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Essential Role of Urease in Germination of Nitrogen-Limited *Arabidopsis thaliana* Seeds[1]

Laura E. Zonia[2], Norm E. Stebbins, and Joseph Carmine Polacco*

Department of Biochemistry and Interdisciplinary Plant Group, University of Missouri, Columbia, Missouri 65211

In *Arabidopsis thaliana*, urease transcript levels increased sharply between 2 and 4 d after germination (DAG) and were maintained at maximal levels until at least 8 DAG. Seed urease specific activity declined upon germination but began to increase in seedlings 2 DAG, reaching approximately 75% of seed activity by 8 DAG. Urea levels showed a small transient increase 1 DAG and then approximately paralleled urease activity, reaching maximal levels at approximately 9 DAG. Urease inhibition with phenylphosphorodiamidate resulted in a 2- to 4-fold increase in urea levels throughout seedling development. Arginine pools (0–8 DAG) changed approximately in parallel with the urea pool. Consistent with arginine being a major source of urea, arginase activity increased 10-fold in the interval 0 to 6 DAG. Allopurinol, a xanthine dehydrogenase inhibitor, had no effect on urea levels up to 3 DAG but reduced the urea pool by 30 to 40% during the interval 5 to 8 DAG, suggesting that purine degradation contributed to the urea pool well after germination, if at all. In aged *Arabidopsis* seeds, there was a correlation between phenylphosphorodiamidate inactivation of urease and germination inhibition, the latter overcome by NH_4NO_3 or amino acids. Since urease activity, urea precursor, and urea increase in young seedlings, and since urease inactivation results in a nitrogen-reversible inhibition of germination, we propose that urease recycles urea-nitrogen in the seedling.

Urease (EC 3.5.1.5., urea amidohydrolase) catalyzes the hydrolysis of urea to ammonia and CO_2. Although it is an abundant seed protein in many members of the Leguminosae, Cucurbitaceae, Asteraceae, and Pinaceae (Bailey and Boulter, 1971), urease is also found at lower levels in the vegetative tissues of most or all other plants (Hogan et al., 1982). Urease has been proposed to function coordinately with arginase in the utilization of seed protein reserves during germination (Thompson, 1980). A second proposed role is the assimilation of urea derived from ureide metabolism (Shelp and Ireland, 1985). Also, urea is an effective foliar fertilizer. For example, within 6 weeks of foliar application of [^{15}N]urea to *Pinus radiata* seedlings ^{15}N was found incorporated in proteins and in new structural growth (Coker, 1991).

A urease-negative soybean mutant, *eu3-e1/eu3-e1*, accumulated considerable levels of urea in all tissues and exhibited necrotic leaf tips (Stebbins et al., 1991). Necrotic leaf tips have been associated with urea burn caused by foliar application of urea plus a urease inhibitor (Krogmeier et al., 1989) and also with a urease-negative phenocopy induced by nickel deprivation (Eskew et al., 1983) in which 2.5% of the dry weight of the necrotic leaf tip was urea. Although a urease-negative trait is not lethal in soybean, we have observed that urease-negative mutants tend to germinate more slowly and at lower frequency.

To help define a role for urease in nitrogen mobilization during germination, we examined urease expression in developing seedlings of *Arabidopsis thaliana*. Urease transcript, activity, and substrate increased during the first 8 d of germination. The contribution of urease activity to seedling development was suggested by the failure of aged *Arabidopsis* seeds to germinate on water in the presence of the urease inhibitor PPD. This inhibition was overcome by nitrogenous compounds. Our results suggest that urease functions to recycle nitrogen bound in urea that accumulates during early seedling development.

MATERIALS AND METHODS

Plant Material and Germination/Growth Conditions

For germination and seedling development studies *Arabidopsis thaliana* Columbia seeds were surface sterilized and evenly distributed on a 9.0-cm Whatman 541 filter paper in a 100- × 15-mm plastic Petri dish containing 3 mL of 1 μM $NiSO_4$ with or without a nitrogen source with or without inhibitor. $NiSO_4$ was added to ensure maximal activity of urease (Polacco, 1977). Plates were sealed with Parafilm; seeds were germinated and grown at 100 μE cm^{-2} s^{-1} constant illumination at 25°C. *Arabidopsis* seeds soaked in dH_2O or 5 mM NH_4NO_3 routinely germinated approximately 50 h after the start of imbibition. Two *Arabidopsis* seed lots were used: fresh seeds were less than 1 year old, and aged seeds were 4 years old and had been stored desiccated at 4°C.

