

Growth, Protein and Pigment Content of Rice Seedlings under Phosphorus Deprivation Condition

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ABSTRACT : Phosphorus (P) is a macronutrient playing important roles in many plant processes. Significant interest has been devoted to search and utilize genotypic variations in P use efficiency in rice but with little effort to understand its physiological and biochemical bases. In this study, we examined responses to P deprivation of some primary and secondary traits in 3-week-old seedlings of the three genotypes, Sobi-byeo (japonica), Dasan-byeo (japonica x indica) and Palawan (indica). In general, percent weight due to root was increased up to 26%, but amounts of root protein and proteins secreted from roots were decreased by 11 to 19% and 31 to 51%, respectively, by 3 to 21 days of P deprivation in the three genotypes. Interestingly, however, responses of Palawan to short-term P deprivation were contrasting to those of Dasan-byeo and Sobi-byeo in seedling weight and contents of shoot protein, chlorophyll and anthocyanin. Seedling weight was not decreased, but shoot protein content was decreased in P-deprived seedlings of Palawan. Contents of chlorophyll in leaves and anthocyanin in roots were increased in Dasan-byeo and Sobi-byeo, but decreased in Palawan. The results suggest that responses of protein and pigment synthesis to P deficiency are different in modern and traditional varieties and the difference may at least in part be due to the selection for high yield under highly fertilized conditions.

Keywords : Anthocyanin, Chlorophyll, Protein secretion, Phosphorus deficiency

Phosphorus (P) plays important structural and regulatory roles in metabolism, growth and development processes. It is a constituent of such compounds as nucleic acids, phospholipids and ATP, and also a metabolite involved in energy transfer, the activation of proteins and the regulation of metabolic processes (Marschner, 1995). The primary source of phosphorus for plants is inorganic phosphate (Pi). Plant growth and development is often limited by the low availability of Pi in soil. Low Pi in many soils throughout the world is a major factor limiting rice yield

(Hedley *et al.*, 1994). Furthermore, more than 80% of the P fertilizer applied to soil may rapidly be bound to Fe and Al, causing low availability of Pi even in fertilized soils (Dobermann *et al.*, 1998). This often requires high inputs of fertilizers to achieve optimum yields and product quality.

Under low-Pi conditions, plant often employs morphological adaptations to increase the acquisition of Pi. Some plant species alter the architecture of their root systems. Plants with highly branched root systems and more root apices are efficient in acquiring Pi (Lynch, 1995). Low Pi availability also stimulates increase in the surface area of the roots in contact with the soil (Ma *et al.*, 2001) and the elongation of root hairs (Bates & Lynch, 1996). Secondary metabolism is also influenced by P-stress leading to accumulation of flavonoids and anthocyanins (Bergmann, 1992).

Low Pi availability also induces biochemical changes in plants. Enhanced secretion of specific proteins and organic acids from roots is a common adaptive strategy to increase Pi acquisition (Duff *et al.*, 1994). Acid phosphatases secreted from root play a role in hydrolyzing Pi from organic P compounds in the soil (Lefebvre *et al.*, 1990). Organic acid excreted from the root aid in the release of Pi from Fe and Al compounds in the soil (Staunton & LePrince, 1996). However, little information is available on the biochemical responses of rice to P deficiency.

Higher yield has been the most important criterion until recently in variety improvement and cultivation in rice. Therefore, much effort has been focused on the selection for yield under highly fertilized soil and application of fertilizer for higher yield (Loneragan, 1997). However, the costs involved and the environmental damage that often results from fertilizer use have directed attention to P-use efficiency (PUE) of the crop plant. Significant genotypic differences in P uptake and use efficiency exist in many plant species including rice. Several reports devoted on screening and selecting genotypes with higher PUE in rice (Fageria *et al.*, 1988; Wissuwa & Ae, 2001). However, little information is available on many developmental, physiological and biochemical processes that determine PUE. Furthermore, no information is available on the physiological and biochemical responses of Korean varieties to

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P stress.

This study was conducted to examine responses to P stress of protein and secondary metabolites synthesis in the three rice genotypes, Dasan-byeo, Sobi-byeo, and Palawan. The results indicate that rice genotypes respond differently in seedling weight, shoot protein content, and chlorophyll and anthocyanin content to short-term P deprivation.

