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Amino Acid Utilization in Seeds of Loblolly Pine during Germination and Early Seedling Growth[1]

I. Arginine and Arginase Activity

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The mobilization and utilization of the major storage proteins in loblolly pine (*Pinus taeda* L.) seeds following imbibition were investigated. Most of the seed protein reserves were contained within the megagametophyte. Breakdown of these proteins occurred primarily following radicle emergence and correlated with a substantial increase in the free amino acid pool in the seedling; the majority of this increase appeared to be the result of export from the megagametophyte. The megagametophyte was able to break down storage proteins and export free amino acids in the absence of the seedling. Arginine (Arg) was the most abundant amino acid among the principal storage proteins of the megagametophyte and was a major component of the free amino acid pools in both the seedling and the megagametophyte. The increase in free Arg coincided with a marked increase in arginase activity, mainly localized within the cotyledons and epicotyl of the seedling. Arginase activity was negligible in isolated seedlings. Experiments with phenylphosphorodiamidate, a urease inhibitor, supported the hypothesis that arginase participates in Arg metabolism in the seedling. The results of this study indicate that Arg could play an important role in the nutrition of loblolly pine during early seedling growth.

In conifer seeds the embryo is enclosed within the corrosion cavity of the megagametophyte, a maternally derived haploid tissue that houses the majority of the storage reserves within the seed (Groome et al., 1991; Hakman, 1993). Proteins and lipids constitute the bulk of the storage reserves in seeds of most of the Pinaceae species examined to date (e.g. Ching, 1966; Simola, 1974; Kovac and Kregar, 1989; Owens et al., 1993), including those of loblolly pine (*Pinus taeda* L.) (Groome et al., 1991). Mobilization of these reserves occurs during germination and the early stages of seedling growth. The breakdown products are exported by the megagametophyte and are taken up by the developing seedling where they are used as a nutritive source. The composition of the chemical milieu exported by the megagametophyte is still largely undefined, as are the details of how metabolites and other molecules are transported from the megagametophyte to the developing seedling.

We have some knowledge of the fate of the products that result from lipid reserve mobilization in the conifer megagametophyte. Free fatty acids are released by lipase-mediated triacylglycerol breakdown (Hammer and Murphy, 1993, 1994). The free fatty acids are converted to Suc in the megagametophyte via β-oxidation, the glyoxylate cycle, and gluconeogenesis (Ching, 1972). Suc is exported to the developing seedling. In pinyon pine (*Pinus edulis* Engelm.) much of the exported Suc is converted to starch, which accumulates mainly within the cotyledons and hypocotyl of the seedling (Murphy and Hammer, 1994).

In contrast, much less is known about the mobilization of storage proteins in conifer seeds following imbibition. Proteolytic enzymes involved in protein reserve breakdown have been partially characterized in Scots pine (*Pinus sylvestris* L.) (Salmia and Mikola, 1976; Salmia et al., 1978; Salmia, 1981a, 1981b) and in lodgepole pine (*Pinus contorta* Dougl.) (Gifford et al., 1989). However, the processes by which the amino acid products of storage protein breakdown are transported from the megagametophyte to the developing seedling are unclear, as are the routes by which amino acids are integrated into metabolic and biosynthetic pathways in the seedling. Arg has been found to be a major component of seed storage proteins in maritime pine (*Pinus pinaster* Ait.) (Allona et al., 1992, 1994) and Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Leal and Misra, 1993), as well as of the soluble amino acid pools in germinated seeds of jack pine (*Pinus banksiana* Lamb.) (Ramaiah et al., 1971) and stone pine (*Pinus pinea* L.) (Guitton, 1964). Assimilation of Arg into biosynthetic and metabolic pathways is thought to occur mainly by conversion to Orn and urea via arginase (L-Arg amidinohydrolase, EC 3.5.3.1), a reaction of the urea (Krebs-Heinseleit) cycle (Bidwell and Durzan, 1975; Thompson, 1980; Bray, 1983). Micallef and Shelp (1989a, 1989b) have demonstrated using radiotracer and enzyme activity studies that both Arg biosynthesis and Arg catabolism occur via the urea cycle in developing soybean cotyledons. There also is some evidence that the urea pathway functions in seedlings of various pine species

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Abbreviations: DAI_2, days after imbibition at 2°C; DAI_{30}, days after imbibition at 30°C; PITC, phenylisothiocyanate; PPD, phenylphosphorodiamidate.

(Naylor, 1959; Guitton, 1964). Arginase activity has been demonstrated in stone pine (Guitton, 1964) and Aleppo pine (*Pinus halepensis* [Mill.]) (Citharel and Citharel 1975).

In this paper, as part of our continuing studies of reserve mobilization in pine seeds, we have examined the fate of amino acids released by protein reserve breakdown in the megagametophyte of loblolly pine during germination and early seedling growth. We have provided evidence that the megagametophyte, which houses the majority of the protein reserves in loblolly pine seeds, is the primary source of free amino acids for the seedling during early seedling growth. We have also tested the hypotheses that Arg is a significant component of the major storage proteins in loblolly pine seeds, and that arginase is involved in the metabolism of the free Arg pools that arise during early seedling growth.

MATERIALS AND METHODS

Loblolly pine (*Pinus taeda* L.) seeds (open pollinated clone 11-9, collected in 1992) were a gift from Westvaco (Summerville, SC). To improve germination, surface-sterilized, mature, desiccated seeds (Groome et al., 1991) were stratified at 2°C between layers of Kimpak for 35 d (35 DAI_2). Germination of 35 DAI_2 seeds was carried out at 30°C under constant 19 μmol m^{-2} s^{-1} light; under these conditions, radicle emergence occurred at 4 DAI_{30}. In this paper the term embryo is used to refer to the sporophyte in mature, desiccated seeds and in stratified seeds. In all other stages of postembryonic growth, the sporophyte is referred to as a seedling. Seedlings were staged using the radicle length criteria outlined by Mullen et al. (1996). Fresh material was used for all of the assays, whereas tissue that had been quick-frozen in liquid N and stored at −70°C was used for the HPLC amino acid analyses.

In organ localization studies whole embryos or seedlings were divided into two parts by a single transverse cut before assaying, just below the point at which the cotyledons diverge from the hypocotyl. The shoot pole segment consisted of the cotyledons and shoot (or shoot apex), whereas the root pole segment was made up of the hypocotyl and root (or radicle).

For in vitro experiments, seed parts were excised from surface-sterilized 35 DAI_2 seeds under aseptic conditions and cultured on 3% (w/v) Bacto-Agar with 60 mM Suc, 15 μg/mL rifampicin, and 2.5 μg/mL amphotericin B. The latter three media components were filter-sterilized prior to addition to the autoclaved agar. Cultures were incubated as described above. Radicle emergence for embryos cultured with intact megagametophytes consistently occurred at 2 d in culture; seedlings developed at approximately the same rate as the seedlings in the germination trays.

For experiments testing the ability of isolated megagametophytes to export amino acids into an artificial sink, megagametophytes were cut along the longitudinal sagittal plane and cultured with the cut surface proximal to the surface of the medium. For these experiments, the medium consisted of 0.8% (w/v) Bacto-Agar with 60 mM Suc, 15 μg/mL rifampicin, and 2.5 μg/mL amphotericin B, in quarter-sectioned Petri plates containing 7.5 mL of agar per section. The agar blocks were melted prior to analysis and treated as an extract.

