Available online at www.sciencedirect.com
ScienceDirect
Plant Science 175 (2008) 2–7
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Shang Fa Yang: Pioneer in plant ethylene biochemistry

Kent J. Bradford *

Department of Plant Sciences, One Shields Avenue, University of California, Davis, CA 95616-8780, USA

Received 19 December 2007; received in revised form 3 January 2008; accepted 4 January 2008
Available online 18 January 2008

Abstract

Shang Fa Yang was born in Taiwan in 1932. After receiving his B.S. and M.S. degrees in Agricultural Chemistry from the National Taiwan University, he came to the United States in 1958 to pursue a Ph.D. degree at Utah State University. Following three postdoctoral years, he was hired at the University of California, Davis, in 1966 where he was a biochemist and professor for 28 years. After retiring early from UC Davis, he subsequently established a research program at the Hong Kong University of Science and Technology and served as Vice President of Academia Sinica in Taiwan. Yang's research achievements included discovering the biochemical pathway for the synthesis of ethylene by identifying the key steps by which *S*-adenosylmethionine (SAM) is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) and subsequently into ethylene. Yang further demonstrated how the methylthio and ribose moieties from SAM were recycled back into methionine in order to sustain high rates of ethylene synthesis, as in ripening fruits. This recycling pathway is now known as the Yang Cycle. Yang also contributed to the isolation, characterization and cloning of ACC synthase and ACC oxidase, the two enzymes in the ethylene biosynthetic pathway, and to the elucidation of their structure and reaction mechanisms. He made important contributions to auxin, cytokinin, cyanide and sulfur metabolism in plants as well. His work formed the basis for subsequent research that has established ethylene as the most thoroughly characterized of the hormonal biosynthesis and signaling pathways in plants.

Keywords: Shang Fa Yang; Ethylene; ACC; Yang Cycle

1. Introduction

Shang Fa Yang (Fig. 1) was born in Taiwan in 1932. He was the youngest child of a large family (five elder brothers and six elder sisters), and his father was a businessman involved in the production maltose from sugarcane. He attended the National Taiwan University and received his B.S. and M.S. degrees in Agricultural Chemistry in 1956 and 1958, respectively. A scholarship brought him to Utah State University to do graduate work with G.W. Miller on the effects of fluoride on plant biochemistry and metabolism [1]. Yang received his Ph.D. in 1962 in Plant Biochemistry and then accepted a postdoctoral position with Paul K. Stumpf at the University of California, Davis, where he worked on lipid biosynthesis in avocado fruits [2]. He then accepted a postdoctoral fellowship with B.N. LaDu at New York University Medical School in 1963–1964, where he met his future wife Eleanor Liu who was studying accounting there. He subsequently returned to California and worked as a postdoctoral researcher with Andrew A. Benson at Scripps Institute of Oceanography in La Jolla during 1964–1965.

Yang was hired in 1966 as an assistant biochemist in the Department of Vegetable Crops at the University of California, Davis, to study the postharvest biochemistry of fruits and vegetables. California ships fresh produce long distances to markets in the eastern US and internationally, making postharvest storage and physiology of fresh produce of considerable importance to the state. He initially shared a laboratory with Harlan K. Pratt, a pioneering researcher in ethylene physiology, in the newly constructed Mann Laboratory, named after Louis Mann. This laboratory was built specifically for postharvest biology studies and was well equipped with controlled temperature chambers and gas flow equipment. In addition, Pratt had custom-built one of the first gas chromatographs that could measure ethylene in the parts-per-million concentrations that are produced by plants. The gaseous compound was found in the early decades of the 20th century to hasten the ripening of fruits and to cause growth

* Tel.: +1 530 752 6087; fax: +1 530 754 7222.
E-mail address: kjbradford@ucdavis.edu.

