

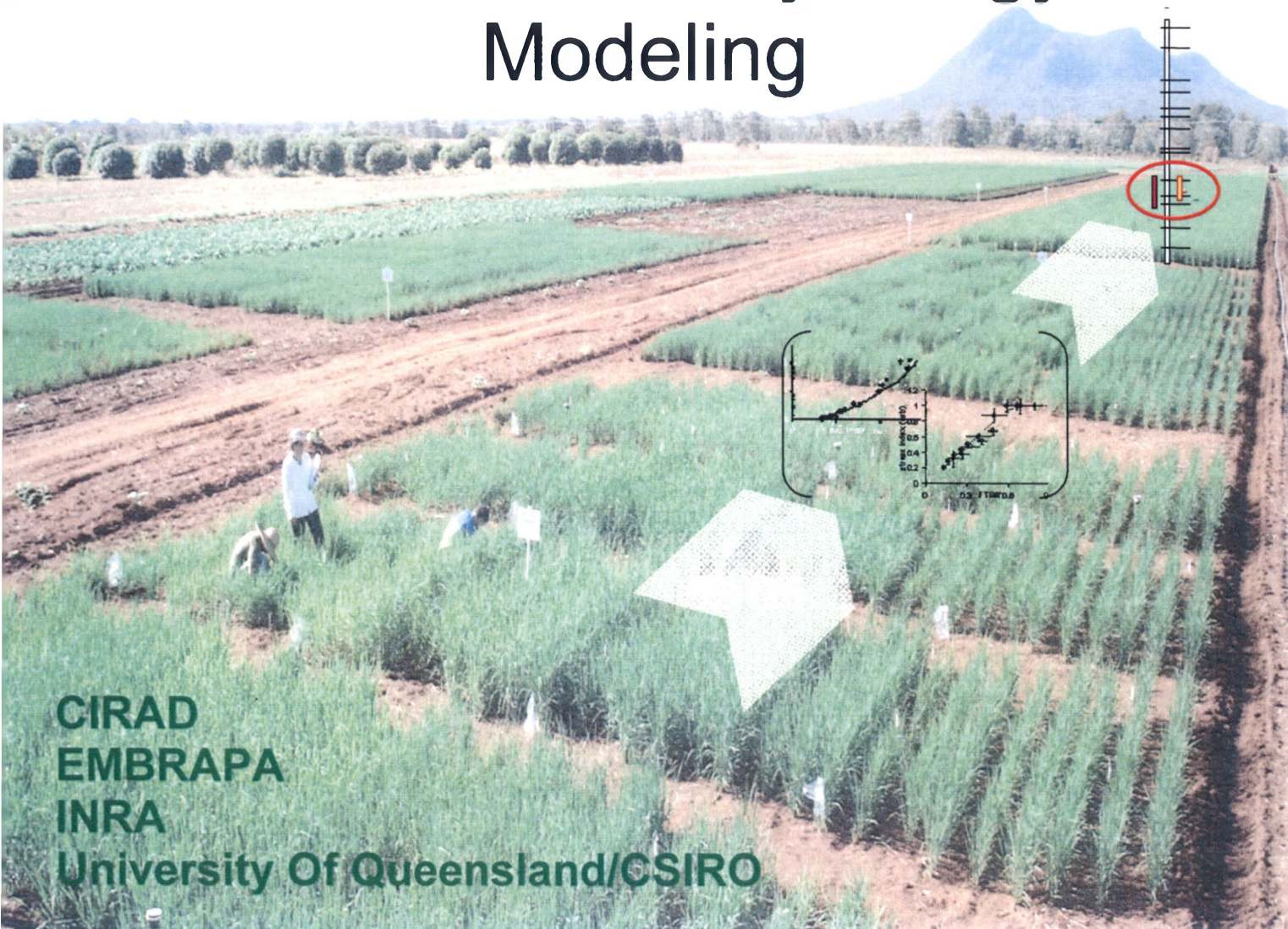
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Commissioned research project 2005#7

Whole Plant Physiology Modeling



Final meeting proceedings
Organized at Pioneer - Johnston, Iowa, USA

PI: Delphine Luquet, Cirad (luquet@cirad.fr)

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Whole Plant Modelling project of the Generation Challenge

Programme (SP 1)

Final Meeting, 4-7th February 2008 - Programme

Meeting hosted by Pioneer (USA), co-organized by Pioneer and Cirad (France)

Project PI: Delphine Luquet, CIRAD

Project participants: Cirad, Embrapa, INRA, University of Queensland / CSIRO

Pioneer contacts: Mark Cooper, Stephen Smith and Leslie Lorenc

Location: Pioneer, Johnston, Iowa, U.S.A.

Dates: 4-7th of February 2008

Participants (WPM Project & invited)

Embrapa (WPM participants; 5)

Camilo Andrade
Reinaldo Gomidie
Cleber Moraes
Edson Bastos
Alexandre Heinemann

INRA (WPM participants; 3)

Claude Welcker
François Tardieu
Karine Chenu

UQ (WPM participants ; 4)

Scott Chapman
Graeme Hammer
Erik Van Oosterom
Greg Mc Lean

Cirad (WPM participants; 4)

Michael Dingkuhn
Delphine Luquet
Nourollah Ahmadi

Invited speakers (3)

José Araus (Cimmyt)
Yves Gibon (Max Planck Institute, Golm)
David Jordan (DPI, Australia)

Pioneer speakers (2)

Mark Cooper
Charlie Messina

Other participants

José Crossa (Cimmyt)

... and participants to be appointed by Pioneer

➤ Day 1

8:00am

- Welcome address by Pioneer (Mark Cooper or Stephen Smith, 15min)
- Introduction and presentation of project components (D Luquet, 15min)

Component 1- Model assisted environment characterization (TPE)

Session chaired by R. Gomide

8:30am: Keynote by David Jordan: "Progress towards the integration of model based environmental characterization in an applied sorghum crop improvement program" (30 min presentation, 15 min discussion).

9:15am: Charlie Messina (Pioneer): "Developing modelling technologies to enhance molecular breeding for drought tolerance in temperate maize" (30 min presentation, 15 min discussion).

*10:00am*****coffee*

10:20am: A. Heinemann: "Characterization of drought stress environments for upland rice and maize in central Brazil" (30 min presentation, 10 min discussion).

11:00pm: M. Dingkuhn – "The West African TPE for sorghum: varietal fit to agro-ecological zones" (30 min presentation, 10 min discussion).

*11:45pm*****Lunch*

1:00pm: Discussion chaired by S. Chapman; Perspectives for model assisted TPE characterization vs. breeding - where we are, where do we go? Methodology usefulness in GCP context and beyond.

*3:00pm*****Coffee*

Component 2: multi-site trial model assisted trait evaluation for breeding

Session chaired by C. Andrade

3:20pm - D. Luquet: Introduction on Component 2 objectives, challenges and difficulties (10min)

3:30pm: Keynote by José Araus – "Maize phenotyping for drought adaptation: contribution of physiological tools". (30 min presentation, 15 min discussion).

4:15pm: Mark Cooper (Pioneer): "Applying modeling technologies within a maize drought molecular breeding program". (30 min presentation, 15 min discussion).

5:00pm end of first day

Evening free

➤ Day 2

Session chaired by C. Welcker

8:00am: C. Andrade, E. Bastos & C. Morais Guimaraes. Experiment design, results & difficulties (75min).

- "Challenges and difficulties of imposing and monitoring crop water stress on field trials." by C. Andrade (20min)
- Rice and maize drought phenotyping for modelling application (E. Bastos & C. Guimaraes) (20min)

9:00am: A. Heinemann: "Crop model assisted characterization of appropriate traits for the rice TPE in Brazilian Cerrados" (30 min presentation, 15 min discussion).

X CIRAD
8

*****9:45am Coffee

10:00pm: debate chaired by C. Welcker. Difficulties of complex/fine trait phenotyping in the field. Usefulness of modelling. Lessons learned and perspectives.

*****12:00pm lunch

Component 3: Model improvement for assisting process based trait phenotyping and connection to genetic information

Session Chaired by A. Heinemann

1:30pm, D. Luquet. Introduction – reminder of Component 3 objectives (10min)

X CIRAD
4

1:40pm: Keynote by Yves Gibon: "Metabolic Phenotyping and Plant Performance" (30min presentation, 15min discussion).

2:25-pm K. Chenu, F. Tardieu, G. Hammer, S. Chapman, C. Welcker :

- 1- "Modelling the GE interaction for leaf and silk growth rates in maize: genetic analyses of time courses and responses to environmental conditions".
- 2- "Modelling the GE interaction in maize: from organ to whole plant and yield". (About 25min each presentation and 30 min discussion)

*****3:45am Coffee

4:15pm: M. Dingkuhn "Phenotyping of sorghum photoperiod responses using heuristics". (20min presentation, 15 min discussion).

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5

5:00pm End of day 2

Evening free

➤ Day 3

Component 3 (2nd session)

Session chaired by G. Hammer

8:30am: D. Luquet, M. Dingkuhn, N. Ahmadi - EcoMeristem model applied to rice phenotyping

- "Modeling drought effects on rice with EcoMeristem: Feedbacks of water and carbohydrate relations on phenotypic plasticity". 1
- "Model assisted phenotyping of a rice mapping population for morphogenesis regulation related QTL detection: proof of concepts". 2
- "Model assisted phenotyping of morphogenetic process diversity within sativa rice" 3

(15 min each presentation; 30 min discussion)

***** 10:30am Coffee

11:30am: debate chaired by G. Hammer. Discussion & perspectives. High throughput phenotyping for complex or opaque traits, association of model derived traits with genomic information: where are we, where do we go?

***** 12:30pm Lunch

2:00pm: General discussion on project outputs, lessons learned and future project objectives

Closure of the workshop (D. Luquet)

*****3:30pm coffee

4:00pm Discussions of potential future involvement of Pioneer in relevant GCP projects that follow on from the current work.

6:00pm end of day 3

Dinner organized by Pioneer

➤ Day 4

8:30am: Visit of Pioneer.

12:00am end of the workshop

*****lunch

1:00 to 4:00pm: departures to the airport to be organized

Component 1

Model assisted characterization
of Target Population of
Environments for breeding (TPE)



***Whole Plant Physiology Modelling Project
Final meeting 4-7 February 2008
Pioneer, Johnston, Iowa (USA)***

Integrating model-based environmental characterization in an applied sorghum crop improvement program

David Jordan¹ and Graeme Hammer²

¹ *Queensland Dept Primary Industries and Fisheries, Hermitage Research Station Warwick QLD 4370 Australia*

² *APSRU, School of Land, Crop and Food Sciences, University of Queensland, Brisbane, Qld 4072, Australia*

Introduction

Comstock (1977) defined the concept of a target population of environments (TPE) associated with a breeding program as the complete set of "types" of environments in which cultivars can be grown within the geographical area targeted by a breeding program. From the plant breeder's perspective the critical factor that separates one environment type (ET) from another is the degree to which the different ETs cause re-ranking of genotypes within trials (ie genotype x environment interaction).

Breeding programs faced with genotype x environment interaction (GxE) have traditionally used multi-environment trials (METs) in an attempt to representatively sample the TPE. This approach can be used to identify genotypes with general adaptation to most of the "types" of environments within the TPE. Alternatively, if types of environments are sufficiently frequent, breeders may also attempt to exploit specific adaptation.

Once ETs have been defined, breeders are interested in the ETs represented in the current year's trials and the frequency and distribution of those ET in the TPE. Weighting trial results based on their ET frequency in the TPE can lead to improved response to selection (Podlich et al., 1999). In the past, various forms of GxE analyses have been used to group trials into relevant ETs. While this approach has the advantage of being relevant to the breeding program, it requires analysis across seasons to achieve relatively unbiased sampling of the TPE. Leaving aside issues to do with sampling variability, such analyses are complicated as they involve unbalanced sets of genotypes and can be influenced by changes in the genetic composition of breeding populations and changes in management systems. The approach of using environmental data either directly (van Eeuwijk et al, 2005) or in a more integrated fashion via biophysical crop simulation modelling (Chapman et al, 2000) has been demonstrated as a way of effectively characterising breeding environments. While this approach is theoretically attractive and cost effective, care must be taken to ensure it reflects the reality of the TPE.

ET's in water limited environments result from the complex interaction of the genotypes under test with uncontrolled biotic and abiotic factors sampled by the trial. This is further complicated by variation in controlled factors, such as crop management. In such situations particular genotype characteristics (e.g. phenology, tillering) change the pattern of water use over the season so that entries in the same trial may experience different ETs. This may explain some of the differences among genotype rankings across trials. Recently simulation modelling has matured to the point where it may be possible to use modelling to explain components of genetic variation at the individual genotype level, thus generating potential to be exploited to increase response to selection (Cooper and Hammer, 2005; Hammer et al., 2006; Hammer and Jordan, 2007). In this presentation we describe our efforts towards using crop simulation to –

- characterise Australian sorghum production environments,
- validate various characterisation approaches, and
- link with analysis procedures to enhance genetic gain.

