Model assisted phenotyping of morphogenetic process diversity within sativa rices

Luquet, D.[1] Dingkuhn, M.[1], Clément-Vidal, A.[1] Mc Nally, K.[2]
[1] Cirad Bios department, Montpellier, France - [2] IRRI, Los Banos, Philippines

Introduction: Reasonably high throughput phenotyping methodologies are needed to search for the genetic determinisms of complex traits, such as physiological process traits. Such measurements commonly rely on time consuming and/or expensive, lab based methodologies (e.g. metabolomics, enzymology; Gibon *et al.* 2004). By formalizing physiological processes involved in complex phenotype responses to environment, plant modelling can assist in phenotyping of such traits, while reducing experimental costs and thus increasing throughput (Dingkuhn *et al.* 2005; Hammer *et al.* 2002).

As presented by Luquet *et al.* (WPM proceeding #C3_5), the model EcoMeristem, which dynamically simulates rice morphogenesis and phenotypic plasticity based on plant internal competition for carbon (Luquet et al. 2006), was recently adapted simulate rice response to drought. The objective of the present study is to test the added value of using EcoMeristem model when used along with metabolomics (sugar) phenotyping on a population of *O. sativa* taken from the GCP core collection. The final goal is to use model parameters for process based traits in genetic association studies, in order to extract from the available genetic diversity genes and alleles involved in rice phenotypic plasticity.

Material and methods: 200 genotypes (covering seven genetic groups, with a majority of indica) were cropped at IRRI experimental station (Los Baños, Philippines) under irrigated upland conditions. Experimental design consisted of 3 replications (blocks); water was fully supplied until first sampling date (S1) at 35 days after emergence (DAE). At this date, main stem leaf number, main stem length (*LLL*) and width of last ligulated leaf, tiller number *TN* and shoot dry weight (*SDW*, separating main stem sheaths (for sugar content analysis) from the rest), were characterized. From S1, water supply was withheld and a second sampling S2 was planned around 45 DAE. Unfortunately a typhoon falling into the stress period made meaningful analysis of S2 data difficult. This paper thus only focuses on rice vegetative morphogenesis under well-watered conditions. Observations at S1 were used to run EcoMeristem in an inverse mode (optimization) to extract genotypic parameters related to morphogenetic processes.

Results: Three categories of phenotypic traits were characterized: direct morphological measurements, model parameters (see detail in WPM proceeding C3_5) and sugar concentrations. In all three cases, some traits showed genotype effects and some (but not always the same) showed genetic-group effects (Table 1). Among sugar parameters, only starch varied among genotypes and sucrose and hexoses did not, probably because the long sampling period (half a day - morning) fell into a period of strong diurnal dynamics of tissue sugar concentration and thus gave high variance. Genetic group effects were also detected in each category of traits (Table 1), sugar and model parameters showing similar classification of genetic groups (not presented). Model parameters and related measurements showed significant correlations (e.g. *Ict* vs. *TN*, *MGR* vs. *LLL;* not presented). Low but significant correlations between sugars and morphological variables, and also between sugars and model parameters, were observed (not presented); in particular, starch was negatively and sucrose (and soluble sugars) positively correlated with vigor-specific variables (*SDW*, tiller number, haun index) or parameters (*MGR, 1/phyllochron, 1/Ict*). This might indicate that sheath sucrose accumulation is associated with low and starch accumulation with low vegetative vigor.

The single trait explaining the most variation in bulk SDW accumulation across genotypes was the model parameter *Ict* (tillering thrshold, explaining 41% of *SDW*). Factorial Discrimination analysis using optimized, genotypic model parameter values (*GDW,LELini MGR, Phyllo, Ict and SLAp*) showed that 2 axes explained 92% of diversity, with tropical japonica and temperate japonica forming distinct clusters (Fig. 1). Interestingly, the model parameters affecting development rate (*Phyllo*) and growth rate of organ size (*MGR*) had effects similar to initial seed and leaf size (*GDW, LEL1*), which are known to affect early vigor. Tillering threshold (*Ict*) and SLA dynamics (*SLAp*) also had similar effects.

