

Fermentation Progression and Quality Attributes of Trinitario and Refractario Cacao (*Theobroma cacao* L.) Hybrid Populations at the International Cocoa Genebank Trinidad (ICGT) – Opportunities for Genetic Branding

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Introduction

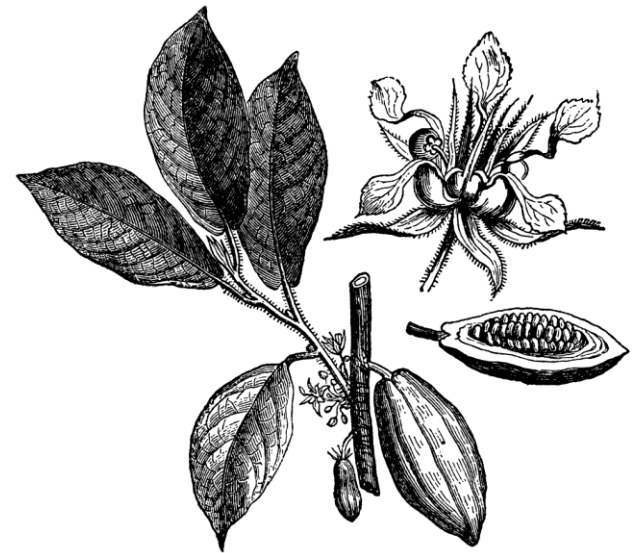
Trinitario and Refractario

- Hybrids originating from the admixing of several of the genetic clusters recognised within cacao.
- Highly regarded in the fine or flavour market segment of the cocoa industry.
- Fermentation behaviour and distinctive flavour/health benefits are not fully understood.
- **Aim: to determine the market potential of these two hybrid populations by assessing attributes of interest.**

Introduction

Trinitario and Refractario

- Methodology
- Results
 - Temperature
 - pH (testa and cotyledon)
 - Bean measurements
 - Polyphenols & alkaloids
 - Flavour volatiles
 - Sensory assessment



Methodology

- Common growing location: The International Cocoa Genebank Trinidad (ICGT).
- Most diverse collection of cacao germplasm in the world.
- Common processing location: CRC Fermentation and Drying Facility, UWI St. Augustine.
- Assessment of the genetic influences on marketable attributes without confounding effects of the environment.



Methodology

- Pods (optimum ripeness) were harvested (verified trees) and not stored for > 3 days
- Fermentation duration: 8 days
- Turned on: days 3 and 5
- Sampling: days 0, 2, 4, 6 and 8
- Drying: sundried on wooden trays
- Trials done each year (at the same time) for 3 years.
- Fermentation in Styrofoam coolers
- Capacity: 30kg