Materials and Methods

Environment Characterisation

A set of locations and management systems (population and row spacing) were selected to represent the main sorghum cropping regions in NE Australia. Locations were matched with soil characterisation data and 100 years of climatic data and crop simulation runs were

conducted using the sorghum model in the APSIM cropping system simulation platform (Keating et al., 2003). Twenty five virtual genotypes were created via a factorial combination of 5 rates of phenological development and 5 tillering propensities. A continuous sorghum cropping system was assumed so that each simulated crop-year reflected the sowing of sorghum following a summer crop the previous year. The APSIM sorghum model was used to generate a simulated crop for each year x location x management x genotype combination. This simulated data set was used for a range of purposes in the study.

Field Trials

A set of sorghum field trials consisting of 21 environments over 4 years and containing ~3000 genotypes (~1000 genotypes/year) was used as a source of phenotypic data (yield, phenology, tillering plant height) for GxE analysis. Nine of these breeding trials were characterised (climate, soil water attributes) to the extent that the sorghum model in APSIM could be run as a “virtual entry” in the trial.

Results and Discussion

Results will be presented to show -

- The nature and frequency of representative “crop perceived” seasonal stress patterns for the target population of sorghum production environments in NE Australia.
- Characterisation of the breeding trial sites (soil, climate, crop performance) and generation of “virtual genotypes” as entries for use with actual breeding data.
- Comparison of various methods of clustering simulated seasonal stress patterns to identify representative environment types that best explain the observed GxE
- Simulation of the differing stress patterns associated with specific entries in the breeding trials, allocation of season type to those entries, and investigation of the potential to remove the impact of explainable phenotypic variability in GxE analysis

Discussion will focus on the benefits and costs to the breeding program via the integration of such model-based approaches

References

- Chapman, S.C., Cooper, M.C., Hammer, G.L., and Butler, D. (2000). Genotype by environment interactions affecting grain sorghum. II. Frequencies of different seasonal patterns of drought stress are related to location effects on hybrid yields. *Australian Journal of Agricultural Research*, 51: 209-222.
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- Hammer, G.L. and Jordan, D.R. (2007) An integrated systems approach to crop improvement. In, J.H.J. Spiertz, P.C. Struik and H.H. van Laar (eds.) *Scale and Complexity in Plant Systems Research: Gene-Plant-Crop Relations*. Wageningen UR - Frontis Series No. 21, Springer, Dordrecht, The Netherlands. pp. 45-61.
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- van Eeuwijk, F.A., Malosetti, M., Yin, X., Struik, P.C., and Stam, P. (2005) Statistical models for genotype by environment data: from conventional ANOVA models to eco-physiological QTL models. *Australian Journal of Agricultural Research* 56: 883-894.



***Whole Plant Physiology Modelling Project
Final meeting 4-7 February 2008
Pioneer, Johnston, Iowa (USA)***

Characterization of drought stress environments for upland rice and maize in central Brazil

¹Alexandre B. Heinemann, ²Michael Dingkuhn, ²Delphine Luquet, ²Jean Claude Combres and ³Scott Chapman

Introduction

In the Cerrado environments of central Brazil, breeding of rainfed upland rice and maize is based on early screens for agronomic traits such as crop duration, plant vigour, panicle and grain weight, commonly characterized under irrigated conditions on research stations. Only once lines reach advanced testing stages, they are evaluated under natural bio-physical constraints that characterize farmers' fields.

The Cerrado is a complex and extensive biome undergoing rapid changes in land use. The rainfall averages about 1200 mm per year, falling mainly (ca. 1000mm) from November to January (cropping season). Upland rice plays an important role in bringing areas into cultivation because of its comparative tolerance to acid, aluminum-toxic soils (Pinheiro et al., 2006). After two or three years of rice cropping in combination with dolomite and phosphate amendments, cropping systems based on intensified pasture and cash crops such as soybean and maize are established.

Meaningful breeding for drought tolerance requires information on the inter and intra-annual probability of drought occurrence, as well as on the characteristics of prevalent drought types such as duration, intensity (which in turn depends on soil depth) and timing with respect to crop phenology. This information can be captured by defining the target population of environments (TPE) for a given crop (rice and maize). The TPE is defined as the set of environments, including spatial and temporal variability, to which improved crop varieties developed by a breeding program need to be adapted.

This study explores possibilities to adjust regional breeding systems to optimally fit the range of environments they are targeted (TPE), using the example of upland rice and maize in Brazil's Cerrados.

Material and Methods

A crop simulation model, from Ecotrop platform (Dingkuhn et al., 2003), was used to determine drought stress patterns for 12 locations and more than 30 environments (6 years x 5 to 6 planting dates) for short and medium duration rice crops (planted in early summer), and for maize grown either as a first or second crop in the summer cycle. Simulation regression analysis, realized to verify drought stress impact on yield for both crops, confirmed the greater drought impact in both crops (quantified as the ratio of water-limited to potential transpiration) when it occurred around the time of flowering and early grain-filling.

To develop a typology of drought patterns for each rice and maize TPE, a four-dimensional matrix consisting of location, sowing date, year and growth phase (100 °C.d period) was established for the mean *cstr* simulated for the 100 °C.d periods. This classification employed a hierarchical agglomerative clustering. For each TPE, the simulated drought stress scenarios were classified into three main groups based on the similarities in the phenological sequence patterns of *cstr*. Similar drought classification procedures were described previously by Chapman et al. (2000).

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Results

For rice, mild mid-season droughts occurred 40 to 60% of the time in virgin (0.4m deep for rice or 0.5m for maize) soils and improved (0.8m for rice or 1.0m for maize) soils, with a yield reduction of <30%. More severe reproductive and grain-filling stress (yield reductions of 50% for rice to 90% for maize) occurred less frequently in rice (<30% of time) and 1st maize crop (< 10% of time). The 2nd maize crop experienced the greatest proportion (75 to 90%) of drought stresses that reduced yield to <50% of potential, with most of these occasions associated with later planting.

The rice breeding station (CNPAF) experiences the same pattern of different drought types as for the TPE, and is largely suitable for early-stage selection of adapted germplasm based on yield potential. However, selection for virgin soil types could be improved by breeding on some less-improved soils in the slightly drier parts of the TPE region. Similarly, the drought patterns at the maize research station (CNPMS) and the other maize screening locations are better suited to selection of lines for the improved soil types. Development of lines for the 2nd crop and on more virgin (acidic) soils would require more targeted selection at late planting dates in drier sites.

Conclusion

For upland rice, both short and medium-duration, the region can be characterized by three different stress patterns. For upland rice short cycle the stress patterns were low (L), mid-season (M) and terminal (T) stress and for medium cycle low stress (L), vegetative stage stress (V) and mid-season to terminal stress (MT). For the scenario with no physical restrictions on root development, the stress level does not limit breeding for potential yield;

For maize as first crop the region also can be characterized by three different stress patterns, nearly environment free of stress (L), a mild, mid-season stress pattern covering both the reproductive and early grain filling periods (M), and severe drought occurring during the late reproductive period (SR). For the scenario with no physical restrictions on root development, the stress level also does not limit breeding for potential yield;

For maize as second crop, the breeding target should be "escaped" (developing of earlier-season cultivars) and/or development of a drought breeding program specific for terminal stress.

References

- Pinheiro, B. da S.; Castro, E. da M. de, Guimaraes, C.M. (2006) Sustainability and profitability of aerobic rice production in Brazil. **Field Crops Research**, 97, 34-42.
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***Whole Plant Physiology Modelling Project
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The West African target population of environments for grain sorghum: Varietal fit to agro-ecological zones

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Problematic. Breeders need tools to translate variable climatic conditions in dry environments into probable crop performance, and to translate genotype profiles into agro-ecological fit. This can be done by model-assisted characterization of target populations of environments (TPEs). West African sorghum cultivars require, in addition to drought tolerance, effective phenological mechanisms for temporal escape from drought and excessive humidity that would favour pests and diseases during sensitive development stages.

Approach. A simulation experiment was conducted to predict the potential and attainable (water limited) growth and yield of three sorghum genotypes differing in plant type and response to photoperiod for combinations of five sowing dates and three sites on the N-S climatic gradient in Mali, for the period from 1971 to 2004. The model used was SARRA-H equipped with the phenological model *Impatience*. Onset and end dates of the rainy season were estimated with the simple soil water balance model BIP. The resulting scenarios were evaluated on the basis of i) escape from drought, ii) escape of grain development phases from periods of high pest and disease pressure and iii) the resulting “safe” periods for sowing. The latter took into account the agronomic advantage associated with early sowing, observed by farmers to minimize weed competition and decreasing soil fertility during the wet season.

Environments. Three sites in Mali representative of different agro-climatic zones were studied, Nara (15°10' N, 7°17' E, 265 m asl; Sahel), Bamako (12°17' N, 7°57' E, 381 m asl; Sudan savannah) and Sikasso (11°21' N, 5°41' E, 375 asl; Guinea savannah). All sites have a hot, tropical climate with a mean, annual, maximal (minimal) daily temperature of 36.0 °C (21.7 °C) at Nara, 34.4 °C (21.9 °C) at Bamako and 33.7 °C (21.0 °C) at Sikasso. Monthly patterns of temperature and potential evapotranspiration (PET) are presented in Table 1. All sites have a mono-modal pattern of rainfall with a distinct rainy season in summer, accounting on average for 394 mm at Nara, 885 mm at Bamako and 1099 mm at Sikasso.

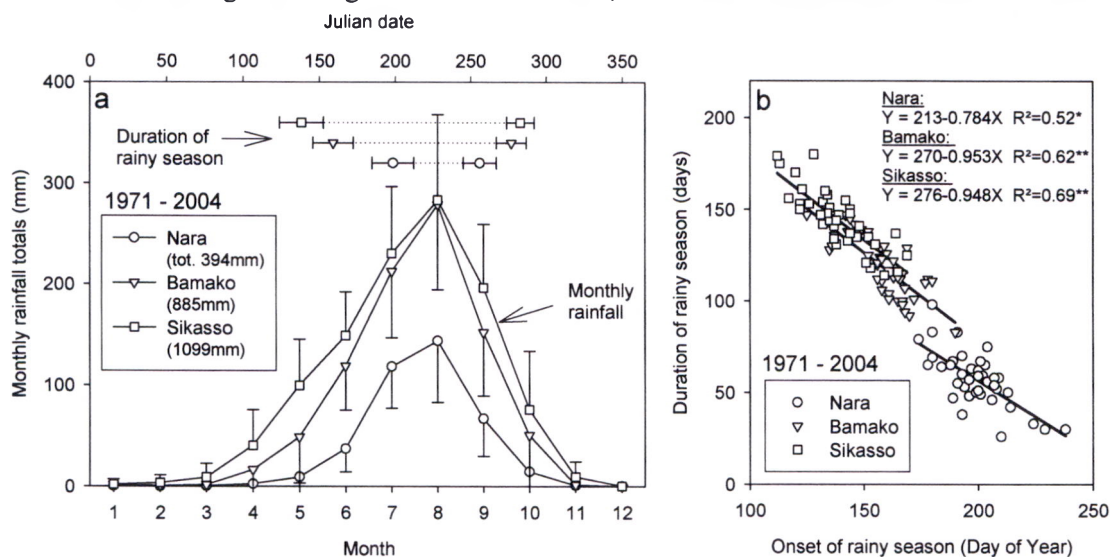


Fig. 1. Rainy season at Nara, Bamako and Sikasso for 1971-2004. **a:** Monthly rainfall (solid lines) and mean duration of rainy season \pm s.d. (dotted). **b:** Duration of rainy season vs. its date of onset.

Genetic materials. Three well characterized (Kouressy et al., 2007) sorghum (*Sorghum bicolor* L.) genotypes were selected for this study to represent different plant types available to farmers in West Africa. V1 is a tall (up to 4.5 m), traditional, highly photoperiod-sensitive *Guinea* landrace from southern Mali with local name *Kendé Ngou*. V1 produces less than 2.0 Mg ha⁻¹ grain but aboveground dry matter can exceed 20 Mg ha⁻¹. V2 is an improved dwarf cultivar (1.7 m) sharing 75% of V1's genome as well as its photoperiod sensitivity. It produces less biomass but more grain (up to 3.5 Mg ha⁻¹) than V1. Both V1 and V2 enable flexible cropping calendars because photoperiodic response makes flowering relatively

independent from sowing date. V3 is a dwarf (1.7 m), early-maturing, photoperiod-insensitive, high-yielding (5.0 Mg ha^{-1}), *Caudatum* hybrid developed by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), coded ICSH89002. V3 must be sown at a specific date in order to synchronize flowering with the end of the wet season (to avoid drought, pest and disease problems during grain filling).

Results & conclusion. Simulation results indicated that potentially high yielding and photoperiod insensitive “modern” cultivars such as V3 have an advantage only in the north where the rainy season is short (Nara, Fig. 2). Sensitive response of flowering to photoperiod was essential for more humid environments having a long wet season, resulting in appropriate timing of flowering and greater flexibility of crop calendars. Traditional V1 was well suited to Bamako and Sikasso, improved V2 only to Bamako and the hybrid V3 only to Nara. These results are supported by experience from multi-location trials. In conclusion, the present methodology can be used to identify phenological and drought response traits required for specific agro-ecological zones, and to target existing genotypes to the most suitable environments.