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[2] Present address: Department of Botany, North Carolina State University, Raleigh, NC 27695–0001.

* Corresponding author; e-mail bcjoecpo@muccmail.missouri.edu; fax 1–314–882–5635.

Abbreviations: AP, allopurinol; DAG, days after germination; dH_2O, deionized, distilled water; PACTPAT, 2-phenoxy-2,4,4,6,6-pentaaminocyclotriphosphazatriene; PPD, phenylphosphorodiamidate.

Nucleic Acid Manipulations

Genomic DNA was prepared using a modification of the protocol described by Dellaporta et al. (1983). An *Mbo*I partial-digest *Arabidopsis thaliana* Landsberg *erecta* genomic DNA library in λEMBL4 (Chang and Meyerowitz, 1986) was probed with soybean urease subclones P9 (Krueger et al., 1987) and JG12 (Torisky et al., 1994) according to the method of Sambrook et al. (1989). Fourteen plaques were detected in a high-stringency screen (65°C, 0.1× SSC) of 200,000 plaques, representing approximately 10 genomic equivalents, and no new plaques appeared in a low-stringency screen. Southern blotting of DNA immobilized on MSI nylon filters (Fisher Scientific) and hybridization were essentially as described by Sambrook et al. (1989). Gel-purified DNA probes were radiolabeled by random priming according to instructions provided with a kit from United States Biochemical (Cleveland, OH).

Preliminary restriction mapping of λEMBL4 clones was performed by hybridizing partial *Bam*HI and *Eco*RI digests with 5′ ^{32}P-labeled oligonucleotides (Collaborative Research, Lexington, MA) homologous to the left or the right cohesive termini of the vector. Distances between restriction sites were determined from sizes of nested fragments after agarose gel electrophoresis and autoradiography.

RNA was purified from pooled stem and leaf tissue according to the protocol of Baulcombe and Key (1980) and centrifuged in 5.7 M CsCl (Chirgwin et al., 1979). Northern analysis was performed on glyoxal-denatured RNA blotted to nylon membranes (MSI, Fisher Scientific) essentially as described by Sambrook et al. (1989).

Enzyme Assays

Protein extracts were prepared from whole seedlings. Seedlings were harvested from filter paper with a cell scraper (Nunc, Thousand Oaks, CA), powdered in liquid N_2 using a mortar and pestle, and homogenized in 3 volumes of extract buffer (0.1 M Tris-maleate, pH 8.0, 1 mM EDTA, 0.25 unit/mL trypsin inhibitor aprotinin, 0.1 mM PMSF) in a 5-mL Potter-Elvehjam tissue grinder. Insoluble material was removed by centrifugation at 16,000*g* for 10 min at 4°C.

Urease activity was measured by the release of $^{14}CO_2$ from [^{14}C]urea. Assays, performed in duplicate at 30°C in 7-mL polypropylene scintillation vials (Fisher Scientific), were initiated by adding 100 μL of protein extract to 900 μL of prewarmed assay reagent (10 mM [^{14}C]urea with specific activity = 400–500 dpm/nmol, 0.1 M Tris-maleate, pH 8.0, 1 mM EDTA, 5% [v/v] *n*-propanol). The vial was immediately capped with a 4-cm length of Scimatco tubing (15-mm i.d., Fisher Scientific) to which another 7-mL scintillation vial had been attached. The bottom of the second vial contained a 1- × 1-cm glass fiber filter (Whatman) saturated with 50 μL of 9 M monoethanolamine. Reactions were stopped by injection of 1 mL of 1 M H_2SO_4 through the Scimatco tubing and allowed to stand for 1 h before disassembling the vials. $^{14}CO_2$ driven off was collected on the monoethanolamine-soaked wick and radioactivity was determined by liquid scintillation counting. Protein was measured by the method of Bradford (1976) using commercial dye reagent (Bio-Rad). Serial dilutions of BSA (Sigma) were used to construct standard curves.

Arginase activity was measured essentially as described by Polacco (1977). The 2-mL reaction, containing 100 μL of Mn^{2+}-activated extract, 20 mM L-Arg, 50 μM PPD, and 90 mM 2-[*N*-cyclohexylamino]ethanesulfonic acid, pH 9.7, was incubated at 30°C and 0.4-mL aliquots were removed for urea determination. Urea production was linear for at least 45 min and was dependent on added Arg.

