Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi **24** (1-2) 401 - 413 (2008)

http://fbe.erciyes.edu.tr/

ISSN 1012-2354

WOUND METABOLISM AND WOUND SIGNALLING IN FRUITS AND VEGETABLES

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ABSTRACT

Fresh-cut fruits and vegetables demonstrate rapid tissue softening and deterioration as compared to intact fruits and vegetables held in similar conditions. Studies have shown a significant role of changes in membrane lipids and lipase activities in the rapid softening and deterioration of fresh-cut (wounded) fruit and vegetables. In this review, we evaluate recent findings on the factors influencing the modifications or degradations of membrane lipids in wounded fruit and other tissues. Moreover, the latest developments in phospholipid-based signaling, including the lipid kinases and phospholipases that are activated, the signals they produce, the domains that bind them, the downstream targets that contain them and the processes they control are discussed.

Keywords: Wounding; Signalling; Fruit; Vegetable; Lipids.

MEYVE VE SEBZELERDE YARA METABOLİZMASI VE SİNYAL İLETİMİ

ÖZET

Benzer koşullar altında taze kesilmiş meyve ve sebzeler kesilmemiş olanlara göre daha hızlı doku yumuşaması ve bozulma gösterirler. Yapılan çalışmalar bu hızlı bozulma ve yumuşamada membrane yağlarında ve lipaz enzimlerinin aktivitelerindeki değişimlerin önemli rol oynadığı ortaya konulmuştur. Bu derlemede, yaralanmış ve diğer meyve ve sebze dokularındaki membrane yağlarının parçalanmasını ve/veya modifikasyonunu etkileyen faktörler hakkında yapılan son çalışmalar ele alınmıştır. Buna ilaveten, fosfolipit temelli sinyaller hususundaki son gelişmeler, uyarılan fosfolipazlar ve lipid kinazlar, ve onların ürettiği sinyaller, bunların bağlandıkları bölgeler, bunları içeren alt kademelerdeki hedefler ve kontrol ettikleri olaylar tartışılmaktadır.

Anahtar Kelimeler: Yaralanma; Sinyal iletimi; Meyve; Sebze; Yağlar.

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ISSN 1012-2354

1. INTRODUCTİON

Fresh-cut fruits and vegetables are highly perishable and show rapid tissue deterioration in correlation with the extent of wounding inflicted on the fruit and vegetables. Rapid textural loss during storage in response to fresh-cut processing has been reported in many fruit and vegetables [1-2,3,4,5-6,7,8]. The increased juice leakage during storage of fresh-cut commodities [5-6,9,10,11] indicates that physiological deterioration of fresh-cut produce possibly results from membrane damage.

Wounding triggers an increase in the endogenous levels of the plant growth regulator jasmonic acid (JA) [12,13,14], and this increase is required for gene activation upon wounding [15,16]. Application of exogenous JA or its methyl ester at physiological concentrations can induce the expression of a variety of genes [17,18]. In potato and tomato, protease inhibitor genes can be activated by oligosaccharide fragments generated from both plant and pathogen cell walls by the action of polygalacturonases [19] and by the 18-amino acid polypeptide systemin [20]. Wounding also induces the production of ethylene, which in turn can lead to responses such as water-soaking, accelerated senescence, or abscission of infected organs, likely brought about through induction and expression of wound-related genes [21,22,23]. Wound-induced ethylene production in fruit and vegetable tissues has been extensively reviewed [24,25,22,23]. The requirement of ethylene in mediating wound-induced gene activation has been demonstrated in tomato [26], in which ethylene acts with jasmonic acid to regulate Pin 2 gene expression.

Additionally, when plants are exposed to wound stress, reactive oxygen species (ROS) including superoxide, hydrogen peroxide (H_2O_2) and the hydroxy radical (+OH), are generated [27,28,29,30,31]. ROS are produced in an oxidative burst as one of the earliest plant responses to abiotic or biotic stress factors. Superoxide and H_2O_2 are relatively non-toxic, but in the presence of transition metals, they are converted to the highly toxic hydroxyl radical via a Fenton-type reaction. Hydroxyl radicals are responsible for the majority of biological oxidative damage associated with oxidative stress [32].