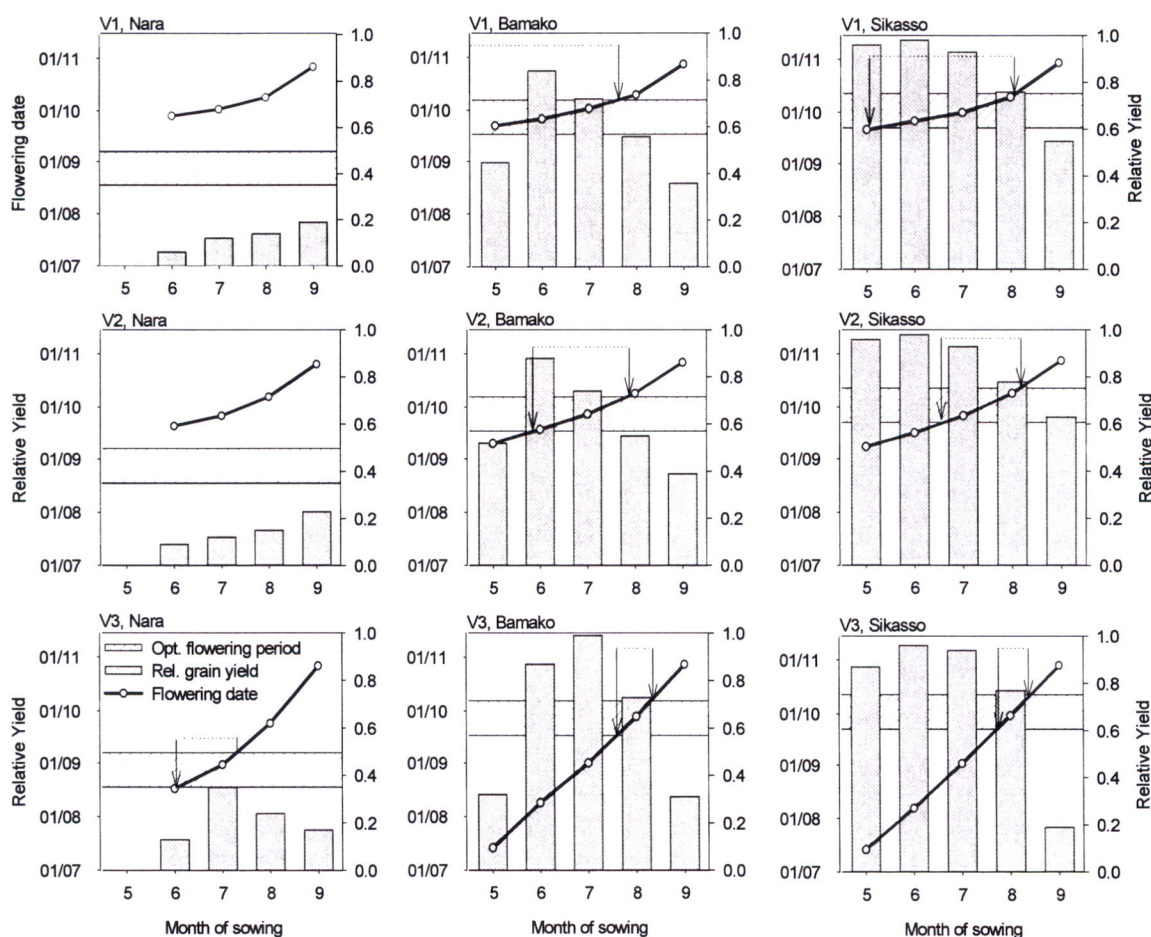


Fig. 2. Simulated date of flowering (circles and solid line) and relative, water limited over non-limited grain yield (vertical bars, refer to scale on right) for 5 sowing dates (01 May to 01 September), 3 sorghum cultivars (V1-V3) and 3 sites (Nara, Bamako and Sikasso). The horizontal bar indicates the optimal period for flowering (last 20 days before end of wet season) for avoidance of biotic stresses, resulting in a cultivar specific “window” for sowing date (marked by arrows).

Articles reporting these results:

- Dingkuhn, M., Kouressy, M., Vaksman, M., Clerget, B., Chantereau, J. 2007. Applying to sorghum photoperiodism the concept of threshold-lowering during prolonged appetence. *European Journal of Agronomy* (in press).
- Kouressy M., M. Dingkuhn, M. Vaksman, A. Clément-Vidal and J. Chantereau. 2007. Potential contribution of dwarf and leaf longevity traits to yield improvement in photoperiod sensitive sorghum. *European Journal of Agronomy* (in press).
- Kouressy M. Dingkuhn M. Vaksman M. Heinemann AB. 2007. Adaptation to diverse semi-arid environments of sorghum genotypes having different plant type and sensitivity to photoperiod. *Agricultural and Forest Meteorology* (in press).

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Component 2

Multi-site trial
Model assisted trait evaluation
For breeding



*Whole Plant Physiology Modelling Project
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Maize phenotyping for drought adaptation: potential contribution of new physiological protocols

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Drought is the main abiotic stress limiting maize productivity in tropical and subtropical regions. A proper phenotyping may help to speed breeding advance. Plant water performance under drought, crop growth and leaf area duration are among the obvious secondary traits to search. A set of 15 contrasting inbred lines from La Posta population, exhibiting a wide range of agronomical performance under drought while no differences in ASI, were grown during the 2007 dry season at Tlaltizapan (State of Morelos, Mexico). In addition another set of 18 single hybrids derived from the same population were also grown. Full irrigation and two different levels of water stress were assayed. Intermediate (IS) and severe (SS) water stress were imposed by stopping irrigation 2 weeks and 3-4 weeks prior anthesis, respectively, and irrigating once again at around 50% anthesis. Crop growth was assessed using a vegetation index (normalized difference vegetation index, NDVI) measured with a portable spectroradiometer and leaf senescence with a portable chlorophyll meter (SPAD). Plant water status was monitored by measuring leaf temperature (LT) and stomatal conductance (g_s), using an infrared sensor and a porometer, respectively. All four traits were measured several times through the crop cycle. The performance of these traits predicting genotypic differences in grain yield (GY) was assessed by principal component and multiple linear regression (stepwise) analyses.

Traits performed differently depending on the trial growing conditions. Under stress, assessments during the second part of the crop cycle (from flowering onwards) of leaf and whole crop senescence using SPAD and NDVI, measurements of stomatal conductance around flowering and canopy temperature all along the crop cycle were the best predictors of grain yield. Genotypes exhibiting higher late values of NDVI, SPAD and g_s and lower LT yielded the more (Fig. 1 left). Under well irrigated conditions high biomass all along the crop cycle together with a low canopy temperature were associated with more grain yield (Fig. 1 right). Single measurements of LT, NDVI and SPAD explained in combination near 75% variability in GY among lines in the most stressed trial, while a combination of a single measurement of g_s and another of NDVI explained near 50% genotypic differences in GY across hybrids (Table 1). Under intermediate stress more than 50% of variability in GY was explained in the lines by g_s measured at two different times of the crop cycle, whereas for the hybrids it was the combination of SPAD and g_s . Under well watered conditions more than 70% of variability across lines was explained by the combination of single measurements of NDVI and SPAD, while for hybrids only a measurement of SPAD at flowering, which explained one third of the genotypic variability, was chosen in the analysis. Methodological problems associated with measuring in very large canopies may explain the lesser performance of these traits in well watered hybrids. The above results along with the heritability of the traits and their genetic correlations with GY (data not shown) support the use of these secondary traits to evaluate genotypic performance under drought.

Figure 1. PCA analysis in maize lines (LPS) grown under WW, well-watered conditions, and SS, severe stress. GY, grain yield; g_s , stomatal conductance; LT, leaf temperature; NDVI, normalized difference vegetation index; SPAD, leaf chlorophyll, SS, severe stress; WW well watered. Numbers following the trait acronyms (LT, g_s , NDVI and SPAD) refer to the days after sowing when measurements were performed.

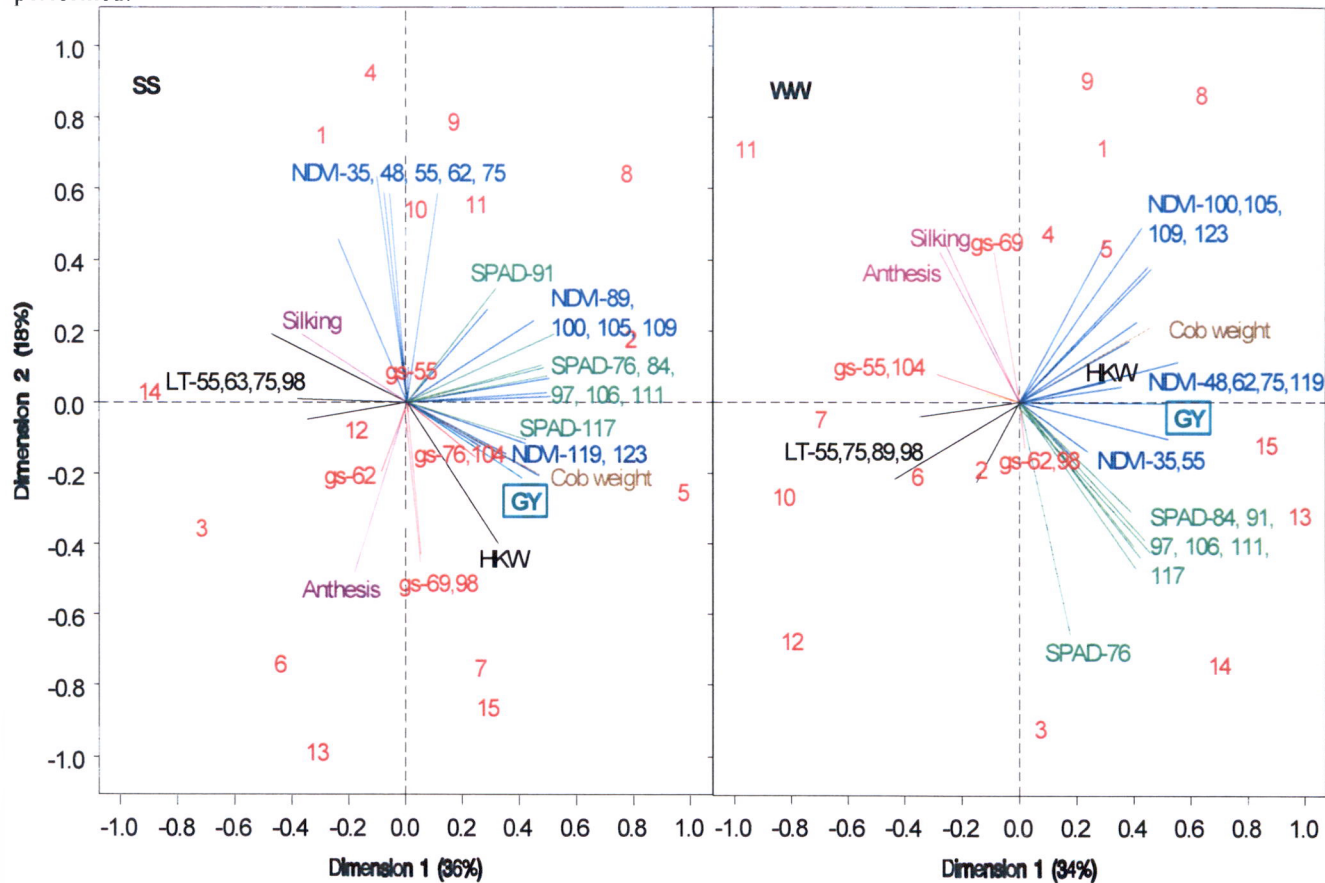


Table 1. Multiple linear regressions (stepwise) to explain GY from physiological traits built from genotype means within each water treatment for both Lines and Hybrids.

	Water treatment	Initial Variable	Initial R^2	Initial MSE	Final Stepwise Model	Final R^2	Final MSE
Lines	SS	LT89	0.40**	78.5	$GY = -28.3 \times LT89 + 4204.6 \times NDVI123 - 16.2 \times SPAD76 + 1068.8$	0.73***	52.7
	IS	gs_{19}	0.24*	180.3	$GY = 4.2 \times gs_{19} + 4.5 \times gs_{104} - 1200.4$	0.54**	141.3
	WW	NDVI109	0.48**	562.7	$GY = 12198.9 \times NDVI109 + 105.6 \times SPAD106 - 9508.9$	0.71**	417.4
Hybrids	SS	gs_{62}	0.33*	157.8	$GY = 2.9 \times gs_{62} + 2591.8 \times NDVI89 - 2003.3$	0.48*	138.6
	IS	SPAD91	0.32**	497.2	$GY = 189.6 \times SPAD91 + 5.8 \times gs_{55} - 7505.7$	0.53**	410.9
	WW	SPAD91	0.34**	931.8	$GY = 230.7 \times SPAD91 - 1035.3$	0.34**	931.8

SS, severe water stress; IS, intermediate water stress; WW, well-watered plants; Initial variable, first variable entering the model; Initial R^2 and MSE, adjusted coefficient of determination (R^2) and mean square error after including the first variable in the model; final R^2 and MSE, adjusted R^2 and MSE obtained with the final stepwise model. GY, Grain yield; LT, Leaf temperature; gs , stomatal conductance; NDVI, Normalized Difference Vegetation Index; SPAD, Chlorophyll content measurement. Numbers following the name of each trait (LT, gs , NDVI and SPAD) represent the data of measurement of each trait in days after sowing.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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Maize drought phenotyping for modelling application

Edson Alves Bastos¹, Milton José Cardoso¹, Everaldo Moreira da Silva² e José Francisco de Carvalho² Reinaldo Lúcio Gomide³

Introduction

Much research has been devoted to the evaluation and selection of drought-tolerant cereals, which can bring enormous benefits to individuals, communities and countries in arid climates. Crop models can be useful tools for breeders, geneticists and physiologists to improve phenotyping methods and protocols. However, field data are necessary to validate these crop models. The objective of this research in “Whole plant physiology modeling of drought tolerance in cereals” project, is to provide growth and development data for maize crops under water deficit vs. no stress conditions, for model application, in edaphic and climatic conditions of Piauí State – Brazil.

