Phenotypic Plasticity of Rice Seedlings: Case of Phosphorus Deficiency

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Abstract: The aim of this study is to characterize the plasticity of root and shoot morphology in rice, the genomic model plant for cereals, using P deficiency as environmental factor causing variability. A phytotron study on Nipponbare (Oryza Sativa L.) seedlings was conducted to analyze the effects of P deficiency on plant organogenesis (tiller and leaf appearance, root apex number) and allometric relationships (root/shoot weight ratio, specific leaf area (SLA) and specific root length (SRL), blade/sheath weight ratio). The results confirmed that the main effect of P deficiency is a reduction of shoot growth for the benefit of the root system. Reduced shoot growth was associated with reduced tiller production, longer phyllochron and reduced leaf elongation rate while final leaf size remained unchanged. The reduced leaf elongation rate might be a primary response to P deficiency and this caused lower phyllochron and tillering by feedback. Allometric parameters such as SLA, SRL, root apex number per unit length and leaf blade/sheath weight ratio remained largely stable under P deficiency. Increased root growth relative to shoot was associated with increased sucrose concentration in roots, and thus possibly resulted from assimilates liberated by shoot growth inhibition. The simple theory of multiple morphological changes resulting from slow leaf expansion under P deficiency requires further experimental confirmation, after which it may serve as a basis for a mechanistic model of rice phenotypic plasticity and certain genotype X environment interactions on morphology.

Keywords: Assimilate partitioning, Oryza sativa L., Phyllochron, Plant architecture, Rice, Root-shoot ratio, Source-sink relationships, Tillering.

According to the functional balance theory, a plant optimizes biomass partitioning between roots and shoots in response to variable access to underground and aboveground resources (Brouwer, 1983). This is to (i) allocate more biomass to organs acquiring the most limiting resource (Bloom et al., 1985) and (ii) adapt plant compartment size (and thus demand) to resource availability (Brouwer, 1983; Marschner and Vetterlein, 1989; Reich, 2001). This dynamic response of morphogenesis to environment is an expression of the plant’s phenotypic plasticity (Dingkuhn, 1996; Dingkuhn et al., 1998; Poorter and Nagel, 2000). Phenotypic plasticity varies among species and genotypes (Wissuwa et al., 2001a, b). The phenotypic plasticity as an adaptation to environmental constraints, as opposed to short-term responses conferring physiological tolerance, has not been studied comparatively well, possibly because of their apparent complexity. Adaptive morphogenesis, ensuring early vigour and early canopy closure, is particularly important during vegetative stages when plants colonize space and resources and are potentially prone to weedy competitors (Asch et al., 1999; Dingkuhn et al., 1999; Suzuki et al., 2002; Caton et al., 2003). Early vigour depends on plant architecture and assimilate partitioning, and has become a major objective in crop breeding, such as upland rice (Dingkuhn et al., 1999) and wheat (Botwright et al., 2002).

Our objective was to identify morphogenetic processes that show strong phenotypic plasticity in the root and shoot of rice, and to describe them in terms of organogenesis and sink dynamics. Phosphorus (P) deficiency was chosen as an environmental factor because it is known to affect both organogenesis and biomass partitioning, generally in favor of the root system (Marschner and Vetterlein, 1989; Poorter and Nagel, 2000). However, the main organogenetic and physiological processes involved in these modifications are poorly understood. We focused on early vegetative development of Nipponbare (Oryza sativa L., japonica type), whose genotype was sequenced by the rice genome project. The study is part of a larger project to develop "gene-to-Phene" (genotype-to-Phenotype) models of crop phenotypic plasticity, simulating plant architecture on the basis of meristem behavior.

Material and methods

Two experiments (Exp) were carried in controlled environments at CIRAD (Montpellier, France) in 2003...
using Nipponbare cv. In Exp 1 we characterized the changes in morphogenesis induced by P deficiency, particularly with respect to root-shoot relationships, and sought to relate them to tissue sucrose and P concentration. In Exp 2 we sought to determine whether the effects of P deficiency on tillering could be explained by the generic, linear relationship between relative tillering rate (RTR) and the relative growth rate (RGR) which is interpreted as evidence that tillering rate is generally a function of assimilate supply (Dingkuhn et al., 1990; Dingkuhn et al., 1999).

