http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (3): 293-297, 2001

Postharvest Changes in Ammonium, Glutamine Synthetase and Glutamate Dehydrogenase in Asparagus Spears during Storage at 20°C

Fabio Gimena Enriquez, Toshiyuki Matsui, Pankaj Kumar Bhowmik
Haruo Suzuki and Kazuhide Kawada
Department of Bioresource Production,
Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan

Abstract: Asparagus (*Asparagus officinalis* L. cv. Welcome) spears deteriorates rapidly after harvest and is associated with an increase in the ammonia content of the spear tip. In other plant systems it is thought that ammonia buildup is toxic and causes tissue deterioration. To further investigate these phenomena the authors held the spears at 20°C after harvest and examined the glutamine synthetase (GS, EC 6.3.1.2) and glutamate dehydrogenase (GDH, EC 1.4.1.2) activities and ammonium content in the spears. GS is the key enzyme responsible for assimilating ammonium while GDH catalyses the amination of oxoglutarate to glutamate (NADHGDH) and the deamination of glutamate to oxoglutarate (NAD^+-GDH). Spears were stored for up to 5 days at 20°C. Accumulation of ammonium increased as the days of storage progressed. The GS activity declined whereas the GDH activities almost doubted after 5 days of storage at 20°C. The increased GDH activities could be caused by a higher ammonium levels in the spears. The rise in GDH activities is linked with a corresponding decline in GS activity. Glutamine and glutamic acid content decreased with days of storage. It may be concluded that the rapid changes in the physiology of asparagus spears are induced in response to harvest, storage and the amino acid loss content are commonly associated with senescence in plant tissue.

Key words: Amino acids, ammonium, *Asparagus officinalis*, glutamate dehydrogenase, glutamine synthetase

Introduction

Fresh asparagus spears are highly perishable. Many physiological and biochemical changes are associated with this postharvest deterioration (Lipton, 1990). Changes are particularly evident in the spear tip wherein sugars are rapidly depleted (Lill *et al*., 1990) and free amino acids and ammonium ions accumulate (King *et al*., 1990). Ammonium is considered to be toxic to plant cells (Givan, 1979) and this toxicity may be a factor in the short shelf life of asparagus. Amino acid and ammonium accumulation are characteristics of mature detached leaves senescing in the dark (Peters and van Laere, 1992). Numerous studies have dealt with the metabolism and enzymology of nitrogen redistribution in both attached (Kamachi *et al*., 1991) and detached (Peters and van Laere, 1992) mature leaves and these studies have been interpreted to be related to natural senescence.

The primary synthesis of amino acids in higher plants may result either from direct amination of 2-oxoglutarate, the reaction being catalyzed by GDH, or it may occur indirectly via glutamine. In the latter case, ammonia is incorporated by GS as the amide group into glutamate, thus producing glutamine. Subsequently, the glutamate synthase (GOGAT) catalyses the reductive transfer of the group from glutamine to 2-oxoglutarate which results in formation of two molecules of glutamate. GDH has a low affinity for ammonia, hence, the pathway via glutamine is accepted as the main pathway of primary biosynthesis of amino acids. However, in conditions of excessive supply of ammonia, appropriate modifications of the metabolism must occur in the plant tissue to prevent the increase of ammonia concentration to a toxic level (Givan, 1979). Enzymes of primary synthesis of amino acids play an important role in the detoxification of ammonia in plant tissues. Consequently, an understanding of the underlying changes that occur in spear after harvest may contribute much to understanding the control of deteriorative process that accompany postharvest storage of asparagus.

To extend our knowledge of the postharvest senescence of asparagus spears and to establish a time sequence of physiological change for later studies, we investigated the respiration rate, some amino acid enzymes and amino acid content that occur in the spears stored for up to 5 days at 20°C.

Materials and Methods

Plant materials: Green asparagus spears (*Asparagus officinalis* L. cv. Welcome) harvested from a commercial crop in Kagawa, Japan were obtained directly from a packing house. Spears were hand harvested and trimmed to approximately 25 cm length. The spears, which were of good quality, straight with closed bracts, were put in plastic bags and held at 20°C for up to 5 days. At harvest (day 0) and subsequent 24 h intervals, the spears were weighed and frozen at -30°C for amino acid and enzyme assays.