Urea and Free Arg Analyses

Mature seed and whole seedlings were extracted using a modification of the protocol described by Stebbins et al. (1991). Urea was measured as ammonia liberated from perchlorate extracts treated with urease. The urease reactions contained 25- and 50-μL aliquots of the perchlorate extract, 2.2 units of type IX jack bean urease (Sigma; 1 unit liberates 1 μmol of NH_3 from urea at 25°C, pH 7.0) in 200 μL of 50 mM KH_2/$NaHPO_4$, pH 7.0. Control reactions were identical except that urease was inactivated by the addition of 0.057 nmol PPD per unit of enzyme. All reactions, prepared in duplicate, were incubated for 30 min at 37°C and then diluted by the addition of 1.8 mL of dH_2O. Ammonia was measured by the phenol-hypochlorite method (Weatherburn, 1967): 200 μL each of phenol nitroprusside and alkaline hypochlorite reagent (Sigma) were added to each reaction, mixed by vortexing, and allowed to stand for 20 min at room temperature, and the A_{625} was determined. A standard curve was constructed using known concentrations of urea that were subjected to perchlorate extraction and urease hydrolysis in the same manner as the samples.

Free Arg was measured as ammonia liberated from perchlorate extracts treated with arginase and urease. Values were corrected for endogenous ammonia and urea as determined above. Arginase reactions (30 min, 37°C) contained 25 or 50 μL of perchlorate extract and 3.4 units arginase (Sigma; 1 unit converts 1 μmol of Arg to urea per min at 37°C, pH 9.5) in 100 μL of 10 mM NaP_2O_7, pH 9.0.

Reagents

PACTPAT was a gift from Dr. G. Erik Peters (International Fertilizer Development Center, Muscle Shoals, AL). PPD was from ICN (Costa Mesa, CA). AP was from Sigma. All other chemicals were reagent or analytical grade and were obtained from either Sigma or Fisher Scientific.

RESULTS

Arabidopsis Seedling Urease Activity

Whole seedling urease specific activity was determined during the first 9 d of *Arabidopsis* development. Seeds were provided 5 mM NH_4NO_3 to ensure that nitrogen was not limiting and 1 μM $NiSO_4$ to ensure that urease, which requires Ni^{2+} for activity, would be fully active. The seed urease activity declined up to 2 DAG but then increased to a maximum (>70% of that of mature seed) in seedlings 8

DAG (Fig. 1). In plantlets 15 and 20 DAG activity was 60 and 25%, respectively, of that of seedlings 8 DAG (Zonia, 1992).

Urease Transcript Increases in Young Seedlings

Urease clones were recovered from an *Arabidopsis* Landsberg *erecta* genomic library (Chang and Meyerowitz, 1986) by a high-stringency screen with subclones JG12 and P9 of soybean urease clone LC4 (Krueger et al., 1987; Torisky et al., 1994). Twelve clones (AU1 and 11 replicas of AU10) appeared to overlap distinct regions of a 13th complete clone, AU14. Sequential hybridizations of the *Arabidopsis* urease clones with subclones JG13, JG12, P7, P9, and P19 of the soybean urease clone LC4 (Torisky et al., 1994) indicated a co-linear hybridization pattern (Fig. 2).

Total RNA was isolated from whole seedlings 2, 3, 4, 6, and 8 DAG, grown identically with plants from which the urease activity data of Figure 1 were derived. A northern blot probed with a mixture of fragments AU1-I and AU10-I (Fig. 2) revealed an approximately 3.5-kb hybridizing transcript that was barely detectable at 2 DAG and had accumulated to higher levels in the 4- to 8-DAG interval (Fig. 3). Thus, the peak of urease activity that occurs at 8 DAG (Fig. 1) is preceded by accumulation of urease transcript at (near) a maximal level at 4 DAG (Fig. 3).

Urea and Free Arg Pools in Developing Seedlings

We measured urea levels in mature seeds and in seedlings 0 to 9 DAG grown in the same medium (5 mM NH_4NO_3 plus 1 μM $NiSO_4$) as plantlets from which urease transcript (Fig. 3) and activity (Fig. 1) data were derived. There was a small peak in seedling urea 1 DAG, followed by a sharp increase beginning 3 DAG and resulting in a maximal or near-maximal level 9 DAG (Fig. 4). Thus, urea appears to be a product of seedling metabolism and accumulates in spite of the presence of seedling urease, whose activity also increases during 3 to 9 DAG (Fig. 1).