0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2008.01.005

Fig. 1. Portrait of professor Shang Fa Yang.

distortions in growing plants, generally associated with leaking gas mains. However, research primarily at the Boyce Thompson Institute in the 1930s showed that plants produce ethylene and that it is broadly involved in regulating plant growth and development, including seed germination, root and shoot growth, responses to environmental stresses, flowering, fruit ripening and senescence or death of plant tissues and organs [3]. However, little was known about the pathway of ethylene biosynthesis in plants, and armed with Pratt's gas chromatograph and his broad knowledge of chemistry and biochemistry, Yang set out to explore plant ethylene biology.

2. Ethylene biosynthesis

Ethylene is a simple gaseous compound containing two carbon and four hydrogen atoms and one double bond (C_2H_4). At the time that Yang entered the field, Morris Lieberman and others had evidence that amino acids, particularly methionine, might be a precursor for ethylene production [4,5]. Various *in vitro* systems were being used to convert methionine and other potential precursors into ethylene, and Yang contributed significantly to these studies, using his knowledge of chemistry to explore different reaction mechanisms [6]. He subsequently utilized both *in vitro* and *in vivo* approaches to explore potential intermediates of and mechanisms for the conversion of methionine to ethylene [7,8]. He also studied the mechanism of formation of ethylene from 2-chloroethylphosphonic acid in plant tissues [9]. This compound, under the generic name of ethephon, enabled the commercial application of ethylene for agricultural purposes, as it is taken up by plants and converted into ethylene. It has been widely used as a fruit-ripening agent, to loosen fruits for harvest and for defoliation of cotton before harvest [3].

Continuing studies on the biogenesis of ethylene showed that methionine was converted to *S*-adenosylmethionine (SAM) and that SAM was a precursor of ethylene. When apple tissues were supplied with ^{14}C-SAM under anaerobic conditions that prevented ethylene formation, a labeled compound accumulated in tissues [10]. This discovery stimulated active competition among various groups to identify the unknown intermediate between SAM and ethylene that culminated in the identification by Adams and Yang of 1-aminocyclopropane-1-carboxylic acid (ACC) as the final *in vivo* precursor of ethylene (Fig. 2; [11]). Yang's group quickly developed a sensitive assay for ACC via its chemical conversion to ethylene [12], which facilitated wide-ranging studies by his group on the regulation of ACC and ethylene biosynthesis in plant growth, fruit ripening, and stress responses [13–15].

The ethylene biosynthetic pathway requires only two specific steps, the conversion of SAM to ACC and of ACC to ethylene (Fig. 2), and attention quickly turned to the identification of the enzymes responsible for them. Yang's group and that of Hans Kende at Michigan State University soon reported the properties of 1-aminocyclopropane carboxylate synthase, the enzyme responsible for the conversion of SAM to ACC [16–18]. The subsequent cloning of ACC synthase, in which the Yang, Kende and A. Theologis laboratories were involved, is described in detail by Kende [19]. Isolation of the ethylene-forming enzyme, or ACC oxidase, was a more difficult task, as the enzyme appeared to be membrane-associated and activity was lost upon cellular disruption and fractionation. The gene coding for the enzyme was eventually cloned by D. Grierson's group based upon expression of a ripening-related cDNA and demonstration that its suppression blocked ethylene synthesis [19–21]. Yang contributed to the characterization of the multiple alleles in the ACC synthase and ACC oxidase families and to structure-function studies of the reaction mechanisms of the two enzymes [22–29].

3. ACC metabolism and the Yang Cycle

Ethylene synthesis exhibits multiple points of regulation, including transcriptional control of multiple ACC synthase and ACC oxidase genes and the activation state and/or stability of the enzymes themselves [30]. For example, light, carbon dioxide, oxygen, and water stress all influence the conversion of ACC to ethylene [31]. The conversion of ACC to ethylene is also stereospecific, as demonstrated by the differential conversion of stereoisomers of 1-amino-2-ethylcyclopropane carboxylic acid to 1-butene [32]. This provided an important test for the enzymatic conversion of ACC to ethylene versus non-specific chemical conversion [33] that was important in the subsequent isolation and cloning of ACC oxidase [34,35]. In addition to being converted to ethylene, Yang's group determined that ACC could be malonylated and that this pool of conjugated ACC was largely unavailable for conversion to ethylene (Fig. 2; [36]).

