The University of Nottingham

UNITED KINGDOM · CHINA · MALAYSIA

Nuclear DNA Content and Genetic Structure of Yams (*Dioscorea* Species, Dioscoereaceae) Cultivated in South Western Ethiopia

Kidist Kibret

Biosciences, University of Nottingham

stxkbki@nottingham.ac.uk

Outline

Introduction

- Dioecious monocots (Figure)
- Cultivated species:
  - *D. bulbifera*, *D. alata*
  - *D. cayenensis* and *D. rotundata* from Ethiopia- *D. cayenensis* species complex (Miege and Demissew, 1997; Hildebrand, 2003)
- Enantiophyllum subgenus
  - *D. alata* domesticated in Asia and *D. cayenensis* species complex domesticated in Africa

- Yams are grown either for their starchy tubers or medicinal properties, are important crops in the tropics and sub-tropics.
- Ethiopia is an isolated centre of yam production in Africa (Westphal, 1975).
- Domestication
  - Central and West Africa (Mignouna & Dansi 2003)
  - South Western Ethiopia (Hildebrand 2003, personal observation)

- Sheko, South Western Ethiopia wild and domestic (Hildebrand, 2003a, b; 2009) Figure
- Cultural reasons for domestication:
  - Nutritional value
  - Introduce variation to domesticated yams (Personal communication with farmers)
  - Bring wild yams closer to home (Hildebrand, 2003a, b; 2009)

- Bench-Maji Zone (Figure) is known for its relatively better natural vegetation cover and diverse traditional cultures and languages.
- Bench and Sheko districts- yam cultivation experts

Objectives

- DNA content estimation,
- Deciphering the relationship within the *D. cayenensis* complex and their relation to others from West Africa using molecular tools and
- Establishing tissue culture techniques

Germplasm collection

- *D. alata*, *D. bulbifera* and a group of yams provisionally placed under *D. cayenensis* complex.
  - Different varieties recognized by farmers

Flow cytometry

- Simplified two step procedure using Otto I and Otto II buffers described by Dolezel *et al.* (2007)
- Quality control
  - β-mercaptoethanol
  - Internal standardization
  - CV≤ 3% in most analyses
  - *Lycopersicum esculentum* as standard

Isolation of nuclei:

- Leaves of both sample and standard were co-chopped containing 0.5 ml of ice-cold Otto I buffer.
- Filtered through 20µm pore size nylon mesh filters.
- 1 ml of Otto II buffer supplemented with Propidium iodide and RNAse both 50 µg/ml and ß-mercaptoethanol (2 µl/ml)
- Analysed by a Becton Dickinson LSR II flow cytometer.
- 5000 particles anlyzed.

42 individuals and 67 analyses including the replications (Figure)

Data analysis

- Sample 2C DNA content =

[(sample G1 peak mean)/ (*L. esculentum* G1 peak mean)] x *L. esculentum* 2C DNA content (pg DNA).

Mean 2C DNA content ± SD graph

- Non-hierarchial clustering grouped data into three species. Table
- The 2C-values of Dioscorea species in the angiosperm database range from 0.95 to 13.50 pg (Bennett and Leitch, 2010).

C-value calculated from overall mean 2C DNA content. 1pg = 980 Mbp (Bennett and Leitch 2010).

Species	C-value (pg)	C-value (Mbp)
D. alata	0.576	564
D. cayenensis complex	0.637	624
D. bulbifera	1.187	1163

Chloroplast Single Nucleotide Polymorphism (SNP)

- DNA extraction
  - Sigma Plant Miniprep Kit
- DNA quantification
  - Spectrophotometer and agarose gel electrophoresis
- PCR
  - 10 ng/µl DNA, 20 µl reaction in Veriti® Thermal Cycler
  - Primers (Scarcelli *et al*., 2011a)

- PCR products sequenced by *Source BioScience LifeSciences*
- Alignment using CLC genomics workbench (v 4.8) sequences from
  - This study
  - NCBI, *D. abyssinica* and *D. praehensilis* (Scarcelli *et al*., 2011a)

Analysis of sequences

- Chlorotypes (Scarcelli *et al*., 2011a)
- *D. praehensilis* with same chlorotypes as *D. cayenensis* complex (this study).
  - The occurrence of wild with same chlorotypes as domesticated has been reported earlier (Terauchi, *et al*., 1992; Chair *et al*., 2005; Scarcelli *et al*., 2011a).

