Amino Acids (1993) 4: 141–155

Amino
Acids

Amino acid metabolism in nongrowing environments in higher plants

S. Sagisaka

The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

Accepted July 3, 1992

Summary. During winter season, growth of biennial and perennial plants was virtually halted. Amino acid analyses of 74 samples of woody and herbaceous plants including grasses and winter wheat showed following results. In innately dormant plants, synthesis and accumulation of free amino acids were completed in fall and next changes occurred in the following spring. In plants under enforced dormancy, a different reaction from that of growing season occurred and continued during wintering under snow.

1. Amino acid pools – From major amino acid contents of the pool, pools were separated into five types: a group which accumulated 1) arginine, 2) arginine and proline, 3) proline, 4) glutamine and glutamate and 5) asparagine, respectively.

2. Inorganic nitrogen assimilation – In *Dactylis glomerata*, about 20 μmoles of NH_3 g^{-1} fresh weight were converted into amide nitrogen of glutamine during winter.

3. Increase of the pool concentrations – In winter wheat (cv. horoshirikomugi), the level in March was more than twice that of November.

4. Changes in the pool composition – (Examples), 1) decrease of arginine in *Agrostis alba*, 2) decrease of asparagine and increase of arginine in *Medicago sativa*, 3) increase of asparagine in winter wheat.

5. Accumulation of particular amino acids — Histidine in *Arctium lappa*, threonine in *Armoracia rusticana* and serine in *Medicago sativa.* Since the reaction appeared to proceed at extremely slow rates over the winter season, amino acid analysis only seemed to be feasible to assess the extent of amino acid accumulation in the pool.

Keywords: Amino acid pools – Arginine pool – Plant amino acids – Proline pool

Introduction

The metabolic transition of woody plants from the growing to the wintering stage in September has already been reported (Sagisaka, 1974b; Sagisaka and Asada, 1981). The increase in the activity of glucose-6-P dehydrogenase (G6PDH) and enzymes involved in ascorbate and GSH metabolism has been noted as one of the characteristics of sequence of events in the twigs (Nakagawara and Sagisaka, 1984). Recent studies revealed that cytological changes in parenchyma cells of poplar xylem began to occur in fall in parallel with the metabolic transition (Asada et al., 1988).

In living cells and tissues, the intermediary reactions of amino acid synthesis are controlled in such a way that they are commensurate with the demands of the cells. As a consequence of the metabolic transition, the homeostatic state of the metabolic responses may shift from the growing to the wintering stage (Sagisaka, 1974b). Conceivably, when the growth and enlargement are virtually halted in fall, a characteristic pool of free amino acids of the non-growing stage is maintained in the cells. This report presents results of studies on the amino acid pools in woody and herbaceous plants at the wintering stage.

Experimental procedures

Materials

Plants were collected from field near the campus of Hokkaido University or from the suburbs of Sapporo City, Hokkaido. Samples were collected from plants growing under ambient temperatures. Winter wheat and rye were sown on September 20 in the field and grown under natural conditions. Apices of the underground stem of *Asparagus officinalis* Linn.

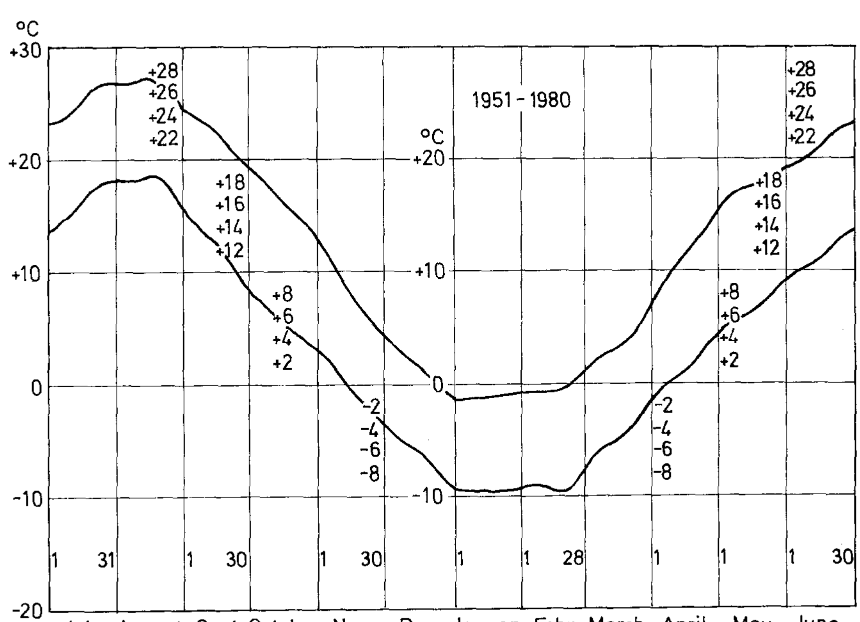

Fig. 1. Mean daily air temperature (°C) in Sapporo throughout the year (1951–1980)

collected in late fall and at an early stage of growth. The date of sampling and the part of plant sampled are listed in the Tables.

