EcoMeristem, a model of morphogenesis and competition among sinks in rice. 2. Simulating genotype responses to phosphorus deficiency

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Abstract. Phenotypic plasticity enables plants to adjust their morphology and phenology to variable environments. Although potentially important for crop breeding and management, the physiology and genetics of plasticity traits are poorly understood, and few models exist for their study. In the previous paper of this series, the structural–functional model EcoMeristem was described and field validated for vegetative-stage rice. This study applies the model to an experimental study on phosphorus deficiency effects on two morphologically contrasting rice cultivars, IR64 and Azucena, grown in controlled environments under hydroponics culture. Phosphorus deficiency caused severe biomass growth reductions in the shoot but not in the root, thus increasing the root / shoot weight ratio. It also inhibited tiller formation and leaf elongation, prolonged the phyllochron, and increased carbohydrate reserve pools in the plant. Analysis aided by the model identified inhibition of leaf extension and tillering as primary effects of the stress. Physiological feedback probably led to longer phyllochron, greater reserve accumulation and root growth stimulation. The main effect of P deficiency appeared to be a reduction in demand for assimilates in the shoot while photosynthetic radiation use efficiency remained nearly constant, resulting in spill-over of excess assimilates into reserve compartments and root growth. The results are discussed in the light of future applications of EcoMeristem for phenotyping and genetic analyses of phenotypic plasticity.

Keywords: carbohydrates, leaf extension rate, Oryza sativa L., phyllochron, root / shoot ratio, tillering.

Introduction

Genotype × environment interaction (G × E) is a term commonly used by breeders and geneticists to describe phenotypic variation that can be neither explained by genotype nor by environment variation alone (Brancourt-Hulmel and Lecomte 2003). It thus quantifies the bulk, unexplained, observed variation but provides no information on the nature and possible adaptive value of this variation. Furthermore, statistical G × E is based on large numbers of genotype and environment combinations, and therefore does not permit extrapolation of individual genotypes’ behaviour to specific environments. Statistical (black box) G × E terms are useful in conventional breeding but are less helpful in the development of gene-specific markers derived from candidate genes having a known function. Researchers investigating phenotypic expression of genes and their polymorphisms prefer describing G × E as phenotypic plasticity (Dingkuhn 1996; Wright and McConnaughay 2002; Luquet et al. 2005a), which is the result of variable expression of the genes concerned, their interaction with the effects of other genes, and physiological interactions among gene products within the plant system.

From a physiological angle, phenotypic plasticity is the environment-induced diversity of phenotypes a given genotype can generate, brought about by the responsiveness of the plant’s metabolic, growth and developmental processes to external and internal signals. This responsiveness may or may not involve changes in the expression patterns of genes, depending on whether the response is actively induced or inherent (constitutive) to the physiological apparatus. Phenotypic plasticity rarely affects the body plan (or basic structure) of the phenotype, but can strongly affect morphology (Dingkuhn 1996; Bos and Neuteboom 1998),

Abbreviations used: CIN, cell wall invertase; DAT, days after transplanting; G × E, genotype × environment interaction; IC, internal competition index; LER, leaf extension rate; MAP, model assisted phenotyping; PAI, plant area index; RSR, root / shoot DW ratio; RUE, radiation-use efficiency; Ta, daily mean air temperature; Tb, base temperature; TT, thermal time.
Phenotyping (MAP) and concluded that advances can be expected from new types of whole-plant models simulating phenotypic plasticity, in order to (i) obtain crop parameters that are less prone to G × E ‘noise’ but instead, explain some of it, and (ii) measure the impact of model parameter variation on plant responses to variable environments (Chapman et al. 2002; Boote et al. 2003; Hoogenboom and White 2003). It is also expected that model parameters for calibrating biological processes, as opposed to parameters forcing phenotype features directly, are functionally closer to gene action. Model-assisted phenotyping may thus give better access to major QTLs or the functional analysis of candidate-gene polymorphisms than phenotyping based on the measurements conventionally made by breeders (e.g. leaf area index or yield). This paper aims to analyse the phenotypic plasticity exhibited by rice under P deficiency, and the capability of the EcoMeristem model to simulate this plasticity on the basis of genotype specific crop parameters. We thereby do not attempt to study or model P uptake and fluxes within the plant. In fact, by using hydroponics, the experimental design imposes levels of plant P deficiency that are less prone to of plant adaptations that may improve P uptake in the field, such as secretion of substances modifying the rhizosphere. Our focus is thus on physiological and morphological responses of the plant to an imposed deficit condition.

