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膜对采后园艺作物衰老的影响

蒋跃明[1]，傅家瑞[2]，徐礼根[3]

（1. 中国科学院华南植物研究所，广东广州 510650；2. 中山大学生命科学学院，
广东广州 510275；3. 浙江大学生命科学学院，浙江杭州 310012）

摘　要：植物衰老是由内在和外部环境因素所诱导和引起的一种主动过程，其中膜的劣变是这种过程早期的表现形式和基本特征。简要评述了膜系统对采后园艺作物的衰老作用。有证据表明膜系统破坏是采后园艺作物衰老的关键事件。膜脂生物化学和生物物理性质的变化、酶促和非酶促的过氧化作用导致了膜特性的改变和功能上的失调（如离子泄漏和细胞去区域化）。这样，反过来又进一步引起细胞代谢异常，并加速衰老进程。目前，膜系统对采后园艺作物衰老的作用过程正逐步得到阐明。

关键词：园艺作物；膜；采后；衰老

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Membrane effects in postharvest senescence of horticultural crops

JIANG Yue-ming[1], FU Jia-rui[2], XU Li-gen[3]

(1. *South China Institute of Botany, Chinese Academy of Sciences*, Guangzhou 510650, China; 2. *School of Life, Zhongshan University*, Guangzhou 510275, China; 3. *School of Life, Zhejiang University*, Hangzhou 310012, China)

Abstract: Senescence is an active progress initiated by internal and environment triggers, and membrane deterioration is an early and fundamental feature of this process. Advances in membrane effects in senescence of postharvest horticultural crops are reviewed. Considerable evidence for senescence of postharvest horticultural crops supports membrane damage as the key event. Biochemical and biophysical changes in membrane lipids and enzymatic and non-enzymatic lipid peroxidation lead to altered membrane properties and defects, such as ion leakage and cellular decompartmentation, which, in turn, result in abnormality in cellular metabolism and accelerated death. A clearer picture of the sequence of occurrences in effects of membranes on postharvest senescence of horticultural crops is now emerging.

Key words: horticultural crops; membranes; postharvest; senescence

1 Introduction

Aging or senescence of higher plants is viewed as a both genetically and environmentally regulated process intimately associated with physiological activities. Postharvest horticultural scientists are interested in determining the mechanisms of senescence in fresh produce in order to find treatments that will allow shelf life to be prolonged. It has been suggested that deterioration of fruits, vegetables, and flowers by

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Author introduction: JIANG Yue-ming (1963-), Male, born in Lanxi city, Zhejiang province, Ph. D. Professor (Ph. D supervisor), working in postharvest biology and technology of horticultural crops.

natural senescence or aging due to physiological abnormalities, share a common mechanism[1~4]. The focus of this process is thought to be alterations in the physical and chemical characteristics of membranes, which, in turn, lead to abnormality in cellular metabolism and accelerated death.

Several reviews on senescence and membrane deterioration in plants[2~4] and on membrane effects in postharvest physiology[9] are available. In this review we shall present, based on select reports, the state of current research on membrane effects in senescence of postharvest horticultural crops.

2 Properties of plant membranes

Cellular membranes are selective, dynamic barriers that play an essential role in regulating biochemical and physiological events. A detailed description of plant membrane composition as well as their physical properties was reviewed by Borochov and Woodson[5], Stanley[4], Leshem[6], and Paliyath and Droillard[7]. Present knowledge of plant membranes identifies them as fluid bilayers of phospholipids containing embedded proteins and sterols. Functional membranes are fluid since phospholipids can move rather freely in lateral dimensions, but the presence of proteins and sterols influences membrane fluidity. The composition of constituent membrane lipids can affect the fluidity of membranes, with unsaturated fatty acid-containing lipids being more fluid than saturated lipids. The concept of fluidity is of great importance to both membrane function and deterioration. During senescence, there is a progressive loss of membrane integrity and changed properties of plant membrane[10].

3 Bulk lipid fluidity

It is now well documented for a variety of senescing tissues, including flowers and fruits, that the plasma and microsomal membranes sustain a decrease in bulk lipid fluidity with advancing age. In senescing tissues that show a climacteric-like rise in ethylene production, the decrease in membrane fluidity, which occurs abruptly, is coincident with or just prior to the rise in ethylene production.[11] The functions of membrane proteins are known to be sensitive to lipid fluidity[13], and thus a change in bulk lipid fluidity sustained by plasma and microsomal membranes during senescence is likely to have a deleterious impact on the functions of membrane-associated enzymes and receptors.