For PPD inhibition studies, seedlings with intact megagametophytes were cultured under aseptic conditions on Kimpak dampened either with water or with 1.1 mM PPD, and 15 μg/mL rifampicin and 2.5 μg/mL amphotericin B. A higher concentration of PPD was used than that reported by Zonia et al. (1995) because the PPD needed first to be taken up by the megagametophyte and then exported to the enclosed embryo in order to inhibit ureases normally active in seedling tissues.

Protein Extractions and Quantification

Proteins not soluble in 50 mM sodium phosphate buffer (pH 7.5), but solubilized by heating pellets in Laemmli buffer (62.5 mM Tris [pH 6.8], 2% [w/v] SDS, and 10% [v/v] glycerol), were prepared using the differential extraction procedure of Gifford et al. (1982). These are referred to as buffer-insoluble protein extracts. For total seed protein, megagametophytes and embryos or seedlings were extracted four times in phosphate buffer and four times in Laemmli buffer, and determined as the sum of the protein content of each of these washes.

Protein content was assayed using the method of Lowry et al. (1951). BSA was used to generate a standard curve. For each data point, three independent replicates were assayed in duplicate.

Storage proteins were quantified by scanning gel densitometry of SDS-PAGE. SDS-PAGE of 18% separating gels was carried out under reducing conditions in the presence of 2-mercaptoethanol, as described by Groome et al. (1991); gels were stained with Coomassie blue R. Gel profiles were quantified by densitometry at 560 nm using a spectrophotometer (DU-65, Beckman). Peak areas were used to determine the amount of protein represented by the individual storage proteins at each stage of development.

Buffer-Insoluble Protein Hydrolysis

To ensure removal of contaminating lipid and carbohydrate from buffer-insoluble extracts prepared for HPLC analysis of storage protein hydrolysate, the Gifford et al. (1982) procedure was modified to include ice-cold chloroform:methanol (2:1, v/v) and 80% (v/v) ethanol washes. Megagametophytes were extracted twice in 1 mL of ice-cold chloroform:methanol (2:1, v/v), twice in 1 mL of 0.05 M phosphate buffer containing 0.1 mM leupeptin, twice with ice-cold 80% ethanol, and once again with 1 mL of phosphate buffer plus leupeptin. Buffer-insoluble proteins were extracted by heating the pellets for 5 min in 500 μL of Laemmli buffer. Proteins were precipitated from 50-μL aliquots of the resulting supernatant with ice-cold 80% (v/v) acetone. The pellet was rinsed twice with 80% (v/v) acetone to remove as much SDS from the sample as possible.

Protein Hydrolysis

Acetone-precipitated pellets were resuspended in 500 μL of constant-boiling 6 N HCl containing 0.1% phenol. Ten percent of each sample was brought up to a final volume of

150 μL of constant-boiling 6 N HCl plus 0.1% phenol for hydrolysis. Hydrolysis was carried out in evacuated, sealed, thick-walled borosilicate glass tubes for 1 h at 160°C. Cooled ampules were opened and samples were dried in a concentrator (Speed-Vac, Savant, Farmington, NY). Samples were stored at −70°C until derivatized. Lysozyme was also analyzed, and the results were compared with published values to ensure the accuracy of the technique.

Free Amino Acid Extraction for HPLC

Free amino acid extracts were prepared for HPLC analysis from approximately 0.25 g (fresh weight) of seed parts by the method of Tuin and Shelp (1994). As an internal standard, 600 nmol of norleucine was added to each sample during homogenization. Extracts were filtered first through a 0.22-μm syringe filter (Millex-GV, Millipore), then through a 10,000 molecular weight cut-off polysulfone membrane filter unit (Ultrafree MC, Millipore) to remove residual proteins. Fifty microliters of filtered extract was vacuum-dried using a concentrator (Speed-Vac, Savant, Holbrook, NY) prior to derivatization.

HPLC Analysis

Samples were derivatized with PITC (Pierce) according to Bidlingmeyer et al. (1984). Free amino acid samples were redried twice with a methanol:1 M sodium acetate:triethylamine (Pierce) (2:2:1, v/v) solution, prior to derivatizing with methanol:water:triethylamine:PITC (7:1:1:1, v/v). Protein hydrolysates were redried twice with ethanol:water:triethylamine (2:2:1, v/v), and derivatized with ethanol:water:triethylamine:PITC (7:2:1:1, v/v).

Samples were analyzed by reverse-phase HPLC on a Waters system equipped with a Waters 712 WISP and a Waters system interface module linking the HPLC to a Baseline 810 workstation. A 3.9 × 300 mm Pico-Tag free amino acid analysis column maintained at 46°C by a Waters temperature-control module was used in accordance with the manufacturer's recommended procedures for physiologic amino acid analysis (Waters). Acidic/neutral and basic physiologic amino acid standards (Sigma) and hydrolysate amino acid standards (Beckman) were used for peak identification and subsequent calculations. Three independent replicates for each developmental stage were analyzed. Selected replicates were analyzed in duplicate to ensure repeatability.

Ninhydrin Soluble Amino Acid Quantification

Amino acids soluble in 50 mM sodium phosphate buffer (pH 7.5) were quantified by the ninhydrin assay (Rosen, 1957). Three independent replicates were analyzed in triplicate for each data point. To minimize protease activity, 0.1 mM leupeptin was included in the phosphate buffer. The presence of leupeptin in the extract did not affect the absorbance values of the ninhydrin reaction.

Arginase Activity Determinations

Five to 10 seed parts were extracted as described by Martin-Falquina and Legaz (1984). The enzyme was activated by preincubating extracts for 60 min at 30°C prior to the assay. Enzyme activity was assayed by incubating 100 μL of either the activated or boiled extract with 0.285 M Arg (pH 9.7), 1 mM $MnSO_4$, and 1 mM maleate in a total volume of 1.5 mL for 30 min at 30°C; the reaction was terminated with 0.7 volume of 87% (v/v) acetic acid (Greenberg, 1955). Urea production was measured by the colorimetric method of Geyer and Dabich (1971). To account for any endogenous urea present in the crude enzyme extract that might artificially inflate the quantity of urea measured in the reaction mixture, the assay was performed with both activated and boiled aliquots subsampled from the same extract. Endogenous urea levels, determined from the boiled controls, were subtracted from total urea levels to obtain a measure of urea evolved by arginase activity, expressed as μmol of urea per minute. Three independent replicates were analyzed in triplicate for each data point. Selected samples were also assayed in the presence of the urease inhibitor PPD (50 μM) (Zonia et al., 1995) to determine if any urease-mediated urea breakdown was occurring in the assay mixture.