MATERIALS AND METHODS

Plant material

Three rice varieties Dasan-byeo (Dasan, japonica x indica type), Sobi-byeo (Sobi, japonica type) and Palawan (indica type) were used in this study. The varieties were selected for the known high (Dasan and Sobi) and low (Palawan) efficiency of nutrient use (Piao *et al.*, 2001; Singh *et al.*, 1998). Seeds were germinated for three days in the dark at 25°C after one-minute surface sterilization in 0.1% HgCl₂ solution (Yoshida *et al.*, 1976). Uniform germinated seedlings were used for solution culture. The experiments were conducted with three replicates, and paired t-tests were conducted for the normal and P-deprivation treatments using the statistical analysis software (SAS 6.12, Cary, USA).

Solution culture

Hydroponic cultures were conducted in a glasshouse in February and March, 2003. The composition and concentration of each element in the standard Yosidas solution are as follows: NaH₄NO₃ (3.6 mM), NaH₂PO₄ (0.4 mM), K₂SO₄ (1.3 mM), CaCl₂ (1.3 mM), MgSO₄ (2.0 mM), FeCl₃ (44.6 μM), H₃BO₃ (25.0 μM), MnCl₂ (11.0 μM), (NH₄)₆MO₇O₂₄ (0.7 μM), ZnSO₄ (0.2 μM), CuSO₄ (0.2 μM), and citric acid (70 μM) (Yoshida *et al.*, 1976). Seedlings were grown in the aerated solutions in a glasshouse at 25°C/18°C (day/night) under 16 h light for 3 weeks. They were then transferred to the P-free nutrient solution and subjected to P deprivation for 3 weeks. The control plants were maintained for the entire period in the standard solution. The P-free nutrient solution was prepared similarly to the standard solution, except NaH₂PO₄ was omitted. The solutions were changed every 3 to 4 days, and the pH was adjusted daily to 5.0 with NaOH. Samples were taken 3, 7, 14, and 21 days after treatment, and kept at -70°C until use.

Protein assay

Intracellular proteins were extracted using the extraction buffer containing 100 mM NaOAc, pH 6.8, 100 mM phenylmethylsulfonyl fluoride, 5 mM dithiothreitol, 10% glyc-

erol, and 0.8% polyvinylpolypyrrolidone. Proteins secreted from roots were obtained by incubating the seedlings in a medium containing 0.025% penicillin for 30 to 60 min at room temperature (Yun & Kaepler, 2001). Protein contents in the solutions were determined by the method of Bradford (1976) with bovine serum albumin as a standard.

Chlorophyll and anthocyanin assay

Chlorophyll in leaves was determined according to the procedure described (Yoshida *et al.*, 1976) by measuring the absorbance of chlorophyll solutions in 80% acetone at 663 and 645 nm. Anthocyanin in roots was assayed according to Lange *et al.* (1971). Anthocyanin was extracted in propa-nol:HCl:H₂O (v/v/v=18:1:18) and the absorbance of the solution was measured at 535 and 650 nm.

RESULTS

Growth responses

Rice seedlings grew normally in the standard P solution.

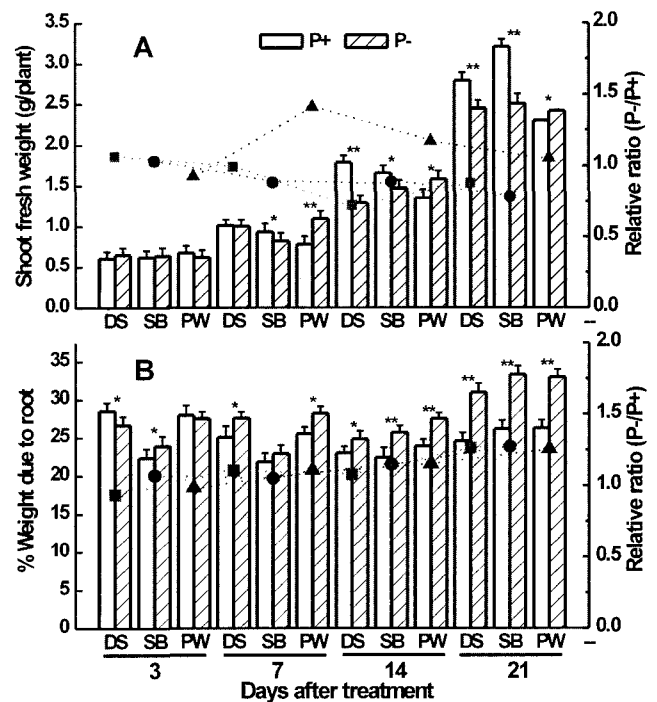


Fig. 1. The responses of shoot weight (A) and percent weight due to root (B) to P deprivation. The relative values of shoot weight in P-deprived plants compared to P-sufficient plants (P-/P+ ratio) for Dasan (■), Sobi (●), and Palawan (▲) are as indicated. Three-week-old seedlings were grown for the additional days as indicated in the standard (P+) or P-free (P-) solutions. * and **: the paired means of the P+ and P- treatments are significantly different at $P < 0.05$ and $P < 0.01$, respectively.