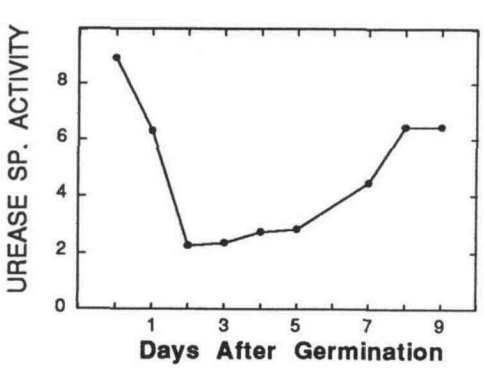

Figure 1. Urease-specific activity (nmol urea hydrolyzed min^{-1} mg^{-1} protein) in mature seed (0 DAG) and in *Arabidopsis* seedlings (1–9 DAG) maintained on 5 mM NH_4NO_3 plus 1 μM $NiSO_4$. Enzyme assays are averages of duplicate reactions; those for 0, 1, and 9 DAG are averages of three separate experiments. Replicates were within 7% of the average values shown.

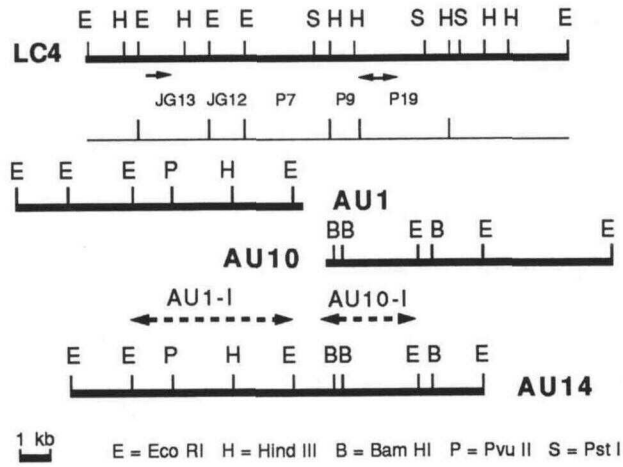

Figure 2. Restriction maps of *Arabidopsis* urease genomic clones. Correspondence to hybridizing regions from soybean urease genomic clone LC4 is indicated. LC4 subclones JG12 and P9 were used in *Arabidopsis* library screenings, and each of the five LC4 subclones was used individually to "walk" down the *Arabidopsis* urease gene. The single and double-headed arrows under the LC4 map indicate the translational start and stop codons, respectively. The dotted double arrows over the AU14 map indicate the AU1-I and AU10-I subclones used as combined probes in the northern blot analysis of *Arabidopsis* seedlings (Fig. 3).

To determine precursors to the seedling urea pool, we first sought to inhibit urea breakdown. Seeds were soaked and grown in PPD, a urease inhibitor (Fig. 5). Seedlings (7 DAG) grown in $\geq$20 μM PPD exhibited a $\geq$80% reduction in urease activity (measured in extracts without added PPD). In agreement, PPD caused at least a 2-fold increase of the urea pool of seedlings grown on water (Fig. 4). In these experiments, seeds were germinated on water to focus on urea generated from seed reserves.

Arg and ureides represent major potential sources of urea (Polacco and Holland, 1993). Ureides are produced by oxidative degradation of purines (Reynolds et al., 1982). To test a purine/ureide source of urea we soaked seeds in 100

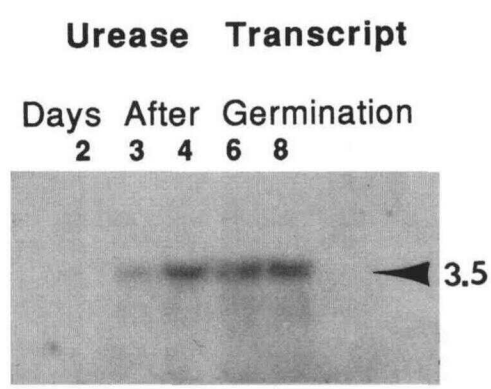

Figure 3. Urease transcript levels during seedling development. Total RNA (15 μg) from whole seedlings 2, 3, 4, 6, and 8 DAG (on 5 mM NH_4NO_3 plus 1 μM $NiSO_4$) was loaded per lane. The northern blot was probed with mixed subclones AU1-I and AU10-I (indicated in Fig. 2).