After seed germination at 33 °C for 4 days, plants were transplanted to 1-litre pots containing quartz sand and watered daily with a nutritive solution, maintained at field capacity (concentrations in mM: KH$_2$PO$_4$=0.21, K$_2$HPO$_4$=0.06, KNO$_3$=1.98, Ca(NO$_3$)$_2$=2.96, CuSO$_4$=6.3*10$^{-5}$, ZnSO$_4$=2.5*10$^{-3}$, H$_3$BO$_3$=7.4*10$^{-3}$, EDTA-Fe=0.206, pH=5.5). Temperature in the culture chamber was 27 °C /23˚C (day/night), relative air humidity was 60%/80% (day/night), and photosynthetically active radiation (PAR, 600 mmol m$^{-2}$ s$^{-1}$) was supplied from halogen lamps during a 15 h photoperiod. The plants were grown in an optimal nutritive solution (P$^+$) and phosphorus deficient solution (P$-$), in which the P concentration was reduced to 1/40 (9 µmol l$^{-1}$). According to Marschner and Vetterlein (1989), P at a concentration lower than 10 µmol l$^{-1}$ affects plant morphology. The P$-$ solution was applied from 7 days after transplanting (DAT) onwards. Until 7 DAT the plants were irrigated with distilled water, assuming that seed reserves provided nutrition during germination. The experiments had four repetitions. Table 1 shows the detail of measurements at roots and shoots levels. Growth and development dynamics were expressed using either thermal time (TT in °D, Eq.1) or DAT as reference.

\[
TT = \sum_{i}^{g}(Ta-Tb), (Eq.1)
\]

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

Data were processed to analyze the following growth traits and their plasticity:
- The phyllochron (time in degree day, °D, separating the appearance of two successive leaves on the main stem), considered as a measure of plant development rate that varies among genotypes but is little affected by environment (Tivet et al., 2001).
- The final leaf lengths and maximal elongation rate, Vmax, estimated by fitting the leaf length (L) versus thermal time (TT) with 3 parameter sigmoid functions:

\[
L = \frac{a}{1+\exp\left(\frac{(TT-TT0)}{b}\right)}, (Eq.2)
\]

where a is the final leaf length, TT0 the thermal time when half the leaf reached an inflexion point and b the maximal elongation rate equal to a/(4*b). (Vmax, in cm °d$^{-1}$)

- Plant Area Index (PAI in cm$^2$ of leaves per plant) was estimated using Eq.3.

\[
PAI = \left[ \sum_{i}^{g}Li*Wi*0.725 \right], (Eq.3)
\]

where Li and Wi are respectively the length and width (cm) of leaf i on a given plant, an allometric coefficient of 0.725 was used to relate L and W to leaf area (Tivet et al., 2001).

- Organs spatial distribution (plant topology) and size (geometry without three-dimensional considerations): Root architecture (apex number, length, diameter) was analyzed using Winrhizo software (pro Regent Instrument, Quebec) applied to scanned images of spread-out root systems previously stained with Toluidine blue for 10 min.

- Dry matter, sucrose and P concentrations of bulk plant parts were determined after lyophilization. Sucrose was measured with a Dionex HPLC (HPAE-PAD detector) after extraction with 80% EtOH at 80°C. Phosphorus was analyzed at a certified laboratory for plant and soil samples.

| Measurement and estimated variables | DAT |
|-------------------------------------|-----|
| Fresh & dry matter - relative growth rate, RGR, SLA | 7, 10, 15, 28 + 35 (Exp2) |
| Blade elongation rate (length L & width w) and Area | Daily |
| Time separating the apparition of two leaves, Phyllochron, °d$^{-1}$ | Daily |
| Tilling : relative tillering rate, RTR, | Daily |
| Plant height | 7, 10, 15, 28 |
| P concentration (shoot and root) | 7, 10, 15, 28 |
| Sugar: sucrose concentration (shoot and root) | 7, 10, 15, 28 |
| Root architecture + specific root length (mm.g$^{-1}$) | 7, 10, 15, 28 |
- Biomass partitioning according to several allometric laws (root/shoot or blade/shoot biomass ratio), root and leaf thickness at plant level, i.e. specific root length (SRL in mm mg\(^{-1}\)) and specific leaf area (SLA in m\(^2\) mg\(^{-1}\)). SLA (Eq.4) was computed using PAI (Eq.3):

\[
SLA = PAI / DW_b, \quad (\text{Eq.4})
\]

where DW\(_b\) is the plant blade dry weight (g).

- The relative tillering rate in tiller tiller\(^{-1}\) day\(^{-1}\) (RTR, Eq.5),

\[
RTR = \left[ \frac{n \cdot TN(t+n)}{TN(t)} \right]^{-1}, (\text{Eq.5})
\]

where TN is the number of tiller at a given time t, and n the number of days separating two observations of TN.