RESULTS

The Major Seed Storage Proteins

Phosphate buffer-insoluble proteins in the megagametophyte made up 72 ± 2% of the total protein in mature, desiccated seeds of loblolly pine. These buffer-insoluble proteins made up 80.3 ± 1.2% of the total protein in the megagametophytes of these seeds. In the megagametophyte the buffer-insoluble proteins with molecular masses of 47, 37.5, and 22.5 kD (Fig. 1B) were previously reported to be seed storage proteins by Groome et al. (1991). An additional buffer-insoluble storage protein with a molecular mass of 13 to 14 kD (Fig. 1B) was identified, which was fully resolved using 18% SDS-PAGE (results not shown). Peak areas obtained by scanning densitometry of triplicate 18% SDS-PAGE profiles indicated that the 47-, 37.5-, 22.5-, and 13- to 14-kD proteins made up approximately 4.5 ± 0.6, 35.8 ± 2.4, 26.3 ± 2.4, and 30.5 ± 3.0% (mean ± SD), respectively, of the buffer-insoluble proteins in the mature megagametophyte. Together, these proteins consistently represented greater than 95% of the total buffer-insoluble fraction.

Quantifying the individual storage proteins either as micrograms of storage protein per megagametophyte (data not shown) or as micrograms of storage protein per micrograms of total seed protein (Fig. 1A) produced similar plots. Little breakdown of these proteins occurred during stratification and germination; notable proteolysis did not occur until after radicle emergence, at 4 DAI_{30}. The 37.5-, 22.5-, and 13- to 14-kD storage proteins shared a similar pattern of disappearance. By 12 DAI_{30}, approximately 96, 86, and 99% of the 37.5-, 22.5-, and 13- to 14-kD proteins, respectively, had been broken down. The 47-kD storage protein, a much smaller contributor to the protein reserves than the 37.5-, 22.5-, and 13- to 14-kD proteins, was broken down more quickly following radicle emergence than the other buffer-insoluble storage proteins. Levels of this protein had declined to below detectable limits by 9 DAI_{30}.

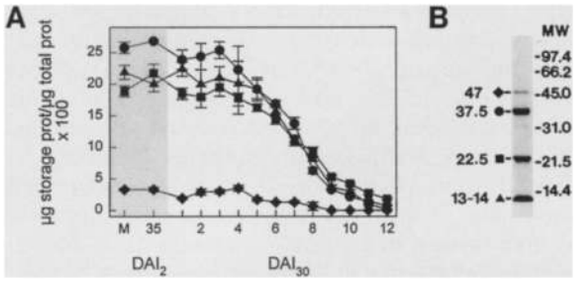

Figure 1. The major buffer-insoluble seed storage proteins in megagametophytes of loblolly pine. A, Quantitative changes in the 47-kD (♦), 37.5-kD (●), 22.5-kD (■), and 13- to 14-kD (▲) buffer-insoluble storage proteins following seed imbibition, determined by scanning densitometry at 560 nm of triplicate Coomassie blue-stained 18% SDS-PAGE profiles run under reducing conditions. The data are expressed as the percentage that each of the storage proteins represents of the total protein content of the megagametophyte plus embryo or seedling, determined as described in "Materials and Methods," at each stage of development. Total protein values are a mean of three independent replicates; the median replicate for each stage was used for the SDS-PAGE. Error bars show ±SE and are not presented when smaller than the symbols. M, Mature, desiccated seed. B, Coomassie blue-stained 12% SDS-PAGE profile of buffer-insoluble proteins extracted from mature megagametophytes under reducing conditions. Symbols to the left of each storage protein correspond to the symbols used in A. Molecular mass markers are indicated to the right of the gel.

Insoluble Storage Protein Analysis

The chloroform:methanol and ethanol pretreatments did not affect the insoluble storage protein yield or SDS-PAGE profile (data not shown) when compared with the standard method for the extraction of buffer-insoluble proteins (Gifford et al., 1982).

HPLC analysis of the hydrolysate prepared from the megagametophyte buffer-insoluble proteins revealed that Arg constituted 23.4 mol% of the amino acid content of the polypeptides, and was the most abundant amino acid in the hydrolysate (Table I). Together, glutamate and Gln, analyzed as Glx, made up 21.0 mol% of the amino acid hydrolysate. If the assumption is made that Glx is composed entirely of Gln, yielding two N per molecule of Glx, then Glx contributes approximately 20.5 ± 1.2% of the total N in the buffer-insoluble storage proteins of the megagametophyte. This leads to the most conservative estimate that Arg, with four N per molecule, constitutes approximately 45.8 ± 0.8% of the N in these proteins (Table I).

Free Acid Pool Quantification

Quantification of soluble amino acid levels by ninhydrin assay indicated that prior to radicle emergence (4 DAI_{30}), the levels of soluble amino acids in the seedling were approximately one-half that found in the megagametophyte (Fig. 2). However, whereas levels of soluble amino acids increased by 8-fold in the megagametophyte following imbibition, corresponding levels within the seedling increased by 152-fold, most notably following radicle emergence. The data demonstrated the same relative changes when expressed on a per milligram soluble protein basis (data not shown).

Table I. *Amino acid composition of the buffer-insoluble proteins extracted from 35 DAI_2 loblolly pine megagametophytes*

Values are the means ± SE of three independent replicates. Proteins were hydrolyzed with 6 N constant-boiling HCl with 0.1% phenol for 1 h at 160°C. Glutamate and Gln were analyzed together as Glx; similarly, aspartate and Asp were analyzed as Asx. Cys and Trp levels were not determined.

Amino Acid	mol%[a]	mol% N[b]
Asx	6.88 ± 0.23	6.72 ± 0.22
Glx	21.00 ± 1.25	20.51 ± 1.21
Ser	6.15 ± 0.07	3.01 ± 0.03
Gly	5.81 ± 0.21	2.83 ± 0.10
His	1.69 ± 0.01	2.48 ± 0.01
Thr	1.76 ± 0.06	0.86 ± 0.03
Ala	5.28 ± 0.10	2.58 ± 0.05
Arg	23.44 ± 0.42	45.80 ± 0.84
Pro	5.15 ± 0.10	2.57 ± 0.08
Tyr	3.15 ± 0.02	1.54 ± 0.01
Val	4.14 ± 0.17	2.02 ± 0.09
Met	1.69 ± 0.09	0.83 ± 0.05
Ile	2.25 ± 0.01	1.10 ± 0.01
Leu	5.19 ± 0.07	2.53 ± 0.03
Phe	1.36 ± 0.33	0.67 ± 0.16
Lys	1.05 ± 0.05	1.03 ± 0.04

[a] Calculations are based on the prediction that Cys and Trp together account for no greater than 4 mol% of the amino acid composition. [b] Based on the most conservative assumption that Glx = 100% Gln and Asx = 100% Asn.

