



Botanical DNA Evidence in a Case of Robbery and Property Crime: Application of High-Resolution Melting Analysis of *Triticum aestivum* L. Grains

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ABSTRACT.

Forensic botany is an emerging discipline that has evolved rapidly during the last few years. Botanical evidence is usually found at crime scenes and sometimes it is the only available element for criminal investigations in cases where other evidence is absent. Different molecular techniques have been applied to analyze botanical evidence. In recent years, research has been focused on DNA barcoding to determine the species of different organisms through sequencing of conserved DNA regions such as cytochrome C oxidase I (COI) or internal transcribed spacer (ITS). Another application of DNA barcoding is the use of real-time PCR combined with high resolution melting temperature (HRM) analysis to discriminate specific conserved DNA regions of closely related botanical species. For melting temperature determination, an intercalating fluorescent dye is added to the real time PCR reaction and a derivative melting curve is generated. Distinct nucleotide sequences of a conserved DNA region will provide different melting temperatures (T_m).

INTRODUCTION.

A robbery and crime property case in a farm was investigated using real-time PCR combined with HRM analysis. The suspects, after burning two yards of a wheat field, stole several tons of a brand new variety of wheat (*Triticum aestivum* L.), transported it in a truck and then distributed among people living in a nearby community. The truck was found by the police and a few wheat grains were collected from the vehicle. The HRM strategy (Reed and Wittwer, 2004) enabled the “molecular traceability” of the wheat grains to the crime scene, demonstrating the usefulness of this approach for the identification of closely related species and its application as a potential forensic molecular tool. Two suspects were arrested and were convicted for the crime.

MATERIALS.

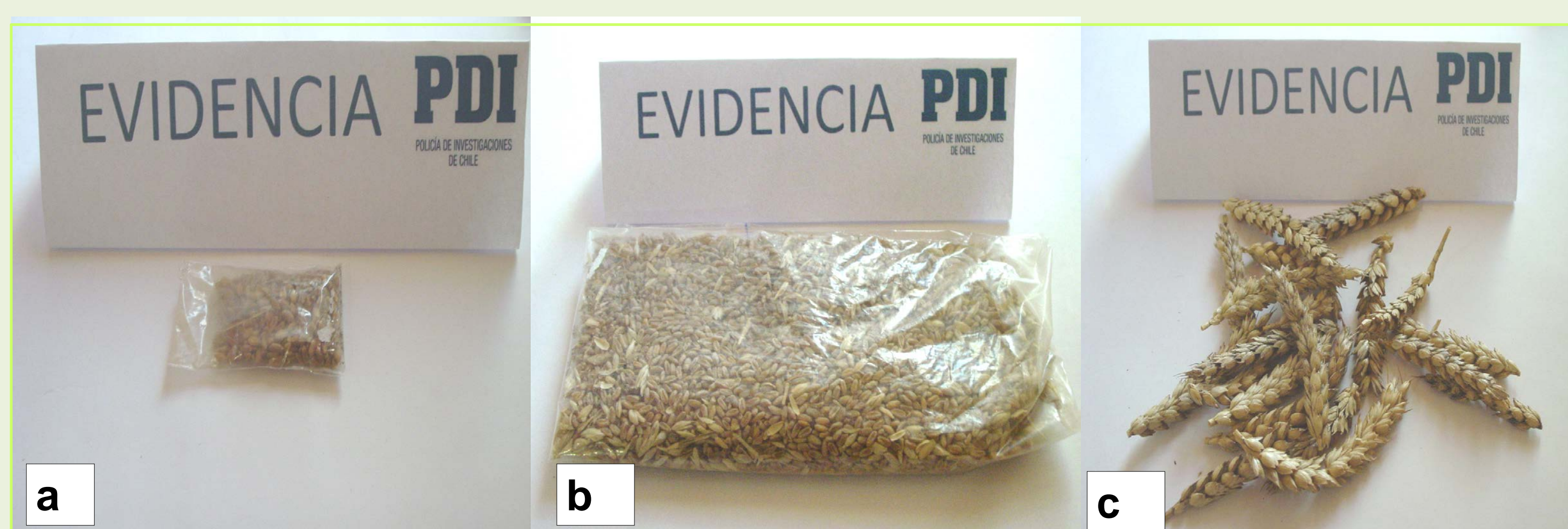


Figure 1. Evidence of wheat found in : a) trailer truck, b) truck and c) farm.



Figure 2. a) wheat mature spikes b) burnt wheat field and c) victim holding burnt spikes in his hands.

METHODS.