4 Lipid composition

The most conspicuous change in the lipid composition of senescing plasma and microsomal membranes is a dramatic decline in membrane phospholipid, which becomes manifest as a membrane sterol: phospholipid ratio increased. The rise in sterol: phospolipid ratio of senescing microsomal and plasma membranes reduces bilayer fluidity[7,14]. For some senescing membranes, there is very little change in saturated: unsaturated fatty acid ratio[1], but for others, including microsomal membranes from senescing carnation flowers, there is a significant decline in the level of unsaturation that can be attributed to membranous lipoxygenase[15,16]. Sterol content in senescing membranes can either increase, as observed in flowers[17,18] and fruits[19,20], or remain constant[21,22]. Changes in phospholipids and sterol contents also bring about increase in membrane microviscosity in apples[19], tomato[23] and broccoli[24].

5 Phase changes

Increased proportions of gel phase lipid appear in the membrane bilayers as senescence intensifies[25]. Thus, senescing membranes may contain a mixture of liquid-crystalline and gel phase lipid domain, and these coexisting domains render the membrane leaky and contribute to the loss of intracellular compartmentation that is a feature of senescence[7]. The formation of gel phase lipid in membranes during senescence is more apparent from measurements of the lipid phase transition temperature. The transition temperature rises progressively with advancing senescence[9].

6 Membrane protein

There is evidence that the progressive destabilization of membrane bilayers accompanying senescence contributes to loss of membrane protein function. Protein degradation is an important feature of the dismantling of membranes, and there is a growing conviction that selective degradation of membrane proteins occurs during senescence[13,26,27]. For example, the number of the receptor protein for ethylene, which is thought to be associated with membranes, decreases with advancing senescence[28,29]. It is also conceivable that free radicals, which produced through membrane-associated lipoxygenase, act directly on proteins[16,8]. With the onset of senescence, the chemical composition changes of membrane lipids may result in lipid phase separations within the bilayer. Evidence is described indicating that lipid metabolites are released from membranes as lipid-protein particles under normal conditions but accumulate in the bilayers of senescing membranes coincident with impairment of lipid-protein particle formation[31].

7 Permeability

As a result of senescence, there is an increased permeability, which, in turn, leads to alterations in *cellular metabolism*[31,32]. *Increased leakage correlates* with increased membrane viscosity and decreased degree of fatty acid unsaturation, which is associated with increased phase transition temperatures of membrane lipids and a decline in fluidity in the senescence of flowers[17,19,32,33]. 1-Methylcyclopropene, an anti-ethylene compound, which inhibits ethylene-induced cellular senescence, results in a delay in an increased electrolyte leakage[13]. These data support what membrane deterioration leading to loss of intracellular compartmentation is an inherent feature of senescence. It is not unreasonable, therefore, to propose that progressive destablization of the bilayer contributes in a major way to membrane leakiness in senescing tissue.

8 Free radicals and peroxidation

Peroxidation of fatty acids with resulting free radical formation has been described as one of the major senescence processes of membranes[1,2,4,35,36]. Enhanced peroxidation, observed in a variety of senescing tissues, leads to gel-phase formation and loss in membrane functionality[1,2,4,7]. Changes in membrane lipids resulting in decreased fluidity will affect the functionality of the associated proteins as well[3]. Various studies demonstrate that fruit ripening and the vase life of flowers can be modulated by radical scavengers[37]. Membranes could be expected to be highly prone to free radical attack inasmuch as unsaturated fatty acids are major components of most membrane lipid bilayers. The consequences of free radical attack on membranes are numerous and include the induction of lipid peroxidation and fatty acid deesterification[4,6]. Of particular interest is the findings that levels of superoxide radical in microsomal membranes of carnation flower increased during senescence[1,7,9]. Additionally, this increased production of superoxide radical during natural aging can be simulated by in vitro aging of isolated microsomal membranes from young tissue in buffer[34]. The formation of superoxide radical by senescing microsomal membranes has been attributed to a membrane-associated lipoxygenase[9]. Baker et al[9]. reported that specific inhibitors of lipoxygenase significantly delay the senescence of cut carnation flowers. Thus, it would appear that superoxide radical is formed as an intermediate during the conversion of polyunsaturated fatty acids to their conjugated hydroperoxydiene derivatives.

9 Ethylene

It is now known that ethylene plays an important role in regulating plant senescence. Ethylene production is regulated by cellular membrane[5,8]. One mechanism is a direct effect on the enzymes that were associated with membranes. The other involves the cellular membrane as a transducer whereby de novo synthesis of the enzymes in ethylene biosynthesis is affected. Microsomal membranes from plant tissues have been found to be capable of catalysing the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene[40]. The conversion of ACC to ethylene by microsomal membranes depends on hydroperoxides generated through membrane-associated lipoxygenase[8,41]. Treatment of microsomes with exogenous phospholipase A_2, which generates fatty acid substrates for lipoxygenase, enhances the production of hydroperoxides as well as the conversion of ACC to ethylene[1]. However, these results do not distinguish between a direct and an indirect effect of ethylene on the peroxidative pathway leading to changes in membrane function.