Results

Temperature profiles

Factor	F Value	Significance
Hybrid	34.97	***
Year	53.37	***
Day	61.32	***

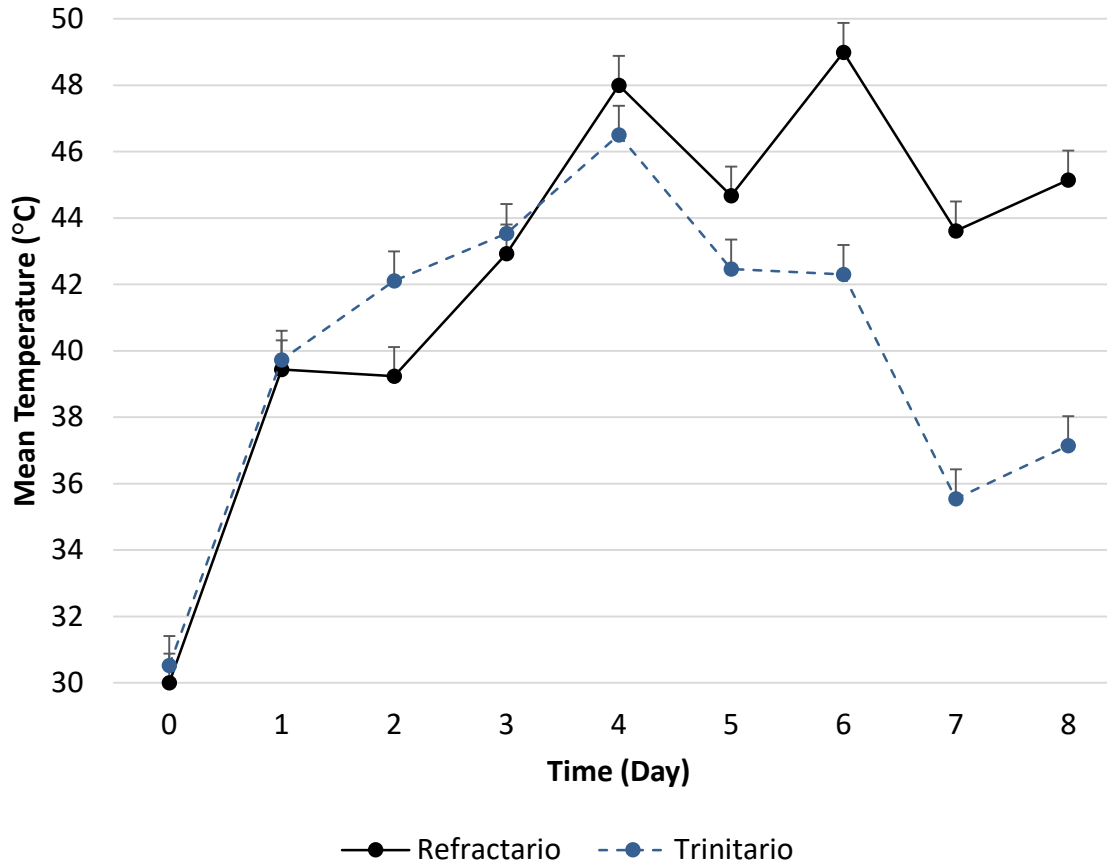
NS, *, **, ***

Nonsignificant or significant at $P \leq 0.05$, 0.01 and 0.001

- Overall Refractario had higher mean temperatures.
- Means for years 1 and 3 were similar (Newman-Keuls Multiple Comparison (N-KMC)Test).

Temperature profiles

Temperature changes during fermentation



- Both hybrids crossed 44°C.

- Temperature peaks for hybrids on day 4 were not significantly different.

- Peaks on day 6 were significantly different (Fisher's Least Significance Difference (LSD) Test).

- Trinitario fermentations should have been terminated on day 6 as temperature was not being sustained which signifies tendency to over fermentation/improper fermentation.

pH profiles

Testa pH			Cotyledon pH	
Factor	F Value	Significance	F Value	Significance
Hybrid	131.92	***	23.94	***
Year	10.41	***	3.70	*
Day	66.81	***	42.54	***