Materials and methods
Experimental design
An experiment was conducted in controlled environments at CIRAD (Montpellier, France) between April and June 2004 with the objectives to characterize morphological and architectural variation (phenotypic plasticity) induced by phosphorus (P) deficiency, relate this plasticity to assimilate partitioning within the plant, and evaluate the capacity of the EcoMeristem model to simulate these phenomena. The experiment had two P levels treatments P− and P+ and four replicates in a randomised block design. Hydroponics was used to ensure that differential P treatments had full impact on plants while minimising the effect on P uptake of specific physiological adaptations such as citrate and phosphate secretion by roots, assuming that these were removed daily by irrigation. Two contrasting rice genotypes were used, IR64 (Oryza sativa L., indica type) having small leaves and producing a large number of tillers per plant during vegetative development and Auscena (japonica type) having larger leaves and a lower tillering rate.

After seeds were germinated for 4 d at 33 °C in an illuminated culture chamber, plants were transplanted in drained, 1-L pots containing quartz sand. Pots were watered daily to field capacity with a culture solution (pH 5.5) containing the following nutrients (concentrations in mM): K2HPO4 = 0.21, KH2PO4 = 0.06, KNO3 = 1.98, Ca(NO3)2 = 2.96, MgSO4 = 0.61, KCℓ = 0.1, (NH4)2SO4 = 0.53, MnSO4 = 2.9 × 10−3, (NH4)2MoO4 = 6 × 10−3, CuSO4 = 6.3 × 10−3, ZnSO4 = 2.5 × 10−3, H3BO3 = 7.4 × 10−3, EDTA-Fe = 0.206. Air temperature in the culture chamber was 28/23 °C (day/night), relative air humidity was 65%/30% (day/night), and PAR was 0.0 MJ m−2 d−1, supplied with halogen lamps during a 14-h photoperiod.

From 12 d after transplanting (DAT) onwards, half of the plant population was subjected to a P deficiency treatment (P−: 0.009 mM PO43−, or 1/30 of control treatment) while the remaining plants continued to receive optimal nutrition (P+: 0.27 mM PO43−). According

In part 1 of this series (Luquet et al. 2006), we introduced and validated the model EcoMeristem, which simulates resource-dependent growth and morphogenesis of rice, on the basis of internal competition relationships among growing organs (sinks). According to this model, organogenetic processes, which create and dimension new sinks, are affected by the balance between supply and demand of plant carbon assimilate, a concept inspired by recent findings on signal transduction (Black et al. 1995; Roitsch et al. 2000). Assimilate supply thus affects demand, and demand feeds back on fresh assimilate production through organ growth. The model was developed to assist phenotyping of complex traits, such as growth responses to environment, for QTL development and association mapping. Such heuristic approaches (Chapman et al. 2002; Hammer et al. 2002) have recently been explored with models describing the physiological processes (Reymond et al. 1995; Roitsch et al. 1997) and phenology (Clerget et al. 1997) and concluded that advances can be expected from new types of whole-plant models simulating phenotypic plasticity, in order to (i) obtain crop parameters that are less prone to G × E ‘noise’ but instead, explain some of it, and (ii) measure the impact of model parameter variation on plant responses to variable environments (Chapman et al. 2002; Boote et al. 2003; Hoogenboom and White 2003). It is also expected that model parameters for calibrating biological processes, as opposed to parameters forcing phenotype features directly, are functionally closer to gene action. Model-assisted phenotyping may thus give better access to major QTLs or the functional analysis of candidate-gene polymorphisms than phenotyping based on the measurements conventionally made by breeders (e.g. leaf area index or yield).
to Marschner and Vetterlein (1989), P in the soil solution strongly affects plant morphology at and below a concentration of 0.01 mM. Leaf and root P concentrations in the deficiency treatment decreased within 3 d after treatment onset to ~1 mg g⁻¹ (DW based) and fluctuated little thereafter. This concentration corresponds to the critical concentration for severe P deficiency in rice (IRRI 2005).

Destructive measurements on roots and shoots were made on five dates (12, 19, 25, 30 and 36 DAT). Details of the sampling procedure and measurements are described in the companion paper (Luquet et al. 2006). Destructive measurements included bulk root and individual leaf sheath and blade dry weight on each culm. The same samples were used for analyses of starch, sucrose, fructose and glucose content by Dionex HPLC (HPAE-PAD detector) after hydrolysis (case of starch) and extraction with 80% ethanol at 80°C. Carbohydrate analyses were only conducted on three of the four available replications.