SAMPLING

Genetic analysis of wheat grains collected from a trailer and truck (Evidence No. #392 and #393) was performed and results were compared to those obtained from wheat plants collected at the farm (Evidence No. #395). Pionero-INIA control wheat grains were also included in the analysis.

PROCEDURE

The wheat seed were germinated to obtained fresh tissue. Wheat DNA was extracted from the leaflets using Plant Dneasy Kit (Qiagen) and quantified by fluorometry. HRM analysis using ITS 2-3 primers was performed in a Real-time PCR system (add model and brand).

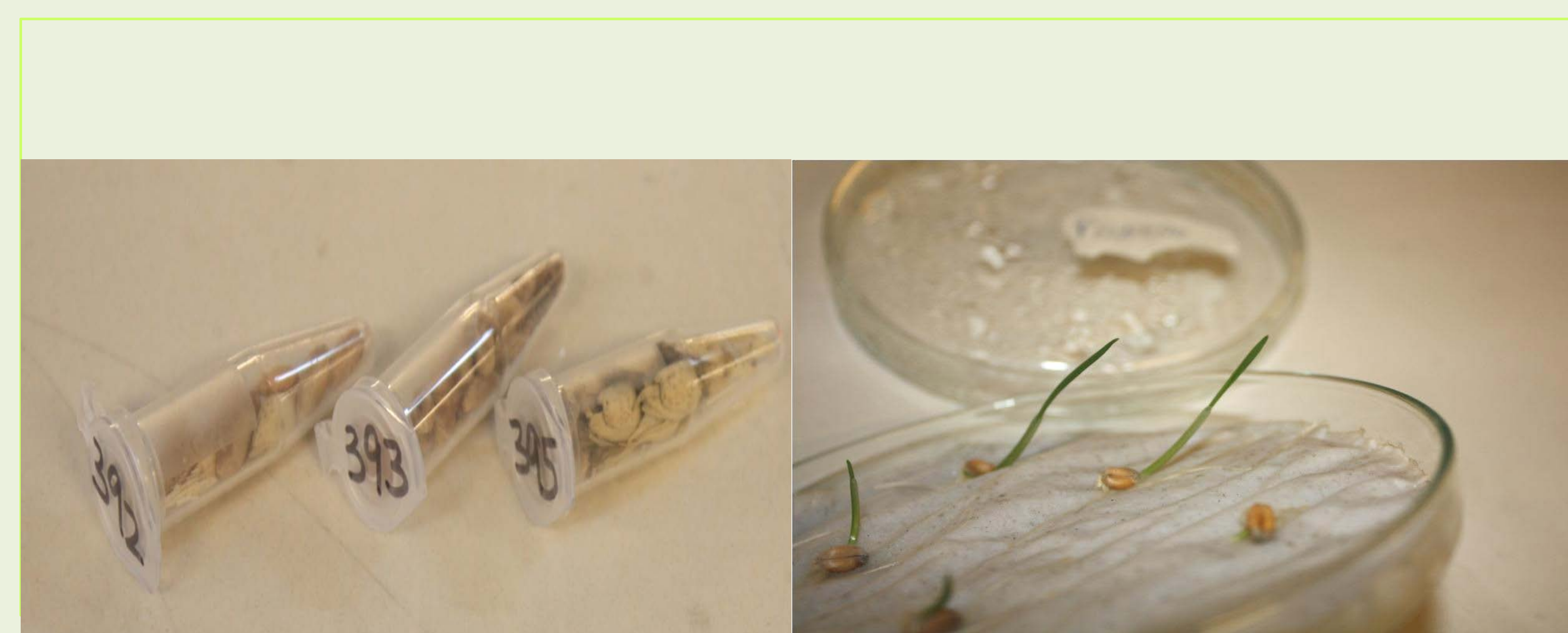


Figure 3. a) wheat grains, b) wheat plants .



Figure 4. Real-Time PCR System. (Brand?)

RESULTS & DISCUSSION.

