



Molecular marker development for the small granule starch trait in cassava

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ISTRTC meeting 2025

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Cassava



Vital staple for millions

1 NO POVERTY



2 ZERO HUNGER



13 CLIMATE ACTION



Industrial cash crop: Supports smallholder livelihoods and a multi-billion-dollar industry in Asia

Specialty starches for different applications

- Global demand for high-value starches for industrial applications is rapidly expanding.
- Starch = Amylose + Amylopectin
- **Structure, morphology**, and relative **ratios** of the components significantly impact the **functional properties** of cassava starch.
- This can be further exploited through breeding to develop new specialty starches targeting specific applications.
- Manipulation of the starch biosynthetic pathways, has led to high- and low-amylose starch genotypes in various crops.



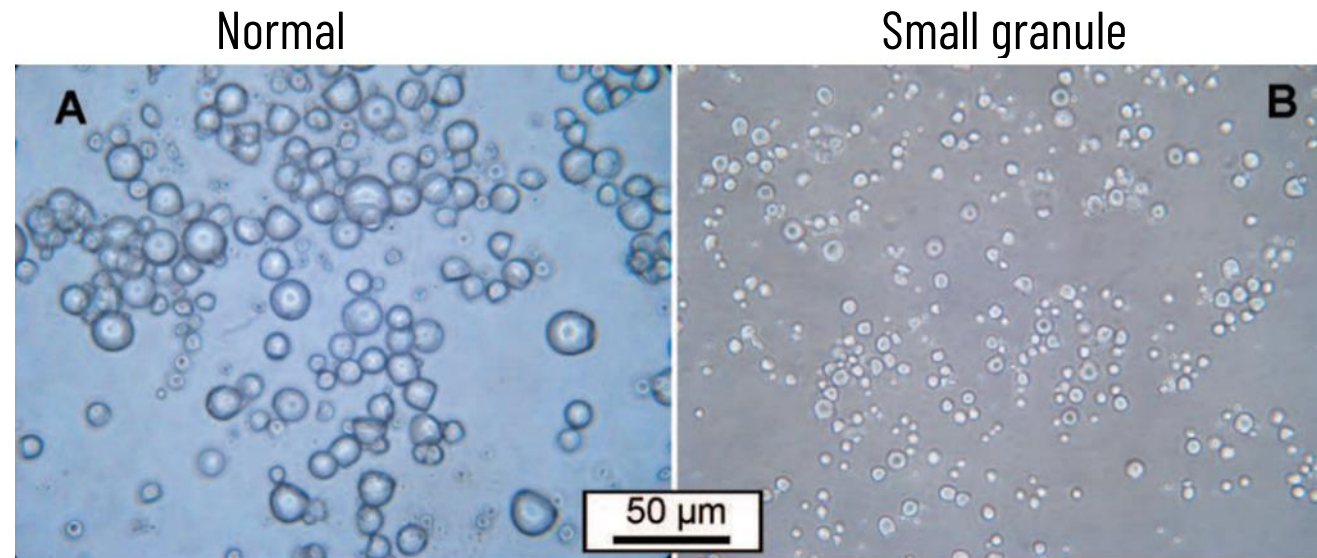
Waxy

Normal

Small granule

Induction of the small granule cassava starch

Mutation breeding: Induced mutation through irradiation with γ rays



40X magnification

	Normal	Small granule
Amylose content (%)	16.8	26
Granule size (μm)	15.1	5.8

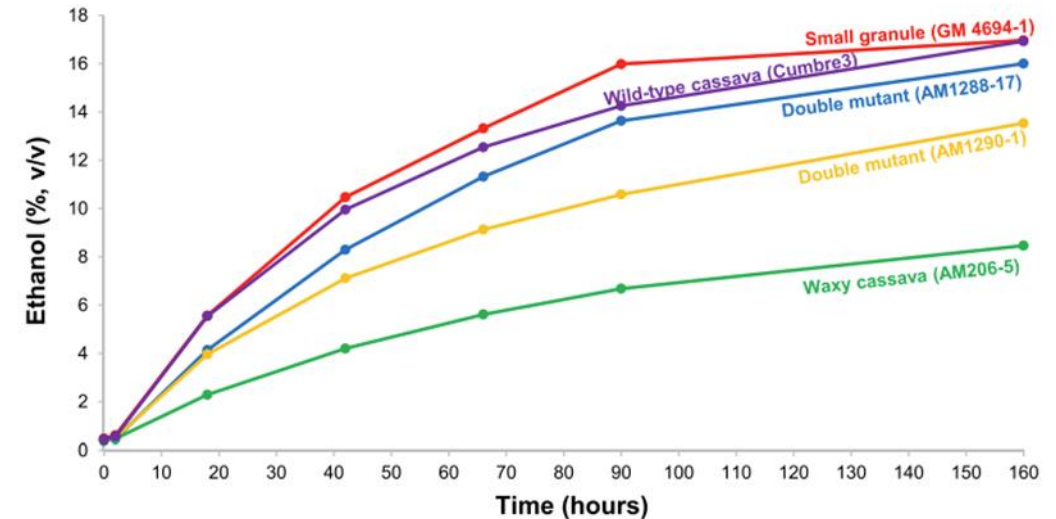
Impact and potential applications

High potential value in several applications due to:

- Reduced granule size = **Increased processing efficiency** due to increased surface-to-volume ratio; more sensitivity to enzymatic hydrolysis
- Higher amylose content
- Low paste clarity

Applications in:

- Bioethanol production
- Pharma and health benefits
- Niche food and industrial sectors due to superior texture and stability