Materials and Methods

Two experiments were carried (2005 wet season and 2006 dry season) out in Teresina, PI, Brazil (05° 05’S, 42° 48’W; 74.4m altitude). Teresina has a tropical climate with mean annual air temperature and humidity of 27.9°C and 69.2% respectively, and annual rainfall of 1299mm, concentrated from February to April (Bastos & Andrade Júnior., 2000). Soil is a sandy Acrisol (*Argissolo Vermelho-Amarelo*, Brazilian Classification System). Focus is made only on 2006 trial.

Four corn genotypes (two varieties: BR106, SINT-TS and two lines: PE01, PE02) were evaluated under two water regimes: water stress and supply during reproductive phase. Experimental design consisted in randomized blocks, with three repetitions. The planting dates were 09/20/06 (stress) and 09/26/06 (no stress), and plants were spaced on 0.8m x 0.25 m. At planting and about 30 days after planting (DAP), fertilization was applied. Irrigation was supplied by sprinkler spaced from 12mX12m, with 1.100 L.h⁻¹. A capacitance probe, Diviner 2000, was used for soil moisture monitoring. Leaf area index, plant phenology and corn biomass per organ type and yield were measured at 4 dates along the experiment to run SARRAH maize model (see Heinemann et al. 2007).

Results and Discussion

Irrigation depth was 412.4mm and rainfall, 124.3mm under no-stress conditions. Under water stress 309.1 mm were applied, with 127.9mm of rain. Time to reach male flowering, female flowering and physiological maturity (Table 1) is in agreement with Cardoso et al. (2006). Corn phenology under water deficit and no-stress was quite similar. This fact can be explained by the fact that water stress began 5 days before male flowering (about 45 DAP) and finished after the female flowering (about 60DAP). Therefore, there was no time for water stress to influence corn phenology.

Table 1. Corn phenology under water deficit and no-stress. Teresina, Brazil, 2006.

Genotypes	Male Flowering 50%		Female Flowering 50%		Physio Maturity 50%	
	No stress	Stress	No stress	Stress	No stress	Stress
PE 01	53.0	54.0	55.7	55.3	101.0	99.7
PE 02	54.7	55.0	58.0	61.3	101.7	100.7
BR 106	51.3	52.3	55.7	60.0	98.7	96.7
SINT TS	45.7	44.3	48.7	51.3	98.3	95.3
Medium	51.2	51.4	54.5	57.0	99.9	98.1

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LAI of corn submitted to water stress (0.3 to 3.5) and no stress (0.44 to 3.6) were similar for a given genotype (Figure 1). Water stress was only in the reproductive phase, in which leaf area is almost defined. Therefore, there was no time for water stress to affect the leaf area index. However, field observations showed that the leaf area index of the corn submitted to water deficit was quite reduced after reproductive phase, due to fast foliar senescence. This reduction can not observed in the Figure 1, since that the leaf area measurement between the flowering phase and the maturity phase was not quantified.

The BR 106 and SINT TS genotypes showed better foliar development than PE 01 and PE 02, because the last ones are lines no adapted to climatic conditions local.

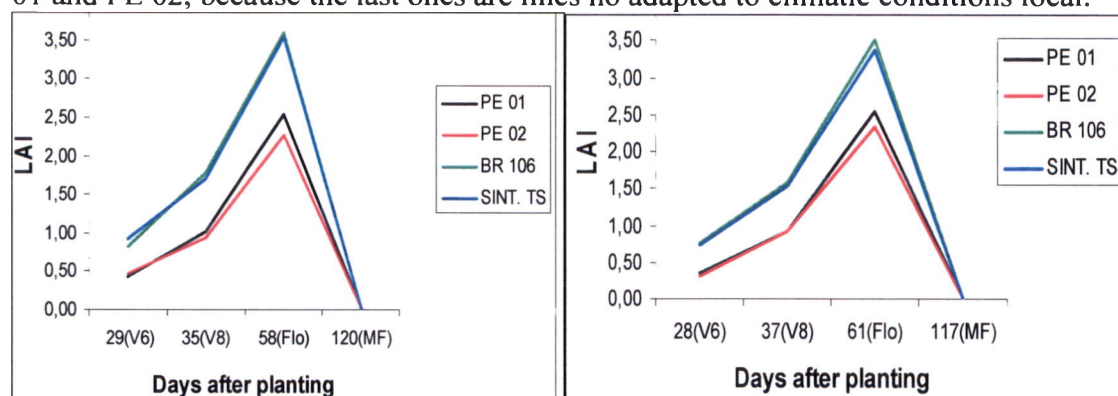


Figure 1. Leaf area index (LAI) of corn under water stress and no-stress

Grain yield showed a significance difference between corns submitted to water stress (average of 1631.8 kg.ha⁻¹) and no stress regime (average of 3532.3 kg.ha⁻¹) (Table 2). Grain yield of corn under water stress was reduced of 53.8%, i.e. more than those observed for Tollenaar & Lee, 2002; Banziger et al., 1999). BR106 (5378 kg.ha⁻¹) and SINT TS (4689 kg.ha⁻¹) varieties showed higher grain yield than PE01 and PE02 lines, as shown for LAI (Figure 1). Therefore, we can conclude that the BR 106 and SINT TS were more tolerant the drought.

Table 2. Grain yield (kg.ha⁻¹) of corn submitted to water deficit and no stress.

Genotypes	No stress	Stress
PE 01	2.486	860
PE 02	1.576	243
BR 106	5.378	1.831
SINT TS	4.689	3.593
Medium	3.532	1.632

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RICE DROUGHT PHENOTYPING FOR MODELING

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Drought occurs generally in major rice producing regions of Brazil, which occasionally causes severe crop losses (Steinmetz et al., 1988). To cope with this problem, it is desirable to develop drought resistant rice cultivars. Increasing knowledge on physiological bases of drought resistance and applying modeling to assist this analysis and find out the effect of main physiological parameters on rice adaptation to drought can increase breeding efficiency for developing adapted cultivars.

Five experiments dedicated to model application (SARRAH, Dingkuhn et al. 2003, and initially planned but not realized: Ecomeristem, Luquet et al. 2006), were conducted during the rainy season or dry period in two phenotyping sites with different environments conditions. Two experiments were conducted at Embrapa Rice & Beans Research Center, Santo Antônio de Goiás-Goiás, where rainy season is from October to April. Genotypes were sown on January 11 2006. Three experiments, with and without drought stress, were conducted during the dry season, at AGENCIARURAL Experimental Station, Porangatu-Goiás. One set of experiments, with drought stress and without drought stress, was seeded on May 12 2006 and repeated on June 11 2006. A last experiment was organized and started in April 2007. In both sites, soil is a Dystrophic Red Latosol (Oxisol).

Experimental plots were made of nine rows of 6m long and 0.35m inter-row at Santo Antônio and 0.4m at Porangatu. Plants were thinned to 20 per linear meter just after emergence. The fertilizer rates applied were 16 kg ha⁻¹ N, 120 kg ha⁻¹ P₂O₅, 64 kg ha⁻¹ K₂O, 20 kg ha⁻¹ FTE and 5 kg ha⁻¹ Zn. The N topdressing was done at the rate of 20 and 30 kg ha⁻¹, 40 and 55 days after sowing respectively at Santo Antônio, and 30 kg ha⁻¹, at pre-heading, at Porangatu. Weeds were controlled by using 1000 g i.a. ha⁻¹ of oxadiazon. There were four replications and the five genotypes tested were: BRS Soberana, BRS Curinga, Primavera, Guarani, and CNA 9019. All of these Sativa, japonica genotypes have similar growth cycle, except BRS Curinga (longer). These genotypes present different plant type growth modern, with erects leaves and traditional, with droopy leaves. In addition, these genotypes also present different response to drought stress.

In well-irrigated treatment, the soil matrix potential was maintained higher than – 0.025 MPa, at 15 cm depth (Stone et al., 1988) during the plant growth, while in drought stress treatment, natural weather conditions were maintained. However, under severe drought stress (dry season Porangatu 2006, 2007) the experiments were well irrigated until 30 days after emergence and then a moderate drought stress was applied, with the application of 50% of water applied to well irrigated plants up to harvest (soil matrix potential about –0.07 MPa).

Three sets of data were collected. The first set concerns fine phenology characterization. These data were collected at weekly interval, from the first week after emergence. Four plants per plot were used for these observations. The phenology data were: number of green leaves of the main stem, number of dead leaves on the main stem, number of ligulated green leaves on the

main stem, % of length of the growing leaf, length of last ligulated leaf, width of the last ligulated leaf, height of the plant, number of tiller and total number of tillers per plant. The second set of data consisted in destructive measurements for growth analysis at about 20, 50, and 75 days after sowing and at physiological maturity. Data were collected on one linear meter (about 20 plants) and concerned: tiller number, blade area, blade dry weight and weight of the rest of the plant. During the fourth reading, number of panicles, number of grain and weight of grain were also included. The crop yield and the 100 seed weight were evaluated in three rows of three meters length. The number of grain and spikelets was determined on 20 panicles.

The third set of data collected concerned soil physicals and chemicals properties: bulk density, % silt, % clay, % sand, water saturation, field capacity, permanent wilt point, C, N, P, K, Al, Ca, Mg, Zn, Cu, Fe, Mn, and CEC (Embrapa, 1997) at 0-20, 20-40, 40-60 and 60-80 cm soil depths.

Soil moisture was determined at weekly interval using just one replication of all genotypes on well irrigated and with drought stress. Data were successfully collected and used to parameterize and validate the crop model Rice06, from ECOTROP platform, for the Brazilian Savanna region.



Figure 1. The front view is the irrigated modeling experiment at AGENCIARURAL Experimental Station, Porangatu-GO.

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Challenges and Difficulties of Imposing and Monitoring Crop Water Stress in Field Trials

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Introduction

Crop water stress is a complex process involving interactions between plant and environment. Characterizing stress in terms of onset, intensity, duration and damage caused to the crop is very important but difficult and can be made easier by using crop growth simulation models like ECOTROP (Sultan et al., 2005), as shown by Heinemann et al. (2007).

Crops grown during the first harvest season in Central Brazil might experiment dry spells (Assad e Castro, 1991) with duration varying from days to weeks (Wolf, 1975). Plants are, usually, subjected to stress at pre-flowering or flowering stage. For crops planted during the second harvest season ("safrinha"), water stress normally occurs at post-flowering or at the end of the cycle and can last longer.

A systematic study of water stress effects on crops has been commonly done by running two simultaneous experiments: one fully irrigated and one with water shortage. Admissible stress level varies with species and may also vary with genotypes. Stress application is imposed by cutting irrigation out for a certain period of time. Plant canopy symptoms, like leaf rolling or senescence are observed and compared to control plants. Stress effect is evaluated by estimating yield reduction. This is a very subjective methodology that requires a lot of practice. Simulation models or even simple spreadsheets (Albuquerque and Andrade, 2000), requiring basic information on soil, crop and climate, can be used to manage irrigation and estimate stress level. The best way of controlling the stress is by using a combination of methods, including some sort of soil, weather and plant monitoring. However, simple indirect approach, based on soil-water potential or soil-water content monitoring, are usually utilized. A description of available methods to monitor soil-water status is found in Andrade et al. (2007). Practical field application of that kind of approach is not simple, though.

The objective of this paper was to address some of the difficulties encountered during field trials carrying out in Brazil.

Materials and Methods

Data from maize and sorghum field trials, carried out in Sete Lagoas, MG, Brazil, were used as a case study. Stressed and non-stressed field experiments were planted in such a way that crops would be fully developed at the beginning of the dry season. Sorghum were sowed on February 27th, 2007 and maize on March 3rd. A spreadsheet (Albuquerque and Andrade, 2000) was used to manage irrigation and to define when to stop irrigation, considering that for maize, stress would be desirable at pre-flowering and for sorghum, just after flowering. After a period of stress, irrigation was re-started in maize crop, while for sorghum stress was maintained until physiological maturity. Maize and sorghum soil-water and yield data, of both fully irrigated and stressed trials, were acquired.

Results and Discussion

The first difficulty faced was to find information on genotypes cycle length when grown at that season, in addition to reliable field-based soil-water data, both required by the monitoring spreadsheet. By using approximate values and considering a forecasted daily evapotranspiration, one could estimate the day for stopping irrigation. Re-watering for maize was difficult to define since there was no information regarding soil-water reduction and stress level reached.