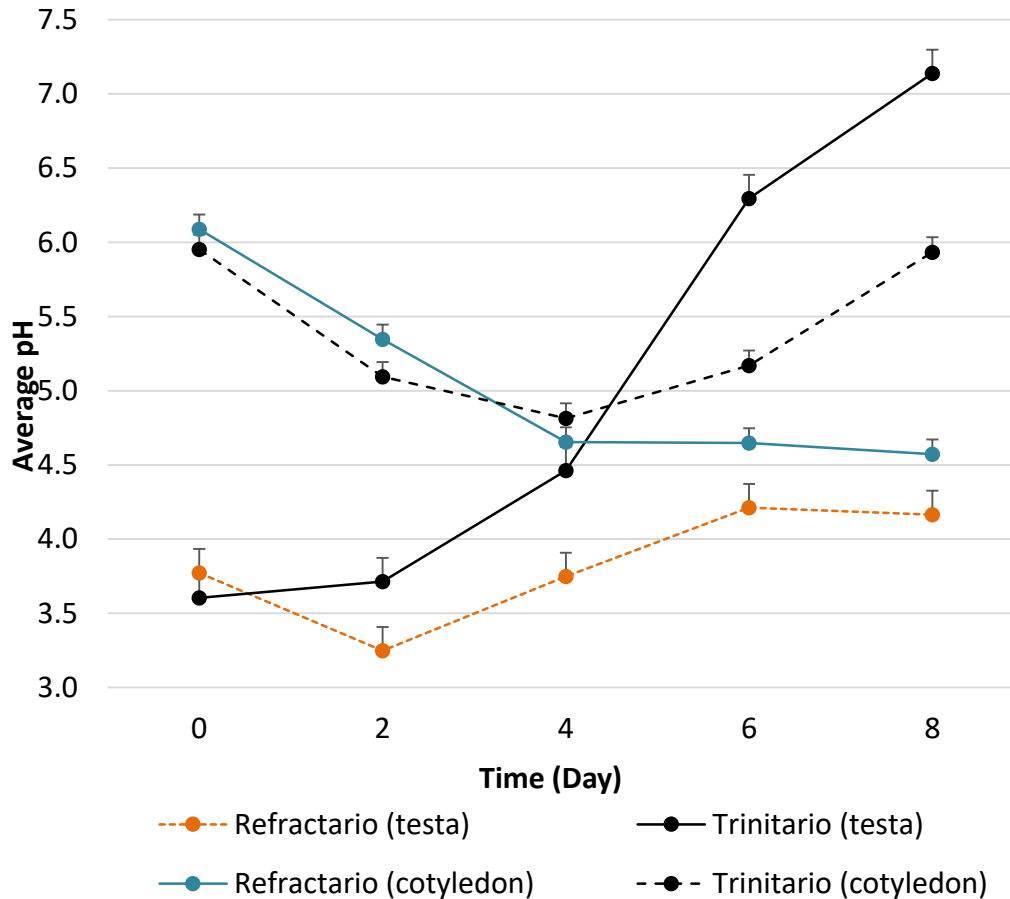
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Nonsignificant or significant at $P \leq 0.05$, 0.01 and 0.001

- Differences between replicates-not significant.
- Significant hybrid $\times$ day interaction.
- Means for years 2 and 3 were similar (N-KMC Test).

pH profiles

pH changes during fermentation



- Hybrids showed similar pH trends- decrease in cotyledon pH and an increase in testa pH as fermentation progressed until day 4.

- On day 6- Trinitario cotyledon pH increased significantly which is indicative of over fermentation/improper fermentation.

- Both hybrids were significantly different in terms of testa and cotyledon pH on day 6 (Fisher's LSD Test).

- Day 6 would have been the time to terminate fermentations for Trinitario, whereas Refractario could have fermented longer.

Bean measurements- count, weight, width, length, thickness

- Data for 3 years was analysed and only hybrid type effect was significant.
- No significant differences between years, replicates and fermentation days.
- **Overall, means for Trinitario were higher than Refractario for measurements of interest.**
- Average bean count and individual bean weight ranged from:
 - 68-72 and 1.39-1.47g -Trinitario
 - 79-84 and 1.19-1.26g -Refractario
- **Trinitario beans had higher individual bean weights and lower bean counts.**

Polyphenols and Alkaloids

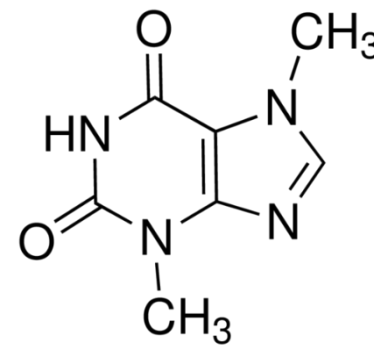
Factor	Theobromine		Caffeine		Procyanidin B2		(+) catechin		(-)-epicatechin	
	F Value	Sig.	F Value	Sig.	F Value	Sig.	F Value	Sig.	F Value	Sig.
Group	26.00	***	146.70	***	14.83	***	27.3	***	0.92	NS
Year	0.69	NS	14.75	***	1.18	NS	0.16	NS	17.75	***
Day	57.06	***	5.88	***	19.47	***	41.17	***	782.8	***
									2	***

NS, *, **, ***

Nonsignificant or significant at $P \leq 0.05$, 0.01 and 0.001

• Overall, means for Trinitario were higher than Refractario for compounds of interest.

