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# Ammonium Inhibition of *Arabidopsis* Root Growth Can Be Reversed by Potassium and by Auxin Resistance Mutations *aux1*, *axr1*, and *axr2*[1]

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**A novel effect of ammonium ions on root growth was investigated to understand how environmental signals affect organ development. Ammonium ions (3-12 mM) were found to dramatically inhibit *Arabidopsis thaliana* seedling root growth in the absence of potassium even if nitrate was present. This inhibition could be reversed by including in the growth medium low levels (20-100 μM) of potassium or alkali ions $Rb^+$ and $Cs^+$ but not alkali ions $Na^+$ and $Li^+$. The protective effect of low concentrations of potassium is not due to an inhibition of ammonium uptake. Ammonium inhibition is reversible, because root growth was restored in ammonium-treated seedlings if they were subsequently transferred to medium containing potassium. It is known that plant hormones can inhibit root growth. We found that mutants of *Arabidopsis* resistant to high levels of auxin and other hormones (*aux1*, *axr1*, and *axr2*) are also resistant to the ammonium inhibition and produce roots in the absence of potassium. Thus, the mechanisms that mediate the ammonium inhibition of root development are linked to hormone metabolic or signaling pathways. These findings have important implications for understanding how environmental signals, especially mineral nutrients, affect plant root development.**

Plant roots display remarkable developmental plasticity in response to environmental signals. Gravity, obstructions, moisture, microbial products, light, and nutrients all serve as signals that have profound effects on root growth and development (reviewed by Feldman, 1984; Marschner, 1986; Schiefelbein and Benfey, 1991; Fisher and Long, 1992). Mechanisms that mediate these effects have been shown to include hormonal signaling, photosynthate partitioning, and ionic signal transduction (Marschner, 1986; Ehrhardt et al., 1992).

One important signal for plants is the essential nutrient nitrogen. For example, the concentration of inorganic nitrogen in the soil (as well as that of other ions) affects the ratio of shoot to root growth (Warncke and Barber, 1973; Maizlich et al., 1980). When nitrogen is scarce, root growth is favored; if the nitrogen level is increased, the shoot to root ratio increases. Soil nitrogen has other effects as well. A localized application of nitrate (1 mM) to barley roots grown with limiting amounts of nitrogen (0.01 mM) stimulates massive proliferation of lateral roots in the zone of application; similar effects have been reported for potassium and phosphate (Drew, 1975). Tuberization and tuber growth in potatoes are inhibited by high levels of nitrogen (Sattelmacher and Marschner, 1978). Exposing apple trees grown on nitrate to 24 h of ammonium during the period of flower bud differentiation will double the percentage of emerged buds that give rise to flowers (from 40 to 80%) (Grasmanis and Edwards, 1974). Thus, both the form (i.e. ammonium versus nitrate) and level of nitrogen differentially affect plant organ growth and development.

Two mechanisms have been proposed to explain the effect of nitrogen on plant development. One mechanism is the differential allocation of photosynthates. Tissues that receive adequate nitrogen supplies are better sinks for acquiring photosynthate and thus grow more rapidly than tissues receiving limited nitrogen (Marschner, 1986). The second mechanism involves hormonal signaling. Inadequate supplies of nitrogen lead to a decrease in cytokinin transport to shoots, a decline in shoot GA levels, and an increase in leaf ABA levels (Marschner, 1986). These hormonal changes affect the growth rates and sink properties of organs. It is still unclear, however, whether nitrogen levels directly influence hormone synthesis and transport or indirectly alter hormone metabolism by influencing growth rates of root primordia and other hormone-producing tissues.

One of the more profound effects of nitrogen on plant development is manifest when plants are provided ammonium as the sole source of nitrogen. The growth of many plants is strongly inhibited by ammonium in the absence of other forms of nitrogen. Part of this effect may be because ammonium absorption is associated with such pronounced acidification of external media as to prove toxic (Maynard and Barker, 1969; Haynes, 1986; Marschner, 1986). These detrimental effects are alleviated by nitrate at concentrations as low as 10% of the ammonium ion concentration (Goyal et al., 1982a, 1982b). Many plants grow best with a mixture of nitrate and ammonium, perhaps because uptake of nitrate depletes protons from the rhizosphere and nitrate reduction in the cell produces hydroxide ions that neutralize the acid

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Abbreviation: 6-BA, 6-benzylaminopurine.

produced by ammonium assimilation (Marschner, 1986; Van Beusichem et al., 1988). Potassium ions can also moderate some toxic effects of ammonium. For example, high potassium levels (2 mM) promote root growth and nitrogen assimilation in shoots of maize seedlings that are grown with 2 mM of ammonium (Xu et al., 1992). How potassium protects some plants from ammonium toxicity is not known.