2. WOUND METABOLİSM

Wounding of plant tissue stimulates biochemical cascades directed at healing and furthering defense responses. Responses to mechanical damage can be local, systemic, or both and hence involve the generation, perception, translocation, and transduction of wound signals to activate the expression of wound-inducible genes. A central role for jasmonic acid (JA) in plant responses is well known [28, 33];

however, many other compounds, including the oligopeptide systemin [20,34,35], diverse oligosaccharides [19] and other phytohormones such as abscisic acid [36] and ethylene [1,23,26], as well as physical factors such as hydraulic pressure or electrical pulses, have also been proposed to play a role in wound signaling cascades [37,38,39]. Enhanced ethylene production is a common response in wounded tissues and is a result of the activation of ACC synthase (ACS) and ACC oxidase (ACO), the two ethylene biosynthetic enzymes. Genes encoding specific isoforms of these enzymes are themselves wound inducible [reviewed in [22,23,40,41]. The induction of various wound-induced defense genes by ethylene [1,41,42], suggests that ethylene may be the mechanism for their activation by wounding. In addition to its direct effect on gene expression, ethylene, along with JA, appear to coordinate gene expression. For example, ethylene regulates Pin gene expression and JA biosynthesis in tomato [26], and Arabidopsis leaves [43], and is involved in pathogen-resistance mechanisms reviewed by [16,40,44]. The promoters of a number of ethylene-induced defense genes contain a sequence element termed the ethylene response element (ERE), or "GCC-box" [45]. The ERE is bound by a class of ethylene-induced transcription factors termed ERE binding proteins (EREPs), or ethylene response factors (ERF) [46]. Several members of this class of transcription factors are rapidly induced in wounded tobacco leaves in an ethylene- and JA-dependent manner, and their subsequent activation in response to ethylene is required for transcription of defense genes [47,48].

JA was originally identified as a potential signal in wounding when its volatile derivative, methyl jasmonate (MeJA), was recognized as a potent inducer of proteinase inhibitor genes in tomato leaves [33]. MeJA and its free acid JA, collectively referred to as jasmonates, are important cellular regulators inducing plant defense responses against a number of pathogens [49] and mechanical or herbivorous insect-driven wounding in soybean hypocothyls [50]. MeJA has become a strong candidate as an airborne signal providing inter-plant communication for defense responses [33]. The biosynthesis of JA is catalyzed by a number of regulated enzymes in several subcellular compartments in reactions collectively known as the octadecanoid pathway [51,52]. JA biosynthesis starts when lipid precursors are released from membranes by the action of phospholipases, most probably the plasma membrane or chloroplast membranes [53]. An increase in free linolenic acid in cultured cells of several plant species challenged with fungal elicitors [54] and in mechanically wounded tomato [54] and castor bean leaves [56] suggests that the release of linoleic acid from membranes is an important step in controlling JA synthesis. A phospholipase A (PLA)-like activity has been proposed to mediate the release of linolenic acid from membranes [33] and a wound-inducible PLA activity has in fact been noted in tomato leaves [57] and other plant species [58]. Recently, phospholipase D (EC 3.1.1.4.4) (PLD) has also been shown to be essential for JA synthesis and JA responses in Arabidopsis plants [53,59]. PLD may generate substrates

for PLA, or directly activate lipoxygenases involved in JA synthesis. Many of the genes encoding JA biosynthetic enzymes are induced by wounding and often also by JA, providing wound response feedback control of JA levels reviewed by [52]. It has been reported that a number of biosynthetic intermediates, isomers, derivatives, and metabolites of the octadecanoid pathway are also powerful cellular regulators, depending on biological systems [60,61]. The mechanism of JA signaling at the molecular level was revealed when the COI1 gene from an Arabidopsis JA-signaling mutant was cloned. The gene encodes an F-box protein, a class of proteins that forms part of a multi-protein complex involved in targeted proteolysis [62]. The authors suggest that JA-responsive genes are repressed by negative regulatory proteins, which can be specifically degraded subsequent to JA perception in a COI1- dependent manner leading to the induction of gene expression. The authors further suggested that the JA-induction of gene expression based on the relief of repression may be common in plants.

