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

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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

The plant itself may be the best indicator of it's own nutritional well being and, indirectly a good

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 5×10^{-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 $CaSO_4$, 5×10^{-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 5×10^{-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***

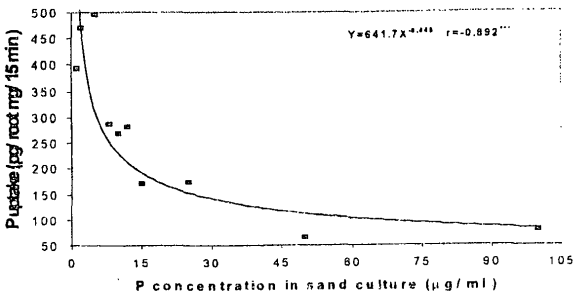

Fig.1. The relationship between the uptake of ^{32}P by roots and the phosphorus concentration supplied to seedlings

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)

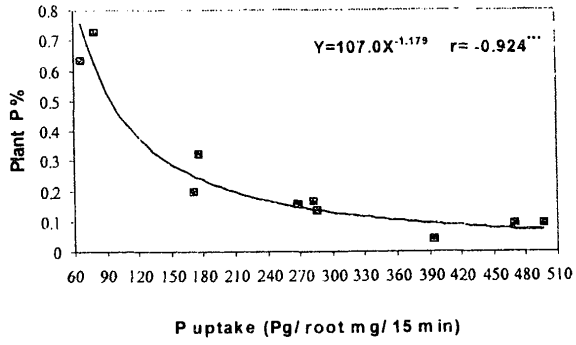

Fig. 2. Relationship between the ^{32}P - labelled P uptake by roots and P content of plants

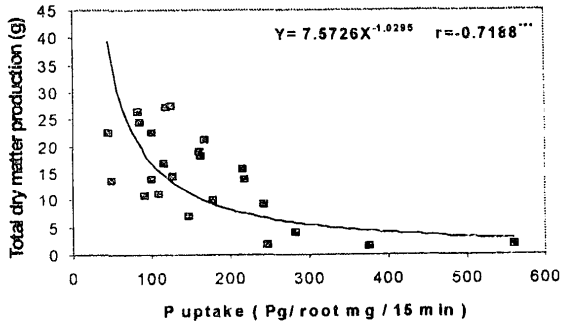

Fig. 3.Relationship between ^{32}P-labelled P uptake by roots and total dry matter production of plants

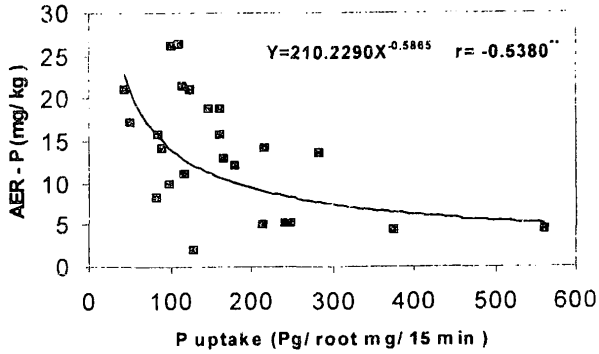

Fig. 4. Relationship between ^{32}P-labelled P uptake by plant roots and AER-P in soil

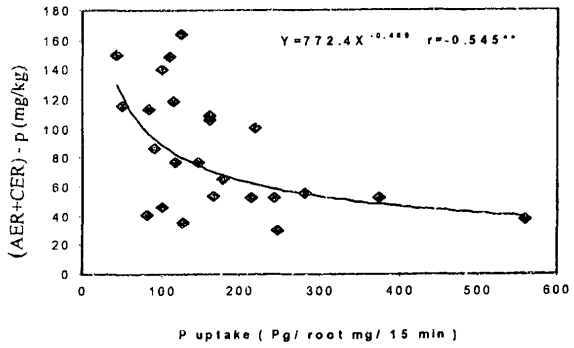

Fig. 5. Relationship between ^{32}P - labelled P uptake by plant roots and (AER+CER) - P in soil

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.

