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Physiological Control of Arginine Decarboxylase Activity in K-Deficient Oat Shoots[1]

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ABSTRACT

The effect of K-deficiency on the putrescine biosynthetic enzyme, arginine decarboxylase (ADC), was investigated by growing oat (*Avena sativa* L. var Victory) plants on a low-K, but otherwise complete nutrient medium in washed quartz sand for up to 18 days. Enzyme activity rose as the concentration of KCl was dropped to 0.6 millimolar or below. However, growth was not inhibited significantly at 0.6 millimolar KCl. ADC activity increased in the whole shoot of K-deficient oats throughout the period of 6 to 18 days, but remained constant in normal plants. At 18 days, ADC activity in entire K-deficient shoots was 6 times greater than in normal shoots, while in the first (oldest) leaf, ADC specific activity increased to more than 30 times the specific activity in the first leaf of normal plants. This effect was due to a moderate rise in total ADC activity in the first leaf between 6 and 18 days, accompanied by a significant decline in protein content. Replacing K^+ with Na^+ or Li^+ significantly inhibited the increase in ADC activity in K-deficient oats, while Rb^+ depressed the specific activity to a level below that in normal plants. An alternative putrescine biosynthetic enzyme, ornithine decarboxylase, was also examined. The specific activity of a pelletable form of the enzyme was increased 2-fold in the shoots of K-deficient oats.

Several forms of environmental stress, including K-deficiency (2, 9), low external pH (10, 13), and osmotic stress (3, 11), induce an accumulation of the diamine, Put[3], and its biosynthetic enzyme, ADC. The largest and most consistent stress-induced changes in PA metabolism have been observed in K-deficient oat and barley seedlings (1). In spite of extensive study, several questions involving the physiological changes associated with ADC have remained unanswered. In particular, neither the relation of increased ADC activity to the age of plant tissue nor the response of ADC to decreasing concentrations of K in the external medium has been thoroughly examined. Moreover, the effects of different replacement monovalent cations for K on ADC activity are unknown. In addition, stress-induced changes in the activity of an alternate Put biosynthetic enzyme, ODC, have not yet been characterized.

A series of experiments, employing oat seedlings germinated and grown for several days in acid-washed quartz sand on a growth medium in which the concentration of K or one of the other monovalent cations was adjusted, was performed to answer these questions. The results demonstrate that ADC specific activity in shoots of K-deficient oats is increased to more than 6 times that in normal plants, that the increase is greatest in older tissue, and that several monovalent cations can inhibit this increase in activity, but to vastly different degrees. Furthermore, the dose-response curve of ADC activity to external K concentration is steep with an RD_{50} for K of 0.35 mM. Finally, the specific activity of a pelletable form of ODC is increased 2-fold by K-deficiency.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. *Avena sativa* L. (var Victory) plants were used in all the experiments described. Seeds were sown in acid-washed quartz sand and grown in controlled growth rooms equipped with 16-h photoperiods at about 350 $\mu E/m^2 \cdot s$ and at 23 ± 1°C for up to 3 weeks. Plants were irrigated every other day by the addition of the appropriate growth medium from above until media began to run out of the bottom of the container.

Clean quartz sand was obtained from a local building supply company. It was rinsed in 3-L lots in tap water until all debris was removed. The sand was then mixed with 1 N HNO_3 for 10 min, washed with tap water for 20 min, and distilled H_2O for 10 min, and finally dried overnight in Pyrex dishes at 110°C.

Several growth media were employed in these experiments. The macronutrient components of the various media are given in Table I. All media contained micronutrients in the following concentrations: FeEDTA, 40 mg/l; NaI, 0.75 mg/l; $MnSO_4 \cdot H_2O$, 0.1 mg/l; H_3BO_3, 0.03 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.02 mg/l, $Na_2MO_4 \cdot 2H_2O$, 0.0025 mg/l; $CuSO_4 \cdot 5H_2O$, 0.00025 mg/l; $CoCl_2 \cdot 6H_2O$, 0.00025 mg/l. In one set of experiments, a series of concentrations of KCl was employed. The details of this experiment are found in "Results."

Determination of PA Levels and Enzyme Activities. PA levels, ADC activity, and soluble ODC activity were determined by the methods described previously (13).

Determination of Pelletable ODC Activity. A pelletable form

Table I. *Composition of Nutrient Media*
All media contained micronutrients as described in the text.