Non-destructive measurements (twice daily) included observations on leaf appearance on all culms to calculate phytochron (thermal time separating the appearance of two successive leaves on the main stem), leaf blade and sheath length and width and tiller number. Plant area index (PAI, total leaf blade area in cm²) was estimated by eqn 1.

\[
PAI = \sum_{i=1}^{n} (L(i) \times W(i)) \times 0.725, \quad (1)
\]

where \(L(i)\) and \(W(i)\) are the length and width (cm), respectively, of blade \(i\) on a given plant, and 0.725 is an allometric coefficient used to relate \(L\) and \(W\) to surface area LA (Tivet et al. 2001).

Specific leaf area (SLA, m² g⁻¹) of individual blades was computed by eqn 2.

\[
SLA = LAb / DWb, \quad (2)
\]

where \(LAb\) and \(DWb\) represent individual leaf blade area (cm²) and dry weight (g), respectively.

Growth and development dynamics were expressed using either thermal time (TT in °C/degree) or DAT as reference.

\[
TT = \sum_{i=1}^{n} (Ta - Tb), \quad (3)
\]

where \(Ta\) is the daily mean air temperature (°C) and \(Tb\) the base temperature below which plant development stops (assumed to be 13°C; Tivet et al. 2001).

**EcoMievroom model**

A detailed description of model structure, parameterisation procedures and sensitivity to input variables and crop parameters is given in the companion paper (Luquet et al. 2006). Only the underlying concepts are repeated here.

EcoMievroom is a whole-plant, deterministic, dynamic, radiation- and temperature-driven crop model (the model also has a soil and plant water balance but these modules were not used in this study). The specificity of the model is its capability to simulate competition for assimilates (aggregate, incremental supply) among growing organs (demand functions). The supply, consisting of daily carbon assimilation minus maintenance respiration, is thereby simulated at the scale of the whole plant (situated within a canopy formed by a homogenous population), whereas demand is simulated at the individual organ level, then aggregated to provide a whole-plant demand term. This procedure allows comparison of plant-level supply and demand for each time step (24 h) and simulation of feedbacks of supply/demand imbalances on organ number (organogenesis), growth rate and final size (organogenesis). Supply/demand relationships are estimated with a state variable called \(Icut\) (Index of internal competition), calculated as aggregate supply divided by aggregate demand for each time step of model execution.

Excess assimilates (\(Icut > 1\)) are reversibly stored as reserves, or if the reserve compartment is saturated, reduce photosynthesis (product inhibition). Deficient assimilates (\(Icut < 1\)) cause two types of adaptive responses. First (\(Icut < 1\)), the current assimilate shortfall for growth is buffered by reserve mobilisation, organ senescence (followed by recycling) and ultimately, delays in organogenetic cycles, in this order; and second, organs that are being initiated are down-sized, leading to smaller demand when they turn into sinks. \(Icut\) conditions also branching events, i.e. tiller appearance in the case of rice. This system of feedbacks stabilises plant carbon balance by adjusting plant development to resources.

The model was parameterised using target files containing morphological observations made on the last sampling date (16 DAT), i.e. 38 d after germination, using statistical optimisation procedures in the case of morphogenetic parameters that cannot be measured directly only the plant (Luquet et al. 2006). Parameterisation was done individually for each treatment and replication in order to calculate SE of parameter values. The objective was thereby not to use the model to predict plant behaviour (extrapolation), but to study treatment and genotype effects on crop parameters (heuristic approach).

**Results**

Treatment and genotype effects on model parameters

The complete set of crop parameters was presented for IR64 in part 1 of this study (Luquet et al. 2006). Initial parameters describing seed and seedling properties differed for Azucena, which had slightly heavier seed (30 mg; IR64: 28 mg) and first leaf (6 mg; IR64: 4 mg), and slightly lower, initial, specific leaf area (SLA) of the first leaf (0.044 m² g⁻¹; IR64: 0.047 m² g⁻¹). Crop parameter values taken from the literature, such as base temperature (TB), PAR extinction coefficient (Kdf) and tissue storage capacity of carbohydrate reserves (STORM), were assumed to have the same values for Azucena and IR64 (details in Luquet et al. 2006).