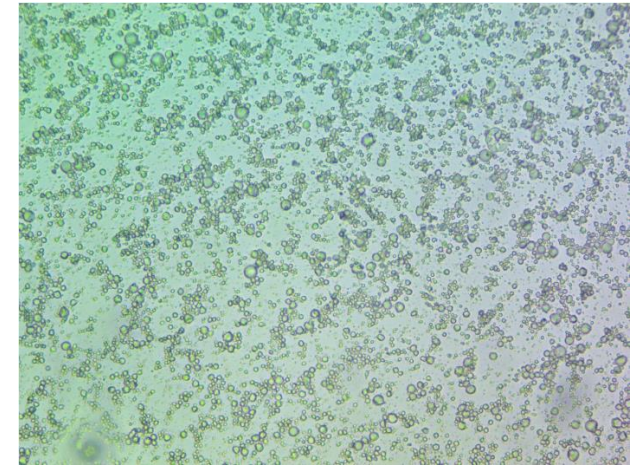
Genetic basis of the small granule trait

The genetic basis is **unknown**

Efficient method for introgression of this trait into commercially competitive varieties would be ideal.

Our aim:

- Determine the **genetic control** of the small granule starch trait in cassava
- **Develop a robust molecular marker** to accelerate breeding of new varieties.



Starch granule size and linked genes

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By JOHN INNES CENTRE NOVEMBER 1, 2023



Wheat starch granules observed under the Scanning Electron Microscope. Large A-type and small B-type granules are visible. Credit: Brendan Fahy/Nitin Uttam Kamble

Gene: Plastidial α -glucan phosphorylase (PHS1/PH01)

JOURNAL ARTICLE

Initiation of B-type starch granules in wheat endosperm requires the plastidial α -glucan phosphorylase PHS1

Nitin Uttam Kamble ✉, Farrukh Makhmadjonov, Brendan Fahy, Carlo Martins, Gerhard Saalbach, David Seung ✉ Author Notes

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Abstract

The plastidial α -glucan phosphorylase (PHS1) can elongate and degrade maltooligosaccharides (MOSs), but its exact physiological role in plants is poorly understood. Here, we discover a specialized role of PHS1 in establishing the unique bimodal characteristic of starch granules in wheat (*Triticum* spp.) endosperm. Wheat endosperm contains large A-type granules that initiate at

Gene function appears conserved in other species

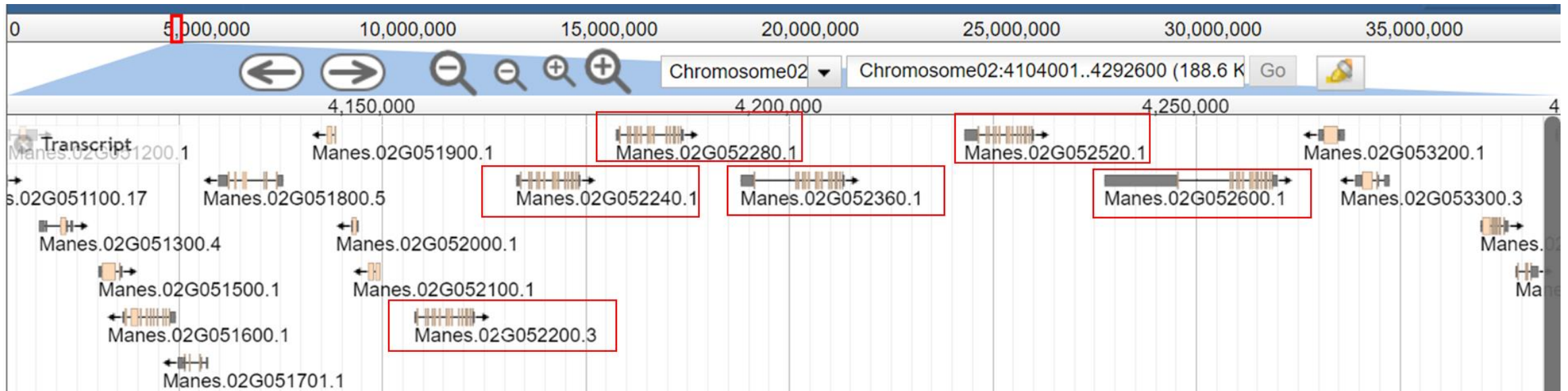
Mutations in the **plastidial α -glucan phosphorylase** (PHS1/PH01) **can alter starch granule size and structure**

