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Nitrogen metabolism in harvested asparagus: No difference between light and dark storage at 20°C

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To extend our knowledge of nitrogen metabolism in asparagus after harvest we monitored protein, amino acid and ammonia levels and glutamine synthetase activity in tip sections (0–30 mm) of asparagus spears (*Asparagus officinalis* L. cv. Limbras 10) stored for up to 48 h in continuous light or dark at 20°C. The patterns of change in light and dark were almost identical. Total protein content increased by 20% 12 h after harvest, before declining to harvest levels by 48 h. Ammonia increased slightly by 48 h. Glutamine synthetase activity increased 10–15% by 18 h then declined to 90% of harvest activity by 48 h. Glutamic acid and glutamine were the most abundant amino acids at harvest, accounting for 42% of the pool. Glutamine and proline levels fell rapidly. By 48 h most amino acids had increased substantially, with asparagine accounting for 28% of the pool. Glutamine levels fell steadily during storage, irrespective of the regime. Our results suggest that postharvest nitrogen metabolism in asparagus spear tips is not modulated by light.

INTRODUCTION

Plant physiology and biochemistry can be seriously disrupted following harvesting, to the detriment of consumer acceptance and nutritive value. Improvement in the longevity of vegetable crops will enhance their usefulness as food items.

In previous publications (King *et al.*, 1990; Lill *et al.*, 1990) we identified postharvest changes in nitrogen metabolism in asparagus spear tips associated with the postharvest deterioration of whole spears. Within 48 h of storage at 20°C in the dark, we observed net protein loss, and accumulation of free amino acids and ammonia.

To mimic commercial practice, studies on postharvest senescence of horticultural crops typically involve harvesting the plant material in the light and storing it in darkness. This approach, however, does not separate the often confounding effects of storage time and light. Storing plant material in darkness can accelerate senescence (Thomas & Stoddart, 1980; Kawakami & Watanabe, 1988). Nitrogen metabolism is particularly affected. The synthesis and accumulation of nitrogen-containing compounds (e.g. asparagine) can be enhanced by darkness (Sieciechowicz *et al.*, 1988). The activity of several enzymes of ammonia metabolism, including glutamine synthetase (GS; EC 6.3.1.2), the primary enzyme responsible for ammonia assimilation (Joy, 1988), is modulated by light (Srivastava & Singh, 1987; Joy, 1988; Schmidt & Mohr, 1989).

Our aims in this paper were (1) to characterise the effect of light on the above indices of postharvest nitrogen metabolism and (2) to initiate studies on GS in asparagus.

MATERIALS AND METHODS

Plant material and storage conditions

Spears (12–15 mm butt diameter) of *Asparagus officinalis* L. (cv Limbras 10) were hand-harvested at Levin Research Centre. Spears were washed, trimmed to 180 mm, placed in clear perforated polythene bags (5 spears/bag) and stored in either continuous light or dark at 20°C for 0, 3, 6, 9, 12, 18, 24 or 48 h. Light was supplied by two Philips HLRG 400 W Hg-vapour lamps at a fluence rate of 90 μmol m^{-2} s^{-1} at spear level. After storage, tip sections (0–30 mm) were excised, frozen in liquid nitrogen and held at −20°C until lyophilisation. Tips obtained at zero time in the field were frozen immediately in liquid nitrogen. Tips of dark treated spears were excised under dim green light. Following lyophilisation, each plot of 5 tips was powdered and stored desiccated at −20°C until analysis.

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Spears were from a single harvest. Each storage time under each regime (light or dark) had 3 replicates.

Assay procedures

Ammonia (NH_4^+ ions), total protein and free amino acids were measured as previously described (King *et al.*, 1990). GS was extracted from duplicate 10-mg samples of lyophilised tips with 1 ml of ice-cold extraction buffer (Vézina *et al.*, 1988) by intermittent vortex mixing over 5 min. GS was extracted from fresh tips in ice-cold buffer (1·5 ml/g) for 30–60 s with an Ultra-Turrax homogeniser. GS activity in the supernatants was measured at 30°C and 540 nm by both the transferase and biosynthetic assays of Donn *et al.* (1984), except that 50 mM Hepes–NaOH was used for the buffers and the $FeCl_3$ reagent was that of O'Neal and Joy (1973). The assays were linear with respect to both time and amount of supernatant. An absorbance of 0·438 corresponded to 1 μmol of 4-glutamylhydroxamate. One unit of activity is defined as the formation of 1 μmol of 4-glutamylhydroxamate in 1 min under the above conditions.

