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A Rapid Method of Estimating the Surface Area of Root System

M.A.M. Chowdhury, and P.E. Weatherley

Dept. of Botany, University of Chittagong,
Aberdeen, AB9 2UD, Scotland, U.K.

ABSTRACT: The surface area of a root system can be estimated from a calibration curve relating the quantity of an acid or stain absorbed on the surface area measured directly on graph paper or with stereo-microscope. The results indicated a linear relationship between the absorbed volume of a IN Hydrochloric acid + 0.01% light green stain and the known surface area of the root sample. The amount of total root length and volume in the sample can also be calculated from the calibration curve.

INTRODUCTION

A quantitative estimation of the amount of root length, volume and its surface area per unit volume of rooted soil and per plant are required in the studies of water and mineral nutrients uptake in various crop plants. Such an estimation of the above mentioned parameters have been well demanded (Gardner, 1960, 1964; Cowan, 1965; Brewester and Tinker, 1971; Herkelrath *et al.*, 1977; Faiz and Weatherley, 1978 and Chowdhury, 1979). Schuurman and Goedewaagen (1970) have discussed elaborately the technique for separating the root system from the soil, and many research workers have developed various methods such as line intersection method (Newman, 1966) radioactive tracer technique (Russel and Ellis, 1968; Baldwin and Tinker, 1970 and Baldwin *et al.*, 1971), resin impregnated block method (Melhuish and Lang, 1968), map measuring wheel method, direct measurement and root instrument method (Rowse and Phillip, 1974) to account the length, volume and surface area of root sample. Several workers (Reicosky *et al.*, 1970; Brewester and Tinker, 1971, and Rowse and Phillip, 1974) have used and compared the results obtained from the line interaction method, direct measurement and root instrument method, and concluded (Recosky *et al.*, 1970) that there was little difference between the co-efficient of variation of each method. The root instrument method was found with more accuracy in direct measurement (Rowse and Phillip, 1974).

In essence, no method is more accurate than the direct measurement. Since the direct measurement is tedious and time consuming, a simple and fast method is more important than that of an accurate one. In such a situation a direct measurement has not been found suitable for fibrous and dense root systems as well. As the root instrument is expensive and there was no such instrument available during this study, attempts were made to introduce a simple, inexpensive but more accurate and less time consuming method than other existing methods. This paper presents a stain and acid adsorption for the estimation of length, volume and surface area of a root system.

MATERIALS AND METHODS

The root system of sunflower (*Helianthus annus*, cv. Tall) plant, grown in sandy loam and water culture (Chowdhury, 1979), and 4 – 7 weeks old were used for the estimation of total root length, volume and surface area of a root system in the present investigation.

Preparation of a root sample for measurement

Root system of a soil grown potted plant was divided vertically into 8 equal sections at right angles through the centre of pots. The vertical sections of the rooted soil column was chosen at random to be used for the estimation of surface area. Each of these sections was placed on a glass sheet horizontally and divided into 4 equal sections – root samples. These root samples were separated carefully from soil particles under gentle spray of water from a wash bottle. The samples were then transferred to large petri dishes and washed repeatedly until free of any dead or debries. The roots of each sample were then grouped according to their diameter into 4 classes – sub samples, and arranged in order of diameter and length into straight lines on petri dishes containing distilled water approximately at 0.25, 0.5, 0.75 and 1.0 mm of depth corresponding to 0.3, 0.6, 1.2 and 1.6 mm diameter of roots.

Direct measurement

The total length of roots in each sub-sample was measured against a graph paper placed below the petri dishes. The roots were focussed from lower side during measurement. The average diameter of all roots in each sub-sample was measured both by stereo-microscope and mm graph paper. The total length and average diameter of roots in each sub-sample was used to calculate the total surface area and volume of each root sample by using formulae $-\pi r^2 + 2\pi rh$ and $\pi r^2 h$ respectively. The total length and surface area of each root sample was calculated individually and plotted on X and Y axis respectively (Figure 1). The same procedure was also followed for water culture grown plant's system.

