Glutamine Synthesis & Translocation in Pine [1]

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Several workers have studied translocation forms of nitrogen in the xylem sap of trees. It is generally assumed that the nitrogen compounds found in the sap are synthesized in the roots and serve as forms in which nitrogen moves to the tops of trees. Various organic compounds (e.g., amino acids, ureides) usually represent most of the nitrogen in the sap, with inorganic forms (e.g., nitrate, ammonia) being present in minor proportions (4, 5, 6, 11, 13).

I have found that glutamine was the major nitrogenous constituent of the saps of seven species of pines, accounting for about 80 % of the total amino acid nitrogen at collection time in July (unpublished data). However, since Bollard's work (5) with apple trees showed considerable seasonal variation in the nitrogen content of the sap, my previous analyses of Pinus saps at one time of the year could not be considered as representative of the composition of the sap at other seasons. Bollard found that the peak concentration of nitrogen in apple xylem sap occurred at the time of blossoming.

The research reported in the present paper had two main objectives: A, to follow the seasonal variation in free amino acids in the xylem sap of loblolly pine (*Pinus taeda*, L.), especially in relation to time of flowering; and B, to conduct preliminary studies of glutamine synthesis and translocation in pine roots and seedlings.

Materials & Methods

► Seasonal Analyses of Xylem Sap. Samples of sap were collected from three 32-year-old loblolly pines, growing on the Duke campus, at monthly intervals from November 1959 to April 1961. All samples were collected between 8 and 9 AM in order to minimize possible diurnal variations in sap composition or concentration. Sap was collected from one branch of each tree, using Bollard's (3) vacuum method. Sap samples were centrifuged to remove debris, and the supernatants were frozen until analyzed.

Qualitative amino acid analyses were carried out by two-dimensional paper chromatography (2). Quantitative determinations of total amino acids in the sap were made by the ninhydrin method of Yemm & Cocking (16).

► Glutamine Synthesis. Isolated roots of pond pine (*P. serotina*, Michx.) and sterile seedlings of loblolly pine were used. The isolated roots were from the clone established in 1955 (1). Sterile loblolly pine seedlings were obtained as described for isolated root cultures of pines (1), except that the entire seedlings were transferred to White's medium in 25 x 150 mm culture tubes and grown for 6 weeks before use. The seedlings averaged 6.8 cm tall when used in the experiment.

Radiotracer techniques were used to study glutamine synthesis. Ammonium glutamate-U-C^{14} (10 μc per μmole) was used as the radioactive substrate. Isolated pond pine roots were cut into segments about 5 mm long, and approximately 30 mg fresh weight of root sections were used in each treatment. The roots were incubated in small glass vials with 0.05 ml of 0.05 M phosphate buffer, pH 6.0, containing 1 μc of ammonium glutamate-U-C^{14}, with or without added NH_4Cl. Loblolly pine seedlings were supplied with solutions of similar composition to those used with isolated roots, except that 0.2 ml volumes containing 2 μc of radioactive glutamate were used. Urea was added to the medium instead of NH_4Cl in one experiment. The seedlings were fed through the roots.

Since the ammonium salt of radioactive glutamic acid was used as a substrate, a small amount of NH_4^+ was added with the tracer in all experiments. The treatments were additions of various amounts of NH_4Cl or urea to the smaller amount of NH_4^+ added with the glutamate.

All feedings were for 24 hours at 23 C. Isolated roots were incubated in darkness, while seedlings were fed in the light (ca. 400 ft-c). All experiments were carried out in duplicate.

After 24 hours, the isolated roots and seedlings were washed with distilled water, and the seedlings were then divided into roots, stems, and leaves (one experiment); or into roots and tops (stems & leaves combined) before analysis. Soluble compounds were extracted with 80 % ethanol, and analyzed by two dimensional chromatography on Whatman No. 1 paper using the phenol: water and butanol: propionic acid: water solvents of Benson et al. (2). Radioautograms were prepared with Kodak No Screen X-ray film, and the radioactivity in compounds was determined by counting directly on the chromatograms with a Geiger-Müller tube (7).

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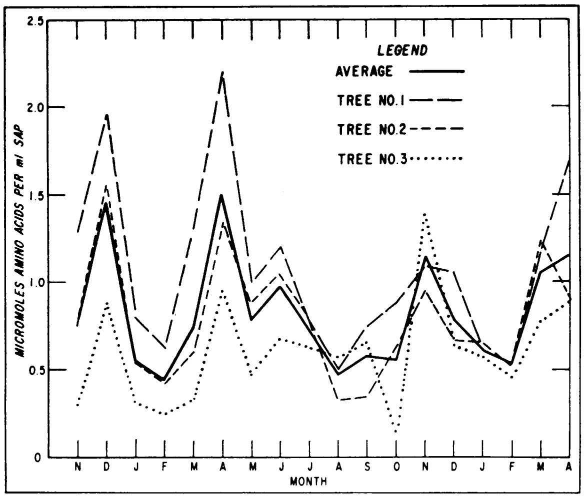

Fig. 1. Seasonal variation in concentration of amino acids in loblolly pine xylem sap, from November 1959 to April 1961.

