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Adding value and minimizing losses: Small granule cassava starch for ethanol production

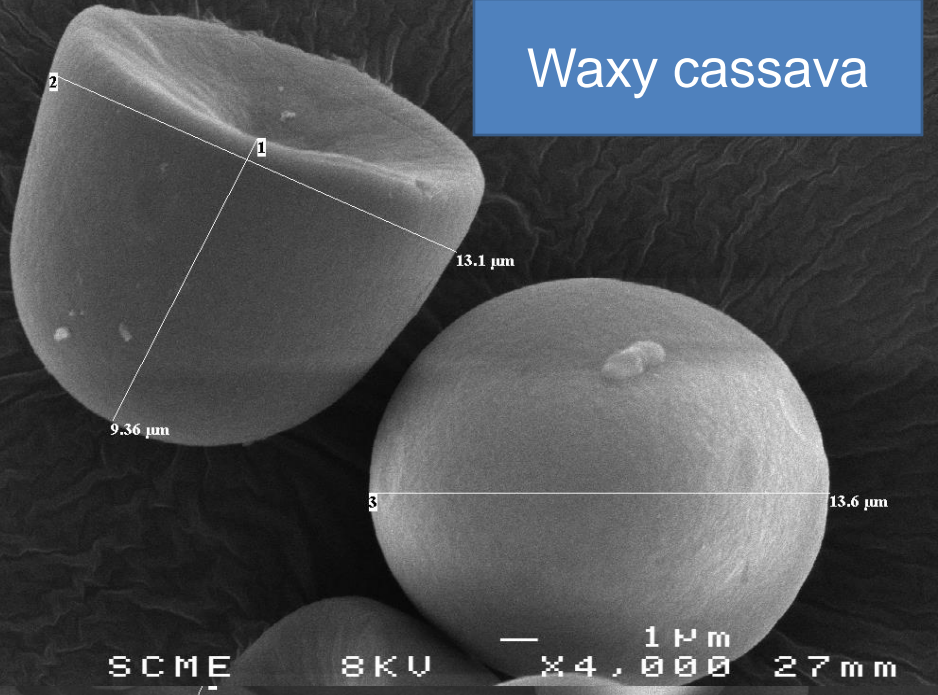
Jhon Larry MORENO, Nelson MORANTE, Xiaofei ZHANG, Thierry TRAN, Hernan CEBALLOS, CHU-KY Son