Stain adsorption method

Each root sample, used to estimate the total length and surface area by direct measurement, was drained to remove excess water and put on a piece of wet foam placed at the bottom of centrifuge tubes. The root sample was then centrifuged for two minutes at 300 rpm and then transferred to 100 ml of 0.01 per cent light green stain contained in a conical flask. Each root sample was shaken separately on a mechanical shaker for 3 minutes, and then drained. The drained root samples were centrifuged for 2 minutes at 300 rpm and transferred to the respective cleaned conical flask containing 100 ml glass distilled water and shaken well on mechanical shaker for 3 minutes, and allowed to reach equilibrium by 2 minutes. The root samples were then transferred to glass distilled water. A 10 ml aliquot of bathed solution was used to measure the absorbance with a colorimeter by using a filter (623 EEL) at 495 nm wave length and 44 percent transmission. The absorbance by definition is $A = \log_{10}. 1/1_0$, where, A = absorbance; 1_0 = intensity of a monochromatic beam transmitted through pure solvent and 1 = intensity of a monochromatic beam transmitted through the solution. The known total length and surface area of each sample, treated with stain, was used to estimate the equivalent of 0.01 percent stain adsorbed by the same amount of roots. The equivalent stain was plotted against the surface area of each sample, treated with stain, was used to estimate the equivalent of 0.01 percent stain adsorbed by the same amount of roots. The equivalent stain was plotted against the surface area of roots for a wide range of values (Figure 3).

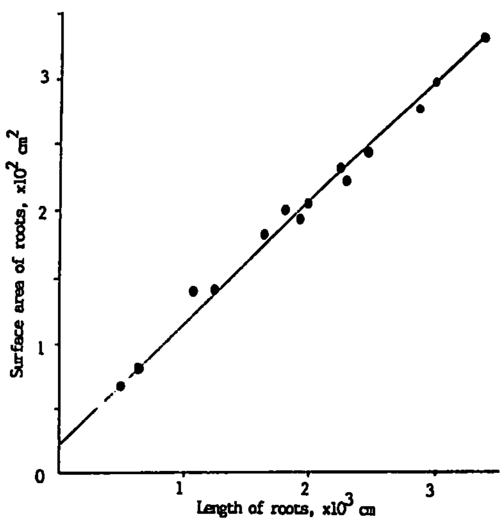

Fig. 1. Relationship between measured length and surface area of roots.

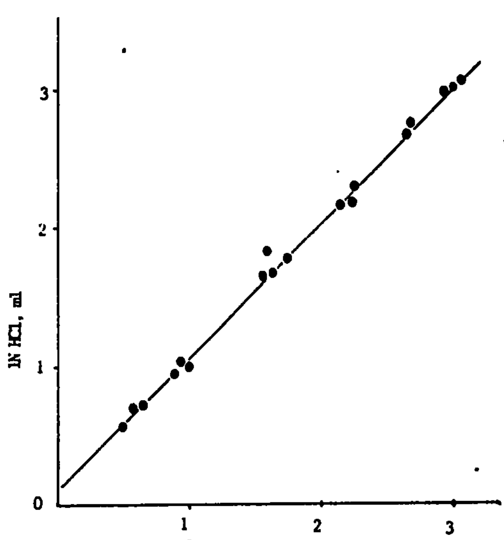

Fig. 2. Relationship between 1N HCl and 0.01 percent light green stain.

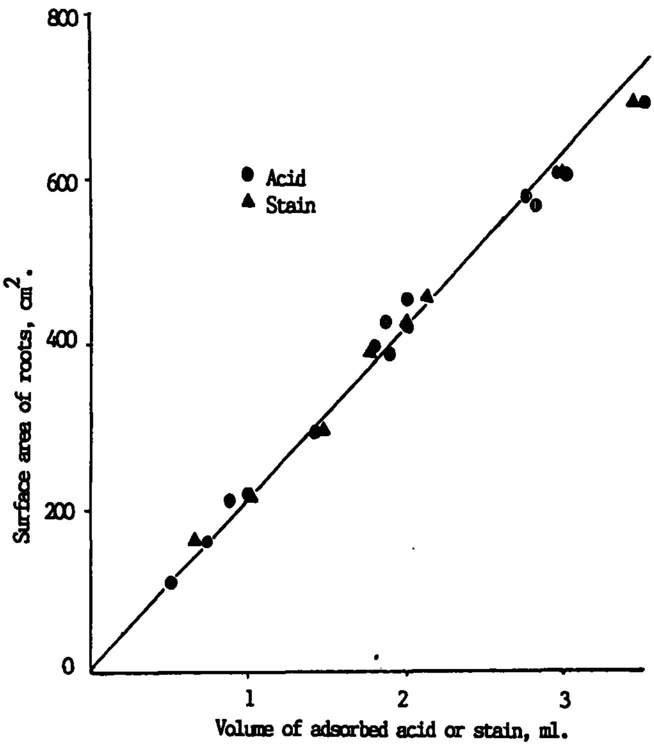

Fig. 3. Relationship between adsorbed acid or stain and measured surface area of roots.