	+K	−K	+Na	+Rb	+Li
			mM		
NH_4NO_3	8.0	8.0	8.0	8.0	8.0
$CaCl_2$	4.0	4.0	4.0	4.0	4.0
$MgSO_4$	1.0	1.0	1.0	1.0	1.0
$(NH_4)H_2PO_4$	1.0	1.0	1.0	1.0	1.0
KCl	6.0	0.006			
NaCl			6.0		
RbCl				6.0	
LiCl					6.0

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[3] Abbreviations; Put, putrescine; PA, polyamine; ADC, arginine decarboxylase; ODC, ornithine decarboxylase; Spd, spermidine; Spm, spermine.

of ODC was found to exist in oats and its activity was determined as follows. Tissue was homogenized at 0.1 g/ml in 0.1 M Tris-HCl buffer, pH 8.0. Homogenates were centrifuged for 10 min at 20,000*g* and the supernatant was decanted. The pellet was resuspended in 0.1 M Tris-HCl buffer, pH 8.0, in a volume equal to the original homogenate. This suspension was sonicated for a total of 6 min, consisting of three 2-min treatments separated by 1-min intervals. Samples were sonicated in a salted ice bath with a Branson Sonifier (Branson Ultrasonic Corporation) at setting 6. The suspension resulting from this procedure was centrifuged for 10 min at 20,000*g* and the ODC activity in the supernatant fraction determined by the method previously described (13).

Each experiment was performed in triplicate and was repeated on at least three separate occasions.

RESULTS

Effect of K-Deficiency on the Growth of Oats. At 6 d after planting, the shoots of −K plants were shorter and less green than those of +K seedlings but equal in fresh weight. No difference in growth rate could be observed until 9 to 12 d, when the fresh weight of +K plants began to increase significantly while the fresh weight of −K plants remained nearly constant (data not shown). By 18 d after sowing, the fresh weight of +K plants was 3 times that of −K plants. At this time, the first leaf of −K plants was totally brown and limp while the second leaf showed white necrotic spots and extensive regions of chlorosis.

Effects of K-Deficiency on PA Levels. Starvation for K induced an enormous increase in the titer of Put in oat seedlings. At 6 d old, Put titer in −K shoots was 15 times greater than in +K plants (Fig. 1a). Put continued to accumulate dramatically in −K oats up to 12 d, at which time the titer of Put was 6300 nmol/g fresh weight. Between d 12 and 18, Put continued to accumulate in −K oats, but at a much slower rate (Fig. 1a). At its peak, Put titer was 20 to 30 times greater in −K than in +K plants.

The signs of necrosis and the increased levels of Put observed in −K oats could be reversed by addition of 6 mM KCl to the irrigating nutrient medium. If KCl was added to 12-day-old −K oats for a period of 6 d, the shoot, particularly the second leaf, became green and much larger than in continuously stressed plants. The appearance of chlorosis and lesions on the first leaf was also inhibited. Similarly, the level of Put declined upon addition of KCl to the nutrient medium, reaching 2800 nmol/g fresh weight at 18 d compared with 7600 nmol/g fresh weight in constantly stressed plants (Fig. 1a). Nevertheless, 6 d of nutrient medium containing KCl was not sufficient to reverse completely the effects of K-deficiency on Put titer, since even after this treatment, levels of Put remained 9 times those in +K plants.

Put is normally converted to Spd and Spm through enzyme-mediated transfer of aminopropyl groups from decarboxylated S-adenosylmethionine (see scheme in Ref. 4). It is interesting, therefore, that Put accumulation in −K plants was not accompanied by large increases in either Spd or Spm. At 15 d, Spd titer was only 50% greater in −K plants, while Spm titer was actually decreased by 3.5-fold in −K oat shoots (data not shown). The reason for this apparent block in the metabolic transformation of Put is not known. Levels of Spd and Spm were also not significantly affected by the addition of KCl to plants which had been deprived of K up until 12 d old.

Effects of K-Deficiency on ADC Activity. The increase in Put titer observed in the shoots of −K oat seedlings was paralleled by a dramatic rise in the activity of the Put biosynthetic enzyme, ADC. ADC activity was slightly higher in −K plants at 6 d after sowing and continued to increase until 18 d (Fig. 1b). In +K oats, on the other hand, the activity of ADC remained essentially constant between days 6 and 18. At 18 d after sowing, ADC activity was 62 pkat/mg protein in the shoots of −K oats, more than 6 times than in +K plants.

