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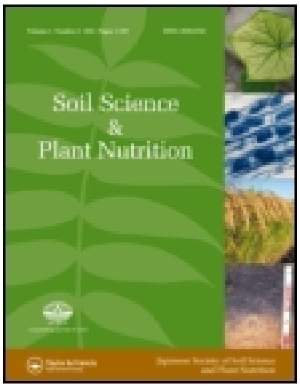

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Potential for Using Plant Xylem Sap to Evaluate Inorganic Nutrient Availability in Soil

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Potential for Using Plant Xylem Sap to Evaluate Inorganic Nutrient Availability in Soil

I. Influence of Inorganic Nutrients Present in the Rhizosphere on Those in the Xylem Sap of *Luffa cylindrica* Roem.

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To examine the availability of nutrients in soil, chemical extraction methods are commonly used. However, such methods require different extractant solutions depending on the types of soil and nutrients being assayed, making it difficult to assay multiple components simultaneously and compare analytical values. We proposed an alternative method, consisting of the collection of xylem sap exuded from plant stems by employing plant roots as a tool for evaluating the levels of available nutrients in soil. We designated this method as the "xylem sap method." We tested the effectiveness of this method by growing *Luffa cylindrica* Roem. seedlings for pre-determined periods of time in pots containing culture medium, removing the aerial parts, and collecting the xylem sap from the cut ends. In a sand culture medium, in which all the nutrients provided were considered to be present in an instantly available form, the concentrations of the nutrients in the xylem sap were found to be proportional to those of the nutrients in the culture solution supplied to the medium, confirming that this technique gives a good index of nutrient availability in the rhizosphere. Furthermore, the contents of the nutrients in the xylem sap were proportional to those of the nutrients accumulated in shoots during the growth period. This shows that, at the seedling stage, nutrient uptake by the aerial parts of *L. cylindrica* during the growth period exactly mirrors the contents in the xylem sap. It was further confirmed that the nutrient contents in the xylem sap were similarly representative of the nutritional status of the plant. However, in a soil-based culture medium, the increase in the total amount of phosphorus and micronutrients in the medium was not reflected in the xylem sap. This was in agreement with existing findings that concentrations of certain specific nutrients are maintained at a fairly steady level in the plant, irrespective of their availability in soluble form in soil. Thus, we concluded that changes in most nutrient concentrations in the xylem sap corresponded to changes in the behavior and availability of nutrients in soil and that the xylem sap method could be used as an accurate indicator of the availability of most nutrients in soil.

Key Words: **availability, evaluation, inorganic nutrients, *Luffa cylindrica*, xylem sap.**

Intensive farming often leads to excessive use of fertilizers. Worldwide, there is a growing concern about the exhaustion of fertilizer resources such as phosphorus, and the environmental problems and threats to human health posed by contamination and eutrophication of underground and river water associated with fertilizer-derived nitrogen and phosphorus (Ugwuegbu et al. 2001; Erickson et al. 2001; Giasson et al. 2002; Ebeling et al. 2002).

The solution of these problems requires a minimum application of fertilizers through strategies designed to replace only the missing nutrients in soil and supply the required nutrition for crops. To enable the use of such strategies, it is thus necessary to determine the actual contents of available nutrients in soil.

The most direct way to determine the nutrient avail-

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ability in soil is to analyze the growth responses of plants in field tests. However, this is a time-consuming procedure and the results tend to be highly area-specific (Marschner 1995). For these reasons, the "extraction method" is chiefly adopted in practice, in which soil samples are shaken in appropriate extractant solutions and the concentrations of the nutrients extracted are used as an indicator of availability. Extractants include water, diluted acids, salts, and complexing agents (Marschner 1995). The extraction method is commonly and widely used because of several advantages, including: simplicity, ease of handling, high reproducibility, and the possibility of completing the extraction itself in a short period of time. Nevertheless, several drawbacks should be considered. The method is based exclusively on chemical analyses, without any way of confirming whether the results obtained are consistent with the nutrient absorption patterns of the plants intended to be grown there. In addition, extracting the same nutrients using different types of solution tends to generate different results. Even if solutions of the same kind are used, the specific values obtained depend on the soil/solution ratio used when the solution of soil are shaken together. In some cases, different solutions need to be used depending on the types of nutrients to be assayed, their abundance in soil, and the physical and chemical properties of soil. Not only can these problems be critical when simultaneous assays of multiple components are needed, but they may also impede comparisons of analytical values. Moreover, in many cases, the extraction method requires air-drying of the soil prior to extraction. Therefore, it is not an appropriate method for the evaluation of nutrients whose availability varies with the oxidation-reduction potential of soil.