On behalf of the Postharvest Quality Lab, Cassava Program

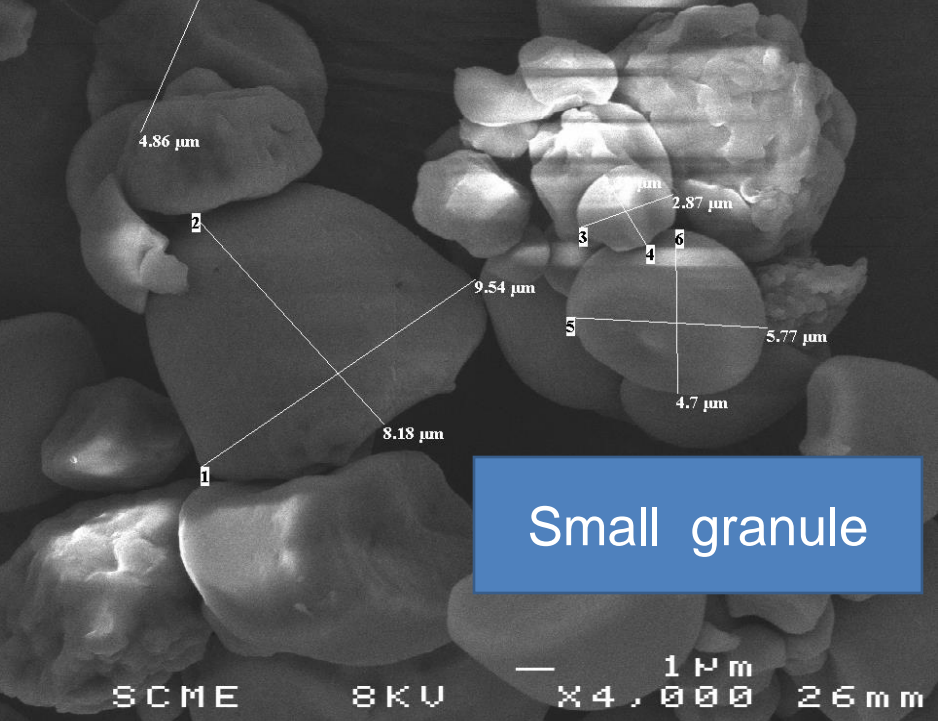
CIAT Palmira, 14/02/2023



Waxy cassava



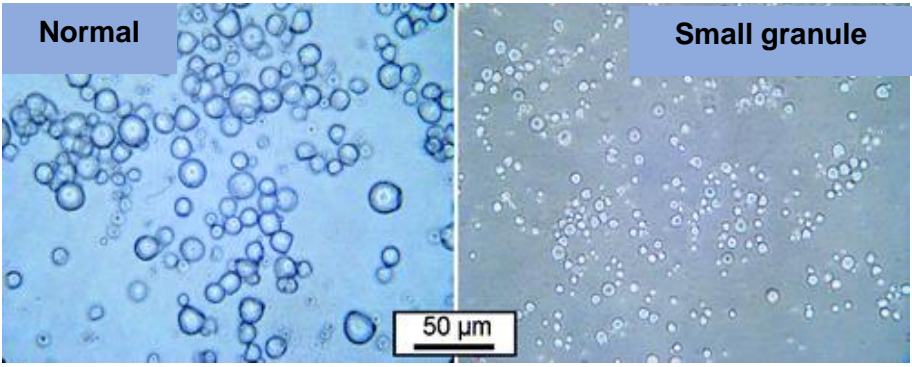
Small granule



Cassava special starches

Unique genotypes developed at CIAT

| | Amylose content (%) | Granule size (μm) | Crystallinity (%) |
|----------------|---------------------|-------------------|-------------------|
| Waxy cassava | 0.0 | 16.0 | 40.0 |
| Small Granule | 26 | 5.8 | - |
| Double mutant | 0.0 | 6.5 | - |
| Normal Cassava | 16.8 | 15.1 | 35.0 |



2003A - Shipment of 6 families of cassava sexual seed

| Cross | Mother | Father | Source | Purpose | No. Seeds |
|---------|-------------|-------------|----------|---------|-----------|
| CM 9331 | SM 1210- 10 | MNGA 1 | GY199590 | Z02 | 300 |
| SM 3015 | MCOL 1505 | | GY199960 | Z01 | 300 |
| SM 3045 | HMC 1 | | GY199962 | Z04 | 300 |
| GM 155 | MTAI 1 | SM 2102- 34 | GY199889 | ASG | 306 |
| C - 4 | | | GY200122 | AFG | 454 |
| C - 127 | | | GY200122 | AFG | 50 |



To be irradiated with Gamma Rays and Neutrons

2003B - Sowing trial F1 of irradiated seed in ICA - Palmira



Self-pollination



- 76 Families of plants from seed irradiated with Gamma Rays - 3616 Seeds
- 26 Families of plants from seed irradiated with Neutrons - 974 Seeds

2005 - Sowing trial F2 (S1) of irradiated seed in CIAT

| Irradiation type | Families | Seeds | Plants in the field | Select plants for Laboratory |
|------------------|----------|-------|---------------------|------------------------------|
| Gamma | 76 | 3616 | 2189 | 1027 |
| Neutrons | 26 | 974 | 553 | 318 |



| Variety | Characteristic |
|-------------|---------------------|
| 5G190- 11 | Low starch |
| 5N14- 5 | Low starch |
| 2G28- 9 | Hollow granule |
| 5G160- 13 | Small granule |
| 5G160- 16 | Small granule |
| 5G160- 18 | Small granule |
| SM3015G43-1 | Small granule ????? |
| 5G108- 3 | PPD resistance |
| 5G108- 4 | PPD resistance |
| 4G15- 1 | PPD resistance |
| 3G77- 4 | PPD resistance |
| 3G77- 5 | PPD resistance |

2007 - Generation of Double Mutant varieties

| Small granule | Waxy |
|---------------|-------------|
| 5G160- 13 | GM 4034- 1 |
| 5G160- 18 | SM 3315- 5 |
| | SM 3316- 24 |
| | SM 3316- 32 |
| | CL 41- 1 |
| | CL 41- 6 |
| | CL 42- 3 |



- 6 Families
- 260 Seeds



2010 - Planting in a crossing nursery

2011/2012 - Generation of Double Mutant varieties
88 Families – 2388 Seeds



| Cross | Families | Seeds | Plants in the field | Select plants for Laboratory |
|--------------|-----------------|--------------|----------------------------|-------------------------------------|
| S1 | 49 | 1932 | 1109 | 263 |
| F2 | 42 | 456 | 279 | 68 |



| Waxy | Small Granule | Double Mutant |
|-------------|----------------------|----------------------|
| 72 | 96 | 6 |

2020 - Planting for crosses with high Dry matter varieties

| Small Granule | Dry Matter |
|---------------|------------|
| 5G 160- 13 | SM2828-28 |
| 5G 160- 16 | TAI8 |
| 5G 160- 18 | CM4919-1 |
| GM 4682- 7 | SM3559-11 |
| GM 4694- 4 | HMC1 |
| GM 4694- 22 | |
| GM 4694- 39 | |

