance
- Charcoal Rot
- Fusarium Wilt
- Bacterial angular leaf spot

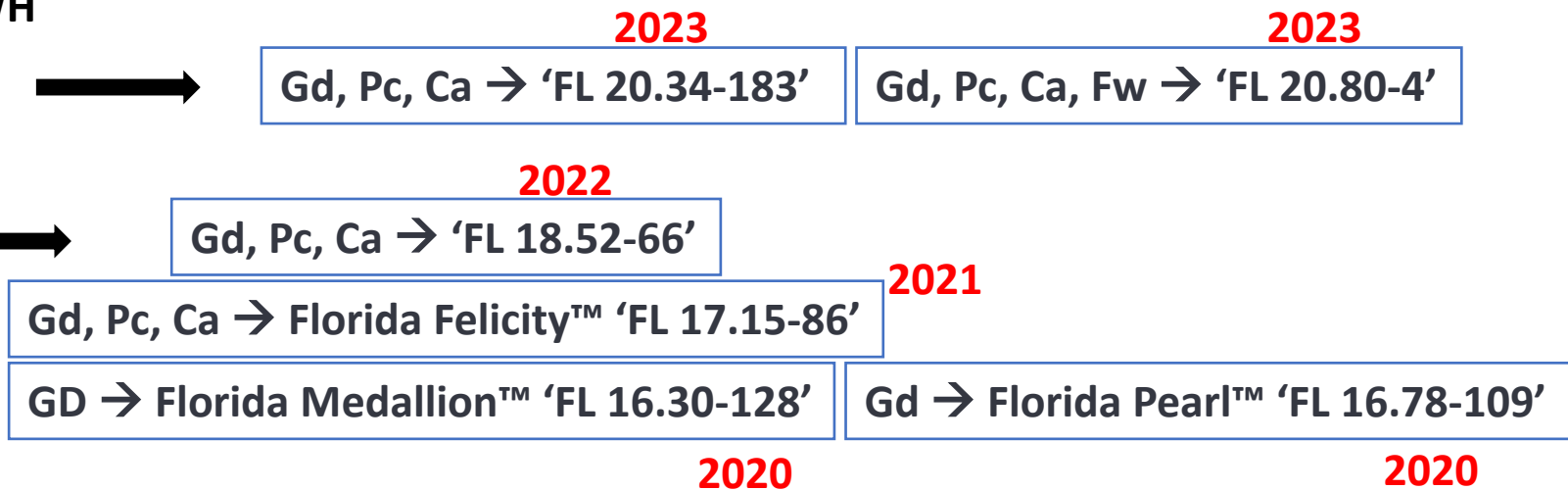
Fruit quality

- Day-Neutrality
- White Fruit
- Flavor - γ -Decalactone
- Flavor - Furaneol and Mesifurane
- Soluble Solid Content



DNA Marker & MASS – Impacts on Florida Strawberry Industry

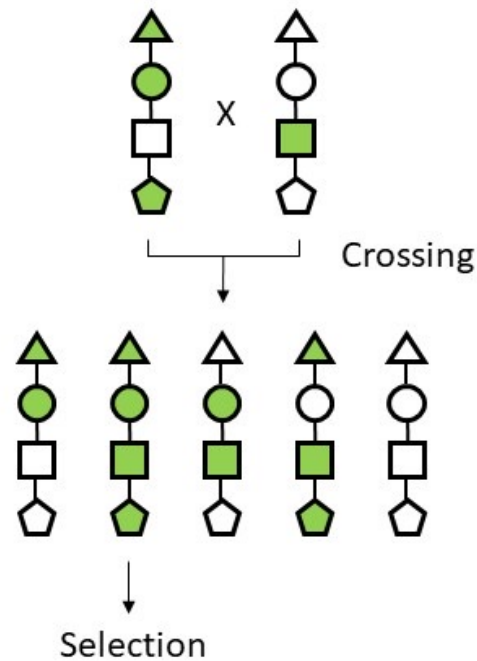
- 2023 – Gd, Pc, Ca, DN, Xf, Cg, Fw, WH, Mp1, FUR, MES, SSC1, SSC2
- 2022 – Gd, Pc, Ca, DN, Xf, Cg, Fw, WH, Mp1
- 2021 – Gd, Pc, Ca, DN, Xf, Cg, Fw, WH
- 2020 – Gd, Pc, Ca, DN, Xf, Cg, Fw
- 2019 – Gd, Pc, Ca, DN, Xf, Cg, Fw
- 2018 – Gd, Pc, Ca
- 2017 – Gd, Pc, Ca
- 2016 – Gd, Pc
- 2015 – Gd