Respiration rate measurement: The spears were weighed and carefully placed in a 6-/ glass jar held at 20°C. Three replicates of 10 spears were used in the experiment. Carbon dioxide production was measured on intact spears, top and bottom portions at 24 hr intervals. Production of CO_2 was measured by taking 10 m/gas samples from the glass jar sealed for 1 hr and injected to a TCD gas chromatograph equipped with a 1 m activated charcoal column at 65°C (GC-BAIT, Shimadzu Co. Ltd.). The results were expressed as $m/CO_2\ kg^{-1}\ hr^{-1}$.

Enzyme extraction: Each spear was cut into two equal halves (designated as the top and bottom portion) just before extraction. Approximately 5 g sample from each portion were homogenized under ice-cold conditions (ca. 0 to 4°) with 1% polyvinylpolypyrrolidone (PVPP), proportional to the sample weight and 1 g of sea sand in Buffer A by using a mortar and pestle. One ml extraction buffer per g fresh weight of plant

materials was used. Extraction was performed according to a method of Hurst and Clark (1993), in which Buffer A contained 50 mM Tris-HCl (pH 7.6), 10 mM $MgSO_4$, 1 mM EDTA, 1 mM dithiothreitol (DTT), 12 mM 2-mercaptoethanol, 5 mM glutamate and 100 m/liter^{-1} glycerol. The homogenate was squeezed through a four-layer cotton cloth and the filtrate was centrifuged at 11 000 × g for 10 min. The residual tissues were re-extracted in 5 ml buffer A and dialyzed with 40 times dilution of the same buffer for 1 h and then centrifuged. The resulting supernatant were mixed together and used for the enzyme assay.

Enzyme assays: The enzymatic activities were assayed in a total volume of 1.0 ml. For GS, 80 mM L-glutamate-Na, 500 mM Tricine-KOH Buffer (pH 7.0), 600 mM NH_2OH, 200 mM $MgSO_4$ $7H_20$, 10 mM Diethylenetriamine pentaacetic acid (DTPA), 80 mM ATP and 800 mM mercaptoethanol were used. After incubating at 35°C for 8 min, the reaction was stopped by adding 1 ml ferric reagent (25 ml $FeCl_3 \cdot 6H_2O$, 50 ml HCl and 20 ml TCA). The activities were monitored using a double-beam spectrophotometer (Shimadzu model UV-150-02) at 540 nm. The GDH activity was determined in both aminating and deaminating directions in a total volume of 1.0 ml. For GDH amination, 20 mM a-ketoglutarate, 100 mM Tris-HCl (pH 8.0), 200 mM NH_4Cl, 1.0 mM CaCl a 0.2 mM NAD(P)H and 100 µl enzyme solution were used. The GDH deamination activities were assayed with a mixture containing 100 mM L-Glutamate, 100 mM Tris-HCl (pH 9.3) 1.0 mM $NAD(P)^+$, 0.5 mM $CaCl_2$ and 100 µl enzyme solution. The GDH amination and deamination activities were monitored using a double-beam spectrophotometer (Shimadzu model UV-150-02) at 350 nm to NADH oxidation or NAD^+ reduction. One unit of GDH activity is defined as the reduction or oxidation of 1 micromole of coenzyme (NADH/NADPH, respectively) per min at 30°C.

Extraction and analysis of amino acids: Triplicate samples of 5 g fresh weight were homogenized under ice-cold conditions with 15 ml of 75%, ethanol then incubated in a water bath shaker at 45°C for 30 min. The solutions were filtered into a flask. The residues were added with 15 ml 75% ethanol and placed again in a water bath at 45°C for 30 min. The filtrate was evaporated under reduced pressure, adjusted to 10 ml with deionized water and centrifuged at 11000 × g for 10 min. Following extraction, the aqueous solution (containing the amino acids) was loaded onto an Ion Exchange Resin (cation-exchange, WAmberlite (R 120) column (1.5 i.d. x 2 cm diameter), eluted and washed with water until pH 6-7. Finally, 25 ml 1N NH_3 was poured through the column to elute the amino acids. Eluates were evaporated as described above and then made up to 10 ml with 0.02 N HCl and filtered through a nitrate cellulose membrane filter (0.45 µm pore size). The ammoniacal eluates were stored in a vial at -20°C until analysis. The amino acid content was determined using an automatic amino acid analyzer (Hitachi L-8500) equipped with a Chromato-Integrator (Hitachi D-2850).