Planting F1-2021

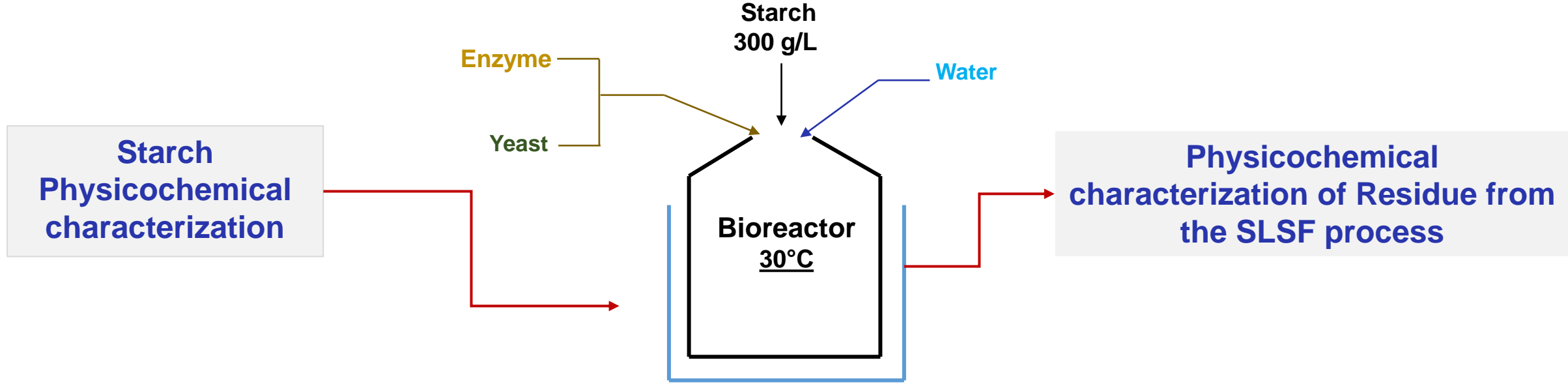


| Family | Mother | Father | Source | Group | No. Seeds | Trasplant. |
|----------|-----------|-----------|----------|-------|------------|------------|
| GM9516A | 5G160-16 | SM2828-28 | GY202003 | SG_MD | 33 | 30 |
| GM13840A | GM4694-22 | SM2828-28 | GY202003 | SG_MD | 33 | 31 |
| GM13843A | GM4694-22 | TAI8 | GY202003 | SG_MD | 33 | 31 |
| GM13901A | 5G160-16 | CM4919-1 | GY202003 | SG_MD | 33 | 30 |
| GM13904A | 5G160-16 | TAI8 | GY202003 | SG_MD | 33 | 31 |
| GM13941A | GM4682-7 | CM4919-1 | GY202003 | SG_MD | 17 | 17 |
| GM13942A | GM4682-7 | SM2828-28 | GY202003 | SG_MD | 33 | 30 |
| GM13944A | GM4694-4 | CM4919-1 | GY202003 | SG_MD | 33 | 33 |
| GM13945A | GM4694-4 | SM2828-28 | GY202003 | SG_MD | 33 | 31 |
| GM13946A | GM4694-4 | SM3559-11 | GY202003 | SG_MD | 9 | 9 |
| GM13947A | GM4694-4 | HMC1 | GY202003 | SG_MD | 4 | 3 |
| GM13948A | GM4694-22 | CM4919-1 | GY202003 | SG_MD | 33 | 32 |
| GM13949A | GM4694-22 | SM3559-11 | GY202003 | SG_MD | 33 | 33 |
| GM13951A | GM4694-39 | CM4919-1 | GY202003 | SG_MD | 28 | 24 |
| GM13953A | GM4694-39 | SM2828-28 | GY202003 | SG_MD | 33 | 27 |
| GM13954A | GM4694-39 | SM3559-11 | GY202003 | SG_MD | 25 | 22 |
| GM13958A | GM4694-39 | TAI8 | GY202003 | SG_MD | 33 | 28 |
| | | | | | 479 | 442 |

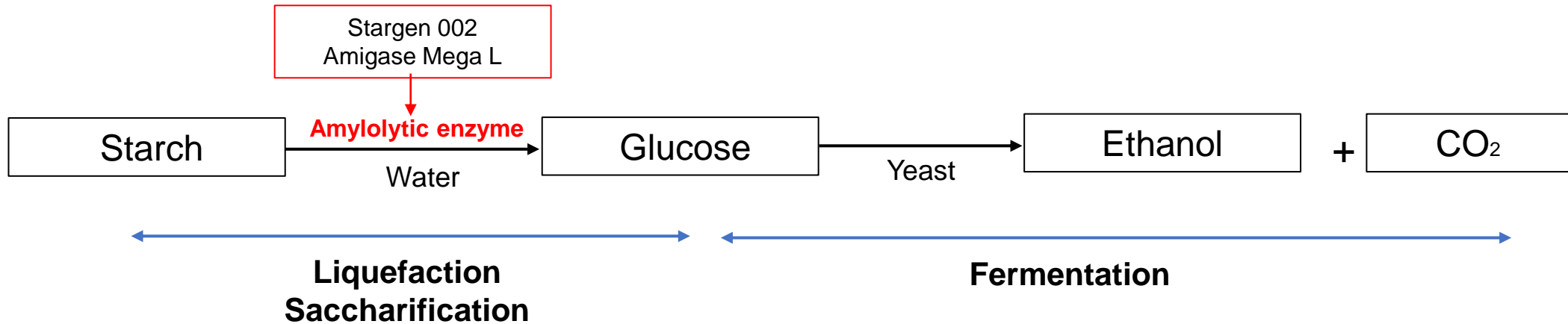
2021 - Planting for crosses with high Dry Matter varieties (2)