Studies have suggested that activation of phospholipase D also may play an important role in mediating wound-induced lipid hydrolysis [58,63]. Wang [59], suggested that multiple forms of PLD were activated in response to wounding in Arabidopsis leaves. Wounding of castor bean leaves rapidly induces PLD-mediated hydrolysis, as confirmed by a rapid increase in phosphatidic acid (PA) and choline [56]. Wound-induced PA production has been detected at both the wound site and at sites distal to wounding in castor bean, tomato, soybean, sunflower, broad bean, pepper [58] and watermelon [64]. The activation in castor bean appears to result from translocation of PLD from cytosol to membranes, mediated by an increase in cytoplasmic Ca^{2+} concentration [34,56]. From analysis of wound activation of PLD and production of various lipid metabolites, Ryu and Wang [65] proposed a working model to account for the role of PLD in wound responses. According to this model, PLD activation may promote the release of polyunsaturated fatty acids through two processes. First, the PLD-mediated formation of PA may start a lipolytic pathway, involving PLD, PA phosphatase and acyl-hydrolysing enzymes. In this pathway, phospholipids are converted sequentially into PA, diacyl glycerol, and free fatty acids. Consistent with this proposed pathway is that wound-induced PA production in castor bean hypocotyls occurs before DAG and linolenic acid are generated [65]. This PLD-initiated process has also been suggested to occur in deteriorating membranes of senescing and aging plant tissues [66,67]. The second path for the production of free fatty acids involves the PA-induced stimulate of acyl hydrolase or PLA activities. In several plant species, the wound induced PA production occurs before the appearance of lysophosphatidylcholine and lysophosphatidyletanolamine, which could result from PLA activity [58,63]. PA has been demonstrated to be an activator of PLA2 in mammalian systems [68,69].

Studies of physically wounded tissues suggest an involvement of polysaccharide degradation in wound metabolism [1,70,71]. Huber and Lee [72] have reported the release of higher amount of pectic oligomers in wounded tomato pericarp compared to intact fruit. The increased production of oligouronides has largely been attributed to PG activity [71]. PG transcripts and activity have been reported to increase in response to wounding, systemin, and pectic fragments in papaya [1,2], tomato [73,74] and pear [75]. The glycan chitosan, a component of fungal cell walls, and fragments derived from the plant cell wall pectins are known to induce Pin gene expression in tomato leaves [76]. Oligogalacturonic acid (OGA) application to tobacco cell suspension cultures [77], carrot protoplasts [78] and tomato leaves [79] results in a rapid depolarization of the plasma membrane and influx of calcium into the cytoplasm, the generation of active oxygen species [80], and the induction of JA [81] and ethylene [26] biosynthesis. In Arabidopsis, OGAs stimulate a class of wound-induced genes expressed predominantly in the local, wounded leaf [82]. OGAs induce not only the local wound response genes, but can inhibit the ability of exogenous JA to stimulate the expression of the systemic class of genes [82]. The authors have suggested that OGAs released following tissue damage contribute to the induction of locally expressed genes but repress the systemic wound-response genes. The suppression of the JA response pathway by OGAs is ethylene dependent, and ethylene alone is sufficient to suppress the JA pathway in local (wounded area) leaves in Arabidopsis [82].

There is also evidence of a role for ABA in wound responses, although the exact nature of this role remains unclear. Pena-Cortes et al., [36] demonstrated that ABA application can directly induce PinII gene expression locally and systemically in potato, and to a lesser extent in tomato and tobacco plants. More direct evidence for ABA involvement in wound response has been reported by Birkenmeier and Ryan [83], who have shown that ABA application induced PinII expression in young tomato plants to a much lesser extent than either wounding or JA application, and further demonstrated that endogenous ABA levels increase significantly in response to wounding only in tissue surrounding the wound site. ABA may also play a role in inducing dehydration-responsive genes locally following wounding. Reymond et al., [84] reported that in Arabidopsis leaves dehydration may directly control wound-gene induction. Analyzing the expression of 150 mechanically and insect driven wound-inducible genes, the authors determined that many wound-induced genes were also stimulated by dehydration and the overall profile of wound-induced gene expression was more similar to that induced by dehydration than by insect feeding [84].

One of the consequences of wounding is pathogen ingress and proliferation. Plants have both preexisting and inducible defense mechanisms against invading pathogens. The latter often includes rapid and

localized cell death, called the hypersensitive response, the activation of a complex array of defense genes, and the production of antimicrobial phytoalexins [85,86,87]. The activation of these defense responses is initiated by the plant recognition of pathogens, either by a gene-for gene-interaction between a plant resistance gene and a pathogen avirulence gene, or by the binding of a non-race specific elicitor such as elicitin to a receptor [86,88]. Signals from such interactions are converted into cellular responses in both host and pathogen [86].

