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Relationship of fluorescent intensity to ion uptake and elongation rates of soybean roots

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Summary The relationship between the fluorescent intensity of individual soybean {*Glycine max* (L.) Merr.} roots or root segments and their nutrient absorption capacity or elongation rate was investigated. Data are reported for the short-term (30 sec) absorption of rubidium, phosphate, and iron by roots of soybean plants which had been cultured in a soil solution system. Results indicate that the rates of absorption of Fe, was directly related to the fluorescent intensity, with the most rapid absorption per unit of root length or surface area occuring in regions of most intense fluorescence. Elongation rate was positively correlated to the intensity of root fluorescence. These relationships should be useful for estimating root elongation rates in soil systems and for evaluating the distribution of growth rates and nutrient absorption activities within a crop root system.

Introduction

The fluorescent nature of plant roots has occasionally been considered in relation to iron nutrition, root exudation, and allelopathy. The roots of nearly all plant species were reported to fluoresce to some extent when illuminated with long-wave (365 nm) ultraviolet light (black light), and the color and intensity of root fluorescence has been tabulated for 135 species[13]. Scopoletin was identified as the major fluorescent constituent of oat (*Avena sativum* L.) roots[14]. Root fluorescence and the exudation of scopoletin have been used to evaluate the allelopathic potential of different oat lines[11].

Several investigations have dealt directly with the fluorescence of soybean roots, including studies of the kinetics of soybean root exudation by assessing the exudation of an unidentified fluorescent substance into water[20,21]. By evaluating the progeny from crosses of the non-fluorescent cultivar 'Minsoy' with the fluorescent cultivars 'Blackhawk' and 'Hawkeye', it was determined that root fluorescence in soybeans is controlled by a single gene with two alleles, with fluorescence dominant to non-fluorescence[12]. Several reports relating to iron absorption by soybean roots have implicated fluorescent phenolic reduction of the ferric iron prior to uptake[1,4,6].

We have observed that the fluorescence was most intense near the growing tip of soybean roots, with the older root segments being weakly fluorescent or non-fluorescent. This observation indicated that root fluorescence may be useful

as an indicator of the relative nutrient absorption activity of different portions of the root, or the growth rate of different roots. To investigate these possibilities, the absorption of phosphate, rubidium, or iron, and rate of elongation of soybean roots, relative to their fluorescent intensity, was evaluated.

Materials and methods

Ion uptake

Soybeans (cultivars 'Davis', 'Forrest', 'Lee 68', and line R-72-346) were grown in a controlled-environment chamber using a root-development growth pan described by Brown and Noggle[3], except that the collodion membrane was replaced with a porous plastic membrane sheeting with 1.2 μm pores. Two shallow metal pans, 40 cm × 15 cm × 1 cm deep, were filled with a (water-saturated) Pea Ridge silt loam soil. A sheet of the membrane material was folded to cover both soil surfaces. The pans were then stood upright with the open surfaces, covered with the membrane, facing together. A 7-day-old seedling was transplanted between the two membrane sheets at the top opening, and the two pans were strapped together with the edges sealed with tape. This method will be referred to as "split-pan". In this way plants could be grown for up to 5 weeks after transplanting with their roots in contact with the soil solution, but never in direct contact with the soil particles. The plants could be removed without injury from the split-pans to be photographed or transferred directly to the test solution for ion absorption tests. Plants in the studies reported were grown in this way for 20 to 30 days.

