Phenotypic evaluation of DREB transgenic rice under water limited conditions

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RATIONALE

Rice yield production highly depends on availability of adequate amounts of water, which is becoming a scarce resource in Latin America (Fig.1A and 1B), and therefore breeding rice for increased water use efficiency must be a priority. Several genes had been demonstrated to be associated with drought tolerance which could lead to improved water use efficiency in an agricultural practice. **D**ehydration **R**esponsive **E**lement **B**inding protein genes (DREB) have been identified as a master switch of stress inducible genes in different plants. Expression of those genes *in planta* have showed increased abiotic stress tolerance, such as for drought, under experimental conditions in different plants.

Our goal for this study is to evaluate rice DREB transgenic lines under water limited conditions close to field environments and establish drought screening protocol.

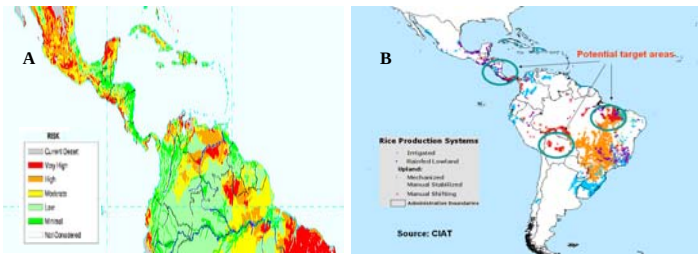

Fig. 1 A. Potential risk areas of drought in Latin America (Caribbean and north of south America). **B.** Potential target areas to introduce DREB technology in Latin America.

MATERIALS AND METHODS

Genetic Transformation. Cica 8, CT6241, and Palmar genotypes were used to generate transgenic rice by *Agrobacterium tumefaciens* mediated transformation, using pBIG *Lip9*::*At*DREB*1A* construct, according with the transformation protocol described by Tabares, E. *et al.* (2004).
The *Lip9*::*At*DREB*1A* contained the Arabidopsis thaliana DREB*1A* transcription factor conferring tolerance to drought and low temperatures (Liu, Q. *et al.* 1998), construct is driven by *Lip9* stress inducible promoter and spliced into the plasmid pBIG, (JIRCAS). *Lip9* corresponds to a rice promoter whose expression is not affected by ABA (Fig.2A and 2B).

A

B

Genotype	Type	Cultivars	Drought tolerance
Cica 8	Indica	Lowland	+
Palmar	Indica	Lowland	++
CT6241	Japonica	Upland	+++

Fig. 2 A. *Lip9*::*At*DREB*1A* gene construct map, some restriction sites are showed. *HPT;* hygromycin resistance gene, *PNos;* nos promoter, *Tag7;* Tag7 terminator, *Lip9;* promoter, *TNos* terminator, *RB* right border, *LB* left border. **B**. Rice genotypes used in this evaluation.

Preliminary evaluation of rice genotypes. In order to find a useful level of drought response which show differences between genotypes, no transgenic plants in vegetative stage were imposed to different levels of field capacity (FC) 100%, 75%, 50%, 25% with intermittent supplement of water and terminal drought (TD) using plastic pots (Fig.3). This material were also evaluated for endogenous *Lip9* gene expression. However due to plants tend to adapt to water levels, we chosen 25-35% of FC and terminal drought treatment, using 2 meters diameter big tray to evaluate large number of transgenic lines at same time.

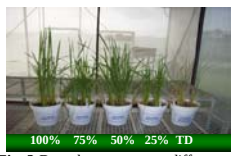

Fig. 3 Drought treatment at different field capacities.

Agronomical evaluation of T_1 transgenic lines. Selected T_1 Transgenic lines with one or two copies of transgene confirmed by southern-blotting (data not shown), were evaluated under intermittent (re-watered) and terminal drought conditions using the big tray at screenhouse. Plant height, leaf number, tiller number, leaf rolling, and dry weight were evaluated. Soil moisture was monitored using a ECH2O soil moisture sensor (EC-5, Decagon Devices, Inc. USA) from approximately three weeks after transplant (Fig. 4).

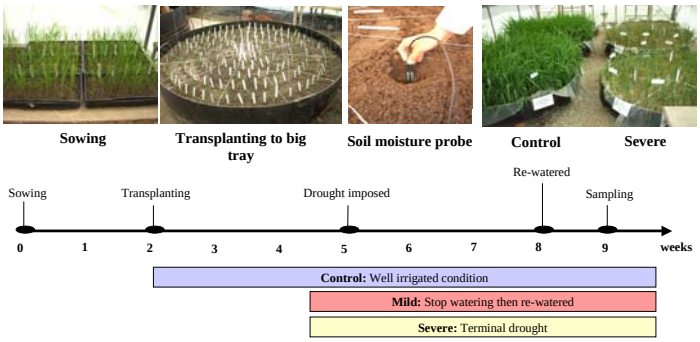

Fig. 4 Experimental set up for 2 meters tray at screenhouse.