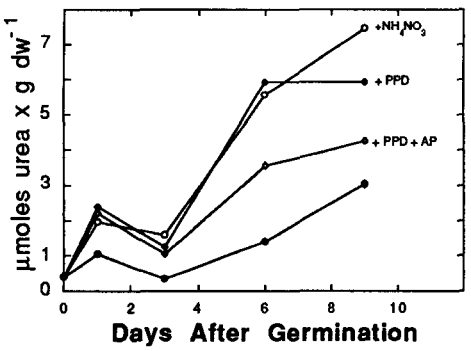

Figure 4. Urea levels in seed (0 DAG) and in seedlings 1 to 9 DAG. *Arabidopsis* seeds were germinated and grown on 1 µM $NiSO_4$ (●) and 1 µM $NiSO_4$ containing 100 µM PPD (◆), 100 µM PPD plus 1 mM AP (◇), or 5 mM NH_4NO_3 (○). All measurements are averages of duplicate reactions. Values for 0, 1, and 3 DAG are averages of three separate experiments. Values for 6 DAG are averages of two separate experiments. Individual values were within 3% of averages shown. dw, Dry weight.

µM PPD plus 1 mM AP. AP is a xanthine dehydrogenase inhibitor (Boland, 1981) that blocks production of ureides (allantoin and allantoate) in active soybean nodules (Triplett et al., 1980) and in soybean seedlings (Fujihara and Yamaguchi, 1978). The urea levels in seedlings soaked in 100 µM PPD or in 100 µM PPD plus 1 mM AP were essentially identical up to 3 DAG. However, AP reduced urea levels by 40 and 30% at 6 and 9 DAG (Fig. 4), respectively, suggesting that purine-derived ureides contribute to the seedling urea pool during this interval.

To test an Arg source for the remainder of the urea pool, we determined seedling Arg levels and arginase activity. Arg (Fig. 6) levels were higher than those of urea but the two pools changed in an approximately coordinate manner. We observed that arginase activity was indeed present

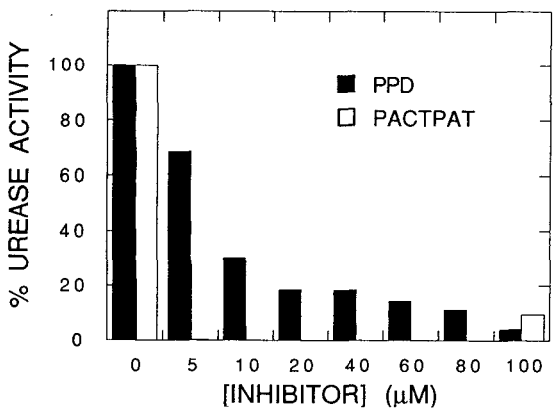

Figure 5. In vivo titration of urease activity with urease inhibitors PPD and PACTPAT. Urease-specific activity was measured in extracts of seedlings 7 (PPD, ■) or 9 DAG (PACTPAT, □) that had been allowed to imbibe and had been germinated and grown in the media containing the inhibitors. Before extracts were made, filters containing seedlings were washed with 100 mL of sterile dH_2O on a Buchner funnel and placed for 4 h in new media without inhibitor. Values are averages of duplicate reactions.

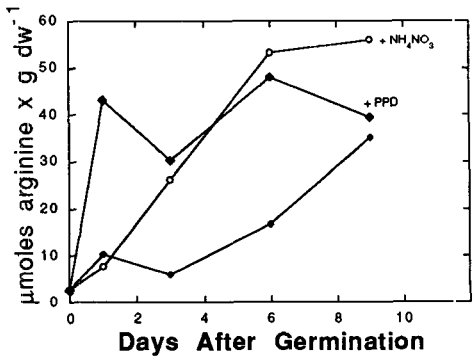

Figure 6. Free Arg levels in seed (0 DAG) and in seedlings 1 to 9 DAG. *Arabidopsis* seeds were germinated and grown on water (●), 100 µM PPD (◆), or 5 mM NH_4NO_3 (○). All media contained 1 µM $NiSO_4$ and all measurements are averages of determinations on duplicate plates. dw, Dry weight.

in whole seedlings and that it increased about 10-fold from 0 to 6 DAG (Fig. 7).

Urease Inhibition Blocks or Delays Germination and Early Seedling Development

We routinely observed that 100 µM PPD caused a 36-h delay in germination of water-soaked seeds, although subsequent development appeared normal. However, PPD effects on a 4-year-old "aged" lot of seeds were much more dramatic: Only 2 to 5% of these seeds germinated in 100 µM PPD in eight separate experiments, one of which is shown in Figure 8A. Of those that did germinate, their small, malformed seedlings never fully developed. Seeds from the same lot had approximately 100% germination on dH_2O (Fig. 8B) or 5 mM NH_4NO_3 (not shown). All treatments contained 1 µM $NiSO_4$.