Extraction of amino acids from plants

Plant tissues of the samples were washed in cold distilled water on the same day, cut into pieces, and homogenized immediately. Homogenization and extraction were carried out as follows; the sample (1.0 g wet weight) was ground in a mortar at 0°C in the presence of 0.3 g sea sand and 2 ml of 0.2 *N* $HClO_4$ containing 2 μmol norleucine. The slurry was transferred to a test tube and the remaining material in the mortar was washed once with 1 ml water. The slurry was centrifuged at 14,000 × g for 5 min and the supernatant was neutralized to about pH 4 with solid $KHCO_3$. The sample was frozen and stored until analysis, before which the remaining precipitates were removed by centrifugation. Glutamine and asparagine were hydrolyzed with 2 *N* HCl for 7 h at 110°C to their corresponding acids, and increase of glutamate and aspartate was measured.

Table 1. Accumulation of arginine in wintering perennials*

Plant	*Malus pumila* Mill.		
	Buds	Living bark	Xylem
(μmol free amino acid/g dry wt)			
Serine	4.1	3.6	1.2
Glycine	0.6	0.2	0.3
Cystine/2	0.3		
γ-Aminobutyrate			
Glutamate	2.9	1.8	3.2
Glutamine	2.3	0.3	0.4
Ornithine	0.1	0.2	0.2
Proline		1.5	
Arginine	12.6	4.3	27.7
Lysine	0.2	0.2	0.2
Alanine	0.6	0.2	1.0
Aspartate	2.7	1.4	1.6
Asparagine	5.3	0.3	29.3
Threonine	1.5	0.5	0.4
Methionine			
Valine	0.6	0.2	0.5
Leucine	0.1		0.1
Isoleucine	0.1		0.2
Histidine	0.6	0.4	0.4
Tyrosine		0.2	
Phenylalanine	0.6	0.9	0.3
Tryptophan		1.4	
Total	35.2	17.6	67.0
NH_3	11.5	6.5	4.0
α-Ketoglutarate family (% of total)	50.9	46.0	47.0

* Sampled on January 12. Blanks in the Table represent the values less than 0.04 μmol g^{-1} dry wt.

Analytical methods

Amino acid analyses were carried out with an amino acid analyzer (Nihon Denshi, model JLC-6H) using a series of citrate buffers (pH 3.20, 4.25 and 7.30), and the concentrations of the various amino acids were calculated on the basis of the internal standard (DL-norleucine) added to each sample.

Results and discussion

A series of visible and invisible events take place consecutively during the 1-year of plant life. In Sapporo, situated in latitude 43° 3′ north, the growth of many trees virtually halted in mid-August. By late October, there was a frost every morning and leaves of deciduous trees have been shed. At this stage, the trees and herbaceous plants were ready for wintering. The ground was covered with snow by late December and the minimum air temperatures at their habitats often reached below ten degrees of cold by this time. After the continuous snow cover, the surface temperature of the ground remained at nearly 0°C until the snow melted in April. In Fig. 1, the mean daily air temperature (°C) in Sapporo is presented.

Accumulation of arginine as the major amino acid

1) Woody plants: arginine was the major amino acid in the pool in the buds and xylem of *M. pumila* Mill. (Table 1), and in the living bark and xylem of *P. communis* Linn. (data not shown). A group which accumulated arginine in their twigs is listed in Table 2. In the twigs of poplar (*Populus gelrica*), a preferential

Table 2. Plants which accumulated arginine at the wintering stage

Woody plants;	
1. *Alnus japonica* Steud.	6. *Malus Sieboldii* Rehd.
2. *Callicarpa japonica* Thunb.	7. *Pyrus communis* Linn.
3. *Enkianthus perulatus* Schneid.	8. *Salix Matsudana* Koid. var. *tortuosa*
4. *Ligustrum tschonoskii* Decaisne	9. *Syringa vulgaris* Linn.
5. *Malus pumila* Mill.	10. *Vaccinium vitis-idaea* Linn.
Herbaceous plants;	
1. *Adonis amurensis* Regel et Radd.	13. *Narcissus tazetta* L. var. *chinensis*
2. *Agrostis alba* Linn.	14. *Oenanthe stolonifera* DC.
3. *Aralia cordata* Thumb.	15. *Paenia obovata* Maxim. var. japonica
4. *Campanula punctata* Lam.	16. *Pettasites japonica* Miq.
5. *Cardiocrinum glehni* Makino	17. *Phellopterus littoralis* Benth. et Hook. fil.
6. *Carex foliusissima* Fr. Schm.	18. *Plantago japonica* Franch. et Sav.
7. *Chloranthus japonicus* Sieb.	19. *Plantago lanceolata* Linn.
8. *Convallaria majalis* Linn.	20. *Rumex acetosella* Linn.
9. *Epimedium macranthum* var. *violaceum* Franch.	21. *Rumex japonicus* Houttuyn
	22. *Saxifraga fortunei* Hook
10. *Geranium nepalense* Sweet	23. *Sedum alboroseum* Baker
11. *Hosta undulata* Bailey	24. *Trfolium pratense* Linn.
12. *Medicago sativa* Linn	25. *Trifolium repens* Linn.

accumulation of arginine in the amino acid pool occurred in fall and the changes in the xylem pool preceded those in the bark. As the changes in the arginine concentration proceeded, the levels of glutamine and glutamate decreased (Sagisaka, 1974a). From these results, it may be concluded that the synthesis and accumulation of major amino acid(s) in the pool were completed by middle October or early November, and their established metabolic pattern lasts until mid April.