An early dilemma in ethylene biosynthesis was that methionine pools were generally too low in plant tissues to sustain the observed rates of ethylene synthesis. Some had suggested that following ACC formation, the methylthio group from methionine would be attached to an existing homocysteine molecule to form a new methionine molecule, thus recycling only the methylthio group. However, Yang's and Lieberman's groups demonstrated that after ACC is released from SAM, the methylthio group and the ribose moiety from the remaining methylthioadenosine (MTA) are both recycled to replenish methionine levels and sustain ethylene biosynthesis [37–39]. This cycle has been christened the Yang Cycle in plant biochemistry texts (Fig. 2; [40]).

4. Other activities and honors

While most widely known for his work on ethylene biosynthesis and action, Yang also maintained active research programs in other areas of plant growth and metabolism, including on auxin metabolism and action [41–43], on cytokinin action [44,45], on the biological effects of sulfite and sulfur dioxide [46,47], and on cyanide generation and metabolism in plants [48].

A theme throughout all of Yang's work is a linkage to practical applications in postharvest biology and plant growth regulation. He applied his knowledge of ethylene biosynthesis to contribute to improvements in postharvest storage conditions [49–52]. Yang figured prominently at many national and international research conferences and served on the editorial boards of leading journals and as a member of several learned societies. He won many awards and honors, including a Guggenheim Fellowship in 1982, the International Plant Growth Substances Association Research Award in 1985, and the Outstanding Researcher Award from the American Society for Horticultural Science in 1992. In 1992, he was named the UC Davis Faculty Research Lecturer, the highest honor given by that institution for excellence in research. He was elected to the US National Academy of Sciences in 1990 and to the Academia Sinica, Taiwan, in 1992. In 1991, Yang received the prestigious international Wolf Prize in Agriculture, which many consider to be the "Nobel Prize" for agricultural research.

After a distinguished career as professor and biochemist at the University of California, Davis, Yang took early retirement in 1994 to accept a Distinguished Professorship at the Hong Kong University of Science and Technology, where he established an active plant research group in the Department of Biology. In 1995, he was recognized as a Distinguished Research Fellow by the Institute of Botany of the Academia Sinica in Taipei, Taiwan, and returned to Taiwan in 1996 to serve as Vice President of Academia Sinica from 1996 to 1999. In this position he directed its numerous research institutes, including the establishment of a new Institute of Agricultural Biotechnology (subsequently renamed the Agricultural Biotechnology Research Center). In both Hong Kong and Taiwan, Yang played important leadership roles in advancing plant biology and agricultural biotechnology. Following his tenure at

