

ARE YOU REALLY MY CLONE?

Identity Verification of the *In-Trust* Sweetpotato Collection at the International Potato Center



David Ellis¹
 Noelle A Barkley¹
 Genoveva Rossel¹
 Ronald Robles¹
 Ana Panta¹
 Jorge Biondi¹
 Awais Khan¹
 Andrzej Kilian²

¹ CIP – Genebank International Potato Center, P.O. Box 1558, Lima 12, Peru

² Diversity Arrays Technology Pty Ltd, University of Canberra, Kirinari St. Bruce, ACT2617 Australia



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Our assumption has been that the genetic integrity in germplasm collections have been pristinely maintained over the decades since collections were made despite vast changes in technology, quality management systems (QMS), improved conservation methods and infrastructure of the genebanks. For clonal genebanks, such as the International Potato Center (CIP), the biggest challenge in the last 40 years has been to maintain quality control in the genetic integrity of the accessions while maintaining a continually living collection long-term. With the advent and application of technologies, such as *in vitro* culture, where the initial goal was to achieve success, financial realities relied more on the success of *in vitro* culture and less on verification of identity of the accession. This was not unique to CIP as this was the status quo for all of us working in the early days of *in vitro* technology thirty years ago. Now, Quality Management Systems (QMS) are in place for process quality assurance, so that we will not repeat the mistakes of the past. At CIP, the sweetpotato collection was initiated in the late 1980's with the donation of *in vitro* cultures from AVRDV and IITA. These collections were of unknown genetic integrity and in many cases with minimal passport data. Parental material existed as potted plants for some accessions, as was the norm for material added to the collection in the 1980's. This poster presents the first public report of a program to access the genetic integrity and to fingerprint the entire *In Trust* global cultivated sweetpotato collection.



Figure 1. Field comparison of sweetpotato *in vitro* material with original mother plants that have been maintained for 30 years. A. Side-by-side field comparison of *in vitro* and mother plants from >400 accessions. B. *In vitro* plantlet. C. Example of morphologically different *in vitro* and mother plant of the same accession. D. Roots showing distinctive differences in flesh and skin color from plants depicted in C.

PROJECT OBJECTIVES: The objective of this project is to generate a unique fingerprint for every accession in the cultivated *In Trust* sweetpotato collection. CIP is fortunate to have mother plants, maintained in the greenhouse which were periodically verified in the field as true-to-type based on original or 20-30 year old passport data from when the collection entered the genebank. These mother plants serve as a positive control for the verification of the identity of the corresponding *in vitro* material. The primary rationale for this project is to verify the identity of each *in vitro* accession and to ensure genetic integrity of material distributed from the genebank. Secondary rationale includes the development of long-lasting tools for long-term monitoring of genetic integrity of the collections and to provide methodology to rationalize other global sweetpotato collections. While SSR markers could provide the unique fingerprints needed in the short-term for accession identification, they do not provide the long-term sequence-based markers which will be needed for future generations; hence, the confirmation by DArTseq. To date, DArTseq has provided unique, reproducible diagnostic markers within and between plates, at different assay times and with different DNA preps. The long-term goal is to provide tools for the research community to better utilize the collections.

