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Use of a root bioassay method to determine phosphorous availability and uptake for some crop species

D.M.A.P. Dissanayake[1], D. Atkinson[2] and A.C. Edwards[3]
[1]Rubber Research Institute, Agalawatta, Sri Lanka.
[2] The Scottish Agricultural College, Edinburgh, EH-9 3JG, U.K.
[3] Macaulay Land Use Research Institute, Aberdeen, AB9 2 QJ, U.K.

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ABSTRACT

A ^{32}P root uptake bioassay method was applied both to sand culture and soil culture grown seedlings to test the potential value of this method for determining the P availability and uptake for some selected crop species. The phosphorus uptake from the bioassay test solution was largely governed by the availability of P in the rooting media and P status of plants. The inverse relationship between P uptake during the bioassay and soil P status means that the method is particularly suited to natural situations with low P conditions. The results of the bioassay appear to provide integrated assessments of the demand for P and the P supply in the rooting environment. The method may be useful to diagnose deficiency of P in tree crops where remedial methods can alleviate the deficiency and increase the yield.

Key Words: ^{32}P, root bioassay, P uptake, P demand, P supply

INTRODUCTION

A range of soil testing methods have been developed and used over the years, in order to determine the nutrient status of soils and assist in making recommendations for fertilier use. But, they have shown varying degrees of success (Sibbessen 1983). In most cases chemical extractants have been used to determine the soil P status. Sometimes, they over estimate the available P in the soil (Olsen and Khasawneh 1980). Even at best, a P - test only provides a relative estimate of the soil P status and consider it as the ability of a soil to release P to the root system of the crop. Analytical values obtained from a particular soil test usually have only a limited value. Their interpretation relates to the conditions under which the extraction was carried out. Also, they have to be evaluated in terms of known crop demand (Sibbessen 1983). In addition, the laboratory values of different P methods could not be expected to reflect variable operations of the numerous factors (environmental, plant physiological as well as soil chemical, physical and biological) which affect phosphate supply and plant growth in the field (Reith *et al.* 1987).

The plant itself may be the best indicator of it's own nutritional well being and, indirectly a good indicator of soil fertility. Therefore, several workers (Martens *et al.* 1969; Welch *et al.* 1957; Zubriski 1971; Cajuste and Kussow 1974) explored the possibility of using plants directly to evaluate soil P status and make fertilizer recommendations. However, to evaluate the P status of the soil via plant analysis, a high positive correlation between plant parameters and some measurement of soil P must exist (Kamprath and Watson 1980). The uptake and translocation of P, plant growth rate and the concentrations of P in the external soil solution are inter-related to the P concentration in the plant tissue. However, in cases where the plant growth rate becomes either limited, or rapidly increases, then these processes are probably operating in a non parallel manner. In such instances, the concentrations of P in the plant will not correlate highly with soil P concentration. Under this situation, plant analysis will not provide reliable information on soil fertility level.

A P deficiency bioassay has been developed based on the rate of metabolic uptake of ^{32}P labelled phosphorus by plant roots from a standardized phosphorus solution containing ^{32}P (Bowen 1970; Harrison and Helliwell 1979). Using this bioassay it has been demonstrated that there is a negative relationship between the rate of uptake of ^{32}P labelled phosphorus and the phosphorus status of a plant. A series of experiments were carried out by using

Abbreviations:* AER - P: Anion Exchange Resin Extractable P, * (AER + CER) - P: Anion and Cation Exchange Resin Extractable P.

annual and perennial crops with the aim of application and modifications for the technique to assess the phosphate requirements of some selected plants.

MATERIALS AND METHODS

Seedlings from different crop species to cover monocotyledons (Maize- *Zea maize*), dicotyledons (sunflower - *Helianthus annus*) and slow growing perennials (birch - *Betula pendular*; rubber - *Hevea brasiliensis*) grown in (a) sand culture (b) P deficient soils either fertilized or unfertilized with phosphatic fertilizers were used.

Sand culture of seedlings

Seedlings (one per pot) of different crops (birch, rubber, sunflower and maize) were grown for 3-4 months in a glass house in pots filled with acid washed phosphate free dry sand (2300 g/pot). Hewitt's (1952) solution with varying amounts of phosphorus (1,2,5,8,10,12,15,25,50 and 100 µg P ml^{-1}) added as $NaH_2PO_4.2H_2O$ was supplied every 2 days. 25 ml of treatment solution was added per plant.