Ideally, any method for determining the availability of nutrients in soil should be the same regardless of differences in analytical conditions, including types of soil and nutrients, thereby overcoming the problems mentioned above. The method should also reflect the nutritional status of the plant.

A nutrient in "available form" refers to a nutrient in a form that can be absorbed by plants. Therefore, to evaluate the nutrient availability to plants in soil, adopting a method which enables to detect only nutrients that can be absorbed by plants would appear to offer a considerable advantage practically.

Nutrients and water in soil are absorbed by the roots, reach the stele, and are then transported to the aerial parts through the xylem vessels. Therefore, the concentrations and composition of the nutrients in the xylem sap probably reflect the nutrient availability in soil and might be used to determine the contents of available nutrients in soil. Movement of substances in the xylem vessels is a one-way traffic from below to above (Marschner 1995). Cutting the stem directly above the roots enables the xylem sap to rise from the roots to be obtained at the cut surface. We have attempted to collect at the level of the hypocotyl the liquid exuded from xylem vessels after removing the aerial parts of the plant, in order to use the plant roots as a tool for extracting available nutrients and to evaluate the contents of available nutrients in soil based on the analytical values obtained.

Up to now, the xylem sap had been studied primarily to elucidate the transfer mechanism of substances from roots to aerial parts (van Beusichem et al. 1988; Cruz et al. 1993; Engels and Marschner 1993). Currently, studies on organic components in the xylem sap, like plant hormones, including abscisic acid and cytokinins (Jackson 2002; Mader JC et al. 2003) are common. However, some reports are particularly interesting: Morita and Abe (1999) and Abe and Morita (2003) utilized the bleeding rate of xylem sap to evaluate the physiological activity of whole root system in lowland rice, and they observed that not only the rate could be measured but also components in the sap could be analyzed. Morita and Toyota (1996) and Yamaguchi et al. (1999) analyzed the nutrients in the bleeding sap of some crops but they did not consider their availability in soils. Schurr (1998) developed a root pressure chamber that enables the continuous collection of xylem sap from intact plants, and Herdel et al. (2001) demonstrated that diurnal variations in the concentrations and fluxes in the xylem are dominated by the internal processes of the plant and that concentrations of the nutrients in the xylem sap are highly but specifically related to each other. However, few studies have been conducted with the objective of using the xylem sap to evaluate the nutrient availability in soil. If our "xylem sap method" proved to be suitable, it may become possible to evaluate the availability of nutrients without tailoring the extraction methods to match different soil conditions and it would enable to consider absorption processes of the plants. The xylem sap method should be suitable both for measuring the growth responses of plants and as an extraction method.

The objectives of the present paper were to examine the relationship between the presence of nutrients and the form they take in the rhizosphere as well as their concentrations in the xylem sap when they are absorbed by the roots, and to determine the potential for using the xylem sap to evaluate the nutrient availability in soil. In a preliminary study, we investigated the influence of the number of seedlings per pot on the volume of the sap exuded from the xylem vessels. The timing of collection of the xylem sap was also investigated to examine the growth conditions required for collecting the xylem sap. As a result, we tested the assumption that the growth of plants cultivated in a limited volume of soil, such as in a

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pot, would be restricted and thus affect the exudation of xylem sap.

MATERIALS AND METHODS

The test plant, *Luffa cylindrica* Roem. cv. Futo-Hechima, was selected due to the ease of collection of the xylem sap.

Growth conditions for collection of xylem sap

Cultivation

Experiment 1: Relationship between the number of seedlings planted and the volume of xylem sap exuded. Two hundred grams of Gray Lowland soil (Table 1) and 200 g of quartz sand (grain size: 0.5–1 mm) were blended and placed in 500-ml plastic pots. Cotyledonous *L. cylindrica* seedlings grown in nursery beds filled with non-fertilized vermiculite, were transplanted to the pots at densities of 3, 6, 9, 12, and 15 plants per pot. A preliminary experiment confirmed that blending quartz sand with soil enabled *L. cylindrica* to continue growing at the seedling stage for a certain period of time even in a relatively small volume of test soil. After transplantation, another 70 g aliquot of quartz sand was added on top of the soil to minimize water evaporation. The seedlings were grown in a greenhouse under natural light, and watered regularly with deionized water to maintain 60% of the maximum water-holding capacity of the soil, determined by pot weight. Five replicate pots were used and the test seedlings were allowed to grow up to the four-leaf stage (approximately 20 d).