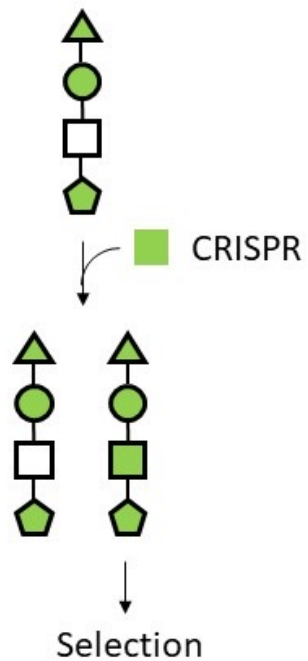
Gd; peach aroma, Pc; Phytophthora, Ca; Anthracnose, DN; Day neutrality, Xf; Angular leaf spot, Cg; Colletotrichum, Fw; Fusarium, WH; White fruit, Mp1; Charcoal rot, FUR; Furaneol, MES; Mesifurane, SSC1&2; Soluble solid content

Methods for Strawberry Breeding

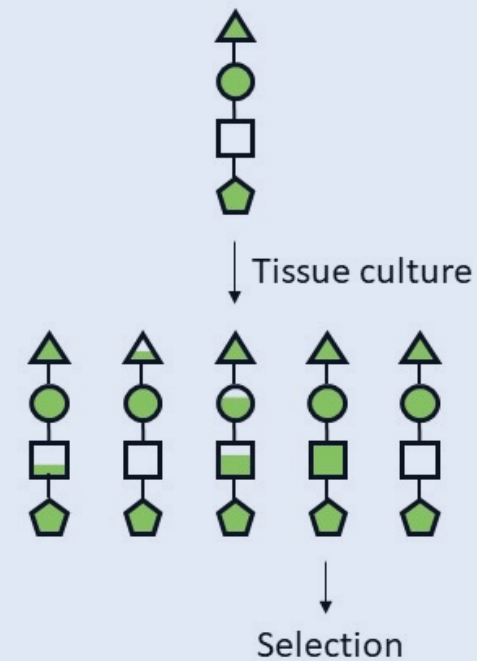
A Selective breeding



B Genetic engineering



C Somaclonal variation



DNA Marker & MASS

USDA Definition – Genetic engineering would not cover traditional breeding techniques, such as marker-assisted breeding, as well as tissue culture and protoplast, or chemical or radiation-based mutagenesis