Soil culture of seedlings

Birch seedlings were grown for 8 months in pots (one per pot) filled with 2.8 kg of air-dried, sieved (<6mm) Scottish soil namely Glentanner which was either fertilized or unfertilized with rock phosphate fertilizers. N, K and Mg were added separately as NH_4NO_3, KCl and $MgSO_4$ at the rate of 50, 128.78 and 19.32 mg/kg soil respectively. Each treatment was replicated twice and pots were kept in the glass house according to the randomized block design. Plants were removed from soil after 8 months and roots were used in the bioassay procedure.

Soil P determination

Soil samples were analyzed for anion exchange resin extractable P (AER-P), anion and cation exchange resin extractable P (AER+CER)-P(Somasiri and Edwards 1992), $CaCl_2$ extractable P (Larsen 1965; Munns and Fox 1976) and acetic acid extractable P (MISR/SAC 1985). Phosphorus contents of the soil extractant were determined colorimetrically (Murphy and Riley 1962).

Phosphorus bioassay

The roots were processed according to the procedure detailed by Harrison and Helliwell (1979) and Harrison *et al.* (1984). After removal of seedlings from the rooting media, roots were washed carefully and placed in a 5 x 10^{-4} M $CaSO_4$ solution for 30 minutes to maintain cell membrane integrity and leach out physically sorbed P in the root-free space. Roots were then transferred to a solution containing the same concentration of $CaSO_4$, 5 x 10^{-6}M KH_2PO_4 and about 0.74 MBq (20 µCi) ^{32}P as orthophosphate $lit.^{-1}$ at 25°C for 15 minutes. One millilitre sample of this initial solution was added to 14 ml of distilled water in the counting vials and counted by Cerenkov radiation in an automatic Packard Tricarb 2425 liquid scintillation spectrometer, prior to the bioassay. When the seedlings were removed from the solution, roots were washed to remove unabsorbed ^{32}P from the root surfaces, and between 10-200 mg samples fresh weight (four per plant) were cut from the terminal ends of lateral roots and placed in counting vials with 15 ml distilled water. ^{32}P in the roots was counted under the same conditions as above. Each root sample was then removed from its vial, blotted and weighed, and the residual ^{32}P recounted under identical conditions. This second count was of ^{32}P which was not metabolically absorbed by the root and which diffused from the root surface into the water of the vial and this was subtracted. The ^{32}P counts (cpm) were corrected for background, decay and percentage counting efficiency. Data were standardized by converting the estimated ^{32}P activities in roots to quantities of phosphorus taken up from 5 x 10^{-6} M phosphate solution, using the following equation based on the initial P and ^{32}P ratio of the bioassay solution and uptake of P and ^{32}P during the bioassay procedure:

$$Y_2 = A\,(C/B)$$

Where,

Y_2 = P uptake by roots (pg P mg $root^{-1}$ 15 min^{-1})
A = 155,000 pg P
B = initial ^{32}P activity (dpm ml^{-1} of assay solution)
C = ^{32}P activity (dpm mg $root^{-1}$)

RESULTS

P uptake and P in the rooting environment

P uptake from the bioassay procedure was largely

Table 1. Relationship of P uptake by roots of tested crop species with P concentration in the rooting media

Crop species	Nature of relationship	r
Birch (Sand culture)	$Y=641.7x^{-0.446}$	-0.892***
Sunflower (Sand culture)	$Y=1079.3x^{-0.397}$	-0.787***
Maize (Sand culture)	$Y=1181.2x^{-0.319}$	-0.946***
Rubber (Sand culture)	$Y=1049x^{-0.347}$	-0.951***

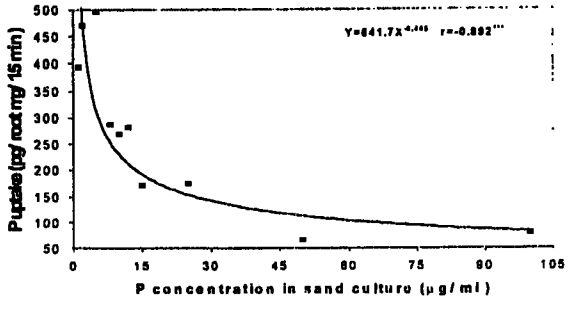

Fig.1. The relationship between the uptake of ^{32}P by roots and the phosphorus concentration supplied to seedlings

governed by the availability of P in the rooting environment (Table 1). A negative exponential relationship was observed for all the crop species (Fig.1). P uptake was high in the plants which grew in the low phosphate level compared to those that grew with high concentration of P. Phosphorus uptake from the bioassay solution declined drastically for plants which received high amounts of phosphate during their growth.