Potential radiation use efficiency (RUEpot), the organogenetic parameters plastochron (PLAS), potential meristem growth rate (MGR), and threshold for tiller initiation (Icut), as well as the ratio of root v. shoot demand for assimilates (RSDdem) were optimised statistically for each combination of genotype, P treatment and replication (Table 1), in order to evaluate genotype, treatment and combined effects on parameter values.

The parameter value for RUEpot was the same for Azucena and IR64 under P⁻ supply (2.9 g MJ⁻¹). It was only very slightly reduced under P⁺ (P = 0.05 for IR64, n.s. for Azucena). The relatively high observed values of RUEpot, compared to 2.0–2.3 g MJ⁻¹ commonly found for rice (Kiniry et al. 2001) were caused by the inclusion of root growth and maintenance respiration in its calculation, processes that are conventionally not included in RUE.

Azucena had a significantly (P < 0.05) higher value for PLAS than IR64, indicating that leaves on the main stem were initiated in slower succession. Phosphorus deficiency further increased PLAS in both cultivars, thus reducing development rate of the plant. The MGR value for Azucena (1.9) was higher than that of IR64 (1.6), indicating that the factor by which
successively produced leaves increased in DW was higher in IR64, and tillering. Phosphorus deficiency further increased $l_{ct}$ for both cultivars, thus inhibiting tiller production. Both cultivars had the same value for $R_{SR\text{dem}}$ under abundant P supply, and thus did not differ in assimilate partitioning to roots. Phosphorus deficiency, however, significantly ($P<0.05$) increased $R_{SR\text{dem}}$ in both cultivars, and particularly in Azucena.

In summary, according to the empirical model parameters, the two cultivars had the same RUE (a measure of photosynthetic capacity) and root/shoot partitioning ratios, but Azucena had lower tillering ability, longer intervals between successively appearing leaves and larger leaves. Phosphorus deficiency had little effect on photosynthetic capacity and none on leaf size but reduced leaf and tiller size was not affected by P deficiency. Consequently, leaf

The measured DW ratio between root and shoot systems was $\sim 0.6$ in both cultivars at the time when germination was completed (first leaf after the coleoptile fully expanded) and the seedlings were transplanted (data not presented). At 12 d after transplanting, the root/shoot DW ratio was between 0.25 and 0.3 and then changed little under abundant P supply, but increased markedly under P deficiency. The model, due to treatment-specific calibration, reproduced these kinetics accurately (Fig. 2).

Absolute DW of root systems per plant at 30 DAT, when P treatment effects were fully expressed, was slightly smaller under P deficiency than in controls (reductions of $\sim 17\%$ in IR64 and $\sim 15\%$ in Azucena) (Table 2). The greater DW fraction allocated to roots under P deficiency ($\sim 32\%$ compared to 21% in controls) did thus not offset the even stronger overall growth inhibition.

Leaf appearance and extension rates

The observed phyllochron (thermal time elapsing between two successively appearing leaf tips) was constant during the developmental stages observed on control plants, as indicated by a linear relationship between leaf number and thermal time (Fig. 3). Leaf appearance rates can be calculated by the slope of this function, which was 0.0169 leaves °Cd−1 for IR64 and 0.0147 leaves °Cd−1 for Azucena under abundant P supply. Phosphorus deficiency increased phyllochron, and conversely, reduced leaf appearance rates to 0.0125 leaves °Cd−1 (IR64) and 0.0104 leaves °Cd−1 (Azucena). The relative reduction of leaf appearance rate induced by P deficiency was 26% for IR64 and 29% for Azucena, indicating that varietal and stress effects were additive, and not interactive.

Despite different leaf appearance rates (and therefore, different elongation durations because in rice, the rapid elongation of leaf $n$ stops when leaf $n+1$ appears; Nemoto et al. 1995; Luquet et al. 2006), leaf blade size was not affected by P deficiency. Consequently, leaf

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IR64 Mean</th>
<th>IR64 SE</th>
<th>Azucena Mean</th>
<th>Azucena SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{UEpot}$ (potential radiation-use efficiency, g m$^{-2}$ J$^{-1}$)</td>
<td>2.88</td>
<td>0.17</td>
<td>2.91</td>
<td>0.05</td>
</tr>
<tr>
<td>$R_{MGR}$ (plastochron, °Cd)</td>
<td>47.3</td>
<td>1.4</td>
<td>55.0</td>
<td>0.6</td>
</tr>
<tr>
<td>$R_{Ict}$ (threshold for tillering, unitless)</td>
<td>1.60</td>
<td>0.08</td>
<td>1.92</td>
<td>0.08</td>
</tr>
<tr>
<td>$R_{RSEm}$ (Root/shoot assimilate demand ratio)</td>
<td>0.31</td>
<td>0.01</td>
<td>0.29</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 1. Empirical values for key parameters of the EcoMeristem model for IR64 and Azucena rice under abundant (P+) and deficient (P−) treatments (means and SE of four replications)
extension rates (LER) observed between leaf tip appearance and ligulation were strongly affected by genotype and P treatment (Fig. 4).