We have been studying potassium uptake in plants (Cao et al., 1992), and during our initial experiments, we discovered that root growth in *Arabidopsis thaliana* is dramatically inhibited if seedlings are deprived of potassium in an otherwise complete nutrient medium. When characterizing this effect, we discovered that the inhibition of root growth is due to the presence of ammonium ions in the media. This inhibition can be reversed by low concentrations of potassium or by mutations that confer resistance to auxin and other hormones that inhibit root growth at high concentrations, *aux1* (Mirza et al., 1984), *axr1* (Estelle and Somerville, 1987), and *axr2* (Wilson et al., 1990). These results show that root development can be disrupted by a specific nutrient imbalance that is somehow linked to hormone metabolism or signaling.

## MATERIALS AND METHODS

### Growth Media

In the initial potassium deprivation experiments, all potassium ions in our *Arabidopsis thaliana* growth medium (Wilkinson and Crawford, 1991) were depleted by replacing 10 mM $KH_2PO_4$ with 10 mM $NaH_2PO_4$ and replacing 5 mM $KNO_3$ with 5 mM $NH_4NO_3$. In addition, 0.4% (w/v) agarose was used instead of 0.7% (w/v) agar because we found that agar was contaminated with potassium. In subsequent experiments, we used the following nutrient medium: 10 mM $NaH_2PO_4$, 5 mM $NaNO_3$, 6 mM $NH_4Cl$, 0.2 mM KCl, 2 mM $MgSO_4$, 1 mM $CaCl_2$, 0.1 mM Fe-EDTA, 50 $\mu$M $H_3BO_3$, 12 $\mu$M $MnSO_4$, 1 $\mu$M $ZnCl_2$, 1 $\mu$M $CuSO_4$, 0.2 $\mu$M $Na_2MoO_4$, 0.5 g $L^{-1}$ Mes, 1% (w/v) Suc, 0.4% (w/v) agarose (pH 5.7 adjusted with 1 N NaOH). When appropriate, hormones were added to the medium after autoclaving. For the comparison of the abilities of different alkali ions to reverse ammonium inhibition of root growth (Figs. 3 and 5), seeds were germinated on media containing 6 mM $NH_4Cl$, 0.5 g $L^{-1}$ Mes, 1% (w/v) Suc, 0.4% (w/v) agarose (pH 5.7 adjusted with 1 N $NH_4OH$). Different alkali ions ($K^+$, $Rb^+$, $Cs^+$, $Li^+$, $Na^+$) as chloride salts were added at the concentrations indicated in the text.

### Seed Germination

Wild-type *A. thaliana* ecotype Columbia and auxin-resistant mutants *aux1-7*, *axr1-3*, and *axr2* (obtained from Dr. Mark Estelle, Indiana University, Bloomington, IN) were used in all experiments. Seeds were surface sterilized for 2 min in 70% ethanol and 15 min in 5% (v/v) commercial bleach with 0.5% (w/v) SDS, then rinsed five times with sterile water, and planted on Petri dishes (30–50 seeds per dish) containing the nutrient medium. Dishes were stored for 2 to 3 d in the dark at 4°C to break dormancy and then incubated at 25°C with continuous illumination from warm-white fluorescent tubes. If the root lengths were to be measured, dishes were oriented vertically so that roots would grow straight along the agarose surface. Root lengths were measured after 5 d of incubation in a growth cabinet.

### Staining of *Arabidopsis* Seedlings

After germination (4–5 d), seedlings were collected from dishes and immersed in 0.1% (w/v) toluidine blue (dissolved in 0.1% [w/v] Na borate) for 30 to 60 s and then rinsed several times with water. Toluidine blue specifically stained roots of young *Arabidopsis* seedlings, probably because of the inability of the stain to penetrate the wax layer covering the hypocotyls and cotyledons; these tissues stain well when they are cut open.