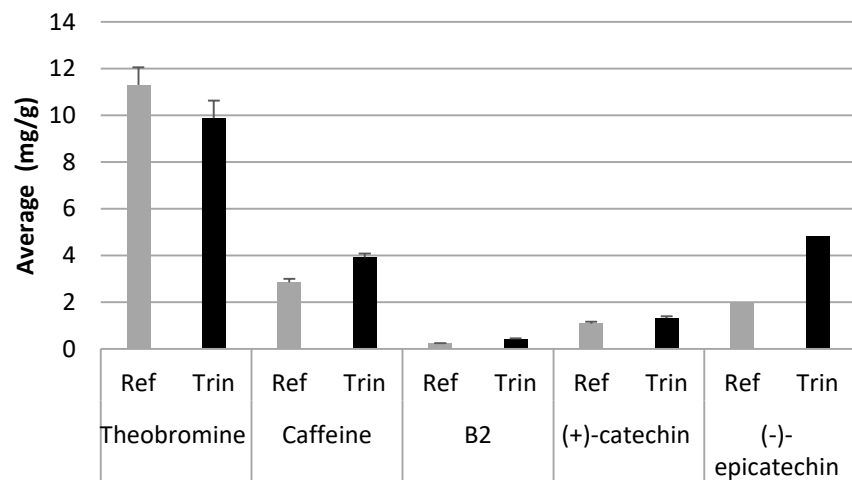
• Means for year 1 were larger for caffeine and (-)-epicatechin.



Polyphenols and Alkaloids

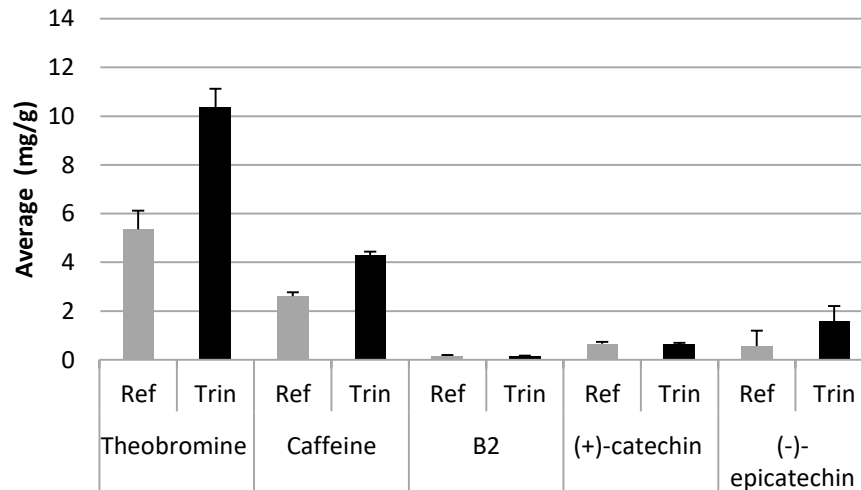
- Higher levels of procyanidin B2, (+)-catechin and (-)-epicatechin on day 6 compared to day 8 for Trinitario beans.
- Refractario also exhibited decreases in these compounds on day 8, normally these compounds decrease with increase in fermentation time.