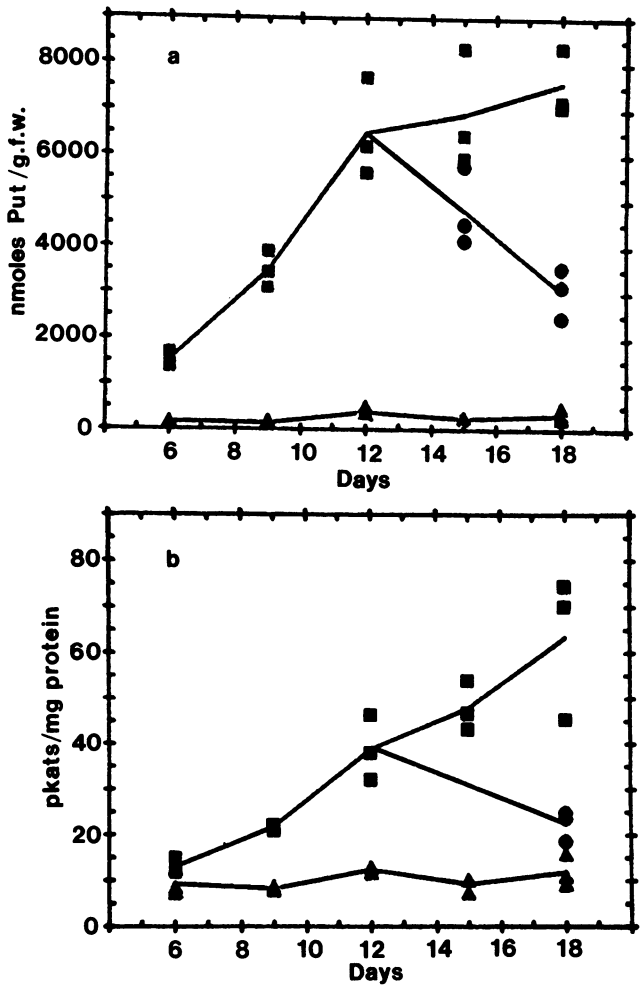

FIG. 1. Effect of K-deficiency on the Put content (a) and ADC activity (b) of oat shoots. (■), Received −K medium throughout; (●), received −K medium up to day 12 and +K medium thereafter; (▲), received +K medium throughout. Solid lines connect the means of the triplicate data points shown.

Just as the accumulation of Put in K-deficient plants was reversible by the addition of K^+, addition of 6 mM KCl beginning at 12 d led to a rapid decline in ADC activity (Fig. 1b). After 6 d of KCl addition, the activity of ADC dropped to 22 pkat/mg protein, 3 times lower than in constantly stressed oats and only twice the level observed in +K plants.

ADC in the First Leaf of K-Deficient Oats. The first leaf expresses the symptoms of K-deficiency first and one would therefore expect the greatest changes in physiology to be observed in this organ. Therefore, ADC activity was determined in the first leaf of −K plants between 6 and 18 d. Starting at 6 d, ADC specific activity increased dramatically, continuing all the way up to 18 d (Fig. 2a), at which time the specific activity of ADC was over 300 pkat/mg protein. It should be noted that, at this age, the first leaf was brown and limp. By comparison, ADC activity was only 62 pkat/mg protein in the whole shoot and less than 50 pkat/mg protein in the second leaf of −K oats. In spite of the dramatic rise in ADC activity in the first leaf, the activity quickly began to decline if 6 mM KCl was added to the growth medium starting at either 12 or 15 d (data not shown).

The reasons for the enormous increase in the specific activity of ADC can be discerned by examining the chnges in total ADC activity and protein content in the first leaf of −K oats. Total ADC activity increased 4-fold in the first leaf between 6 and 9 d