| GY202112 | | | | | | | | | |
|----------|-------|-------------|-------------|---------|---------|--------------|--------------|---------------|-----------|
| Entrada | Prior | Madre | Padre | Prop. 1 | Prop. 2 | Flores Total | Semilla Esp. | Prop. General | Prop. M+P |
| | | GM 4682- 7 | GM 4682- 7 | S1 | SG | 4 | 2 | SSG | S1_SG |
| | | AM 1305- 21 | SM 2828- 28 | SG | DM | 66 | 40 | GDM | SG_DM |
| | | AM 1305- 21 | SM 3559- 11 | SG | DM | 15 | 9 | GDM | SG_DM |
| | | AM 1305- 42 | SM 1219- 9 | SG | DM | 30 | 18 | GDM | SG_DM |
| | | AM 1305- 42 | SM 2828- 28 | SG | DM | 31 | 19 | GDM | SG_DM |
| | | AM 1305- 42 | SM 3559- 11 | SG | DM | 4 | 2 | GDM | SG_DM |
| | | GM 4679- 2 | SM 1219- 9 | SG | DM | 39 | 23 | GDM | SG_DM |
| | | GM 4679- 2 | SM 2828- 28 | SG | DM | 46 | 28 | GDM | SG_DM |
| | | GM 4679- 2 | SM 3134- 73 | SG | DM | 11 | 7 | GDM | SG_DM |
| | | GM 4679- 2 | SM 3559- 11 | SG | DM | 4 | 2 | GDM | SG_DM |
| | | GM 4682- 7 | SM 1219- 9 | SG | DM | 23 | 14 | GDM | SG_DM |
| | | GM 4682- 7 | SM 2828- 28 | SG | DM | 15 | 9 | GDM | SG_DM |
| | | GM 4694- 11 | SM 3559- 11 | SG | DM | 31 | 19 | GDM | SG_DM |
| | | GM 4694- 22 | GM 579- 13 | SG | DM | 233 | 140 | GDM | SG_DM |
| | | GM 4694- 22 | SM 2773- 32 | SG | DM | 242 | 145 | GDM | SG_DM |
| | | GM 4694- 22 | SM 2828- 28 | SG | DM | 59 | 35 | GDM | SG_DM |
| | | GM 4694- 22 | SM 3134- 73 | SG | DM | 31 | 19 | GDM | SG_DM |
| | | GM 4694- 22 | SM 3559- 11 | SG | DM | 84 | 50 | GDM | SG_DM |
| | | AM 1305- 21 | SM 3386- 49 | SG | DM_CQ | 44 | 26 | GDM | SG_DM_CQ |
| | | GM 4694- 11 | SM 3386- 49 | SG | DM_CQ | 21 | 13 | GDM | SG_DM_CQ |
| | | | | | | 1033 | 620 | | |

Very High Gravity (VHG): 30% dry matter



**Simultaneous Liquefaction,
Saccharification and Fermentation (SLSF)**



Chemical characterization of native starches

| Sample | Characteristic | Amylose (% db) | Starch content (% db) | | | |
|------------|----------------|---------------------------|---------------------------|----------------------------------|-----------------------------|-----------------------------------|
| | | | TS | RDS | SDS | RS |
| GM 4694-1 | Small granule | 21.95 ± 0.05 ^e | 94.06 ± 1.88 ^a | 48.01 ± 0.64 ^d | 43.14 ± 2.05 ^{ef} | 2.91 ± 1.41 ^{ab} |
| AM 1288-17 | Double mutant | < LQ | 97.70 ± 1.48 ^a | 46.04 ± 0.14 ^d | 47.96 ± 1.01 ^{fg} | 3.70 ± 0.87 ^{ab} |
| AM 1290-1 | Double mutant | < LQ | 95.39 ± 1.29 ^a | 54.36 ± 2.61 ^e | 36.14 ± 2.96 ^{cd} | 4.89 ± 0.35 ^{ab} |
| AM 206-5 | Waxy | 1.59 ± 0.10 ^a | 96.93 ± 0.57 ^a | 16.91 ± 0.85 ^b | 40.26 ± 2.25 ^{cde} | 39.77 ± 3.10 ^c |
| CUMBRE-3 | Normal | 16.05 ± 0.09 ^c | 96.70 ± 3.37 ^a | 16.95 ± 0.93 ^b | 35.92 ± 1.08 ^{cd} | 43.83 ± 2.01 ^{cd} |
| MTAI-8 | Normal | 20.01 ± 0.13 ^d | 91.02 ± 1.49 ^a | 11.16 ± 0.31 ^a | 33.90 ± 0.41 ^{bc} | 45.96 ± 0.10 ^d |
| RICE | Waxy | 4.33 ± 0.07 ^b | 95.90 ± 0.76 ^a | 70.47 ± 1.42 ^g | 24.78 ± 1.76 ^a | 0.65 ± 0.34 ^a |
| MAIZE | Normal | 23.60 ± 0.42 ^f | 94.96 ± 0.59 ^a | 40.76 ± 0.29 ^c | 53.49 ± 0.18 ^g | 0.71 ± 0.11 ^a |

<LQ: less than the limit of quantification; TS: total starch; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch. Data are shown as the mean ± standard deviation from two determinations. Value followed by different letter in the same column are significantly different (P<0.05).