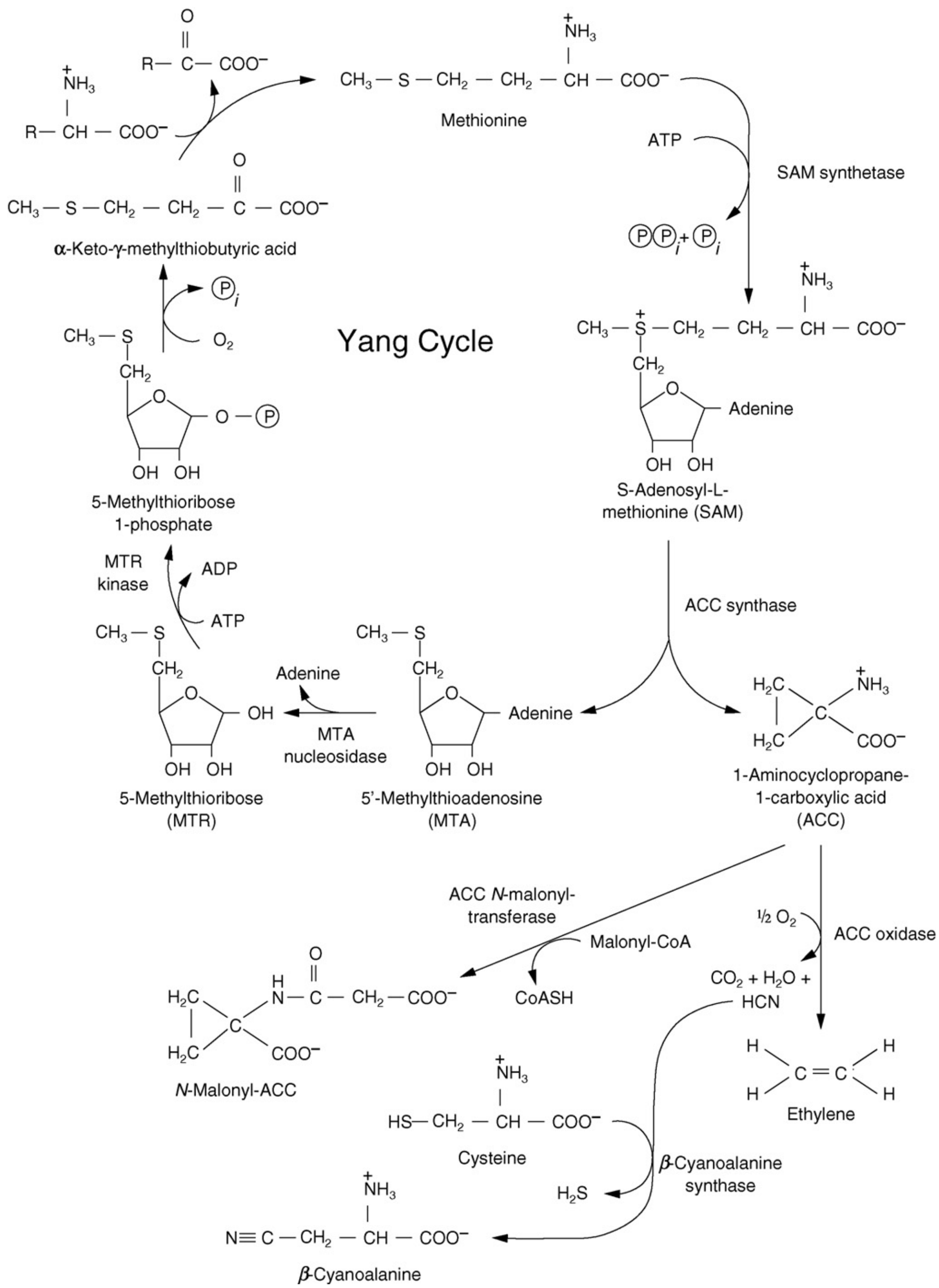

Fig. 2. The Yang Cycle and formation of ethylene and other products from ACC. See text for details.

Academia Sinica, Yang retired to Davis, California in 1999, although he continued to actively publish additional scientific work as recently as 2007.

Shang Fa Yang passed away suddenly and unexpectedly from complications of pneumonia on February 12, 2007 at the age of 74 years. As was his wish, the Shang Fa and Eleanor Yang Scholarly Exchange Endowment was established in October 2007 to support exchange of scholars in the agricultural, biological and chemical sciences between UC Davis and Academia Sinica, Taiwan, two institutions that Yang served with distinction.

5. Concluding remarks

Shang Fa Yang's pioneering studies in the biosynthesis and action of ethylene have ensured his legacy in the history of plant biology. Future plant biologists will know of him through his discovery of ACC and the biosynthetic pathway for ethylene biosynthesis, for the Yang Cycle and for his many other contributions to our field that are described in more than 225 journal articles and book chapters that he published during his career. He was known for his clarity of thought and his ability to identify and design critical experimental tests of hypotheses.