Each plant was removed from the split-pan and immediately transferred with its roots immersed to a complete nutrient solution labelled with the radioactive element under study. Elemental concentrations in the solution were: 1200 ppm N (NO3), 95 ppm P, 235 ppm K, 228 ppm Ca, 23 ppm Mg, 97 ppm S, 8 ppm Fe, 0.05 ppm Zn, 0.20 ppm Mn, 0.05 ppm B, 0.02 ppm Cu, and 0.01 ppm Mo. Test elements were ^{32}P as orthophosphoric acid, ^{86}Rb as rubidium chloride, and ^{59}Fe as ferric chloride. In each test, 0.5 mCi of the label was added 1.0 litre of the nutrient solution. The roots were immersed in this test solution for either 30 sec or 5 min. The plants were then de-capitated to stop transpiration and the roots were rinsed twice for 15 sec in 10^{-4} *N* HCl in deionized water. The roots were examined under black light and 3 groups of samples were taken corresponding to 3 fluorescent intensities: non-fluorescent, weakly fluorescent, and strongly fluorescent. Two samples of each intensity class were taken per plant. The two samples were treated as repetitions, and were averaged. Each plant served as a replication. The number of plants used varied from 10 to 18. The root samples were immediately weighed for fresh weight, and ranged from 20 to 100 mg. Samples were washed in H_2SO_4 and dissolved in 1 ml of 1 *N* HCl and 15 ml of scintillation cocktail. Radioactive decay of the label elements was measured by liquid scintillation with adjustment for background and quenching.

To convert from root fresh weight to root length and surface area, coefficients were developed relating these parameters. A Bausch and Lomb Omnicon Alpha 500 image analysis instrument was used to estimate the length and area of roots of the three fluorescent intensity classes after they had been weighed. The coefficients obtained and their standard errors are given in Table 1. Using these coefficients the conversion was made from root fresh weight to length (cm) and area (cm^2).

Elongation rate

Soybean plants were grown either by the split-pan method or in 7 cm square plexiglass 'soil-tubes', 100 cm long. Each tube was filled with Pea Ridge silt loam, planted with a single soybean plant, and covered with aluminium foil to prevent exposure of the roots to light. The tubes were then placed vertically, at a slight incline, in a greenhouse. Root elongation rate was estimated by repeatedly photographing the same positions of the root system over time. In either growth system the roots were photographed under both visible and black light at 3- to 5-day intervals. From these

Table 1. Coefficients for conversion from root fresh weight to length and surface area

Root fluorescent intensity	Conversion from	
	gram to cm	gram to cm^2
Strong	524 ± 64	123 ± 24
Weak	945 ± 293	251 ± 90
None	988 ± 150	262 ± 51

photographs the change in length (change in position of root apex) per day of roots was determined. The visible-light photographs were evaluated in pairs which were sequential in time. The difference in length of single roots between those two photographs was taken to be the elongation over that time period. The black-light photograph which had been taken at the same time as the first photograph in the pair being evaluated was used to classify the fluorescence of the apical portion of the root. The fluorescent intensity was determined visually and grouped into: non-fluorescent, weakly fluorescent, moderately fluorescent, strongly fluorescent, and very strongly fluorescent. A total of 188 observations were made for the split-pan group and 219 observations for the soil tube group.

For the determination of elongation rate of roots in the soil tubes, only those roots which encountered the plexiglass surface and later re-entered the soil were used. Those roots which followed the plexiglass surface for more than 5 cm were not chosen because their elongation rate may have been abnormally high due to the reduced resistance to elongation at the soil—plexiglass interface.

To quantitatively compare the rate of root elongation to fluorescent intensity the soil-tube photographs were re-evaluated by use of the image analysis instrument. For these determinations the fluorescent intensity in the black-light photographs was determined as the difference in contrast of the fluorescent root segment and its surrounding background. The elongation rates of these roots were again determined using the same procedure described previously. Fifty-four observations were made in this manner.

In both the visual and quantitative fluorescence determinations, a combination of taproot, first-order, and second-order laterals from different plants were evaluated. The visual ratings were analyzed by a one-way analysis of variance for an unbalanced design. In the quantitative determinations made with the image analyzer, the daily increase in root length was regressed linearly on fluorescent intensity.