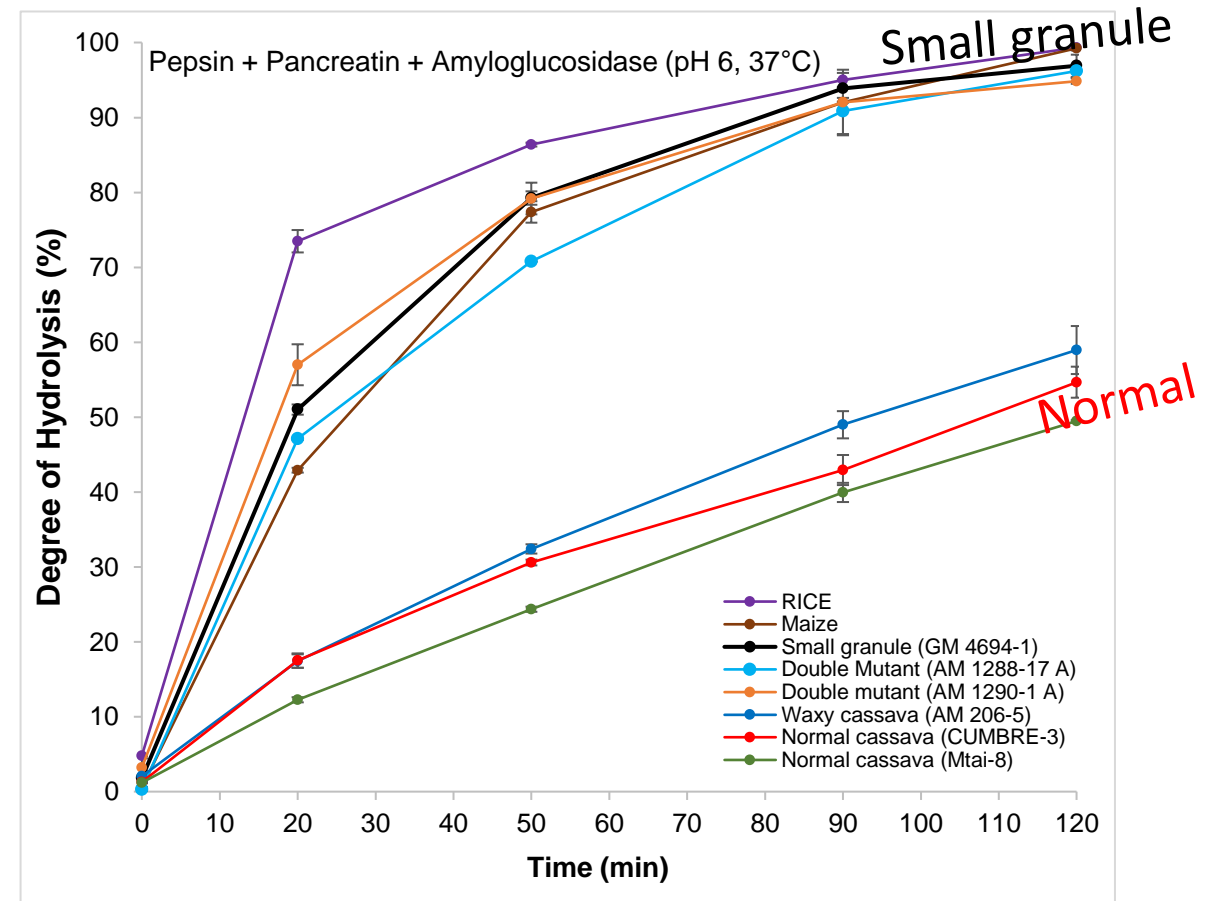
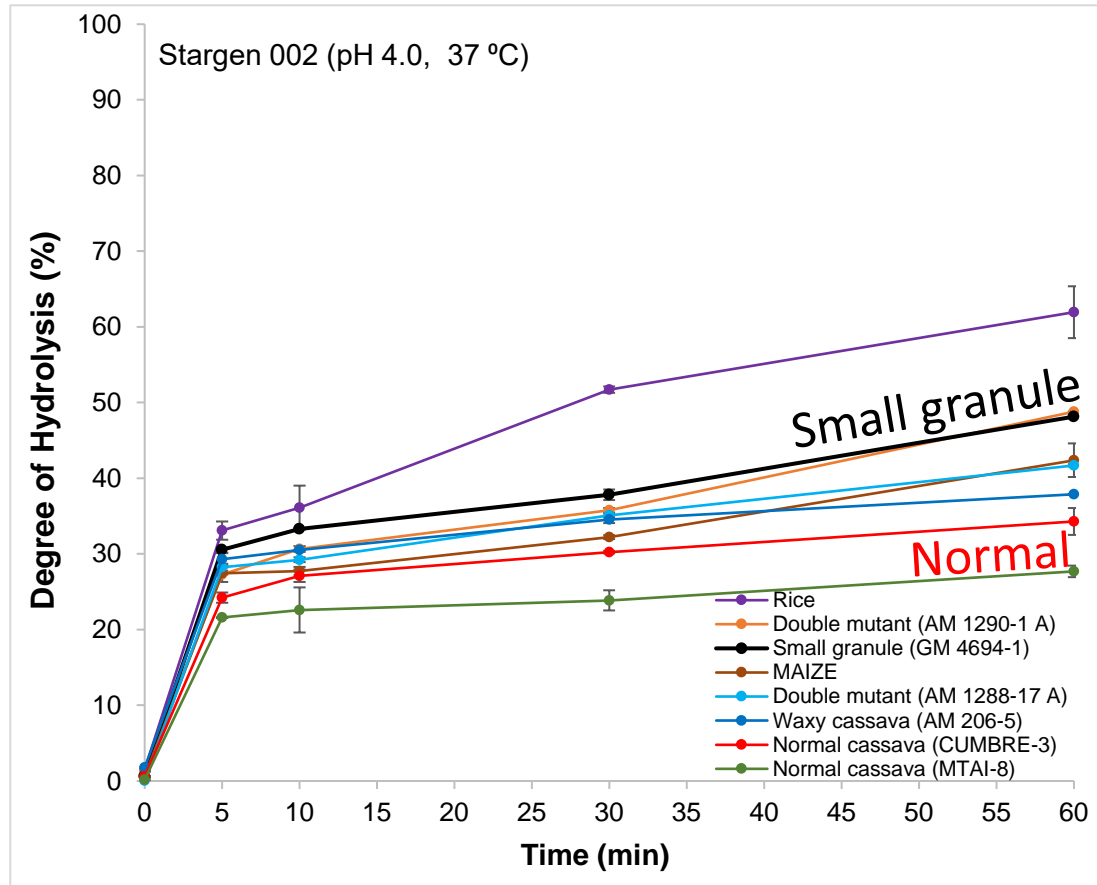