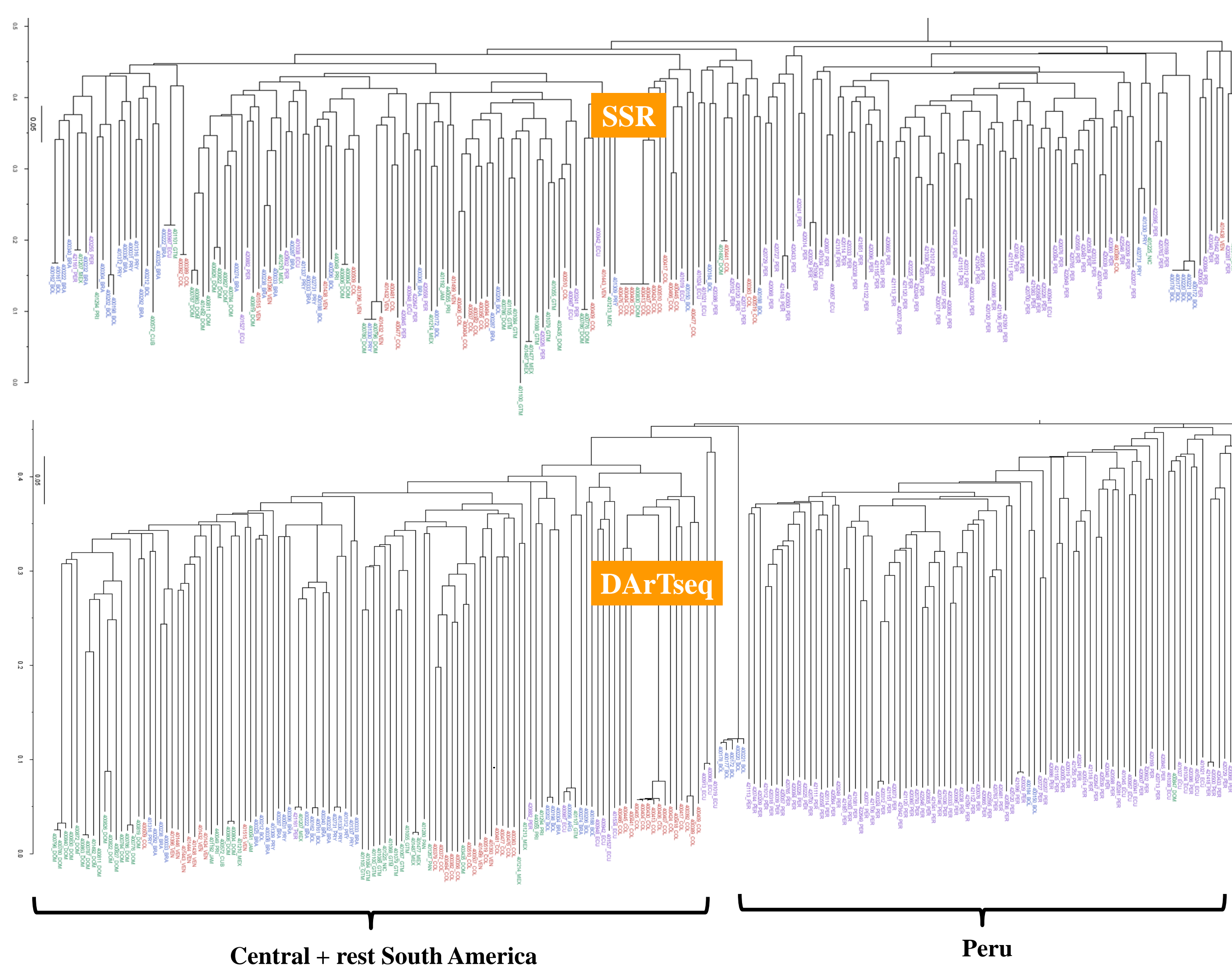


Figure 2. Dendrograms based on country of origin from SSR and DArTseq marker analysis of 213 accessions from the CIP *In Trust* cultivated sweetpotato collection. Neither marker method resolved the central American and northern south American (Colombia and Venezuela) accessions well.