- Algae *C. reinhardtii*: mutant lacking *PH01* = reduced starch content, higher amylose, and abnormal morphology of starch granules
- Rice: Loss of *PH01* = accumulation of smaller starch granules and altered amylopectin structure
- Wheat: *PHS1* required for the initiation of B-type (small, spherical) granules, with loss of the gene only affecting the formation of B-type granules and not A-type (large discoid) granules
- Potato (*Solanum tuberosum* L): *PH01a* gene is tandemly duplicated and knock out of the genes led to smaller, more spherical granules, although the amylose content was decreased



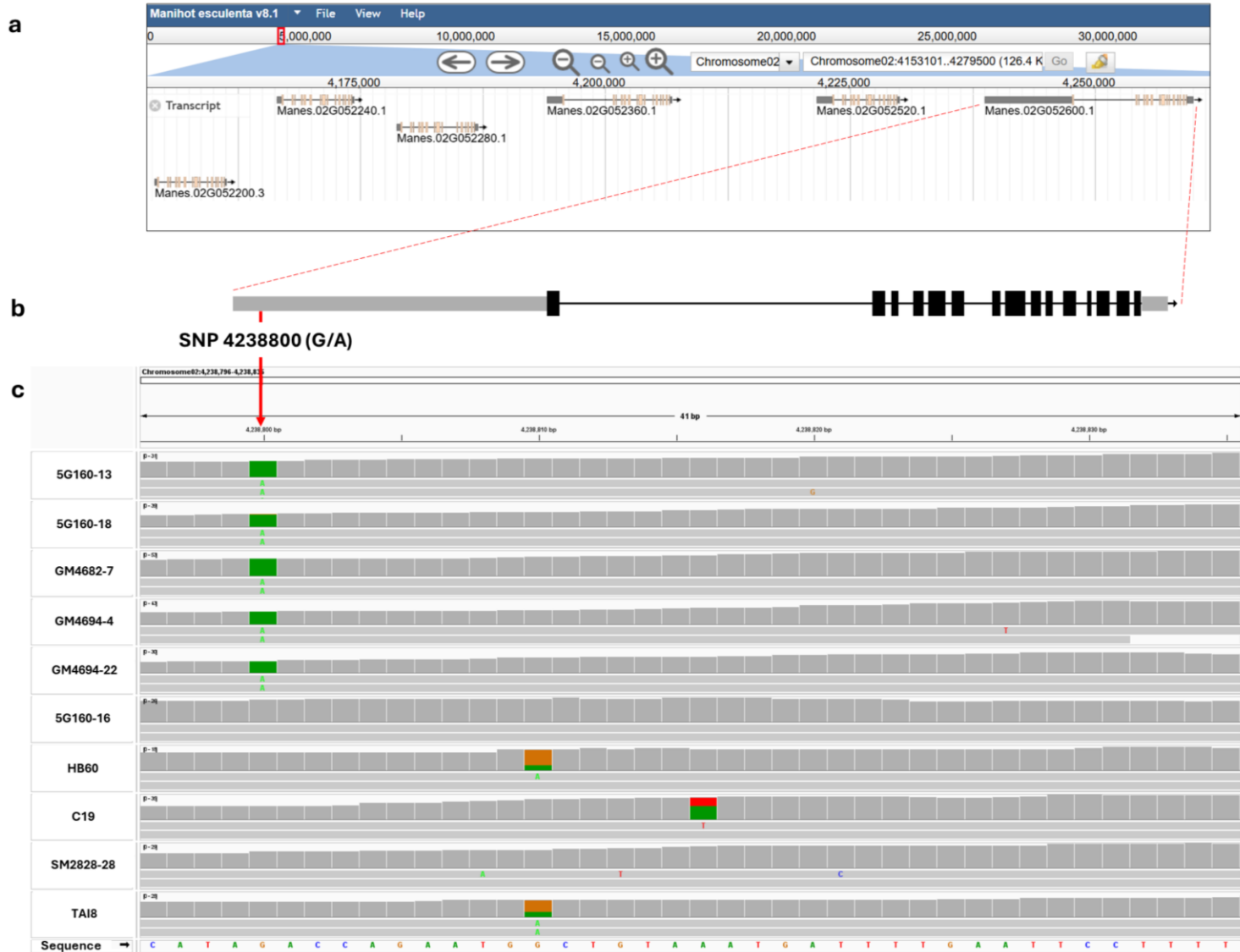
Cassava PHS1/PH01: Tandem duplication (cluster of 6)

