# Deciphering the genetic and environmental control of leaf expansion under drought in rice (*Oryza sativa* L.)

Leaf expansion is an important parameter in determining the ability of a plant to intercept light and convert it into biomass. It is also one of the most sensitive processes to water deficits. To dissect the underlying mechanisms controlling tissue expansion under water deficits we used a multidisciplinary approach, combining whole-plant phenotyping, QTL mapping, and gene expression studies.

## Methods

### Dynamics of leaf elongation and growth to environmental variables

The dynamics of leaf expansion, emergence, and growth were measured in a series of greenhouse experiments. Progressive water deficits was applied using the fraction of transpirable soil water (FTSW) as the stress covariable.

### Identification of QTL for leaf extension and growth

The Vandana/Moroberekan backcross (BC) population was evaluated in the field over three seasons (2005-2007). The linkage map and genotype information was provided by Dr Brigitte Courtois (CIRAD). Leaf expansion and morphogenesis were measured and the QTL identified.

### Transcript profiling

Changes in gene expression of candidate genes related to cell elongation and drought response were investigated in the zone of leaf expansion at four levels of stress intensity (1, 0.6, 0.4, and 0.1).

## Results

### Response of leaf elongation to water deficits

Leaf elongation was highly sensitive to water deficits, consistently starting to decrease at higher FTSW values than transpiration in all genotypes (Fig. 1). Reduction in leaf elongation rate (LER) was greater as stress intensity increased (Fig. 2).

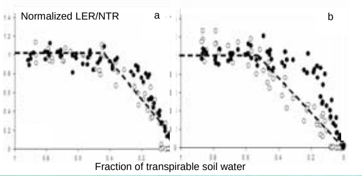

Fig 1. Normalized LER (open circles) and transpiration rates (black circles) to soil drying (FTSW) in a) Apo (drought-tolerant) and b) IR72 (drought-sensitive).

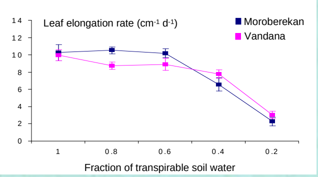

Fig. 2. LER in Moroberekan and Vandana after 1 week at target levels of drought stress (FTSW).

### Response of leaf morphogenesis to water deficits

Under severe stress (FTSW 0.2) leaf initiation and development was delayed (Fig. 3). A decrease in LER affected number of leaves, leaf area and biomass (Fig. 4). There was significant genotypic variation in biomass partitioning to leaves under drought between Moroberekan and Vandana (Fig. 5). Under drought stress, Moroberekan partitioned greater biomass leaves, whilst Vandana partitioned greater biomass to stems.

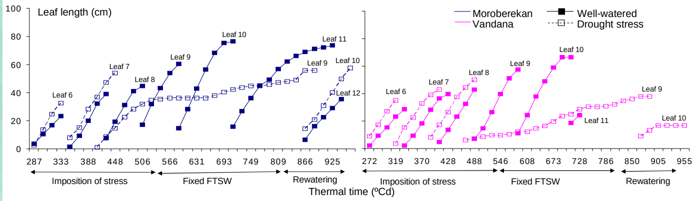

Fig. 3. Leaf emergence and elongation in Moroberekan and Vandana, a progressive water stress was applied until the target FTSW was reached and maintained for 10 days, and was then rewatered.

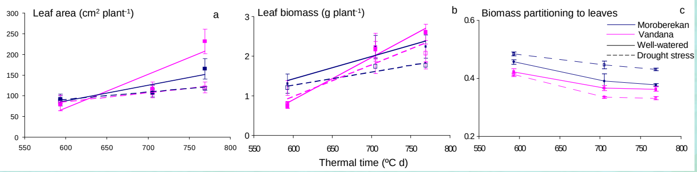

Fig. 4. The effect of field drought stress on a) total leaf area, b) leaf biomass and c) biomass partitioning to leaves in Moroberekan and Vandana. Error bars represent ±1 s.e.

Although Moroberekan LER decreased at a higher threshold FTSW value relative to Vandana, Vandana showed a greater reduction in parameters related to leaf growth than Moroberekan (Table 1).