All radioactive spots on the chromatograms were counted; however, for brevity, only the data for certain compounds of interest are presented in the tables in this paper. Data are given both as total cpm and as percentages of the total radioactivity on chromatograms. The total cpm data should not be regarded as strictly quantitative, since the extraction procedures were kept relatively mild to minimize glutamine breakdown. Also, variations in size of seedlings and root samples used in the experiments affected total cpm data. All results given are average values for the two root samples or seedlings of each treatment.

Results

Chromatographic analyses of xylem sap samples showed that glutamine was the most abundant amino acid present at all times of the year. Visual inspection of chromatograms indicated that glutamine accounted for about three-fourths of the total amino acids, with much smaller amounts of glutamic acid, aspartic acid, serine, glycine, alanine, and γ-aminobutyric acid also present. Analyses of total free amino acids in the sap indicated two seasonal peaks in concentration (fig 1). One peak, occurring about April 1, coincided with the time of flowering. The second peak came in November or December.

Glutamine synthesis from carbon-labeled ammonium glutamate was demonstrated in both isolated roots and seedlings. In isolated pond pine roots (table I), increasing the concentration of NH_4^+ in the medium from 2 to 202 mM had three main effects: A, radioactivity in glutamine increased from 7 to 30 % of the total activity; B, radioactivity in unmetabolized glutamic acid decreased from 13 to 5 % of the total; and C, radioactivity in the organic acids decreased from 12 to 2 %. In all treatments, γ-aminobutyric acid accounted for the majority of the radioactivity in root extracts.

Results obtained with loblolly pine seedlings showed different labeling patterns in roots and tops. Increasing the NH_4^+ concentration from 1 to 101 mM resulted in the following patterns of radioactivity (table II): A, glutamine increased in roots (16–55 %) and tops (11–40 %); B, glutamic acid decreased in the roots (9–3 %) but remained about the same in the tops; and C, organic acids decreased in the roots (18–5 %) and remained relatively constant in the tops. The greatest treatment effect on glutamine content occurred between 11 and 101 mM NH_4^+; the differences between the 1, 2, and 11 mM NH_4^+ concentrations were much smaller. There was no marked accumulation of γ-aminobutyric acid in any part of the seedlings, in contrast to the results with isolated roots.

Although fairly high concentrations of NH_4Cl were used in these experiments, neither the isolated roots nor seedlings showed any visible symptoms of toxicity. However, it is not known how well the NH_4^+ penetrated the root tissues, although radioactive glutamate apparently was taken up readily.

The addition of urea (25 mM) to the medium had the same general effect on levels of glutamine, glutamic acid, and organic acids as did the addition of NH_4Cl (table III). Although more radioactive glutamine was found in the tops of seedlings treated with urea than in the seedlings treated with NH_4Cl,

Table I

Effect of Ammonium Concentration on Metabolism of Ammonium Glutamate-U-C^{14} by Isolated Pond Pine Roots

Compounds	Total ammonium concentration (mM)							
	2		4		22		202	
	cpm	% Total	cpm	% Total	cpm	% Total	cpm	% Total
Glutamine	1,837	7	1,225	6	2,661	11	5,946	30
Glutamic acid	3,540	13	1,605	8	2,360	10	1,081	5
Organic acids	3,238	12	1,820	9	2,426	10	484	2
γ-Amino-butyric acid	16,998	60	14,337	70	14,357	59	11,172	57
Other	2,455	8	1,372	7	2,326	10	1,172	6
Total	28,068	100	20,359	100	24,130	100	19,855	100

Table II

Effect of Ammonium Concentration on Metabolism of Ammonium Glutamate-U-C^{14} by Loblolly Pine Seedlings

Compounds	Total ammonium concentration (mM)							
	1		2		11		101	
	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops
	cpm/*Seedling*							
Glutamine	3,497	1,083	1,483	926	686	1,819	6,667	7,217
Glutamic acid	2,018	1,966	1,120	2,012	224	2,696	336	3,253
Organic acids	3,997	1,445	1,799	1,709	628	2,101	651	1,864
Other	13,005	5,630	7,008	3,849	2,150	5,685	4,528	5,613
Total	22,517	10,124	11,410	8,496	3,688	12,301	12,182	17,947
	% *Total radioactivity*							
Glutamine	16	11	13	11	19	15	55	40
Glutamic acid	9	19	10	24	6	22	3	18
Organic acids	18	14	16	20	17	17	5	10
Other	57	56	61	45	58	46	37	32

the higher concentration of added urea, in comparison to the added NH_4Cl, does not allow a direct comparison of the two sources of nitrogen.