Although soluble amino acid levels in seedlings cultured with an intact megagametophyte increased 62-fold after 10 d in vitro, levels in isolated seedlings increased by only 8-fold during the same period (Fig. 3A). In contrast to isolated seedlings, isolated megagametophyte halves exhibited a 28-fold increase in soluble amino acids, whereas amino acid pools in intact megagametophytes cultured with seedlings increased by 8-fold (Fig. 3B). Soluble amino acid accumulation by isolated megagametophyte halves cultured with the cut surface proximal or distal to the agar was nearly identical (data not shown). The same relative changes were seen

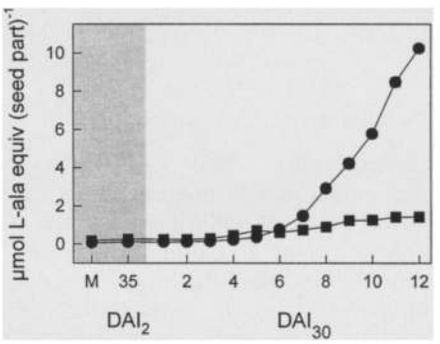

Figure 2. Quantitative changes in the soluble amino acid content of seedlings (●) and megagametophytes (■) from intact seed following imbibition, determined by ninhydrin assay. Values are means ± SE of three independent replicates; error bars are not evident because SE values are smaller than the symbols.

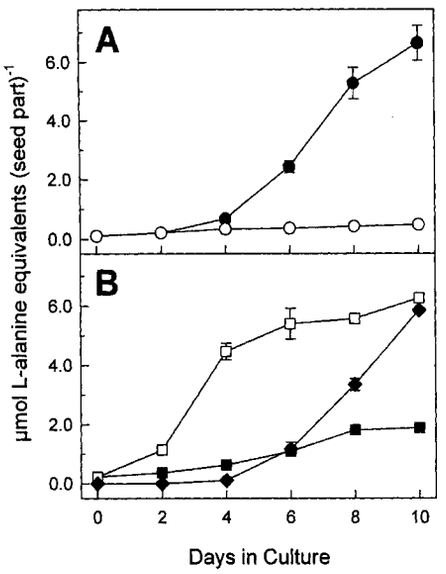

Figure 3. The effect of one seed part on soluble amino acid accumulation by the other. Excised seed parts were cultured on 3% (w/v) agar, except where noted, with 60 mM Suc, 15 µg/mL rifampicin, and 2.5 µg/mL amphotericin B. A, Seedlings cultured with (●) or without (○) intact megagametophytes. B, Megagametophytes cultured intact with seedlings (■) or as isolated megagametophyte halves cut along a median longitudinal sagittal plane and placed with the cut surface proximal to the surface of the agar (□). Amino acid export from isolated megagametophyte halves into 0.8% (w/v) agar (◆) is also shown. Values are means ± SE of three independent replicates; where error bars are not evident, SE values are smaller than the symbols.

when the in vitro amino acid data were expressed on a per milligram soluble protein basis (data not shown).

The isolated megagametophyte halves also exported amino acids in the absence of the seedling (Fig. 3B), in a pattern similar to that of the accumulation of amino acids by seedlings incubated with an intact megagametophyte (Fig. 3A). Preliminary experiments demonstrated that isolated megagametophyte halves will also export amino acids when cultured with the cut surface distal to the agar, i.e. in the absence of a sink, and that exudate from the corrosion cavity has a high amino acid content (data not shown).

HPLC Free Amino Acid Pool Analysis

Relatively minor changes in the proportions of amino acids or the overall free amino acid pool size occurred during stratification, as demonstrated by a comparison of the amino acid profiles of mature, desiccated, and 35 DAI_2 embryos (Table II) and megagametophytes (Table III). Aspartate, glutamate, Asn, Arg, and Pro were the major amino acids in the mature, desiccated, and 35 DAI_2 embryos; aspartate, glutamate, Asn, and Arg were major amino acids in the mature, desiccated, and 35 DAI_2 megagametophytes. Aspartate, glutamate, and Arg levels displayed the greatest relative increases in the embryo over the course of stratification. Levels of glutamate demonstrated the largest increase in the megagametophyte during this period.

Glutamate, Gln, aspartate, and Arg were prominent in 3 DAI_{30} seedlings. Glutamate, Gln, and Arg demonstrated the largest increases in the seedling from 35 DAI_2 to 3

Table II. *Soluble amino acid composition and pool size of loblolly pine seedlings following seed imbibition*

Amino acid values are expressed as nmol/seedling. Total protein, quantified as described in "Materials and Methods," is expressed as µg/seedling, and is included for comparison. All values are the means ± SE of three independent replicates.

Amino Acid	Mature Desiccated	35 DAI_2	3 DAI_{30}	6 DAI_{30}	9 DAI_{30}	12 DAI_{30}
Asp	2.46 ± 0.35	4.82 ± 0.72	9.17 ± 1.86	6.74 ± 0.24	30.3 ± 1.4	80.1 ± 10.3
Glu	5.67 ± 0.51	10.6 ± 1.6	28.5 ± 5.6	50.5 ± 8.7	138 ± 15	248 ± 23
Ser	0.74 ± 0.02	0.77 ± 0.13	2.00 ± 0.16	13.7 ± 2.3	53.2 ± 4.2	152 ± 2
Asn	2.88 ± 0.15	3.05 ± 0.64	6.12 ± 0.13	742 ± 48	4388 ± 519	12924 ± 1042
Gly	0.25 ± 0.01	0.19 ± 0.01	0.75 ± 0.07	1.15 ± 0.41	4.70 ± 1.09	ND[a]
Gln	1.07 ± 0.09	0.68 ± 0.14	10.1 ± 1.5	92.1 ± 9.9	905 ± 157	1699 ± 286
His	0.21 ± 0.02	0.27 ± 0.04	0.97 ± 0.12	12.3 ± 1.3	61.4 ± 1.8	195 ± 13
Thr	0.22 ± 0.06	0.57 ± 0.04	0.78 ± 0.09	5.82 ± 0.42	17.3 ± 1.8	45.5 ± 5.7
Ala	1.25 ± 0.01	0.63 ± 0.11	5.47 ± 0.78	39.2 ± 3.2	76.1 ± 7.1	174 ± 13
Arg	4.29 ± 0.25	7.09 ± 1.11	15.2 ± 0.6	141 ± 14	755 ± 53	2326 ± 193
Pro	3.12 ± 0.08	4.77 ± 0.95	6.95 ± 1.00	13.3 ± 2.9	32.2 ± 4.0	38.7 ± 7.6
Tyr	0.47 ± 0.04	0.92 ± 0.13	1.39 ± 0.19	14.2 ± 2.1	7.68 ± 0.25	19.0 ± 7.6
Val	0.39 ± 0.01	0.95 ± 0.06	1.50 ± 0.07	39.7 ± 0.5	140 ± 2	254 ± 12
Met	0.08 ± 0.01	0.07 ± 0.01	0.25 ± 0.02	1.68 ± 0.10	2.52 ± 0.71	4.85 ± 1.28
Cys	BDL[b]	BDL	BDL	0.15 ± 0.01	14.6 ± 7.7	66.7 ± 7.4
Ile	0.16 ± 0.01	0.46 ± 0.01	0.46 ± 0.04	13.9 ± 1.1	32.4 ± 0.9	63.4 ± 3.3
Leu	0.11 ± 0.01	0.45 ± 0.07	0.57 ± 0.05	14.9 ± 2.5	26.5 ± 5.5	35.5 ± 2.3
Phe	0.21 ± 0.01	0.53 ± 0.19	0.40 ± 0.03	6.92 ± 0.17	15.4 ± 1.7	17.0 ± 1.2
Trp	0.16 ± 0.01	0.17 ± 0.02	0.19 ± 0.02	3.18 ± 0.10	17.2 ± 0.9	45.9 ± 3.5
Lys	0.10 ± 0.01	0.10 ± 0.02	0.28 ± 0.09	2.27 ± 0.25	3.19 ± 0.69	12.7 ± 3.1
Total	23.9 ± 0.3	38.5 ± 4.2	91.1 ± 2.0	1215 ± 57	6699 ± 667	18330 ± 1423
Total protein	210 ± 10	210 ± 10	225 ± 8	500 ± 16	1306 ± 65	2079 ± 68

[a] ND, Not determined. [b] BDL, Below detection limit.