Ammonium assay: Five g samples from each portion were extracted with 10% Tricholoroacetic acid and centrifuged at 11000 × g for 10 min. The ammonium content was assayed as described. Ammonium content of the supernatant was determined from a triplicate 500 µl samples by adding 200 µl 0.5 M Tris-buffer (pH 8.0), 100 µl 0.1 M 2-oxoglutarate solution (pH 7.4), 30 µl 8 mM β-NADH solution and 150 µl distilled water. The absorbance of the samples was recorded at 340 nm against a reagent blank.

Statistical analysis: A randomized complete block design was used with three replications. The level of significance was calculated from the F value of ANOVA.

Results

Weight loss: Figure 1 shows the weight loss (%) of asparagus spears during storage. The weight loss increased as the days of storage progressed. During the first 3 days of storage, the weight loss was minimal but it increased significantly after 4 to 5 days of storage.

Respiration rate: The respiration rate of asparagus (intact spears, top and bottom portions) after harvest at 20° is shown in Fig. 2. The pattern of change in respiration rate in intact, top and bottom portions of the spears declined after 1 day of storage and increased subsequently until the end of the storage period. The asparagus spears respiration rate was significantly different in the top and bottom portion and intact spears. However, no significant difference was found between the bottom portion and intact spears. The top portion was over 1 or 2 times greater than the bottom portion and intact spears. The increased respiration rate was significantly higher in the top portion by about 40% and 45% than the bottom portion and intact spears, respectively, at the end of 5-day storage.

Enzyme activities: GS and GDH activities on the top and bottom portions of the spears during storage at 20° are shown in Fig. 3 and 4, respectively. The GS activity increased in both portions 1 day after storage. However, the activity in the top and bottom portions declined to about 22 and 24% respectively, of the initial levels after 5 days (Fig. 3). The GS activity in the top portion was significantly higher than the bottom portion of the spears. The GDH amination (NADH-dependent) and deamination (NAD^+-dependent) activities both in the top and bottom portions increased throughout the storage period (Fig. 4). The amination activity of the top portion was consistently higher than the bottom portion throughout the storage period. On the other hand, the deamination activity in the top portion was found to be significantly different from the the bottom portion of the spears after 3 to 5 days storage. Both amination and deamination activities of GDH almost doubled over the 5-day postharvest period both in the top and bottom portions of the spears.

Ammonium content: The ammonium content in the top and bottom portions of spears reached to about 40% after 72 h storage and increased to about 60% of the initial levels at the end of the storage period (Fig. 5). The top portion was significantly higher than the bottom portion of the spears.

Glutamine and glutamic acid content: Figure 6 shows the glutamine and glutamic acid level of asparagus spears on the top and bottom portions of the spears held at 20° for 5 days. Both glutamine and glutamic acid content were significantly higher in the top portion than in the bottom portion of the spears during storage up to the 4th and 3rd day after harvest, respectively. Glutamine content in both portions increased 1 day after storage and then declined

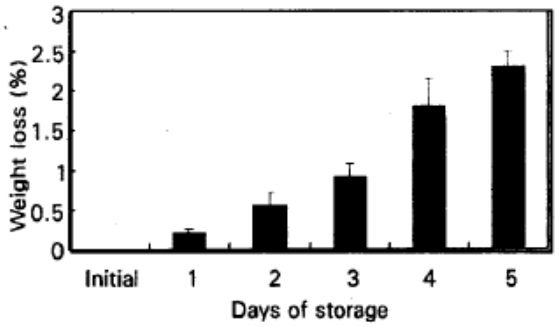

Fig. 1: Weight loss (%) of asparagus spears held at 20°C. Vertical bars indicates SE

