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

The control of leaf development and expansion under water deficit contributes to early vigor and maintenance of growth and biomass accumulation, which may be important components of crop drought resistance. 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 under water deficits

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

Gene expression of candidate genes in the zone of leaf expansion at four FTSW levels (1, 0.6, 0.4, and 0.1) was determined.

Results

Response of leaf elongation and morphogenesis to drought stress

Leaf elongation was highly sensitive to water deficits, consistently starting to decrease at FTSW than transpiration (Table 1). Significant genotypic variation in the FTSW threshold value, at which leaf elongation rate (LER) decreased, was also observed. The reduction in elongation rate was higher as the level of stress intensity increased (Fig. 1).

Table 1. FTSW thresholds for leaf extension and transpiration of five rice accessions

	FTSW thresholds	
Variety	Leaf extension	Transpiration
Apo	0.418	0.242
IR64	0.455	0.354
IR71525-19-1-1	0.376	0.218
IR72	0.445	0.204

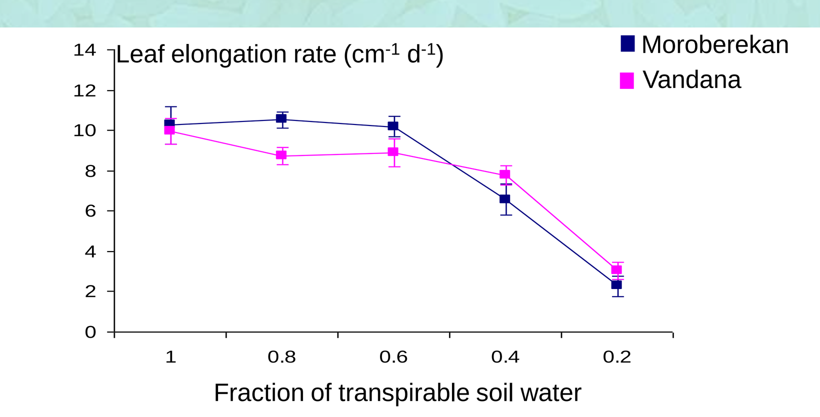

Fig. 1. Leaf extension rate in Moroberekan and Vandana after 1 week at target levels of drought stress (FTSW).

In both field and greenhouse experiments, drought stress decreased leaf elongation rate, leaf area, and biomass (Fig 3). Leaf elongation and morphogenesis were severely inhibited when water deficit (FTSW 0.2) was maintained for 10 d (Fig. 2).