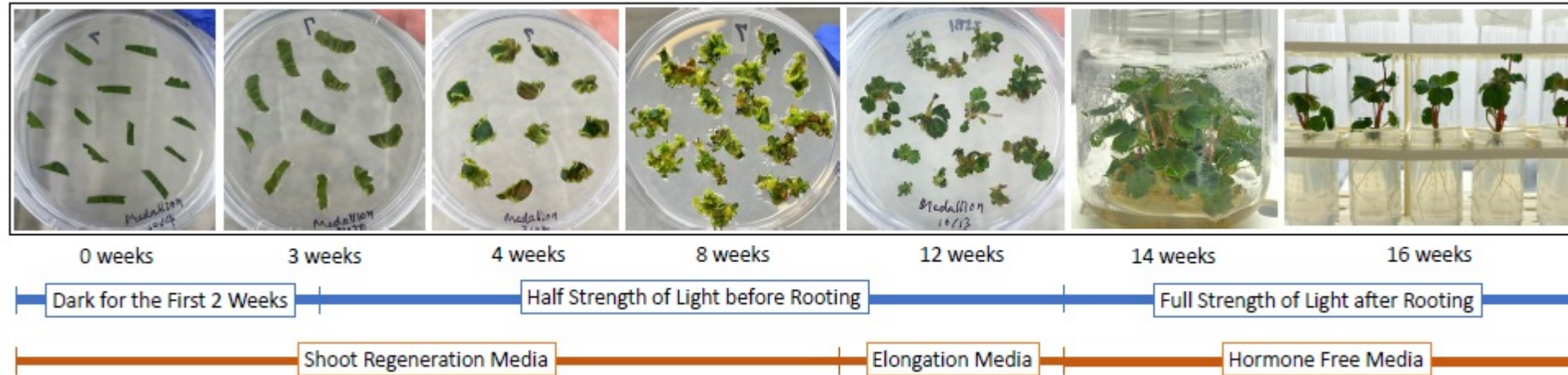
GMO in EU

No Regulation

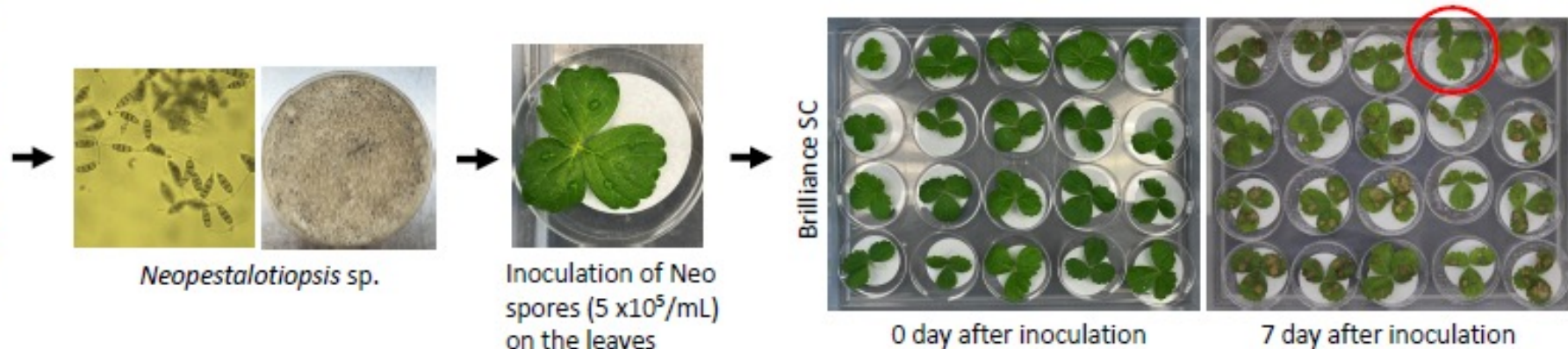
<https://edis.ifas.ufl.edu/publication/HS1448>



Somaclonal Variations - Neopestalotiopsis Resistance 'Florida Brilliance' & 'Florida Medallion'