RESULTS AND DISCUSSION

We found no major differences between light and dark storage in the patterns of change for protein, amino acid and ammonia levels and glutamine synthetase activity over 48 h at 20°C. This suggests that the changes detected are associated with deterioration *per se* rather than with a changed light environment.

The pattern of change for protein during storage (Table 1) confirmed our previous work (King *et al.*, 1990). Protein content increased 10–20% 6–12 h after harvest, then declined to the harvest level (300 mg g^{-1} dry wt) by 48 h. Ammonia was maintained at harvest level (12 μmol g^{-1} dry wt) for up to 24 h after harvest (Table 2). By 48 h the level was 15 μmol g^{-1} dry wt, considerably less than 160 μmol g^{-1} dry wt we reported previously (King *et al.*, 1990). This disparity may be explained by interseasonal variation in the timing of the defined patterns of change.

GS activity (mean ± SEM) of freshly harvested spear tips was 11·6 ± 0·7 units g^{-1} fresh weight when measured by the transferase assay and 1·2 ± 0·1 units g^{-1} fresh weight by the biosynthetic assay. The respective GS activities of lyophilised tissue were 130 ± 8 and 12 ± 1 units g^{-1} dry weight. Table 3 details the changes in the transferase activity during storage. The activity remained relatively constant for 24 h postharvest, with possibly a slight transient increase at 18 h. By 48 h, however, the activity had begun to decline. There were no significant differences between light and dark storage. Cooney *et al.* (1980) reported on activity of 82·5 μmol glutamine h^{-1} mg^{-1} protein for GS in asparagus seedlings which, assuming biosynthetic assay was

Table 1. Total protein content [mg g^{-1} dry wt, (SEM)] of asparagus spear tips during 48-h storage at 20°C in continuous light (L) or continuous dark (D) conditions

At harvest		Time after harvest (h)						
		3	6	9	12	18	24	48
301 (8·6)	L	321 (2·0)	349 (10)	340 (3·0)	360 (8·7)	308 (8·3)	315 (3·4)	306 (5·6)
	D	298 (3·9)	347 (6·1)	351 (8·7)	356 (5·7)	322 (19)	330 (10)	283 (17)

Table 2. Ammonia (NH_4^+) content [μmol g^{-1} dry wt, (SEM)] of asparagus spear tips during 48-h storage at 20°C in continuous light (L) or continuous dark (D) conditions

At harvest		Time after harvest (h)						
		3	6	9	12	18	24	48
12·9 (0·8)	L	12·2 (0·3)	12·2 (0·9)	11·3 (0·8)	12·4 (0·2)	12·0 (0·8)	13·3 (0·8)	15·9 (0·7)
	D	12·0 (0·9)	11·1 (0·8)	12·0 (0·7)	10·7 (0·8)	12·9 (0·7)	12·2 (0·3)	14·1 (1·3)

Table 3. Glutamine synthetase activity [units g^{-1} dry wt (SEM)] of asparagus spear tips during 48-h storage at 20°C in continuous light (L) or continuous dark (D)

At harvest		Time after harvest (h)						
		3	6	9	12	18	24	48
130 (8)	L	141 (1)	142 (2)	146 (2)	145 (2)	157 (4)	142 (4)	122 (7)
	D	133 (6)	140 (7)	140 (2)	145 (6)	153 (3)	147 (7)	118 (4)

Table 4. Free amino acid content [μmol g^{-1} dry wt (SEM)] of asparagus spear tips during 48-h storage at 20°C under either continuous light (L) or continuous dark (D) conditions