Acid adsorption method

Root surface area was estimated by acid adsorption method (Wilde and Voigt, 1949, and Baxter and West, 1977). In the present study the same procedure was followed but with slight modification. This modified method was similar to that of stain adsorption method except that the root sample was treated with 1N HCL for 15 seconds. Other procedures and precautions were the same as in stain adsorption method. A 10 ml aliquot of the bathed solution was titrated with N/100 NaOH solution to determine acid equivalent. The volume of adsorbed 1N HCL by each root sample was calculated from the equivalent N/100 NaOH solution. The length and corresponding surface area of each root sample treated with 1N HCL was used for the estimation of equivalent adsorbed acid. The equivalent acid values were plotted against the respective surface area of roots (Figure 3).

RESULTS AND DISCUSSION

Figure 1 shows that there was a linear relationship between the measured length and corresponding surface area of roots for a wide range of values with Y axis intercept. A similar relationship was also obtained between 1N HCl and 0.01 per cent light green stain (Figure 2), while a plot of the relationship between known root surface area and its equivalent stain or acid showed a linear relationship but without Y axis intercept (Figure 3).

The measured surface area of known root samples and their length equivalent were compared and it is evident that 1000 cms of root produced about 125 cm^2 of root surface area and vice versa. It is revealed from Figure 2 that 1 ml of 1N HCL was equivalent to 1.09 ml of 0.01 per cent light green stain. The same values for both acid and stain was obtained in Figure 3, and it was estimated that 1 ml stain or 0.9 ml acid equivalent to about 264 cm^2, 2100 cm and 26.4 cm^3 was correspondent to surface area, length and volume of roots respectively.

The effect of stain and acid on roots was examined by treating the same root sample successively for 4 – 5 times with 0.01 per cent light green stain and 1N HCl. In each treatment the washing out of both stain and acid were tested by colorimeter and acid titration method respectively. It was invariably found that the same root sample in each treatment adsorbed about the same but with a difference of $\pm$ 3 and $\pm$ 5 percent change in values both for stain and acid respectively. Microscopic examination of both acid and the stain treated roots just after washings revealed that there was no change of root's configuration in stain but became slightly soft in acid. It was also observed that the roots did not adsorb the stain permanently even when treated for 3 minutes, since the adsorbed stain was found to disappear from the root surface as soon as dipped into known amount of distilled water. The action of 1N HCL on roots even for 15 seconds was observed to cause softening effect. Hence, the respective changes both in adsorbed stain and acid by $\pm$ 3 and $\pm$ 5 percent could be attributed to both interfibrillar and systematic errors. It is, however, clear that as long as the same procedure was used in all estimation the values of stain and acid were found to be fairly satisfactory, reproducible and comparable.

It was observed that the same roots in the sample did not take uniform staining along the length and most probably the same with acid, which is thought to be due to the diameter and age of each root sample. But the randomized sampling technique minimized the errors within the samples. Once the root surface area of the samples was calibrated these factors did not seem to affect the method. The calibration curve obtained between the measured root surface area and its equivalent stain acid were verified and found suitable to be used as standard curve during the measurement of sunflower root system. So the present method claims as an inexpensive, simple, fast and accurate one over the existing ones. This sort of attempt is new and very little work is found in literature on root surface area measurement. Further works are in progress to confirm whether these methods can hold good to be use as a standard one for the estimation of total length, surface area and volume of roots for other field grown, specially crop plants.

Precautions

1. Roots of heterogeneous sizes (*i.e.* assorted diameter and length) were used in each sample;

2. Samples of different sizes for the direct measurement of root length and surface area were used;

3. Fixed time factor was maintained for each treatment in all estimation.

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