Effects of Seed P-enrichment and Localized P-fertilizer Application on Soil-grown Wheat

Nobuhito Sekiya¹, Ryosuke Fukuju² and Katsuya Yano²

Abstract: We previously reported two methods of phosphorus (P) supply that improve crop growth. One is to apply P-fertilizer locally into soils and the other is seed P-enrichment by soaking seeds in P-solution. Here, we examined which of the two methods or a combination of the two is more effective for wheat grown in pots. Each method increased dry matter to a similar extent but with a different time interval. The effect of localized P-application was observed at 25 d after sowing (DAS), while that of seed-P enrichment was observed at 50 DAS. Despite the positive effects of both methods, their combination had no additive effect. At 50 DAS, the growth variations were explained by P content, which depended on root length. We therefore assume that increasing root length may be the common and biologically relevant feature of both methods and that either application alone is sufficient to achieve this effect.

Key words: Localized application of fertilizer, Phosphorus availability, Phosphorus use efficiency, Seed P-enrichment, Seed priming.

Phosphorus (P) is an essential element for plant growth. However, soil-grown plants are usually subjected to P deficiency because the concentrations of P dissolved in soil solution are very low (typically less than 1 μM) due to bonding of P by the cations aluminum, calcium, and iron (Raghothama and Karthikeyan, 2005). Thus, P-fertilizer application is necessary to attain satisfactory crop yields. In addition, soil P-sorption almost immediately immobilizes most of the applied P (often 90% or more), meaning that the rate of P application far exceeds crop demands (Stevenson and Cole, 1999). In addition to the eutrophication of lakes and rivers caused by this deposit of P in soils (Sharpley, 1995), there is a serious concern that phosphate rock, which is the raw material of P-fertilizers, will be depleted within this century (Runge-Metzger, 1995). Therefore, it is important to improve the efficiency of P-fertilizer, that is, reduce the excessive consumption of P-fertilizer without decreasing crop yields.

We previously reported that spatially or temporally localized P-supply to crops could improve the efficiency of P-fertilizer. In the former case, P-fertilizer is applied locally instead of uniformly, which results in enhanced P acquisition and growth of maize plants via increased root length, especially in fertile soils (Yano and Kume, 2005; Kume et al., 2006). In the latter case, P-solution is used to soak seeds directly rather than application to soil. We found that P-enriched wheat seeds require 60% less P-fertilizer not only due to enriched-P in the seeds but also because of enhanced P-uptake capacity via increased root length (Sekiya and Yano, 2010).

In this study, we examined the performance of each method (i.e., the localized application of P-fertilizer in the soil and seed P-enrichment) and their combination on the growth of wheat (Triticum aestivum L. cv. Norin 61) and determined 1) the time interval and magnitude of the effect on growth, and 2) the effectiveness of each of the 3 approaches to promote growth.

Materials and Methods

The seed treatment was performed according to the procedure reported by Sekiya and Yano (2010). A potassium phosphate solution was prepared by dissolving a mixture of 30 g of KH₂PO₄ and 10 g of K₂HPO₄ in 760 mL of distilled water (pH: 6.1, EC: 16.71 dS m⁻¹ in a 50% diluted solution). Distilled water was used as the control. Both the solution and distilled water were aerated using...
chloride (0.12 g K$_2$O). Twenty pots were then subjected to ammonium sulphate (0.12 g N) and 0.21 g of potassium. The soil in each pot was uniformly mixed with 0.55 g of superphosphate as used in P-uniform was applied to a half section of the soil column (P-rich), and the other half received no P-fertilizer (P-poor).

pumps. Wheat (cv. Norin 61) seeds placed within net bags were dipped into the solution (P-enriched seeds) and distilled water (non-enriched seeds) and were incubated at 30°C for 24 hr. After the incubation, 70 – 80% of seeds were swollen, while ~1mm seminal roots emerged from the rest of the seeds. Then, those swollen seeds were subjected to the study.

Forty plastic pots (160 mm in diameter, 200 mm in depth) were filled with Andosol (2.3 kg of dry soil per pot) that contained a very low level of P (0.1 mg Truog-P kg$^{-1}$). The soil in each pot was uniformly mixed with 0.55 g of ammonium sulphate (0.12 g N) and 0.21 g of potassium chloride (0.12 g K$_2$O). Twenty pots were then subjected to uniform application of P-fertilizer, and the remaining pots were subjected to localized treatment (Fig. 1). In the “uniform” group, the soil in each pot was uniformly mixed with 0.46 g of superphosphate (0.08 g P$_2$O$_5$) (P-uniform), which is known to cause moderate P-deficiency in wheat (Sekiya and Yano, 2010). In the “localized” group, the same amount of superphosphate as that used for the “uniform” group was applied to a half section of the soil column within each pot (P-rich), and the other half received no P-fertilizer (P-poor).

On 9 August 2007, five P-enriched and five non-enriched seeds were sown 0.02 m deep at the center in a pot with either the uniform or the localized P-fertilizer, giving a total of 4 treatments: The non-enriched seeds in the uniform P-fertilizer (Non-enriched-uniform), the P-enriched seeds in the uniform P-fertilizer (Enriched-uniform), the non-enriched seeds in the localized P-fertilizer (Non-enriched-local), and the P-enriched seeds in the localized P-fertilizer (Enriched-local). Each treatment had ten replicates. The pots were arranged in a glasshouse in Nagoya University in a randomized, complete block design. No differences were observed in germination rate among the treatments. The pots were irrigated at 3 d intervals throughout the experimental period to maintain 40% of field capacity in the soil. Seedlings were thinned to one stand in each pot 10 d after sowing (10 DAS) and were grown for the next 40 d.