- **Nine** homologous genes identified in the *Manihot esculenta* genome
- **Six** of the genes in tandem duplication on **chromosome 2**



- Three of the other genes in other locations (Chr 2, 5 and 17)

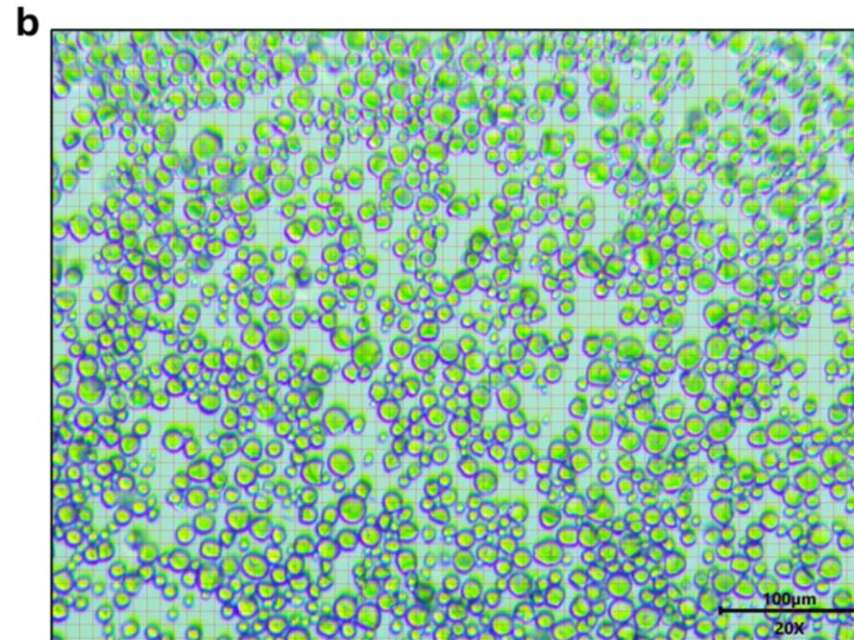
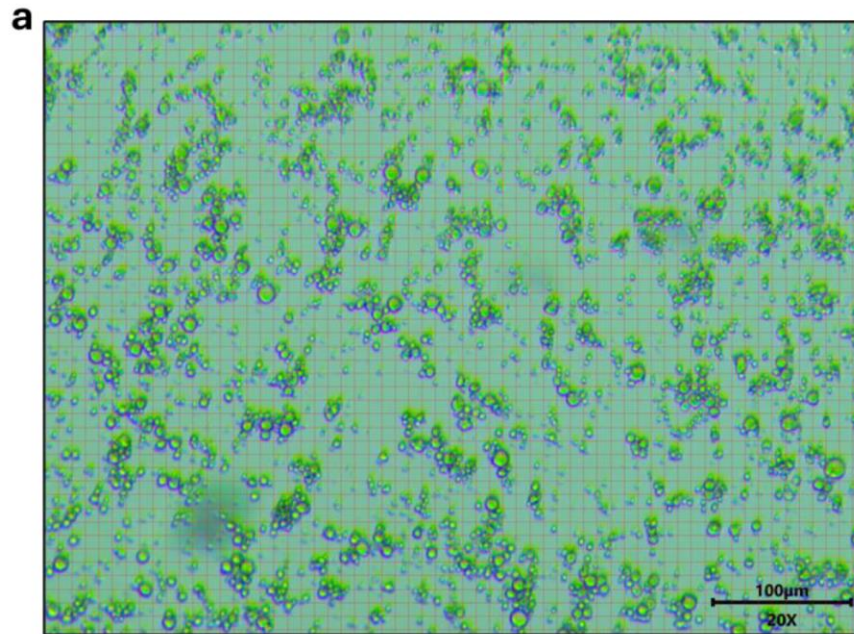
In silico analysis focusing on gene cluster