Yang maintained an open mind and was willing to challenge accepted ideas, even his own, when they proved untenable in the face of experimental data. As a mentor, Yang was demanding and rigorous yet positive, encouraging and supportive. Many graduate students and postdoctoral associates benefitted greatly from both his mentorship and his subsequent support for their careers. Students and colleagues will remember and miss his humor, his humanity, and his sparkling intellect. This special issue is a fitting tribute to Shang Fa Yang's pioneering contributions to our understanding of the role of ethylene in plant biology.

Acknowledgements

The author is grateful to Eleanor, Albert and Bryant Yang for sharing personal information and thanks Yee-Yung Charng, Neil E. Hoffman, John H. Miyazaki, Galen D. Peiser, Mikal E. Saltveit and Wing Kin Yip for contributing additional information.

References

[1] S.F. Yang, G.W. Miller, Biochemical studies on the effect of fluoride on higher plants. 1. Metabolism of carbohydrates, organic acids and amino acids, Biochem. J. 88 (1963) 505–509.

[2] S.F. Yang, P.K. Stumpf, Fat metabolism in higher plants. XXI. Biosynthesis of fatty acids by avocado mesocarp enzyme systems, Biochim. Biophys. Acta 98 (1965) 19–26.

[3] F.B. Abeles, P.W. Morgan, M.E. Saltveit, Ethylene in Plant Biology, second ed., Academic Press, San Diego, 1992.

[4] M. Lieberman, Biosynthesis and action of ethylene, Annu. Rev. Plant Physiol. 30 (1979) 533–591.

[5] M. Lieberman, A.T. Kunishi, L.W. Mapson, D.A. Wardale, Ethylene production from methionine, Biochem. J. 97 (1965) 449–459.

[6] S.F. Yang, H.S. Ku, H.K. Pratt, Ethylene production from methionine as mediated by flavin mononucleotide and light, Biochem. Biophys. Res. Commun. 24 (1966) 739–743.

[7] A.H. Baur, S.F. Yang, Precursors of ethylene, Plant Physiol. 44 (1969) 1347–1349.

[8] A.H. Baur, S.F. Yang, Methionine metabolism in apple tissue in relation to ethylene biosynthesis, Phytochemistry 11 (1972) 3207–3214.

[9] S.F. Yang, Ethylene evolution from 2-chloroethylphosphonic acid, Plant Physiol. 44 (1969) 1203–1204.

[10] D.O. Adams, S.F. Yang, Methionine metabolism in apple tissue—implication of *S*-adenosylmethionine as an intermediate in conversion of methionine to ethylene, Plant Physiol. 60 (1977) 892–896.

[11] D.O. Adams, S.F. Yang, Ethylene biosynthesis: identification of l-aminocyclopropane-l-carboxylic acid as an intermediate in the conversion of methionine to ethylene, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 170–174.

[12] M.C.C. Lizada, S.F. Yang, Simple sensitive assay for 1-aminocyclopropane-1-carboxylic acid, Anal. Biochem. 100 (1979) 140–145.

[13] N.E. Hoffman, S.F. Yang, Changes of 1-aminocyclopropane-1-carboxylic acid content in ripening fruits in relation to their ethylene production rates, J. Am. Soc. Hort. Sci. 105 (1980) 492–495.

[14] Y.B. Yu, S.F. Yang, Biosynthesis of wound ethylene, Plant Physiol. 66 (1980) 281–285.

[15] K.J. Bradford, S.F. Yang, Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged tomato plants, Plant Physiol. 65 (1980) 322–326.

[16] Y.B. Yu, D.O. Adams, S.F. Yang, 1-Aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis, Arch. Biochem. Biophys. 198 (1979) 280–286.

[17] T. Boller, R.C. Herner, H. Kende, Assay for and enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid, Planta 145 (1979) 293–303.

[18] H. Kende, Enzymes of ethylene biosynthesis, Plant Physiol. 91 (1989) 1–4.

[19] H. Kende, Ethylene biosynthesis, Annu. Rev. Plant Physiol. Plant Mol. Biol. 44 (1993) 283–307.

