Root surface area measurements based on adsorption and desorption of nitrite

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Abstract

A method, based on the negative adsorption of NO$_2^-$, has been developed to determine surface area of roots. Young roots of 3–4 year old plants of Acacia catechu, Eucalyptus camaldulensis and Leucaena leucocephala were up-rooted and cut into 18 cylindrical pieces. Each root piece was immersed individually for 10 sec in 0.05 M, 0.10 M and 0.15 M aqueous NaNO$_2$ solution and the excess solution on the root surface allowed to drain off. It was then transferred to conical flask containing distilled water and shaken for 15 min for desorption of nitrite. A known quantity of this aliquot was reacted with 1% acidic sulphanilamide and 0.02% NED HCl. A pink colour developed, and its optical density was read at 540 nm. A positive linear correlation was noted between colour density and root surface area. The respective correlation coefficient values for 0.05 M, 0.10 M and 0.15 M NaNO$_2$ solutions were 0.973, 0.963 and 0.964 for Acacia catechu, 0.933, 0.903 and 0.898 for Eucalyptus camaldulensis and 0.968, 0.976 and 0.972 for Leucaena leucocephala (significant at $p < 0.001$). The method was successfully adopted to determine the root surface area of seedlings of Albizia lebbek, A. procera, Acacia auriculiformis, A. nilotica, Dalbergia latifolia and D. sissoo.

Introduction

Surface area of plant root system is an important parameter for most models developed for absorption of water and nutrients (Bouldin, 1961; Nye, 1973; Passioura, 1963; Silberbush and Barber, 1983). However, there is no direct method for measuring surface area of an irregular object such as plant root system. The indirect methods include surface adsorption of acid (Wilde and Voigt, 1949) or basic organic dye (Donham 1958), gravimetric loss of concentrated salt solution (Carley and Watson, 1966) and photoelectric measurement (Morrison and Armson, 1968).

Such methods have not found wide application, however, and because of lack of calibration results, provide only relative data about root surface area (Bohm, 1979). The surface adsorption of acid or basic organic dye depends upon pH and cation exchange capacity of roots which varies from species to species. For instance, calibration lines obtained with safranin adsorption/desorption method are found to be non-linear and regression parameters depend on crop species as well as root age (L K Wiersum, pers. commun.). Moreover, the basic organic dye at the high pH ionizes cellulosic structural component and leads to its fixation on roots (Carman, 1982). High salt concentration (5.77 M) used in gravimetric method, roping together of fine roots (Pearson, 1974), and photoelectric measurement, excluding translucent fine roots (Bohm, 1979), provide spurious data about the surface area.

The present paper, however, describes a new method that is calibrated with standard reference roots. It is based on the principle of negative adsorption (Schofield, 1947). The method utilizes very dilute solution of nitrite which spreads as an aqueous thin layer on root surface. Later, this nitrite is desorbed in water and estimated colorimetrically, employing colour reaction of nitrite with diazo-coupling reagents, sulphanilamide and N-(1-naphthyl)-ethylene dihydrochloride (Wray and Fido, 1990).
Materials and methods

Young roots of 3–4 year old plants of *Acacia catechu* (L.f.) Willd., *Eucalyptus camaldulensis* Dehn. and *Leucaena leucocephala* (Lam.) de Wit were dug out, washed thoroughly with distilled water and cut randomly into 18 cylindrical pieces. The length and diameter of each piece of the reference roots were measured at several points by Vernier calipers. Average length (*l*) and diameter (*d*) was worked out to compute surface area of each piece with the help of the following formula:

\[ \text{Root surface area} = \pi \ d \left( \frac{d}{2} + 1 \right) \]

After having been immersed individually in 0.05 M aqueous NaNO₂ solution for 10 sec, each root piece was held vertically for 5 min in the air by clipping on a string, allowing the excess solution on its surface to drain off. It was then transferred to an Erlenmeyer flask containing 200 mL distilled water and shaken for 15 min on an electric shaker. A 0.4 mL aliquot of this solution containing desorbed nitrite was taken in a test tube and 0.3 mL each of 1% (w/v) sulphanilamide (made in 3 M HCl) and 0.02% (w/v) N-(1-naphthyl)-ethylene dihydrochloride (NED HCl) added. A pink colour developed. It was diluted to 5 mL with distilled water and kept for 20 min for maximum colour development. The optical density (OD) of the colour was read at 540 nm on a UV-vis spectrophotometer (Systronic, India).

The procedure was repeated, taking 0.10 M and 0.15 M NaNO₂ solution for immersing the root pieces and 400 mL distilled water for desorption of nitrite. The pH values for 0.05 M, 0.10 M and 0.15 M NaNO₂ were noted to be 7.41, 7.51 and 7.99 respectively.

For a comparison, pieces of *Leucaena leucocephala* root were subjected to known methods of titration, estimating surface desorbed dilute acid (Wilde and Voigt, 1949) and gravimetry, quantifying surface adhered concentrated (5.77 M) calcium nitrate solution (Carley and Watson, 1966). Two runs of each method were made.

Statistical analysis

Simple correlation coefficients and regression equations for straight lines were calculated to establish relationship between computed surface area and desorbed nitrite. The significance of correlation coefficients was tested at \( p < 0.001 \). The regression equations obtained for the reference roots at 0.05 M, 0.10 M and 0.15 M NaNO₂ were employed to calculate the total surface area of the root system of three month old seedlings of six species, namely *Acacia auriculiformis*, *A. nilotica*, *Albizia lebbek*, *A. procera*, *Dalbergia latifolia*, and *D. sissoo*, taking 10 samples of each. The mean values of root surface area of each tree seedling were compared among themselves by employing covariance and Duncan's multiple range significance test (Gomez and Gomez, 1984).

Results

The concentrations of nitrite desorbed from the surface of the root pieces of the reference plants (*Acacia catechu*, *Eucalyptus camaldulensis* and *Leucaena leucocephala*) exhibited very strong positive correlation \( (p < 0.001) \) with their surface area (Fig. 1). The regression equations obtained for the three reference roots at 0.05 M, 0.10 M and 0.15 M aqueous sodium nitrite solution, when employed to compute total root surface area of six 3 month old seedlings of six species.