10　Ca^{2+}

An increasing amount of evidence points to Ca^{2+} regulating plant senescence[13,42,43]. Calcium or the calcium-calmodulin complex can activate lipid-degrading enzymes. Furthermore, the Ca^{2+} calmodulin complex is required for protein phosphorylation[16]. Phosphorylation of proteins promoted by the Ca^{2+} calmodulin complex is reduced in senescent apples and tomatoes, and this could alter the biochemical processes in the cell and participate in the onset of senescence[44]. As numerous authors have shown a relationship between ethylene and the induction of senescence[29,45], research to determine if Ca^{2+} is able to translate the ethylene signal and initiates membrane lipid degradation has been undertaken[45]. To date, the "trigger" through which calcium is released into the cytosol and senescence is initiated is still unclear.

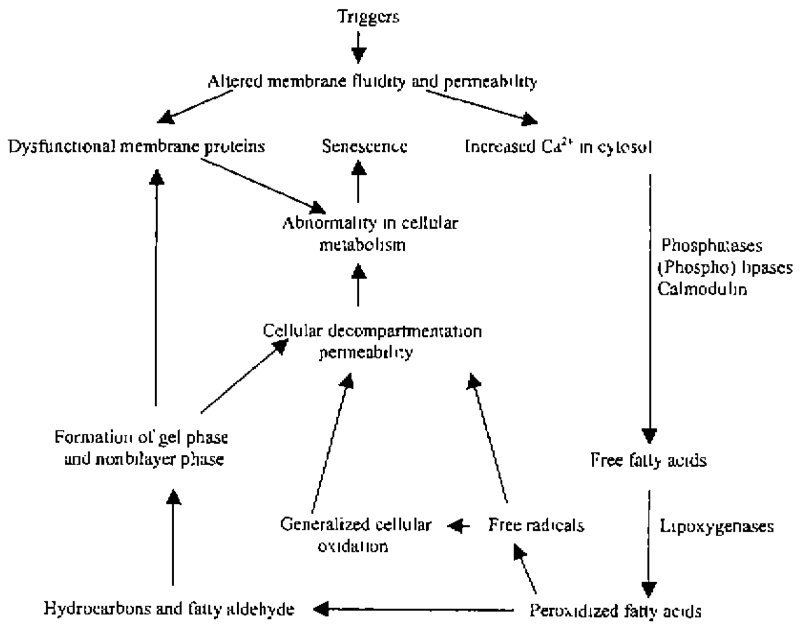

Fig. 1　Generalized scheme to account for membrane deterioration in postharvest horticultural crops due to senescence. Adapted in part from Marangoni *et al.*[5] and Thompson *et al.*[1]

11　Nonbilayer lipid

There is evidence for the induction of nonbilayer lipid configurations following peroxidation. Indeed, many of the products of peroxidation are not cylindrically shaped because they have bulky side groups (e.g., hydroperoxy, cyclic peroxide, and epoxide groups), and, thus, could promote the conversion to nonbilayer configurations. Lipid peroxidation has been shown to increase the rate of transbilayer lipid migration[46]. Moreover, trace amount of hydroperoxide derivatives of fatty acids may act as fusogens, and oxidized di- and tri-enoic fatty acids serve as Ca^{++} ionophores in model bilayers[47,48]. Thus, it is highly conceivable that lipid peroxidation, which occurs during senescence, promotes the formation of nonbilayer lipid configurations in sensescing membranes.

Recently, a modern approach to the study of senescence is related to gene expression of enzymes involved in lipid metabolism[31,49,50]. These authors found that membrane-associated enzymes concerning lipid biosynthetic potential decreased during senescence and that second messenger synthesis continued, maintaining the tissue responsiveness to the environment and hormones. However, more work in this area is greatly needed.

12 Concluding remarks

In light of information available, there appear to be distinguishable mechanisms of senescence for membranes. These processes produce destabilization and loss of functionality of membrane via biochemical and biophysical changes in lipids and/or proteins. In all cases, attack by enzymes such as phospholipase and lipoxygenase as well as free radical formation has been reported. Specifically, the enhanced free radicals initiates lipid peroxidation and estabilization of the membrane bilayer, which, in turn, leads to leakage, an increased proteolytic activity, and a generalized loss of membrane function. Thus, senescence of microsomal members appears to be lipid driven. A pattern of membrane deterioration accompanying a series of events during senescence of postharvest horticultural cops has been summarized schematically in Figure 1.

As membranes have an important effect on postharvest senescence of horticultural crops, it is desirable for food and horticultural scientists to find treatments that will allow shelf life to be prolonged. The prospects of postharvest life extension of fresh produce by regulating membrane functions appear to be promising, either by exogenous chemical application or genetic manipulation.

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