Experiment 2: Relationship between the timing of xylem sap collection and its volume. Six plants per pot were transplanted and each was allowed to grow to the 1, 2, 3, 4, 5, 6, and 7 leaf-stages. Xylem sap was collected from each pot at each stage. The other conditions were the same as those in Experiment 1.

Collection of xylem sap

After the harvest stage was reached in Experiments 1 and 2, the stem was cut at the level of the hypocotyl just below the cotyledons (approximately 1 cm above the ground) and the aerial parts were removed. One end of a soft plastic tube was placed over the end of the remaining hypocotyl, with the other end leading to the test tube. Hardel et al. (2001) showed that element concentrations in the xylem sap display diurnal variations, rising at night. This may be because the transpiration rate and water absorption rate of roots are higher than during the daytime (Hirasawa 1998), although Herdel et al. (2001) rejected this simple hypothesis, suggesting that plant internal processes were the dominant factor. Though the test plants kept their aerial parts, through which the transpiration stream is generated and removed during xylem sap collection, the above aspects were taken into account. The plant stems were cut early in the morning and the xylem sap was collected continuously for 24 h.

Measuring the volume of roots in a pot

To determine the root/soil volume ratio in a pot, the volume of the roots was measured. All the roots, including rootlets, were collected and the attached soil rinsed off. They were then placed in a measuring cylinder filled with an appropriate volume of water. The total volume of the roots was measured by the apparent change in the water volume. The volume of soil represented the total volume before blending with quartz sand.

Relationship between nutrient behavior in the rhizosphere and nutrient concentrations in xylem sap

Cultivation

Experiment 3: Culture media in which culture solutions at different concentrations were added to quartz sand. Five hundred milliliter plastic pots were filled with 400 g of quartz sand (grain size: 0.5–1 mm). The basic concentrations of the culture solutions (see Table 2), and the 0.75-fold, 0.5-fold, and 0.25-fold concentrated culture solutions, respectively, were added to the pots to a volume equal to 60% of the maximum water-holding capacity of the quartz sand. Cotyledonous *L. cylindrica* seedlings grown in nursery beds filled with non-fertilized vermiculite, were transplanted to the pots, with six plants per pot. The seedlings were grown in a greenhouse under natural light and the above-described culture solutions were added regularly to maintain 60% of the maximum water-holding capacity of the quartz sand, determined by pot weight. Five replicate pots were used.

Table 1. Properties of soil used.

$pH(H_2O)$	Truog-P ($mg\ P_2O_5\ kg^{-1}$)	CEC	Exchangeable cations		
			K	Ca	Mg
			($cmol_c\ kg^{-1}$)		
5.5	39.4	17.5	0.5	6.0	2.6

Table 2. Composition and concentrations of the components of the basal culture solution used in Experiment 2.

NO_3-N	NH_4-N	P	K	Ca	Mg
($mg\ L^{-1}$)					
225	28	62	394	120	48
Fe	B	Mn	Zn	Mo	Cu
($mg\ L^{-1}$)					
0.6	0.5	0.5	0.05	0.05	0.02

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Experiment 4: Culture media in which soil and quartz sand were blended at different ratios. Culture media were prepared by blending Gray Lowland soil (Table 1) with quartz sand at weight ratios (w/w% of soil/quartz sand) of 20/80, 40/60, 50/50, 60/40, 80/20, and 100/0. Four hundred grams of each was placed in 500-ml plastic pots. Cotyledonous *L. cylindrica* seedlings grown in nursery beds filled with non-fertilized vermiculite, were transplanted to the pots, with six plants per pot. After transplantation, another 70 g aliquot of quartz sand was added on top of the soil. Fertilizers were not supplied. The seedlings were grown in a greenhouse under natural light and watered regularly to maintain 60% of the maximum water-holding capacity of the soil, determined by pot weight. Five replicate pots were used in this experiment.

Collection of xylem sap

After the test seedlings had been allowed to grow up to the four-leaf stage (approximately 20 d), the xylem sap was collected according to the protocol described above.

Analysis of nutrients in xylem sap and plant aerial parts

The phosphorus concentration in the xylem sap was measured by the molybdenum blue colorimetric method (microflow spectrophotometer UV-730; Shimadzu Corporation, Kyoto, Japan), and the concentrations of potassium, calcium, magnesium, manganese, zinc, and copper were measured by atomic absorption spectrometry (atomic absorption spectrophotometer A-2000; Hitachi, Ltd., Tokyo, Japan). Nitrogen, another important nutrient, was excluded from the analysis to be examined in more detail later separately, specifically for the nitrate form and the ammonium form.