	At harvest		Time after harvest (h)													
				3		6		9		12		18		24		48	
Alanine	24	(1·8)	L	17	(2·3)	17	(1·8)	18	(1·2)	16	(0·3)	15	(1·3)	16	(0·3)	24	(0·9)
			D	17	(0·6)	17	(0·3)	15	(1·8)	14	(0·9)	14	(1·5)	17	(0·9)	20	(1·2)
Arginine	39	(2·2)	L	67	(15)	38	(2·0)	72	(5·0)	39	(6·8)	64	(7·0)	49	(11)	63	(11)
			D	61	(9·9)	38	(2·2)	72	(5·4)	75	(4·1)	51	(9·9)	53	(4·5)	42	(4·7)
Asparagine	48	(2·1)	L	28	(2·7)	32	(2·0)	38	(3·8)	38	(1·5)	54	(4·7)	97	(3·5)	190	(9·5)
			D	32	(1·8)	39	(0·3)	38	(0·7)	42	(3·5)	53	(5·1)	87	(4·4)	167	(12)
Aspartic acid	38	(1·5)	L	21	(2·5)	39	(1·7)	31	(2·2)	44	(1·0)	25	(0·6)	20	(2·0)	29	(0·9)
			D	22	(2·1)	44	(1·8)	30	(0·9)	34	(4·0)	29	(2·9)	32	(0·7)	28	(1·8)
Cysteine	1·0	(0·12)	L	0·8	(0·12)	0·8	(0·06)	0·7	(0·09)	0·6	(0·15)	0·7	(0·10)	0·7	(0·09)	0·8	(0·07)
			D	0·9	(0·09)	0·9	(0·09)	0·6	(0·07)	0·6	(0·06)	0·8	(0·12)	0·8	(0·15)	0·8	(0·10)
Glutamine	101	(7·3)	L	73	(7·7)	54	(4·6)	69	(4·8)	68	(3·4)	40	(3·3)	36	(2·0)	28	(2·9)
			D	71	(5·8)	69	(2·6)	53	(3·2)	51	(1·2)	45	(4·1)	36	(3·8)	25	(3·4)
Glutamic acid	94	(5·8)	L	74	(5·2)	89	(8·4)	85	(4·4)	77	(0·9)	66	(1·5)	65	(0·9)	65	(1·7)
			D	66	(6·5)	91	(4·4)	72	(9·0)	77	(5·0)	70	(5·0)	75	(6·2)	69	(4·6)
Glycine	6·3	(0·2)	L	3·8	(0·5)	4·3	(0·2)	4·1	(0·15)	4·5	(0·15)	3·3	(0·2)	6·0	(0·4)	12	(0·3)
			D	3·8	(0·3)	4·8	(0·09)	3·3	(0·2)	3·7	(0·07)	3·4	(0·4)	5·6	(0·1)	9·1	(0·6)
Histidine	5·8	(1·1)	L	2·6	(0·6)	4·6	(0·5)	2·9	(0·5)	4·7	(0·4)	3·0	(0·2)	10	(1·5)	20	(1·9)
			D	2·3	(0·6)	6·3	(1·2)	3·5	(0·3)	5·5	(0·7)	2·9	(1·1)	7·2	(1·8)	14	(0·3)
Hydroxyproline	0·73	(0·13)	L	0·5	(0·03)	0·7	(0·1)	0·7	(0·03)	0·8	(0·03)	0·6	(0·03)	0·7	(0·03)	0·6	(0·03)
			D	0·7	(0·06)	0·9	(0·07)	0·6	(0·01)	0·8	(0·03)	0·5	(0·09)	0·7	(0·07)	0·7	(0·06)
Isoleucine	1·3	(0·1)	L	0·6	(0·07)	1·1	(0·09)	1·3	(0·03)	2·3	(0·15)	3·3	(0·15)	7·6	(0·3)	29	(1·7)
			D	0·7	(0·06)	1·6	(0·5)	1·0	(0·03)	1·7	(0·3)	2·5	(0·5)	6·0	(0·4)	18	(0·9)
Leucine	3·0	(0·1)	L	1·9	(0·3)	1·5	(0·2)	1·5	(0·1)	2·7	(0·01)	4·0	(0·07)	8·9	(0·7)	24	(1·0)
			D	1·6	(0·2)	2·3	(0·4)	2·5	(0·1)	2·6	(0·2)	3·4	(0·6)	7·1	(0·5)	18	(1·2)
Lysine	1·3	(0·03)	L	0·6	(0·19)	1·3	(0·07)	1·8	(0·2)	2·7	(0·4)	3·3	(0·3)	9·1	(0·7)	25	(1·0)
			D	0·8	(0·15)	1·8	(0·6)	1·1	(0·1)	2·1	(0·1)	3·1	(0·3)	7·3	(0·4)	17	(1·2)
Methionine	4·9	(0·4)	L	2·3	(0·3)	3·1	(0·6)	2·1	(0·4)	2·7	(0·1)	1·2	(0·06)	3·6	(0·3)	7·5	(1·0)
			D	2·7	(0·4)	4·1	(0·4)	2·2	(0·1)	3·7	(0·4)	2·0	(0·2)	3·6	(0·5)	4·9	(1·1)
Phenylalanine	2·3	(0·03)	L	1·7	(0·4)	2·0	(0·15)	1·6	(0·15)	2·1	(0·2)	2·6	(0·4)	7·0	(0·3)	22	(1·2)
			D	1·9	(0·2)	2·2	(0·3)	1·3	(0·1)	2·1	(0·1)	2·4	(0·5)	5·6	(0·5)	14	(0·7)
Proline	36	(4·2)	L	16	(0·3)	10	(1·4)	10	(1·6)	8·1	(0·2)	6·6	(0·6)	6·1	(0·9)	15	(1·8)
			D	25	(1·0)	12	(1·8)	11	(1·4)	6·7	(1·5)	5·1	(0·8)	5·8	(0·9)	11	(1·4)
Serine	36	(2·5)	L	21	(1·4)	27	(2·6)	30	(0·3)	34	(1·0)	29	(1·2)	40	(1·2)	69	(2·7)
			D	23	(0·9)	34	(1·2)	25	(1·2)	30	(1·3)	25	(2·7)	41	(1·5)	56	(2·5)
Threonine	7·4	(0·4)	L	5·7	(0·5)	8·5	(0·8)	9·8	(0·2)	12	(0·6)	9·6	(0·2)	15	(0·7)	20	(0·3)
			D	5·8	(0·3)	11	(1·2)	7·7	(0·3)	10	(0·3)	9·1	(1·2)	15	(0·6)	19	(1·3)
Tryptophan	0·16	(0·01)	L	0·14	(0·01)	0·18	(0·02)	0·20	(0·03)	0·38	(0·05)	0·7	(0·05)	1·5	(0·08)	4·5	(0·4)
			D	0·16	(0·04)	0·16	(0·02)	0·18	(0·01)	0·31	(0·03)	0·6	(0·03)	1·1	(0·13)	3·4	(0·3)
Tyrosine	0·40	(0·03)	L	0·23	(0·03)	0·40	(0·01)	0·43	(0·03)	0·6	(0·1)	0·8	(0·09)	1·7	(0·1)	3·1	(0·3)
			D	0·23	(0·03)	0·50	(0·15)	0·30	(0·01)	0·6	(0·03)	0·8	(0·13)	1·7	(0·1)	3·0	(0·3)
Valine	18	(0·6)	L	9·1	(1·0)	9·7	(0·7)	10	(0·3)	12	(0·7)	12	(0·3)	20	(0·9)	55	(2·9)
			D	10	(0·6)	12	(0·6)	9·4	(0·3)	10	(1·1)	11	(1·4)	19	(0·7)	37	(1·9)
Total	466	(15)	L	348	(36)	344	(26)	391	(14)	372	(16)	343	(15)	425	(22)	707	(29)
			D	349	(23)	392	(12)	349	(22)	374	(13)	333	(25)	427	(20)	577	(36)