[20] C.J.S. Smith, A. Slater, D. Grierson, Rapid appearance of an mRNA correlated with ethylene synthesis encoding a protein of molecular weight 35000, Planta 168 (1986) 94–100.

[21] A.J. Hamilton, G.W. Lycett, D. Grierson, Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants, Nature 346 (1990) 284–287.

[22] J.G. Dong, W.T. Kim, W.K. Yip, G.A. Thompson, L.M. Li, A.B. Bennett, S.F. Yang, Cloning of a cDNA encoding 1-aminocyclopropane-1-carboxylate synthase and expression of its messenger RNA in ripening apple fruit, Planta 185 (1991) 38–45.

[23] W.T. Kim, S.F. Yang, Structure and expression of cDNAs encoding 1-aminocyclopropane-1-carboxylate oxidase homologs isolated from excised mung bean hypocotyls, Planta 194 (1994) 223–229.

[24] W.-K. Yip, T. Moore, S.F. Yang, Differential accumulation of transcripts for four tomato 1-aminocyc1opropane-1-carboxylate synthase homologs under various conditions, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 2475–2479.

[25] D.C. Olson, J.H. Oetiker, S.F. Yang, Analysis of LeACS3, a 1-aminocyclopropane-1-carboxylic acid synthase gene expressed during flooding in the roots of tomato plants, J. Biol. Chem. 270 (1995) 14056–14061.

[26] Y. Charng, S.J. Chou, W.T. Jiaang, S.T. Chen, S.F. Yang, The catalytic mechanism of 1-aminocyclopropane-1-carboxylic acid oxidase, Arch. Biochem. Biophys. 385 (2001) 179–185.

[27] N. Li, S. Huxtable, S.F. Yang, S.D. Kung, Effects of N-terminal deletions on 1-aminocyclopropane-1-carboxylate synthase activity, FEBS Lett. 378 (1996) 286–290.

[28] J.F. Shaw, Y.S. Chou, R.C. Chang, S.F. Yang, Characterization of the ferrous ion binding sites of apple 1-aminocyclopropane-1-carboxylate oxidase by site-directed mutagenesis, Biochem. Biophys. Res. Commun. 225 (1996) 697–700.

[29] H.Q. Zhou, H.W. Wang, K. Zhu, S.F. Sui, P.L. Xu, S.F. Yang, N. Li, The multiple roles of conserved arginine 286 of 1-aminocyclopropane-1-carboxylate synthase. Coenzyme binding, substrate binding, and beyond, Plant Physiol. 121 (1999) 913–919.

[30] C.T. Argueso, M. Hansen, J.J. Kieber, Regulation of ethylene biosynthesis, J. Plant Growth Reg. 26 (2007) 92–105.

[31] S.F. Yang, N.E. Hoffman, Ethylene biosynthesis and its regulation in higher plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 35 (1984) 155–189.

[32] N.E. Hoffman, S.F. Yang, A. Ichihara, S. Sakamura, Stereospecific conversion of 1-aminocyclopropanecarboxylic acid to ethylene by plant tissues—conversion of stereoisomers of 1-amino-2-ethylcyclopropanecarboxylic acid to 1-butene, Plant Physiol. 70 (1982) 195–199.

[33] T.A. McKeon, S.F. Yang, A comparison of the conversion of 1-amino-2-ethylcyclopropane-1-carboxylic acid stereoisomers to 1-butene by pea epicotyls and by a cell-free system, Planta 160 (1984) 84–87.

[34] J.G. Dong, D. Olson, A. Silverstone, S.F. Yang, Sequence of a cDNA coding for a 1-aminocyclopropane-1-carboxylate oxidase homolog from apple fruit, Plant Physiol. 98 (1992) 1530–1531.

[35] A.J. Hamilton, M. Bouzayen, D. Grierson, Identification of a tomato gene for the ethylene-forming enzyme by expression in yeast, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 7434–7437.