REFERENCES

Bowen GD 1970 Early detection of phosphate deficiency in plants. Soil Sci. Plant Anal. 1 (5): 293-298.

Cajuste LJ and Kussow WR 1974 Use and limitations of the North Carolina method to predict available phosphorus in some oxisols. Trop. Agric. (Trinidad) 51:246-252.

Harisson AF and Helliwell DR 1979 A bioassay for comparing phosphorus availability in soils. J. Appl.Ecol. 16: 497-505.

Harisson AF, Dighton J and Smith MR 1984 The phosphorus deficiency bioassay: Sampling and data handling procedures. Merlewood Research and Development Paper No.103. Institute of Terrestrial Ecology, Grange - over- sands.

Harisson AF, Hatton JC and Taylor K 1986b Application of a root bioassay for determination of P-deficiency in high altitude grasslands. J. Sci. Food Agric. 37: 10-11.

Harisson AF, Dighton J, Hatton JC and Smith MR 1986a A phosphorus deficiency bioassay for trees and grasses growing in low nutrient status soils. Proc. VIth Int Coll, Optimization of Plant Nutrition, Vol III (Ed. M. Prevel) pp.957-963 AIONP/ GERDAT, Montpelier, France.

Hewitt EJ 1952 Sand and Water Culture Methods Used in the Study of Plant Nutrition. Technical communication No.22. Commonwealth Agricultural Bureau, UK.

Kamprath EJ and Watson ME 1980 Conventional soil and tissue test for assessing the phosphorous status of soils. In: F.E. Khasawneh, E.C. Sample and E.J. Kamprath (Eds.) The Role of Phosphorus in Agriculture. 16: 433-469.

Larsen S 1965 The influence of calcium chloride concentration on the determination of lime and phosphate potentials of soils. Soil Sci. Soc. Am. J. 16:No 2.

Martens DC, Lutz JA and Jones GD 1969 Forms and availability of phosphorus in selected virginia soil as related to available phosphorus tests. Agron. J. 61:616-621.

MISR/SAC 1985 Macaulay Institute for Soil Research and Scottish Agricultural Colleges - Advisory Soil and Interpretation Bulletin 1 - Aberdeen, Scotland,U.K.

Munns DN and Fox RL 1976 The slow reaction which continues after phosphate adsorption: Kinetics and equilibrium in some tropical soils. Soil Sci. Soc. Am .J. 40 :640-646.

Murphy J and Riley JP 1962 A modified single solution method for the determination of phosphates in natural waters. Analytica Chimica Acta .27: 31-36.

Olsen SR and Khasawneh FE 1980 Use and limitations of physical chemical criteria for assessing the status of phosphorus in soils. In: F.E. Khasawneh, E.C. Sample and E.J. Kamprath (Eds.) The Role of phosphorus in Agriculture, Am. Soc. Agron. Maidson, Wisconsin, U.S.A., 361 - 140.

Reith JWS, Inkson RHE, Scott NM, Caldwell KS, Ross JAM and Simpson WE 1987 Estimates of soil phosphorus for different soil series. Fertilizer Research 11: 123-142.

Sibbesen E 1983 Phosphate soil tests and their suitability to assess the phosphate status of soil. J. Sci.Food Agric. 34:1368-1374.

Somasiri LLW and Edwards AC 1992 An ion exchange resin method for nutrient extraction of agricultural advisory soil

samples. Comm. Soil Sci Plant Anal. 23 (7 & 8): 645-657.

Welch LF, Ensminger LE and Wilson CM 1957 The correlation of soil phosphorus with the yield of Ladino clover. Soil. Sci. Soc. Am. Proc. 21:618-620.

Zubriski JC 1971 Relationships between forms of soil phosphorus, some indexes of phosphorus availability and growth of sudan grass in greenhouse trials. Agron. J. 63: 421-425