JARQ 28, 237-241 (1994)

Effect of Spear Removal on ABA Levels in Adjacent Buds of Asparagus Spears

Masaji KOSHIOKA*, Toshikazu UESUGI, Takaaki NISHIJIMA* and Hiroko YAMAZAKI***

* Department of Applied Physiology, National Research Institute of Vegetables, Ornamental Plants and Tea (Ano, Age, Mie, 514-23 Japan)
** Nagano Vegetable and Ornamental Crops Experimental Station (Oomuro, Matsushiro, Nagano, 381-12 Japan)

Abstract

Effect of spear removal by cutting on abscisic acid (ABA) level in buds of asparagus spears was investigated. ABA level in buds was determined by an enzymelinked immunosorbent assay after a pre-purification procedure. ABA level in F-buds which included the buds adjacent to stems of cladophylls or spears drastically decreased to 38.0% of the control level at 12 hr after cutting and reached the minimal ABA level of 10.6% of the control at 48 hr after cutting. On the other hand, F-buds started to elongate just 12 hr after cutting and then grew to over 40 mm height in 72 hr. ABA level in S-buds which included the remaining buds also decreased after cutting although no significant elongation of S-buds was observed. Thus, it is assumed that the ABA level in buds drastically decreased by a stimulus or stress of cutting before the buds started to grow.

Discipline: Horticulture
Additional key words: abscisic acid, Asparagus officinalis

Introduction

Young spears of asparagus (*Asparagus officinalis* L.) are one of the commercially important vegetables although the volume of harvest is small compared to that of major crops. However, the import volume of fresh asparagus spears has recently increased up to nearly 50% (about 15,000 t) of the domestic volume of harvest due to the increase of consumption. Under these circumstances, it is important to develop an effective technique for stimulation of asparagus bud sprouting. To achieve this objective, the physiology and mechanism of bud sprouting of asparagus were analyzed.

Young spear of asparagus grows very rapidly, as much as 12 cm in height/day at 30.8°C[9], to become a cladophyll within a month after sprouting. Under these temperature conditions, buds adjacent to the first spears emerge in about 20 days. However, the removal of a cladophyll or a young spear stimulates the sprouting of an adjacent bud within 2~3 days. This phenomenon is a response similar to the loss of apical dominance. Although the role of abscisic acid (ABA) in the regulation of apical dominance has not yet been clarified, it was suggested that the effect of ABA on the growth of lateral buds could be both inhibitory[2,14], or stimulating[2-4]. In asparagus, endogenous levels of ABA in buds at the

developing stages were found to be highest in the resting buds[9]. However, the effect of spear removal on ABA levels in adjacent buds of the spears is not clear. In this report, we investigated the changes of ABA levels in asparagus buds after stem of cladophyll and spear removal by cutting.