[36] N.E. Hoffman, S.F. Yang, T. McKeon, Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor in higher plants, Biochem. Biophys. Res. Commun. 104 (1982) 765–770.

[37] J.H. Miyazaki, S.F. Yang, Metabolism of 5-methylthioribose to methionine, Plant Physiol. 84 (1987) 277–281.

[38] J.H. Miyazaki, S.F. Yang, The methionine salvage pathway in relation to ethylene and polyamine biosynthesis, Physiol. Plant 69 (1987) 366–370.

[39] S.Y. Wang, D.O. Adams, M. Lieberman, Recycling of 5′-methylthioadenosine ribose carbon into methionine in tomato tissue in relation to ethylene production, Plant Physiol. 70 (1982) 117–121.
[40] A. Crozier, Y. Kamiya, G. Bishop, T. Tokota, Biosynthesis of hormones and elicitor molecules, in: B. Buchanan, W. Gruissem, R. Jones (Eds.), Biochemistry & Molecular Biology of Plants, American Society of Plant Biologists, Rockville, MD, 2000, pp. 850–929.
[41] N. Aharoni, S.F. Yang, Auxin-induced ethylene production as related to auxin metabolism in leaf disks of tobacco and sugar beet, Plant Physiol. 73 (1983) 598–604.
[42] Y.B. Yu, D.O. Adams, S.F. Yang, Regulation of auxin-induced ethylene production in mung bean hypocotyls—role of 1-aminocyclopropane-1-carboxylic acid, Plant Physiol. 63 (1979) 589–590.
[43] Y.B. Yu, S.F. Yang, Auxin-induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion, Plant Physiol. 64 (1979) 1074–1077.
[44] L.F.O. Chen, J.Y. Hwang, Y.Y. Charng, C.W. Sun, S.F. Yang, Transformation of broccoli (*Brassica oleracea* var. *italica*) with isopentenyltransferase gene via *Agrobacterium tumefaciens* for post-harvest yellowing retardation, Mol. Breed. 7 (2001) 243–257.
[45] O. Lau, S.F. Yang, Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production—suppression of auxin conjugation, Plant Physiol. 51 (1973) 1011–1014.
[46] G.D. Peiser, S.F. Yang, Ethylene and ethane production from sulfur dioxide injured plants, Plant Physiol. 63 (1979) 142–145.
[47] G.D. Peiser, M.C.C. Lizada, S.F. Yang, Sulfite-induced lipid peroxidation in chloroplasts as determined by ethane production, Plant Physiol. 70 (1982) 994–998.
[48] G.D. Peiser, T.T. Wang, N.E. Hoffman, S.F. Yang, H.W. Liu, C.T. Walsh, Formation of cyanide from carbon 1 of 1-aminocyclopropane-1-carboxylic acid during its conversion to ethylene, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 3059–3063.
[49] H. Hyodo, H. Kuroda, S.F. Yang, Induction of phenylalanine ammonia lyase and increase in phenolics in lettuce leaves in relation to development of russet spotting caused by ethylene, Plant Physiol. 62 (1978) 31–35.
[50] S.F. Yang, J.H. Oetiker, Molecular biology of ethylene biosynthesis and its application in horticulture, J. Jpn. Soc. Hort. Sci. 67 (1998) 1209–1214.
[51] L.C. Huang, U.L. Lai, S.F. Yang, M.J. Chu, C.I. Kuo, M.F. Tsai, C.W. Sun, Delayed flower senescence of *Petunia hybrida* plants transformed with antisense broccoli ACC synthase and ACC oxidase genes, Postharvest Biol. Technol. 46 (2007) 47–53.
[52] C.Y. Yang, F.H. Chu, Y.T. Wang, Y.T. Chen, S.F. Yang, J.F. Shaw, Novel broccoli 1-aminocyclopropane-1-carboxylate oxidase gene (BoACO3) associated with the late stage of postharvest floret senescence, J. Agric. Food Chem. 51 (2003) 2569–2575.