Calculation of LER from plant observations was done in two different ways. The mean LER was obtained by dividing the final leaf blade length by the time elapsing between tip and ligule appearance (corresponding to a phyllochron), and the maximal LER was calculated from daily observations on the length of the appeared portion of the leaf. In both cases, it was assumed that the previous leaf’s sheath, from which the tip of the new leaf appeared, had already attained its final length, and that no internode elongation took place, which would have pushed the apex upwards and caused an erroneous LER. Measurements of sheath length and apex position confirmed these assumptions (data not presented).

Mean LER increased approximately linearly with leaf position, whereas maximum LER increased linearly for the first four leaves and then levelled off, or even decreased. These findings are not necessarily conflicting because they integrate different periods of observation. As an observation common to both methods applied, Azucena generally had higher LER than had IR64, and P deficiency reduced LER for both cultivars. The model accurately reproduced the observed differences in mean LER, but it simulated a non-linear (progressive) increase in LER with leaf position, as opposed to the nearly linear pattern observed. It will therefore require some modifications in the way the MGR parameter is implemented (which governs size relationships among leaf positions), particularly for simulations of longer periods of growth that involve larger number of leaves.

Carbohydrate dynamics

Observed patterns of carbohydrate distribution and dynamics were quite similar in both cultivars and we present details only for Azucena (Fig. 5). Sucrose concentration in leaf blades was constant across sampling dates at approximately 70 mg g⁻¹ and was not affected by P treatment. Glucose concentration in leaf blades, which was much lower than that of sucrose, increased steadily during plant development. This increase was strongly inhibited by P deficiency, a phenomenon that was probably related to the reduced growth rate of leaves observed at the same time. Fructose concentration was generally proportional to glucose concentration, the ratio being ~1:1 in shoot organs (leaf blades and sheaths) and 2:1 in roots (Fig. 5, inset graph).

In the leaf sheaths, sucrose concentrations were less stable than in blades (Fig. 5). They showed a decreasing trend in control plants, but not in P-deficient plants, resulting in higher sucrose concentrations relative to controls. A similar, but less significant increase in starch concentration was also observed in sheaths under P deficiency.

Glucose concentrations in roots varied little during the experiment and were not significantly affected by P treatment. Sucrose concentrations in roots, however, were significantly increased by P deficiency. This applied also to starch, although concentrations of this compound were extremely
In summary, leaf blades whose growth was inhibited by P deficiency had markedly decreased concentrations of hexoses. Such a decrease was not observed in roots, whose growth was stimulated by P deficiency in relative terms (compared with shoot growth). However, P deficiency caused a marked increase in sucrose and starch concentration in sheaths and roots.

The model does not permit simulation of specific carbohydrate compounds, but it distinguishes between assimilate incorporated into structural biomass (supply) and reserves (here defined as carbohydrates that are not immediately invested in structural growth). According to the model, assimilate resources (and thus, \( k_s \)) are high initially due to seed reserves (Fig. 6). \( k_c \) then attains a steady-state equilibrium, fluctuating between 0.8 (deficit) and 2 (abundance), the oscillations being caused by organogenetic (and thus carbon demand) cycles that produce cohorts of leaves and tillers. This steady-state level is slightly higher for P-deficient plants than for control plants because of general carbon demand decrease due to lower leaf initiation rates and a higher \( k_c \) threshold for tillering. As a result, higher levels of transitory assimilate reserves in the shoot are simulated for P deficient plants. The simulated patterns of reserves resembled those of observed starch and sucrose concentration in the sheath, although the model seemed to over-estimate the reserve compartment. The measurements of starch and sucrose, measured in the morning, however, represent only trend information because diurnal peak concentrations are not known and other compounds may also have reserve function.