The plants were harvested from five replicates in each treatment at 25 DAS and from the remaining five replicates at 50 DAS. Shoots were cut at the soil surface, and roots were gently washed free of soil under running water using a 3 mm sieve. The root sample collected from each pot was divided into 2 sub-samples: one for measuring dry matter weight and the other for measuring root length, and the latter was preserved in 70% ethanol. The shoot and root samples were dried at 80°C for 48 hr, weighed, and then ground using a vibrating mill. The milled samples were then dry-ashed at 550°C for 4 hr and dissolved in hydrochloric acid. P concentrations in the extract solutions were determined using a colorimetric method (Murphy and Riley, 1962). P content was calculated by multiplying plant dry matter weight with P concentration, and P use efficiency (PUE) was obtained as a reciprocal of P concentration. The sub-samples of roots preserved in 70% ethanol were spread on a transparent sheet, without overlap. Digitized images were obtained using a scanner with a resolution of 300 dpi and an output format of 256 grey-scales. The root length was determined by the diameter class, by using WinRHIZO Basic LA 2400 (Regent Instrument, Canada).

The data were analyzed using two-way analysis of variance (ANOVA), in which the variation sources consisted of the seed treatment, the P-fertilizer, and their interactions. The mean separation between treatments was then determined using the Tukey-Kramer honestly significant difference test. Regression analysis was performed with dry matter weight as the dependent variable and P content or PUE as the independent variable. Correlations between P content and root length were evaluated using the Pearson correlation coefficient test.

**Results and Discussion**

Both the localized P-fertilizer and seed P-enrichment significantly enhanced the dry matter weight of wheat plants, but each effect appeared at different times (Fig. 2). While the results of ANOVA showed that the effect of localized P-fertilizer (Uniform vs. Local) was significant both at 25 DAS ($P = 0.006$) and 50 DAS ($P = 0.014$), that of seed P-enrichment (Non-enriched vs. Enriched) was not significant at 25 DAS ($P = 0.260$) but significant at 50 DAS ($P = 0.036$). The delayed effect of seed P-enrichment is consistent with our previous observations (data not shown). Therefore, the effect of seed P-enrichment should be examined at both early and later growth stages.

The multiple comparisons show that dry matter weight
at 50 DAS was increased to a similar extent by the single application of seed P-enrichment (Enriched-uniform) and of localized P-fertilizer (Non-enriched-local) (Fig. 2b). However, combined application (Enriched-local) did not increase dry matter weight to a greater extent than either treatment alone (Fig. 2b). On the basis of these results, we see no reason to recommend combination treatment at present.

Plant dry matter weight (g plant$^{-1}$) is a function of P content (mg P plant$^{-1}$) and PUE (g DM mg$^{-1}$ P). Thus, we used regression analyses to determine which factor, P content or PUE, could explain variations in dry matter weight across the treatments (Fig. 3). At 25 DAS, variations in dry matter weight could not be explained by P content (Fig. 3a, $R^2 = 0.001, P = 0.926$) but by PUE (Fig. 3b, $R^2 = 0.546, P < 0.001$). This suggests that factors other than P limited plant growth, at least during the early growth stage. It is well established that plants increase PUE through re-translocation and re-utilization of stored P (Wang et al., 2010). The remobilization of P is not necessarily associated with senescence and occurs even in young plants (Peng and Li, 2005; Wang et al., 2010). Thus, during the early growth stage, the active P remobilization might have influenced the plant growth. In contrast, at 50 DAS, dry matter weight obviously responded to P content (Fig. 3c, $R^2 = 0.813, P < 0.001$) but not to PUE (Fig. 3d, $R^2 = 0.002, P = 0.864$), revealing that P was the limiting factor at 50 DAS.
Fig. 4 shows that P content at 50 DAS was correlated with root length across the treatments ($R = 0.658$, $P = 0.002$). P uptake is strongly dependent on root length (Barber, 1995). Thus, each method should have increased P content, and hence dry matter weight, through an increase in root length. Initially, we expected that the 2 methods (localized P supply and seed-P-enrichment) would influence wheat growth independently, and therefore combining the two methods (enriched-local) would yield highest plant growth. However, this expectation was not supported by the data (Fig. 2b); the results of ANOVA detected a significant interaction between the effects of the two method ($P = 0.029$).

We assumed that each of the 2 methods, when applied alone, increases root length and P content, but the combination did not increase root length further. Thus, increasing root length might be the common and biologically relevant effect of both methods. Indeed, numerous evidences have been accumulated that plants respond to a spatially localized P-supply by developing longer roots in the P-rich soils (Drew, 1975; Yano and Kume, 2005; Kume et al., 2006). Coordination of local and systemic signaling networks may be involved in the root proliferation under the localized P supply (Chiou and Lin, 2011), and has been studied at the molecular level (Chiou and Lin, 2011; Abel, 2011). Little information is available as to how seed P-enrichment increases root length except for our previous study (Sekiya and Yano, 2010). However, we speculate that seed P-content might be a major factor connecting the two events (root length increase and P-enrichment) because a strong positive relationship has been observed between seed P-content and root length (Koide and Lu, 1995; White and Veneklaas, 2012). Thus, the non-additive effect of the combination on plant growth is likely due to its insignificant effect on root length and hence P content. Elucidation of whether the insignificant effect is caused by the plant internal (plant phosphorus demand) or the external (soil phosphorus availability) factor might provide an opportunity for deriving the potential of the combination treatment.

In conclusion, both the localized P-supply and seed P-enrichment methods enhanced wheat growth to a similar extent. The effect of the former was observed at the early growth stage and the latter at the later growth stage. Because of a significant interaction between the two methods, which contributes to the absence of an additive effect, their combined use is not recommended.

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