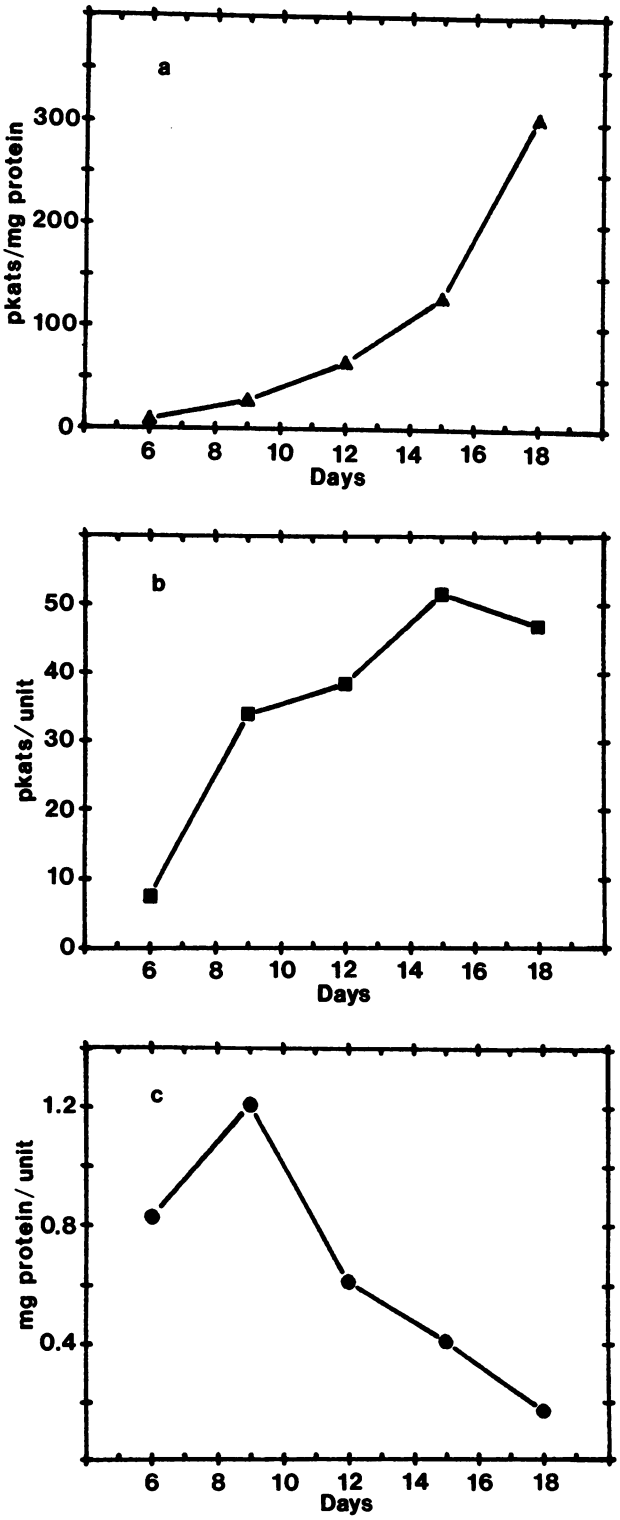

FIG. 2. Effect of K-deficiency on ADC specific activity (a), ADC total activity (b), and protein content (c) in the first leaf of oat plants. (▲), ADC specific activity; (■), ADC total activity; (●), protein content. Each data point represents the mean of triplicate samples.

(Fig. 2b) but much more slowly between 9 and 18 d. Protein content in the first leaf, on the other hand, peaked at 9 d and then declined rapidly up to 18 d, at which time the level of protein was only 17% of what it had been at 9 d (Fig. 2c). Thus, the apparent increase of over 30-fold in the specific activity of ADC between days 6 and 18 in the first leaf can be explained by a moderate increase in total ADC activity coupled with a steep decline in protein content.

Effects of Monovalent Cations on Growth and ADC. Previous studies have shown that addition of Rb or Na to K-deficient plants resulted in the rapid disappearance of Put (2, 6). In one study (6), Na caused complete disappearance of Put while Rb decreased Put content by 75% within 9 d. By contrast, addition of Li reduced Put content by only 25%. Therefore, the effects of various monovalent cations on the Put biosynthetic enzyme, ADC, were determined in K-deficient oat plants.

The addition of monovalent cations had a significant effect on the growth of oats grown in a K-free medium (Table II). The addition of RbCl or NaCl partially improved growth to a condition intermediate between +K and −K oats. These two cations resulted in a different pattern of growth, however. While the relative size of the first and second leaves in plants receiving NaCl was similar to that observed in +K oats, the growth of the second leaf was markedly inhibited by the addition of RbCl. Also, the shoots of +Rb plants were a much darker shade of green than either +K or +Na plants. Plants receiving LiCl, on the other hand, were even smaller in stature than −K plants, though this difference was not observed on the basis of protein content (Table II). Furthermore, the leaves of +Li plants showed none of the white lesions characteristic of −K plants.