Table 1. Percent reduction in tillering, leaves and leaf area accumulated in stress compared to well watered

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| Variety | Tiller number | Leaf number | Leaf area (cm² plant⁻¹) | Total dry matter (g plant⁻¹) |
|---|---|---|---|---|
| Morobereken | 13.15 | -1.21 | 13.3 | 53.61 |
| Vandana | 37.93 | 33.3 | 61.84 | 68.28 |

**Acknowledgments:** We thank A Reyes, N Turigan, N Sadiasa, A Los Anes, C Oca, K Ng, L Holongbayan, and N Driz for their technical support. This work is part of the Generation Challenge Program Special Funded Project 15 "*Determination of a common genetic basis for tissue growth rate under water-limited conditions across plant organs and genomes.*"

### Response of leaf elongation to vapour pressure deficit

LER is also a function of temperature and VPD. As VPD increases LER was reduced under both well watered and drought stressed plants (Fig. 5)

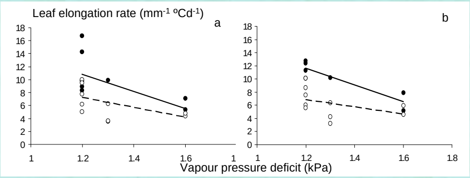

Fig. 5. Response curves of LER to vapour pressure deficit in a) Apo and b) IR72. The black circles represent well-watered (75% field capacity) and the white circles represent drought stressed plants (50% field capacity).

### QTL mapping of leaf expansion and growth under water deficits

Traits associated with leaf expansion and growth were measured under rainfed (wet season, 2005) and drought stress (dry season 2006, stress only; dry season 2007, stress and control) in the field. Within the BC population large phenotypic variation was observed for all traits. In 2005-06, a total of 20 significant QTL were identified for leaf elongation and morphology (Fig. 6). In 2007 a total of 34 QTL were identified in 18 regions for elongation, leaf area, leaf dry weight, and biomass partitioning. Preliminary analysis of QTL x environment interaction showed two common regions (Table 2). These regions on chromosome 1 and 2 were common across water treatments suggesting limited cross-over interaction between environments.

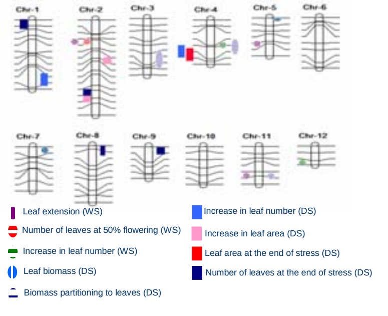

Fig 6. QTL identified for leaf growth and development in the dry season (DS) and wet season (WS). Boxes and circles represent ±1 LOD. For boxes and circles on the left of the chromosome the Moroberekan allele increased the trait.

Table 2. Common QTL from the same donor identified across years.

| Chr | Position (cM) | Trait | | |
|---|---|---|---|---|
| | | 2005 | 2006 | 2007 |
| 1 | 11-13 | | Number of leaves[a] | Leaf dry weight[a] (well watered) |
| 2 | 26-28 | Leaf extension[b] | | Leaf dry weight[a] (control) |
| | | Number of leaves[c] | | Leaf dry weight[a] (stress) |

[a]Measured at the end of the stress period, [b]measured on the 4th leaf, [c]measured at 50% flowering

### Genetic control of leaf extension

Expression profiles of four expansins in the leaf elongation zone of two cultivars (Apo and IR64) were determined. Highly significant differences were observed in expression patterns of genes between cultivars (Fig. 7). There was a significant interaction with the level of stress intensity (FTSW). Many expansins had higher expression at FTSW 0.4 corresponding to when leaf elongation began to decline. EXPA2 had the highest expression of the four expansins. Large scale expression profiling is currently under-way using an Affymetrix chip system.

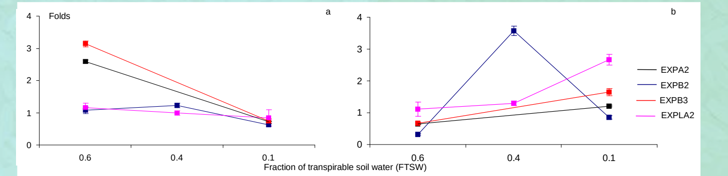

Fig 7. Average relative expression of expansins in the elongating zone of leaves under three levels of stress intensity (FTSW) in a) Apo, and b) IR64

## Conclusions

The mechanisms of leaf growth maintenance under water deficits are being dissected by whole plant phenotyping, QTL mapping and transcript profiling. LER was highly sensitive to environmental variables (water deficits and VPD). The FTSW dry-down system allowed the response of LER to be characterized across different levels of stress intensity. Highly significant genotype, stress intensity and genotype x stress intensity interaction were identified from gene expression studies. These data will be used to analyse genotype x environment interactions on leaf growth regulation under water deficits. Although QTL were not validated for LER across environments several regions were identified for leaf growth traits.

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