In Experiment 3, the removed aerial parts of the plants were subjected to wet-digestion after drying, and the concentrations of all the examined nutrients were measured using the same methods as those for xylem sap analysis.

RESULTS AND DISCUSSION

Growth conditions for collection of xylem sap

Relationship between the number of seedlings planted and the volume of xylem sap exuded [Experiment 1]. The relationship between the number of seedlings planted per pot and the growth of the plants is shown in Fig. 1A. The root weight per pot increased with the number of seedlings. In contrast, the shoot weight increased when the number of seedlings increased from three to six plants, but leveled off thereafter. Per plant weight of both roots and shoots decreased with an increasing number of seedlings.

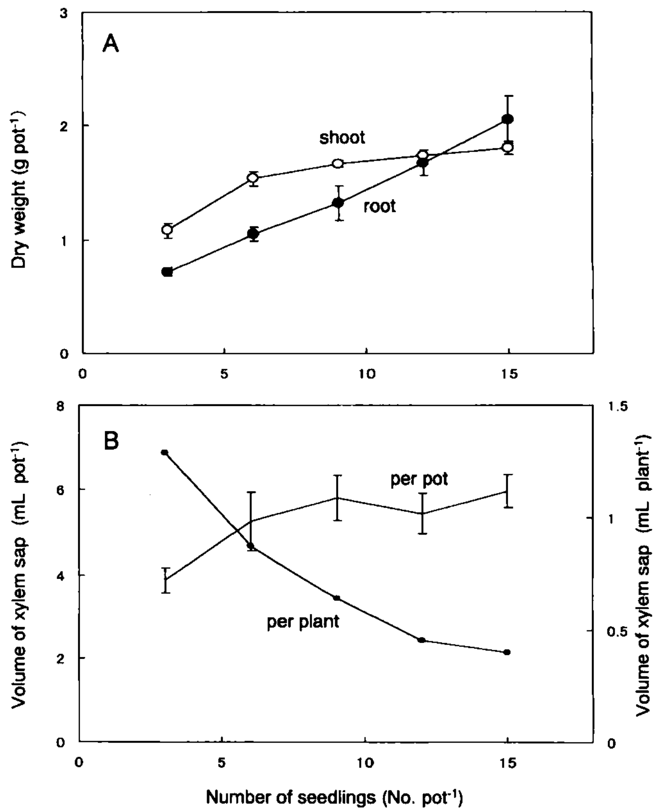

Fig. 1. Relationship between number of seedlings and (A) their growth and (B) volume of xylem sap exuded. Bars indicate standard deviation.

The relationship between the number of seedlings planted and the volume of xylem sap exuded is shown in Fig. 1B. The volume of xylem sap exuded per pot increased when the number of seedlings increased from three to six plants, but leveled off thereafter. The volume of xylem sap exuded per plant decreased with an increasing number of seedlings.

These results clearly showed that if the plants were grown at high planting densities, competition among plants would prevent the aerial parts from growing successfully. Consequently, the exudation of xylem sap was limited because the growth of the aerial parts was restricted. We concluded that since growing six plants would maximize the volume of xylem sap collected under our experimental conditions, it was appropriate to grow 6 plants per pot.

Relationship between the timing of xylem sap collection and its volume [Experiment 2]. The relationship between the plant stage at which the xylem sap was collected and its volume was examined with reference to the number of plant leaves. The relationship between the number of leaves and plant growth (Fig. 2A) or the volume of xylem sap (Fig. 2B) at the time of xylem sap collection is shown. Shoot weight and root weight increased as plants grew, and the volume of