Fresh shoot weight was increased gradually in all varieties, and was higher in Dasan and Sobi than in Palawan in the standard P solution. The response of shoot growth to P deficiency has become apparent as early as 7 days after treatment (DAT). However, the response was contrasting among the genotypes tested. After 14 days of P deprivation, shoot weight was significantly decreased in Dasan and Sobi, but it increased in Palawan. The relative weight of shoots in P-deprived plants compared to P-sufficient plants (P-/P+ ratio) was 0.89, 0.88, and 1.16 for Dasan, Sobi, and Palawan, respectively. However, the P-/P+ ratio for Palawan was decreased to 1.05 at 21 DAT and no significant difference was indicated between the treatments.

The fresh root weight of 3-week-old seedlings was lower in Sobi than Dasan and Palawan, but it became similar in the three genotypes from 5-week-old seedlings. Percent fresh weight due to roots was increased in seedlings in P-free solutions from 7 DAT similarly in the three genotypes and it was increased gradually as growth stages advanced. Twenty-one days after treatment, the percent fresh weight due to roots was about 26% higher in the seedlings in the P-free solutions than in the those in the control solution in the three varieties (Fig. 1B).

Protein content and secretion

Soluble protein content in shoots and roots was affected by the developmental stage and it was decreased after four weeks of germination. Protein content per unit shoot fresh weight was affected at the earlier stages of P deprivation. The ratio of P-/P+ in shoot protein was increased in Dasan and Sobi, but decreased in Palawan at 7 and 14 DAT. The ratio became around 1.0 in the three genotypes at 21 DAT (Fig. 2A). Protein content per unit root fresh weight was decreased in all genotypes by P deprivation from 7 DAT. The ratio of P-/P+ in root protein was 0.89, 0.81, and 0.81 for Dasan, Sobi, and Palawan, respectively, indicating 11 to 19% decrease in protein content by P deprivation (Fig. 2B). Proteins secreted from the root per seedling was increased but that per unit root fresh weight decreased as growth stages advanced. Amount of proteins secreted per unit root fresh weight was higher in Dasan and Sobi than in Palawan in 3-week-old seedlings, but the difference became smaller in the seedlings older than 4 weeks. The P-/P+ ratio in secreted proteins at 3 DAT was 0.53, 0.49 and 0.69 for Dasan, Sobi and Palawan, respectively, indicating 31 to 51% decrease in the amount of secreted proteins by P deprivation. The ratio was gradually decreased to near 0.30 after a little transient increase at 7 DAT (Fig. 2C).