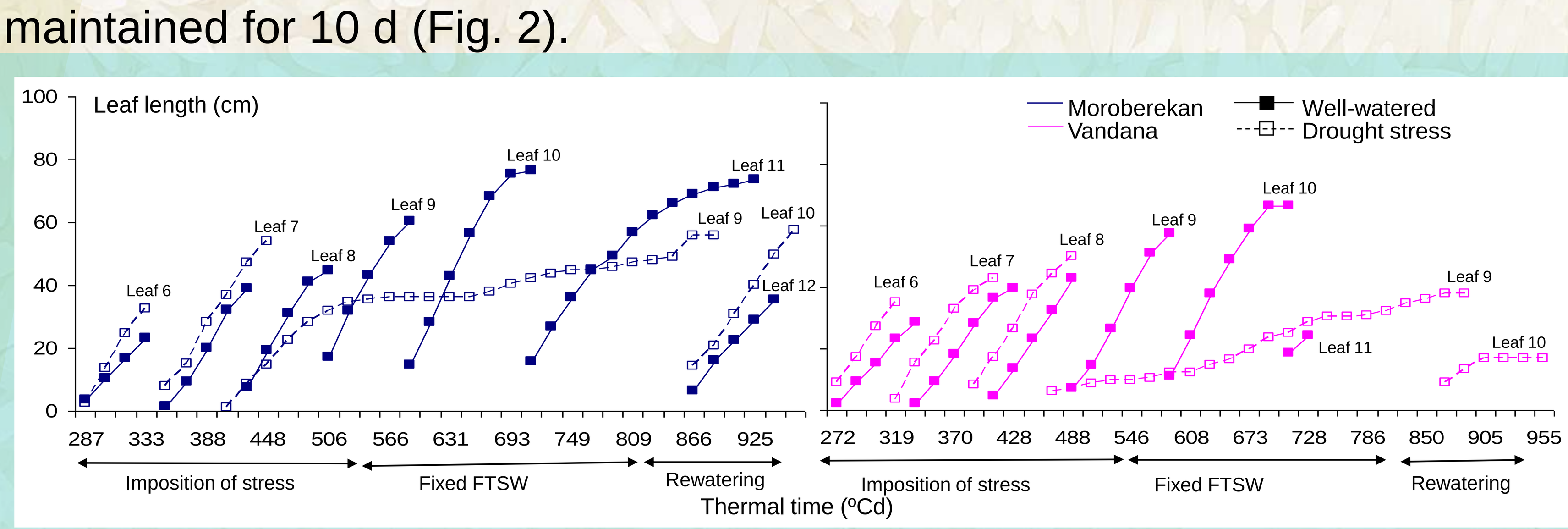

Fig. 2. Leaf emergence and elongation in Moroberekan and Vandana, a progressive water stress was applied until the target FTSW was reached and maintained for 10 days, and was then rewatered.

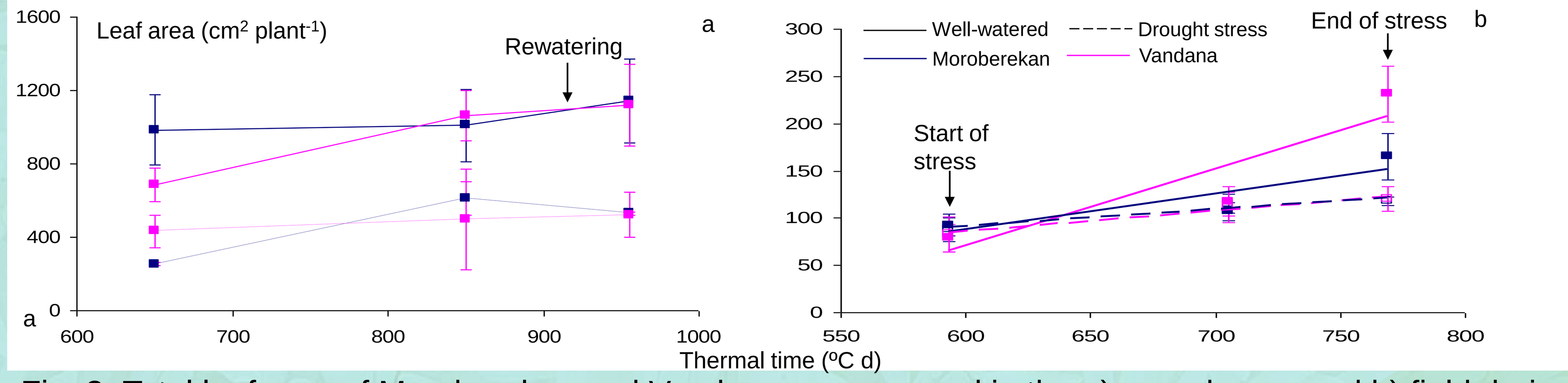

Fig. 3. Total leaf area of Moroberekan and Vandana as measured in the a) greenhouse and b) field during drought stress. Error bars represent 1 s.e.

