Combined phenotypic and transcriptomic approaches to evaluate drought-stress response in banana (*Musa* spp.), a non-model crop.

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Introduction

With a production exceeding 122 million tons in 2012 (FAOSTAT), banana (*Musa* spp.) is a very important crop and a staple food for millions of people, especially in the least developed countries. Drought is the main abiotic constraint restricting banana production. To gain insight into the response of banana to water deficit, we created a lab model to simulate drought and performed an RNA-seq experiment on three different genotypes. To confirm the mRNA-seq results, an independent experiment was set up with more biological replicates and putative drought-responsive genes were selected for RT-qPCR validation. To confirm our lab model and earlier phenotypic characterizations carried out in our greenhouse model, a field trial has been established at IITA-Arusha (with a long dry season of 5 months and a short dry season of 1.5 months). Physiological and growth/yield-related parameters are being evaluated during 2 growth cycles together with the expression level of a subset of genes up-regulated under drought-induced conditions in the lab model. Simultaneously, drought response is measured with Thermal Imaging.

Figure 1. Drought-induced stress in banana. A) Lab model: *in vitro* osmotic-stress test. Three triploid banana genotypes with contrasting drought tolerance (ABB: tolerant; AAA: intermediate; AAAh: sensitive) were grown autotrophically under stress (5% PEG 8000=-0.5 Bars) and control conditions (0% PEG). Samples from root and leaf tissues were collected at 3 days and used for the RNA-seq experiment (with 3 biological replicates). **B) Field trial established in IITA-Arusha.** Complete Randomized Design with 4 accessions: 2 presumed drought tolerant (ABB, AA[1]) and 2 drought sensitive (AAAh, AA[2]), 10 replicates per accession and 2 treatments (irrigated and not irrigated). AAAh: highland banana. AA[1] and AA[2] are two different diploid accessions.

Results

Phenotypic evaluation of drought-stress response in the field

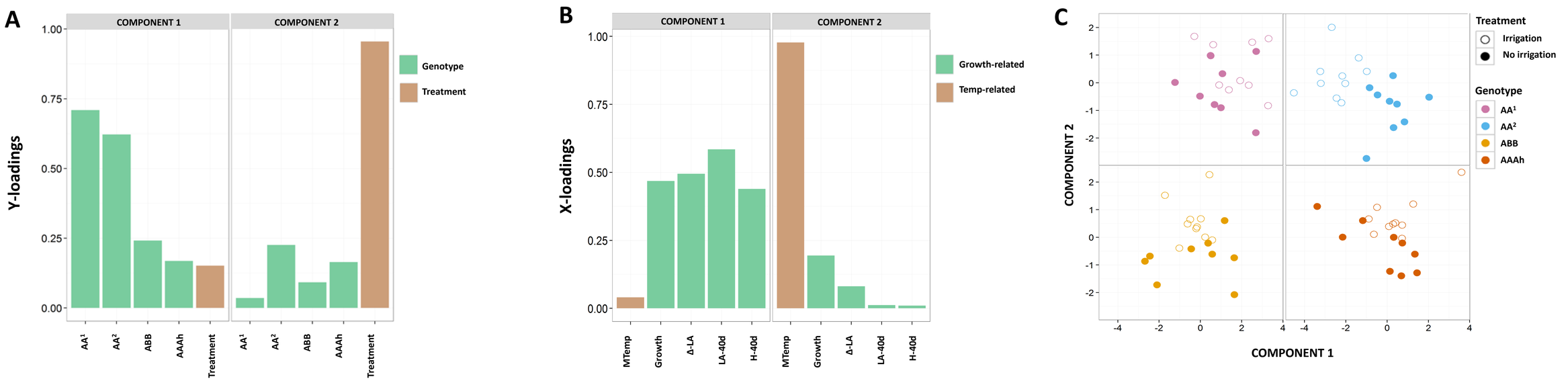

Figure 2. Partial Least Squares (PLS) analysis of phenotypic variables measured during drought-induced conditions in the field. A) PLS Y-loadings (absolute values) for components 1 and 2. The Y-loadings express the weight that a component exerts on the response variables in the PLS. Treatment= irrigation/no irrigation. Number of biological replicates: n=10/8 (AA[1] irrigated/non-irrigated), 10/9 (AA[2] irrigated/non-irrigated), 10/10 (ABB irrigated/non-irrigated), 10/10 (AAAh irrigated/non-irrigated). **B) PLS X-loadings (absolute values) for components 1 and 2.** The X-loadings express the weight that the predictor variables exert on the PLS components. MTemp: Mean Leaf temperature (measured with Thermal Imaging, as described in [1]); Δ-LA: total leaf area increase during 30 days of water deprivation; LA-40d: leaf area measured 40 days after the treatment started; H-40d: plant height measured 40 days after the treatment started. **C). Representation of PLS X-scores for each genotype separately.** Predictor variables: H-40d, growth, LA-40d, Δ-LA and MTemp. Response variables corresponding to the genotypes (AA[1], AA[2], ABB and AAAh) and the treatment (irrigated/non-irrigated).