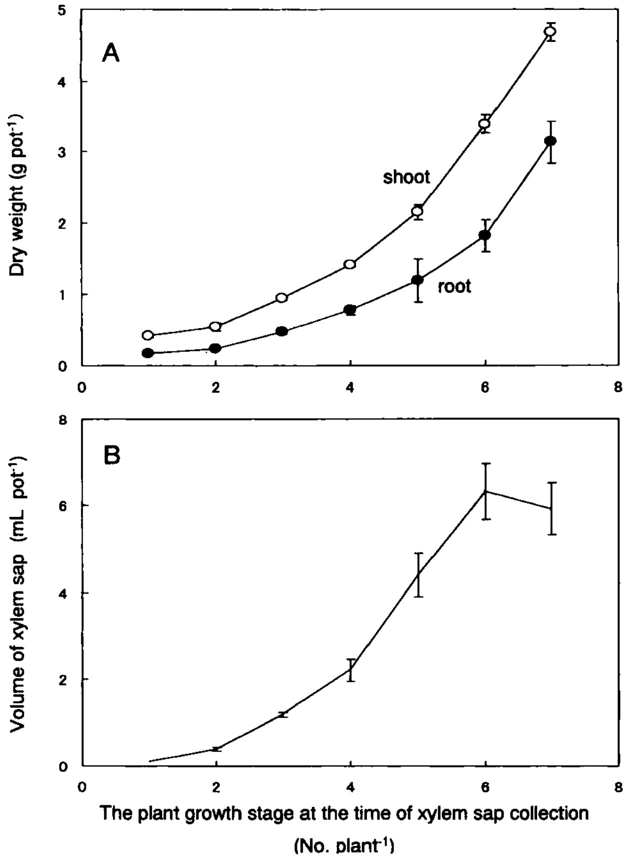

Fig. 2. Relationship between timing of xylem sap collection and (A) plant growth stage and (B) volume of xylem sap exuded. Bars indicate standard deviation.

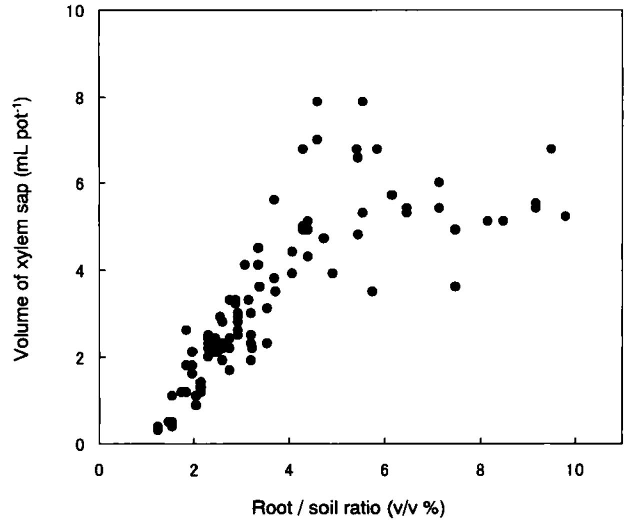

Fig. 3. Relationship between root/soil ratio in pot and volume of xylem sap exuded.

xylem sap exuded increased up to the six-leaf stage. However, at the stage of development of the seventh leaf, the color of the plants became pale, and the volume of xylem sap exuded leveled off at the stage of development of the sixth leaf.

Under the conditions used in this experiment, it appears that continuation of growth up to the seven-leaf stage led to the exhaustion of the nutrients in soil in the pots, resulting in early development of nutrient deficiency. Consequently, we collected the xylem sap at the six-leaf stage for the remaining experiments because normal growth was maintained only up to that stage. In practice, it may be desirable to adopt the shortest duration of the period that enables the collection of a sufficient volume of xylem sap needed for analysis.

The results of Experiments 1 and 2 showed it is obvious that growing plants in a limited soil volume restricted not only of growth, but also the volume of xylem sap exuded, which was affected especially by the planting density. Use of the nutrient absorption function of plants to evaluate the availability of nutrients present in soil requires that such restrictions be minimized. Comparison of the volume of xylem sap exuded and the volume of soil water revealed that the xylem sap collected for 24 h accounted for a maximum of 4–5% of soil water. Because the absorption of water from soil by roots is closely proportional to the area of contact between the roots and soil, the relationship between the root/soil volume ratio and the volume of xylem sap exuded was examined using the data from Experiments 1 and 2 (Fig. 3). The results indicated that the volume of xylem sap exuded increased up to the point when the root/soil volume ratio reached a value of 5%, but then leveled off. This indicates that the volume of xylem sap exuded generally increased with the increase of the contact area of roots with soil, but that there was an upper limit to the volume of xylem sap that could be obtained from a plant whose root growth was restricted, such as when growing in a pot. In other words, these results indicated that when the root/soil volume ratio and the volume of xylem sap increased proportionally, an almost constant volume of xylem sap was consistently obtained for a specified root/soil ratio, number of seedlings planted per pot, and planting period. We consider that this is important for standardizing the collection of xylem sap.

Based on the above examination, we decided to use growth conditions where 200 g of test soil and 200 g of quartz sand were blended and placed in a 500-ml pot. Six *L. cylindrica* plants, with developed cotyledons, were transplanted to each pot. The plants were allowed to grow to the four-leaf stage and the aerial parts were then removed to allow xylem sap collection.