Materials and methods

Seeds of Welcome asparagus cultivar were sown in March, 1991, and grown in plastic pots (13 *l*/pot) containing Andosol fertilized with 0.25(N)-0.36(P)-0.25(K) kg/m^3 in a greenhouse (latitude 34°7′N, longitude 136°5′E), and a temperature regime of 30°C/20°C day/night was used. Watering was performed as necessary. Stems of cladophylls and spears were removed by cutting from crowns of the young plants in September, 1991. For the uncut control, crowns which contained young spears were selected. Three to four crowns from treated plants were harvested at 0 to 72 hr, and 3-4 crowns from control plants were harvested at 0, 12, and 48 hr intervals. Each crown contained 3-4 spear bud clusters. The bud clusters were excised and separated into two groups: an F-bud group which included the buds adjacent to stems of cladophylls or spears, and an S-bud group, which included the remaining buds. The length of the buds in both groups was measured. Usually, F-buds grew rapidly after cutting. A 5 mm section was excised from the spear top, because the ABA concentration in the spear apex of asparagus was reported to be much higher than that in the subtending tissues[8]. All samples were frozen in liquid nitrogen immediately after excision and lyophilized. For ABA extraction, 3-4 buds were combined (10-20 mg dry weight or 100-200 mg fresh weight) and thoroughly homogenized in 1 m*l* of 80% methanol using a glass homogenizer, and the homogenate was centrifuged at 3,000 g for 10 min. The supernatant was collected in a 10 m*l* test tube. The precipitate was again homogenized with 80% methanol and the process was repeated twice. The supernatants were combined in the 10 m*l* test tube and dried in a vacuum centrifuge. The residue was dissolved in 1 m*l* of phosphate buffer (pH 8.3), and the solution was washed three times by partitioning with 1 m*l* of hexane in the test tube by mixing on a test tube mixer. After the hexane phase was removed, the buffer phase was further washed by partitioning with 1 m*l* of ethyl acetate (EtOAc) in the test tube three times. After the EtOAc phase was removed, the buffer phase was adjusted to pH 2.5-3.0 with a few drops of 1N HCl. Then, ABA was extracted by partitioning the buffer phase with 1 m*l* of EtOAc three times. The EtOAc phase was combined in a 10 m*l* test tube. Finally, the combined EtOAc phase was dried over anhydrous sodium sulfate and then in a vacuum centrifuge. The residue was dissolved in 5 m*l* of 25 mM tris buffer saline. A 100 μ*l* aliquot of the solution was used for ABA analysis. The quantitative immunoassay of ABA was performed by using the PHYTODETEK-ABA KIT (Idetek, Inc. USA) according to the manual. The detection was optimum between 5×10^{-14} and 5×10^{-12} mol of ABA per well. The quantitative experiment was conducted at least three times. The averaged ABA values of uncut F- and S-buds at 0, 24 and 72 hr were considered to represent the control levels of ABA, respectively. The quantitative means within each bud-sample were examined by t-test at $P = 0.05$ between control and samples.

Results and discussion

The ABA amounts determined by the immunosorbent assay were often overestimated compared with those measured by gas chromatography with an electron capture detector or mass spectrometer (GC-ECD/MS)[10,13]. However, Soejima et al. recently reported a good correlation between the results obtained

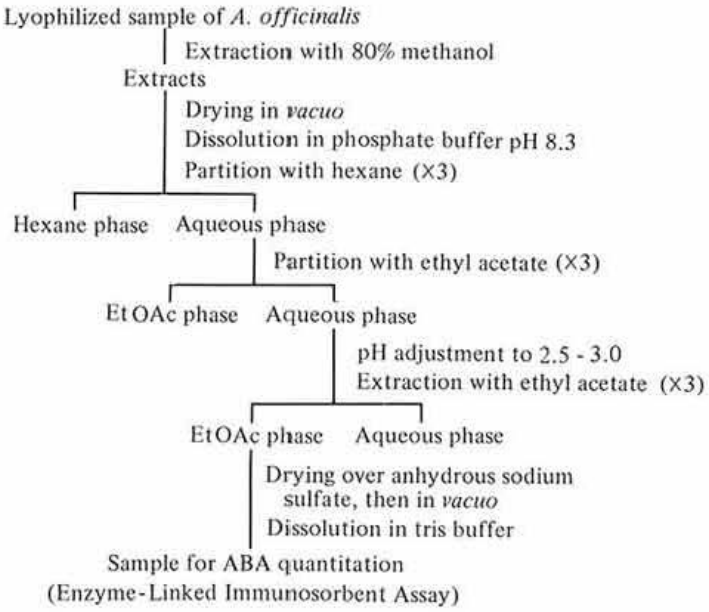

Fig. 1. Fractionation procedure of ABA

by using the enzyme-linked immunosorbent assay with the PHYTODETEK-ABA KIT and gas chromatographic analysis in apple organs after appropriate purification steps such as solvent partitioning and TLC separation[11]. Therefore, we decided to use the enzyme-linked immunosorbent assay with the PHYTODETEK-ABA KIT for the analysis of ABA in asparagus buds. As a pre-purification procedure for a small quantity of sample less than 20 mg dry weight or 200 mg fresh weight, fractionation by solvent partitioning was carried out as shown in Fig. 1. For the examination of the pre-purification procedure, 8.4 Bq of [^{14}C]ABA (2.04×10^2 MBq/mmol) was added to the F- and S-bud samples and the recovery was tested three times, respectively. The averaged recovery rates in the standard addition test were 90.1 ± 6.05% in F-buds and 91.1 ± 0.86% in S-buds. Furthermore, the values of ABA obtained by the enzyme-linked immunosorbent assay after the pre-purification procedure were almost the same as those by GC-ECD and no interfering substances were observed in the extracts. We, therefore, concluded that the analytical procedure used in this paper was suitable for the estimation of ABA in such a small quantity of sample.