Table 2. Relationship of P uptake by roots of tested crop species with plant P content.

Crop species	Nature of Relationship	r
Birch (Sand culture)	$Y=107.0x^{-1.179}$	-0.924***
Sunflower (Sand culture)	$Y=124.9x^{-1.103}$	-0.808***
Maize (Sand culture)	$Y=7807x^{-1.670}$	-0.886***
Rubber (Sand culture)	$Y=6911x^{-1.955}$	-0.936***
Birch (Soils)	$Y=127.1x^{-0.849}$	-0.770***

*** significant at P<0.001

P uptake and plant P

The relationships of P uptake in the bioassay tests with plant P contents of different crop species are shown in Table 2. A negative exponential relationship was observed for all the crop species (Fig.2)

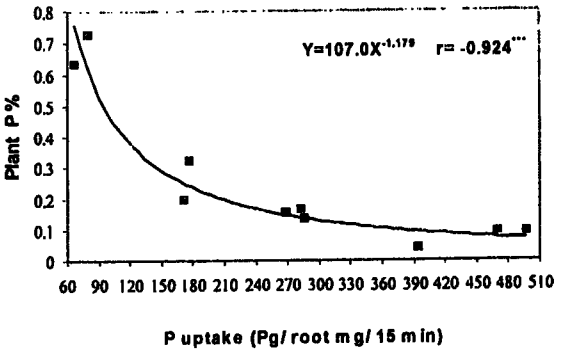

Fig. 2. Relationship between the ^{32}P - labelled P uptake by roots and P content of plants

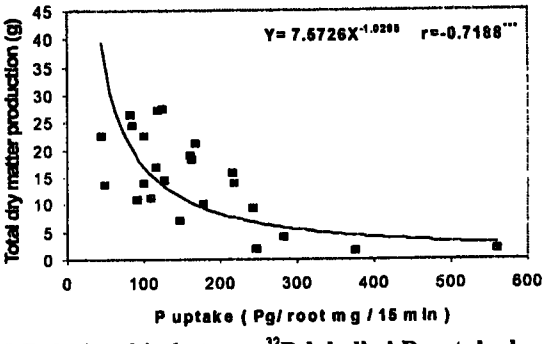

Fig. 3.Relationship between ^{32}P-labelled P uptake by roots and total dry matter production of plants

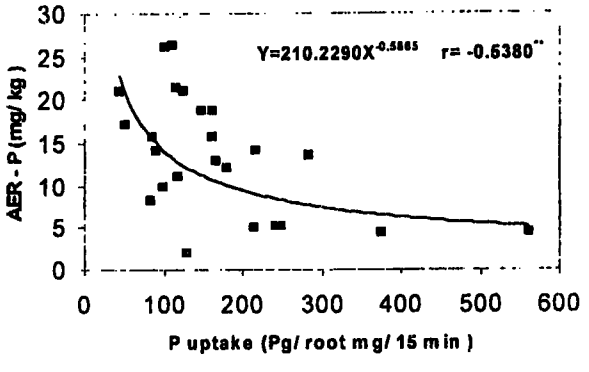

Fig. 4. Relationship between ^{32}P-labelled P uptake by plant roots and AER-P in soil

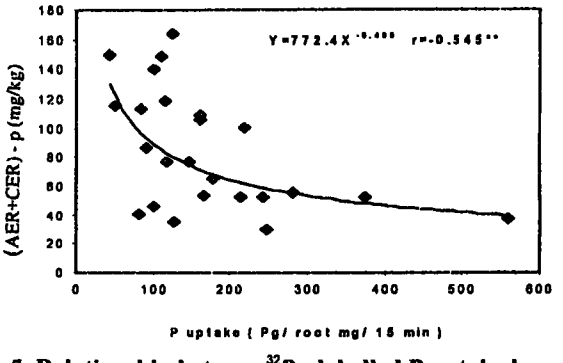

Fig. 5. Relationship between ^{32}P - labelled P uptake by plant roots and (AER+CER) - P in soil

Where higher rates of P uptake occur with low p status plants than with those grown under higher P levels.

P uptake and plant productivity

The rates of ^{32}P labelled P uptake form the bioassay test showed an inverse relationship with the plant yield (Fig. 3).