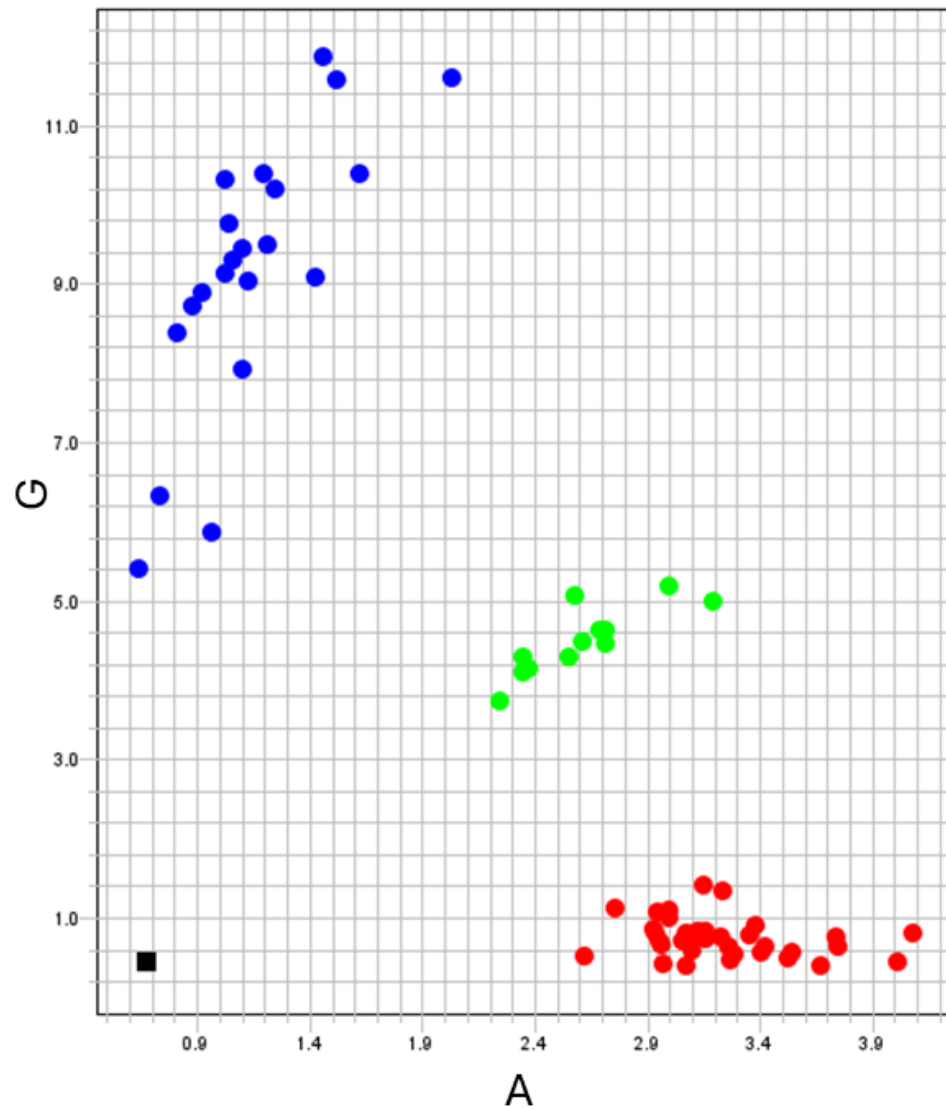
- Whole genome sequence (WGS) available for 392 accessions (breeding progenitors + landraces)
- Many SNPs and indels in the region, but one SNP perfectly distinguished **small (11)** vs **normal (381)** granule accessions
- SNP of interest on gene promoter region
- Designed **KASP marker** for SNP
- Tested on progenitors to compare with results from WGS

Multiparent population development for validation

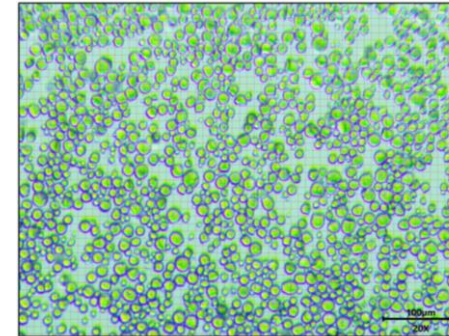
- Developed from contrasting parents (Small vs Normal granule)
- **962** accessions phenotyped using microscopy