### Relationship between root / shoot ratio and sugar concentrations

The modelling results indicated that P-deficient plants accumulated more carbohydrate reserves because demand for assimilates in growing shoot organs (tillers and leaves) was reduced. This might also explain why root \( \times \) shoot DW partitioning was enhanced in these plants, and we therefore compared sugar concentrations in various compartments with root / shoot DW ratios (RSR) (Fig. 7). Across P treatments,

**Table 2.** Absolute root DW and sugar (glucose, fructose, sucrose and starch) content per plant at 30 DAT for IR64 and Azucena rice under abundant P supply (\( P^+ \)) and deficient supply (\( P^- \)).

<table>
<thead>
<tr>
<th>Compartment</th>
<th>IR64 P+</th>
<th>IR64 P-</th>
<th>Azucena P+</th>
<th>Azucena P-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root DW (g plant(^{-1}))</td>
<td>0.290 ± 0.002</td>
<td>0.247 ± 0.014</td>
<td>0.312 ± 0.010</td>
<td>0.260 ± 0.019</td>
</tr>
<tr>
<td>Root DW (% of plant DW)</td>
<td>21.5</td>
<td>32.4</td>
<td>20.1</td>
<td>31.9</td>
</tr>
<tr>
<td>Root sugar content (mg plant(^{-1}))</td>
<td>7.97 ± 1.31</td>
<td>10.59 ± 1.24</td>
<td>17.04 ± 1.22</td>
<td>16.46 ± 2.05</td>
</tr>
<tr>
<td>Root sugar content (% of plant sugar content)</td>
<td>7.5</td>
<td>15.8</td>
<td>10.2</td>
<td>17.0</td>
</tr>
</tbody>
</table>
a negative correlation was observed between leaf blade glucose concentration and RSR, and a positive correlation was observed between sheath reserve (starch + glucose) concentration and RSR. These relationships, which were similar for the two genotypes, showed an increasing slope as the plants developed, and were significant (P < 0.05) only for the later sampling dates (25 and 30 DAT).

**Discussion**

**Mechanism of P deficiency effects on morphology seen through the model**

On the basis of the concept of inter-organ competition for assimilates realised in the model (which is in part hypothetical) and the simulation results using the fitted model, a coherent theory of P deficiency effects on growth and development processes at the scale of the whole plant emerges. It strongly supports the recent finding that under P deficiency, assimilate resources are abundant in rice (Wissuwa et al. 2005). We suggest that P deficiency reduces demand for carbon in the leaves (through inhibition of leaf appearance rate, leaf extension rate and tiller production).

Lower demand for assimilates in growing leaves is associated with reduced glucose concentrations in the blades, which can be seen as an indicator of smaller sink activity (Black et al. 1995; Yang et al. 2003; Roitsch and Gonzales 2004).

The release of glucose from sucrose through cell wall invertase (CIN) activity in juvenile tissues is a rate-limiting step for growth (Black et al. 1995; Roitsch et al. 2000). The predicted increase of assimilate storage pools, known to be predominantly located in leaf sheaths (Luquet et al. 2005a), is supported by measurements. In contrast, no increase in glucose concentration was observed in roots, although their growth was stimulated by P deficiency (at least, relative to shoot growth), whereas sucrose concentration in roots increased significantly.

These observations suggest that root growth was not stimulated in terms of increased sink activity (which would probably be associated with an increase in glucose concentration), but more passively by the spill-over of excess assimilates from the shoot to the root. The increase of assimilate storage in sheaths and the build-up of sucrose in the roots support this hypothesis. In fact, Burleigh and Harrison (1999) and Shane et al. (2003) suggested that P deficiency is sensed in shoot organs and may only indirectly affect root growth behaviour.

The available data and hypotheses now enable us to propose a comprehensive theory of how the various morphological and phenological effects of P efficiency may come about and interact at the whole-plant level (Fig. 8). Phosphorus deficiency is sensed in the leaves when concentration drops below a critical value (Burleigh and Harrison 1999; Shane et al. 2003). This triggers at least three physiological responses induced by an unknown signal,
Fig. 4. Extension rates of leaf blades on the main stem as a function of leaf position (in chronological order), genotype (Azucena or IR64) and P treatment (deficient or adequate). Left: mean leaf extension rate computed from final leaf length and the corresponding, presumed duration of extension (tip appearance to ligule appearance). Centre: maximal leaf extension rate computed from daily observation on the length of the visible (exserted) part of the leaf blade. Right: simulated, mean leaf extension rates.

which may be hormonal (Burleigh and Harrison 1999) and may involve sugar signalling (Roitsch et al. 2000). These responses are (i) a decrease of the sensitivity of tillering to assimilate availability, (ii) a decrease in leaf extension rates and a proportional increase in leaf extension duration, while final leaf size remains unaffected, and (iii) the stimulation of citrate and phosphatase secretion by roots (Raghothama 1999; Lim et al. 2003; Shane et al. 2003; not measured in this study).