P uptake and soil P assessing methodologies

The uptake of ^{32}P by birch plants from the bioassay solution was negatively related to the soil fertility level measured as anion-resin extractable P ($r = -0.538^{**}$) and mixed resin extractable P ($r=-0.545^{**}$). The rate of P uptake declined as the soil fertility level increased (Fig.4 and 5). However, such significant relationships were not found with acetic acid extractable P($r=-0.256$) and $CaCl_2$ extractable P ($r = -0.211$).

DISCUSSION

P uptake in the bioassay procedure was largely governed by the availability of phosphorus in the rooting media which is in agreement with findings of Harrison and Helliwell (1979), Harrison *et al.* (1986 a,b and 1991). The relationship was sound and the method was able to estimate phosphorous availability in the rooting media for all the plant species. Experimental evidence indicates that the bioassay method seems to be applicable to plants irrespective of their species as it provides information on P in both plant and rooting environment. Also, it shows that the method is applicable to tested range of plant species. This indicates that the technique is likely to be a physiological reaction common to most of plants irrespective of the species. As this bioassay procedure provides information on both plant and soil P status, it could be considered that the method is useful to predict the future performances of the plant.

Although all the tested plant species behaved in a similar way in response to ^{32}P uptake in the bioassay test, their uptake rates were different. This may be due to the variability of the demand for P in different species as reported by Harrison and Helliwell (1979). This indicated that the method is closely associated with both P supply of the rooting media and the plant demand for phosphorus. On the other hand, the relationship of P uptake with plant P concentration indicated that the method provides information on plant P status and it is highly sensitive to changes in plant P status.

The relationship observed between plant P and P uptake in the bioassay indicated that when plants are deficient in P, their uptake rates are higher. At this stage, P concentration of plants were varied among species and this indicated that the P stress condition is species related. Generally, at the P deficient level, the P concentration of the plant is 0.3% for birch; 0.1% for rubber; 0.2% for sunflower and 0.25% for maize. This shows that the bioassay method provides information on plant P deficiency and therefore could be used in correcting P deficiency as proposed previously (Bowen 1970; Harrison & Helliwell 1979).

The inverse relationship between P uptake during the bioassay and soil P status means that the method is particularly suited to natural situations with low phosphorous conditions which are difficult to assess using more conventional soil P tests, as indicated previously by Harrison and Helliwell (1979). In application of the method for plants grown in soil, it showed that the uptake from the bioassay solution was negatively related to both AER-P and (AER+CER) - P, but not with other conventional soil analytical methods. Sibbesen (1983) indicated that the resin method is the most suitable test for P and Smith (1979) concluded that AER method imitates the depleting action of plant roots by removing readily available P from soil solution. The high correlation with the resin methods further support the suitability of the bioassay procedure in assessing the soil P status. In the present study, bioassay of P uptake was not correlated with the acid extractions for P and this was in agreement with the findings of Sibbesen (1983), who classified all the acid extractions as the most unsuitable methods in soil phosphate determination due to poor relationship with plant P uptake.

Although, conventional soil P test values were not significantly related to the total dry matter production of plants, P uptake from the bioassay was negatively related to the plant productivity. This suggests that the plant productivity is a function of plant P content and it is largely determined by the phosphate availability in the soil. This was in agreement with the findings of Harrison *et al.* (1986b). The resin extractable soil P was linearly related to plant productivity. This illustrates the suitability of the bioassay technique to predict the potential growth response of trees in relation to fertilizer application.

A major drawback in the bioassay method is that it only provides the information on nutrient deficiencies after the plant is affected. However, a

soil test indicates broad changes in soil P fertility and therefore allows rapid remedial action to be carried out. For this reason, the applicability of the bioassay technique to short term crops may not be useful where the lost yield due to deficiency is never regained. In contrast, for long term crops like birch and rubber, the technique may be suitable because there is enough time to correct the diagnosed deficiency before the yield is severely affected.

Results of this study show that generally the root bioassay method could be considered as a technique which relates soil and plant P through a physiological uptake mechanism, including the growth of pot, grown seedlings and it has a potential for use in field. However, its applicability has to be evaluated in controlled experiments by considering the factors which could affect the sensitivity and accuracy of the method. Among these factors, the effects of mycorrhizal association of plant roots may be important in determining phosphate uptake rates.

In addition to growth rate of trees, the effect of other elements on the P status, the age of the tree, the age of the root, response time to fertilizer application and method of collection of root samples could be considered to influence the accuracy of the method and subsequent interpretation of P uptake data. The reproducibility of the results have to be tested, especially in the field situation before it is used for any advisory purposes. Therefore considering these limitations, future studies should focus on improving the technique as a field tool in measuring the P availability.

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