Somaclonal plants in soil



Florida Medallion



**Detached Leaf Assay
- 300 Somaclones
from Each Variety**

**Clonal Propagation
& Field Test in 2023**

Final selected somaclones

19

10

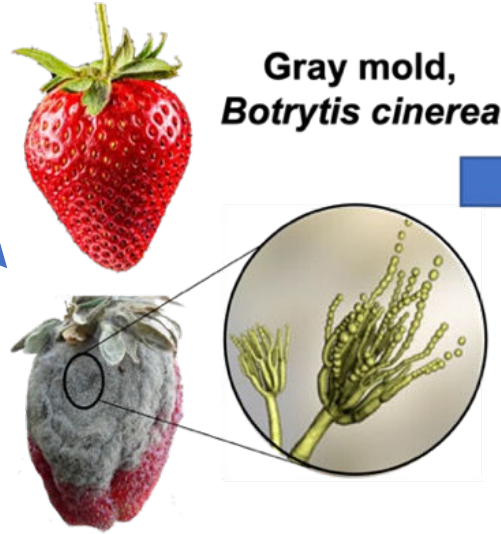
Florida Brilliance



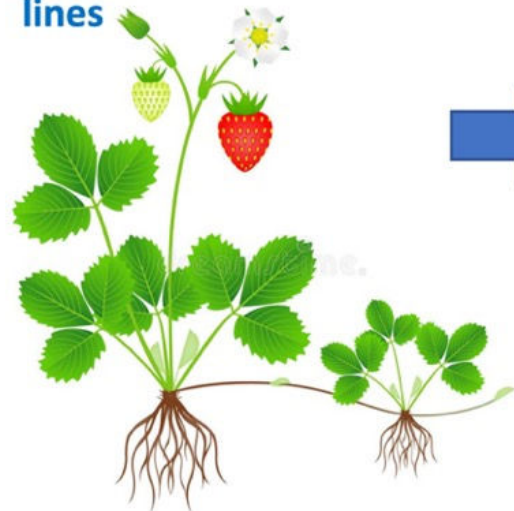
Updates of CRISPR GE in Strawberry

WRKY25

A Development of gray mold (*Botrytis cinerea*) resistant lines

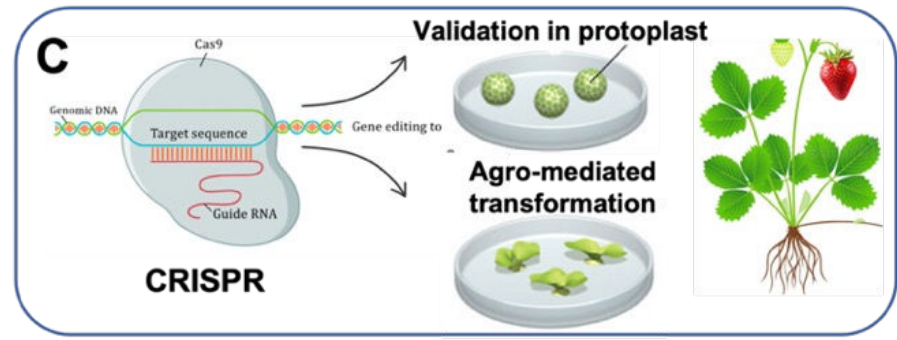


B Development of runnerless lines