2) Herbaceous plants: plants were collected from October to November. As a typical example, the amino acid composition of *A. alba* was presented in Table 3. At this stage, in 25 out of 44 plants, arginine was a major amino acid, together with appreciable amounts of glutamine plus glutamate and/or asparagine plus

Table 3. Accumulation of arginine, arginine and proline or glutamate and glutamine in plants at the wintering stage

Plants	*Agrostis alba* L.		*Armoracia rusticana* Gaertn.		*Dactylis glomerata* L.	
Date of sampling	Oct. 18	Apr. 18	Nov. 17	May 22	Oct. 25	Apr. 18
	(μmol free amino acid/g wet wt)					
Serine	0.9	1.6	9.1	4.4	4.5	2.1
Glycine		0.4		0.2	0.2	0.2
Cystine/2		0.2		0.3		0.2
γ-Aminobutyrate		0.5		0.2	0.3	0.2
Glutamate	2.8	7.1	13.5	5.4	26.3	6.5
Glutamine	2.2	5.3	5.8	4.6	26.3	48.7
Ornithine	2.8				0.1	
Proline			19.0	1.5	2.4	0.1
Arginine	23.4	2.9	98.9	68.8	3.4	5.0
Lysine	0.9	0.6	4.7	2.6	0.9	0.3
Alanine	1.1	3.4	2.0	1.2	1.5	3.8
Aspartate	0.8	3.4	9.1	5.2	1.5	3.5
Asparagine	4.4	1.6	1.6	1.5	3.5	2.0
Threonine	0.7	1.9	10.2	4.6	5.1	5.7
Methionine					0.1	0.1
Valine	0.3	1.1	7.3	1.5	1.6	4.5
Leucine	0.3	0.2	3.6	1.4	0.4	0.8
Isoleucine	0.3	0.3	5.5	0.7	0.9	2.5
Histidine	0.3	1.3	4.8	3.9	3.0	1.8
Tyrosine		0.1	2.4	0.8	0.1	0.2
Phenylalanine		0.3	2.7	0.7	0.2	0.5
Tryptophan	0.3	0.3		0.4	0.5	0.3
Total	41.5	32.5	207.4	110.0	82.9	89.0
α-Ketoglutarate family (% of total)	75.2	48.6	66.2	73.2	71.0	68.0
NH_3	2.5	1.2	5.6	1.2	4.6	1.6
Note	coleoptile		stem and root		coleoptile	

Blanks in the Table represent the values less than 0.04 μmol g^{-1} wet wt.

aspartate. The proline concentration in this group was less than 10% of the α-ketoglutarate family of amino acids (Mattoon, 1963).

Occurrence of arginine and proline as the major amino acids

1) Woody plants: arginine and proline coexisted in the buds, living bark and xylem of *M. bombycis* Koidz., *R. sinanense* F. Maekawa and *M. glyptostroboides* Hu et Cheng (Tables 4 and 5). The highest concentration of arginine and/or proline was found in the pool of *M. bombycis* Koidz. and *P. sargentii* Rehd.

2) Herbaceous plants: the amino acid pool of the group which accumulated arginine and proline is presented in Tables 3 (*A. rusticana* Gaertn) and 6 (*A. lappa* Linn.). Nine samples out of 44 contained both arginine and proline (Table 5).

Table 4. Examples of high levels of arginine and proline in wintering perennials*

Plants	*Morus bombycis* Koidz.			*Prunus sargentii* Rehd.		
	Buds	Living bark	Xylem	Buds	Living bark	Xylem
	(μmol free amino acid/g dry wt)					
Serine	2.6	1.2	1.5	3.4	0.3	0.3
Glycine	0.2	0.1	0.2	0.3		0.2
Cystine/2	0.2		0.4			
γ-Aminobutyrate		0.6	0.2			
Glutamate	8.4	10.0	7.7	7.5	3.5	2.5
Glutamine	#	#	#	5.5	3.4	0.8
Ornithine	0.2	0.4	0.1		0.1	0.2
Proline	86.7	110.8	50.5	125.4	4.1	1.8
Arginine	17.4	60.3	10.7	12.4	88.1	32.7
Lysine	0.3	0.5	0.4			0.2
Alanine	0.8	0.6	0.4	1.2		0.2
Aspartate	2.9	2.8	0.1	4.3	2.5	1.6
Asparagine	15.1	6.3	0.6	7.7	1.0	2.5
Threonine	2.6	1.2	0.4	1.3	0.3	0.2
Methionine						
Valine	1.0	0.2	0.4	0.9	0.3	0.3
Leucine	0.1					0.1
Isoleucine	0.2			0.3		0.2
Histidine	0.4	0.9	0.2	1.7	2.1	0.2
Tyrosine	0.2					
Phenylalanine	0.2					0.2
Tryptophan				0.2	0.1	
Total	139.5	195.9	73.8	172.4	105.8	44.7
NH_3	10.9	16.1	7.1	25.1	9.7	4.7
α-Ketoglutarate family (% of total)	80.8	93.0	93.8	87.5	93.8	85.0

* Sampled on January 12.