If PPD inhibited *Arabidopsis* germination and development because of a critical deprivation of urea-nitrogen, then this inhibition should be overcome by adding nitrogenous compounds to the imbibition medium. Indeed, addition of 5 mM NH_4NO_3 (Fig. 8C) or 1 mg mL^{-1} casamino acids (Fig. 8D) overcame the effects of PPD. In separate tests NH_4^+ or NO_3^- did not block PPD root uptake and

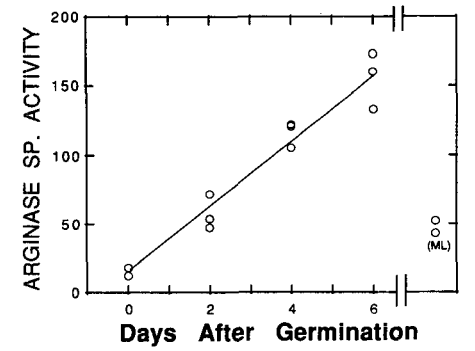

Figure 7. Arginase activity (nmol urea produced min^{-1} mg^{-1} protein) in whole seedlings. ML indicates the arginase activity of mature leaves pooled from soil-grown flowering plants. Points are individual replicate determinations.

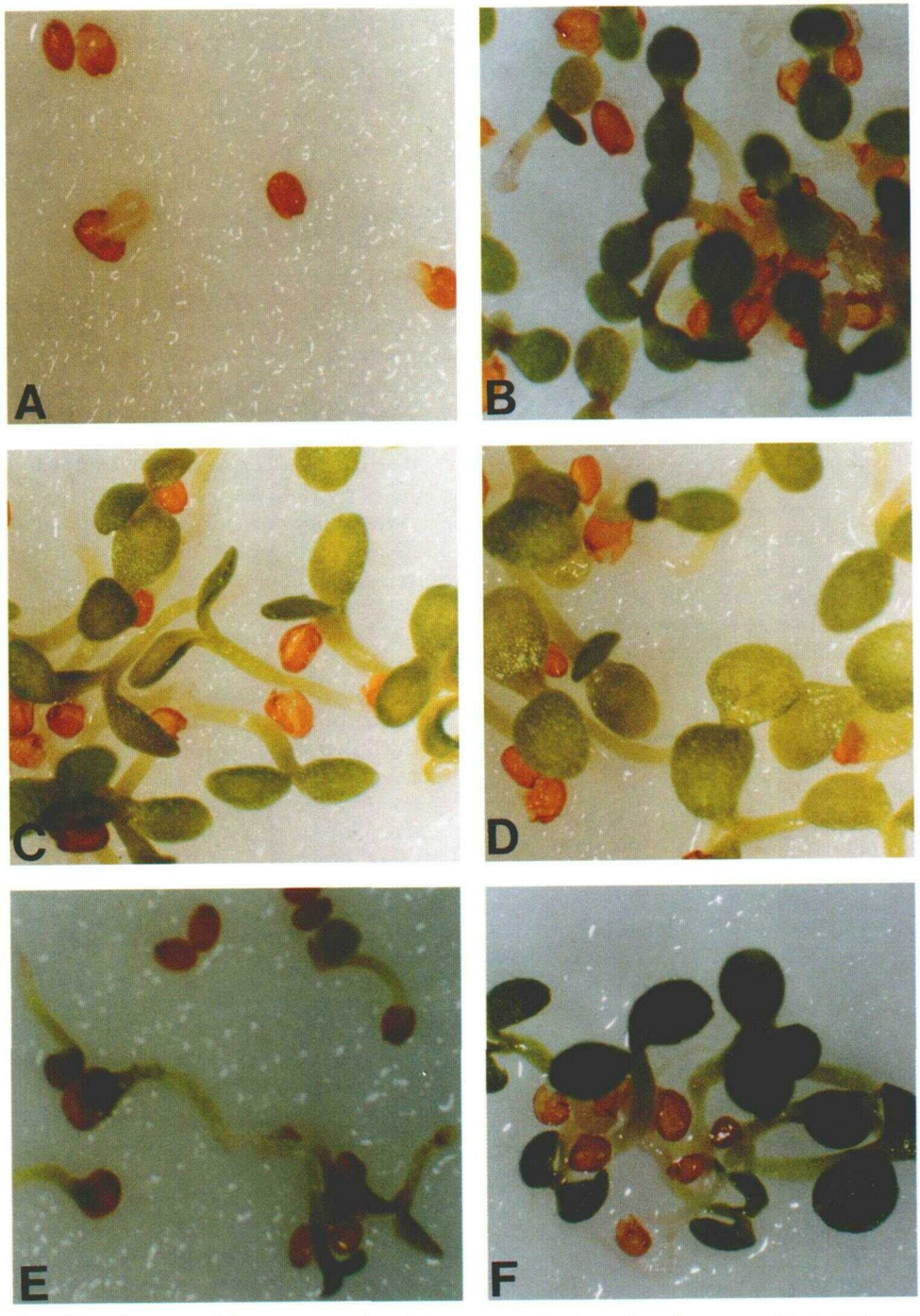

Figure 8. The effect of urease inhibitors PPD and PACTPAT on germination of aged (4 years old) *Arabidopsis* seeds and reversal of the effect by addition of nitrogenous compounds. Seeds are shown following imbibition on the following media (all containing 1 μM $NiSO_4$): A, 100 μM PPD (6 DAI); B, dH_2O (5 DAG); C, 100 μM PPD plus 5 mM NH_4NO_3 (4 DAG); D, 100 μM PPD plus 1 mg mL^{-1} casamino acids (4 DAG); E, 250 μM PACTPAT (5 DAG); F, 250 μM PACTPAT plus 5 mM NH_4NO_3 (5 DAG).

inactivation of leaf urease. Furthermore, PPD is not generally toxic, since seedlings transferred to 100 μM PPD at 5 to 9 DAG all showed continued growth and development indistinguishable from nontreated controls. This indicates also that urease activity is most important prior to 5 DAG.

When seeds were soaked in 250 μM PACTPAT, a second urease inhibitor (Peters et al., 1988) reported to be approximately 80% as effective as PPD in inhibiting microbial urease (Savant et al., 1988), germination of aged *Arabidopsis* seeds decreased by 50 to 70% (Fig. 8E). Surviving seedlings never fully developed and in many cases showed various malformations. As with PPD, inhibition by PACTPAT was overcome by 5 mM NH_4NO_3 (Fig. 8F). Like PPD, PACTPAT exhibited dose-dependent urease inhibition in extracts of