Small granule

RDS: Rapidly digestible starch

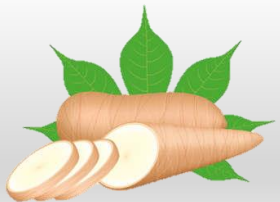
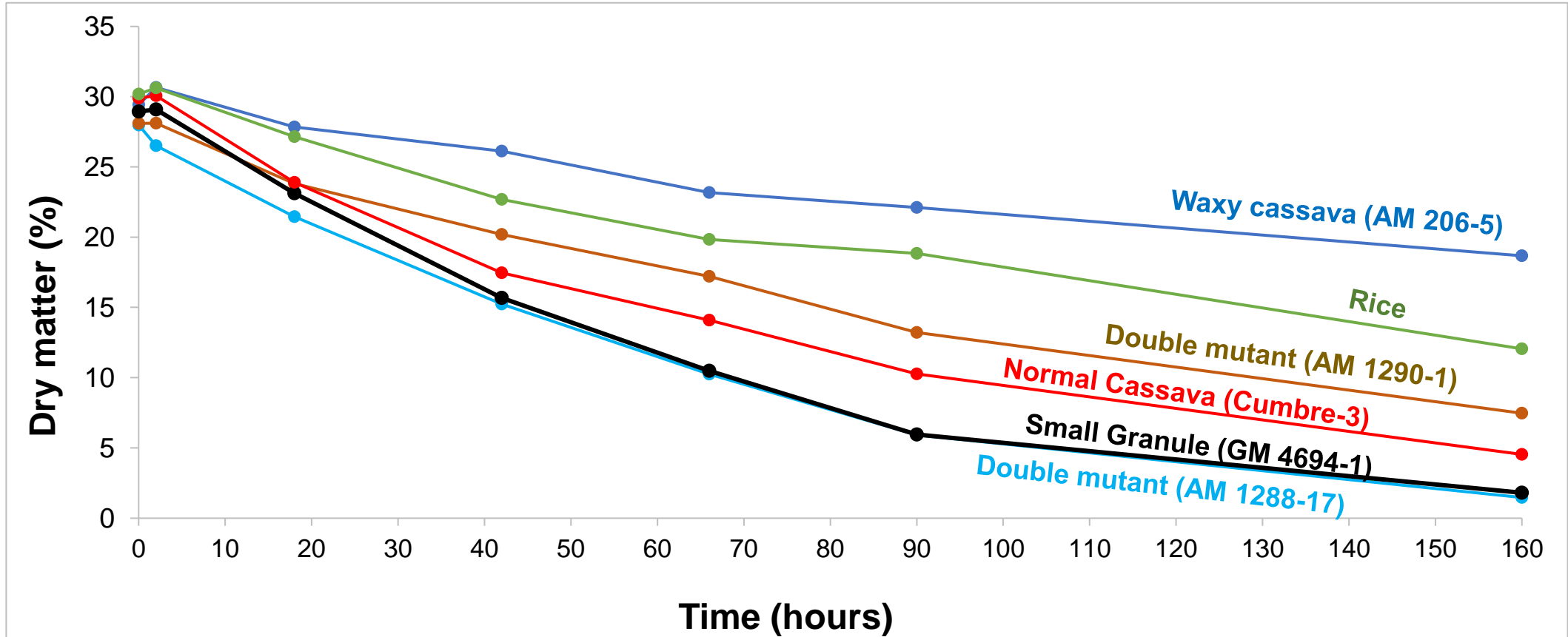
RS: Resistant starch