Relationship between nutrient behavior in the rhizosphere and nutrient concentrations in xylem sap

Culture media in which culture solutions at different concentrations were added to quartz sand [Experiment 3]. No differences were observed

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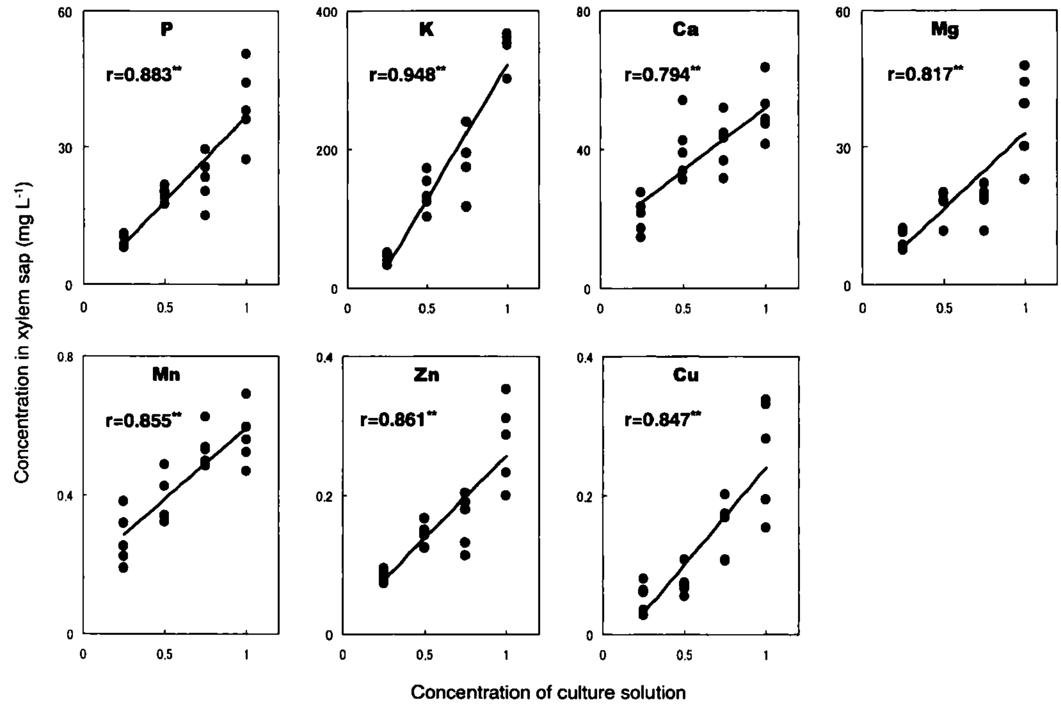

Fig. 4. Relationship between culture solution concentrations and nutrient concentrations in xylem sap. **: Indicates significance at 1% level.

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in the biomass produced for roots and shoots as well as the volume of xylem sap exuded between the basic concentration and 0.75-fold concentration. After further reduction of the concentration, however, both the biomass produced and the volume of xylem sap decreased: at 0.25-fold concentration, both decreased to nearly 70% of the basic concentration (data not shown).

The relationship between the culture solution and nutrient concentrations is shown in Fig. 4. Concentrations of phosphorus, potassium, calcium, magnesium, manganese, zinc, and copper in the xylem sap were directly proportional to those in the culture solutions, and the correlation coefficient between the xylem sap and culture solutions for all the elements was 0.8 or higher. Because nutrient absorption by quartz sand is negligible, the rhizosphere itself retains the culture solution, suggesting that almost all the nutrients given were in an ionic (i.e., available) form. Nutrient levels in the xylem sap also changed in proportion to the changes in the concentration of each nutrient in the culture medium. These results clearly indicated that the changes in the nutrient concentrations in the xylem sap accurately reflected the changes in the concentrations of available nutrients in solution in the rhizosphere.

Meanwhile, xylem sap nutrient concentrations were lower in concentration ranges of 0.25-fold and 0.5-fold, where growth and the volume of xylem sap exuded were reduced. This suggests that there was a close association between the nutrient concentrations in the xylem sap and the viability of plants. Thus, to elucidate the relationship between the nutrient contents in the xylem sap and the nutritional status of plants, we examined the relationship between the nutrient contents in the xylem sap obtained and those in the aerial parts (i.e., the relationship between the total content of each nutrient in the xylem sap obtained within 24 h and the contents of each nutrient taken up by the shoots during the 20-d growth period). The results are shown in Fig. 5.