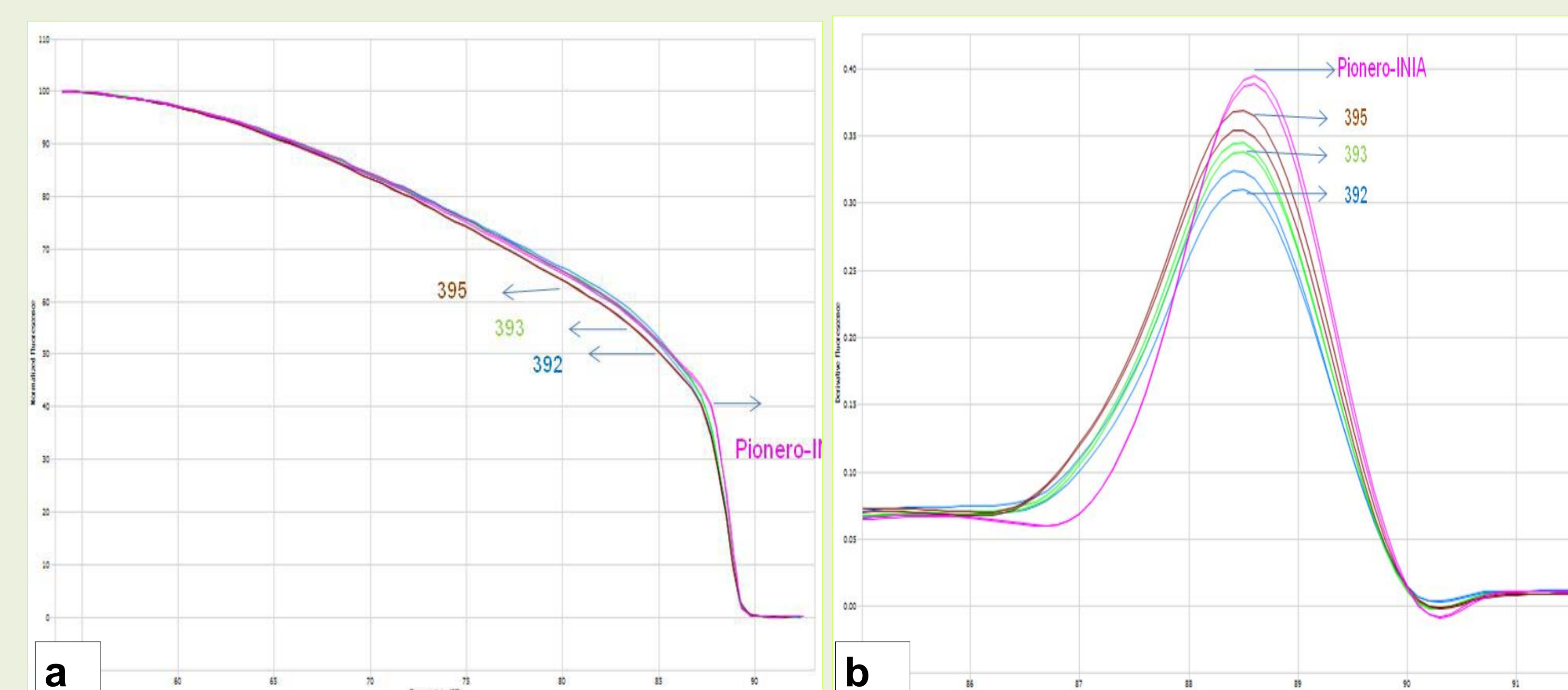


Figure 5. a) Normalized melt curve and b) Derivative melt curve with HRM and ITS approaches for wheat samples (Evidence samples: #392, #393, #395 and control).

RESULTS & DISCUSSION.

Melting temperatures peaks for each sample were within the range 191 ° F to 192 ° F. All wheat evidences samples (#392, #393 and #395) produced one maximum peak, which was different from that of the control wheat (Pionero-INIA). The melting curves of wheat evidences—samples were similar in shape, all presented ΔT_m intersample below 0.34 ° F, with ΔT_m of wheat samples approximately 0.36 ° F lower than wheat control Pionero-INIA. These results suggests that the unknown wheat samples (#392, #393 and #395) are genetically indistinguishable for the targeted locus. These results agree with those obtained by Ganopoulos *et al.* (2011; 2013).

EVIDENCE SAMPLE	SOURCE OF DNA	PEAK (°F) ± SD
Trailer (392)	leaflet tissue	191.12 ± 0,00
Truck (393)	leaflet tissue	191.03 ± 0.45
Farm (395)	leaflet tissue	191.21 ± 0.45
Pionero-INIA (Control)	leaflet tissue	191.48 ± 0.00

Table 1. Variability for the melting peak of the amplicons from wheat sample with ITS by high-resolution melt (HRM).

CONCLUSION.

The wheat (*Triticum aestivum* L.) samples studied, showed genetic correspondence between the grains collected from the truck and trailer with the seeds found in the field in La Laguna farm. The results allowed to link the wheat seeds found in vehicles with the crime scene (farm). The genetic study of botanical evidence present at the crime scene contributed to the reconstruction of the event

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