Root Morphological Plasticity for Heterogeneous Phosphorus Supply in *Zea mays* L.

Katsuya Yano and Takashi Kume

(Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan)

**Abstract**: The morphological plasticity of roots in nutrient-enriched patches of soil is regarded as an adaptive response in plants, but its functional efficiency is still debatable. We examined whether the efficiency is dependent upon the patch size, or the amount of phosphate (P) supplied in maize (*Zea mays* L.). Two levels of P-input (high and low) and three patch sizes (large, medium and small) were used in various combinations in containers filled with soil. Irrespective of the P-inputs, P uptake and biomass were greatest in large patches together with root proliferation restricted to the soil inside patches, indicating that the effect of P-patch size was stronger than the amount of P supplied. Due to the fine root proliferation (about 0.5mm-width) of higher specific root length, the root length promoted was not accompanied by more biomass investment inside the patches. For the medium and small patches, such a localized root proliferation disappeared, resulting in impaired plant growth with limited P acquisition. It was concluded that the efficiency of the root plasticity on P acquisition depends on the size of P patches more strongly than the inputs.

**Key words**: Nutrient patches, Phenotypic plasticity, Phosphorus uptake, Resource foraging, Root proliferation, Soil heterogeneity, *Zea mays*.

Soil nutrient heterogeneity in a field is found to occur at an individual plant scale, where available phosphate varied 3-fold and nitrate did 12-fold around individual plants (Jackson and Caldwell, 1996). Even at a single-root scale, plant roots usually experience heterogeneous environments in soil, rather than homogenous ones found in the laboratory and solution culture. An apparent disparity in plant performance between heterogeneous and homogenous systems exists, with heterogeneous application of nutrients resulting in superior growth and nutrient acquisition by plants in such soils (Birch and Hutchings, 1994; Fransen et al., 1998). There is considerable evidence that many plants respond to this heterogeneity by employing phenotypic plasticity as a ‘behavioral adaptation’ to enhance their acquisition of essential resources (Grime, 1994; Robinson, 1994; Jackson and Caldwell, 1996; Tanaka et al., 2000).

Drew (1975) demonstrated that heavy branching of lateral roots occurs in the seminal root axis of barley when nitrogen or phosphorus was supplied locally. Elevated uptake kinetics of the roots has also been observed to precede changes in root morphology in patchy, nutrient-enriched microenvironments (Drew and Saker, 1975; Jackson et al., 1990; Burns, 1991; Caldwell, 1994; van Vuuren et al., 1996). Nutrient acquisition, with plastic responses of roots, has been simulated using mathematical models based on the mobility of each nutrient (Jackson and Caldwell, 1996; Robinson, 1996), and attempts have been made to assess the plastic root responses on competitive ability (Hodge et al., 1999; Bliss et al., 2002; Genney et al., 2002).

However, considering the various costs associated with phenotypic plasticity (DeWitt et al., 1998), the plastic responses of root systems may not always bring about advantages for individual plants. Fitter (1994) assumed that, while the proliferation of roots within a nutrient patch would appear to be an adaptive response, if the patch is short-lived, or supplies only limited resources, the costs associated with the investment in new roots may not be repaid. Using a clonal plant species, Wijesinghe and Hutchings (1997) demonstrated that plant biomass decreased in proportion to the size of the compost even if the same amount of compost was applied in each container. Although the report did not include data on the root morphological responses, the growth depression observed might result from a failure of root plasticity to the smaller patches.

In this study, we have attempted to test the hypothesis that the functional significance of root morphological plasticity depends on the nature of heterogeneous nutrient distribution, such as scale and quantity of the resource in a patch. Phosphate (P) was chosen to test the hypothesis since P is immobile in soil and its acquisition by plant is principally determined by morphological traits such as root length and width, rather than physiological kinetic parameters (Barber, 1995). Maize plants were used to address better P
utilization in arable lands, concerning depletion of available P resources in near future. The objective of the study was to identify the extent to which morphological root plasticity is adaptive in maize plants using combinations of three patch sizes and two levels of P supply.

Materials and Methods

1. Plant growth

A plastic container (30 cm in length and width, 10 cm in depth) was filled with 3.0 kg of subsoil of Humic Andosol (pH 6.0) that has negligible concentrations of available P (40 µg Truog-P kg⁻¹ soil). Previously, 2.5 g of (NH₄)₂SO₄ and 0.83 g of KCl were amended into the soil and distributed uniformly in every container. Two P treatments were prepared: High-P consisting of 5.78 g of super phosphate per container, and low-P consisting of 0.58 g super phosphate per container. Three patch sizes (large, medium and small) were prepared for each of the two P treatments (Fig. 1). The design of patches was similar to that used by Wijesinghe and Hutchings (1997). Consequently, six treatment combinations were thus possible using the two P-levels and three patch sizes. Four replicate containers were prepared for each combination.