The contents of the nutrients in the shoots were proportional to the nutrient contents in the xylem sap, indicating the presence of a significant correlation between them. The 24-h exudate from the xylem vessels was based on absorption caused only by root pressure because the plant did not have aerial parts at this time. By contrast, the nutrients contained in the shoots during the 20-d growing period had been translocated from the roots to the aerial parts using the transpiration stream as the main driving force. Because the absolute amounts of nutrients transported from the roots by these different driving forces were proportional to each other, at least for *L. cylindrica* at the seedling stage, the nutrient contents of the 24-h exudate from the xylem vessels essentially comprised a 'snapshot' of nutrient uptake by the aerial parts during the 20-d growth period. Thus, our method of evaluating the nutrient availability in soil using nutrient concentrations in the xylem sap also reflected the nutritional status of the plants. Nutrient contents in the shoots are expressed as the sum of the

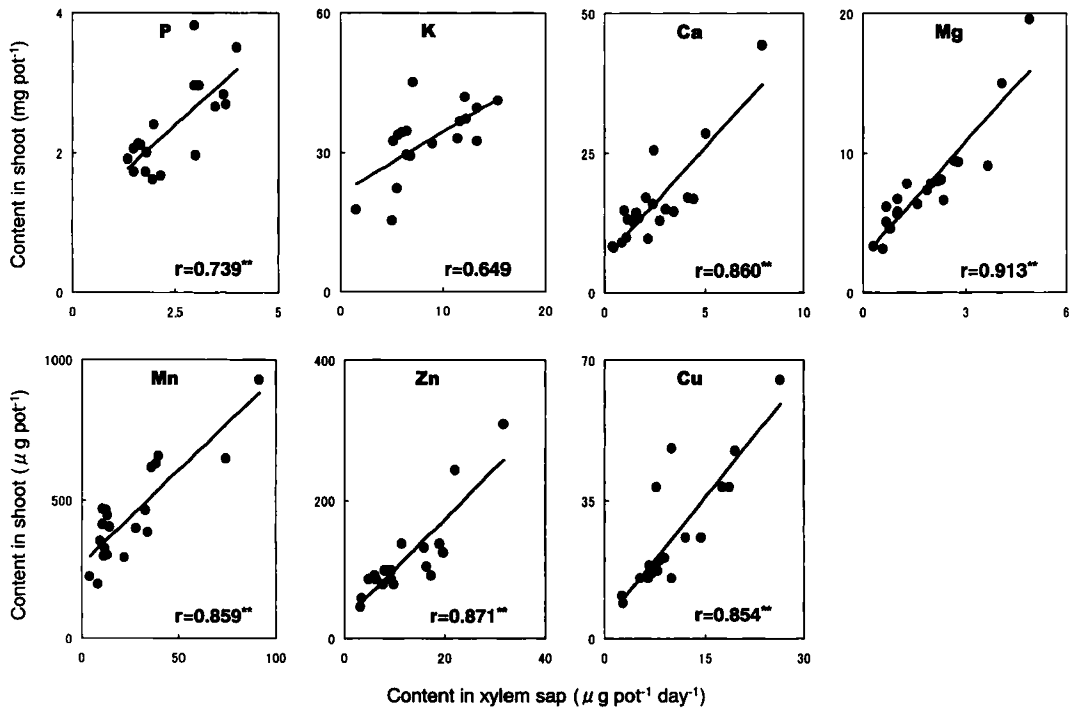

Fig. 5. Relationship between nutrient contents in xylem sap and those in shoots. **: Indicates significance at 1% level.

contents of nutrients in the xylem sap collected over 24 h. Conversely, it was confirmed that the analysis of the nutrients in the xylem sap obtained over 24 h enabled to infer the accumulation of nutrients up to that stage.

Culture media in which soil and quartz sand were blended at different ratios [Experiment 4]. The growth of the roots and shoots remained almost constant, regardless of the changed in the blending ratios of soil and quartz sand. The volume of xylem sap exuded decreased when soil alone without added quartz sand was used, but no differences were observed with the other treatments (data not shown). These results support those of the preliminary test in which blending of quartz sand with soil enabled *L. cylindrica* to grow in a relatively small volume of soil. They also indicated that the addition of a certain amount of quartz sand to soil facilitated xylem sap collection.

The influence of the blending ratios of soil and quartz sand on the element concentrations in xylem sap is shown in Fig. 6. Unlike in Experiment 3, in which culture solutions were added to quartz sand, obvious differences were observed for each element. Concentrations of phosphorus and of micronutrients such as manganese, zinc, and copper in the xylem sap remained almost constant or showed only a small increase, as the proportion of soil in the culture medium increased. By contrast, the concentrations of macronutrient cations such as potassium, calcium, and magnesium in the xylem sap increased significantly with increasing proportion of soil in the culture medium.

