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Correlations between Potassium Uptake and Hydrogen Efflux in Barley Varieties[1]

A POTENTIAL SCREENING METHOD FOR THE ISOLATION OF NUTRIENT EFFICIENT LINES

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ABSTRACT

Rates of hydrogen ion secretion and potassium (^{86}Rb) absorption by intact roots of twenty-four barley varieties were measured in solutions containing K_2SO_4 (1×10^{-4} to 1×10^{-3} molar) plus 5×10^{-4} molar $CaSO_4$, at initial pH values in the range 5.3 to 5.5. Fluxes of H^+ and K^+ were strongly correlated in short-term experiments (up to 15 minutes) as well as in long-term experiments (lasting 24 hours). The observed correlations provide the basis for a preliminary screening method, designed to segregate varieties with high rates of potassium uptake by the use of an acid-base indicator (methyl red).

The high crop yields currently attained through Western-style agricultural systems are attributable, in large measure, to the selection of strains of crop plants which provide maximum yield responses under conditions of high fertilizer application. Unfortunately, the selection of these strains appears to have been made with little regard for the efficiency of plant fertilizer utilization. Plant efficiency, which in the present context may be defined as yield per unit of available inorganic nutrient, has been demonstrated to vary enormously within populations of our major crop plants (3, 5, 14).

At the root-soil interface, one important determinant of nutrient efficiency is the capacity for absorption of nutrient ions from soil solution. This capacity, which is genetically determined, varies extensively among and within crop species (9, 14, 15). A second component of nutrient efficiency is the effectiveness with which the absorbed nutrient is subsequently utilized by the plant. Here also, utilization coefficients (yield per unit of absorbed nutrients) have been shown to vary considerably according to species or cultivar (3, 5, 14).

It has been suggested that the ever increasing costs of fertilizers now make it imperative that breeders be able to isolate improved strains of crop plants which are better adapted to exploit available inorganic resources (8). The present absence of appropriate screening techniques makes the isolation of such plants an extremely slow and difficult process. Our woeful ignorance regarding the genetics of ion transport in higher plants is yet another indication of these difficulties. A major task which faces the crop physiologist and plant breeder, therefore, is to develop rapid and simple procedures to facilitate the screening of large numbers of plants for improved nutrient efficiency. This paper describes a method which may prove to be feasible for the isolation of lines of plants which are characterized by high rates of potassium absorption.

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MATERIALS AND METHODS

Seeds of barley varieties employed in this study were germinated in sand and transferred, after 18 h, to hydroponic growth facilities. Plants were grown for 5 more days in an 18-h day, 6-h night at a temperature of 26 ± 2 C. In most experiments, roots of these plants were bathed in aerated 0.01 strength Johnson's modified inorganic nutrient solution (2), in which potassium was maintained at approximately 60 μM constant infusion from a peristaltic pump. As an alternative, in short-term influx studies plants were grown in 0.5 mM $CaSO_4$ solution. In the initial experiments and the long-term study, discs bearing 100 plants were transferred to 2-liter Plexiglas vessels for the purposes of flux determinations. This provided a root weight to solution volume ratio of approximately 1 g:200 ml. For the short-term flux measurements, roots of 10 plants (per disc) were immersed in 50 ml solution, providing a root weight to solution ratio of 1 g:5 ml solution. Potassium uptake by intact roots of the barley varieties was determined either by estimating the ^{86}Rb uptake of roots, as described previously (3), or by measuring the rate of removal of ^{86}Rb from uptake media. The former method was generally employed for short-term flux measurements, whereas the latter method was used for measuring net uptake rates in longer experiments (<24 h).