For maize, the last irrigation was applied 43 days after planting (dap) and a 76 mm rainfall occurred at 47 dap (Figure 1A). One can notice that possibly the crop started to suffer some stress at about 66 dap, when the soil-water dropped under 50% of available water. This indicates that stress occurred later than the desirable pre-flowering stage. Stress intensity seemed to have been adequate, as grain yield reduction for the six genotypes, varied from 47.3 to 75.3%.

For sorghum (Figure 1B), last irrigation was applied on 51dap and a 76 mm rainfall occurred on 54 dap. Crop started to suffer some stress by 100 dap, i.e. too late. Stress effects were diminished

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by the 76 mm rainfall. Yield reduction varied from 2.8 to 15.7%, confirming that stress onset, duration and intensity was not enough to cause significant damage to sorghum crop.

Other difficulties faced during data collection campaign were the strong labor demand associated with inappropriate equipments and instruments. Soil water content was monitored by gravimetric method, that is time consuming and labor intensive. Soil spatial variability was a problem requiring replicated samples to be collected. After cutting the irrigation, it was difficult to auger soil and soil layers could be mixed up. The volume of plant samples to be processed for leaf area and dry matter, considering all genotypes and replications of the two experiments (stressed and irrigated) and for the two crops was enormous, demanding lots of labor and large lab ovens.

Conclusions and Recommendations

The methodology to impose and control water stress in field trials needs to be refined; more research is needed to access the effects of different stress levels (onset, duration and intensity) into crops growth and yield; plant and soil indicators of water stress have to be better defined and correlated to crop growth and yield reduction; some sort of automated data collection is required to improve data quality and reduce labor involved with soil and plant sampling.

Research on genotypes rooting system development, specially considering water or multiple stresses, is strongly recommended.

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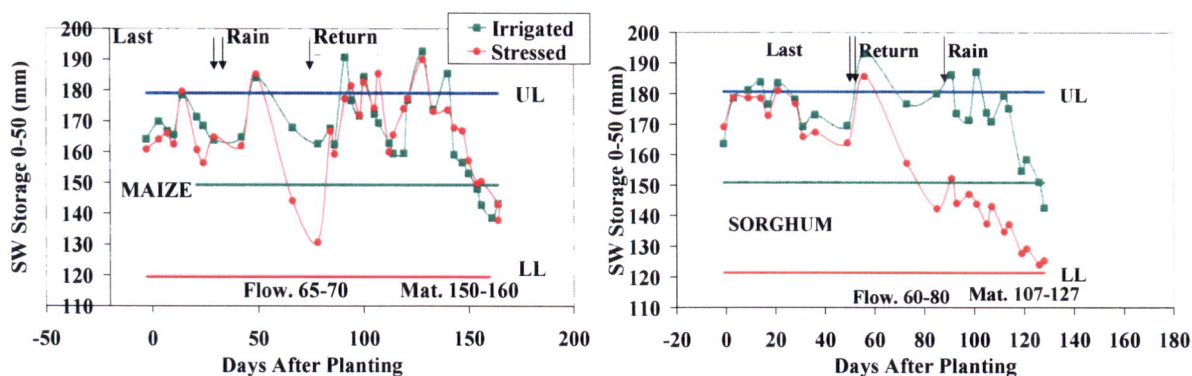


Figure 01 – Soil-water storage along maize (left) and sorghum (right) crop cycle and field-determined upper and lower limits of available water.

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Crop model assisted characterization of appropriate traits for the rice TPE in Brazilian Cerrados

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Introduction

Many agronomic traits of interest such as yield, but also specific leaf area and biomass partitioning can be used to enhance the rate of genetic improvement for rice. Trait expression in upland rice is affected by abiotic stresses such as drought, temporally and spatially changing the means and variances of grain yield.

Crop simulation models have captured much of the understanding of plant growth over nearly 40 years of plant system research (Sinclair & Seligman, 1996), providing a dynamic framework for the physiological dissection of complex growth and development traits. In spite of limited success of early efforts to use agronomic crop models for genotypic characterization based on heuristic approach (Hammer et al., 2005), it is now possible to use crop models to simulate different phenotypic traits and their respective impact on yields for a wide range of TPEs.

The Cerrado region, specifically Goiás state, is characterized by three different drought stress patterns for short upland rice: low, mid-season and terminal stress (Heinemann et al., 2007), low stress being predominant, when there is no restriction (deep soil) for root development due to *Al*-induced acidity in deeper soil layers. However, for shallow soil condition there is a predominance of mid-season stress occurrence. For a plant breeding program, it would be desirable to know which phenotypic traits could further improve plant adaptation for targeted environments.

The objective of this study is to estimate the added value of combining specific phenotypic traits, such as duration of vegetative phase, specific leaf area, response to soil water status to maintain or improve grain yield in the upland rice target population of Goiás state.

Material and Methods

The crop model RICE06, derived from the generic model SARRAH and implemented in the ECOTROP modeling platform of Cirad, was parameterized and validated for one upland rice short cultivar (Guarani). For this purpose, field experiments were carried out at Porangatu (two planting dates - 12/05/2006 and 11/06/2006) and Goiânia (11/01/2006). Based on the “standard genetic coefficients” obtained through the parameterization, 17 virtual genotypes were created by modifying and combining the parameters: maximum and minimum specific leaf area (SLA_{max} and SLA_{min}), depletion P factor (threshold value of soil water content from which the crop begins to experience water stress) and vegetative period duration. SLA_{max} and SLA_{min} (input data) were modified in plus and minus 20%, P factor values were 0.2, 0.35 and 5.0, 0.35 being reference value, and values for vegetative period were 490 and 700 degree days, 490 being the reference. Leaf area index (LAI = SLA * Leaf Biomass) is calculated based on daily values of SLA (calculated based on Michaelis model using SLA_{max} and SLA_{min} as an input data).

The crop model was run for all genotypes in the TPE defined by Heinemann et al. (2007) for two scenarios: deep and shallow soil.

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Results

Results shown here are just preliminary tests (to check model sensitivity to chosen parameters) for one site (CNPAF station of Embrapa), for a no stress year (2002) and two environments, deep and shallow soil. For deep soil, Figure 1a, shows that the increase of vegetative period does not lead to a gain in relative yield (relation between yield obtained from virtual genotype and yield obtained by the standard genotype). For a higher vegetative period, the increase of specific leaf area (increase on the difference of SLAmin and SLAmax as an input data) decreases the relative yield. For shallow soil (Figure 1b) an increase in vegetative period could provide higher relative yields. Apparently, this result is the opposite of expected in the region, since in shallow soils it is expected that short-cycle cultivars have better performance. However, simulation must be done for all sites and planting dates in TPE to verify this trend.

Conclusions

This study will demonstrate, for a region where TPEs were characterized in detail, how crop modelling can assist ideotype definition and thus facilitate preliminary choices for a breeding programme.

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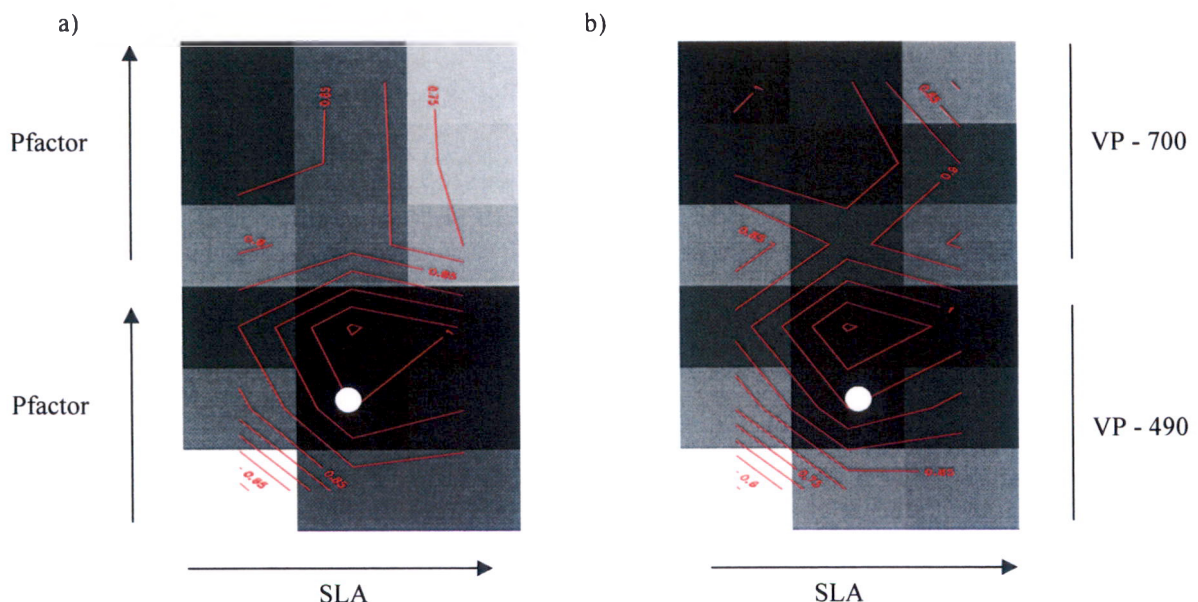


Fig. 1. Effect of P factor, specific leaf area based on the difference of SLAmin and SLAmax as an input data (SLA) and vegetative period (VP) on the relative yield (relation between yield obtained from virtual genotype and yield obtained by the standard genotype) for a) deep soil and b) shallow soil. The white circle represents the standard genotype (Guarani).

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Component 3

Model improvement for
process based trait phenotyping
& connection to genetic information



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Metabolic Phenotyping and Plant Performance

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Our work aims at the identification of genes involved in the orchestration of the metabolism, at an integrated understanding of gene regulation and metabolism, and ultimately at linking metabolism and plant performance, i.e. biomass production and quality. A full understanding of metabolism requires quantitative data about transcripts, proteins or enzyme activities, and metabolites. Numerous efforts have been undertaken to sequence various organisms and as a result, transcript profiles are now routinely performed at the genome level, and many proteins can be detected and even quantified. Recent developments in mass spectrometry also provide information about levels and fluxes accessible for almost every primary metabolite and many secondary metabolites. In contrast and despite the fact that linking properties of macromolecules with phenotypes is a major aim in fundamental and applied biology, catalytic properties of enzymes have not yet been included into this equation. Enzymes have been studied for more than a century, but traditionally they have been purified from various organisms or tissues and subjected to various *in vitro* experiments in order to study the corresponding reaction mechanisms [1, 2, 3, 4] and eventually determine their constants (K_m , K_i , k_{cat} , v_{max}). Properties and responses of enzymes now need be understood in their physiological context.

We have developed a robotized platform which allows the determination of up to 50 enzymes in a range of tissues and plant species, with a throughput of up to 10.000 determinations per week. As a first application we have shown that changes in transcript abundance are not necessarily reflected by changes in enzyme activity in rosettes of *Arabidopsis thaliana* grown under various environmental conditions [5, 6, 7]. Typically activity changes are strongly delayed and less pronounced, indicating the importance of post-transcriptional regulation and suggesting that changes in transcripts are integrated over time. These results were confirmed by performing metabolite profiles which integrate the activities of hundreds of enzymes [8]. We hypothesized that such a metabolic acclimation facilitates an adjustment of metabolism to mid-term shifts in growth conditions, while ignoring noise due to diurnal changes and day-to-day fluctuations [9]. In some cases, enzyme activities change independently of, and even reciprocally to, the encoding transcripts. This presumably reflects regulation of translation and/or protein degradation.

These approaches identified enzymes that consistently respond to environmental challenge. Although we have used reverse genetic approaches, this has not yet allowed us to understand these responses [unpublished results]. A further striking result is that most of the 40 enzyme activities measured in leaves of 100 *Arabidopsis* accessions, grown under controlled conditions and showing large differences in growth rates, were found to be significantly correlated with each others [Sulpice et al., in preparation]. In other words, we could not identify any specific enzymatic phenotype that would be associated with biomass production. In that context, Junker et al [10] have recently shown using canola embryos that most enzyme activities are actually in large excess

relative to the requirements of the fluxes observed *in vivo*, which suggests that changes in total activities will have less influence on fluxes than changes in catalytic properties such as K_m or K_i . This is also inherent to the finding that most enzyme activities can be strongly decreased by antisense without affecting fluxes. Variations in catalytic properties may indeed have important consequences on metabolism but also on plant form and function.

Changes in catalytic properties can result from the expression of specific isoforms exhibiting specific properties, or from post-translational events such as the phosphorylation of serine or tyrosine residues and the reduction or oxidation of cysteine residues. Several enzymes like nitrate reductase [11] and ADP-glucose pyrophosphorylase [12, 13, 14] have been intensively investigated, revealing highly complex regulations, but to our knowledge no “untargeted” approach has been undertaken so far to investigate the extent of alterations in catalytic properties as related to changes in growth conditions. By performing a high throughput approach in maize, we actually identified several enzymes showing unexpected shifts in their properties in response to growth conditions.

Variation in the sequence of a given structural gene may also affect the properties of the corresponding enzyme, and depending or not on growth conditions and developmental stages, affect the phenotype. Thus, the introgression of an apoplastic invertase with a higher affinity for sucrose, from a wild tomato species into the cultivated tomato, resulted in a higher content of soluble organic compounds in the ripe fruits [15]. Predicting that many more of such relationships will be found by screening genotypes (natural populations or mutants) for alterations in kinetic properties of key enzymes, we are presently screening accessions of *Arabidopsis* and maize for alterations in such properties.

