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KINETICS OF ZINC ABSORPTION BY EXCISED ROOTS OF TWO SUGARCANE CLONES *

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SUMMARY

Sugarcane clones differ in regard to susceptibility to zinc (Zn^{2+}) deficiency in the field. Excised roots of clone H57–5174 actively absorbed Zn^{2+} at a rate twice that of H53–263 roots. The maximum rates of Zn^{2+} uptake (Vmax) and the Km values also differed markedly between these two clones. H53–263 roots have a 6-fold greater affinity for Zn^{2+} than do those of H57–5174. H57–5174 readily develops Zn^{2+} deficiency symptoms in the field but H53–263 rarely does so. A partial explanation for these varying responses appears to lie in these data.

INTRODUCTION

Accumulation of individual nutrients in plant tissues has been shown to differ among varieties, clones, and cultivars of single species [2 3 5 8 10 11]. These observations were interpreted as evidence that ion absorption by plants is under genetic control, and that frequently such differences are apparently governed by a single gene pair since phenotypic expression was one of two widely differing extremes. As examples, single gene pairs regulate magnesium absorption in celery [7], boron absorption in celery [8] and tomato [3 10], and iron uptake in soybeans [2 11].

Most reports of variations in absorptive capacities of varietes or clones were based upon observations of differences in nutrient accumulation by intact plants grown under controlled conditions. In only one case have the kinetics of ion absorption been examined in these plants. Phillips, Baker, and Clagett [6] studied the kinetics of phosphate absorption by excised roots and leaves of two corn

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hybrids known to accumulate different levels of phosphorus in the ear leaves. No differences were found insofar as absorption by excised roots was concerned, but P absorption by excised leaves did differ between the low and high P accumulating hybrids. The variation between hybrids was sufficient to account for a 1.6-fold difference in P accumulation [6].

From previous studies (Ref. [9] and Bowen, unpublished data) it was suspected that differences occurred in regard to either zinc (Zn^{2+}) requirements or absorptive capacities for Zn^{2+} in Hawaiian sugarcane clones. In preliminary work at the Hawaiian Sugar Planters' Experiment Station it was observed that growth of sugarcane clones H57–5174 and H58–1545 was severely affected by Zn^{2+} deficiency [9]. Contrarily, growth of clone H53–263 was apparently insensitive to depletion of the Zn^{2+} supply [9]. The present study was conducted to investigate the basic physiological reasons for these differences.

METHODS

The two sugarcane clones used in these studies were H53–263, thought to have a low Zn^{2+} requirement, and H57–5174, considered to require larger amounts of Zn^{2+} for optimum growth. Both clones are derived from interspecific crosses of *Saccharum* spp. Setts were cut from commercially grown plants, dipped in phenylmercuric acetate (diluted 1:1600) for 20 mins at 50°C, and planted in vermiculite in flats in the greenhouse. Each flat was irrigated with tap water daily and 5 g of a 16:16:8 fertilizer were applied on days 1 and 8. After 14 days, the germinating setts were removed from the vermiculite avoiding root damage; then were washed, and apical 1–cm segments of the roots were excised and rinsed in distilled water. The maximum time lapse between excision of the roots and the start of an experiment was 15 mins.

In initial experiments, root sections (approximately 100 mg fr. wt.) were placed for one hour in continuously aerated solutions containing 0.5 mM $CaSO_4$, non-radioactive Zn^{2+}, as $ZnSO_4.7H_2O$ (0.008 to 0.080 mM), and $^{65}Zn^{2+}$ (0.75μc $^{65}Zn^{2+}$/μmole non-radioactive Zn^{2+}; sp. act. of $^{65}Zn^{2+}$ was 178 μc/μmole). It was found that the Zn^{2+} absorption system in root apices of clone H53–263 was saturated at an external Zn^{2+} concentration of 0.08 mM, whereas that of H57–5174 was not saturated at this concentration. Therefore, in those experiments from which the kinetic parameters were calculated for the latter clone, the external Zn^{2+} concentration was increased to 0.5mM. The pH of all solutions was 5.7 $\pm$ 0.2 and the temperature was 30 $\pm$ 0.5°C. After one hour, root sections were rinsed three times for 1 min each in flowing distilled water, and then desorbed for 30 mins in aerated 0.5 mM $CaSO_4$ at 4°C. Thus. the reversibly adsorbed fraction was eliminated, and the Zn^{2+}

absorption rates reported herein pertain only to metabolically-mediated active absorption. The roots were then dried overnight at 70°C, weighed, and ashed at 400°C for 12 hours. The ash was redissolved in 0.1 *N* HCl, and a 1-ml aliquot was assayed for radioactivity with a scintillation probe equipped with a NaI (T1) crystal (3 cm diameter × 2.5 cm thick) connected to a scaler.