Pigment content

Chlorophyll content per fresh weight at 21 DAT was

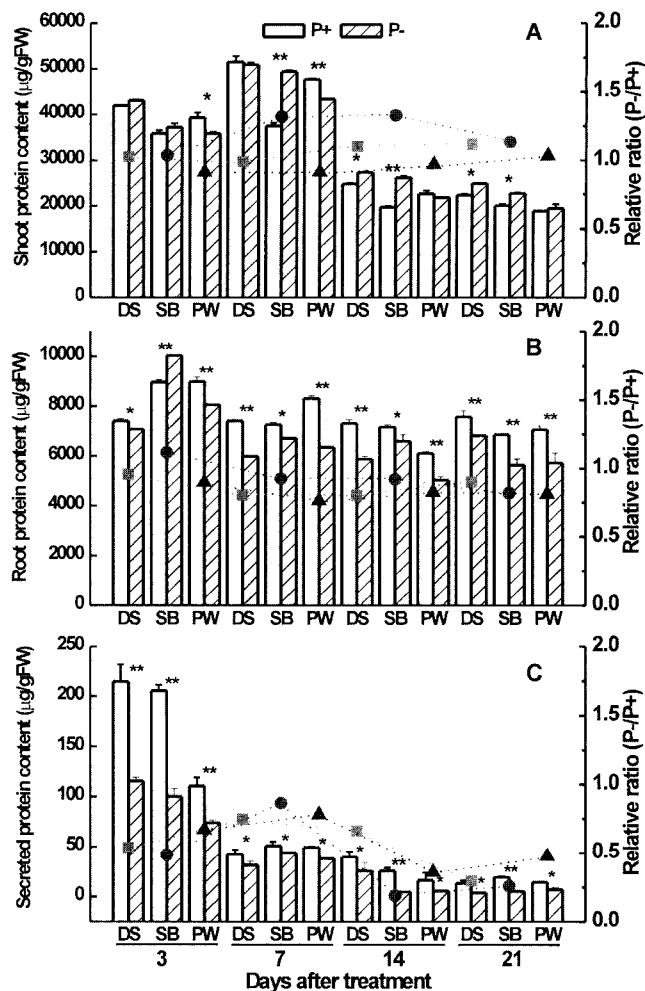


Fig. 2. The responses of contents of shoot protein (A), root protein (B), and protein secreted from roots (C) to P deprivation. The relative values of P-deprived plants compared to P-sufficient plants (P-/P+ ratio) for Dasan (■), Sobi (●), and Palawan (▲) are as indicated. Three-week-old seedlings were grown for the additional days as indicated in the standard (P+) or P-free (P-) solutions. * and **: the paired means of the P+ and P- treatments are significantly different at $P < 0.05$ and $P < 0.01$, respectively.

increased in Dasan and Sobi, but decreased in Palawan by P deprivation (Fig. 3A). The P-/P+ ratio in chlorophyll content was 1.23, 1.25 and 0.94 for Dasan, Sobi and Palawan, respectively. Anthocyanin content in roots also responded similarly as in chlorophyll. Anthocyanin content at 21 DAT was increased in Dasan and Sobi, but decrease in Palawan by P deprivation. The P-/P+ ratio in anthocyanin content was 1.13, 1.15 and 0.75 for Dasan, Sobi and Palawan, respectively (Fig. 3B).

DISCUSSION

P is one of the macronutrients required for many plant

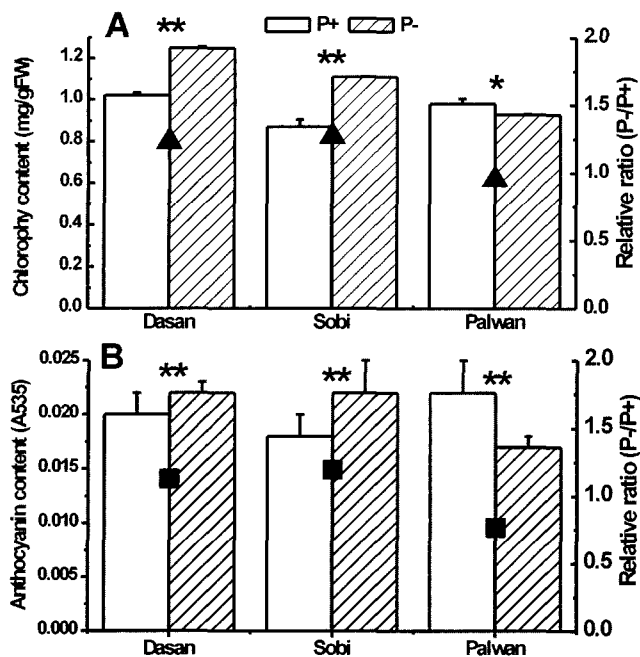


Fig. 3. The responses of contents of chlorophyll in leaves (A) and anthocyanin in roots (B) to P deprivation. The relative values of P-deprived plants compared to P-sufficient plants (P-/P+ ratio) for chlorophyll (\blacktriangle) and anthocyanin (\blacksquare) are as indicated. Three-week-old seedlings were grown for the additional days as indicated in the standard (P+) or P-free (P-) solutions. * and **: the paired means of the P+ and P-treatments are significantly different at $P < 0.05$ and $P < 0.01$, respectively.