To conclude, responses of primary metabolism to environmental or even developmental challenges can probably be achieved in large part without an in depth reprogramming of the enzyme machinery. Instead, catalytic properties may call the tune more often than expected, and thus be important players of plant performance.

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Short-term responses of leaf growth rate to water deficit scale up to whole-plant and crop levels. An integrated modelling approach in maize.

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Physiological and genetic studies of leaf growth often focus on short-term responses, leaving a gap to whole-plant models that predict biomass accumulation, transpiration and yield at crop scale. To bridge this gap, we propose here a model that combines (i) a short-term model of maize leaf expansion that capture genetic and environmental variations (Reymond *et al.*, 2003; Welcker *et al.*, 2007), with (ii) a new model coordinating the development of all leaves of a plant and (iii) the crop model APSIM (Wang *et al.*, 2002; Keating *et al.*, 2003) which takes account of the complex interactions between plants and their environment. The model was tested against final length of all leaves of the plant, leaf area index, biomass accumulation and yield for contrasting field environments.

MATERIAL AND METHODS

Maize seeds (*Zea mays* L., hybrid Dea) were sown in 12 field experiments in North (Grignon) and South (Montpellier) of France under contrasting temperatures, evaporative demands and soil water conditions. Ten plants were tagged at the appearance of leaf 3 and their visible and ligulated leaves were counted every second or third day. The final length and width of each leaf of these plants were measured. In addition, five to eight plants with similar development stages were sampled every second or third day in order to record the number of initiated leaves and the lamina length of all leaves of a plant. Based on these experimental data, the model estimated leaf initiation, appearance, ligulation and the beginning and end of linear expansion rate for each leaf. Responses of leaf elongation rate to meristem temperature, meristem-to-air vapour pressure deficit (VPD) and soil water deficit (predawn leaf water potential) were determined from growth chamber and greenhouse experiments (Ben Haj Salah and Tardieu, 1995; Reymond, 2001; Reymond *et al.*, 2003). Maximum leaf width of each leaf was estimated as a function of leaf position and the width of the widest leaf on the plant. Lamina area was calculated as the product of lamina length by maximal width, corrected by a shape factor of 0.75 (Zhang and Brandle, 1997).

The leaf model was incorporated as a replacement module for canopy leaf development in the APSIM-Maize model of the APSIM platform (Wang *et al.*, 2002; Keating *et al.*, 2003). A new micrometeorological module was added to APSIM to calculate weather data at an hourly time step and estimate environmental conditions as sensed by leaves (meristem temperature, meristem-to-air VPD and predawn leaf water potential).

The ability of the new model to predict biomass accumulation was tested in three field experiments in Gatton (Australia) with the hybrid Hycorn 53 (Pacific Seeds, Toowoomba, Australia) (Lemaire *et al.*, 2007). Leaf, grain and whole-plant biomass were sampled on 10 plants every 3 to 4 weeks. Leaf area index (LAI) was estimated from measurements of leaf weight and specific leaf area on these plants.

RESULTS AND DISCUSSION

Stable patterns of leaf development were observed over a large range of field conditions. A stable thermal time was required for initiation of successive leaves, their beginning of linear expansion or their emergence from the whorl. The pattern for cessation of elongation and leaf ligulation was more complex, as thermal time required for these processes decreased for leaves growing after floral transition and nullified for the uppermost leaves.

Stable responses of leaf elongation rate were observed for leaf 6 in growth chamber and greenhouse experiments. Leaf elongation rate linearly increased with meristem temperature. When expressed per thermal time unit, the leaf elongation rate linearly decreased with meristem-to-air VPD in well-watered conditions and it linearly decreased with predawn leaf water potential in the absence of evaporative demand during the night, as previously observed in maize (Ben Haj Salah and Tardieu, 1996 and 1997; Reymond *et al.*, 2003). Large effects of evaporative demand and water deficit were also observed on final leaf area in field experiments (observed data points in Fig 1), consistent with effect on elongation rate in controlled conditions.

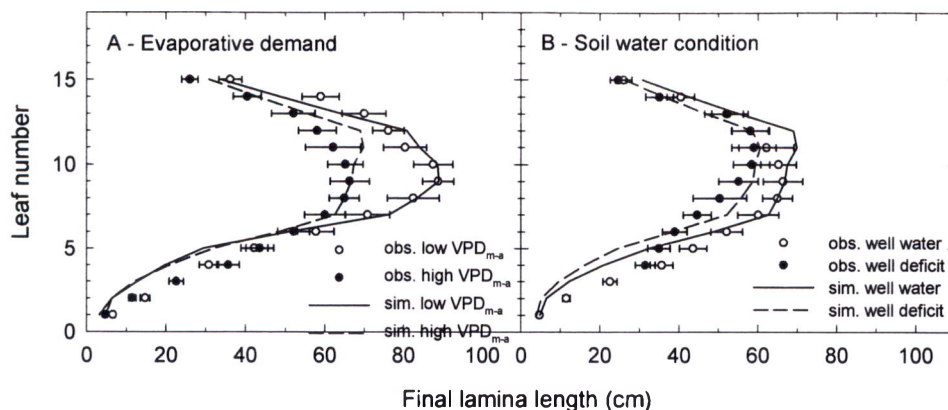


Figure 1. Observed (points) and simulated (lines) final lamina lengths in field plants grown under contrasting evaporative demands (A) and soil water conditions (B). Data for well-watered conditions with meristem-to-air vapour pressure deficit (VPD_{m-a}) of 1.1 (○) and 2.6 kPa (●) averaged over the vegetative period (A); well-watered (○) and water deficit (●) conditions (B). Error bars, standard deviations; $n = 10$.

The leaf model resulted from a combination between a model that coordinates the development of all leaves of the plant (13 parameters estimated from a single field experiment and data from Andrieu *et al.*, 2006) and a model that predicts leaf 6 elongation rate as affected by environmental conditions (4 parameters estimated from growth chamber and greenhouse experiments). The leaf-coordinating model estimated the beginning and end of linear elongation for all leaves and predicted the variation in leaf elongation rate among leaves of a plant. It thus fixed the time frame of expansion for all leaves, while the leaf growth model simulated the leaf elongation rate as affected by the leaf environmental conditions (meristem temperature, meristem-to-air VPD and predawn leaf water potential) that were estimated using the interface with the APSIM model.

The model was used to predict (with a single set of parameters) the final length of all leaves of a plant in the 12 field experiments carried out in France. Examples of simulations are given for final leaf length of plants grown under contrasting VPD (Fig. 1A) and soil water conditions (Fig. 1B). Effects of changes with time in soil water potential were accurately simulated for reduction in length of individual leaves. Only leaves exposed to water deficit during their development (either hidden in the world or partly emerged) had a reduced final area in the model as in the field experiments. Overall the model adequately simulated the final lamina length for effects of both the environment and leaf position on the stem (for all the tested environments: $y = 1.018 x$, $r^2 = 0.922$, $Cve = 0.147$).

Tests over the crop cycle were performed in the three Australian experiments using a local hybrid. This genotype displayed similar leaf growth than Dea with nevertheless lightly longer lamina for first leaves. Interfacing the leaf model with the crop model APSIM allowed estimation of integrated phenotypes at canopy level. The model adequately predicted leaf area index ($y = 0.919 x$, $r^2 = 0.619$, $Cve = 0.347$), vegetative biomass ($y = 0.839 x$, $r^2 = 0.955$, $Cve = 0.177$) and grain yield ($y = 0.986 x$, $r^2 = 0.849$, $Cve = 0.432$) under well-watered conditions that varied in seasonal temperature and VPD profiles across contrasting sowing dates.

The study presented here shows that it is possible to integrate a leaf growth model with a time scale of hours into a canopy model with a time scale of months. Furthermore the parameters used in the leaf model are stable characteristics of a genotype over a wide range of environments, and can be related to QTL independent of environment (Reymond *et al.*, 2003; Welcker *et al.*, 2007). This study thus opens the way for modeling genetic variability at the whole-plant level under fluctuating conditions and should help in the evaluation of the contribution to yield of QTL for individual leaf traits.

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Modelling the GE interaction for leaf and silk growth rates in maize: genetic analyses of time courses and responses to water deficit

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Plants subjected to water deficit undergo rapidly fluctuating conditions. Those are day-to-day fluctuations for soil water status, with soil drying or rapid re-watering, and hourly fluctuations for evaporative demand, with changes in light or in vapour pressure deficit during the day. Even in water deficit, plants experience a relatively favourable water status at the end of the night, and loose water during the day so their water status in the afternoon is unfavourable. Consequently, the plant growth rate and photosynthesis rate have rapid fluctuations, with different time course for each genotype (Sadok et al. 2007). Because of this complexity, it is tempting to define tolerance to water deficit at the temporal scale of the whole cycle, by comparing overall performance of genotypes in network of field experiments which buffer the short term fluctuations. This view has allowed considerable progress in drought tolerance in the last 30 years (Campos et al. 2004), but makes it difficult to refine the adaptation of genotypes to changing climatic conditions, or to allow local adaptations to target populations of environments (Chapman et al. 2002).

We have used a different strategy, in which the phenotype is not defined by a plant trait at a given time, but as the sensitivity of the considered genotype to soil water status or to evaporative demand, established over a range of environmental conditions (Reymond et al. 2003; Sadok et al. 2007). It consists, first, of expressing all rates per unit thermal time, thereby obtaining temperature-independent rates (Sadok et al. 2007). Second, the major environmental conditions involved in leaf development were identified for short time intervals (minutes to hours). Temperature, evaporative demand and soil water deficit had an overriding effect on leaf growth rate, whereas light and plant carbon balance had minor effects (Ben Haj Salah and Tardieu 1997; Sadok et al. 2007). Third, response curves of leaf elongation rate to temperature, evaporative demand and soil water status were established and the parameters of these responses were analysed genetically (Reymond et al. 2003; Welcker et al. 2007). This allowed simulation of leaf growth in novel inbred lines as defined by their alleles at QTL (Reymond et al. 2003; Sadok et al. 2007). This also allowed comparison of the genetic determinisms of several organs of the plant, namely leaves and silks (Welcker et al. 2007).

1. Modelling the time courses of the elongation rates of leaves and silks.

The leaf elongation rate of a given maize leave is constant over 5 to 7 days in the absence of environmental changes. This period of time is that necessary for the next two leaves to appear above the whorl (e.g. for leaf 6, until the appearance of leaf 8). This has been observed under stable temperature in a growth chamber (Ben Haj Salah and Tardieu 1995), and is observed under naturally fluctuating conditions provided that rates are expressed per unit thermal time (Sadok et al. 2007). In the latter case, leaf elongation rate is constant during the night, and consistent over 5-7 nights and over different experiments for a given genotype. This baseline leaf elongation rates is, therefore, a genotypic characteristic, which can be analysed genetically in mapping populations.

Night-time leaf elongation rate decreases between nights when the soil water status declines with soil water depletion. The relationship between soil water potential and night time leaf elongation rate is common to several experiments for a given genotype. It is, therefore, a second stable genotypic characteristic. In the same way, elongation rate decreases in the morning with increasing evaporative demand, and a stable relationship is observed for a given genotype between the leaf elongation rate and the vapour pressure difference between meristem and air (VPD_{ma}). Both effects are approximately additive so we can predict leaf elongation rate in any combination of temperature, evaporative demand and soil water status.

Silk elongation essentially follows the same pattern, but with a continuously declining trend. Silk elongation rate is maximum during the night and a gap is observed during the day, which is proportional to VPD_{ma} . Night time silk elongation rate declines linearly with soil water potential, in the same way as leaves. This suggests that common processes may drive the elongations of silks and leaves, with a likely predominance of hydraulic mechanisms. In both organs, the fluctuations of elongation rate with evaporative demand are very rapid, suggesting a physical mechanism rather than mechanisms involving cell cycle or cell wall properties. In both cases, the amount of sugars in the growing zone is equal or higher in plants subjected to water deficit than in well watered plants. Combined with the fact that elongation rate is faster during the night than during the day, this makes unlikely a mechanism based on the availability of carbon to growing cells.

2. Genetic analysis of the sensitivity of leaf growth to water deficit or evaporative demand

The responses of leaf elongation rate to water deficit and evaporative demand have been studied in 100 to 220 recombinant inbred lines of three mapping populations, namely two crosses of temperate parents (F-2 x Io and F-

2 x F252) provided by A. Charcosset (INRA), and one cross between two tropical parents identified by JM Ribaut (CIMMYT) as having contrasting behaviours under water deficit. Each population was studied in 5-8 different experiments, and response curves were common to all experiments for each recombinant inbred line (Reymond et al. 2003, Sadok et al. 2007, Welcker et al. 2007). Heritabilities ranged from 0.5 for the lowest cases to 0.8 for the highest. Surprisingly, the ranges of sensitivities did not appreciably differ between mapping populations, although tropical lines originated from a dry region and were selected for drought tolerance.

QTLs were identified for the sensitivities to soil water deficit and to evaporative demand. In each population, 5 to 7 QTLs were identified for each sensitivity. Most QTLs of sensitivity to evaporative demand tended to co-localise with those of the sensitivity to soil water status, in spite of the fact that they were determined in different experiments, *i.e.* well watered and day-time measurements for the sensitivity to evaporative demand, and water deficit and night - time measurements for the sensitivity to soil water potential.