Tiller production of rice depends on the one hand on available buds (topological sites, equivalent to leaf axils; Hanada 1993; Tivet et al. 2001) and on the other hand on available resources (Dingkuhn 1996; Dingkuhn et al. 1999). Bud number usually does not limit tiller production. This was also the case in this study (data not presented), and the longer phyllochron observed under P deficiency can therefore not explain the reduction in tillering. In fact, excess production of assimilates observed in this treatment should theoretically favour tiller production. The opposite was the case, indicating that tillering was more specifically inhibited by P deficiency.

We hypothesise that the presumed, direct effects of P deficiency on leaf extension rates and tillering caused some other, more indirect effects. The increased extension duration of leaf blades, associated with a delay in ligule appearance, may be responsible for the longer plastochron observed under P deficiency. In rice, ligule appearance from the previous leaf’s sheath generally coincides with cessation of leaf blade extension and the appearance of the next leaf tip (Nemoto et al. 1995). In fact, Fournier et al. (2003) and Bos and Neuteboom (1998) showed for wheat that ligule appearance is the trigger for both events, enabling a coordinated morphogenesis of the culm. It is therefore likely that leaf extension duration, which depends on extension rate, feeds back on leaf appearance rate (which is equal to phyllochron$^{-1}$). However, the opposite causalities can be hypothesised as well, namely, that plastochron (leaf initiation rate$^{-1}$) governs phyllochron that governs leaf extension duration. Further studies are necessary to determine whether P deficiency directly affects leaf extension rate and duration, or plastochron, or both.

The stimulation of root growth relative to shoot growth under P deficiency may also be an indirect effect, caused by under-utilisation of assimilates in above-ground organs. The accumulation of sucrose and starch in the leaf sheaths, generally considered a storage organ, clearly indicates that supply exceeded demand. Although leaf blade sucrose content remained remarkably constant among sampling dates and P treatments, glucose (Fig. 5) and fructose, which are cleavage products of sucrose and occurred at a ratio of nearly 1:1 in the shoot (data not presented), decreased sharply under P deficiency. We interpret this as an indication of reduced sink activity. CIN is considered a key enzyme for the regulation of sink activity of growing tissues (Black et al. 1995;
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Fig. 5. Bulk organ concentrations of glucose (a, d, g), sucrose (b, e, h) and starch (c, f, i) in blades (a, b, c), sheaths (d, e, f) and roots (g, h, i), observed on Azucena rice under abundant (P+) and deficient (P−) phosphorus supply, on four consecutive sampling dates. Vertical error bars indicate standard error of three replicates. Inset: linear regressions between fructose and glucose concentrations in roots (Y = −1.36 + 1.97X, R²=0.78) and shoot organs (blade or sheath; Y = −0.25 + 0.93X, R²=0.98), for all dates, treatments and the two cultivars confounded.

Yang et al. 2003) and may have been down-regulated in shoot organs by the stress. In drought stressed rice, for example, the reduced expression of organ-specific cell wall invertase genes is associated with growth inhibition (Ji et al. 2005).

If local hexose concentration is indeed an indicator of growth related sink activity, the sinks in the root system were not stimulated by P deficiency. In fact, root growth did not increase in absolute terms, but only relative to the shoot. But the substantial increase in sucrose concentration in the root system under P deficiency suggests that more sucrose was transported to the roots. Consequently, growth of the root system was probably not stimulated by signals, but simply benefited from greater assimilate supply. This
**Fig. 6.** Simulated kinetics of index of internal competition (Ic) and shoot carbohydrate reserves (fraction of dry matter) for Azucena rice under differential P supply (P+, P−). Left: tillers (↑) are initiated when Ic exceeds the physiological threshold Ict (horizontal line), which is higher under P deficiency (P−), i.e., on average, higher under P− (1.37) than under P+ (1.25). Right: simulated reserves decrease over time, following a pattern similar to that of the observed sugar concentrations in the sheath. Oscillations are caused by organogenetic cycles. Results for IR64 rice were similar. Observed sucrose and starch reserves in the leaf sheaths are plotted for comparison.