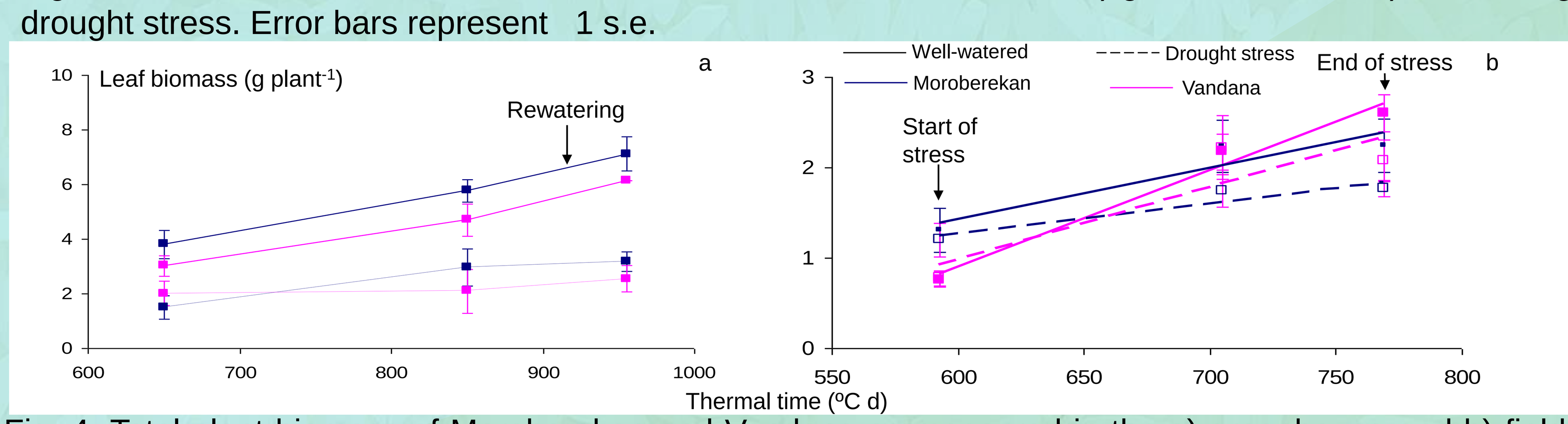

Fig. 4. Total plant biomass of Moroberekan and Vandana as measured in the a) greenhouse and b) field during drought stress. Error bars represent ±1 s.e.

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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 (Fig. 5). 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.

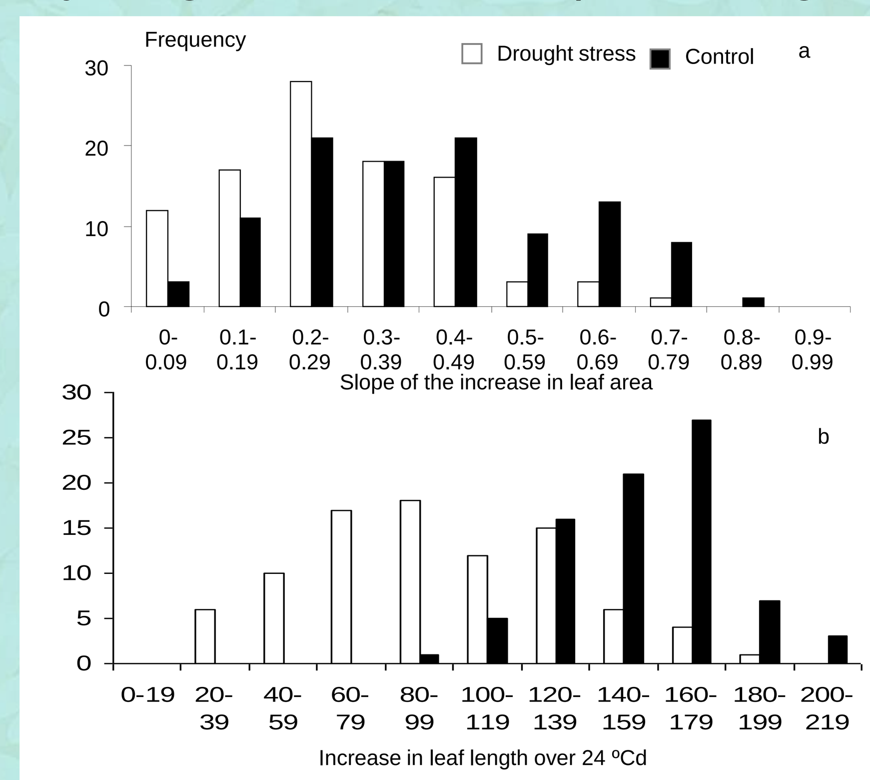

Fig. 5. Phenotypic variation in the backcross lines for a) increase in leaf area, and b) increase in leaf length (2006, dry season)