Table III. *Soluble amino acid composition and pool size of loblolly pine megagametophytes following seed imbibition*

Amino acid values are expressed as nmol/megagametophyte. Total protein, quantified as described in "Materials and Methods," is expressed as μg/megagametophyte, and is included for a comparison. All values are the means ± SE of three independent replicates.

Amino Acid	Mature Desiccated	35 DAI_2	3 DAI_{30}	6 DAI_{30}	9 DAI_{30}	12 DAI_{30}
Asp	7.14 ± 0.71	8.53 ± 1.09	24.1 ± 0.9	16.7 ± 4.0	20.7 ± 4.1	17.6 ± 2.7
Glu	12.1 ± 1.4	21.7 ± 3.9	52.8 ± 1.2	73.7 ± 3.1	80.1 ± 10.7	50.4 ± 13.2
Ser	3.10 ± 0.54	1.85 ± 0.12	6.76 ± 0.46	14.7 ± 1.8	22.7 ± 1.8	49.7 ± 4.3
Asn	14.6 ± 3.5	10.3 ± 0.1	10.8 ± 1.0	27.7 ± 3.0	35.6 ± 2.6	45.3 ± 5.4
Gly	0.90 ± 0.09	0.86 ± 0.22	1.71 ± 0.21	4.69 ± 0.92	8.74 ± 0.30	22.8 ± 1.9
Gln	4.82 ± 0.61	2.87 ± 0.57	14.8 ± 4.8	34.2 ± 5.3	28.5 ± 6.9	77.2 ± 4.3
His	0.72 ± 0.18	0.91 ± 0.14	1.85 ± 0.15	5.99 ± 0.56	11.6 ± 0.9	21.3 ± 1.8
Thr	1.03 ± 0.30	1.66 ± 0.59	1.94 ± 0.13	6.83 ± 0.80	12.6 ± 0.6	30.4 ± 1.0
Ala	1.75 ± 0.57	1.60 ± 0.13	10.8 ± 4.6	14.0 ± 3.8	12.8 ± 0.4	26.7 ± 3.1
Arg	8.94 ± 1.60	10.9 ± 0.3	19.4 ± 2.3	73.0 ± 2.7	213 ± 14	241 ± 16
Pro	2.41 ± 0.76	2.55 ± 0.24	9.38 ± 0.64	26.5 ± 2.0	47.9 ± 3.6	114 ± 7
Tyr	1.76 ± 0.35	2.33 ± 0.14	5.42 ± 0.45	15.3 ± 3.4	20.1 ± 0.4	28.4 ± 2.3
Val	1.94 ± 0.26	2.64 ± 0.24	5.99 ± 0.27	15.4 ± 2.8	21.6 ± 1.1	44.7 ± 2.6
Met	0.50 ± 0.02	0.42 ± 0.03	1.37 ± 0.25	2.77 ± 0.06	3.19 ± 0.13	5.40 ± 0.41
Cys	BDL[a]	BDL	BDL	BDL	0.73 ± 0.40	1.44 ± 0.16
Ile	1.07 ± 0.12	1.85 ± 0.18	2.72 ± 0.02	6.29 ± 0.35	11.0 ± 0.4	21.8 ± 0.7
Leu	1.09 ± 0.09	2.57 ± 0.32	3.92 ± 0.25	8.35 ± 0.43	18.4 ± 1.8	26.7 ± 1.0
Phe	0.63 ± 0.10	0.95 ± 0.07	1.37 ± 0.36	5.01 ± 0.46	12.8 ± 0.4	15.4 ± 0.8
Trp	1.02 ± 0.10	1.37 ± 0.11	1.57 ± 0.09	4.55 ± 0.67	13.5 ± 0.5	16.0 ± 3.5
Lys	0.37 ± 0.02	0.47 ± 0.06	0.53 ± 0.09	1.26 ± 0.26	2.74 ± 0.15	5.71 ± 0.87
Total	65.9 ± 6.0	76.4 ± 4.4	177 ± 10	344 ± 17	598 ± 32	862 ± 61
Total protein	1816 ± 78	1772 ± 76	1797 ± 93	1904 ± 115	1489 ± 63	831 ± 83

[a] BDL, Below detectable limit.

DAI_{30}. Aspartate, glutamate, and Arg were the major amino acids in the megagametophyte at 3 DAI_{30}; these same amino acids also showed the greatest increase from 35 DAI_2 to 3 DAI_{30}.

The most pronounced changes in the amino acid profiles in the seed occurred following radicle emergence, i.e. in 6 DAI_{30}, 9 DAI_{30}, and 12 DAI_{30} seedlings. Large increases in the amounts of a few amino acids, rather than uniform increases in many amino acids, accounted for much of the dramatic rise in amino acid content of the seedling following radicle emergence (Table II). The same was also true, although to a lesser degree, for the megagametophyte (Table III). Levels of Arg and Gln continued to increase in 6 DAI_{30}, 9 DAI_{30}, and 12 DAI_{30} seedlings; these amino acids were the second and third most abundant amino acids, respectively, in seedlings from these stages of development (Table II). Levels of Asn, which was not especially prevalent in the earlier stages of development, escalated following radicle emergence to make it the most abundant amino acid in 6 DAI_{30}, 9 DAI_{30}, and 12 DAI_{30} seedlings. Asn made up 70% of the free amino acids in the 12 DAI_{30} seedling, whereas Arg accounted for 13% of the free amino acid pool in 12 DAI_{30} seedlings. Levels of glutamate increased in the seedling through all of the stages examined, but represented a smaller percentage of the free amino acid pool in 9 DAI_{30} and 12 DAI_{30} seedlings than during the earlier stages of development. Arg constituted the highest percentage of the free amino acid pool in 9 DAI_{30} and 12 DAI_{30} megagametophytes (Table III), making up approximately 28% of the free amino acid pool in the 12 DAI_{30} megagametophyte, a time at which storage protein hydrolysis was well advanced (Fig. 1A). Glutamate was also a major amino acid in the megagametophyte at 6 DAI_{30}, 9 DAI_{30}, and 12 DAI_{30}, even though levels of glutamate declined from 9 DAI_{30} to 12 DAI_{30}. Levels of Pro and Gln increased quite substantially from 9 DAI_{30} to 12 DAI_{30}; these amino acids represented the second and third largest components, respectively, of the free amino acid pool in the 12 DAI_{30} seedlings. Asn did not constitute a large proportion of the amino acid pool in the megagametophyte.

Gly concentrations were not determined for 12 DAI_{30} seedlings because the very large Asn peak did not permit satisfactory resolution of the small Gly peak.