Day 6



Alkaloids & Polyphenols

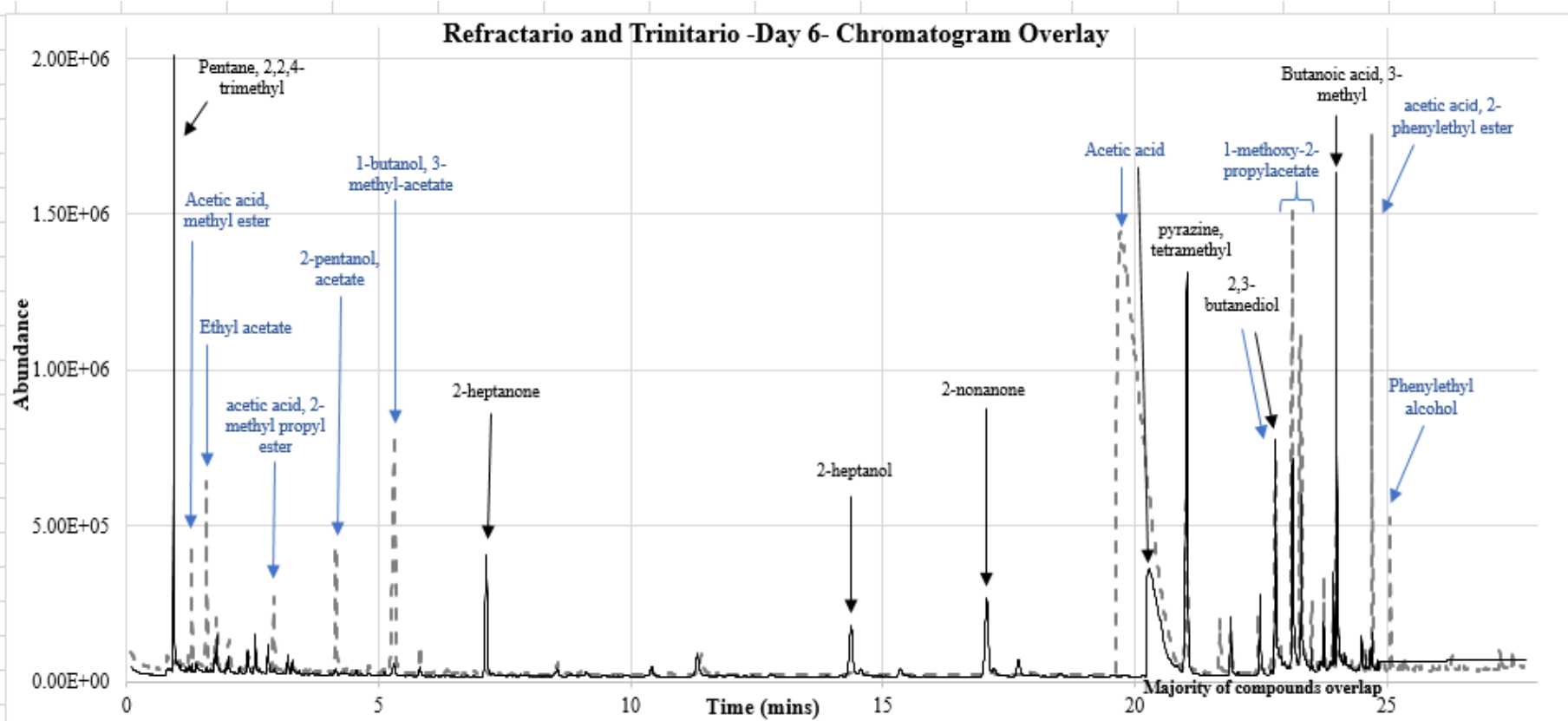
Day 8



Alkaloids & Polyphenols

- On day 8 Trinitario beans contained more theobromine, caffeine and (-)-epicatechin and Refractario beans had higher quantities of (+)-catechin and procyanidin B2.

Flavour volatiles



Some volatile compounds identified and their associated sensory attributes based on literature include:

- Trinitario: 2-Heptanol- citrusy (odour quality), fruity (sensory perception); 2-Heptanone- fruity, floral (odour quality), fruity, floral (sensory perception).
- Refractario: ethyl acetate- pineapple (odour quality), fruity (sensory perception).

These perhaps attribute to the fresh fruit and floral scores in sensory assessment.

Sensory assessment

- This was done for 3 years, however only year 1 data was analysed.
- Statistical analyses revealed that the hybrids were only significantly different in terms of acidity ($P \leq 0.01$; F value = 6.43).
- All other flavour attribute differences were not significant.