### $^{86}Rb^+$ Uptake Studies

Seedlings (5–7 d old), grown on nutrient medium supplemented with 6 mM $NH_4Cl$ and 0.2 mM KCl, were floated on an uptake solution (10 mL) consisting of 0.2 mM $CaCl_2$ and 2 mM Mes [pH 5.7 adjusted with 1 M $Ca(OH)_2$] with $NH_4^+$ and alkali ions (as chloride salts) added as indicated. Seedlings (15–20) were used for each datum point. Uptake was initiated by addition of radioactive $^{86}Rb^+$ (as RbCl; Amersham) to a final concentration of 0.4 $\mu$Ci $mL^{-1}$. Nonradioactive RbCl was added to give a final concentration of 50 $\mu$M. After 1 h of incubation at room temperature with illumination from warm-white fluorescent tubes, seedlings were harvested, washed three times for a total of 20 min with ice-cold desorption solution containing 1 mM $CaCl_2$ and 20 mM KCl, blotted, and then separated individually into Eppendorf tubes. The accumulation of $^{86}Rb^+$ was quantified by Cherenkov measurement in a liquid scintillation counter. The uptake of $^{86}Rb^+$ was linear for at least 100 min (data not shown).

### $^{13}NH_4^+$ Uptake Studies

Seedlings (5–7 d old), grown on nutrient medium supplemented with 0.2 mM KCl but without $NH_4Cl$, were placed in a treatment solution consisting of 0.2 mM $CaCl_2$ and 0.2 mM Mes (pH 6.0) with or without 200 $\mu$M KCl. For each treatment, five replicates with 15 seedlings per replicate were used. The seedlings were floated on a treatment solution for 10 min before the radiolabeled solution was added. $^{13}NH_4^+$ was provided at approximately 0.015 $\mu$Ci $mL^{-1}$, at a final concentration of either 1 or 10 mM $NH_4Cl$. The $^{13}NH_4^+$ influx was for 10 min at 25°C under diffuse light from fluorescent tubes. After the 10-min exposure of the seedlings to tracer, the radioactive solution was removed by aspiration and replaced with identical nonlabeled solution for 2 min. The wash solution was removed by aspiration, and the 15 plants were transferred immediately to a scintillation vial. This was counted for 0.5 min in a $\gamma$ counter (Minaxi 5000; Packard) along with blanks and the uptake solution for specific activity determination. After the specific activity was counted, the plants were weighed after they were blotted gently.

## RESULTS

### Ammonium Inhibition of Root Growth

This study began with a search for mutants defective in potassium uptake. To design a screen for such mutants, we first examined how *Arabidopsis* would respond to potassium deprivation. *Arabidopsis* seeds were germinated on a complete medium minus potassium (a modified growth medium in which all potassium ions were replaced with $Na^+$ and ammonium ions, and agar was replaced with agarose). When plated on this medium, *Arabidopsis* seedlings displayed a dramatic inhibition of root growth. After germination (3–4 d), seedlings grown in the absence of potassium showed almost no root growth, whereas cotyledons and hypocotyls looked normal (Fig. 1A). Seedlings grown on a complete medium with potassium had normal, elongated roots as expected (Fig. 1B). Further incubation on a medium minus potassium led to yellowing of cotyledons and failure to develop true leaves. Because inhibition of root growth was the first observable effect of potassium deficiency under these conditions, the factors leading to this effect were examined further.

Initially, the inhibition of root growth induced by potassium deprivation was a puzzling result because root growth proceeds normally when *Arabidopsis* seedlings are germinated in water (i.e. with no potassium present). Therefore, we checked whether a component of the potassium-free growth medium might be inhibiting root growth. Specific ions were either left out of the complete growth medium or added back to water. When ammonium ions were omitted from the growth medium in addition to potassium, normal root growth was observed (Fig. 1C); when ammonium ions (with chloride, nitrate, or succinate) were added to water or nutrient media without potassium, then roots did not form (data not shown).