- SNPs from *ndhH-Exon, ycf1-rrn5 and rrn4,5-trnN* to study not only diversity of the crop-wild relatives' complex of *Dioscorea* (Scarcelli *et al*., 2011a) but also *D. alata* and *D. bulbifera*
- SNPs already reported by Scarcelli *et al*. (2011a) and new in this study

- UPGMA analysis of aligned sequences
- *D. bulbifera* (Opsophyton) was separated from the rest (Enantiophyllum) (Onueme, 1978; Wilkin *et al*., 2005).

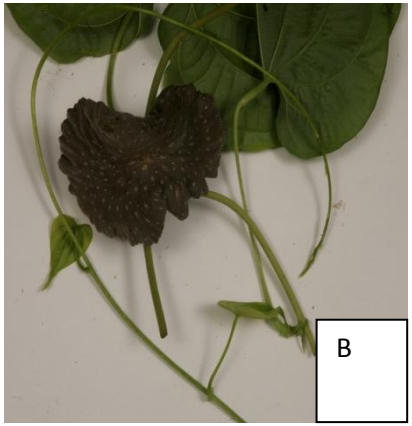
Morphological characterization

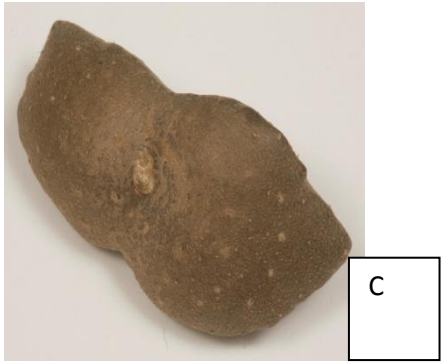
- Glasshouse, 25-30 degree Celsius
- IPGRI/IITA (1997) similar to farmers classification into varieties
- *D. cayenensis* complex
  - young and mature stem colour,
  - young and mature leaf colour,
  - mature stem waxiness and presence/absence of spines


A
B
C

- *D. alata*
  - colour differences of different parts

- *D. bulbifera*





Tissue culture

- Murashige and Skoog medium (MS), MS with charcoal and Woody Plant medium (WP) with charcoal
  - All three species grew roots, shoots and aerial tuber

In addition to MS medium (Mignouna *et al*., 2009a), woody plant medium with charcoal (this study)

Species	Media	Percentage
D. alata	MS	60
	WPC	69.2
	MSC	37.5
D. bulbifera	MS	33.3
	WPC	60
	MSC	25
D. cayenensis complex	MS	30.8
	WPC	50
	MSC	44.5

Conclusion

- Certain morphotypes of *D. praehensilis* and *D. abyssinica* have same chlorotypes as *D. cayenensis/rotundata* complex
- *D. cayenensis* complex same DNA amount as *D. praehensilis* and *D. abyssinica*

➢ suggest that they are same taxonomic entity.

Future work

- Additional primers are needed to relate between *D. abyssinica, D. praehensilis,* and *D. cayenensis/rotundata* complex
- More *Dioscorea* spp. from other countries need to be included to compare with Ethiopian, including wild
- Relation of morphology, genetic diversity and the cause of change in morphology during domestication need further investigation
- Chromosome count of a representative individual is needed to relate DNA amount with chromosome number.

Acknowledgement

- Dr. Sean Mayes, Dr. Mike Davey, Prof Sebsebe Demissew
- Dr Katie Mayes
- Dr. Sue Fox