\# Unable to calculate, owing to an increase of threonine content after acid hydrolysis. Blanks in the Table represent the values less than 0.04 μmol g^{-1} dry wt.

Table 5. Plants which accumulated arginine and proline at the wintering stage

Woody plants;

1. *Abies sachalinensis* Masters
2. *Acer saccharum* Marsh.
3. *Betula ermanii* Chamisso
4. *Daphne kamtschatica* var. jezoensis
5. *Fraxinus mandshurica* var. *japonica* Maxim.
6. *Larix kaempferi* Carr.
7. *Metasequoia glyptostroboides* Hu et Cheng
8. *Morus bombycis* Koidz.
9. *Photinia glabra* Maxim.
10. *Picea glehnii* Masters
11. *Prunus sargentii* Rehd.
12. *Qercus mongolica* var. *grosseserrata* Rehd. et Wils.
13. *Ribes sinanense* F. Maekawa
14. *Taxus cuspidata* Sieb. et Zucc.
15. *Ulmus davidiana* var. *japonica* Nakai

Herbaceous plants;

1. *Arctium lappa* Linn.
2. *Armoracia rusticana* Gaertn.
3. *Asparagus officinalis* Linn.
4. *Cacalia hastata* Linn.
5. *Crocus sativus* Linn.
6. *Liriope graminifolia* Baker
7. *Lysimachia japonica* Thunb.
8. *Taraxacum officinale* Weber
9. *Tulipa gesneriana* Linn.

Occurrence of proline as the major amino acid

1) Woody plants: the buds and living bark of *G. biloba* Linn. contained proline as the major amino acid, whereas proline and arginine coexisted in the xylem (Table 7). Five out of 30 contained predominantly proline (Table 8).

2) Herbaceous plants: four plants out of 44 contained proline as the major amino acid (Table 8) and the composition of *B. oleracea* var. *capitata* Linn. is presented in Table 6.

Accumulation of glutamine and glutamate

Four out of 44 herbaceous plants accumulated glutamine and glutamate as the major amino acids (Table 9). After five months under the snow and at about three weeks before the onset of active growth, glutamine and glutamate were the major amino acids in *D. glomerata* Linn. (Table 3) and in *P. pratense* Linn. (Table 10).

Increase of asparagine and aspartate concentrations

M. sativa Linn., *P. pratense* Linn., *T. pratense* Linn. (Table 10) and *T. repens* Linn. (data not shown, similar concentrations of each amino acid in the pool as those of *T. pratense* Linn.) synthesized large amounts of asparagine in fall. After five months of the life under snow, asparagine was no longer the major amino acid of the pool and was replaced by arginine (*M. sativa* Linn., *T. pratense* Linn. and *T. repens* Linn.) or glutamine (*P. pratense* Linn.), depending on the plant. Winter wheat (Table 11) and rye (data not shown) synthesized large amounts of asparagine and aspartate during wintering.

Table 6. Composition of amino acid pools of *Arctium lappa* Linn. and *Brassica oleracea* var. *capitata* Linn.

Plants	*A. lappa* L.	*B. oleracea* var. *capitata* L.
Date of sampling	Nov. 18	Dec. 12
(μmol free amino acid/g wet wt)		
Serine	1.7	3.3
Glycine	0.1	0.1
Cystine/2		
Glutamate	4.1	15.3
Glutamine	0.8	17.4
Proline	39.9	46.4
Arginine	17.8	0.6
γ-Aminobutyrate	0.2	0.4
Lysine	0.5	0.4
Alanine	0.8	3.0
Aspartate	2.3	5.8
Asparagine	8.7	
Threonine	1.4	2.3
Methionine	0.1	0.6
Valine	1.3	0.4
Leucine	1.2	0.4
Isoleucine	1.3	1.4
Histidine	5.5	6.7
Tyrosine	0.5	
Phenylalanine	0.4	0.4
Tryptophan		
Total	88.6	104.9
NH_3	2.0	5.3
α-Ketoglutarate family (% of total)	70.9	76.4
Note	stem	leaves

Blanks in the Table represent the values less than 0.04 μmol g^{-1} dry wt.