- Biomass production expressed by the relative growth rate in g g\(^{-1}\) day\(^{-1}\) (RGR, Eq.6).

\[
RGR = \left[ \frac{DM(t+n)}{DM(t)} \right]^{-1}, (\text{Eq.6})
\]

where DM\(_t\) is the total or aboveground dry matter at time t, and n the number of days separating two observations of DM.

Fig. 1. (a) Average leaf number on the main stem (MS) along thermal time in P\(^-\) and P\(^+\) (Exp1 until 28 DAT). (b) Average final blade length in P\(^+\) vs. P\(^-\) for given leaf ranks on the main stem and primary tillers (Exp1).

Fig. 2. (a) Time course of average plant tiller number normalized by leaf number on the main stem (MS) in Exp1 (vertical bars indicate SE). (b) Relationship between average relative growth rate (RGR) and relative tillering rate (RTR) in Exp2; broken lines indicate 95% confidence interval of correlation.
Results and Discussion

1. Leaf appearance and tillering rates

Under P deficiency, phyllochron significantly (P < 0.05) increased by 20% relative to control (Exp1: Fig. 1a). This might be due either to slower development (increase in plastochron) or to slower leaf elongation prior to tip appearance. Whether or not P deficiency affected the plastochron as shown in wheat by Rodriguez et al. (1998) remains uncertain.

Tillering rate was significantly (P < 0.05) reduced by P deficiency, both when calculated on the basis of thermal time or even when taking into account the slower development rate in P− plants by normalizing

| Final leaf length (cm) Vmax (cm.°d⁻¹) | Average | SE   | Average |
|--------------------------------------|---------|------|---------|
| Leaf 5                               | P+      | 18.37| 0.22    | 0.53    |
|                                      | P−      | 18.83| 0.46    | 0.52    |
| Leaf 6                               | P+      | 22.21| 0.96    | 0.65    |
|                                      | P−      | 21.62| 0.31    | 0.51    |
| Leaf 7                               | P+      | 27.14| 0.12    | 0.67    |
|                                      | P−      | 25.31| 1.41    | 0.33    |

Table 2. Average (and standard error SE) final leaf lengths and maximal elongation rate (Vmax) for leaves at position 5, 6 and 7 on the main stems of plants in optimal and P deficiency conditions.

Fig. 3. Kinetics of average main stem leaf elongation (for leaves 5, 6, 7, with SE) respectively in P− and P+ treatments.

Fig. 4. Average plant SLA (Specific Leaf Area) and SRL (Specific Root Length) expressed respectively along (a) shoot and (b) root growth; (c) SLA and (d) SRL expressed along plant development (leaf number on the main stem) in P+ and P− conditions (Exp1 at 7, 10, 15 and 28 DAT).
the number of emerged tillers by the number of leaves appeared (Fig. 2a). Rice tillering was previously shown to strongly depend on aboveground relative growth rate RGR (Dingkuhn et al., 1990), and the question is here whether the effect of P deficiency on tillering is a result of growth reduction or a specific inhibition of tiller initiation. The RTR/RGR plot (Exp2; Fig. 2b) suggests that the effect of P deficiency on tillering rate is trophic, i.e., related to growth and therefore assimilation, although the large experimental error leaves room for other hypotheses.

2. Leaf extension rate and final length

Final leaf length was not affected by P deficiency (Fig. 1b, for blade length), whereas the maximal leaf elongation rate (Vmax) was significantly (P < 0.05) reduced at leaf positions 6 and 7 on the main stem (Fig. 3 and Table 2). This might indicate that final leaf length was determined soon after leaf initiation (Bos et al., 1998ab; Rodriguez et al., 1998) and thus the condition before P deficiency could affect it, whereas leaf elongation rate responded rapidly to the stress. The longer phyllochron in P- could thus be a result of lower leaf elongation rate and the longer time for a leaf to attain its final length, which delays the appearance of a new leaf.

3. Root and shoot allometric relationships

Considering roots and shoots separately, several allometric relationships remained unaltered under P deficiency; for example SLA and SRL plotted against shoot and root biomass, respectively (Fig. 4ab). SLA plotted against leaf number on the main stem (Fig. 4c) or thermal time (not presented) was also not affected by P deficiency. SRL plotted against leaf number as an index of development, however, varied greatly hindering the evaluation of the effects of the P deficiency (Fig. 4d). Other allometric relationships that remained unaffected by P deficiency were mean root diameter (Fig. 5a), total root volume (Fig. 5b) and root apex number (Fig. 5c), vs. total root length respectively. The dry weight ratio of blade vs. shoot root diameter (Fig. 5a), total root volume (Fig. 5b) and root apex number (Fig. 5c), vs. total root length respectively. The dry weight ratio of blade vs. shoot