Arginase Activity

To address concerns that urease present in the extract might break down urea evolved by the activity of arginase, resulting in an underestimate of arginase activity, selected samples were assayed in the presence of the urease inhibitor PPD. There was no significant difference in levels of urea determined in the presence or absence of PPD in these samples at $\alpha = 0.05$ (data not shown). Since the pH optima for ureases in other species (see Torisky and Polacco, 1990) are lower than the pH optimum for loblolly pine arginase (9.7; data not shown), urease activity may have been reduced below detectable limits in the assay mixture.

When expressed as specific activity, arginase activity increased 6-fold in the seedling following imbibition, and peaked at 8 DAI_{30}. A 4-fold increase in arginase specific activity was observed in the megagametophyte, reaching a maximum at 6 DAI_{30} (Fig. 4A). Peak arginase specific ac-

tivity was 3.6-fold greater in the seedling than in the megagametophyte. When expressed on a per-seed-part basis, there was a 43-fold increase in arginase activity in the seedling from mature, desiccated seed to peak activity at 11 DAI_{30}; arginase activity in the megagametophyte increased a modest 6-fold following imbibition, reaching a peak at 12 DAI_{30} (Fig. 5A). The increase in arginase activity in the seedling coincided with the accumulation of soluble amino acids in the seedling (Fig. 2), particularly the increase in Arg (Table II). In 35 DAI_2 embryos, 70% of the total specific activity of arginase was located in the shoot pole segment (Fig. 4B). At 8 DAI_{30}, 91% of the specific arginase activity of the seedling was found in the shoot pole segment. On a per-seed-part basis, the percentage of arginase activity localized in the shoot pole segment increased from 63% in 35 DAI_2 embryos to 94% in 12 DAI_{30} seedlings (Fig. 5B). Arginase activity did not increase in seedlings cultured in the absence of the megagametophyte (Fig. 6).

The development of seedlings with intact megagametophytes grown in the presence of the urease inhibitor PPD was compromised. After 8 d in culture, the average radicle length of seedlings cultured on Kimpak saturated with 1.1 mM PPD was 7 ± 6 mm (mean ± SD, n = 90), whereas that of seedlings cultured on water-saturated Kimpak was 56 ± 31 mm (mean ± SD, n = 90). The accumulation of Tris buffer-soluble proteins was also lower in seedlings cultured with PPD than in seedlings cultured with water; PPD-treated seedlings contained 72.6 ± 9.2 μg soluble protein mg^{-1} dry weight, whereas control seedlings contained 97.7 ± 8.8 μg soluble protein mg^{-1} dry weight. Levels of urea per milligram of dry weight, as determined by colorimetric assay of heat-inactivated Tris-soluble ex-

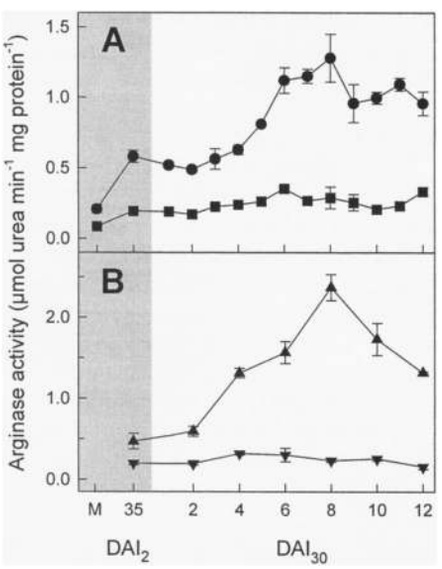

Figure 4. Arginase-specific activity (μmol urea min^{-1} mg^{-1} protein) in loblolly pine seed following imbibition. A, Arginase activity in seedlings (●) and megagametophytes (■) from intact seed, and B, in seedlings from intact seed bisected into root pole (▼) and shoot pole (▲) segments prior to assaying. Values are means ± SE of three independent replicates; where error bars are not evident, SE values are smaller than the symbols. M, Mature, desiccated seed.

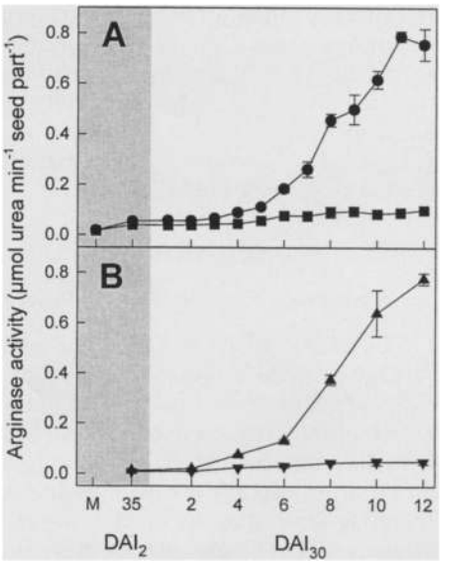

Figure 5. Arginase activity (μmol urea min^{-1} seed $part^{-1}$) in loblolly pine seeds following imbibition. A, Arginase activity in seedlings (●) and megagametophytes (■) from intact seed, and B, in seedlings from intact seed bisected into root pole (▼) and shoot pole (▲) segments prior to assaying. Values are means ± SE of three independent replicates; where error bars are not evident, SE values are smaller than the symbols. M, Mature, desiccated seed.

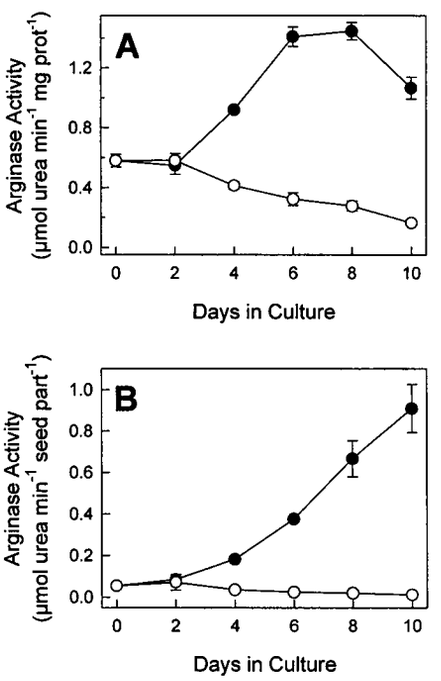

Figure 6. Effect of the megagametophyte on arginase activity in loblolly pine seedlings. Seedlings were cultured with (●) or without (○) intact megagametophytes. A, Arginase activity expressed as μmol urea min^{-1} mg^{-1} protein. B, Arginase activity expressed as μmol urea min^{-1} seed $part^{-1}$. Values are means ± SE of three independent replicates; where error bars are not evident, SE values are smaller than the symbols.

tracts, were generally higher in PPD-treated seedlings than in control seedlings (Fig. 7). The increase in urea pool size in seedlings in which urease activity was inhibited demonstrates that urease is normally active in loblolly pine during early seedling growth. Arginase activity was markedly reduced in seedlings with intact megagametophytes that were cultured with 1.1 mM PPD (Fig. 8).