seedlings 7 DAG. The seedlings had germinated on the inhibitor. At 100 and 500 μM, PACTPAT reduced urease activity to 9 (Fig. 5B) and 4%, respectively, of that in extracts of nontreated seedlings.

DISCUSSION

The *A. thaliana* urease gene AU14 (Fig. 2) was recovered by its homology to soybean urease clone LC4, which contains the structural gene for the ubiquitous urease isozyme (Torisky et al., 1994). LC4 and AU14 subclones showed hybridization co-linearity. Nonoverlapping AU14 subclones, AU1-I and AU10-I, each mapped to a single locus on the *Arabidopsis* restriction fragment length polymorphism map and showed tight linkage (Hauge et al., 1993), suggesting that they fall within a single urease structural gene locus.

Northern blots probed with AU1-I plus AU10-I revealed that urease transcript accumulated to maximal or near-maximal levels by 4 DAG and remained high at least until 8 DAG (Fig. 3). Both urea and urease activity, like urease transcript, increased after germination and showed related patterns of accumulation: Urease activity had maxima in mature seed (0 DAG) and in seedlings 8 to 9 DAG; its substrate, urea, reached maximal levels at 1 and 9 DAG. These patterns suggest a urease role in recycling urea-nitrogen during *Arabidopsis* germination and early seedling development. The potential importance of this role was indicated by the failure of aged seeds to germinate in the presence of the urease inhibitor PPD (Fig. 8A), seeds that had a germination frequency of close to 100% on dH_2O (Fig. 8B). PPD germination inhibition was overcome by nitrogenous compounds, 5 mM NH_4NO_3 (Fig. 4C) or 1 mg mL^{-1} casamino acids (Fig. 4D), observations supporting a urease role in turning over seedling nitrogen reserves. PPD is a specific (Held et al., 1976; Kobashi et al., 1985) inhibitor of plant (Kerr et al., 1983) and microbial (Martens and Bremner, 1984) ureases. The jack bean urease-PPD complex is quite stable, with a half-life of 6.4 h (McCarty et al., 1990).