Inorganic nitrogen assimilation on wintering

D. glomerata Linn. contained an equal amount of glutamate and glutamine in late October. By the following April, the amount of glutamate in the pool had decreased and glutamine was the major amino acid. During this period, about 20 μmol of NH_3 g^{-1} fresh weight were converted to organic nitrogen in the form of glutamine in the plant (Table 3). Large amounts of asparagine accumulated in winter wheat during wintering (Table 11). *Urtica dioica* Linn. accumulated asparagine and arginine during winter in the subterranean parts (Rosnitschek-Schimmel, 1985).

Table 7. Increase of proline in winter followed by a decrease in an early stage of budding in *Ginkgo biloba* Linn.

Part of twig sampled and date of sampling	Buds Apr. 6	Buds May 8	Living bark Apr. 6	Living bark May 24	Xylem Apr. 6	Xylem May 24
	(μmol free amino acid/g dry wt)					
Serine	2.9	3.7	3.2	2.2	1.6	1.2
Glycine	0.2	0.4	0.1	0.2	0.1	0.1
Cystine/2						
γ-Aminobutyrate	0.3	1.1	0.3	1.8	1.3	2.1
Glutamate	4.7	8.2	7.8	8.8	2.8	1.4
Glutamine	4.2	6.7	2.6	3.9	2.6	4.7
Ornithine				0.1		0.1
Proline	59.1	66.2	20.5	2.6	11.6	1.4
Arginine	0.2	0.2	2.4	0.9	13.4	
Lysine	0.2	0.4	0.5	0.2	0.6	
Alanine	1.7	2.5	1.8	1.9	0.7	0.7
Aspartate	2.3	3.2	2.9	1.8	1.4	1.6
Asparagine	0.3	5.0	1.7	0.3	0.1	0.8
Threonine	1.0	1.3	0.9	1.2	0.5	0.7
Methionine						
Valine	0.8	0.7	1.0	1.2	0.6	0.6
Leucine	0.2	0.2	0.3	0.4	0.2	0.3
Isoleucine	0.3	0.2	0.3	0.3	0.2	0.2
Histidine	1.2	1.1	0.8	0.8	0.5	0.3
Tyrosine	0.1			0.1	0.1	0.1
Phenylalanine	0.2	0.2	0.9	0.3	0.4	0.2
Tryptophan	0.2	0.2	0.1	0.7	0.1	0.1
Total	80.1	101.5	48.1	29.7	38.8	16.6
NH_3	4.4	6.5	7.0	3.4	5.0	1.2
α-Ketoglutarate family (% of total)	85.5	81.2	69.9	60.9	81.7	58.4

Blanks in the Table represent the values less than 0.04 μmol g^{-1} dry wt.

Table 8. Plants which increase their proline concentration on wintering

Woody plants;	**Herbaceous plants;**
1. *Cytisus scoparius* Link.	1. *Brassica oleracea* var. *capitata* Linn.
2. *Forsythia koreana* Nakai	2. *Calanthe tricarinata* Lindl.
3. *Ginkgo biloba* Linn.	3. *Lolium perenne* Linn.
4. *Rosa acicularis* Lindl.	4. *Sasa senanensis* Rehd.
5. *Rosa rugosa* Thunb.	

Increase of the pool concentration on wintering

In winter wheat (Table 11) and rye (data not shown), the level in March was more than twice that of November.

Table 9. Plants which accumulated glutamine and glutamate or asparagine and aspartate at the wintering stage

Herbaceous plants;

A. Plants which accumulated glutamine and glutamate.

1. *Dactylis glomerata* Linn.
2. *Oreorchis patens* Lindl.
3. *Phleum pratense* Linn.
4. *Veronica arvensis* Linn.

B. Plants which accumulated asparagine and aspartate.

1. *Secale cereale* Linn. cv. *12 rye*
2. *Triticum aestivum* Linn. cv. horoshirikomugi

Table 10. Changes in the amino acid composition in the crops during wintering

Plants	*Medicago sativa* L.		*Phleum pratense* L.		*Trifolium pratense* L.	
Date of sampling	Nov. 15	Apr. 14	Oct. 25	Apr. 18	Oct. 25	Apr. 14
	(μmol free amino acid/g wet wt)					
Serine	3.1	8.3	2.6	3.8	2.3	3.8
Glycine	0.2	0.9	0.1	0.2	0.1	0.4
Cystine/2	0.7	0.7	0.1	0.1		0.7
γ-Aminobutyrate	1.5	16.1	0.3	0.6	0.9	1.6
Glutamate	6.5	9.5	2.1	3.5	2.7	2.8
Glutamine	2.1	3.3	6.7	13.2	2.0	3.0
Ornithine			0.1		0.1	
Proline	4.4	5.0	0.7	0.5	2.4	
Arginine	7.9	27.1	3.4	4.1	12.5	34.0
Lysine	0.3	0.7	0.1	2.3	0.1	1.0
Alanine	2.0	18.2	1.0	2.4	0.8	1.3
Aspartate	5.9	0.7	0.9	1.6	5.4	6.1
Asparagine	28.5	8.3	16.6	5.0	36.5	2.5
Threonine	1.7	0.9	1.4	1.1	1.2	1.4
Methionine		0.1	0.1	0.1		0.1
Valine	0.5	1.0	1.2	1.2	0.7	1.6
Leucine	0.3	0.9	0.2	0.1	0.4	1.2
Isoleucine	0.3	0.7	0.5	0.1	0.4	1.0
Histidine	2.1	8.2	0.7	2.3	2.2	3.6
Tyrosine	0.8	0.6	0.1	0.1		0.4
Phenylalanine	0.8	0.3	0.3	0.2	0.1	0.6
Tryptophan		0.3	0.5	0.4	0.2	0.3
Total	69.6	111.8	39.7	42.9	71.0	66.1
α-Ketoglutarate family (% of total)	32.2	54.6	33.5	51.0	20.8	62.6
NH_3	1.1	2.9	1.2	0.9	1.1	2.2
Note	coleoptile		coleoptile		stem and leaves	