Results and discussion

Ion uptake

The data indicate that each ion tested was absorbed at a faster rate per cm by strongly fluorescent roots than by non-fluorescent segments during the 30-sec exposure treatment (Table 2). However, when the additional error of determination resultant from the weight to length and area transformations is considered, the differences for P and Rb may not be significant. The data from the 5-min exposure were quite different. This difference may result from transport of the isotopes into the older, non-fluorescent tissue from the fluorescent tissue, and inhibition of the metabolic activity of the young, fluorescent tissue after

Table 2. Relative uptake of Rb, P, and Fe by soybean roots of different fluorescent intensity

Test element	Time of exposure	Number of plants		Relative counts per minute			
				Root fluorescent intensity			
				None	Weak	Strong	LSD.05
^{86}Rb	30 sec	10	cm^{-1}	1.00	0.74	1.36	0.47
			cm^{-2}	1.00	0.74	1.53	0.48
^{86}Rb	5 min	12	cm^{-1}	1.00	0.60	0.79	0.30
			cm^{-2}	1.00	0.59	0.89	0.31
^{32}P	30 sec	10	cm^{-1}	1.00	0.85	1.60	0.23
			cm^{-2}	1.00	0.85	1.81	0.24
^{32}P	5 min	18	cm^{-1}	1.00	0.77	0.74	0.32
			cm^{-2}	1.00	0.77	0.83	0.33
^{59}Fe	30 sec	10	cm^{-1}	1.00	1.86	2.98	1.08
			cm^{-2}	1.00	1.86	3.37	1.11

prolonged submersion. It is assumed that the shorter period is more representative of the true uptake activity of the various root segments as they grew in the split-pan soil system.

The uptake pattern observed with 30 sec exposure was similar to several previous reports of absorption of Rb, P, and Fe by different root segments, which indicated that uptake of these elements was most rapid in the apical few cm of the root[2,8,9,10,15,17,18]. Most rapid ion uptake normally occurs near the root tip and near the root base[2,10]. The rapid absorption near the tip has been attributed to the high metabolic activity and lack of suberization of these tissues, while the enhanced uptake near the base is thought to be associated with the proximity of this region to the above-ground plant parts which serve as a nutrient sink, and with the prescence of second-order lateral roots. In these experiments, the strongly fluorescent tissue corresponded to the apical 2 to 5 cm of some root tips and had no second-order lateral branches, while the non-fluorescent samples were composed largely of the basal portions of first-order laterals which had numerous second-order lateral branches.

The pattern of uptake relative to fluorescence was different from that for Rb and P, in that the non-fluorescent tissue had the lowest mean rate of uptake. The pattern of Fe uptake corresponds well with the reported pattern of iron reduction capacity of the soybean root, where the greatest reduction of ferric iron occurred between the regions of root elongation and root maturation[1], which would normally correspond to the fluorescent region. This same region was found to be

Table 3. Elongation rates (mm/day) of soybean roots of different fluorescent intensities with pairwise least significant differences

Root fluorescent intensity		Plant culture method			
		Split-pan		Soil-tube	
None		0.23		0.21	
	$lsd_{.05}$		0.24		0.47
Weak		1.13		0.58	
	$lsd_{.05}$		0.29		0.41
Moderate		3.17		1.58	
	$lsd_{.05}$		0.31		0.44
Strong		5.27		2.64	
	$lsd_{.05}$		0.32		0.54
Very strong		8.84		4.64	

most active in the reduction of ferric iron by tomato roots[5]. A similar distribution of iron uptake and translocation activity was found for barley roots, with the majority of the iron uptake occurring between 1 and 5 cm from the root tip[9]. The relationship between iron uptake and fluorescence is much stronger than the relation with Rb and P uptake, with Fe uptake by the strongly fluorescent root being three times that by non-fluorescent roots.