DISCUSSION

Mobilization of Megagametophyte Protein Reserves

The 47-, 37.5-, 22.5-, and 13- to 14-kD phosphate buffer-insoluble storage proteins of the megagametophyte represent approximately 80% of the total protein in the megagametophytes of mature, desiccated loblolly pine seeds. From this, it may be inferred that the majority of the free amino acids resulting from storage protein hydrolysis in the megagametophyte arise from the breakdown of these proteins. Consequently, the breakdown of these storage proteins contributes substantially to free amino acid pools in the seed during early seedling growth. These buffer-insoluble storage proteins in the megagametophyte were broken down primarily following radicle emergence, which occurs at 4 DAI_{30}. The disappearance of these proteins correlated with the increase in soluble amino acids in the megagametophyte, as well as in the seedling.

The in vitro soluble amino acid pool experiments provide several lines of evidence to indicate that the majority of the free amino acids produced in the megagametophyte, predicted to arise from storage protein breakdown, are exported to the developing seedling. First, the pool of soluble amino acids accumulated by isolated seedlings after 10 d in vitro was much smaller than that accumulated by seedlings incubated 10 d in vitro with an intact megagametophyte (Fig. 3A). Second, isolated megagametophytes accumulated a far larger pool of soluble amino acids than megagametophytes from intact seeds (Fig. 3B). And third, the megagametophyte was able to export the soluble amino acids that were generated in the absence of the seedling (Fig. 3B). The relatively small size of the soluble amino acid pool in the megagametophyte from intact seed compared with that of the seedling from intact seed suggests that amino acids are exported by the megagametophyte as they are produced via storage protein hydrolysis, rather than sequestered for later transport.

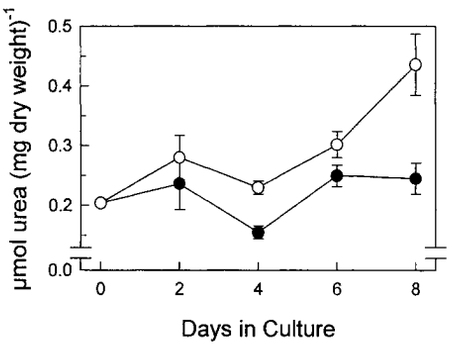

Figure 7. Effect of the urease inhibitor PPD on the accumulation of urea by loblolly pine seedlings. Seedlings with intact megagametophytes were cultured on Kimpak moistened with water (●) or 1.1 mM PPD (○); 15 µg/mL rifampicin and 2.5 µg/mL amphotericin B were also included. Values are means ± SE of three independent replicates; where error bars are not evident, SE values are smaller than the symbols.

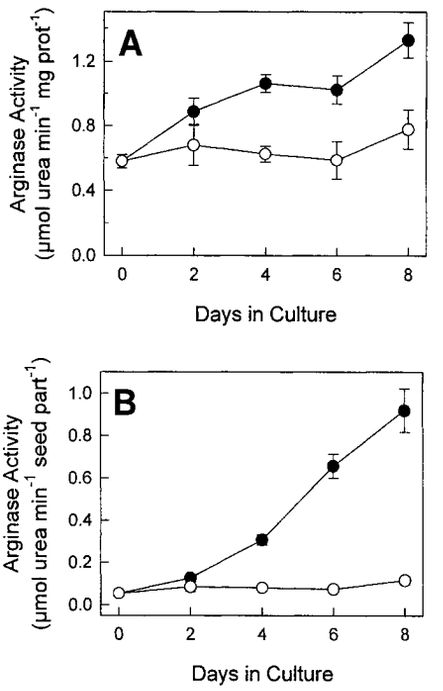

Figure 8. Effect of the urease inhibitor PPD on arginase activity in loblolly pine seedlings. Seedlings were cultured with intact megagametophytes on Kimpak dampened either with water (●) or with 1.1 mM PPD (○); 15 µg/mL rifampicin and 2.5 µg/mL amphotericin B were also included. A, Arginase activity expressed as µmol urea min^{-1} mg^{-1} protein. B, Arginase activity expressed as µmol urea min^{-1} seed part^{-1}. Values are means ± SE of three independent replicates; where error bars are not evident, SE values are smaller than the symbols.

Storage protein mobilization in the megagametophyte can occur in the absence of the seedling. Loblolly pine megagametophytes cultured either with or without seedlings have similar, although not identical, patterns of storage protein breakdown (J.E. King and D.J. Gifford, unpublished data). Furthermore, megagametophytes appear to be autonomous in their ability to export the amino acid products of protein reserve breakdown, even so far as not to require a sink for export to occur. The ability of isolated megagametophytes to export products of reserve breakdown also has been demonstrated in ponderosa pine (*Pinus ponderosa* Laws.) by Ching (1970).

The accumulation of larger free amino acid pools by isolated megagametophyte halves compared with megagametophytes with intact seedlings (Fig. 3B) indicates that export by the megagametophyte into an artificial sink is not as complete as export to the seedling. Likewise, there are subtle differences in the disappearance of storage proteins in megagametophytes cultured with and without seedlings (J.E. King and D.J. Gifford, unpublished data). Therefore, even though the seedling is not required to initiate storage

protein breakdown and subsequent amino acid export by the megagametophyte, it may play a role in the export process. The seedling may be a more efficient sink than agar, since the seedling is closely appressed to the surface of the megagametophyte (S.L. Stone and D.J. Gifford, unpublished data) and is able to take up amino acids that are exported by the megagametophyte into the corrosion cavity, effectively removing them from the surface of the megagametophyte. Alternatively, the seedling may play more of an active role in the export process, involving some form of communication between the seedling and the megagametophyte.

Other studies with conifers have demonstrated that events associated with reserve mobilization in the megagametophyte can occur in the absence of the seedling, although they also are modulated to a greater or lesser degree by the presence of the seedling (Bilderback, 1974; Murray and Adams, 1980; D.J. Gifford, unpublished data). The embryonic axis has also been shown to play a role in storage reserve mobilization in diverse angiosperm species such as cucumber (*Cucumis sativus* L.) (Davies and Chapman, 1979a, 1979b, 1980), castor bean (*Ricinus communis* L.) (Gifford et al., 1984; Mullen and Gifford, 1995), mung bean (Kern and Chrispeels, 1978), sunflower (*Helianthus annuus* L.) (Allen et al., 1984), and barley (*Hordeum vulgare* L.) (Thomas and Rodriguez, 1994; Jacobsen et al., 1995). In some instances, there is hormonal regulation by the embryonic axis. In other cases, the axis acts as a sink to inhibit feedback regulation of mobilization enzymes. However, there are few species in which embryonic axis control over reserve mobilization in storage tissues is well understood (Bewley and Black, 1994). The conifer seed provides an elegant system with which to study such interactions, since the megagametophyte and developing seedling are physically unconnected and are easy to manipulate.

