IDENTIFYING THE PHYSIOLOGICAL AND GENETIC TRAITS RELATED TO DROUGHT TOLERANCE IN CASSAVA

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important staple foods in the human diet in the tropics, cultivated in areas considered marginal for other crops. Because of its remarkable tolerance to drought and great ability to survive uncertain rainfall patterns, it is considered a contributor to food-security against famine, requiring minimal inputs and making it an important crop for drought prone areas of tropical and sub-tropical Africa, Asia and Latin America.

When water is available, cassava maintains a high stomatal conductance with high internal CO_2 concentration; but when water becomes limiting, the stomata are closed in response to even small decreases in soil water potential. In addition, leaf area growth is decreased in response to water stress and is rapidly reversed following the release from stress. This suggests that the ability to regulate numerous plant processes to rapidly change course is the key for cassava success unfavorable weather.

This project aims to determine the best traits to be used in breeding programs for drought tolerance by elucidating the mechanisms of cassava's remarkable tolerance to drought and to identify trait-marker associations for the development of a more cost-effective breeding process for drought tolerance that can be used for cassava and other crops.

Specific Objectives:

1. Improve the understanding of the drought tolerance mechanisms in cassava.
2. Select contrasting cassava genotypes for tolerance to drought environments.
3. Identify molecular markers for drought tolerance traits via QTL and candidate gene mapping in cassava
4. Assess the benefits of transgenic over-expression of a gene for improved leaf retention introduced into the *Manihot esculenta* gene pool.
5. Strengthen the partnership among institutions involved with plant breeding programs for drought tolerance

METHODS / ACTIVITIES

To understand the cassava drought tolerance mechanisms:

1. Selection of contrasting genotypes for drought tolerance
2. Evaluations (field and greenhouse) under controlled conditions

Parameters:

- Leaf conductance; transpiration; photosynthesis
- Solutes accumulation
- Accumulation of specific low molecular weight proteins
- Abscisic acid (ABA) and phaseic acid (PA) accumulation
- Accumulation of nonstructural carbohydrate
- Leaf area; leaf retention; root depth; # of roots; root dry matter; starch; productivity; tolerance to cassava green mites (CGM) and other pests

Identification of cassava genotypes with wide diversity for drought tolerance traits:

1. Crosses 20 Tolerants (TOL) vs 10 Susceptibles (SUS); and self of TOL
2. Selection of the 4 best parentals combinations and progenies evaluation
3. 200 seeds of each cross germinated *in vitro* and multiplied to produce 40 copies → shared with Brazil, Colombia, Ghana and Tanzania
4. At each local: *in vitro* multiplication (rapid propagation) to evaluate the segregating populations.
5. In the segregating populations and parentals: evaluation of water deficit effects
6. Identification of genotypes with good drought tolerance → incorporate into breeding program or directly used by farmers

Identification of molecular markers associated with drought tolerance genes:

1. Four segregating populations (F_1); progenies from selfing (S_1) and parentals → evaluated in the replicated trials. The target sites will be in drought prone regions of Brazil, Colombia, Ghana and Tanzania (**Fig. 1**)
2. Segregant parentals lines → genotyped with >600 SSR markers from cassava genetic map
3. Polymorphic markers evaluated in the F_1 and S_1
4. Phenotypic and genotypic data analyzed to identify genes involved
5. Candidate genes mapped
6. Identification of associations between markers and QTL for drought

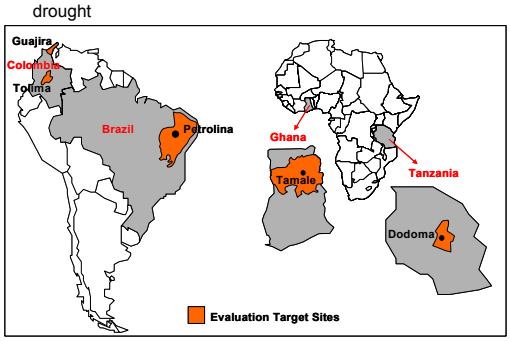

Fig. 1 – Target sites in Colombia, Brazil, Ghana, and Tanzania, where segregating populations will be evaluated for drought tolerance.

Assessing the effect of the leaf retention gene for improving drought tolerance:

- At ETH-Zurich were developed transgenic cassava plants, expressing the isopentenyl transferase (*ipt*) gene encoding cytokinin production under the control of the senescence-regulated *SAG12* promoter from *Arabidopsis*
- Seven transgenic lines from clone TMS 60444 were obtained → two lines with significant reduction of senescence
- Transformed and non-transformed plants → evaluated in the field and greenhouse (Colombia) → to analyze the effect of transformation on drought tolerance attributes

ACCOMPLISHED ACTIVITIES/RESULTS:

Studies on physiological and yield atributes related to cassava drought tolerance

To evaluate hypothesized mechanisms that cassava might use to regulate growth and carbohydrate partitioning during water deficit, a study was conducted at Cornell University. The results showed that the stem and petiole total non-structural carbohydrates (TNC) serve as a substantial carbohydrate buffer that may contribute to cassava's hability to withstand extended droughts during which photosynthate production is stopped (**Fig. 2**).