Fig. 6 (a) Time course of average (and SE) Root/Shoot and Blade/Shoot dry weight ratios in P+ and P− treatments (Exp 2); (b) Average (and SE) sucrose concentration ratio between root and shoot in P+ and P− treatments (Exp1).
(blade/shoot ratio, Fig. 6a) was constant among leaf positions and unaffected by P treatment, whereas root-shoot mass ratio (R/S) was positively (P < 0.05) affected by P-. (Fig. 6a). This might indicate that root architectural and morphological development was more related to the size (total length) of the root system than to crop development stage (leaf number on main stem). Meanwhile root growth (biomass) and development (apex number) were maintained under P deficiency whereas shoot growth and development were diminished.

We suggest that the increase of R/S, resulting from modifications of assimilate partitioning, indirectly affected tillering through an over-proportional reduction of aboveground growth under P deficiency. This might, in turn, have affected some other root-shoot allometries, such as root apex number per tiller, which increased significantly under P−. This phenomenon resembles the development of cluster roots under P deficiency observed in other species (He et al., 2003; Shane et al., 2003). These morphological changes were correlated with growth rates of the respective organs, (e.g. tillering, Fig. 2a-b, or root architecture, Fig. 5a-c), and were thus at least partly under trophic control. On the other hand, Wissuwa et al. (1998) and Wissuwa and Ae (2001b) reported that R/S is controlled by one or several genes induced by environmental factors such as P deficiency. It is unknown whether these inducible genes affect assimilate partitioning through stimulation of root sinks or inhibition of shoot sinks for assimilates, and by what mechanism.

4. **Bulk organ P and sucrose content**

In both treatments, the shoot P concentration decreased after 10 DAT, but more rapidly in P− plants (Fig. 7). The root P concentration responded more rapidly to the P deficiency. Root and shoot sucrose concentration decreased under P deficiency at 10 DAT, simultaneously with shoot P concentration. Thereafter, under P− conditions, the shoot sucrose concentration decreased gradually while that of roots increased towards the level observed under P+ condition. The resulting root/shoot sucrose concentration ratios (Fig. 6b) decreased under P+ and strongly increased under P−, probably indicating enhanced transport of sucrose to the root. Shane et al. (2003) suggested that a critical shoot P concentration exists below which root growth is stimulated. Our results suggest that root growth stimulation under P− (relative to shoot) is brought about by lower assimilate demand of the shoot, and thus greater export, as opposed to increased sink activity in the root (in which case root sucrose concentration would decrease). This interpretation is in line with the observed lower extension rate of leaves under P−, and thus lower consumption of assimilates. We therefore suggest that the morphological changes induced by P deficiency in rice seedlings are primarily a consequence of lower leaf extension rates, associated with longer phyllochron and reduced tillering, thereby reducing the demand for assimilates in the shoot. The assimilates thus liberated increase the sucrose concentration in the roots and enable greater root growth. In fact, Shane et al., (2003) concluded from a split-root experiment using differential P resources in *Lupinus* that growth of the root system responds not to ambient P concentration but to deficiency occurring in the shoot. Further studies are needed to show whether increased root growth under P deficiency can indeed be explained by a simple inhibition of leaf expansion, and whether the inducible genes identified by Wissuwa et al. (1998) and Wissuwa and Ae (2001b) are involved.

**Conclusion**

This study aimed at analyzing the phenotypic plasticity of rice seedlings induced by variable P resources, a factor that is known to change plant morphology. The results confirmed that one of the main effects of P deficiency is a reduction of shoot growth and thus assimilate demand, while root growth is not inhibited by P deficiency. It seems that this phenomenon, apparently brought about by a reduction in leaf elongation rate without changing final leaf size, explains all or much of the other changes in development, morphology and architecture. Under P deficiency, the phyllochron was prolonged because leaves took longer to expand and the tillering rate was low because it is largely under trophic control, and shoot growth was strongly reduced to the benefit of roots. Allometric parameters such as SLA, SRL, root apex number or volume per unit root length and leaf blade/sheath weight ratios remained stable. As a general result, both growth and development were slowed by P− at the shoot level,
while root growth was maintained. Measurements of sucrose concentrations supported the hypothesis that morphogenetic effects of P− were a result of modified relationships among sinks, and specifically, inhibition of leaf expansion. Studies extended to later growth stages, additional parameters such as plastochron and other stresses are under way to test the generality of the present results. They will serve to develop ecophysiological models of rice phenotypic plasticity and guide the search for genes controlling it. The identification of a generic, inducible mechanism controlling root-shoot sink ratios would be of great interest for crop improvement.