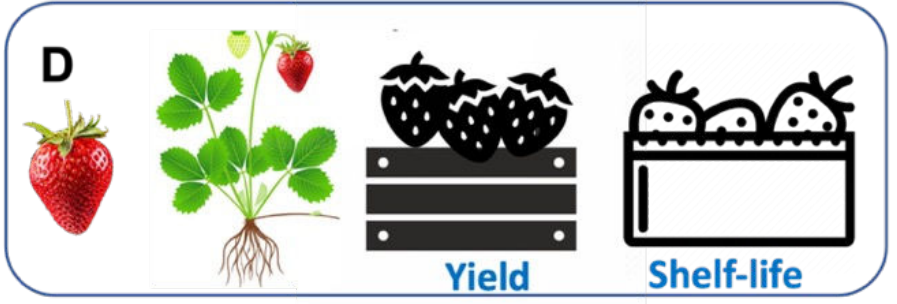


GAOX and LAM

AIM1. CRISPR/Cas9 editing in strawberry



AIM2. Quality evaluation of the CRISPR lines

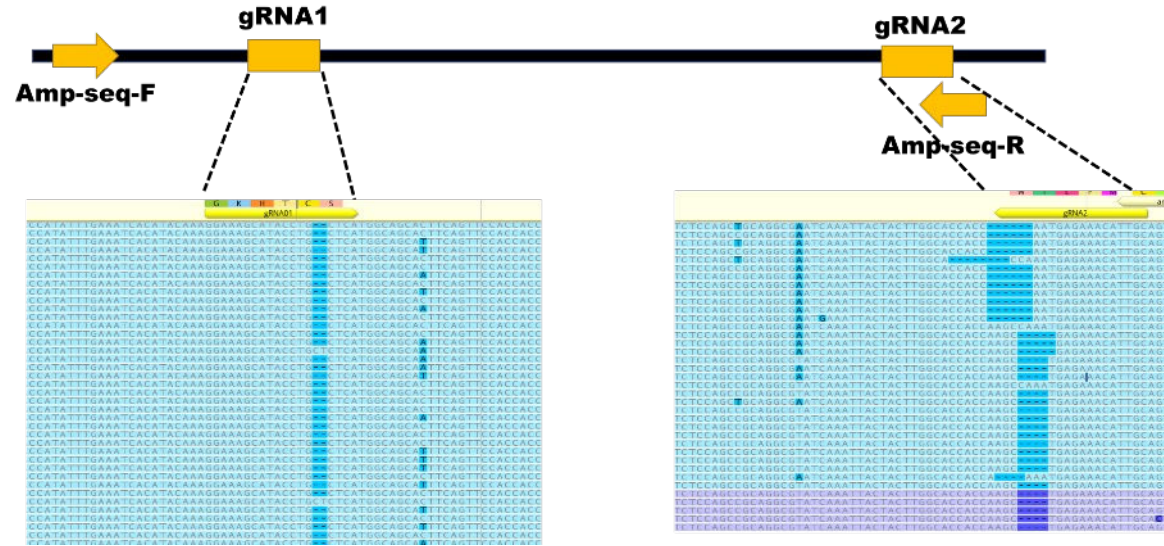
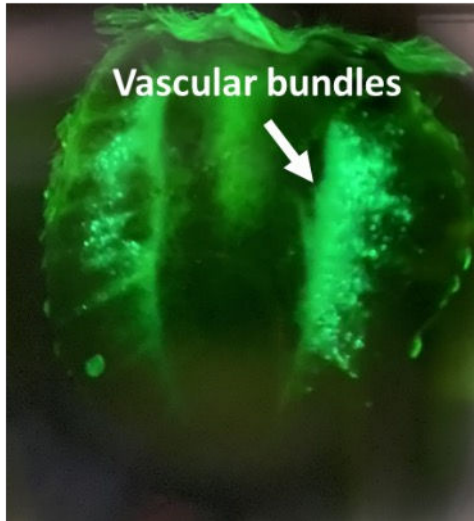


AIM3. Integration of CRISPR lines to breeding

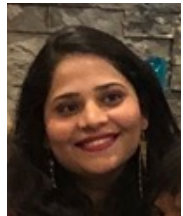


CRISPR GE System in UF Strawberry Program

Amplicon sequencing

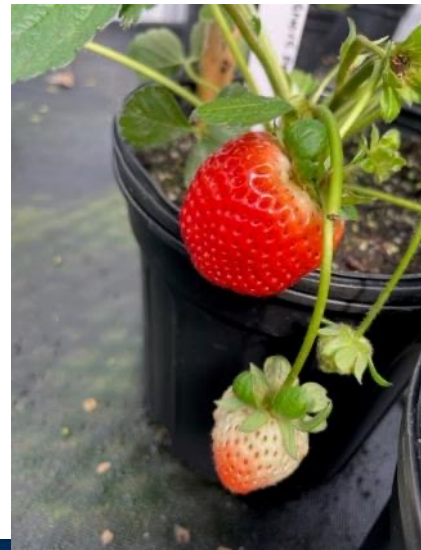


Manbo Lee



Kanika Saxena

Edited - 'Florida Brilliance'
(White Fruit)