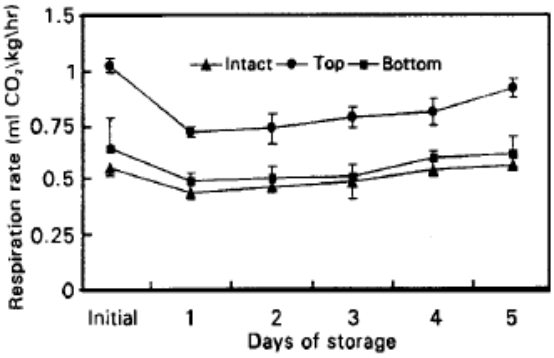

Fig. 2: Respiration rate of intact spears, top and bottom portions of the spears held at 20°C. Each point represents the mean of 3 replications. Vertical bars indicate SE

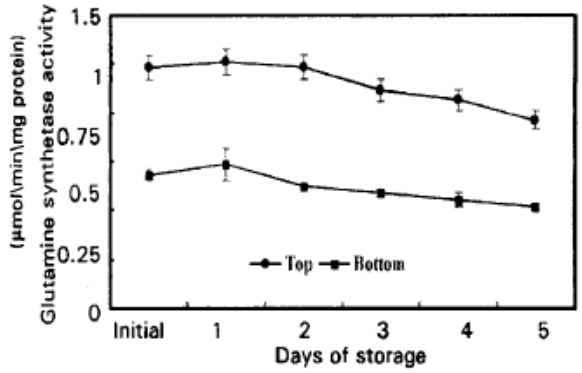

Fig. 3: Glutamine synthetase activity in the top and bottom portions of the spears held at 20°C. Each point represents the mean of 3 replications. Vertical bars indicate SE

until the end of the storage period. Glutamic acid in the top portion increased 1 day after storage and decreased subsequently at the end of the storage period. The glutamine and glutamic acid content ranging from 84.9 to 497.8 nmol g^{-1} and from 73.4 to 319.4 nmol g^{-1} fresh weight, respectively were observed after 24 to 72 h of storage. At the end of the storage period both glutamine and glutamic acid declined to almost at the same lower level both in the top and bottom portions of the spears.

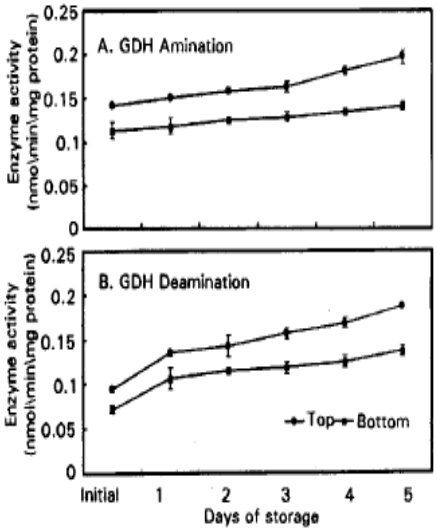

Fig. 4: Changes in the activities of (A) amination and (B) deamination glutamate dehydrogenase enzyme in the top and bottom portions of the separs held at 20°. Each point represents the mean of 3 replications. Vertical bars indicate SE

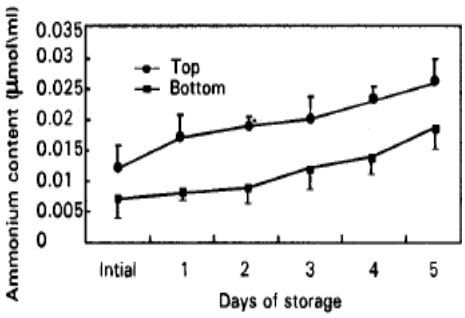

Fig. 5: Ammonium content in the top and bottom portions of the spears held at 20°C. Each point represents the mean of 3 replications. Vertical bars indicate SE

Discussion

Changes occur in GS and GDH activities and ammonium levels in harvested asparagus spears stored at 20°C for 5 days. The GS activity decline whereas GDH activities almost doubled after the 5-day storage period. The increased GDH activities could be caused by a higher ammonium levels in the spears. The rise in GDH activities is linked with a corresponding decline in GS activity.