Generally, when phosphorus and micronutrient concentrations decrease in a soil solution, which is the main area for plant roots to obtain nutrients, these elements are liberated from soil to maintain an almost constant concentration. Absorption of these elements by the roots is considered to depend to a large extent on mass flow and diffusion. Therefore, changing the proportion of soil in the culture medium is likely to result in only relatively small changes in the availability of such elements in the rhizosphere soil solution. By contrast, the absorption of macronutrient cations depends not only on mass flow and diffusion from the rhizosphere soil solutions, but also on exchange reactions between H^+ on the root surface. Thus, an increased amount of soil in contact with the roots should enhance the amounts of macronutrient cations in the rhizosphere and their availability. The concentrations of the nutrients observed in the xylem sap in proportion to the changes in the blending ratio of soil in the culture medium, were consistent with these assumptions.

Overall, we conclude that the nutrient contents in the culture medium were affected by the changes in the concentrations of the culture solutions added (Experiment 3) and the changes in the proportions of blended soil (Experiment 4). In Experiment 3, in which soil was not used, the nutrient concentrations in the xylem sap changed in proportion to the nutrient levels in the cul-

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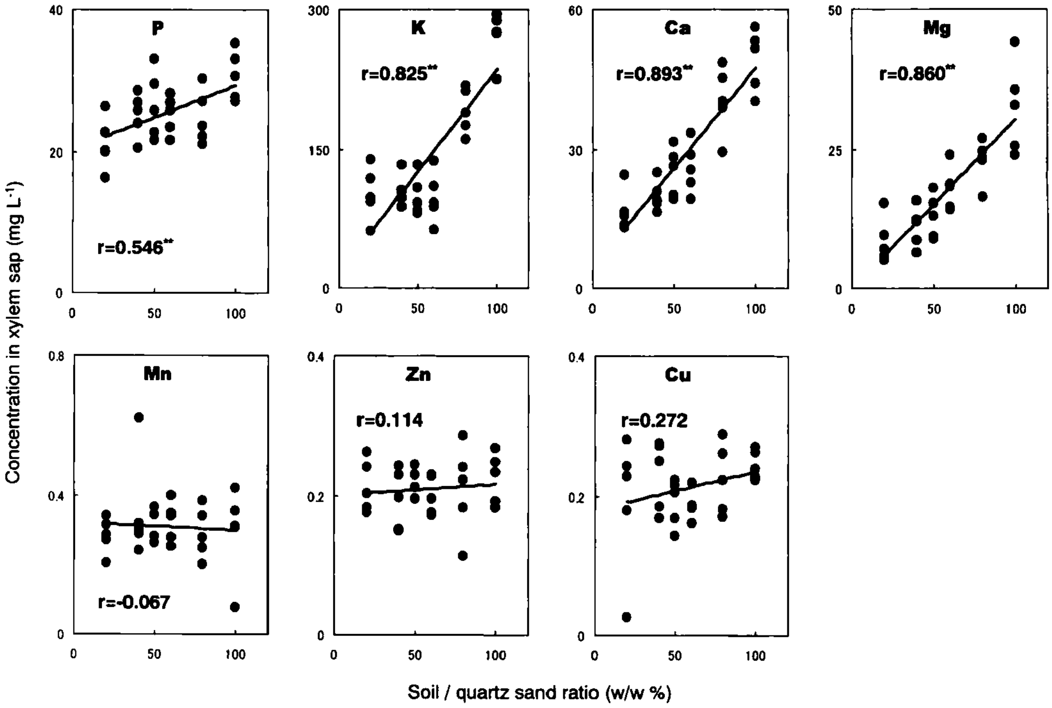

Fig. 6. Influence of soil/quartz sand blending ratio on nutrient concentrations in xylem sap. **: Indicates significance at 1% level.

ture medium. In Experiment 4, however, where soil was used, the concentrations of only same nutrients changed in proportion to the nutrient contents in the culture medium. These results accurately reflected the behavior of the nutrients present in the rhizosphere during nutrient absorption by the roots from soil. Furthermore, it was confirmed that the nutrient contents in the xylem sap reflected the nutritional status of the aerial parts of the plant. The fact that in the xylem sap method multiple nutrients can be simultaneously extracted from soils with plant roots would be an advantage over the extraction method. A relatively larger amount of testing solution could be obtained by the xylem sap method, compared with the use of pressed juice of plants. Therefore, based on our findings, we conclude that using nutrient concentrations in the xylem sap should represent an effective method for evaluating the nutrient availability in soil under the conditions used in our experiments. We will compare the xylem sap method with the extraction method in the following paper.

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