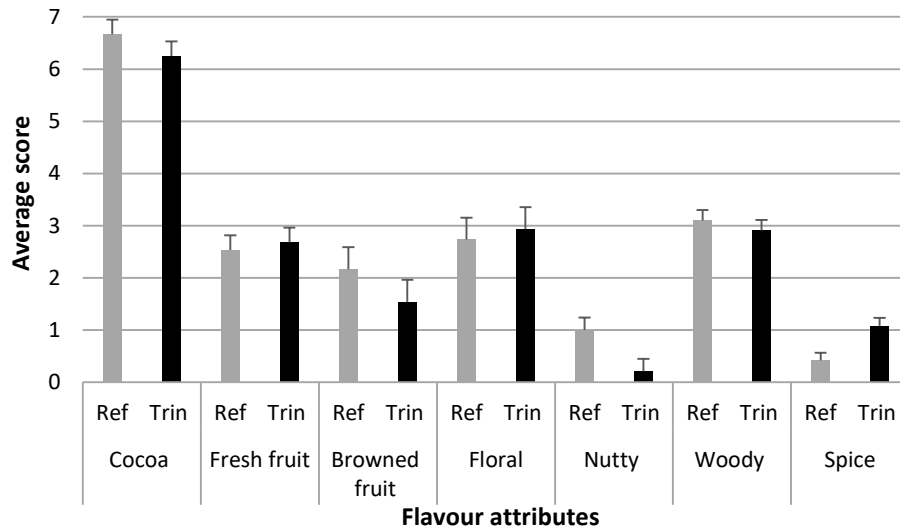


Cocoa
Liquor and
Chocolate
Flavour
Wheel

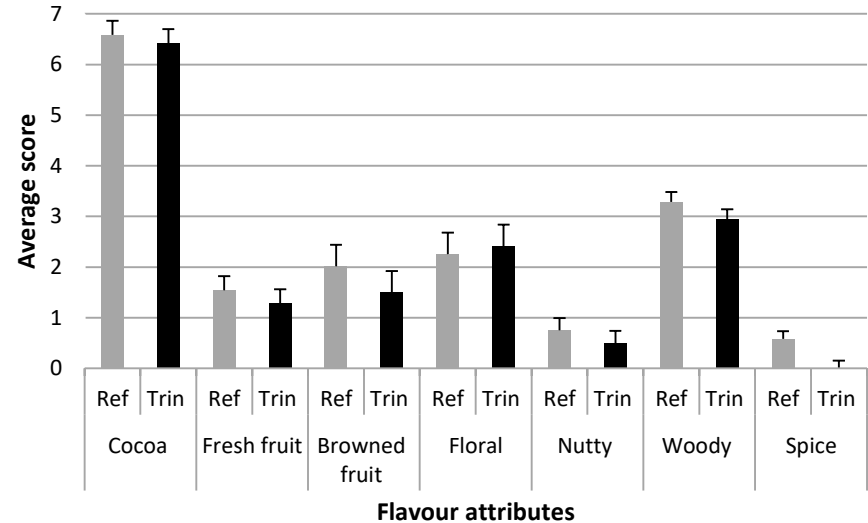
CAOBISCO/ECA/FCC, (2015).
"Cocoa Beans: Chocolate and
Cocoa Industry Quality
Requirements". (End, M.J. and
Dand, R., Editors). Adapted from
the Cocoa Research Centre,
Sensory Training Guide, (2015).
Cocoa Research Centre, UWI.

Sensory assessment

Day 6



Day 8



- Hybrids were significantly different in terms of scores for Spice on day 6 and on day 8 (Fisher's LSD Test).

- There was a significant decrease from day 6 to day 8 for:
 - Fresh fruit in Refractario
 - Fresh fruit and Spice notes in Trinitario (Fisher's LSD Test).

Conclusion

- Based on temperature, pH, chemical and sensory analyses data, 6 days would be optimum fermentation time for Trinitario.
- For Refractario, temperature and pH trends indicated the possibility of longer fermentation (7 days), which only negatively impacted fresh fruit notes.
- The study has demonstrated the potential marketable uniqueness of the hybrids in relation to variability in physical, sensory and chemical characteristics.
- **The results underscore the importance of genetics and fermentation time on quality, which can be exploited to develop genetic branding strategies for ICGT. As well as the need for customised postharvest processing protocols.**

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