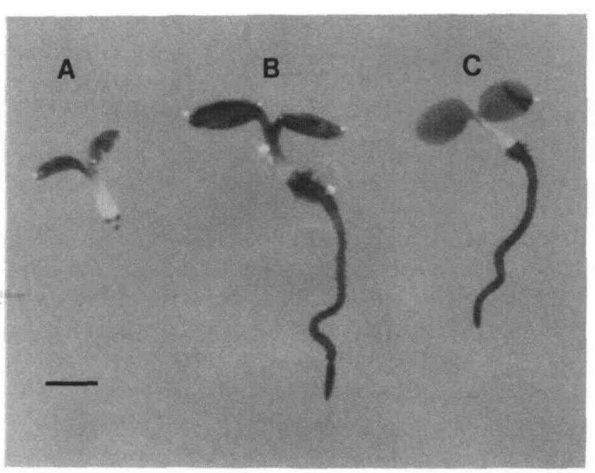


**Figure 1.** Inhibition of root development by high levels of ammonium in the absence of potassium. *Arabidopsis* seeds were germinated on nutrient media supplemented with 6 mM $NH_4Cl$ (A), 6 mM $NH_4Cl$ plus 0.2 mM KCl (B), or without $NH_4Cl$ and KCl (C) as described in "Materials and Methods." Seedlings were stained with toluidine blue to help visualize the root and photographed 4 d after germination. Bar = 2 mm.

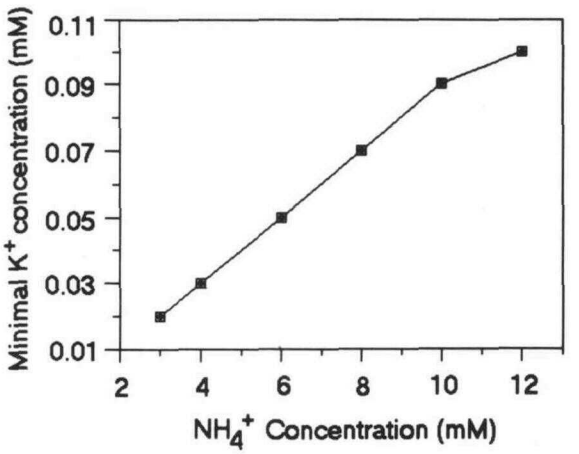


**Figure 2.** Dependence on ammonium concentration of the minimal potassium concentration required to reverse the ammonium inhibition. The minimal potassium concentrations (determined as the concentrations that restored root growth to more than 50% of seedlings) were plotted as a function of ammonium concentration in the growth medium.

Thus, the dramatic inhibition of root growth seen in the absence of potassium is produced by ammonium ions in the growth medium.

### Effect of Alkali Ions on Root Growth Inhibition

The concentration of potassium needed to reverse the ammonium inhibition of root growth is linearly related to the concentration of ammonium ions present in the medium, as shown in Figure 2. This result suggests that potassium protects plants by competing with ammonium, perhaps for a transporter or an enzyme. If this is true, other ions that mimic potassium should also reverse the ammonium inhibition. The closest analog of potassium is $Rb^+$, which is used extensively to study potassium transport (Epstein et al., 1963; Kochian and Lucas, 1982). $Rb^+$ cannot, however, serve as a nutritional replacement for potassium during plant growth (Marschner, 1986). When testing the effect of $Rb^+$ on root growth in the absence of potassium, we found that this ion reversed the ammonium inhibition just as effectively as potassium, as shown in Figure 3. Other alkali ions were also tested. $Cs^+$, another inhibitor of potassium uptake (Epstein and Hagen, 1951; Sheahan et al., 1992), was more effective than potassium at low concentrations (10–50 μM) in reversing ammonium inhibition but was ineffective at higher concentrations (100–500 μM) (Fig. 3), presumably because it is toxic to cells at these concentrations and inhibits, by itself, root elongation (data not shown). It is interesting that $Na^+$ and $Li^+$ do not alleviate the ammonium inhibition even at concentrations as high as 10 mM (Fig. 3 and data not shown).

To confirm that potassium, $Rb^+$, and $Cs^+$ are all effective competitive inhibitors in *Arabidopsis*, we examined the effect of each ion on $Rb^+$ uptake, as shown in Figure 4. As expected, potassium inhibited $Rb^+$ uptake (50% inhibition at 250 μM, Fig. 4A), matching the unlabeled $Rb^+$ dilution of $^{86}Rb^+$ uptake (50% inhibition at 280 μM, Fig. 4B). $Cs^+$ was tested next and found to strongly inhibit $Rb^+$ uptake (50% inhibition at 160