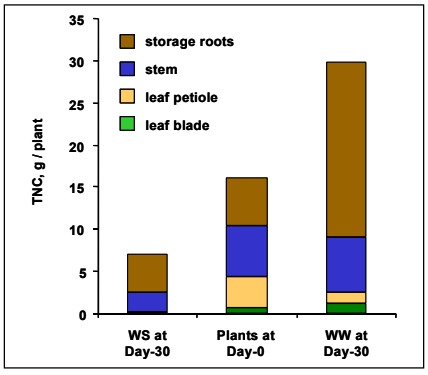

Fig. 2 - Total non-structural carbohydrates (TNC) in cassava plants at the beginning of the study (Day-0) and after 30 days of water deficit (WS) or well-watered treatment (WW).
Source: Duque & Setter, 2005

Identification of contrasting drought tolerant cassava varieties

Based on the outcomes from the cassava breeding programs carried out by Embrapa (Brazil) and CIAT (Colombia), in the last 15 years, 40 cassava varieties were identified as contrasting for drought tolerance attributes, being 28 tolerants and 12 susceptibles.

In vitro multiplication of the contrasting drought tolerant varieties

The selected contrasting varieties have been multiplied by *in vitro* micropropagation procedure at CIAT. Individual copies of each contrasting genotype were produced and shipped to Brazil (Embrapa). These copies were *in vitro* multiplied and acclimated in greenhouse to produce plants for field evaluation.

Establishment of a crossing block

The selected contrasting genotypes were planted in September 2005 in a crossing block at CIAT for pair-wise crosses to produce segregating populations for genetic mapping of traits associated with drought tolerance.

Establishment of field trails in the target sites

To evaluate drought tolerance related traits, field experiments were established in the target sites in Brazil, Colombia, Tanzania, and Ghana, using drought tolerance contrasting cassava varieties:

Brazil: Two 5-plant groups of 60 contrasting varieties were planted (May/2006) in an irrigated field in Petrolina. 3-4 months after planting (MAP), the irrigation, in one group, will be stopped and the growth and physiological parameters will be evaluated (**Fig. 3**).

Fig. 3 – Constrasting cassava varieties under controlled water deficit. in Petrolina, Brazil.

Colombia: Two 7-plant groups of 36 varieties were planted (Nov/2005) in an irrigated field in two sites of the Tolima Department. Two months after planting (Day 0) the irrigation, in one group, was stopped and sampling were at Days 0, 15, 30, 45, 60. The last sampling will be at harvest time (11-12 MAP). The parameters/samples were: plant height; leaf temperature, leaf retention, leaf and stem samples for ABA and sugars. At harvest, the production parameters will be added.

At CIAT, 15 contrasting varieties, planted (Mar/2006) in 60kg plastic bags, were evaluated in a screenhouse under control and water deficit (no irrigation after Day 0 = 2 MAP). The sampling were at Days 0, 15, 30, 45, 60. At each Day, 5 plants were harvested. The parameters were: soil moisture; plant height; leaf retention; leaf relative water content; total biomass (FW); leaf, stem and root tips samples for ABA and sugars); root biomass (FW and DW); # of storage roots. The roots were storaged for volume and lenght analysis using the WInRHIZO software. For the same 15 varieties, two 3-plant groups were planted (Jun/2006), under open sky, in 60kg plastic pots. Two MAP the irrigation, in one group, was stopped and the pots covered with a plastic film. The parameters and samples will be collected at the harvest time, around 10-11 MAP.

The data and samples collected in Colombia are in process of statistical and lab analysis at Cornell University.

Tanzania: Nine contrasting local and improved varieties were selected from two yield trials conducted in 2005 at Hombolo site in Dodoma. These varieties were planted (Feb/2006) and the harvest is scheduled for 12 MAP. In the meantime, the following parameters is being collected: plant establishment; disease and pest incidence; plant height.

Ghana: Ten contrasting cassava genotypes, selected from SARI germplasm and from farmers, were planted (Aug/2005). The data recorded include: dry weight (leaves, petioles, stem, adventitious roots, tuber roots); # of leaves, leaf scars, tuber roots; length of adventitious and tuber roots; plant height; width of plant canopy.

Cited Reference

Duque, L.O., and Setter, T.L. (2005) Response of cassava (*Manihot esculenta*, Crantz) to terminal water stress: ABA, sugar and starch accumulation/partitioning and root growth under different water regime treatments. L 5.09, Interdrought II, September 24 to 28, 2005, Rome, Italy

Generation

Embrapa
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