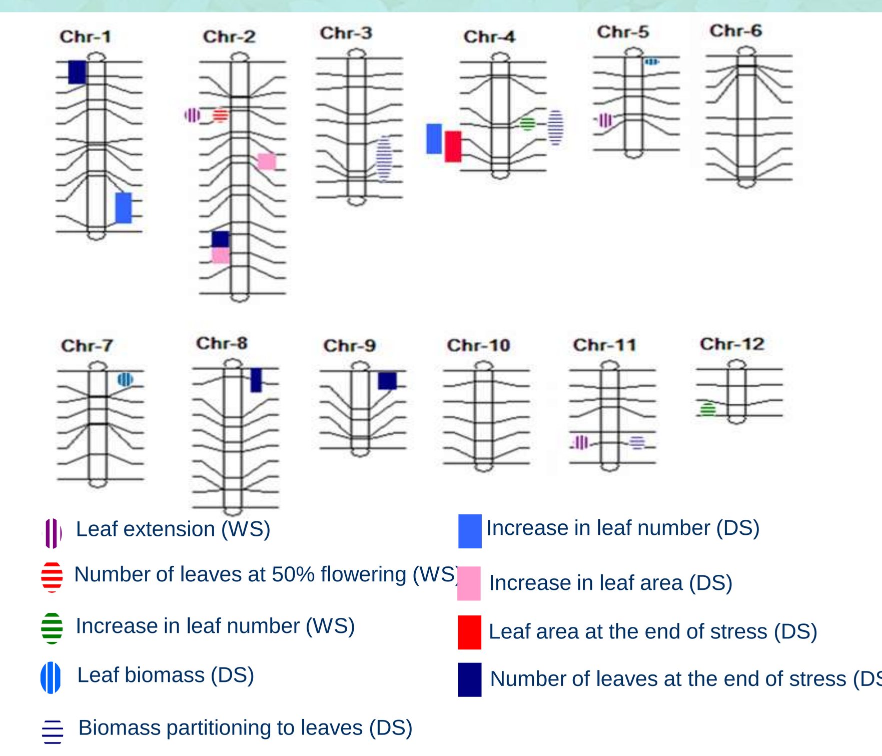

Fig 6. QTLs identified for leaf growth and development in the dry season (DS) and wet season (DS). Boxes and circles represent 1 LOD score. For boxes and circles on the left of the chromosome the Moroberekan allele increased the trait.

Preliminary analysis of QTL x environment interaction showed two common regions (Table 2). These regions were common across water treatments suggesting limited cross-over interaction between environments.

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

Chromosome	Position (cM)	Trait 2005	Trait 2006	Trait 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 and five drought candidate genes in the non-elongating zone of leaves in two cultivars (Apo and IR64) were determined. Highly significant differences were observed in expression patterns of genes between cultivars (Fig. 7 and 8) and interaction between stress intensity and cultivar. Large scale expression profiling is currently under-way using an Affymetrix chip system.