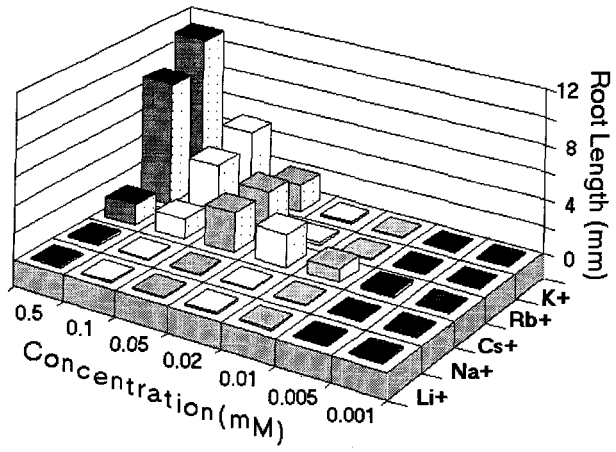


**Figure 3.** Effect of different alkali ions on ammonium inhibition of root growth. *Arabidopsis* seeds were germinated on media containing different alkali ions (as chloride salts) and 6 mM $NH_4Cl$ as described in "Materials and Methods." Root lengths were measured 5 d after germination. The average root lengths taken from 20 seedlings are plotted as a function of alkali ion concentration in the media.

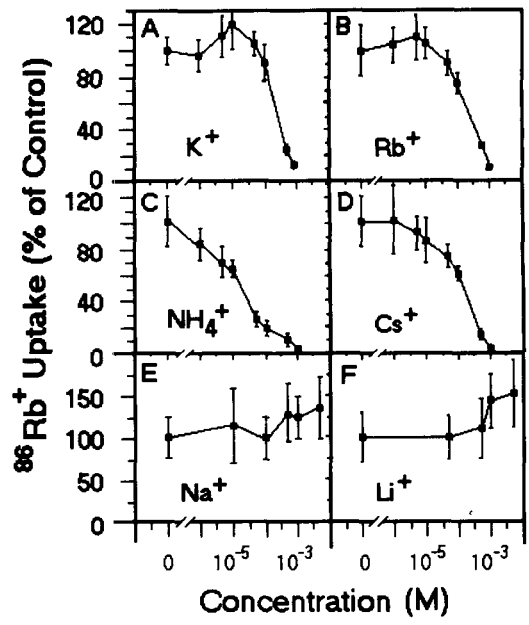


**Figure 4.** Effect of different alkali ions and ammonium ions on $^{86}Rb^+$ uptake. *Arabidopsis* plants were placed in $^{86}Rb^+$ uptake solution containing different cations as described in "Materials and Methods." The percentage of $^{86}Rb^+$ uptake relative to control plants is plotted as a function of the cation concentration. The average hourly $^{86}Rb^+$ uptake rate of the control plants incubated in uptake solution with no additional cations was 0.10 ± 0.02 μmol $g^{-1}$ fresh weight $h^{-1}$. The vertical bars at each datum point indicate the SD in measurements taken from 15 to 20 seedlings.

μM, Fig. 4D). In contrast, $Na^+$ and $Li^+$ did not decrease $Rb^+$ uptake (Fig. 4, E and F). Similar results were also reported by Smith and Epstein (1964). Thus, the ability of an alkali ion to reverse the ammonium inhibition is strongly correlated with its ability to inhibit $Rb^+$ uptake (i.e. mimic potassium). These results support the hypothesis that ammonium and potassium competitively interact in plants, and an imbalance (excessive ammonium to potassium ratio) leads to inhibition of root growth. In this light, it is interesting that ammonium ions are the most effective inhibitor of $Rb^+$ uptake that we tested (50% inhibition at 30 μM, Fig. 4C).

One hypothesis explaining how potassium protects seedlings from ammonium inhibition is that potassium decreases uptake of ammonium to a level that is not inhibitory for root growth. We examined the effect of low concentrations of potassium on ammonium uptake in *Arabidopsis* using [$^{13}N$]-ammonium and found no inhibitory effect (Table I). Thus, potassium is not reversing the root growth inhibition by decreasing the influx of ammonium. In addition, if rootless seedlings maintained for 5 to 7 d on ammonium without potassium are transferred to a complete medium with potassium, they then resume normal root growth (Fig. 5). Root growth was not restored, however, if other alkali ions were used instead of potassium or if potassium was left out with or without ammonium (Fig. 5).