Blanks in the Table represent the values less than 0.04 μmol g^{-1} wet wt.

Table 11. Composition of amino acid pools of *Triticum aestivum* L. cv. horoshirikomugi in late fall and winter

	Leaves		Stem		Crown	
Date of sampling	Nov. 11	Mar. 14	Nov. 11	Mar. 14	Nov. 11	Mar. 14
	(μmol free amino acid/g dry wt)					
Serine	6.8	22.3	5.8	34.2	5.4	16.6
Glycine	0.4	0.9	1.3	4.1	1.3	1.0
Cystine/2	1.3	1.4	0.9	1.6	0.9	1.6
Glutamate	24.6	28.8	26.8	26.9	17.0	29.1
Glutamine				16.1	7.6	2.1
Proline	0.8	2.8	16.5	16.6	16.1	5.7
Arginine		13.5	1.8	3.1	3.6	31.1
γ-Aminobutyrate		0.5	0.6		2.3	1.6
Lysine	0.4	8.8	1.8	2.1	3.6	15.0
Alanine	7.6	8.4	9.8	44.0	18.3	26.4
Aspartate	11.4	16.3	13.4	17.1	4.5	14.5
Asparagine	1.7	66.0	21.0	98.0	22.8	92.7
Threonine	1.7	3.7	3.6	6.7	6.7	6.2
Methionine						
Valine	1.3	11.6	4.0	20.2	6.3	11.4
Leucine	0.4	3.7	3.1	4.7	5.8	8.3
Isoleucine	0.4	8.4	2.2	9.8	2.7	8.3
Histidine	0.8	11.2	2.2	11.4	4.9	35.8
Tyrosine		0.9			0.5	0.5
Phenylalanine	0.4	5.1	0.9	1.6	1.8	4.7
Total	60.0	214.3	115.7	318.2	132.1	312.6
NH_3	29.2	31.6	32.1	40.4	29.0	38.9
α-Ketoglutarate family (% of total)	42.3	21.3	39.5	19.7	35.3	22.3
Aspartate plus asparagine (% of total)	21.8	38.4	29.7	36.2	20.7	34.3

Blanks in the Table represent the values less than 0.04 μmol g^{-1} wet wt.

Accumulation of particular amino acids

In fall, *A. lappa* Linn. and *B. oleracea* var. *capitata* Linn. (Table 6) accumulated histidine and high levels of threonine so far assayed were present in *A. rusticana* Gaertn (Table 3). On wintering, the histidine level rose several-fold: a particularly high increase of serine was detected in *T. aestivum* Linn. (Table 11).

Changes in the pool composition

1) Wintering stages: in fall, arginine was accumulated in *A. alba* Linn. and at the last stage of snow thawing of next year, arginine was no longer the major amino acid in the pool concomitant with the increase of other amino acids such as glutamate (Table 3). In *M. sativa* Linn., *T. pratense* Linn. (Table 10) and *T. repens* Linn. (Table 2), the accumulated asparagine in the pool had decreased by the following April and arginine was the major amino acid in the plants (Table 10).

2) At the early stage of growth in spring: in fall, arginine which was accumulated in poplar buds as the major amino acid, decreased in spring in these buds at the early stage of budding concomitant with the increase of glutamine and glutamate. After budding in May, relative portions of glutamine and glutamate decreased, while asparagine and aspartate were predominant in the latter stage in the apex (Fig. 2). In the stem of garden asparagus, arginine and proline predominated during winter (Table 12). During the early stages of the development, the decrease of arginine and proline proceeded and concomitantly the levels of many of the amino acids remained almost constant. The level of asparagine increased in the latter stage of the stem growth.