**Fig. 7.** Relationships between bulk organ sugar concentrations on DW basis (a, b: glucose in leaf blades; c, d: starch and sucrose in sheaths) and the root/shoot dry weight ratio at three sampling dates for Azucena (a, c) and IR64 (b, d) rice. Three replications and two P treatments confounded.

can be modelled as a spill-over of abundant resources from the shoot to the root, causing a modified biomass partitioning pattern.

**Differences between genotypes seen through the model**

Although P deficiency decreased dry matter growth by nearly 60% in both cultivars (Fig. 1), radiation efficiency (RUE) was reduced by only 13% in IR64 and 6% in Azucena (Table 1). Both cultivars had the same RUE in the control treatment. This indicates that dry matter production can be extremely variable even for a given light environment and given RUE, depending on how rapidly and efficiently assimilates are re-invested in leaf area production (Luquet et al. 2006).

The two cultivars differed strongly in morphology, Azucena having larger leaves, longer phyllochron and fewer tillers than IR64. Accordingly, the genotypes differ in the MGR, PLAS and Ict parameters, but not in the RUEpot and RSRdem parameters (Table 1, control columns). Under P deficiency, some of these parameters change little (RUEpot and MGR), but others increase strongly (PLAS, Ict and RSRdem). As discussed in the previous section, the increase in RSRdem, stronger for Azucena (+58%) than for IR64 (+35%) (Table 1), can actually be explained with a spill-over of excess assimilates into root growth, suggesting that RSRdem, and thus root demand for assimilates, was not truly affected by P deficiency. This also explains why the model apparently over-estimated the transitory reserve pool while simulating its dynamics and treatment effects quite well (Fig. 6).

The P deficiency effect on PLAS was greater in Azucena (+38%) than in IR64 (+23%), and its effect on Ict was greater in IR64 than in Azucena (+88 and 18%, respectively,
Reduced P uptake
Shoot P deficiency
Physiol. signal(s)
Slower leaf extension
Delayed ligulation
Longer plastochron
Longer phyllochron
Fewer leaves (and fewer tiller buds)
Reduced assimilate demand for shoot growth
Inhibition of tillering
Reserve build-up in shoot organs
Citrate and phosphatase secretion
Root growth stimulation (rel. to shoot)

Italicics = observed in this study

Fig. 8. Hypothetical diagram integrating various morphological and physiological effects of P deficiency in rice.

Table 1). These stress-induced changes in parameter values indicate that the parameters, which can be considered traits in the context of genotype comparison or phenotyping, are probably subject to G × E interactions. More studies are needed to evaluate the robustness of the model's crop parameters. In general, it can be expected that they are more stable (less prone to G × E) than direct observations on plants, such as plant biomass, tiller number or leaf area at a given date and situation. But stresses, such as severe P deficiency, can obviously change plant reaction norms, necessarily involving changes in model parameter values. Such heuristic analyses (Hammer et al. 2002; Dingkuhn et al. 2005) can help distinguish constitutive and inducible, phenotypic plasticity. Constitutive plasticity translates environment variability into phenotype variability using constant reaction norms (or constant crop parameter values), and inducible plasticity is the result of changing reaction norms (or changing parameter values). However, as the example of root/shoot partitioning showed (change in root sink activity or spill-over of shoot assimilates?), the distinction between constitutive and inducible plasticity depends in part on model structure, and can thus be artificial. Proof of concept can only be obtained by relating genetic parameters, such as QTLs or candidate-gene polymorphisms, to model parameters and their variability. Such analyses are currently in progress.

Conclusion
Morphological and developmental effects of P deficiency were analysed for two contrasting rice varieties during vegetative growth, in order to test the EcoMeristem model's capacity to capture genotypic differences and stress induced, phenotypic plasticity. The model proved to be of great value in relating to each other the various morphological and phenological changes the stress induced. According to EcoMeristem, two direct effects of the stress (inhibition of tillering and leaf blade extension rate) can sufficiently explain the remaining stress effects observed (reduction of biomass growth, longer plastochron, increased assimilate partitioning to roots, increase in plant carbohydrate reserves). The model also enabled measurement determining genotype-specific parameter values, a heuristic application. These might be of future use in model assisted phenotyping for process-based traits, which are difficult to estimate without the help of models.

Acknowledgment
The authors thank the Generation Challenge GCP of the CGIAR for partially funding this research.

References


Manuscript received 31 October 2005, accepted 18 January 2006

http://www.publish.csiro.au/journals/fpb