### Hormonal Effects on the Inhibition of Root Growth

Because phytohormones have profound effects on root growth and development (Feldman, 1984; Klee and Estelle, 1991; Schiefelbein and Benfey, 1991), we asked whether plant hormones might be playing a role in mediating the ammonium inhibition of root growth. The effects of several mutations that are known to impair hormone signaling were tested. The most dramatic effect was observed with the auxin-resistant mutants *aux1*, *axr1*, and *axr2* (Mirza et al., 1984; Estelle and Somerville, 1987; Wilson et al., 1990). These mutants were originally selected by their ability to produce roots in the presence of inhibitory levels of auxin and have recently been shown to be resistant to several hormones (Pickett et al., 1990; Wilson et al., 1990; M.A. Estelle, personal communication). These mutants were found to produce roots when grown on media containing high levels of ammonium and low levels of potassium (Fig. 6A) and, thus, were resistant to the ammonium inhibition. The *axr2* mutant displayed the strongest resistance, having the longest roots, whereas *aux1* and *axr1* showed intermediate levels of resistance.

To gain more insight into the linkage between hormone effects and the ammonium inhibition, we tested the effect of exposing wild-type *Arabidopsis* seedlings to individual hormones at different concentrations. As expected, we found that some of the hormones inhibited root growth; however,

**Table I.** *Effect of potassium ion on ammonium uptake*
Ammonium uptake (±SD) was derived from five measurements.

| External Ammonium Concentration | Ammonium Uptake | |
|---|---|---|
| | 0 μM KCl | 200 μM KCl |
| mM | μmol $g^{-1}$ fresh wt $h^{-1}$ | |
| 1 | 7.8 ± 0.8 | 8.4 ± 1.0 |
| 10 | 40.7 ± 3.9 | 45.0 ± 6.0 |

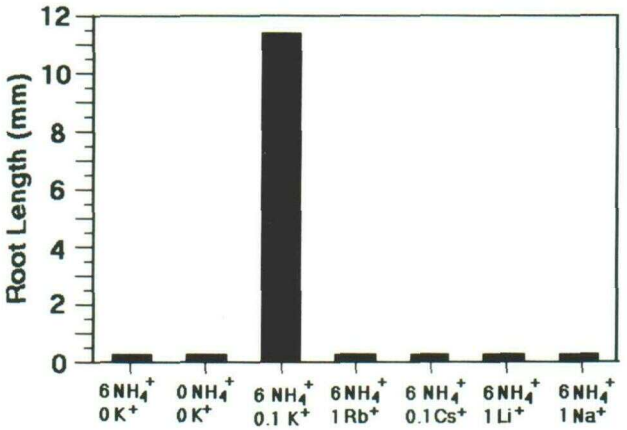


**Figure 5.** Rescue of root growth in ammonium-inhibited seedlings. Seeds were soaked for 5 d on a nutrient medium supplemented with 6 mM $NH_4Cl$ but no KCl to generate "rootless" seedlings. Rootless seedlings were then transferred to growth media supplemented with different cations (as chloride salts) in the mM concentrations indicated. Root lengths plotted in histogram were measured 4 d after transfer.

no single hormone treatment produced seedlings that resembled those seedlings from ammonium inhibition. High levels of $GA_3$ (up to 20 μM) did not inhibit root growth (data not shown). ACC (an ethylene precursor) and cytokinin (as 6-BA) at high concentrations (up to 50 μM) partially inhibited root growth but not as effectively as ammonium treatment (Fig. 6, B–D, and data not shown). $Ag^+$, which blocks ethylene inhibition (Yang and Hoffman, 1984; Eliasson et al., 1989), had no effect on the ammonium inhibition (data not shown). ABA (1 μM) dramatically inhibited root growth but also inhibited cotyledon expansion unlike the ammonium treatment (data not shown). IAA treatment produced seedlings that most resembled ammonium-treated plants (Fig. 6, E and F). High levels (20 μM) inhibited root growth; however, some subtle differences from ammonium-treated seedlings were noted. The ratio of root to hypocotyl length and the swelling at the base of the hypocotyls were greater for IAA-treated than for ammonium-treated seedlings (Fig. 6, F and H).

Next, combinations of hormones were tested. It was found that a combination of cytokinin (1 μM 6-BA) and auxin (0.5 μM IAA) produced seedlings that most closely resemble ammonium-treated seedlings (Fig. 6, G and H). The combination of hormones inhibited root growth at much lower levels than if single hormone treatments were used. When the auxin-resistant mutants (*aux1*, *axr1*, and *axr2*) were tested, they showed partial resistance to the auxin plus cytokinin treatment, with *axr2* displaying the greatest root growth (Fig. 6I). These results show that root growth inhibition by hormonal treatment or by ammonium treatment can be partially reversed by the auxin-resistant mutations and suggest a linkage between the mechanisms that mediate these two effects.