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References
Asch, F., Sow, A. and Dingkuhn, M. 1999. Reserve mobilization, dry matter partitioning and specific leaf area in seedlings of African rice cultivars differing in early vigor. Field Crops Research. 62 : 191-202.
Bloom, A.J., Chapin, F.S. and Mooney, H.A., 1985. Resource limitation in plants - An economic analogy. Annual Review of Ecology and Systematics. 16 : 363-392.
Bos, H. J. and Neuteboom, J. H. 1998a. Morphological analysis of leaf and tiller dynamics of wheat (Triticum aestivum L.): Responses to temperature and light intensity. Annals of Botany. 81 : 131-139.
Bos, H. J. and Neuteboom, J. H. 1998b. Growth of individual leaves of spring wheat (Triticum Aestivum L.) as influenced by temperature and light intensity. Annals of Botany. 81 : 141-149.
Botwright, T.L., Condon, A.G., Rebetzke, G.J. and Richards, R.A. 2002. Field evaluation of early vigour for genetic improvement of grain yield in wheat. Australian Journal of Agricultural Research 53: 137-145.
Brouwer, R. 1983. Functional equilibrium: sense or nonsense? Netherlands Journal of Agricultural Science 31: 335-368.
Caton, B. P., Cope, A. E. and Mortimer, M. 2003. Growth traits of diverse rice cultivars under severe competition: implications for screening for competitiveness. Field Crop Research. 83 : 157-172.
Dingkuhn, M. 1996. Modelling concepts for the phenotypic plasticity of dry matter and nitrogen partitioning in rice. Agricultural Systems. 52 : 383-397.
Dingkuhn, M., Johnson, D., Sow, E.A. and Audebert, A.Y. 1999. Relationships between upland rice canopy characteristics and weed competitiveness. Field Crop Research. 61: 79-95.
Dingkuhn, M., Jones, M. P., Johnson D. E. and Sow, A. 1998. Growth and yield potential of Oryza sativa and O. glaberrima upland rice cultivars and their interspecific progenies. Field Crop Research. 57 : 57-69.
Dingkuhn, M., Schnier, H. F., Datta, S. K. D., Dormling, K., Javellana, C. and Pamplona R. 1996. Nitrogen fertilization of direct-seeded flooded vs. transplanted rice: II- Interactions among canopy properties. Crop Science 30 : 1284-1292.
He, Y., Liao, H. and Yan, X. 2003. Localized supply of phosphorus induces root morphological and architectural changes of rice in split and stratified soil cultures. Plant and Soil. 248 : 241-248.
Marschner, M. and Vetterlein, D. 1989. Fertilizer effect on root growth and drought resistance. University of Hohenheim Institute of Plant Nutrition Eds. (Stuttgart, Germany): 15pp.
Poorter, H. and Nagel, O. 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. Australian Journal of Plant Physiology. 27 : 595-607.
Reich, P. B. 2001. Root-shoot relations: optimality in acclimation and adaptation or the ‘emperor’s new clothes’? Plant roots, the hidden half. E. Y. a. K. U. Weisel Y. New York, Marcel Dekker : 205-220.
Rodriguez, D., Pomar, M. C. and Goudriaan, J. 1998. Leaf primordia initiation, leaf emergence and tillering in wheat (Triticum Aestivum L.) grown under low Phosphorus conditions. Plant and Soil. 202 : 149-157.
Shane, M. W., Vo, M. D., Roock, S. D. and Lambers, H., 2003. Shoot P status regulates cluster-root growth and citrate exudation in Lupinus albus grown with a derived root system. Plant, Cell and Environment. 26 : 265-273.
Suzuki, T., Shiraiwa, T. and Horie, T. 2002. Competitiveness of four rice cultivars against Barnyardgrass, Echinochloa oryzicola Vasing, with reference to root and shoot competition. Plant Production Science. 5 : 77-82.
Tivet, F., Pinheiro B. D. S., and Dingkuhn, M. 2001. Leaf blade dimensions of rice (Oryza sativa L. and Oryza glaberrima Steud.). Relationships between tillers and the main stem. Annals of botany. 88 : 507-511.
Wissuwa, M., Yano, M. and Ae, N. 1998. Mapping of QTLs for phosphorus deficiency tolerance in rice (Oryza sativa L.). Theoretical and Applied Genetics. 97 : 777-783.
Wissuwa, M. and Ae, N. 2001a. Further characterization of two QTLs that increase phosphorus uptake of rice (Oryza sativa L.) under phosphorus deficiency. Plant and Soil. 237 : 275-286.
Wissuwa, M. and Ae, N. 2001b. Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. Plant Breeding. 120 : 43-48.