The present amino acid analyses indicated that arginine and proline play a role as a major storage amino acid in the wintering plants. In April, the last stage of snow thawing, the plants were considered to be in an early stage of development and therefore required an abundant pool of amino acids for protein synthesis. At this stage, syntheses of general amino acids proceeded first at the expense of the major amino acids present in the pool. Thus, metabolically mobile

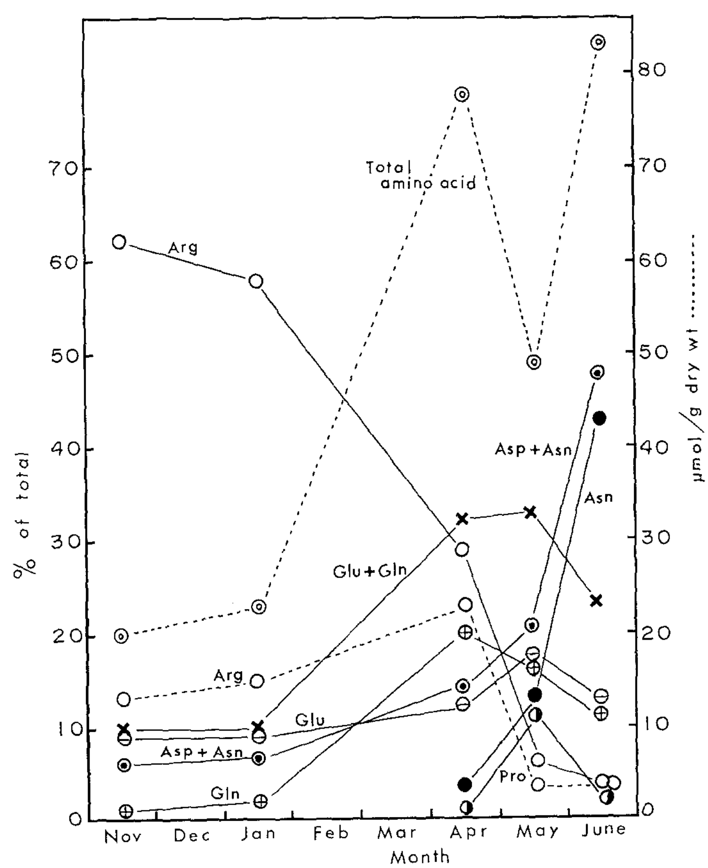

Fig. 2. Changes in the relative amount of arginine and other amino acids in the buds and apices of *Populus gelrica* from November to June. The bud opening occurred in early May. (——), % of total amino acids; -○-, Arg; -×-, Glu plus Gln; -⊖-, Glu; -⊕-, Gln; -⊙-, Asp plus Asn; -●-, Asn; - -○- -Arg, (μmol/g dry weight); - -◎- -, total amino acid (μmol/g dry weight)

Table 12. Composition of amino acid pools of the apex of *Asparagus officinalis* Linn. in late fall and during an early stage of growth

		(b)**	(c)**	(d)	(e)
		Growing			
	(a)*	stem length (cm)			
Sample	Wintering	0.5	1.5	12	40
	(μmol free amino acid/g wet wt)				
Serine	2.9	4.3	4.1	4.5	4.8
Glycine	0.7	0.8	0.6	0.6	0.4
Cystine/2				0.5	0.5
γ-Aminobutyrate	0.2				
Glutamate	7.8	6.0	5.6	6.0	6.1
Glutamine	7.7	7.0	7.1	9.0	8.9
Proline	19.9	104.6	82.0	28.3	2.0
Arginine	11.9	5.7	1.3	0.9	3.9
Lysine	1.1	2.0	1.7	1.6	0.3
Alanine	0.7	3.8	1.2	2.1	1.4
Aspartate	3.1	3.7	3.5	3.6	3.1
Asparagine	5.3	2.4	2.1	1.7	8.8
Threonine	1.3	1.9	1.7	1.6	1.8
Methione	0.7		0.5	0.3	0.4
Valine	1.6	3.5	2.0	1.6	1.2
Leucine	1.4	0.6	1.1	0.9	0.3
Isoleucine	0.7		0.5	0.4	0.5
Histidine	0.9	0.8	0.7	0.5	0.7
Tyrosine	0.2	0.5	0.5	0.4	0.4
Phenylalanine	0.8	0.9	0.7	0.5	0.3
Tryptophan	0.2				
Total	66.0	148.5	116.9	65.0	45.8

* Wintering underground. ** Growing underground.
(*a*), sampled on November 17; (*b*) and (*c*), sampled on April 28.
Blanks in the Table represent the values less than 0.04 μmol g^{-1} wet wt.

fractions of stored nitrogen were believed to contribute to the initial stage of rapid growth in wintering plants.

The earlier report of Durzan (Durzan, 1968) indicated that *Picea glauca* accumulated arginine and proline. Poplar twigs accumulated arginine in the wintering period (Sagisaka, 1974a). In this study, it is evident that the accumulation of arginine and/or proline in perennial plants from fall through midwinter occurred annually and appeared to be a normal physiological event in the metabolism of stored nitrogen. The recent progress in amino acid metabolism in higher plants has been reviewed by Miflin and Lea (Miflin and Lea, 1977). Results of the present study suggested that reactions involved in arginine and proline syntheses appear to operate in plants in milieu of midwinter.