Hydrolysis of native starches



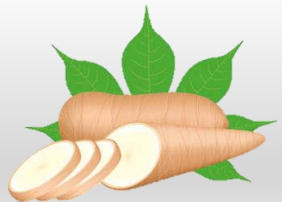
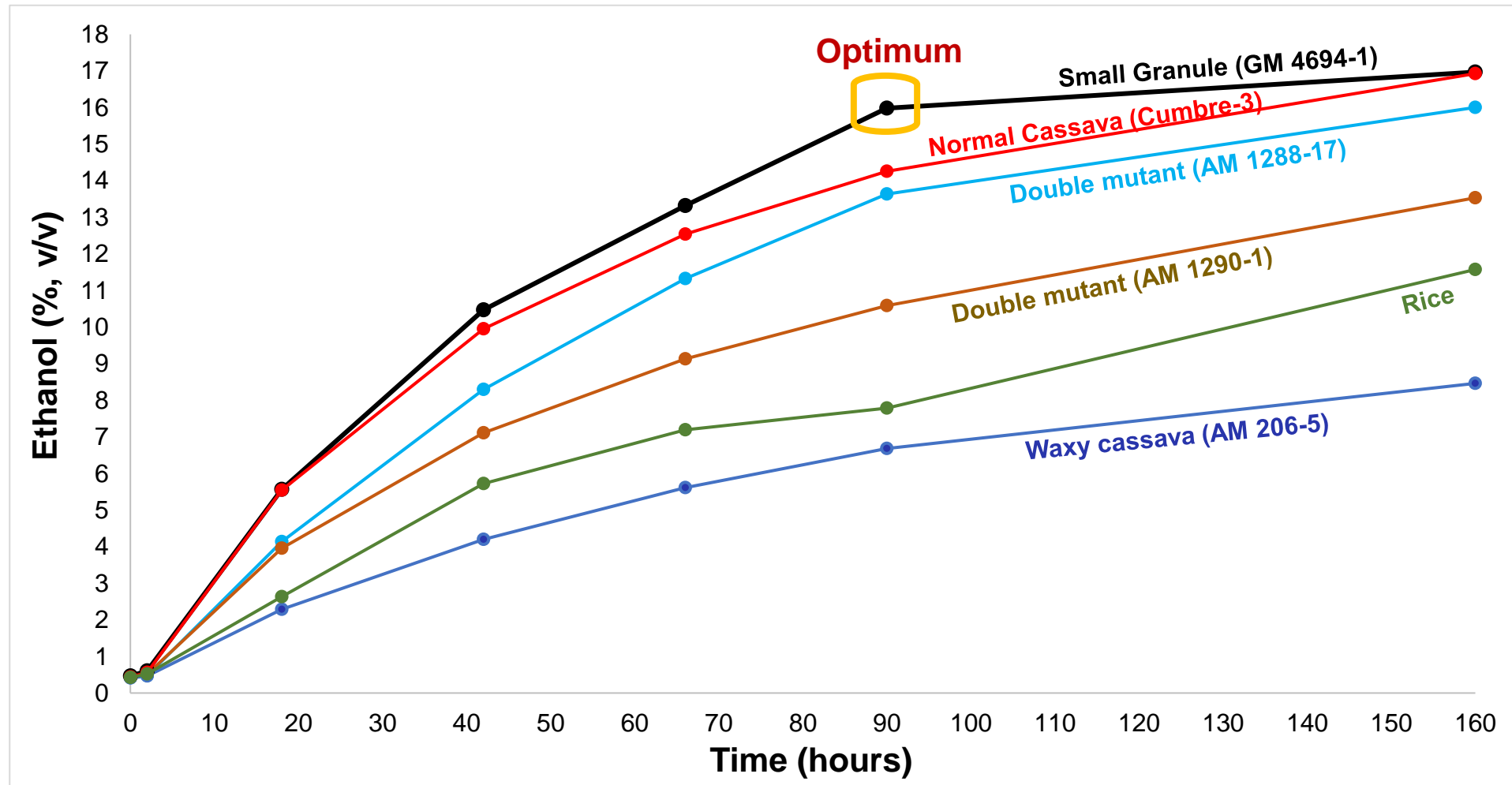
Small granule