Florida Brilliance



GE – T1 selfing



GE – T1 selfing



GE – T1 selfing



CRISPR GE for Neopestalotopsis

Florida Brilliance



Florida Brilliance – OCP3



Gene
Knockdown
Assay- OCP3



Gene
Knockout by
CRISPR- OCP3



CRISPR GE for Runnerless In Strawberry

Medallion without GA

Medallion with GA 50ppm



**Genes for Regulation of Runner Development with Gibberellic Acid – GAox and LAM
(LOSS OF AXILLARY MERISTEMS)**

CRISPR GE for Runnerless In Strawberry

Florida Medallion

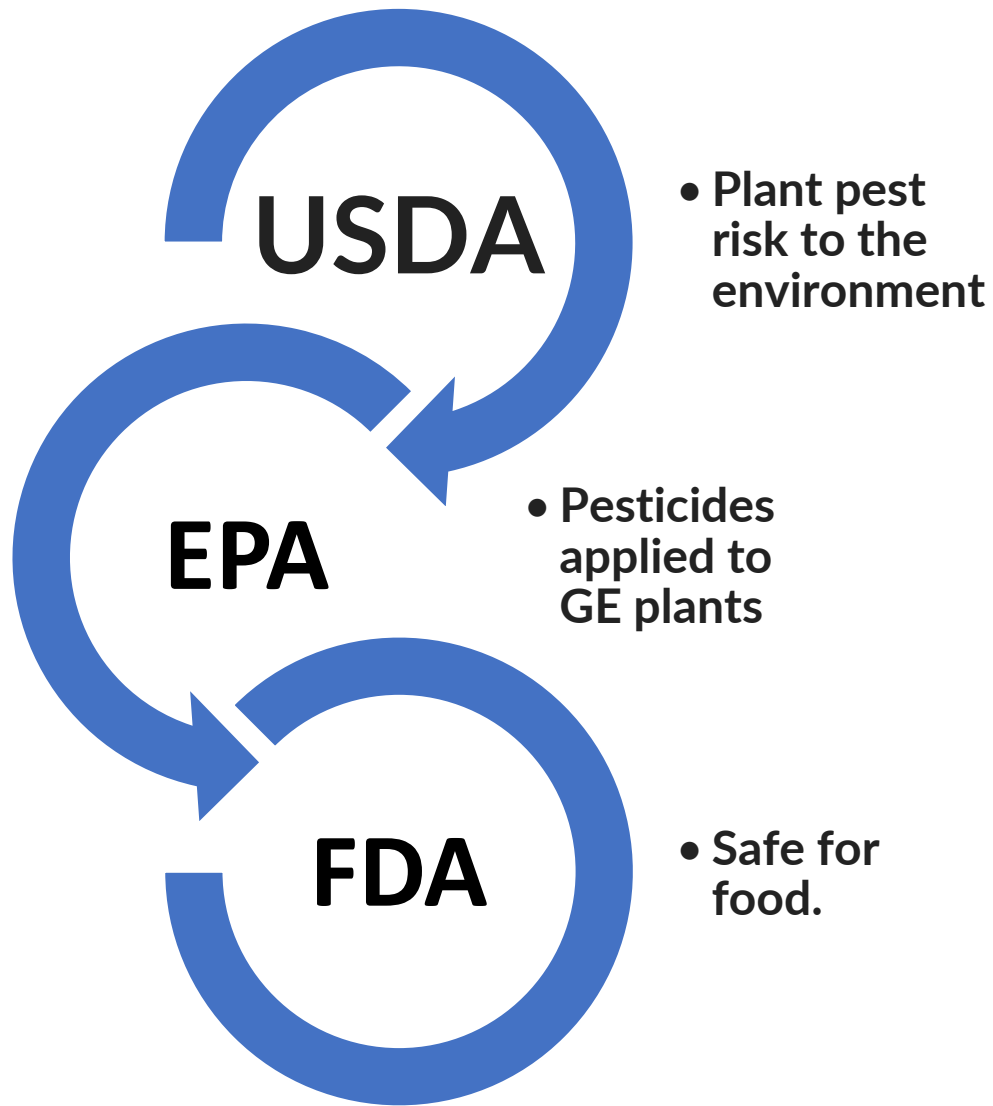
GE

Florida Brilliance



- Gene Editing for Runnerless Strawberry – GAox and LAM (LOSS OF AXILLARY MERISTEMS)

Current Regulation for CRISPR GE in the US



Sections 340.4 and 340.5 are applicable beginning April 5, 2021

Under USDA's final rule,

- Certain GE plants will not need regulatory approval from APHIS **if they otherwise could have been developed through conventional breeding.**
- GE plants will not be subject to regulation **if they have plant-trait combinations that are the same as other plants.**
- Other GE plants are not subject to regulations **if they are greenlighted by APHIS under an inquiry process.**



Questions