In total, 7 "hot zones" were determined over the three mapping populations. The effect of each of those was studied in 3 populations of insertion lines, (i) an advanced backcross of a tropical line (CML 444) in the temperate line F252, (ii) BC5-S3 insertion lines of the early flint line Gaspé in B73, provided by R. Tuberosa (U. Bologna), (iii) two sets of reciprocal insertion lines derived from a dent by flint F1 population, provided by Biogemma. Each of the 7 targeted zones was confirmed by the magnitude of their effects in insertion lines, in spite of the fact that those lines had different genetic backgrounds than the mapping populations. Interestingly, the lines which had been chosen for QTLs of sensitivity to evaporative demand also contrasted for the sensitivity to soil water status, and *vice versa*.

This shows that an appreciable genetic variability exists for the sensitivities to water deficit and evaporative demand, which can be synthesised in the effect of 7 zones of the genome.

3 Have sensitivities of leaf and silk growths to water deficit a common genetic determinism ?

Leaf growth and Anthesis-Silking Interval (ASI) are the main determinants of source and sink strengths of maize *via* their relations with light interception and kernel number, respectively. They depend on the abilities of leaves and silks to expand under fluctuating environmental conditions, so we tested the possibility that they may have a partly common genetic determinism. This was first tested in a subset of lines of the tropical mapping population presented above, in which both silk and leaf elongation rates were measured as a function of soil water status or of evaporative demand. The rank of genotypes was common for the responses of both organs (O. Turc, unpublished).

The whole population was then studied, but the silk elongation rate was indirectly evaluated *via* ASI which was measured in 3 and 5 fields under well-watered conditions and water deficit, respectively, by JM Ribaut at CIMMYT. Half of the QTLs for ASI under well-watered conditions co-localised with QTLs for leaf elongation rate measured in the absence of water deficit, and half of the QTLs for ASI under stressed conditions co-localised with QTLs for the response of leaf elongation rate to soil water deficit (Welcker et al. 2007). The allele for leaf growth maintenance was in all cases that for shorter ASI (maintained silk elongation rate) under water deficit. Hence, source and sink strengths may have a partly common genetic determinism.

4. Toward the modelling of virtual genotypes

The results presented here suggest that (i) it is possible to genetically analyse the sensitivities of growth to evaporative demand and to soil water deficit, even in naturally fluctuating conditions. (ii) Because the genetic analysis was carried out on parameters of a model, it is possible to simulate the growth of the considered organ for any genotype of a mapping population under any environmental condition. This was tested for new lines, known by their alleles only (Reymond et al 2003). (iii) Different organs of a plant share part of the genetic determinism of the sensitivity to soil water status and of evaporative demand. This was the case for leaves and silks (Welcker et al. 2007), but also between different leaves of a plant (unpublished). It is, therefore, possible to "pyramid" alleles which determine the reactions of different organs. For this reason, a whole plant model has been designed, which combines the model of leaf growth presented here and a model coordinating the developments of all leaves of a plant (see Chenu et al, same volume). We believe that this approach may have important consequences in the research of better adaptations of genotypes to target populations of environments.

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Phenotyping of sorghum photoperiod responses using heuristics

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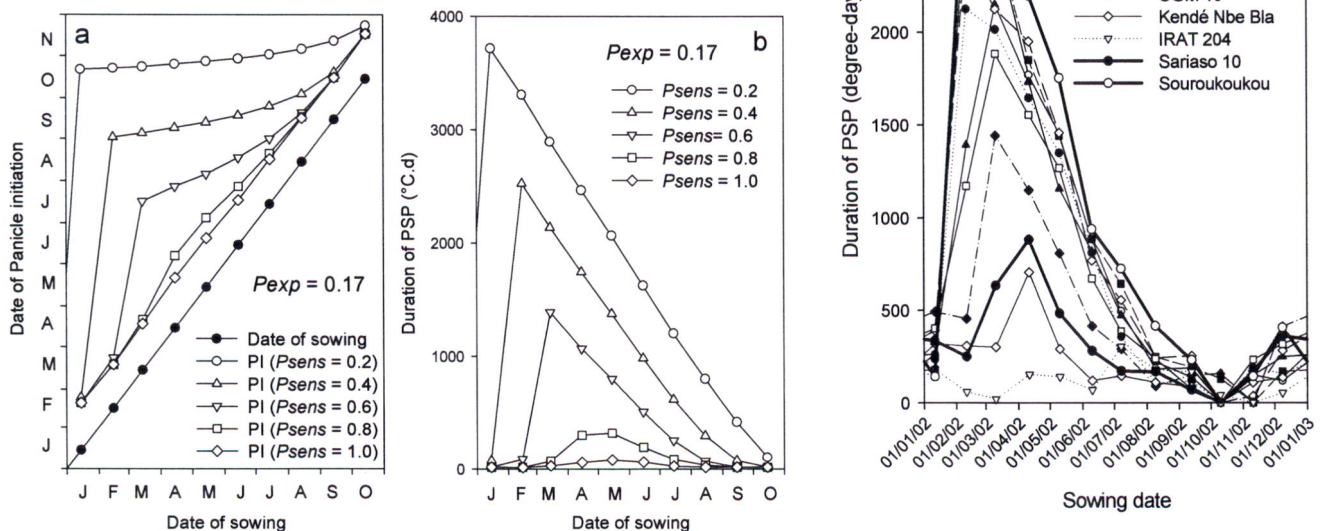
Problematic. Effects of photoperiod (PP) on panicle initiation (PI) of West African sorghums are essential for agro-ecological adaptation. Conventional models based on fixed (genetic) PP thresholds (qualitative response) or signal accumulation (quantitative response), are able to predict flowering for only a limited range of conditions. Accurate phenotyping requires a model having broader validity.

Approach. An alternative model is proposed in which PP thresholds that vary with plant age. The generic algorithm was called “*Impatience*” because it implements decreasing day length requirements during prolonged wait states, or appetite, during the photoperiod sensitive phase (PSP). The model was applied to field data obtained from sowing date experiments in Mali, in order to validate it and evaluate the range of genotypic responses that can be explained. On this basis, a heuristic model-assisted phenotyping approach was developed for field conditions using 3 sowing dates and fitting of 3 parameters (P_{exp} , P_{sens} and BVP). Phenotyping for the 3 parameters requires data on flowering or flag leaf ligulation dates for the 3 dates and daily min/max temperature.

Genetic materials. The model was tested with extensive data sets for 20 very diverse genotypes including traditional and improved *caudatum*, *guinea*, *guinea margaritifera*, *durra-kafir* and *durra-caudatum* materials. The phenotyping methodology was evaluated for a subset of 5 genotypes (E35-1, *caudatum*; Kendé, *guinea margaritifera*; Wassoulou, *guinea*; Souroukoku, *caudatum*; CSM 388, *guinea*).

Results. Genotypes having diverse sensitivity to PP showed a common pattern of variability of PSP vs. sowing date (Fig. 1). PSP was shortest for October sowings and consistently longer for earlier sowings, unless the crop was sown before a critical, genotype specific date associated (break point).

Fig. 1. Duration of the photoperiod sensitive phase (PSP) observed for 12 monthly sowing dates in 2002/3 at Samanko near Bamako, for 11 contrasting sorghum genotypes. →



↑ **Fig. 2.** Sensitivity of the *Impatience* model to variation of the P_{sens} parameter for the Bamako site. a: variation of date of PI; b: variation of PSP.

Figure 2 shows the sensitivity of the *Impatience* model to the parameter P_{sens} . Variation of P_{sens} explained much of the genetic diversity of patterns observed in Fig. 1. Specifically, the model predicted accurately that (1) PI does not occur at any genotype specific day length, but instead, on increasingly long

days as the PSP is extended; (2) PI occurs predominantly when day length decreases, or after summer solstice; (3) PSP increases linearly (but not always proportionally) when crops are sown earlier in the year; (4), a genotype specific sowing date exists in winter (cool season) or spring (hot dry season) after which PSP suddenly increases by up to 160 days (“break point”); and (5), the largest variance of PSP occurs near the break point. However, the model tended to underestimate the duration of PSP when it fell into the cool season, which might be explained by specific thermal effects or a specific inhibitory effect of increasing day length on PI in some genotypes.

The ability of the model to discriminate among genotypes was used to devise a simple, model assisted phenotyping methodology. Genotypic parameters were determined by measuring one model parameter (BVP) and by fitting two others (*Psens* and *Pexp*) to measured dates of flag leaf ligule appearance for three sowing dates. Thus parameterized, the model predicted accurately the phenological patterns of contrasting materials observed in different experiments and years (Fig.3).

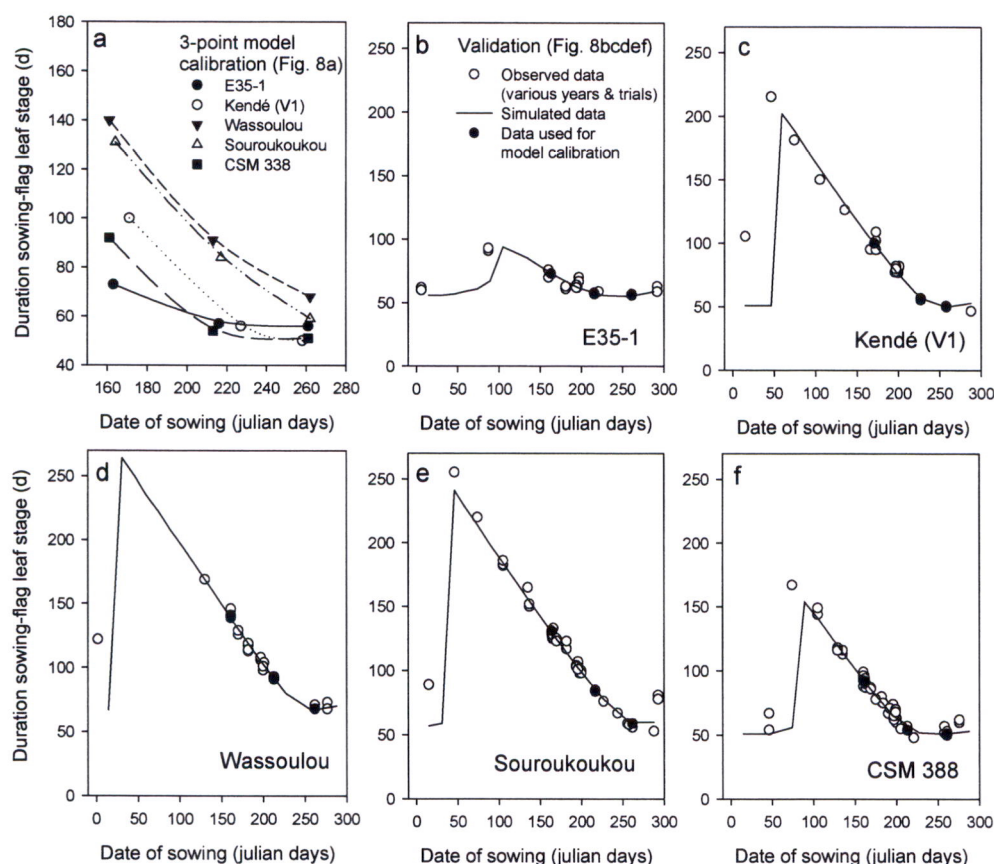


Fig. 3. Model assisted phenotyping of 5 sorghum genotypes having contrasting phenology: Calibration of the *Impatience* model based on observed date of flag leaf ligulation for 3 sowing dates (Fig. 6a) and validation of model for a larger range of sowing dates (Fig. 6b-cdef).

Conclusion. By varying a single model parameter, the essential genotypic differences in PP response could be simulated (including “quantitative” and “qualitative” response), but 3 model parameters had to be parameterized to achieve fit to all observed patterns. In an environment comparable to that of Bamako, 3 sowings (June, August, and September) and simple observations of flowering or flag leaf ligulation date are sufficient to phenotype sorghum accessions with the help of the *Impatience* model. Further research will show whether and how genotypic variation of model parameters correlates with genetic information in the context of QTL or association studies. Further studies are also planned to explore the validity of the model for a broader range of latitudes and climatic conditions.

Articles reporting these results:

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Modeling drought effects on rice with *EcoMeristem*: Feedbacks of water and carbohydrate relations on phenotypic plasticity

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Introduction

Technologies created by molecular biologists for accelerating crop improvement are permanently progressing. The challenge today is to provide to geneticist traits of interest as well as related phenotyping methodologies, in order to find out underlying genes and alleles through association or QTL mapping approaches. Phenotyping methods are needed that enable addressing physiological, process based traits that are closer to gene action than conventional, descriptive methods that are subject to large GxE effects. This is particularly the case regarding plant response to abiotic stresses such as drought, involving adaptive biological processes that constitute phenotypic plasticity. Most of these processes are difficult and expensive to access experimentally. Modelling has an important role to play here: if a model formalizes correctly biological processes to simulate the phenotype, then it is possible to use the model as a phenotype analyzer (heuristic approach; Dingkuhn *et al.* 2005; Hammer *et al.* 2002). The model thereby serves to provide genotypic parameter values obtained by model optimization against observed data. If observations used to parameterize the model are easy to measure, this permits high throughput applications within genetic studies.