Each experiment contained 2 replicates and was run 3 times. The kinetic constants were determined by the double reciprocal plot method [4], and were reproducible within ± 10%. The graphs were plotted by linear regression.

RESULTS AND DISCUSSION

Active Zn^{2+} absorption by excised root apices from each clone was linear with time for at least 1 h (Fig. 1), but roots of H57–5174 absorbed Zn^{2+} at a rate approximately twice that of H53–263 roots. Thus, a striking difference was manifested by excised roots of these two clones in their capacity to absorb this micronutrient. The maximum rates of Zn^{2+} uptake (V_{max}) and the external Zn^{2+} concentrations at which the absorption rate was $\frac{1}{2}$ V_{max} (K_m) also differed markedly between the two clones tested (Table 1). The kinetic parameters calculated for Zn^{2+} uptake by excised roots of H53-263 compared favorably with the V_{max} and K_m values reported

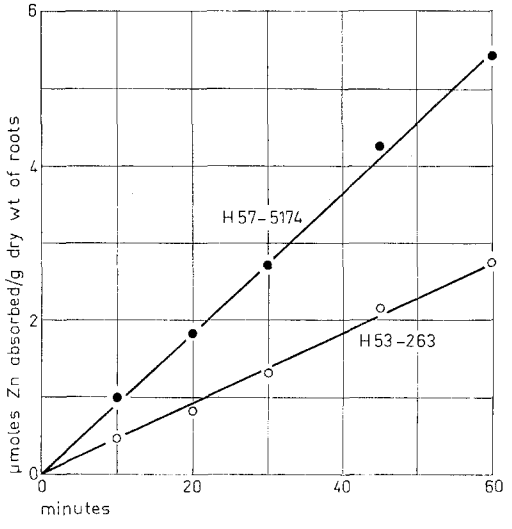

Fig. 1. Absorption of zinc by excises root tips of sugarcane clones H53–263 and H57–5174 as a function of time. (External Zn concentration, 0.08 m*M*; Ca^{2-} concentration, 0.5 m*M*; pH, 5.7 ± 0.2; temperature 30 ± 0.5°C).

previously [1] for Zn^{2+} absorption by leaf tissue of this clone (Table 1).

The maximum rate of active Zn^{2+} absorption by excised root apices of H57–5174 was approximately four-fold greater than that of H53–263 in these experiments. The initial Zn^{2+} concentration in root apices from H53–263 was 1.0 μmole per g dry wt. whereas those from H57–5174 contained 1.4 μmoles Zn^{2+} per g dry wt. The effect of this small difference upon subsequent rates of Zn^{2+} absorption and the estimated V_{max} values is unknown at present, but it is doubtful that the effect would be significant.

TABLE 1

Apparent K_m and V_{max} values for absorption of zinc by sugarcane root apices and leaf tissue at pH 5.7 and 30°C in the presence of 0.5 m*M* $CaSO_4$.

Clone	Tissue	K_m (*M*)	V_{max} (μmoles absorbed/g dry wt. hour)
H53–263	Leaf *	1.1×10^{-5}	5.9
H53–263	Root	1.8×10^{-5}	4.0
H57–5174	Root	10.3×10^{-5}	18.3

* Data from Ref.[1] included for comparison.

The observed differences in the apparent K_m values of the two sugarcane clones is likely to be more significant, however, since this kinetic parameter would be independent of the initial Zn^{2+} content of the tissues. The customary premise that ion absorption occurs through the formation of reversibly- dissociable ion-carrier intermediates was adopted herein. The reversible association of the Zn^{2+} ion and the membrane-bound carrier is considered to occur very rapidly in comparison to its rate of dissociation and the concomitant deposition of Zn^{2+} into the 'inner space'. A further premise inherent to this discussion is the consideration of the K_m values as being approximately equal to the dissociation constant of the Zn^{2+} carrier complex. Thus, the reciprocals of the K_m values are interpreted herein as an approximation of the affinity of the carrier for Zn^{2+}. From this viewpoint, then, root apices from clone H53–263 have about a 6–fold greater affinity for Zn^{2+} than do those of clone H57–5174.

It has been generally considered that H53–263 requires less Zn^{2+} for optimum growth in the field than does H57–5174. Furthermore, H57–5174 developed symptoms of Zn^{2+} deficiency quite readily in

hydroponic culture in the greenhouse when Zn^{2+} was withheld, whereas the contaminant Zn^{2+} concentration – 0.09 mg Zn^{2+} per litre – prevented the appearance of characteristic Zn^{2+} deficiency symptoms on H53–263, thus confirming the earlier report [9]. A partial explanation for these varying responses appears to lie in the data presented herein.

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