Three germinating maize seeds (Zea mays L. cv. Robust 30-71) were sown into the center of each soil container on June 18, 1999, with plants being thinned to one 7 days later. Containers were weighed at two-day intervals to calculate the amount of water lost via evapotranspiration, and tap water was supplied so as to maintain soil moisture content at 40% (w/w). The containers were kept in a greenhouse for 55 days and all of the plants were harvested on August 12, 1999.

2. Sampling procedure

Shoots were partitioned into the leaf blade and the stem including the leaf sheath. Leaf blade area was measured using a leaf area meter (LI 3100, Li-Cor, Lincoln, Nebraska). Each root system was sampled by the pin-board method modified by Kono et al. (1987). This allows sampling with minimum disturbance of the configuration of the intact root system. Using this technique, each root sample was separated into two parts: one growing inside the P-patch, and the other growing outside the P-patch. Each of these root samples on either side of the P-patch was further divided into sub-samples of similar fresh weight: one for measurement of the dry matter and P content, and the other for the measurement of the root length described below.

3. Determination of P content

Samples of the leaf blade, stem, and roots from both inside and outside the patches, were dried at 80°C for 48 hours before being weighed. Each dried sample was then ground and P concentrations were determined colorimetrically using the phosphovanado-molybdate method (Hanson, 1950) once the plant material for each tissue sample had been mineralized with nitric acid and perchloric acid.

4. Measurement of root length and width

Sub-samples of roots previously preserved in FAA (formalin: acetic acid: 70% ethanol, 1:1:18 by volume) were stained with 0.2% methylene blue for 48 hours. The stained roots were then sandwiched between two transparent sheets taking care to prevent any overlap between roots. A digitized image was acquired using a desktop scanner at 200 dpi and 256 grayscale. Using a macro-program developed by Kimura et al. (1997), root length and width were measured from these images using NIH Image (version 1.60).

5. Statistics

The analyses of patch size, at three levels, were combined in a factorial arrangement with the two levels of P treatment. The containers were arranged in a randomized complete block design with four replicates. The data were analyzed by two-way analysis of variance (ANOVA), in which the variation sources consisted of the P-input level and the patch size.
treatments. The mean separation was determined using Fisher’s protected least significant difference method (LSD) when significant effects were detected by ANOVA. For the root parameters, the paired-sample t-test between the roots inside and outside the P patches was performed.

Results

Given that similar results were obtained for small P patches and treatments in which P was applied uniformly in a preliminary experiment, we omitted assessing the effect of uniform P application (no patches) on root morphology. This is confirmed again with another experiment (Kume et al., 2001).

1. Root proliferation

Root dry matter (DM) obtained for the whole root system was greatest in the large-patch plants at both P levels (Table 1). Looking at root DM between inside and outside patches, no significant contrasts could be detected between them for any of the treatments. For root length, however, the significant contrasts were detected in plants from the large-patches at both P levels. While the contrasts in root length were not significant, roots tended to be longer inside the patches when compared to those from outside the patches in the medium and small patches in the low-P treatment. The total root length of the whole root system was similar among the three patch sizes in the high-P plants. Conversely, in the low-P plants, the total root length from the large-patch exhibited an approximately 3-fold increase by when compared to those from the medium- and small-patches. As a result, the low-P plants in the large-patch had the total root length comparable to those of the high-P plants as no significant differences were detected.

In contrast to root length, the specific root length was greater in the low-P plants (66m g⁻¹ DM on average) than in the high-P plants (35m g⁻¹ DM on average) for the whole root system. In consistent with root length, only the plants from the large-patches

![Fig. 2. Root length distribution along the root width. Roots inside the patch (●) or outside the patch (○). Large (a), medium (b) or small (c) patch size with high-P input, large (d), medium (e) or small (f) patch size with low-P input. Probability using ANOVA](http://example.com/tr.png)

### Table 1. Root weight and length of 55-day-old maize plants grown in soil where P was supplied with different input levels and patch sizes.

| P level | Patch size | Root dry matter (g plant⁻¹) | Root length (m plant⁻¹) | Specific root length (m g⁻¹ DM) |
|---------|------------|------------------------------|-------------------------|--------------------------------|
|         | Whole Inside vs. Outside patch Whole Inside vs. Outside patch Whole Inside vs. Outside patch |
| High    | Large      | 9.68                         | 292.0                   | 30.7                             |
|         | Medium     | 6.06                         | 240.8                   | 40.1                             |
|         | Small      | 7.43                         | 252.2                   | 34.7                             |
| Low     | Large      | 3.00                         | 218.3                   | 73.5                             |
|         | Medium     | 1.23                         | 73.9                    | 57.6                             |
|         | Small      | 1.13                         | 72.7                    | 65.9                             |
| LSD (0.05) |          | 1.92                         | 84.6                    | 12.8                             |
| ANOVA P level |          | P<0.001                      | P<0.001                 | P<0.001                          |
| Patch size |          | P=0.002                      | P=0.004                 | P=0.758                          |
| Interaction |          | P=0.346                      | P=0.156                 | P=0.029                          |

† ns and * indicate no significance and significance at the 5% level between inside and outside the patches by the paired-sample t-test, respectively.
at both P levels exhibited significant contrasts in the specific root length between the roots from inside and outside the patch, indicating that the localized alteration of root length was attributed to the specific root length, not to dry weight increase.