The pattern of change in respiration rate in the top and bottom portions or intact spears showed a decline during the first day after harvest. This pattern of respiration rate change is very similar to that reported in asparagus spears (King *et al.*, 1988; Enriquez *et al.*, 2000). Harvesting asparagus immediately removes the source of respiratory substrates and may lead to substrate limitation of respiration rate (Rabe, 1984). Trippi *et al.* (1988) associated a decline in respiratory activity to nucleotide metabolism. It seems probable that the supply and control of metabolic energy is vulnerable to disruption which quickly leads to loss of normal respiratory function. The respiration rate of the top portion was over 1 or 2 times greater than the bottom portion or the intact spears. This reflects the major gradient in metabolic activity along the

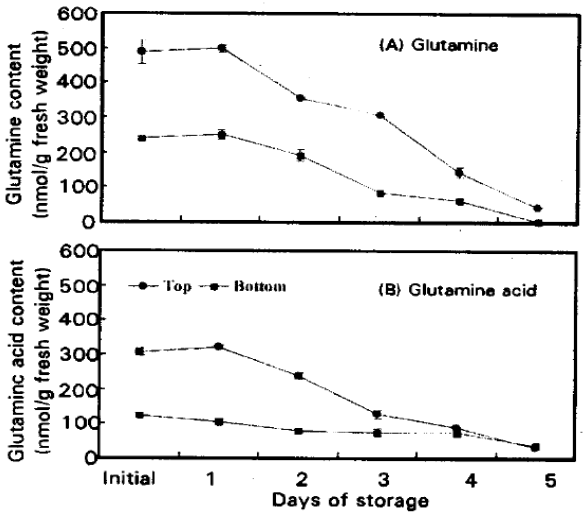

Fig. 6: Glutamine and glutamic acid contents in the top and bottom portions of the spears held at 20°C. Each point represents the mean of 3 replications. Vertical bars indicate SE

spear (Saltveit and Kasmire, 1985) with the apical tissue involved in active cell elongation showing the highest respiration rate (Lill *et al.*, 1990). Changes in ammonium levels in harvested asparagus spears both in the top and bottom portions more than doubled after 5 days of storage. The trend of postharvest ammonium accumulation of the spears increases with the time of storage. Ammonia within the plant cell may be generated by a number of different mechanisms. In the root or leaf, ammonium ions may be produced by the reduction of nitrate or by the conversion of glycine to serine in photorespiration (Lea *et al.*, 1989). Ammonium in asparagus spears could possibly arise from either deamidation of asparagine or glutamine, from phenylalanine ammonia lyase activity, which releases ammonia as the first step in the production of lignin, flavonoids and other secondary compounds (Joy, 1988). Ammonium is toxic to plant cells at high concentrations, and is normally assimilated if produced within the plant (Miflin and Lea, 1980). The accumulation of ammonium in senescing leaves has been shown to coincide with almost complete disappearance of GS (Peters and van Laere, 1992). Recent results, followed the same trend in which GS activity declined with an increased ammonium accumulation to about 60% after 3 to 5 days. The decline of GS activity after harvest, perhaps as a consequence of the general metabolic decline occurring at this time (King *et al.*, 1993) or maybe the consequence of autophagic processes (Journet *et al.*, 1986) induced by carbohydrate deprivation in harvested asparagus spears. It seems unlikely, therefore; that the rise in ammonium is due to reduced GS activity.

Accumulation of amino acids and other nitrogen-containing compounds is typical of plants subjected to environmental stress (Rare, 1990). Those stress conditions which include altered water relations, nutrient depletion and ammonium toxicity are likely to be important during the postharvest period. Postharvest losses of protein and amino acid have been described in asparagus (King *et al.*, 1990). Although glutamine is known to be the principal N-translocator, the rise in glutamine level measured here during the first 24 h was of little importance, which corresponds with the decrease in GS activity. In some cases, glutamine did not accumulate substantially (Malik, 1982).

The increasing GDH activities after harvest seem to be a common feature of senescence (Peters and van Laere, 1992) and in some cases, appear to parallel ammonium accumulation. Our results and that of ammonium feeding experiments have led to the hypothesis that GDH is induced by ammonium to combat its toxicity. The physiological shift towards increasing the GDH activities may be to prevent principally ammonia toxicity. The kinetic properties of GDH from higher plants suggest that it is unsuited for the assimilation of low ammonia concentrations but that it may be more suited for dealing with rapid increases in intracellular ammonia. The GS reaction has a high affinity for ammonia (O'Neal and Joy, 1973) and GS is generally regarded as a scavenger of ammonia. Asparagus spears appears to have evolved a mechanism whereby, when the reduction in the potential to assimilate toxic concentrations of ammonia was reduced by repression of GS, this was compensated for by an increase in GDH level. The results of the present study showed that a rise in GDH activity is linked with a corresponding decline in GS activity. It may be concluded that the rapid changes in the physiology of asparagus spears are induced in response to harvest, storage and the amino acid loss content are commonly associated with senescence in plant tissue.