In *Arabidopsis*, large seeds produce seedlings that survive longer than those produced by small seeds when both are germinated and grown in dH_2O (Krannitz et al., 1991), suggesting that the larger seeds contain greater stores, which support growth in the absence of exogenous nutrients. Large seeds also tend to germinate at a higher frequency than small seeds, as demonstrated in *Lupinus texensis* (Schaal, 1980) and wild radish (Stanton, 1984). By depriving the seedling of that portion of its nitrogenous reserves catabolized to urea, PPD effects may mimic those observed with smaller seed size. In the presence of PPD, seedlings accumulated about 6 μmol urea g^{-1} dry weight (Fig. 4), which, as indicated below, is one-tenth of the urea that can be generated from arginase-catalyzed breakdown of all free and protein-bound seed Arg.

Ureides and Arg are possible sources of urea during germination and early seedling development (Polacco and Holland, 1993). We propose that all urea generated during the first 3 DAG is Arg derived, since AP did not diminish urea pools in PPD-treated seedlings up to 3 DAG (Fig. 4). Arginases appear to break down significant quantities of Arg released from bound reserves in germinating pea (de Ruiter and Kollöffel, 1983) and pumpkin (Chou and Splittstoesser, 1972). Deduced amino acids of the *Arabidopsis* 12S globulin (Pang et al., 1988) and 2S albumin (Krebbers et al., 1988) seed storage proteins contain 7.0 and 6.9 mol% Arg, respectively, whereas an "average" protein contains 3.0 mol% Arg (VanEtten et al., 1963). We recalculated the data of VanEtten et al. (1967): 11% of *Arabidopsis* seed nitrogen is in Arg, which is present (bound plus free) at close to 60 μmol g^{-1} dry weight. We found 2 μmol g^{-1} dry weight free Arg in the dry seed, suggesting that subsequent large increases in free Arg (Fig. 6) were due to proteolytic release of protein-bound reserves. Arginase activity, which increased 10-fold from 0 to 6 DAG (Fig. 7), could potentially release 60 μmol g^{-1} dry weight urea from Arg. Obviously, at least a portion of seed Arg has other fates, e.g. incorporation into new protein or generation of polyamines.

We cannot currently explain PPD-induced elevation of Arg pools. It is possible that deprivation of Arg nitrogen by urease inhibition stimulates increased Arg release from reserves. In any case, if Arg is catabolized to urea, arginase levels would be expected to increase during reserve mobilization. That urea accumulated to moderately high levels in seedlings germinated in dH_2O suggests that the site of urea production is compartmentalized so that urease, which is cytosolic in jack bean (Faye et al., 1986), does not have access to its substrate. Plant arginases are intramitochondrial (Taylor and Stewart, 1981; Polacco and Holland, 1993); thus, arginase-derived urea may not freely diffuse to cytoplasmic urease.

Although *Arabidopsis* leaves were reported to have no detectable arginase (Ludwig, 1993), we found leaf arginase at a level approximately 25% of that of the seedling at 6 DAG (Fig. 7). We assayed Arg-generated urea accumulating in the presence of the urease inhibitor PPD. Thus, we detected an activity that may not have been detectable by measuring Arg-generated Orn (Ludwig, 1993), which can be consumed in other reactions. We have recently cloned an *Arabidopsis* arginase (Krumpelman et al., 1995).

Degradation of ureides to urea by plants has been debated (Polacco and Holland, 1993). We observed a 30 to 40% reduction of urea pools by AP in *Arabidopsis* seedlings 5 to 8 DAG. AP is an effective xanthine dehydrogenase inhibitor in navy bean nodules (Boland, 1981) and inhibits ureide accumulation in soybean nodules (Triplett et al., 1980) and seedlings (Fujihara and Yamaguchi, 1978). If AP blocks ureide buildup from purines in *Arabidopsis* seedlings, then diminution of urea pools could indicate that a portion of urea is ureide derived in the interval 5 to 8 DAG.

In summary, increases in urea and urease activity during *Arabidopsis* seedling development are consistent with a urease role in recycling urea generated from catabolism of nitrogenous reserves. Much urea appears to be derived from Arg, since Arg pools (Fig. 6) are large and arginase activity (Fig. 7) increases sharply during seedling development. Ureide catabolism may have generated a portion of the urea pool during the interval between 5 and 8 DAG, since AP reduced the pool by 30 to 40%. The observed

increases in urea levels in PPD-treated seedlings and nitrogen-reversible PPD inhibition of germination of aged seeds both support the hypothesis that urease has an important role in seedling nitrogen metabolism.

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