ABA level in F-buds drastically decreased to 38.0% of the control level at 12 hr after cutting, and reached the minimal ABA level of 10.6% of the control at 48 hr after cutting as shown in Fig. 2. The ABA level in the F-buds was the same at 72 hr. On the other hand, F-buds started to elongate just 12 hr after cutting and then grew to over 40 mm height in 72 hr although bud growth in the control plants was not observed in 72 hr as shown in Fig. 3. In S-buds, the ABA level also decreased after cutting although the rate of decrease was lower than that in F-buds, and the minimal ABA level of 25.0% of the control was reached at 24 hr after cutting. The level slightly increased in the following 48 hr, although not significantly. At 72 hr after cutting no significant elongation of subsequent buds in S-buds was observed.

Hoad reported that ABA can be transported

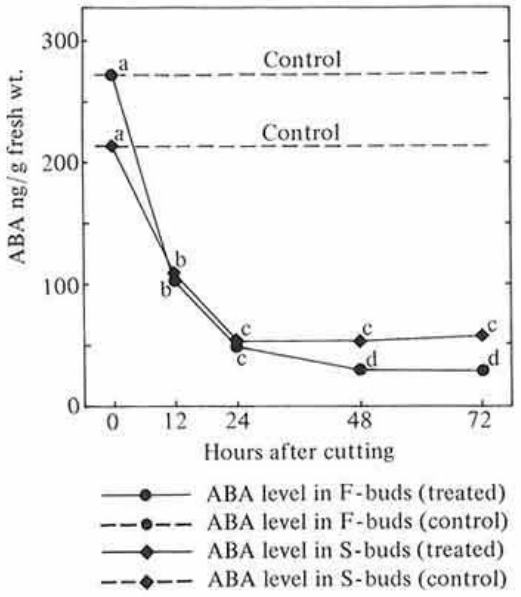

Fig. 2. Changes in ABA level in asparagus buds during sprouting after spear removal by cutting
Letters for each bud show significance at P=0.05 with t-test.

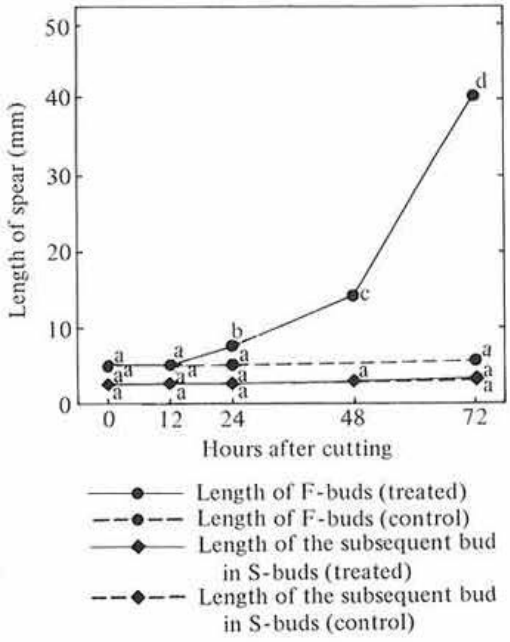

Fig. 3. Changes in length of asparagus buds during sprouting after spear removal by cutting
Letters for each bud show significance at P=0.05 with t-test.