Proline was accumulated in plants under water stress (Barnett and Naylor, 1966; Dix and Pearce, 1981; Taylor et al., 1982; Stewart and Lee, 1974). When young plants which are unable to adapt to chilling temperatures were placed in such an unfavorable condition, the growth was retarded and they accumulated several kinds of amino acids such as proline, alanine, glutamate and so forth (Chu, et al., 1978; Draper, 1972; Özturk and Szaniawski, 1981; Patterson et al., 1981; Taylor et al., 1972). The prominent difference in the pool between the plants in the wintering stages and the growth retarded ones was manifested by the accumulation of arginine alone or the coexistence of arginine and proline in the former plants. The occurrence of proline alone in the pool of wintering plants was very rare.

Tissues of wintering twigs contain about 0.3 M sucrose (Parker, 1958), which functions as a natural cryoprotectant (Sakai, 1961). Proline alone enhanced the recovery of cultured maize cells after freezing (Withers and King, 1979). Heber et al. (Heber et al., 1971) showed that thylakoid functions were protected from freezing inactivation by added proline and arginine and so forth. Therefore, arginine and proline played a role not only in the storage of nitrogen, but also, in combination with sucrose, in the protection of cells from freezing damage.

References

Asada M, Ahn YH, Sagisaka S (1988) Changes in parenchyma cells of poplar xylem during transition from growing to wintering stages. Plant Cell Physiol 29: 243–246

Barnett NM, Naylor AW (1966) Amino acid and protein metabolism in Bermuda grass during water stress. Plant Physiol 44: 1222–1230

Chu TM, Jusaitis M, Aspinall D, Paleg LG (1978) Accumulation of free proline at low temperatures. Physiol Plant 43: 254–260

Dix PJ, Pearce RS (1981) Proline accumulation in NaCl-resistant and sensitive cell lines of *Nicotiana sylvestris*. Z Pflanzenphysiol 102: 243–248

Draper SR (1972) Amino acid changes associated with low temperature treatment of *Lolium perenne*. Phytochemistry 11: 639–641

Durzan DJ (1968) Nitrogen metabolism of *Picea glauca* I. Seasonal changes of free amino acids in buds, shoot apices, and leaves, and the metabolism of uniformly labelled ^{14}C-L-arginine by buds during the onset of dormancy. Can J Bot 46: 909–919

Heber U, Tyankova L, Santarius KA (1971) Stabilization and inactivation of biological membranes during freezing in the presence of amino acids. Biochim Biophys Acta 241: 578–592

Mattoon JR (1963) The biosynthesis of amino acids. In: Bernfeld P (ed) Biogenesis of natural compounds. Pergamon Press, London, pp 1–30

Miflin BJ, Lea PJ (1977) Amino acid metabolism. Annu Rev Plant Physiol 28: 299–329

Nakagawara S, Sagisaka S (1984) Increase in enzyme activities related to ascorbate metabolism during cold acclimation in poplar twigs. Plant Cell Physiol 25: 899–906

Ozturk M, Szaniawski RK (1981) Root temperature stress and proline content in leaves and roots of two ecologically different plant species. Z Pflanzenphysiol 102: 375–377

Parker J (1958) Changes in sugars and nitrogenous compounds of tree barks from summer to winter. Naturwiss 45: 139

Patterson BD, Pearson JA, Payne LA, Ferguson IB (1981) Metabolic aspects of chilling resistance: Alanine accumulation and glutamate depletion in relation to chilling sensitivity in passion-fruit species. Aust J Plant Physiol 8: 395–403

Rosnitschek-Schimmel I (1985) Seasonal dynamics of nitrogenous compounds in a nitrophilic weed II. The role of free amino acids and proteins as nitrogen store in *Urtica dioica*. Plant Cell Physiol 26: 177–183

Sagisaka S (1974a) Effect of low temperature on amino acid metabolism in wintering poplar. Plant Physiol 53: 319–322

Sagisaka S (1974b) Transition of metabolism in living poplar bark from growing to wintering stages and vice versa. Plant Physiol 54: 544–549

Sagisaka S, Asada M (1981) Coordinate and noncoordinate changes in enzyme activities in pentose phosphate cycle in poplar: A control of enzyme activities in differentiated xylem. Plant Cell Physiol 22: 1459–1468

Sakai A (1961) Effect of polyhydric alcohols on frost hardiness in plants. Nature 189: 416–417

Stewart GR, Lee JA (1974) The role of proline accumulation in halophytes. Planta 120: 279–289

Taylor AO, Jepsen NM, Christeller JT (1972) Plant under climate stress III. Low temperature, high light effects on photosynthetic products. Plant Physiol 49: 798–802

Taylor AA, De-Felice J, Havill DC (1982) Nitrogen metabolism in *Poterium sanguisorba* during water stress. New Phytol 90: 19–25

Withers LA, King PJ (1979) Proline: A novel cryoprotectant for the freeze preservation of cultured cells of *Zea mays* L. Plant Physiol 64: 675–678

Authors' address: S. Sagisaka, The Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan.

Received January 28, 1992