Type	Variable name	Line effect	Genetic group effect
Observed	Tiller nb	Y	Y
Observed	Shoot dw	Y	Y
Observed	Haun index	N	N
Observed	LLL / rank	Y	Y
model	MGR	Y	Y
model	Phyllo	N	N
model	Ict	Y	Y
model	SLAp	N	N
Biochem	Glucose	N	N
Biochem	sucrose	N	Y
Biochem	starch	Y	Y

Table 1: ANOVA for genotypic and genetic group effects regarding sugar ("biochem"), measurements ("observed") and model parameters ("model"). "Y" = significant and "N" = non significant effects (P>0.05). LLL: last ligulated leaf length; MGR "Meristem Growth Rate", controlling successive leaf dimensioning; Ict, controlling tillering through carbon supply, SLAp, slope parameter to compute SLA; phyllo for phyllochon).
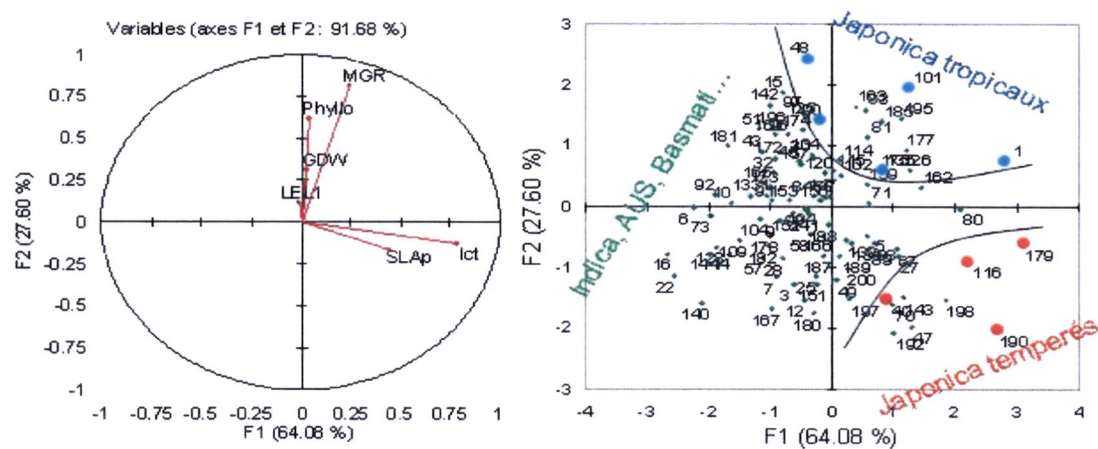

Figure 1: Factorial discriminative analysis of genotypes and genetic groups using EcoMeristem model parameters: LEL1, 1st leaf length; GDW, seed dry wt; MGR, meristem growth rate; Ict, threshold for tillering;, SLAp, slope parameter for change in specific area; Phyllo, phyllochrone. Left: parameters explaining main axes. Right: distribution of genotypes (blue: tropical japonica, red: temperate japonica and green: indica + others).

Conclusion: Results demonstrated the relevance of model parameters for phenotyping rice populations for morphogenetic process traits. Tillering threshold parameter *Ict* was most predictive of SDW across genotypes. Model parameters showed significant correlations with related measurements but also with independently measured sugar concentrations, indicating that the morphogenetic parameters bear a relationship with sink-source relationships. Model parameters discriminated to some extent among genetic groups, and this observation deserves further study. Model parameters process based traits seem thus to be a promising approach for the phenotyping of rice populations in the context of association studies. But this work needs to be further extended to drought.

References

Dingkuhn M, Luquet D, Quilot B, Reffye PD (2005) Environmental and genetic control of morphogenesis in crops: Towards models simulating phenotypic plasticity. *Austr J. Agric. Res.* **56**, 1-14.

Gibon Y, Blaesing OE, *et al.* (2004) A Robot-Based Platform to Measure Multiple Enzyme Activities in Arabidopsis Using a Set of Cycling Assays: Comparison of Changes of Enzyme Activities and Transcript Levels during Diurnal Cycles and in Prolonged Darkness. *The Plant Cell* **16**.

Hammer GL, Kropff MJ, Sinclair TR, Porter JR (2002) Future contributions of crop modelling - from heuristics and supporting decision making to understanding genetic regulation and aiding crop improvement. *Euro.J. Agron.* **18**, 15-31.

Luquet D, Dingkuhn M, Kim HK, Tambour L, Clément-Vidal A (2006) EcoMeristem, a Model of Morphogenesis and Competition among Sinks in Rice. I. Concept, Validation and Sensitivity analysis. *Funct. Plant Biol.* **33**, 309-323.