The differences in the extent of proliferation in roots from either inside or outside the P patches were also apparent in the distribution of the root length in combination with root width, particularly in fine roots of approximately 0.5 mm in diameter (Fig. 2.). The main effect of patch was significant in both large patches of the high-P (P<0.001) and the low-P (P=0.078). For the former, the interaction between patch and root width was also significant (P=0.019), indicating that the roots of particular width (0.3 to 0.5 mm) responded to the patch specifically.

2. Plant growth and P acquisition

At the time of harvesting, a 2- to 4-fold increase in leaf area was observed in high-P plants when compared to plants grown under conditions of low-P (Table 2). The largest leaf areas were found in plants grown in large patches at both P treatments, with no significant differences detected between the medium and small patch in high-P plants. DM, including the roots, was significantly affected by the P treatment (P<0.001) and slightly by the patch size (P=0.071). As a result, the DM of the whole plant was also greater in the high-P plants than in the low-P specimens, and within each P treatment the highest DM was observed from plants in the largest patches. P content was significantly affected not only by P level (P<0.001) but also by the patch size (P=0.028); larger the size resulted in greater P content.

Discussion

1. Greater P acquisition in large patches

The results show that the maize plants exhibited defined responses to P-patches in the soil. This was particularly apparent in large-patches and is manifested as localized root proliferation (Table 1 and Fig. 2). Given that the local root response was observed in plants subjected to both high-P and low-P treatments, it would seem that plants are able to differentiate between the P concentrations inside and outside P-patches, even at the low P concentrations. Given that P acquisition and DM was greatest in the large-patches at both the P levels (Table 2), it seems likely that P acquisition by the plant is strongly determined by the size of the patch and rather than the amount of P in the soil.

Furthermore, as local root proliferation was attributed mainly to the fine roots, the root DM from plants inside the patches was similar to that outside the patches (Table 1). These results contrast to the findings of Fransen et al. (1999) and Hodge et al. (1999) who observed increased root biomass in nutrient patches without alterations in the specific root length in several grasses. Conversely, alterations in the specific root length with root proliferation were found in other studies (Robinson and Rolison, 1983; Eissenstat and Caldwell, 1988; Eissenstat, 1991; Yano and Kojima, 1998).

DM and P acquisition in plants grown in medium and small patches were observed to be consistently lower than those observed in large-patches for both P levels (Table 2). Thus it must be concluded that P application so as to make smaller patches, including uniformly application (Kume et al., 2001), would be wasteful for the maize plants regardless of the amount of supply.

2. Limitation of the root plasticity and its implication to improve P fertilizer efficiency

In both of the high- and low-P plants, root
proliferation was unclear in smaller the patch sizes, as assessed by the contrasts between inside and outside patches in terms of the specific root length (Table 1) or the root length for each root width (Fig. 2). The results revealed that the plants could not adapt with the root plasticity to these smaller sizes of P-patches. Probably due to this effect, the similar results were obtained when P was applied uniformly in a preliminary experiment.

Looking at the root length of whole root system (Table 1), however, the cause of failure to adapt would be different between the high- and low-P plants; the formers (241 and 252 m) had the similar root length to their large patch (292 m) but the latters (74 and 73 m) were very poor compared to the large patch (218 m). In the high-P plants, decreased root length inside the patches correlated with an increased length outside the patches in both medium and small patches (Fig. 2). It therefore seems possible that the rapid root proliferation observed under conditions of high-P occurred because these plants were unable to restrict elongation and branching to within the smaller P-patches. Such an excessive root proliferation could not likely contribute to P acquisition despite of the costs, since mobility of P is quite low in soil due to the strong binding (Barber, 1995). Perhaps impaired balances of the cost-benefit would likely result in the growth depression. In order to confirm this, an investigation of carbon costs, not only in the root tissues, but also in the respired carbon under conditions of heterogeneous P distribution, needs to be undertaken.

In contrast to the high-P plants, the plants subjected to the low-P treatment showed sever P-deficiency especially in both medium and small patches, resulting in poor root growth as a whole. Considering carbon cost for the root plasticity as described above, the P-deficient plant may be in a dilemma that a limited amount of carbon must be invested to roots for acquisition of P which is limiting carbon acquisition. The results obtained here suggested the plant in such situation seems to decline carbon investments to the root plasticity.

We found that the functional significance of phenotypic plasticity of maize roots in P-enriched soil is primarily dependent upon patch size and not P levels. This would imply that it may not always be beneficial for plants to exhibit root plasticity. The success of the response is thus dependent upon the spatial attributes of nutrient-patches and also the mobility of nutrients in the soil. Our results would explain the rationale about the advantage of localized P fertilization for crop cultivation as known experimentally, and moreover suggest a significance of the localizing scale. In so far as this affects considerations of the depletion of P resources, more efficient means of P fertilization need to be employed to emulate the effect of the large patch P dynamics as demonstrated in this study.

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