## DISCUSSION

Ammonium ion is a central intermediate in the metabolism of nitrogen in plants. Ammonium is produced during nitrate assimilation, nitrogen fixation, deamination of amino acids from storage proteins, and photorespiration (reviewed by Miflin and Lea, 1990; McGrath and Coruzzi, 1991; Last, 1993). Ammonium can also be acquired by direct uptake from soil. The assimilation of ammonium (i.e. fixation into carbon) requires carbon skeletons, energy, Gln synthetase, and glutamate synthase. Excessive and unbalanced ammo-

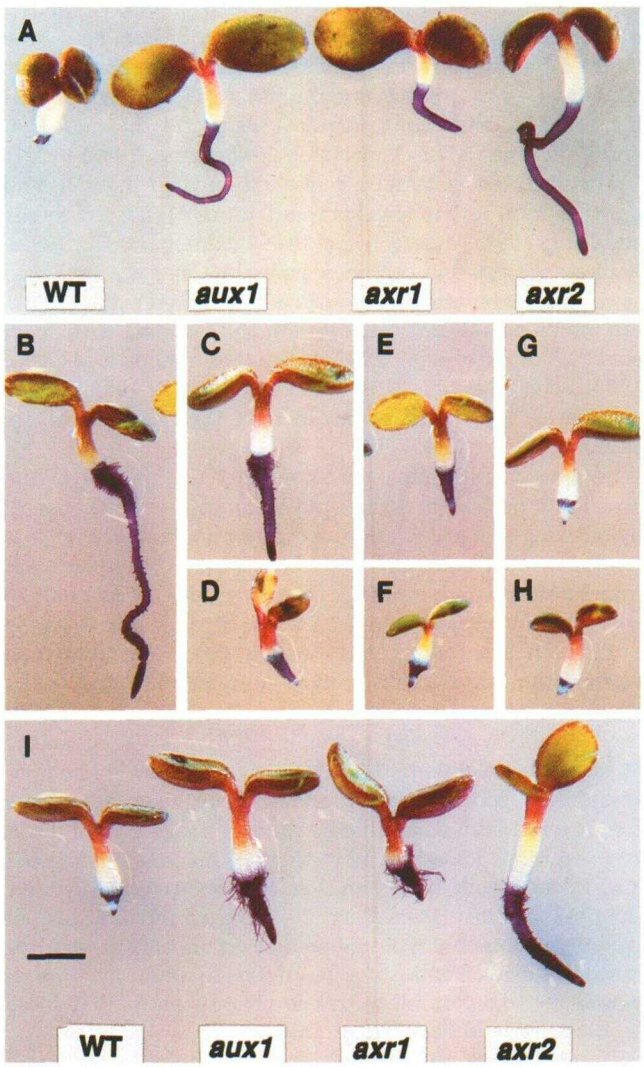


**Figure 6.** Ammonium and hormone inhibition of root growth for wild-type and three auxin-resistant mutants. A, Seeds from wild-type (WT) and three auxin-resistant mutants (*aux1*, *axr1*, *axr2*) were germinated on nutrient media supplemented with 6 mM $NH_4Cl$ and 20 μM KCl, which inhibits root growth in wild-type seedlings. B–H, Wild-type seeds were germinated on nutrient media supplemented with 6 mM $NH_4Cl$, 0.2 mM KCl (B–G) plus 1 μM 6-BA (C), 50 μM 6-BA (D), 0.5 μM IAA (E), 20 μM IAA (F), or 0.5 μM IAA plus 1 μM 6-BA (G). For comparison, wild-type seedlings grown on media with 6 mM $NH_4Cl$ and limiting levels of KCl (20 μM) are shown (H). I, Seeds from wild-type (WT) and three auxin-resistant mutants were germinated on nutrient media supplemented with 6 mM $NH_4Cl$, 0.2 mM KCl, 0.5 μM IAA, and 1 μM 6-BA. Seedlings were stained with toluidine blue and photographed 5 d after germination. Bar = 2 mm.

nium uptake and assimilation have been shown to have detrimental effects on cellular metabolism by causing acidification and inhibition of cation uptake (e.g. potassium and $Ca^{2+}$) (for reviews, see Haynes, 1986; Marschner, 1986). These detrimental effects are reversed if nitrate is also present; assimilation of nitrate produces hydroxide ions and may compensate for the acidification induced by ammonium assimilation (Marschner, 1986; Van Beusichem et al., 1988). Potassium also plays a key role in cellular metabolism, but it is not metabolized. Instead, potassium is critical for activating at least 60 enzymes in plant cells, for sustaining protein synthesis, for controlling turgor pressure and stomatal opening, and for transporting organic acids in the phloem (Leigh and Wyn Jones, 1985; Marschner, 1986; Glass, 1989).