Amino Acid Composition of the Storage Proteins and Free Amino Acid Pools

Seed storage proteins are often rich in the amide amino acids Gln and Asn (Shotwell and Larkins, 1989), but also may be rich in Arg (Derbyshire et al., 1976; Higgins, 1984). All three of these amino acids have a high N-to-carbon ratio, making them particularly suited for storage of N (Bray, 1983). Arg is the major amino acid in the buffer-insoluble storage proteins of loblolly pine, accounting for 23.4 mol% of the amino acids and nearly one-half of the N in these proteins. Arg has been shown to be a prominent amino acid in other conifer storage proteins, both by amino acid analysis of maritime pine (*Pinus pinaster* Ait.) 2S and 11S globulin storage polypeptides (Allona et al., 1992, 1994), and by deduced amino acid sequence of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) 11S globulin cDNA clones (Leal and Misra, 1993). The Arg content of the buffer-insoluble megagametophyte proteins of loblolly pine is higher than that of the seed proteins in any of the 200 angiosperm species surveyed by Van Etten et al. (1963). For example, *Arabidopsis thaliana* 2S albumin and 12S globulin seed storage proteins are made up of 6.9 and 7.0 mol% Arg, respectively (Zonia et al., 1995); soybean (*Glycine max* L.) 11S and 7S globulins are made up of 5.6 and 8.8 mol% Arg, respectively (Derbyshire et al., 1976).

The breakdown of the Arg-rich storage proteins in the megagametophyte correlated with the large influx of Arg in the free amino acid pools of the megagametophyte and also of the seedling (Fig. 1; Table III). High levels of free Arg also were found in both the seedlings and megagametophytes of jack pine (*Pinus banksiana* Lamb.) (Ramaiah et al., 1971) and stone pine (*Pinus pinea* L.) (Guitton, 1964) following imbibition. Guitton (1964) presented tenuous data that indicated that the increase in free Arg pools in stone pine was the result of proteinase action on seed proteins; however, solid evidence to support this supposition is still lacking.

The substantial rise in the free Arg content of the seedling following radicle emergence raises the possibility that Arg is exported from the megagametophyte to the seedling without conversion to another form for transport. If this is the case, then perhaps other amino acids arising from storage protein breakdown in the megagametophyte also are exported without prior metabolic interconversions. This is in contrast to many dicotyledonous species, such as the legumes, in which the amino acid products of storage protein hydrolysis undergo major metabolic interconversions in the cotyledons prior to transport to the embryonic axis (Lea and Miflin, 1980; Bray, 1983). In cereal species, amino acids and small peptides are exported from the endosperm to the developing seedling with very few modifications (Bray, 1983). However, the cereal endosperm is nonliving at maturity, whereas the conifer megagametophyte is a metabolically active tissue; thus, the two systems are not analogous. Clearly, the export of amino acids from the major site of storage reserve deposition to the seedling in conifers follows a process unique from that described in dicots or monocots and deserves further study.

Gln and/or glutamate are also prominent amino acids in the loblolly pine buffer-insoluble seed storage proteins. The acid-hydrolysis technique used in this study did not permit differentiation between Gln and glutamate. Therefore, the proportion of Gln to glutamate in these proteins, and thus the relative contributions of Gln and glutamate to the free amino acid pool, cannot be determined from these data. However, even if the most conservative assumption is made, that Glx is composed entirely of Gln, yielding two N per molecule of Glx, the proportion of N contained in Glx is still only one-half of the N found in the Arg of these storage proteins (Table I). Thus, Arg would appear to be a more important repository of N in the major storage proteins in loblolly pine than either Gln or glutamate.

Like Arg, the high percentage of Gln/glutamate in the major storage proteins of the megagametophyte suggests that the breakdown of these proteins might be accompanied by substantial increases in the levels of Gln and/or glutamate in the megagametophyte, which may then be transported to the seedling. The relatively high levels of Gln in both the seedling and the megagametophyte at 9 DAI_{30} and 12 DAI_{30} support this hypothesis. Gln is frequently used as a transport compound (Lea and Miflin, 1980), and the increase in Gln pool size in the seedling during the latter stages examined may also reflect this role.

In contrast to Gln, glutamate is more prevalent in the free amino acid pools prior to 9 DAI_{30}. However, it must be recognized that these data estimate the free amino acid steady-state pool size and not the rate of flux through amino acids. Therefore, if the role of Gln and glutamate released by storage protein hydrolysis is primarily as metabolic cycle intermediates in a high state of flux, then the importance of these amino acids in loblolly pine early seedling growth may be underrepresented in the steady-state data presented in this paper.

Asn is also an important transport amino acid in plants (Lea and Miflin, 1980), and has been demonstrated to be a major amino acid in cotton seedlings, particularly in the vascular exudate (Capdevila and Dure, 1977). The abundance of Asn in the later stages of seedling growth, contrasting with the relatively low concentrations of Asn in the nonvascularized megagametophyte, suggests that Asn may be the form in which amino acid N is transported from the cotyledons to other parts of the seedling in loblolly pine. Radiotracer experiments would verify this hypothesis. It is interesting that Dilworth and Dure (1978) provide evidence for the flow of N from Arg to Asn during germination in cotton.

Correlation of the Rise in Free Arg Content with Increases of Arginase Activity in the Seedling

Arg constitutes a significant proportion of the free amino acid pools of both the megagametophyte and the seedling of loblolly pine during germination and early seedling growth, and as such may be important in the nutrition of the developing seedling. Arginase has been proposed to be one of the major routes of Arg assimilation into metabolic and biosynthetic pathways in both angiosperm and conifer species (Durzan and Steward, 1983). The correlation between the increase in free Arg pools and arginase activity in the seedling suggests that arginase plays a role in Arg metabolism during loblolly pine early seedling growth. Evidence that Arg is metabolized by arginase in the seedling is provided by the experiments conducted with PPD, an inhibitor of urease. If arginase is metabolizing significant quantities of Arg during early seedling growth, then an active urease would be required to break down urea produced by the arginase reaction. The generally higher level of urea accumulated by seedlings with intact megagametophytes cultured in the presence of the urease inhibitor PPD compared with seedlings with intact megagametophytes cultured in water (Fig. 7) indicates that urease is normally functional in loblolly pine during early seedling growth. Blocking urea breakdown via urease resulted in marked impairment of seedling development, demonstrating that N flow via urea is important for early seedling growth in loblolly pine. The magnitude of difference in urea accumulation by control and PPD-treated seedlings was not as great as that reported in Arabidopsis seedlings by Zonia et al. (1995), perhaps because arginase activity was considerably lower in PPD-treated seedlings than in control seedlings (Fig. 8). These experiments raise the possibility that arginase activity in the seedling could be regulated via feedback inhibition by elevated urea levels or possibly by decreased ammonia production.

The localization of most of the seed's arginase activity in the shoot pole segment of the young seedling implies that the cotyledons and/or the epicotyl are likely to be the primary site of Arg metabolism. Furthermore, arginase activity in the seedling was greatly reduced in the absence of the megagametophyte, indicating that some input from the megagametophyte is required to induce arginase activity in the seedling. These observations support the proposed model that free Arg arising from storage protein breakdown in the megagametophyte is transported without metabolic interconversion to the developing seedling, where arginase participates in the assimilation of Arg into metabolic and biosynthetic pathways, primarily within the cotyledons and epicotyl. The results presented in this paper lay a solid foundation for more in-depth investigations into the transport and metabolism of Arg, as well as the regulation of arginase gene expression, in loblolly pine seedlings.

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