H^+ efflux was measured using a Radiometer (model PHM 84), Radiometer Ltd, Copenhagen, Denmark or a Fisher (Accumet model 610) pH meter (Fisher Scientific Co., Ltd., Pittsburgh, PA) to determine the change of pH of the unbuffered uptake media. For short-term experiments, the electrode was suspended in the medium so that a continuous recording of pH values could be made. Generally, rates of acidification of the media in the range from 5.5 to 5.0 were measured. In longer experiments (<24 h) with large numbers of samples, pH values of the media were obtained, *in situ*, by placing the electrode into the uptake vessel at the end of the experiment. Uptake media, consisting of various concentrations of K_2SO_4 together with 0.5 mM $CaSO_4$ and labeled with ^{86}Rb (10^{-2} Ci mol^{-1}), were maintained in Plexiglas vessels at 30 C throughout the uptake period. Solutions were aerated continuously and well mixed by means of a magnetic stirring device. Acid-base indicators employed to indicate the extent of pH changes in the uptake media were prepared according to recipes located in a standard laboratory handbook (1). Generally, 1 ml methyl red indicator was added to 10-ml aliquots of uptake media.

RESULTS AND DISCUSSION

Differences among species and among varieties of crop plants in rates of nutrient uptake, translocation, and utilization of the acquired nutrients are known to be substantial (3, 9, 15). Previous

reports concerning K^+ influx in barley reveal that fairly large differences among varieties may be detected as early as 6 days after germination (5). In agreement with these findings, we observed that K^+ influx into $CaSO_4$-grown roots of the 24 varieties employed in this study ranged from 13.16 ± 0.69 to 6.83 ± 0.89 × 10^{-6} mol g^{-1} h^{-1}. In long-term estimates of uptake, based upon 24-h experiments (Table I), net uptake values of roots previously grown in balanced inorganic nutrient solutions also varied to a similar extent. Certain varieties, *e.g.* Fergus and Mingo, which demonstrated high rates of K^+ uptake in this experiment, also demonstrated high flux values when grown in $CaSO_4$ or in 1 μM K^+ (data not shown). By contrast, other varieties, *e.g.* Herta and Trebi, were extremely sensitive to the previous nutrient regime. Such environmental interactions, while complicating the analysis, must warrant consideration and further exploration in attempts to select varieties for particular soil-nutrient regimes.

Having established fairly clear-cut differences in rates of K^+ uptake among selected varieties, we attempted to explore the well documented correlations between K^+ uptake and H^+ secretion (6, 7, 10, 11, 13) as a means of screening for K^+ uptake.

Figure 1 shows the rates of acidification of 0.1 mM K_2SO_4 plus 0.5 mM $CaSO_4$ solutions by intact roots of the varieties Fergus and Betzes. These varieties were selected on the basis of previous trials, from among the group of 24 varieties, as representative high- and low-influx varieties. In the absence of K^+ in the external solution, pH values remained essentially constant (about 5.3) in $CaSO_4$ solution. In control experiments without plants, solutions initially at pH 5.3 slowly alkalinized over several h, becoming stable around pH 6.5. Reported influx values reflect not only varietal differences in ion uptake but also internal potassium status, since it is well known that potassium influx is negatively related with root potassium status (4, 12). Under the growth conditions employed in this experiment, in which K^+ was continuously provided at 60 μM, Fergus consistently demonstrated higher influx values than did Betzes (10.08 ± 0.29 and 6.81 ± 0.35 × 10^{-6} mol K^+ g^{-1} h^{-1}, respectively). Figure 1 demonstrates that Fergus, in keeping with expection, caused a much more rapid acidification of external media than did Betzes. Hydrogen ion efflux values were estimated at 12.76 ± 0.41 and 4.27 ± 0.59 × 10^{-6} mol H^+ g^{-1} h^{-1}, respectively.

Table I. *Hydrogen Fluxes (ϕ_{H^+}), Final pH Values for Media, and Potassium Fluxes (ϕ_{K^+}) for the 24 Varieties Based upon a 24-h Experiment*

Each variety was assigned a rank on the basis of ϕ_{H^+} and ϕ_{K^+}. Plants had previously been grown in 0.01 strength Johnson's medium (2). All fluxes × 10^{-6} mol g^{-1} h^{-1}. Spearman rank correlation coefficient for ϕ_{H^+} and ϕ_{K^+} ranks, 0.91 (significant at $P < 0.001$).