The uptake of potassium is likely to involve channels and perhaps transporters (Kochian and Lucas, 1982; Schroeder and Fang, 1991; Anderson et al., 1992; Sentenac et al., 1992). Because the size and charge of potassium ions are similar to those of ammonium ions (Buurman et al., 1991), ammonium can inhibit potassium uptake (Smith and Epstein, 1964; Ajayi et al., 1970; Rufty et al., 1982; Deane-Drummond and Glass, 1983; Scherer et al., 1984; Vale et al., 1987, 1988) and is transported by a potassium channel (Schachtman et al., 1992).

In the present study, we show that ammonium in the absence of potassium dramatically inhibits root development by blocking root growth during the germination of *Arabidopsis* seeds. This ammonium effect is novel, being different from known ammonium toxic effects because (a) it cannot be alleviated by nitrate or succinate, (b) it does not resemble cation deficiency symptoms because seeds germinated in water with no added salts produce normal roots, (c) it can be alleviated by low levels of potassium (20–100 $\mu$M), and (d) it can be partially reversed by mutations that confer resistance to hormone treatment. *Arabidopsis* is normally tolerant to ammonium, germinating and setting seeds when grown on a complete medium with ammonium as the sole form of nitrogen and with potassium at concentrations greater than 0.2 mM. In most soils, potassium concentrations typically vary from 0.2 to 5 mM (Marschner, 1986). The inhibition of root growth by ammonium described here occurs only at very low concentrations of potassium in a defined culture medium. It is unlikely except under extreme circumstances that these conditions would be found in nature.

To inhibit root growth, ammonium must be blocking cell division or expansion. Perhaps, in the absence of potassium, ammonium accumulation within the cell is sufficient to disrupt metabolic pathways, osmoregulation, or ion and pH balances, the latter leading to the uncoupling of oxidation and photophosphorylation. High levels of ammonium or $NH_3$ in the cell are known to have toxic effects in plant cells (Marschner, 1986). Whatever the mechanism, the inhibition is reversible, because root growth can be restored in inhibited seedlings if potassium is subsequently provided.

The protective effect of potassium (and $Rb^+$ and $Cs^+$) may be mediated via several different mechanisms. Potassium may be activating enzymes essential for growth that were otherwise inhibited by ammonium. Both Ajayi et al. (1970) and Xu et al. (1992) have shown that potassium enhances ammonium assimilation. Potassium may also be lowering intracellular ammonium concentration by activating protein synthesis. Typically, 30 to 40% of incoming nitrogen is assimilated into proteins (MacKown et al., 1982). Potassium may serve to restore the ionic balance and pH of the cell by exchanging for protons across the plasma membrane. We do know that the protective effect of potassium is not due to inhibition of ammonium uptake.

The other interesting finding from this study is that *aux1*, *axr1*, and *axr2* mutants are resistant to the ammonium inhibition and produce roots in the absence of potassium. These results suggest that hormone synthesis, transport, or signaling is disrupted during the ammonium treatment. Perhaps too much auxin and cytokinin are being delivered to roots, or the root meristem has become more sensitive to hormones. In support of this hypothesis, we show that treating seedlings with low levels of auxin plus cytokinin produces an inhibitory effect very similar to the ammonium effect. In addition, we show that the *aux1*, *axr1*, and *axr2* mutants are resistant to the auxin plus cytokinin treatment. Our results, however, do not rule out other hypotheses. For example, the ammonium ions may be disrupting the normal control of ion transport so that cell expansion is impeded; the *aux1*, *axr1*, and *axr2* mutations may compensate for this defect, thus allowing for normal cell expansion. Whatever the mechanism, the nutrient imbalance caused by ammonium treatment in the absence of potassium is linked to hormone physiology and severely inhibits the proper development of *Arabidopsis* roots. To investigate further the ammonium inhibition, we have isolated additional mutant plants that produce roots in the presence of ammonium and the absence of potassium (our unpublished results). We hope these mutants will provide insights into factors that control root growth and development.

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