The addition of monovalent cations also had a profound effect on ADC in plants grown in K-free medium (Table II). The addition of either NaCl or LiCl suppressed the increase in ADC normally observed in −K oats. ADC activity was more than 500% greater in −K than in +K oats, but only 74% and 46% greater in +Na and +Li plants, respectively. Surprisingly, +Rb plants contained ADC activity more than 3 times lower than in +K oats. Monovalent cations can therefore be ordered with respect to their ability to suppress ADC activity in oats, as follows: Rb, K, Li, Na.

Response of ADC Activity to KCl Concentration. It is clear that growth is inhibited and ADC activity induced by growing oats on a medium containing only 0.006 mM compared with plants receiving 6 mM KCl. An experiment was therefore performed to determine the changes in growth and ADC activity in plants receiving intermediate levels of KCl. The results, shown in Figure 3, demonstrate that as the concentration of KCl is lowered from 0.6 to 0.06 mM, growth (as expressed by mg protein/shoot) decreased 2-fold. Concentrations of KCl lower than 0.06 mM did not result in any additional depression of growth nor did concentrations of KCl greater than 0.6 mM result in increased growth.

ADC activity, on the other hand, was greater in oats grown at all concentrations of KCl less than 6 mM, though concentrations of KCl less than 0.06 did not result in any additional increase in its activity (Fig. 3). Also, concentrations of KCl greater than 6 mM did not result in lower ADC activity (data not shown). The concentration of KCl in the external medium which caused 50%

Table II. *Effect of Various Monovalent Cations on the Arginine Decarboxylase Activity of 12-Day Oat Seedlings*

Oat plants were grown for 12 d in acid-washed quartz sand. Plants were irrigated every other day with one of the five media described in Table I.

Cation Added	Protein	% of Control	ADC Activity	% of Control
	mg/seedling	%	*pkat/mg protein*	%
K	3.06	100	4.12	100
Rb	2.10	69	1.23	30
Na	2.46	80	7.32	178
Li	1.55	51	5.29	128
None	1.49	49	24.47	594

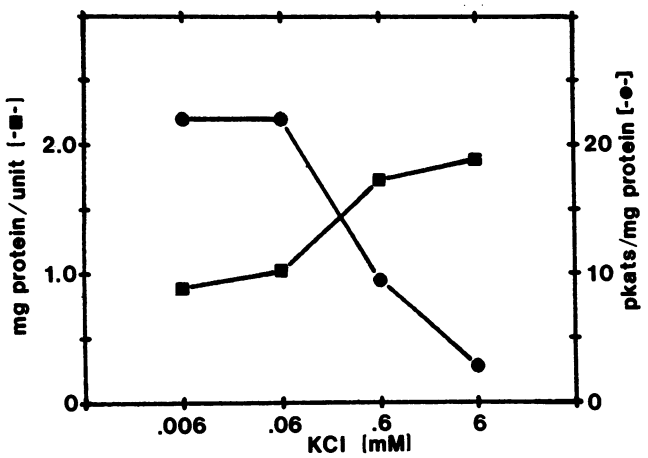

FIG. 3. Response of protein content and ADC activity to ambient K concentration in 12-d-old oat shoots. (●), ADC activity; (■), protein content. Each data point represents the mean of triplicate samples.

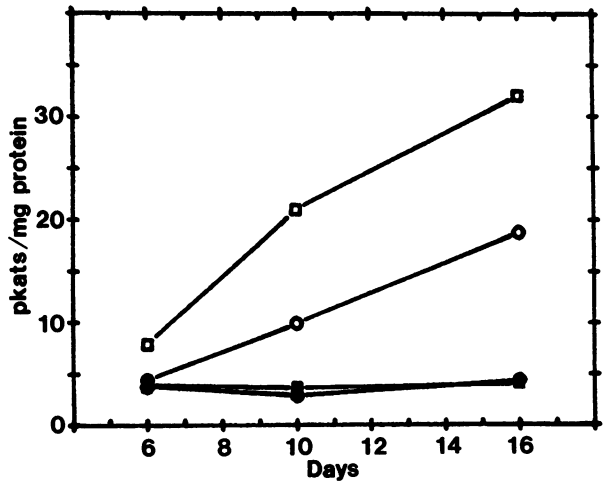

FIG. 4. Effect of K-deficiency on soluble and pelletable ODC activity in oat shoots. (■), Soluble activity in −K shoots; (●), soluble activity in +K shoots; (□), pelletable activity in −K shoots; (○), pelletable activity in +K shoots. Each data point represents the mean of triplicate samples.