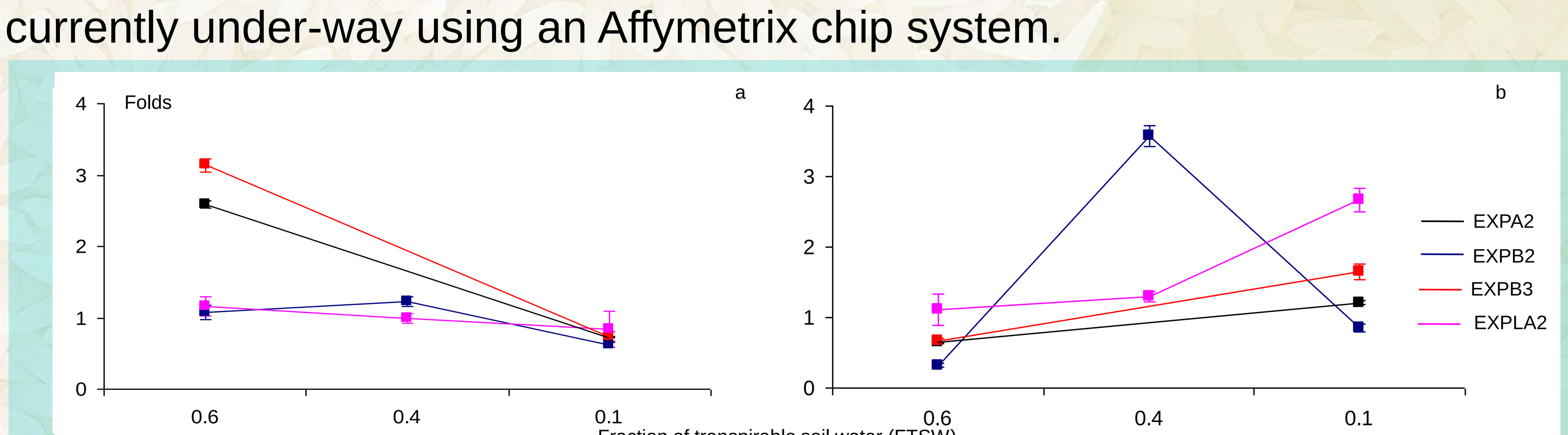

Fig 7. Average relative expression of expansins in the elongating zone of leaves under three levels of stress intensity (FTSW) in a) Apo, and b) IR64

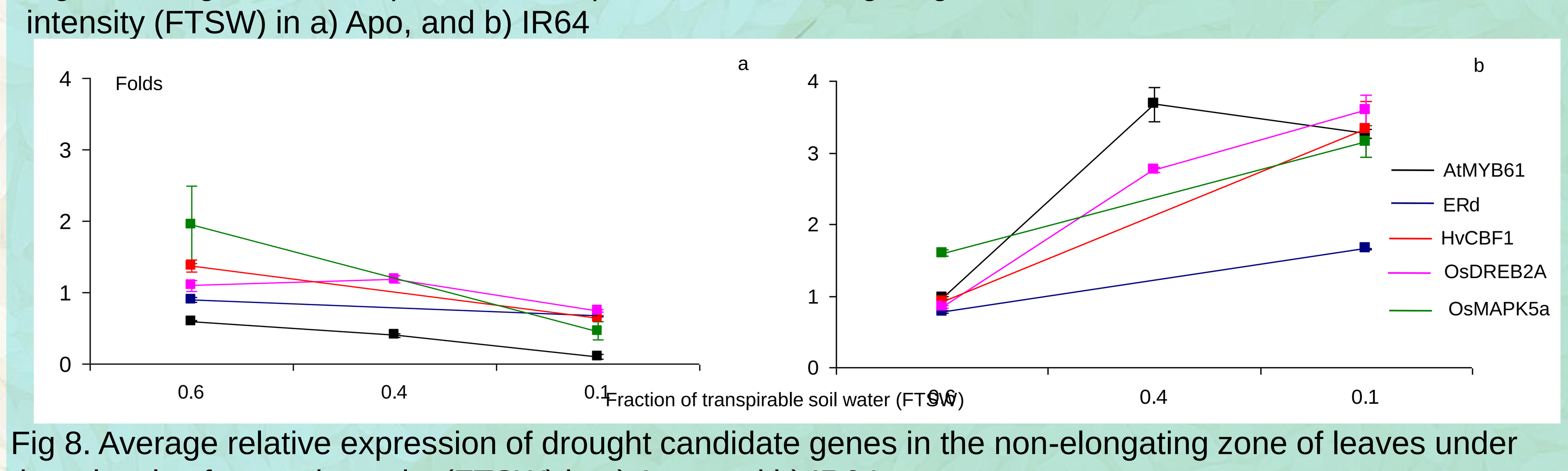

Fig 8. Average relative expression of drought candidate genes in the non-elongating zone of leaves under three levels of stress intensity (FTSW) in a) Apo, and b) IR64

Conclusions

The mechanisms of leaf growth under water deficits are being unraveled using QTL mapping, dissecting the response of leaves in the field and greenhouse, and transcript profiling. LER response to FTSW confirms genotypic variation. Although QTL were not validated for LER across environments several regions were identified for leaf growth traits. The FTSW dry-down system was used for gene expression analysis. These data will be used for analysing the genotype x environment interactions on leaf growth regulation under water deficits.

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.*"

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