Acknowledgement

I would like to thank the Ministry of Education, Science, Sports and Culture for the scholarship grant.

References

Enriquez, F.G., T. Matsui and K. Kawada, 2000. Changes in the activities of glutamine synthetase and glutamate dehydrogenase of asparagus spears after harvest. Food Preserv. Sci., 26: 95-101.

Givan, C.V., 1979. Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry, 18: 375-382.

Hurst, P.L. and C.J. Clark, 1993. Postharvest changes in ammonium, amino acids and enzymes of amino acid metabolism in asparagus spear tips. J. Sci. Food Agric., 63: 465-471.

Journet, E.P., R. Bligny and R. Douce, 1986. Biochemical changes during sucrose deprivation in higher plant cells. J. Biol. Chem., 261: 3193-3199.

Joy, K.W., 1988. Ammonia, glutamine, and asparagine: A carbon-nitrogen interface. Can. J. Bot., 66: 2103-2109.

Kamachi, K., T. Yamaya, T. Mae and K. Ojima, 1991. A role for glutamine synthetase in the remobilization of leaf nitrogen during natural senescence in rice leaves. Plant Physiol., 96: 411-417.

King, G.A., D.C. Woolard, D.E. Irving and W.M. Borst, 1990. Physiological changes in asparagus spear tips after harvest. Physiol. Planta, 80: 393-400.

King, G.A., K.G. Henderson, E.M. O'Donoghue, W. Martin and R.E. Lill, 1988. Flavour and metabolic changes in asparagus during storage. Scient. Hortic., 36: 183-190.

King, G.A., P.L. Hurst, D.E. Irving and R.E. Lill, 1993. Recent advances in the postharvest physiology, storage and handling of green asparagus. Postharvest News Inform., 4: 85-89.

Lea, P.J., R.D. Blackwell, A.J.S. Murray and K.W. Joy, 1989. Recent Advantances in Phytochemistry. Plenum Press, New York, USA., pp: 157-189.

Lill, E.R., G.A. King and E.M. O'Donoghue, 1990. Physiological changes in asparagus spears immediately after harvest. Scient. Hortic., 44: 191-199.

Lipton, W.J., 1990. Postharvest biology of fresh asparagus. Hortic. Rev., 12: 69-155.

Malik, N.S.A., 1982. Senescence in detached oat leaves I. changes in free amino acid levels. Plant Cell. Physiol., 23: 49-57.

Miflin, B.J. and P.J. Lea, 1980. Ammonia Assimilation. In: The Biochemistry of Plants, Stumpf, P.K. and E.E. Conn (Eds.). Vol. 5, Academic Press, New York, pp: 169-202.

O'Neal, D. and K.W. Joy, 1973. Glutamine synthetase of pea leaves. I. Purification, stabilization and pH optima. Arch Biochem. Biophys., 159: 113-122.

Peters, K.M.U. and A.J. van Laere, 1992. Ammonium and amino acid metabolism in excised leaves of wheat (*Triticum aestivum*) senescing in the dark. Physiol. Planta., 84: 243-449.

Rabe, E., 1984. Physiology of asparagus (*Asparagus officinalis*) as related to the production of the crop. N. Z. J. Exp. Agric., 12: 251-260.

Rare, E., 1990. Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds. J. Hortic. Sci., 65: 231-243.

Saltveit, M.E. Jr. and R.F. Kasmire, 1985. Changes in respiration and composition of different length asparagus spears during storage. HortScience, 20: 1114-1116.

Trippi, V.S., A. Paulin and A. Pradet, 1988. Effect of oxygen concentration on the senescence and energy metabolism of cut carnation flowers. Physiol. Planta, 73: 374-379.