Variety	ϕ_{H^+}	Rank	Final pH	ϕ_{K^+}	Rank
Fergus	1.69	1	3.78	3.74	2
Trebi	1.30	2	3.96	3.93	1
Paragon	1.25	3	3.89	3.20	3
Galt	1.25	3	3.86	2.84	6
Mingo	1.06	5	3.97	3.03	5
Bonanza	1.06	5	4.10	2.75	7
Betzes	0.95	7	4.02	2.63	9
BT 334	0.86	8	4.06	2.53	11
Fairfield	0.86	8	4.06	3.13	4
Steptoe	0.66	10	4.08	2.59	10
Lion	0.40	11	4.37	1.99	18
Compana	0.36	12	4.45	2.40	14
Himalaya	0.32	13	4.63	2.72	8
Vantage	0.28	14	4.63	2.49	12
Olli	0.27	15	4.54	2.44	13
Hannchen	0.18	16	4.71	2.27	16
Herta	0.17	17	4.65	2.15	17
Klondike	0.09	18	4.88	1.34	24
Summit	0.08	19	4.90	2.28	15
Excelsior	0.07	20	5.10	1.85	19
Keystone	0.07	20	4.97	1.53	22
Conquest	0.04	22	5.16	1.65	21
Hector	0.04	22	5.04	1.35	23
Odessa	0.01	24	5.46	1.70	20

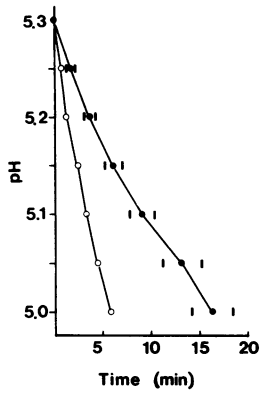

FIG. 1. Acidification of 0.1 mM K_2SO_4 plus 0.5 mM $CaSO_4$ solution by intact roots of Fergus (○) and Betzes (●). Each value shown is the mean of three replicates together with the SE of the mean.

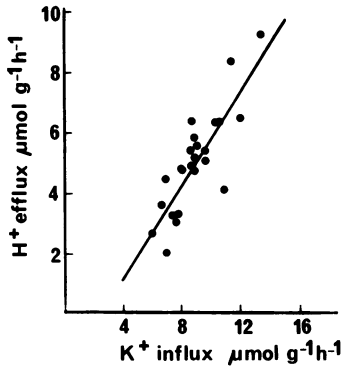

FIG. 2. A plot of H^+ efflux against K^+ influx for the 24 varieties used in the study. Plants were previously grown in 0.5 mM $CaSO_4$. Duration of the uptake period in 0.5 mM K_2SO_4 plus 0.5 mM $CaSO_4$ was 15 min. Correlation coefficient = 0.83 (significant at $P < 0.001$).

Having established the potential correlation between H^+ efflux and K^+ influx in these varieties, we proceeded to examine the relationship within the group of 24 varieties. Plants which had previously been grown in 0.5 mM $CaSO_4$ solutions (initial pH, 5.3) for 15 min. Figure 2 shows the plot of K^+ uptake against hydrogen secretion derived from this experiment.

The regression

$$\phi_{H^+} = -1.868 + 0.791\ \phi_{K^+}$$

was highly significant (r = 0.83; significant at $P < 0.001$). A comparison of ranks assigned to each cultivar on the basis of K^+ uptake and H^+ efflux also gave significant correlation (Spearman rank correlation coefficient, 0.80; significant at $P < 0.001$). Similar results were obtained when plants, previously grown in a balanced inorganic nutrient medium in which external K^+ was maintained at approximately 60 μM were allowed to absorb K^+ from 1 mM K_2SO_4 plus 0.5 mM $CaSO_4$ solution duirng a 24-h experiment. Figure 3 shows a plot of H^+ efflux against K^+ uptake (fluxes averaged over 24 h) for the 24 varieties. The regression was highly significant (r = 0.88; significant at $P < 0.001$), and a Spearman ranking correlation coefficient of 0.91 was obtained for the comparison of K^+ uptake and H^+ efflux ranks (Table I).