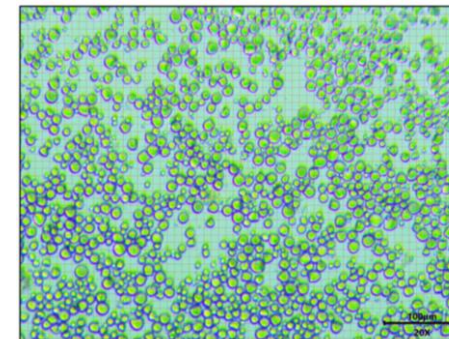
KASP marker testing and validation



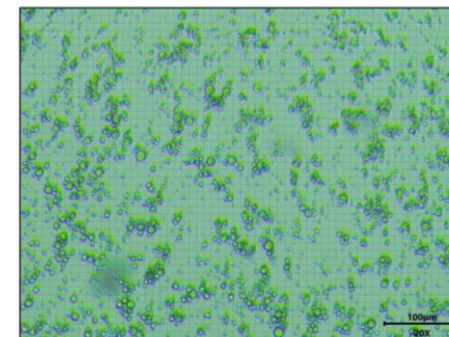
GG: Normal granule



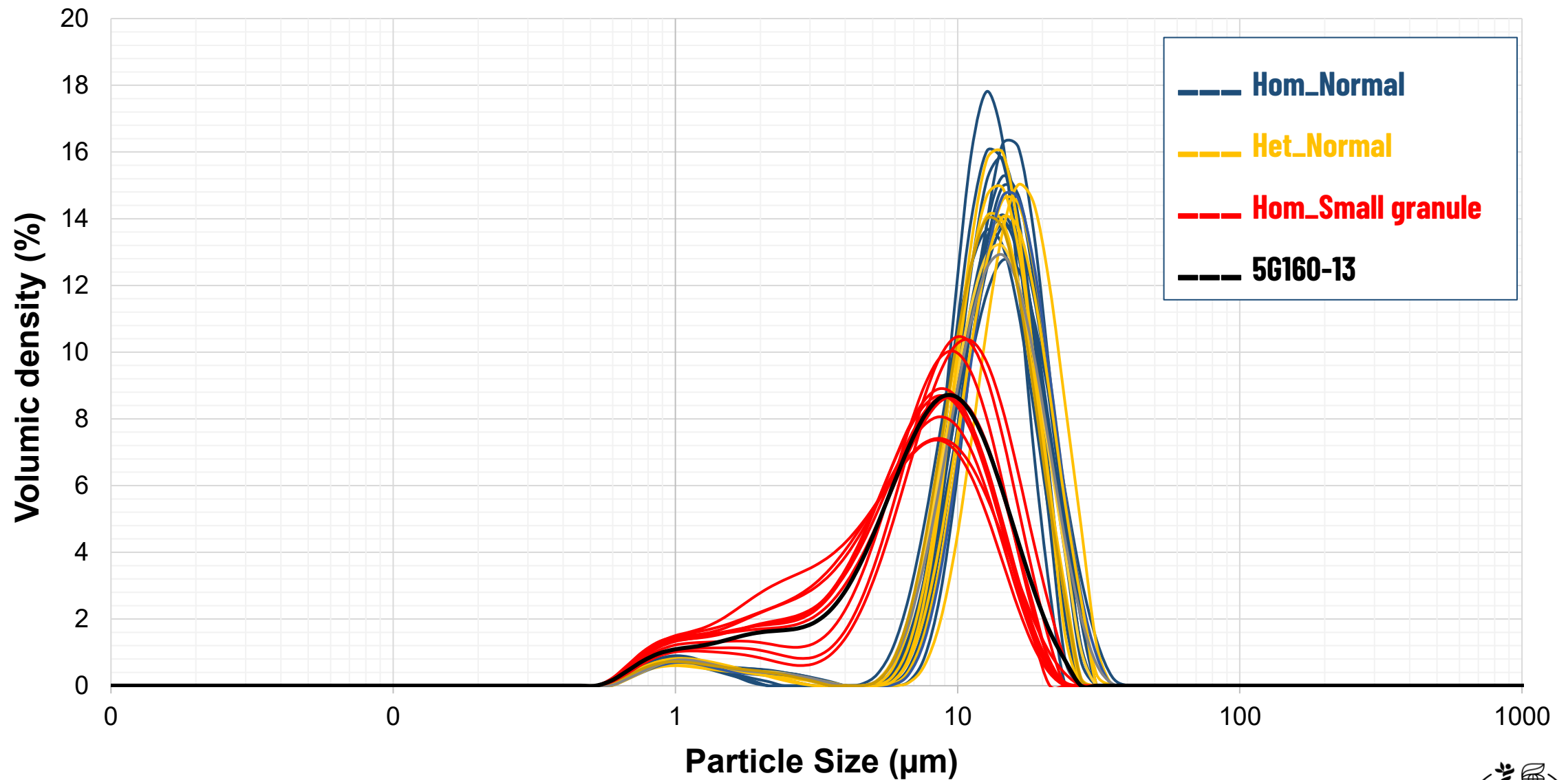
AG: Normal granule



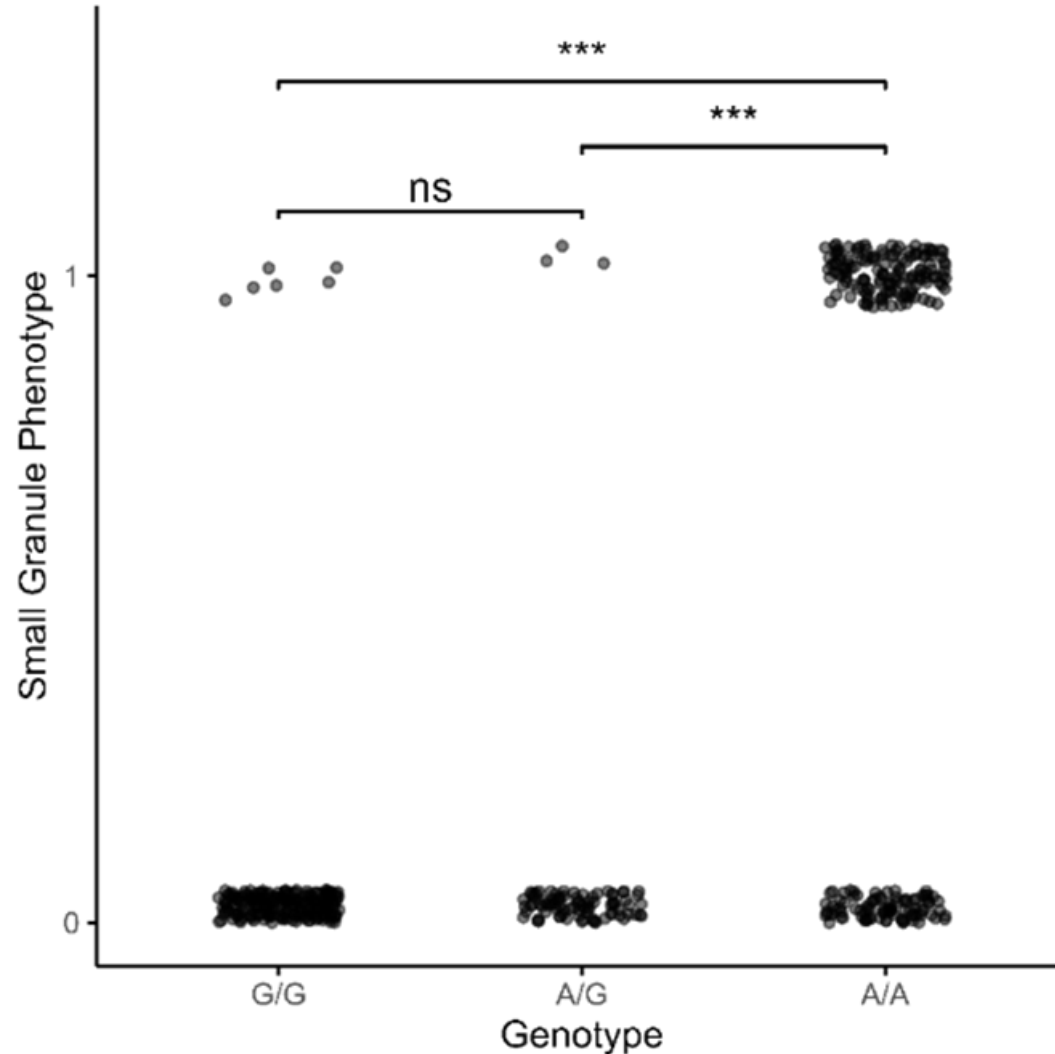
AA: Small granule



Particle analysis by laser diffraction



KASP marker - trait association on segregating population

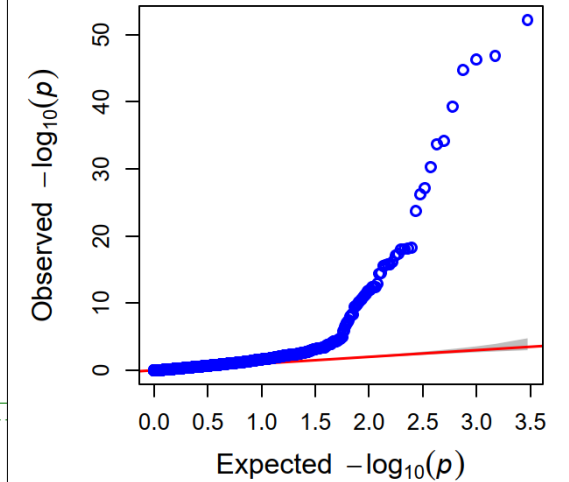
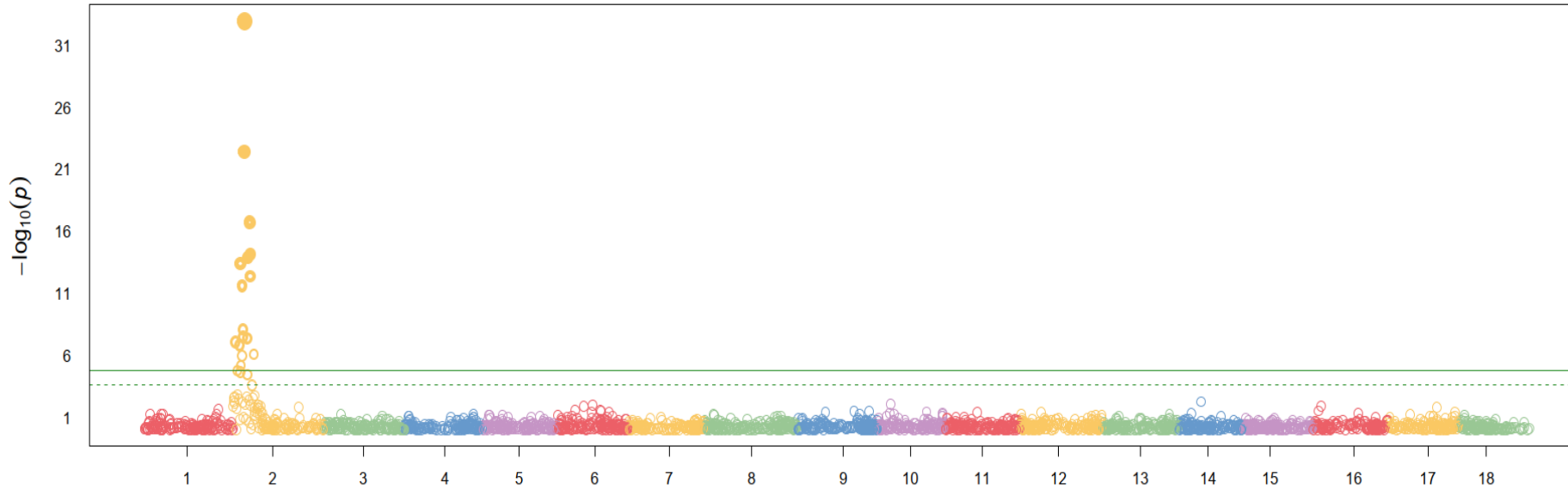


High association with trait:

Accuracy = 81.9% with some
False positives

$R^2 = 48\%$

GWAS as a validation on segregating population (DArTag 3k panel)



- The same region on **chromosome 2**, was identified as significant ($p = 1.71E-20$; PVE = 4.65), validating our hypothesis and initial analyses, with the **most significant marker 49 Kb from our KASP marker**

Conclusions

- A **key genomic locus** controlling the small granule cassava starch trait was identified.
- The **first molecular marker** for this trait was developed.
- This novel marker provides a **cost-effective, high-throughput tool for MAS**, which will significantly accelerate the development of specialty starch varieties.
- Future studies can explore whether the small granule phenotype in cassava requires **all the α -glucan phosphorylase** homologs or if **a single homolog** is primarily responsible for the effect.

- Gene homology studies may enable a better understanding of inheritance patterns for other important traits in cassava.
- Study also validates the informativeness and high resolution of the DArTag panel for trait discovery.



THANKS!



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