of the maximal increase in ADC activity (the RD_{50}) was estimated to be 0.35 mM. Of particular interest is the fact that at 0.6 mM KCl, ADC activity was more than 2 times greater than at 6 mM KCl, but growth was not significantly depressed at this concentration. This result suggests that oats respond to decreases in ambient K concentrations with enhanced ADC activity before plants suffer negative effects on growth due to K-deprivation.

Effects of K-Deficiency on ODC Activity. Even though ADC activity increases dramatically in K-deficient oats, it is possible that the other Put biosynthetic enzyme, ODC, is also affected by K-deficiency stress. Therefore, soluble and pelletable ODC activity were determined in +K and −K oat seedlings (Fig. 4). No difference could be observed in soluble ODC activity between +K and −K oats at any age. Pelletable ODC activity, on the other hand, was twice as great in −K oats than in +K oats throughout the experimental period, even though ODC activity increased in both −K and +K plants (Fig. 4).

DISCUSSION

These experiments demonstrate that ADC activity, along with the diamine, Put, increase massively and reversibly in K-deficient oat seedlings. Older tissue is most severely affected by stress and it is in this tissue that ADC increases most. An alternate Put biosynthetic enzyme, ODC, is also increased in K-deficient plants. This is the first report of a stressed-induced increase in pelletable ODC activity. While the magnitude of the response is not nearly as great as with ADC, increases in pelletable ODC in response to stress may be physiologically important, considering the differing compartmentation of these two Put biosynthetic enzymes. ODC is known to be localized in the nucleus (5) while ADC appears to be in the cytoplasm (9, 12).

Increases in ADC activity in K-deficient cereals have been described before in the studies of Smith (8, 9). In 26-d-old K-deficient barley shoots, ADC activity was enhanced 2.2-fold over normal nutrition plants and in 21-d-old K-deficient oat shoots, ADC activity was enhanced 4.4-fold (9). Surprisingly, Smith did not observe a difference in ADC activity between K-deficient and normal nutrition oat shoots until 18 d after sowing, while in the experiments described in this report, significant differences could be observed in only 9 d. It is possible that this discrepancy is due to the fact that Smith included 4 mM NaCl in his growth medium while we have found that NaCl depresses ADC activity in K-deficient oats.

An increase in ADC or ODC activity in K-deficient plants might result from increased biosynthesis of enzyme, decreased degradation, or by relief from direct inhibition by K. In a study of K-deficiency in *Escherichia coli* (7), K at 200 mM was inhibitory to ODC activity, suggesting a direct effect by this ion. This interpretation is not the case with the increase in ADC in K-deficient oat leaves since ADC activity is undiminished in extracts including as much as 400 mM KCl (Young, unpublished results). Instead, increased biosynthesis or decreased degradation seems more likely since the labeling of ADC by [^{35}S]methionine in acid-stressed and osmotic-stressed oat leaf segments is increased by 30% (Young and Galston, in preparation). The cause of the increase in pelletable ODC activity is unknown.

While monovalent cations can reverse the effects of K-deficiency on ADC activity, they do so to vastly different degrees. Of particular interest is Rb, which reduces ADC activity to a level three times lower than the activity in +K plants, while increasing the protein content by 41% compared to −K plants. Thus, factors other than increased ADC activity must be involved in growth enhancement in the presence of Rb. By contrast, addition of Li to the growth medium, which partially reverses the increase in ADC activity due to K-deficiency, does not improve growth. The cause of growth diminution in +Li plants, therefore, is not a result of increased ADC activity. Moreover, since monovalent cations control the expression of ADC activity differentially, the control mechanism for this enzyme must be very specific.

At 0.6 mM KCl, an intermediate concentration of K in the growth medium, an interesting phenomenon is observed. While the growth of 12-d-old oat seedlings is not depressed at this concentration of K, ADC activity is enhanced 2-fold. This response suggests that increased ADC activity is compatible with normal growth in mildly K-deficient oats. This result supports models for a role for Put in K-deficient plants as a physiological adaptation to ionic stress (2).

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