Field experiment. We identified potential field locations in Colombia to develop drought screening: CIAT headquarters, Aipe (high temperature and low humidity) and Santa Rosa (high temperature and high humidity)(Fig. 5A). A movable rainout shelter has been implement, which is designed to protect a certain area of land against receiving precipitations so that an experimentally controlled drought stress can be imposed on that area. We will able to evaluated promising transgenic lines selected by the big tray screening at reproductive stage under controlled field conditions (Fig. 5B).

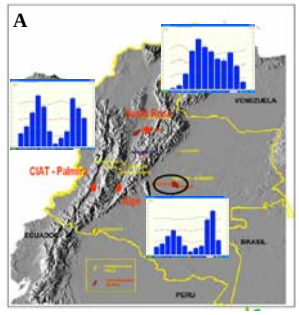

Fig. 5 A. Potential locations for drought screening in Colombia. **B.** Movable rain out shelter system for drought screening in field conditions.

Endogenous *Lip9* gene expression. Aiming to understand the expression patterns of genes responding to abiotic drought stress, we develop a first evaluation of untransformed plants to see endogenous levels of response of drought stress responsive gene *Lip9*.
Total RNA was extracted from leaves with TRIzol® reagent (Invitrogen) using manufacturer specifications. Residual DNA was eliminated using Deoxyribonuclease I (Invitrogen). 500ng of total RNA was reverse transcripted into first strand cDNA using the Superscript™ III reverse transcriptase (Invitrogen).
Primers for expression analysis of *Lip 9* were designed over Genebank accessions AB011367. Real Time PCR analysis was performed in an OPTICON II equipment (MJ research) using the Brilliant® SYBR® Green QPCR Master Mix (Stratagene). Relative expression analysis was performed using r18S as internal housekeeping gene, data were analyzed using QGENE software.

RESULTS AND DISCUSSION

T_1 transgenic lines preliminary screening. There were no clear difference of the agronomic traits between Palmar non-transgenic and transgenic lines. Line AA50 (Cica 8) showed differences in leaf rolling under severe condition and dry weight (Fig. 6A and 6B), but there was not difference in plant height, leaf number and tiller number (data not shown).

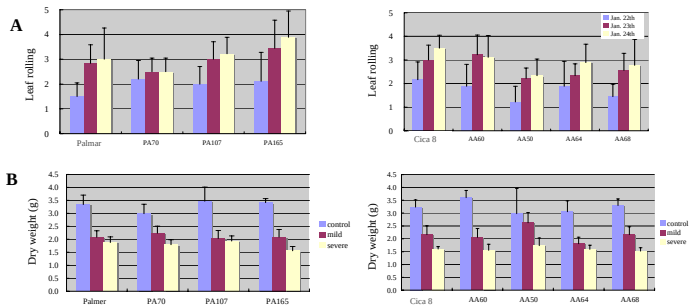

Fig. 6 T_1 transgenic lines preliminary drought screening: T_1 transgenic lines (PA70, PA107, PA165, AA60, AA50, AA64, AA68), non transgenic genotypes (Palmar and Cica 8). **A.** Leaf rolling under severe condition. **B.** Dry weight.

Endogenous *Lip9* gene expression. Palmar and Cica 8 (low drought tolerance genotypes) showed high endogenous expression of *Lip9* gene at 100% FC. At 50% FC, CT6241 and Palmar showed a change in expression levels of *Lip9* gene. Cica 8 showed high *Lip9* expression under terminal drought treatment (Fig.7). We conclude that the induction of *Lip9* promoter should be evaluated lower than 50% Field Capacity. The low expression levels found in CT6241, can be explained due to its high drought tolerance.

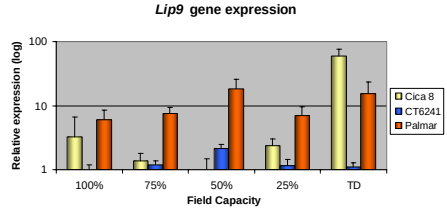

Fig. 7 Relative expression of endogenous *Lip9* gene in three rice genotypes under different field capacities. TD: Terminal Drought

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