over long distances in plants via the phloem and xylem[5–7]. Zhang and Davies also observed the ABA movement from the roots to the shoots in *Pisum sativum* and *Cammelina communis*, even under conditions of low transpirational flux[15]. We suggest that the rapid decrease of the ABA level in asparagus may be due to the ABA movement from the buds caused by a stimulus or stress of cutting (spear removal), because the decrease was too rapid to be attributed to the metabolism of ABA during growth. On the other hand, Dorffling reported that the ABA levels in lateral buds of *Acer pseudoplatanus* and *Syringa vulgaris* were higher than in the stem or petiole tissues and that the ABA concentration decreased when the lateral buds grew, although the total amount of ABA of the buds did not change appreciably[1]. In *Phaseolus vulgaris*, fruit removal led to axillary bud growth and the ABA content of the buds did not change substantially[12]. These results suggest that the decrease of the ABA concentration occurred during bud growth. In our results, however, the ABA level in buds drastically decreased before the buds started to grow, indicating that the decrease of the ABA level in asparagus may not be due to bud growth. Although the same phenomena had been observed in *Elytrigia repens* (personal communication from Dr. D. Pearce, the University of Calgary), the effect of ABA on bud dormancy and sprouting remains to be clarified. However, the decrease of the ABA level may indicate that sprouting was induced, although the reason why the S-buds did not elongate in spite of the decrease of the ABA level has not been clarified yet.

References

1) Dorffling, K. (1976): Correlative bud inhibition

and abscisic acid in *Acer pseudoplatanus* and *Syringa vulgaris*. *Physiol. Plant.*, **38**, 319-322.
2) Hartung, W. & Funfer, C. (1981): Abscisic acid and apical dominance in *Phaseolus coccineus* L.: the role of tissue age. *Ann. Bot.*, **47**, 371-375.
3) Hartung, W. & Steigerwald, F. (1977): Abscisic acid and apical dominance in *Phaseolus coccineus* L. *Planta*, **120**, 299-305.
4) Hillman, J.R. (1970): The hormonal regulation of bud outgrowth in *Phaseolus vulgaris* L. *Planta*, **90**, 222-229.
5) Hoad, G.V. (1973): Effect of moisture stress on abscisic acid levels in *Ricinus communis* L. with particular reference to phloem exudate. *Planta*, **113**, 367-372.
6) Hoad, G.V. (1975): Effect of osmotic stress on abscisic acid levels in xylem sap of sunflower (*Helianthus annuus* L.). *Planta*, **124**, 25-29.
7) Hoad, G.V. (1978): Effect of water stress on abscisic acid levels in white lupin (*Lupinus albus* L.) fruit, leaves and phloem exudate. *Planta*, **142**, 287-290.
8) Kojima, K. et al. (1992): Distribution and changes in ABA contents during elongation of spears of asparagus. *J. Jpn. Soc. Hort. Sci.*, **61(S2)**, 296-297 [In Japanese].
9) Matsubara, S. (1980): ABA content and levels of GA-like substances in asparagus buds and roots in relation to bud dormancy and growth. *J. Am. Soc. Hort. Sci.*, **105**, 527-532.
10) Rosher, P.H., Jones, H.G. & Hedden, P. (1985): Validation of a radioimmunoassay for (+)-abscisic acid in extracts of apple and sweet-pepper tissue using high-pressure liquid chromatography and combined gas chromatography-mass spectrometry. *Planta*, **165**, 91-99.
11) Soejima, J. et al. (1990): Good correlation between enzyme-linked immunosorbent assay and gas chromatographic analysis of abscisic acid in apple organs. *J. Jpn. Soc. Hort. Sci.*, **58**, 819-826.
12) Tamas, I.A. et al. (1979): Effect of fruits on dormancy and abscisic acid concentration in the axillary buds of *Phaseolus vulgaris* L. *Plant Physiol.*, **64**, 615-619.
13) Walton, D., Dahek, W. & Galson, E. (1979): A radioimmunoassay for abscisic acid. *Planta*, **146**, 139-145.
14) White, J.C. & Mansfield, T.A. (1977): Correlative inhibition of lateral bud growth in *Pisum sativum* L. and *Phaseolus vulgaris*: studies of the role of abscisic acid. *Ann. Bot.*, **41**, 1163-1170.
15) Zhang, J. & Davies, W.J. (1990): Changes in the concentration of ABA in xylem sap as function of changing soil water status can account for changes in leaf conductance and growth. *Plant Cell Environ.*, **13**, 277-285.

(Received for publication, March 18, 1994)