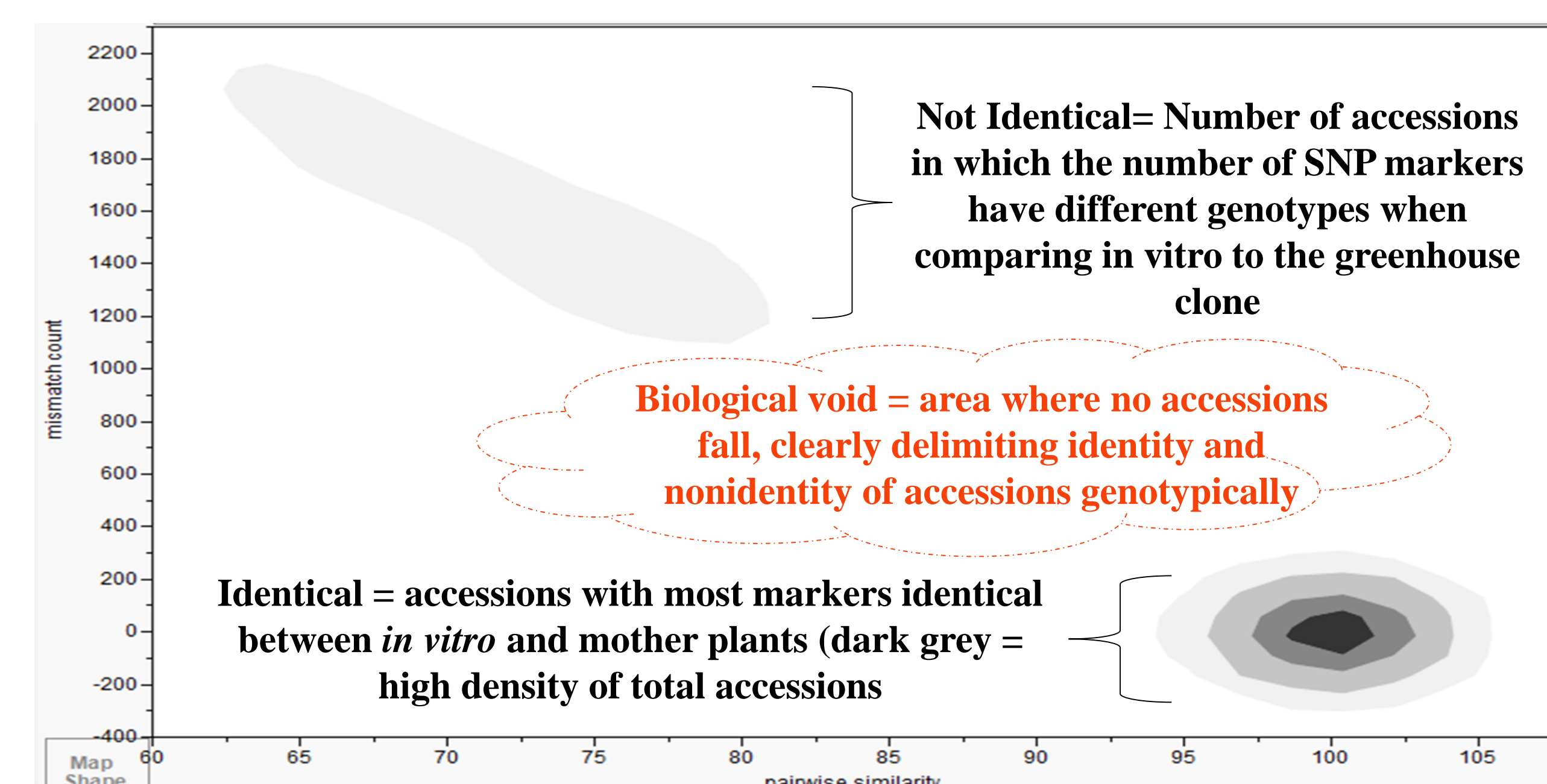


Figure 3. Density plot showing comparison between *in vitro* and mother plants. Accessions called as matches contain >95% (most are within 1%) of the total SNP markers identical to each other. When they are different, they have 100's to 1000's (>10%) of markers different. This clearly facilitates the identification of accessions in the *in vitro* collection which are not true-to-type.

SUMMARY:

- 88% of the *In Trust* collection is genetically identical to greenhouse maintained mother plants based on SSR and/or DArTseq analysis.
- The *In Trust* cultivated sweetpotato collection will be 99% fingerprinted by July 2016.
- Identity analysis, confirmed by morphological analysis, will be completed in 2019-2021, after which all accessions will be available for distribution.
- QMS are now in place to ensure integrity into the future. These include:
 - ISO accredited procedures and standards
 - Continual review and audit of written protocols
 - Barcode labeling of all plants and processes
 - Scheduled training/audits of all staff