processes, and plant growth and development is often limited by P deficiency. P-deficiency symptoms are usually noted on rice seedlings as stunted growth, reduced tillering, and delayed plant development (Wells *et al.*, 1994). It is believed that many unknown developmental, physiological and biochemical changes underlie the symptoms. Reduced seedling growth is a general observation in P-deficient plants (Marschner, 1995). Our results with Dasan and Sobi that showed about 11% reduction in shoot weight at 21 days of P deprivation is consistent with the general observation. However, shoot weight of Palwan was not reduced during the period of 7 to 21 days of P deprivation, indicating genotypic difference in response to P deficiency. Since P uptake, storage and remobilization processes determine overall P use efficiency, it is likely that P storage and remobilization efficiency could affect shoot growth during the short-term P deprivation (Inthapanya *et al.*, 2000; Wissuwa and Ae, 2001). One of the developmental changes generally observed in P-deficient plants is the increased root to shoot ratio (Andrews *et al.*, 1999; Yun & Kaepler, 2001). The root growth under P starvation is favored in a short period and reduced over longer periods, as a feedback effect of reduced shoot growth and reduced photosynthesis (Mollier

& Pellerin, 1999). Percent fresh weight due to roots was about 26% higher in P-stressed seedlings at 21 DAT in the three varieties, indicating little genotypic difference in relative increase in root fresh weight under P deprivation. These results suggest that the response mechanism to short-term P deficiency is likely different between the shoot and root in Palwan.

Genotypic difference in soluble proteins was greatest in the amount of proteins secreted from the root in younger seedlings. Dasan and Sobi secreted more proteins than Palwan. The effect of P deprivation in soluble protein content and secretion was generally similar in the three varieties except in protein content per unit shoot fresh weight at 7 and 14 DAT. Protein content in shoot was increased in Dasan but decreased in Palwan in P-stressed plants during the period. This difference has a similarity with the observation in shoot weight. In maize seedlings, soluble protein content is changed little during the short-term P deficiency (Yun & Kaepler, 2001). However, protein content is generally decreased by the long-term P deficiency (Andrews *et al.*, 1999).

The accumulation of chlorophyll and anthocyanin has been observed as P-deficiency symptoms in many plant species (Bergmann, 1992; Halsted & Lynch, 1996; Yun & Kaepler, 2001). However, our results indicate genotypic difference in the response of secondary metabolism to P deficiency in rice. The chlorophyll content per fresh weight in P-deprived seedlings was increased over 23% in Dasan and Sobi, but decreased about 6% in Palwan. Interestingly, the anthocyanin content in roots showed a similar genotypic response. Anthocyanin content per fresh weight in P-deprived seedlings was increased over 13% in Dasan and Sobi, but decreased about 25% in Palwan. Chlorophyll content is generally increased in P-stressed rice seedlings in conjunction with the reduced growth rate (Wells *et al.*, 1994). Increased chlorophyll content and reduced shoot weight in P-deprived Dasan and Sobi seedlings are consistent with the general observations of P stress in many plant species (Bergmann, 1992; Halsted & Lynch, 1996; Yun & Kaepler, 2001). However, decreased chlorophyll content and increased shoot weight in P-deprived Palwan seedlings are quite contrary to the general observations.

It has been reported that there is genotypic variation in tolerance to P deficiency in rice. Traditional varieties are usually more tolerant to P deficiency than modern varieties (Inthapanya *et al.*, 2000; Wissuwa & Ae, 2001). Therefore, it is highly possible that the contrasting results in protein and pigment synthesis also may be a parallel responses to P deficiency observed in modern and traditional varieties. Possibility of biotype response is excluded since the responses of Dasan (japonica x indica) are much similar to Sobi (japonica) but little to Palwan (indica). The relevance of these

findings could be deduced from the previous reports in other crop species that macronutrients effects are primarily mediated through their influence on protein synthesis and growth (Andrews *et al.*, 1999). Efficiency of P uptake and storage in P-sufficient conditions and reutilization of stored P during P stress may be involved in the genotypic variation. Partitioning of carbon and P between tissues may be altered in P-deficient plants, resulting in many developmental and biochemical changes (Andrews *et al.*, 1999; Smith, 2001). Consequently, the genotypic differences observed in responses of shoot weight, shoot protein, and contents of chlorophyll and anthocyanin under short-term P deprivation are likely related to the efficiency of P storage and reutilization.

It is generally agreed that tolerance to P deficiency is complex in nature. However, dissection of the complexity of the trait into major physiological and biochemical factors should be preceded for further understanding and improvement of the trait. The results from this study suggest that responses of protein and pigment synthesis to P deficiency are different in modern and traditional varieties and the difference may at least in part be due to the selection for high yield under highly fertilized conditions during variety development. Further investigation on the possible involvement of the protein and pigment synthesis with P uptake and utilization will contribute to the understanding of P-deficiency tolerance in rice.

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