Dry matter content during SLSF process



Small granule

Ethanol production during SLSF process



Small granule

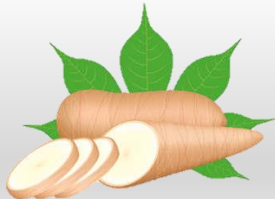
Optimum reached 3 days faster with small granules cassava, meaning higher production capacity

Highest rate of hydrolysis with Small Granule Cassava (GM 4694-1) and Double Mutant (AM 1290-1)

Combination of:

- 1) **Small granule variety**
- 2) **Very high gravity fermentation process**

Enables high ethanol yield at lower costs and shorter time – nearly 3 days faster for same ethanol production as the control.



**Small
granule**

Cost savings of hydrolyzing small granule cassava at low temperature compared to normal starch at 90°C

Small granules cassava starch are more sensitive to amylase hydrolysis, and can be nearly fully hydrolyzed (95-99%) by incubation at 40°C, i.e. without gelatinization. No gelatinization means lower viscosity, allowing for higher concentration of starch (up to 35%), thus reducing the volume of water needed.

In contrast, to obtain the same hydrolysis with normal cassava starch, gelatinization is necessary with incubation at 90°C. Gelatinization means higher viscosity, limiting starch concentration to 10-15%.

| | <i>Unit</i> | Conventional 90°C + normal cassava | Low temperature 40°C + small granule cassava |
|--|-------------|---|---|
| Temperature of hydrolysis incubation | °C | 90 | 40 |
| Starch concentration | % | 12 | 35 |
| Weight of water to incubate 1 kg starch | kg | 7.3 | 1.9 |
| Energy necessary to heat water from 30°C to incubation temperature | kJ | 1843.6 | 77.8 |
| Diesel to incubate 1 kg of starch (with heat transfer & losses factor 30%) | L | 0.06193 | 0.00261 |
| Diesel cost to incubate 1 kg of starch * | USD | 0.093 | 0.004 |
| Diesel cost to incubate 1 t of starch * | USD | 92.89 | 3.92 |

* For this evaluation, diesel price was set at 1.5 USD/L



granule

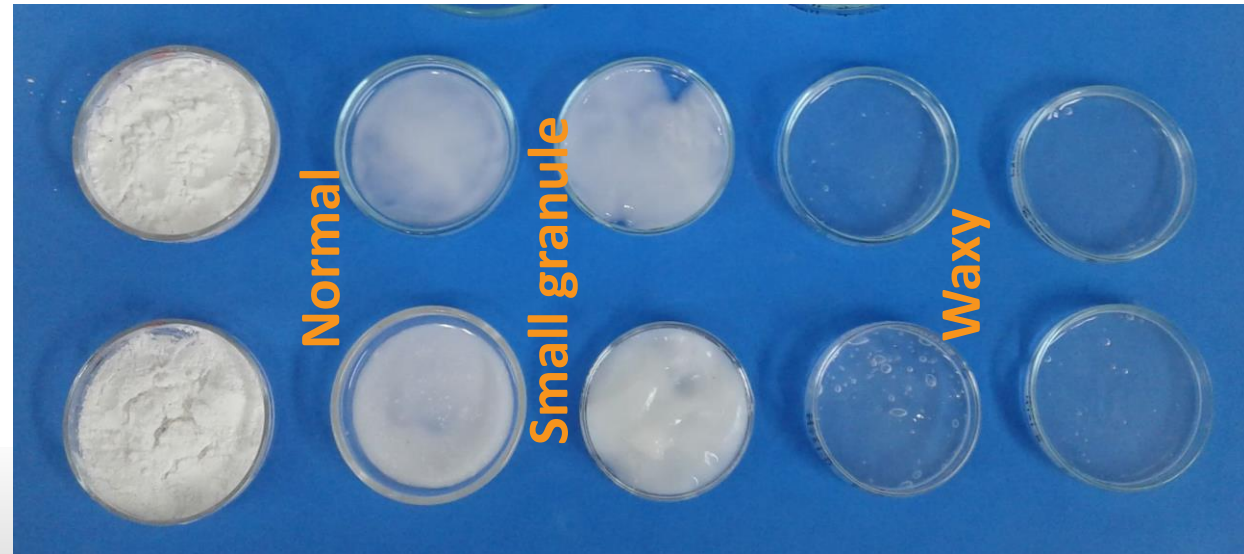
Other potential uses of small granules

Production of glucose syrup

Other fermentations than ethanol: Lactic acid, glutamate, etc.

Direct applications as food ingredient / chemical:

- Low viscosity during gelatinization
- Opaque gels
- High solubility and high swelling power
- Bioplastics



Small granule

Gracias! Thank you!

CIAT Cassava Program

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