used, is equivalent to $1{\cdot}4 \times 10^{-3}$ units mg^{-1} protein. Our GS activity at harvest, similarly expressed, is $3{\cdot}6 \times 10^{-2}$ units mg^{-1} protein. On a fresh weight basis, our GS transferase activity is comparable to that of pea-leaf and pod tissue reported by Storey and Beevers (1978), who also used a transferase assay.

Changes in the amino acid complement during storage (Table 4) also confirmed the trends of the previous work (King *et al.*, 1990). Initially, amino acid pools fell while protein increased and then rose in parallel with protein loss. At harvest, glutamic acid and glutamine were almost equally abundant and accounted for 42% of the pool. Arginine, asparagine, aspartic acid, proline and serine combined accounted for a further 42%. After 48-h storage, asparagine was most abundant and accounted for 28%. Arginine, glutamic acid, serine and valine added up to a further 36%. The concentrations of most amino acids (e.g. asparagine, glycine, histidine, leucine, tryptophan) either fell slightly or remained constant for 12–24 h, then increased markedly by 48 h. Others (arginine, aspartic acid, cysteine, glutamic acid, hydroxyproline) remained relatively constant throughout. Proline fell dramatically within 6 h, steadied, then followed the general trend and increased by 48 h. Glutamine was unusual in that levels steadily declined throughout storage. Clearly, a major shift in nitrogen metabolism occurs in asparagus spears after harvest. Amide accumulation (glutamine and asparagine) occurs when plants detoxify large amounts of ammonia such as during leaf senescence (Givan, 1979), or during

periods of environmental stress (Rabe, 1990). Asparagine accumulates dramatically in detached pea shoots when darkened, but not in the light (Joy *et al.*, 1983). Asparagus spear tips, obviously, behave quite differently from pea shoots.

In conclusion, we have demonstrated that light and dark storage at 20°C have no differential effects on nitrogen metabolism in asparagus spear tips during early postharvest senescence. This differs markedly from other systems. We conclude that the observed shifts in nitrogen metabolism are storage-related rather than light-modulated.

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