Discussion

The results of seasonal analyses showed that glutamine was the most abundant amino acid in the xylem sap at all times of the year. Although two seasonal peaks in amino acid concentration of the sap were noted, the importance of these peaks has not been determined. The peak occurring about at the time of flowering (April 1) is not necessarily related to flowering, as suggested by Bollard's work. In April loblolly pine breaks dormancy and resumes general growth in North Carolina. No explanation has been found for the late-fall peak in amino acid concentration; however, one phenological event occurring at this time of the year is the shedding of 2-year-old needles; the increase in amino acid concentration at this time may be related to the movement of nitrogen from old needles back into the branches.

Measurements of the concentration of amino acids in xylem sap, as reported in this study, are not directly indicative of the total amount of amino acids being translocated, since the rate of transport was not determined. The time of greatest translocation of amino acids in pine xylem sap may well occur during the summer months when the rate of transpiration is maximal.

Results of tracer studies showed that glutamic acid was a precursor of glutamine in pine tissues. Although the exact steps in glutamine synthesis, or the significance of synthesis from glutamate as compared with other possible precursors, have not been determined, the data suggest the presence of a glutamine

Table III

Effect of Added NH_4Cl or Urea on Metabolism of Ammonium Glutamate-U-C^{14} by Loblolly Pine Seedlings

Compounds	Compound added to incubation medium*								
	None NH_4^+ = 1 mM			NH_4Cl (10 mM) NH_4^+ = 11 mM			Urea (25 mM) NH_4^+ = 1 mM		
	Roots	Stem	Leaves	Roots	Stem	Leaves	Roots	Stem	Leaves
	cpm/*Seedling*								
Glutamine	8,128	182	701	17,319	579	1,451	1,854	672	7,562
Glutamic acid	2,885	89	1,270	1,881	100	1,951	318	77	2,774
Organic acids	1,740	56	515	1,056	103	799	173	62	1,170
Other	7,559	222	860	5,902	109	1,408	617	212	2,798
Total	20,312	549	3,346	26,158	891	5,609	2,962	1,023	14,304
	% *Total radioactivity*								
Glutamine	40	33	21	66	65	26	63	66	53
Glutamic acid	14	16	38	7	11	35	11	8	19
Organic acids	9	10	15	4	12	14	6	6	8
Other	37	41	26	23	12	25	20	20	20

* All treatments contained ammonium glutamate-U-C^{14} at a concentration of 1 mM.

synthetase system (10, 15). Naylor and Tolbert (12), in their study of glutamic acid metabolism in barley, found labeling patterns from glutamate-U-C^{14} which were similar to my results with pine tissues.

Glutamine synthesis also was increased by added urea. Hydrolysis of urea and subsequent use of the liberated NH_3 by glutamine synthetase, or direct incorporation of urea into glutamine, as suggested by Steward and Pollard (14), could explain the results.

The radioactivity appearing in organic acids, both in isolated and seedling roots, decreased as the NH_4^+ concentration increased. These results suggest that conditions which favor glutamine synthesis suppress metabolism of glutamate to α-ketoglutarate. High concentrations of ammonia also may enhance the conversion of α-ketoglutaric acid to glutamic acid and glutamine and thus influence organic acid metabolism.

The hypothesis of Kursanov (9), Kretovich (8) and other Russian workers is that organic acids (e.g. α-ketoglutarate) serve as nitrogen acceptors in plant roots, and that the acceptor acids, on combination with nitrogen from the soil, give rise to translocation forms of nitrogen (e.g., glutamine). My results are in general agreement with this hypothesis. In my experiments with seedlings, translocation of C^{14}-labeled compounds to the tops (as estimated from total cpm data) was increased by adding NH_4Cl or urea to the feeding solutions. Although the treatments did increase the amount of radioactive glutamine in the tops, it was not shown that glutamine represented a main transport form for radioactivity moving from roots to tops.

Summary

Glutamine was the most abundant amino acid in loblolly pine xylem sap at all seasons of the year. Seasonal peaks in total amino acid content of the sap occurred in April and in November to December.

Glutamic acid served as a precursor of glutamine in isolated roots of pond pine and in loblolly pine seedlings, apparently via the glutamine synthetase reaction. Adding NH_4Cl or urea to incubation mixtures containing ammonium glutamate-U-C^{14} increased the amount of radioactive glutamine formed; and, in loblolly seedlings, also increased the amount of C^{14} translocated from roots to tops.

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