Effect of drought on leaf temperature

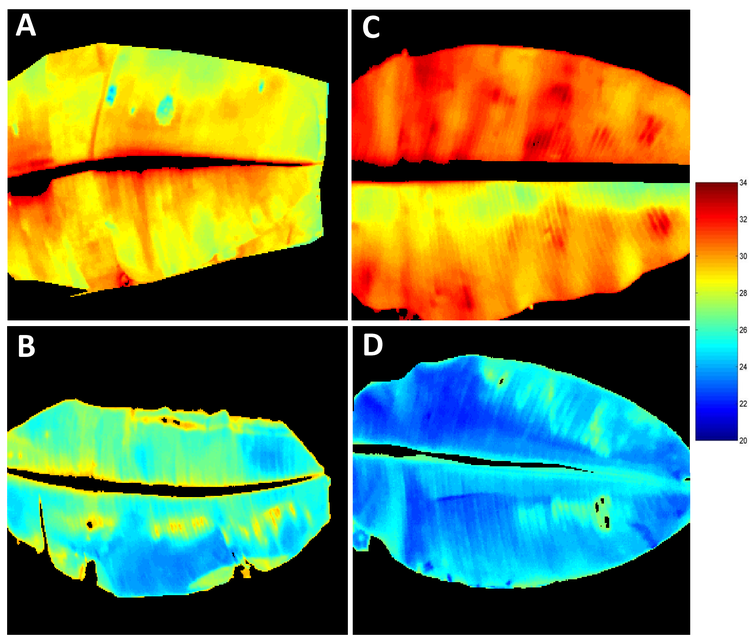

Figure 3. Lamina temperature of two triploid banana genotypes grown in the field under irrigation and no irrigation. A) Non irrigated ABB. B) Irrigated ABB. C) Non irrigated AAAh. D) Irrigated AAAh.
Images obtained with infrared thermography. Higher temperatures indicate lower stomatal conductance and *vice versa*. Leaf temperature set as described in [1].

Transcriptomic analysis of putative drought-responsive genes in lab and field models

Table 1. ANOVA results showing the significance level of the genotype, treatment and genotype*treatment effects for selected tissue-specific and drought-induced genes. [a]Abbreviation and gene annotation according to the *Arabidopsis* gene with highest similarity to the corresponding *Musa* gene. [b]Calculations based on RT-qPCR results of an independent *in vitro* osmotic-stress test to validate former RNA-seq results. Genotypes used: ABB, AAA and AAAh. Number of biological replicates (stress/control): n=6/6. ** p<0.05, ***p<0.01, ****p<0.001. Grey line separates leaf-specific genes (1-3) from root-specific genes (4-8).

Abbreviation[a]	Gene annotation[a]	*P*-value		
		Genotype[b]	Treatment[b]	Genotype*Treatment[b]
1. MYB94	Myb-type transcription factor 94	n.s.	****	n.s.
2. NCED3	9-Cis-epoxycarotenoid dioxygenase 3	****	**	n.s.
3. YABBY5	YABBY-type transcription factor 5	****	**	n.s.
4. ALAAT2	Alanine aminotransferase 2	**	****	n.s.
5. biLTP	Bifunctional inhibitor, lipid transfer family protein	****	****	n.s.
6. ETHE1	ETHE-1 like protein	****	****	n.s.
7. LTP	Lipid transfer family protein	****	****	n.s.
8. Per59	Class-III peroxidase Per59	**	***	n.s.

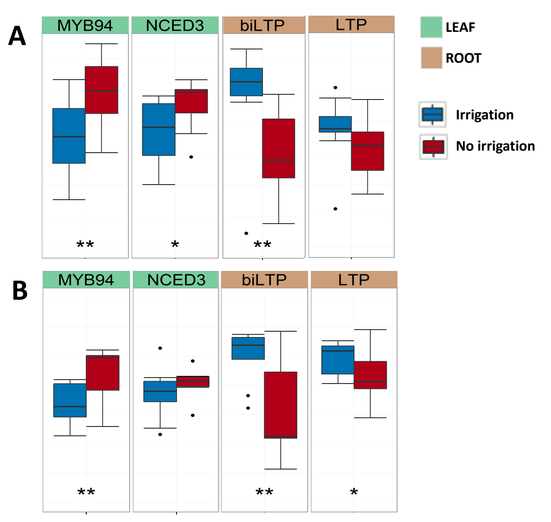

Figure 4. RT-qPCR validation of tissue-specific and drought-induced genes on field samples of genotypes ABB (A) and AAAh (B). NRQ=Normalized Relative Quantities. Significant level of t-test indicated: *p<0.1, ** p<0.05. Outliers indicated as dots. Number of biological replicates for ABB genotype (Irrigated/non-Irrigated): 9/9 (MYB94), 9/9 (NCED3), 10/10 (biLTP), 10/10 (LTP). Number of biological replicates for AAAh genotype (Irrigated/non-Irrigated): 10/6 (MYB94), 10/6 (NCED3), 9/9 (biLTP), 9/9 (LTP).

Conclusions

- Although the field trial was characterized by big phenotypic variation, PLS analysis clearly differentiated between irrigation and non-irrigation in one of the tested genotypes (AA[2]).
- Thermal imaging is a promising technique for drought-stress monitoring at leaf level in the field.
- The lab model approaches the field situation better for leaf than for root tissue, as up-regulation in water limiting conditions was validated for MYB94 in ABB and AAAh genotypes, and for NCED3 in ABB genotype.

References

[1] Jones, H.G. (2002). *Use of infrared thermography for monitoring stomatal closure in the field: application to grapewine*. Journal of Experimental Botany, 53 (378): 2249-2260.

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