Elongation rate

The results of visual evaluation of fluorescence for both the split-pan and soil-tube culture methods are reported as the mean elongation rate for each fluorescence type with their pairwise least significant differences (Table 3). The elongation rate was very strongly and significantly related to the intensity of the fluorescence. Visual evaluation of the fluorescence was adequate to separate different classes of roots and estimate their elongation rates. The higher values of elongation rate obtained with the split-pan method relative to the soil-tube method were thought to be the result of the reduced resistance to penetration experienced by the roots as they grew between the membrane sheets.

Elongation rates of the soil-tube roots were also evaluated relative to a quantitative estimation of the fluorescence (Fig. 1). The higher elongation rates were again associated with the higher relative fluorescent intensities. A linear regression on fluorescent intensity, was highly significant (probability of a greater $F < 0.0001$), accounted for 82% of the variation (R^2) in the elongation rates, and had a non-significant intercept. The regression coefficient was estimated as 0.054. There was insufficient data at the high levels of fluorescent intensity to accurately indicate whether a significant curvature was present in the

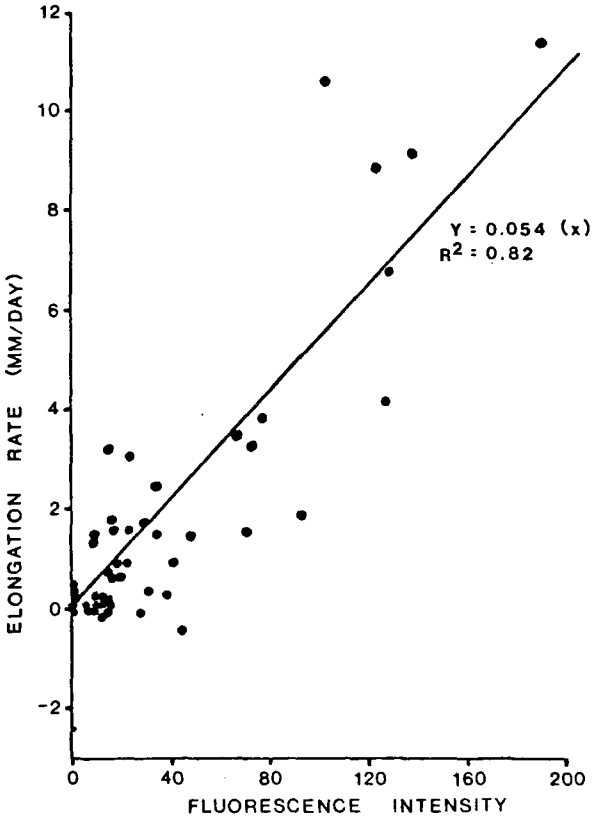

Fig. 1. Relation between elongation rate of soybean roots and their fluorescent intensity as measured by an image analyzer.

relation. The small number of data points at these higher levels is characteristic of the fact that relatively few roots are ever observed to have such rapid elongation in soil.

In both the visual and quantitative fluorescence determinations no significant difference was found between cultivars or between tap-root, first-order, and second-order laterals. Although the regression coefficient may differ, a similar fluorescence—elongation relationship probably exists for most other crop roots.

The relationship between root fluorescence and elongation permits estimation of the growth of various roots by a single assessment of root fluorescent intensity under black light. Previously, determinations of elongation rate of roots have been made by a number of methods: the time required for the roots to pass through a known length of soil[7,22,24], the length of roots removed from soil cores after a given growth period[16,25], or root elongation observed along a face of a transparent box containing the soil and seedlings[23]. While each of these methods has been reliable, they are time-consuming, tedious, or not well suited to field observation of root elongation. The fluorescence relation, however, could be used with plants grown in observation boxes, or rhizotrons, as well as in the field

using the fiber-optic scope technique[19]. This method would eliminate the difficult task of estimating root elongation rate by making repeated observations to evaluate change in root length per time interval.

Root fluorescence in soybean appears to be useful for identifying the regions of most active uptake Fe and for estimating the rate of root elongation. These relations should be very useful in estimating the growth or nutrient uptake activity of various roots, or the distribution of active elongation and absorption within a root system.

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