The objective of this study was to adapt *EcoMeristem*, a crop model simulating rice morphogenesis and phenotypic plasticity during vegetative stage, to the case of drought. This paper describes and tests the new model formalisms based on experimental data.

Material and methods

Experiment: Two experiments (Exp1, 2) were carried out on one rice genotype, IR64 (*Oryza sativa* L., indica group), to analyse rice morphogenesis, sugar metabolism and water status variations under drought. The setup was based on a replicated dry-down design using potted plants in a phytotron, with gravimetric monitoring of soil moisture and plant transpiration, to compute FTSW (Fraction of Transpirable Soil Water: $FTSW = AWS/TTSW$, with AWS, available soil water content and TTSW, total transpirable soil water content). Stressed plants were re-watered after a severe stress level was attained.

Observed dynamics in stressed and control plants of transpiration and leaf expansion rates, plant morphogenesis, sugar content, leaf rolling and senescence, were used to develop and test algorithms implemented in *EcoMeristem*.

Model presentation: *Ecomeristem* (Dingkuhn *et al.* 2006; Luquet *et al.* 2006; Luquet *et al.* 2007) simulates rice morphogenesis based on (i) algorithms and parameters controlling leaf initiation rate (*phyllchron*) and pre-dimensioning (*MGR*, Meristem Growth Rate and *SLAp*, slope of the relation computing structural specific leaf area vs. leaf rank), tillering (*Ict*), radiation use efficiency (RUE) and, as a proportional consequence, root growth; and (ii) daily carbon sink to source balance estimation based on a plant internal competition index, *Ic*, (C supply/demand ratio, demand being the sum of assumed organ daily growth and supply, daily assimilation). Depending on genotypic parameters, plant responds to *Ic* by adjusting organ size and number (phenotypic plasticity). The model is specifically dedicated to model assisted phenotyping, i.e. for optimizing parameters as a means to quantify genotypic, process based traits. For this purpose the model was recently implemented in modelling software, named *EcotropV4* (developed in Delphi language), facilitating model development or modification and implementing optimization and sensitivity analysis tools.

Results

Main experimental results: Water stress reduced transpiration and leaf expansion rates when FTSW dropped below 0.69 and 0.56, respectively (Figure 1). Plant growth and development were slowed (Figure 2). Sugar metabolism was differently affected regarding sink (young leaves, root) and source (mature leaves) organs: starch and sucrose increased in sink organs with and hexoses decreased, while the opposite was observed in source organs (not shown). However, bulk, whole plant carbohydrate concentration was not affected (Figure 1, middle graph).

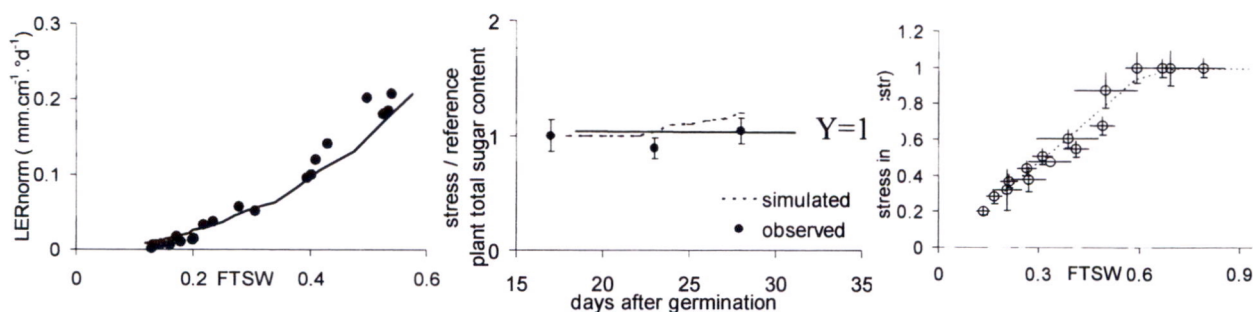


Figure 1 : comparison between observed and simulated (left) RLER (leaf expansion rate normalized by final potential length), (middle) plant total sugar content (stress by reference ratio) and (right) cstr (actual by potential transpiration rate) response to drought in Exp2.

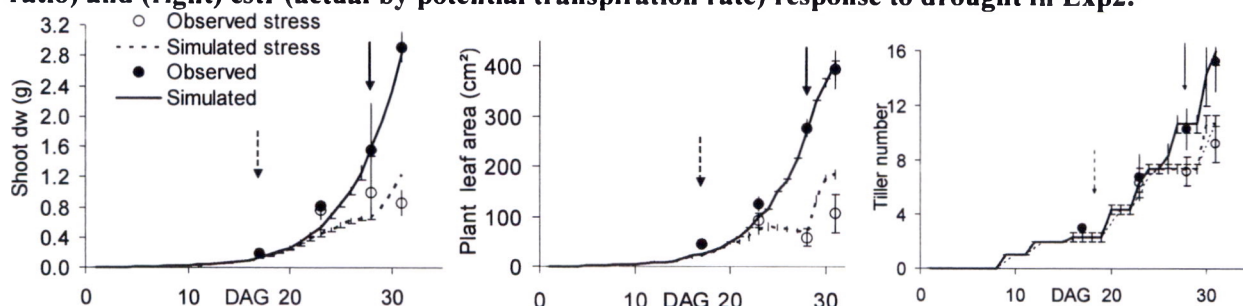


Figure 2 : comparison between observed and simulated shoot dry weight, plant leaf area and tillering dynamics in Exp2. Arrows indicate stress set up (dashed) and re-watering date (plain).

Model adaptation and modeling results: the new formalisms implemented in Ecomeristem consisted first of a water stress index, cstr, equal to the ratio between actual and potential transpiration, function of FTSW and a genotype dependent threshold parameter defined experimentally. Based on cstr as state variable, a set of equations formalizing water stress impact on transpiration, potential carbon assimilation, LER and leaf rolling were implemented.

The improved model simulated well growth response to drought, water use, organogenetic and morphological dynamics while using a common set of model parameters for both drought and control treatments in Exp1 and 2 (Figure 1, 2, results for Exp2). Source-sink relationships between mature and developing organs, tillering and leaf senescence were accurately simulated. The model also reproduced correctly whole plant carbohydrate balance stability from control to stressed treatment, indicating carbon non-limiting conditions of rice plants under drought (Figure 1). However, details metabolic dynamics in source and sink organs were not implemented in the model.

Conclusion

EcoMeristem model adaptations to drought have been successfully implemented. The model is now being applied to a large range of genotypes, to assist in QTL analyses and association mapping for rice.

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Model assisted phenotyping of morphogenetic process diversity within sativa rices

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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 threshold, explaining 41% of *SDW*). Factorial Discrimination analysis using optimized, genotypic model parameter values (*GDW*, *LELin*, *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*, *LELI*), 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	lct	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; lct, controlling tillering through carbon supply, SLAp, slope parameter to compute SLA; phyllo for phyllochon).

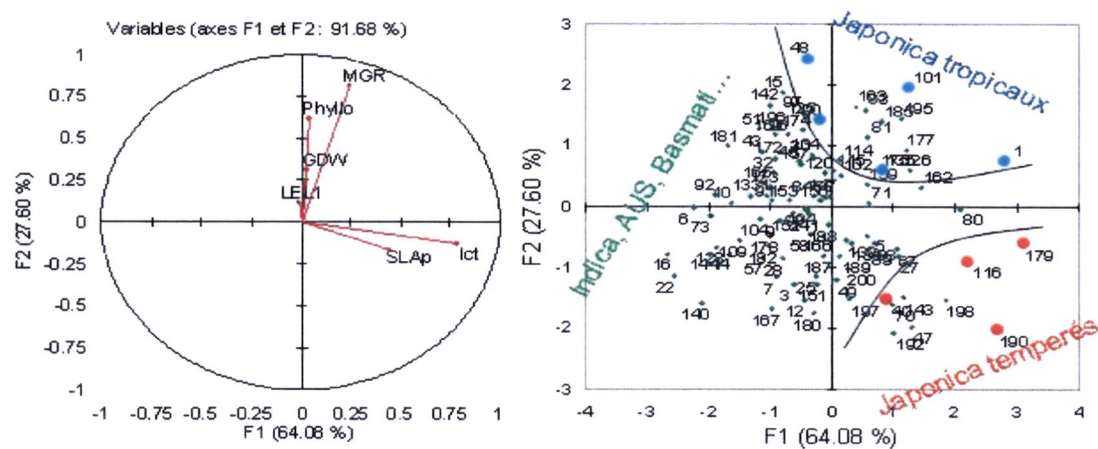


Figure 1: Factorial discriminative analysis of genotypes and genetic groups using EcoMeristem model parameters: LEL1, 1st leaf length; GDW, seed dry wt; MGR, meristem growth rate; lct, threshold for tillering; SLAp, slope parameter for change in specific area; Phyllo, phyllochrone. Left: parameters explaining 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 *lct* 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.

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Mapping QTL involved in rice early morphogenesis as described by the process based parameters of *EcoMeristem* model

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Introduction: Plant models describing organ or whole plant growth and development as a set of response processes to environment are of great potential for the genetic dissection of complex traits. Indeed, related model parameters can be considered as ‘process based’ traits and are expected to (1) have less complex genetic determinisms than related complex traits accessible experimentally and (2) be less prone to uncontrolled environment effects since model equations separate genotypic parameter (G) from environmental variable (E) effects (Dingkuhn et al., 2005). Proof of this concept was provided for response processes at organ level, e.g. by Reymond et al. (2004) for maize leaf expansion rate response to drought. Recently, the model *Ecomeristem* was developed to address early morphogenesis processes involved in whole plant phenotypic plastic expression in response to E. *Ecomeristem* main originality is to formalize in a simple way and based on a few parameters, the daily control of plant morphogenesis by meristems in response to E. E is mainly characterized at plant level by a state variable I_c , plant internal competition index for carbon, daily computed as the ratio between plant C supply (assimilation) and demand (sum of organ daily demand for growth). Main process based genotypic parameters controlling morphogenesis in the model are MGR (Meristem Growth Rate, pre-dimensioning newly created organs), I_{ct} , a threshold parameter regulating tillering depending on I_c state variable, SLAP (controlling leaf structural thickness depending on its rank) and phyllochron (PHYLm, controlling leaf appearance rate). The objective of the present study is to compare QTLs detected by *Ecomeristem* model parameters and by measured related phenotypic complex traits, for then analysing the stability of parameter-QTLs across different growth conditions.

Materials and methods: A mapping population, composed of 178 recombinant inbred lines (RIL) of the IR64 / Azucena cross (Ahmadi et al. 2005), was grown in a phytotron in hydroponic conditions with either optimal or phosphorus deficient (P) nutritive solution, using a alpha lattice design with 2 replications. Twenty-three days after sowing phenotypic traits related to early morphogenesis were measured: plant height (PH), main stem leaf number (LN), tiller number (TN), main stem last ligulated leaf length (LLL), width (LLW) and dry biomass (LLDW), shoot dry weight (SDW), maximum root length (MRL), primary root number (PRN) and root dry biomass (RDW). Data obtained under P conditions are indicated with a p subscript. PH, TN, LN, SDW and RDW data were used as targets to apply *Ecomeristem* model in a inverse mode, i.e. to optimize for each line in the population four of its main morphogenetic parameters described above: MGR, I_{ct} , SLAP, PHYLm. Composite Interval mapping procedures of the MapQTL software package (Van Ooijen, 2006) were used for QTL detection.

Results: Model parameters, MGR, I_{ct} and PHYLm, showed normal distribution within the RILs population and transgressive segregations were observed. Significant genetic variation was observed for almost all parameters, under both growth conditions. Significant correlations were observed between model parameters and measured data. Broad sense heritability of model parameters did not exceed the one of measured traits. At least one QTL was identified for each parameter (except SLAP) under both growth conditions, but the percentage of genetic variation explained by each QTL was low, seldom higher than 10%. Under optimal growth conditions, two MGR QTLs, out of 3, were mapped on chromosome 1, near the *Sd1* locus involved in meristem growth via the biosynthesis of GA. This chromosomal area very

often exhibits a QTL for plant height with a bimodal LOD distribution. Interestingly MGR allowed detecting 2 QTLs in this chromosomal segment by contrast with PH. The QTLs for lct were all mapped in chromosomal areas involved in tillering ability. The 2 QTLs for PHYLM co-localised with QTLs involved in leaf number, tillering ability and vegetative growth duration but didn't co-localise with known QTLs for phyllochron and plastochron. QTLs involved in MGR and PHYLM were mapped in same chromosomal area under the two growth conditions, which was not the case for QTLs involved in lct.

Conclusion: QTLs mapped for model parameters MGR, lct and PHYLM were consistent with the ones involved in morphogenesis related complex traits directly measured, i.e. plant height, biomass accumulation, tillering number and leaf number. To the exception of PHYLM, they were also consistent with the literature regarding genetic bases of related traits. Model parameters allowed identifying other genomic areas involved in morphogenesis compared to direct observations. The unexpected instability of lct QTLs across growth conditions, expression of an inducible phenotypic plasticity, needs further confirmation.

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