In addition, equilibrium pH values in the long-term experiments

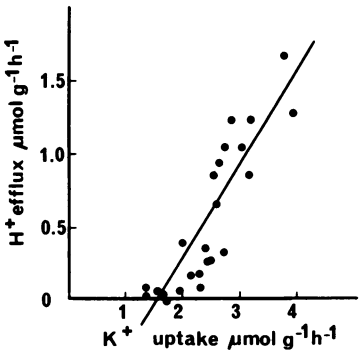

FIG. 3. A plot of net H^+ efflux against K^+ uptake for the 24 varieties. Plants, previously grown in a balanced inorganic medium containing 60 μM K^+ were exposed to 1 mM K_2SO_4 plus 0.5 mM $CaSO_4$ for 24 h. Correlation coefficient = 0.88 (significant at $P < 0.001$).

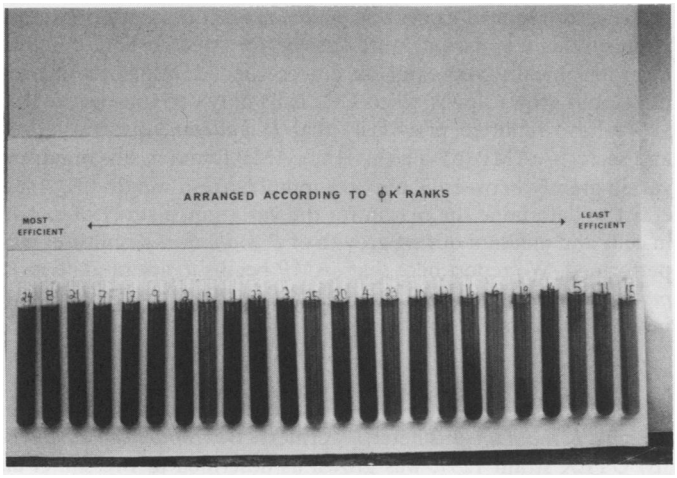

FIG. 4. Arrangement of 10-ml aliquots of uptake media after the 24-h period of potassium uptake (Fig. 3) and hydrogen efflux by the 24 barley varieties and after the addition of 1 ml methyl red. Tubes are arranged from left to right in order of decreasing potassium uptake by the varieties.

were correlated with rates of potassium uptake. For example, varieties Trebi, Fergus, and Paragon (K^+ uptake rates > 3.2 μmol g^{-1} h^{-1}) reduced the values of uptake media to below pH 4. By contrast, varieties whose uptake rates were lower than 1.85 μmol g^{-1} h^{-1} failed to lower pH values significantly below pH 5. Such large differences might readily be revealed by means of an appropriate acid-base indicator. Methyl red was found to be ideal for this purpose. The addition of 1 ml 0.1% solution of this indicator to 10-ml aliquots of the uptake media after 24 h of root activity provided an immediate visual segregation of cultivars. Figure 4 shows the arrangement of aliquots of the uptake media derived in the experiment shown in Figure 3 and Table 1. Tubes treated with methyl red were arranged from left to right in decreasing order of K^+ uptake. This arrangement revealed a clustering of the red colored solutions (high K^+ uptake, low pH) at the left hand side of the figure. Thus, as a preliminary method for screening large numbers of varieties in a breeding program, this method holds considerable promise. In addition, the rapidity of the method might permit the isolation of progeny of specific intervarietal crosses with a view to unravelling the details of the inheritance of ion transport characteristics. *A priori,* there is no assurance that the varietal differences observed at the seedling stage will necessarily apply throughout the entire life cycle. Clearly, more extensive work is necessary in this area. Nevertheless, in a previous long-term study (5), varieties which demonstrated high rates of K^+ uptake at seedling stages tended to have higher tissue K^+ content at later stages of development and even higher seed K^+ levels.

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