Pharmacological studies with inhibitors of protein kinases and phosphatases suggested a participation of protein phosphorylation and dephosphorylation in the induction of defense responses including the generation of ROS, activation of defense genes, and hypersensitive cell death [89]. Increasing evidence demonstrates that mitogen-activated protein kinases (MAPK) like proteins are one of the key regulators in the signaling pathways in plants including tobacco cell suspension cultures and leaves [48,90] and tomato leaves [35,91]. The MAPK modules function as molecular switches to turn on the expression of genes and cellular responses [92]. Salicyclic-acid-induced protein kinase (SIPK) and wounding-induced protein kinase (WIPK), and their orthologs in several plant species are also suggested to be involved in plant defense signaling [48,93,94,95]. SIPK was first identified as salicylic acid-induced protein kinase and was later shown to be responsive to a number of biotic and abiotic stresses, including pathogen or pathogen-derived elicitors, ozone, wounding, salt, and osmotic stresses [96]. The activation of a second MAPK, WIPK, accompanies the activation of SIPK in resistant tobacco suspension cells treated with fungal elicitins [41]. The authors have proposed that transient activation of these MAPKs leads to various defense responses, and allows the cells to adapt to adverse environments. However, persistent activation of these signal modules causes in apoptosis [97,98,99]. In tobacco cell suspension cultures, transient activation of SIPK has been linked to L-phenylalanine ammonia lyase gene activation induced by fungal elicitors [41].

Inoculation of plant tissues with pathogen, or treatment of cell cultures with microbial elicitors leads to an oxidative burst characterized by the rapid production of hydrogen peroxide [reviewed in 100,101]. Mechanical stimulation of isolated parsley cells [102] the treatment of tobacco cell suspension cultures with cell wall-derived OGAs [103,104] generate H_2O_2. H_2O_2 can act as a local signal for hypersensitive cell death and also as a diffusible signal for the induction of defensive genes in adjacent cells [105]. Immediately after wounding, plants transiently produce ROS, such as the superoxide anion, locally in the damaged tissue and H_2O_2 both locally and systemically throughout the plant [106]. Because wound-induced OGAs transiently elicit ROS production, they are considered to be one of the primary signals of tissue damage [37]. Moreover, both OGAs and fungal-derived chitosan can elicit ROS. However, OGAs

have partial mobility and are also induced by systemins [107], and they probably characterize a local, intermediate step in signaling following systemin production, rather than a mobile main signal [37]. It has been proposed that the NADPH oxidase complex on the plasma membrane plays a critical role for ROS generation [108]. Involvement of protein kinases and phosphatases [109], G-proteins [103,108], phospholipase C [PLC; [103], have been suggested to participate in the regulation of the oxidative burst in plants. Similarly, the roles of phospholipid signaling in the oxidative burst, involvement of PLA or PLC in the elicitor-induced oxidative burst in cultured soybean cells were proposed by Legendre et al., [103] and Chandra and Low [109], respectively.

A model for the expression of defense-related genes in tomato leaves in response to wounding and systemin has been presented [33]. In this model, systemin initiates a cascade of intracellular events leading to the activation of a cytoplasmic phospholipase that releases linolenic acid from membranes, linolenic acid is converted to JA. PG was demonstrated to be among the early-expressed genes in tomato leaves [74], raising questions regarding its role in the signal transduction pathway, because it was known to produce OGAs from plant cell walls that are activators of both the defensive genes [33] and of the production of H_2O_2 in tomato leaves [104]. When considering local gene expression, OGAs seem to be the primary elicitors initiating wound responses [reviewed in [40]. In addition to the wound-induced polygalacturonase activity [82], an analysis of the Arabidopsis genome sequence shows that there are many genes encoding proteins with predicted functions in pectin degradation [63]. Perhaps some of these enzymes are rapidly activated or their catalytic activity is greatly enhanced in response to wounding, releasing OGAs that activate the wound response.

Systemic signaling requires vascular connections and distribution [110]. Some reports have suggested that the systemic signal is carried in the phloem. For example, an electrical signal associated with Pin gene expression [111] and systemin [57] in tomato are carried by the phloem. Studies on transmissible electrical activity induced by wounding suggested that this electrical activity may be the systemic signal responsible for gene expression in tomato plant [35,112]. The authors demonstrated that the systemic signal generated by mechanical damage was not carried by the phloem. Furthermore, the systemic accumulation of proteinase inhibitors correlated with an electrical signal with the characteristics of an action potential, and applied electrical signals generating action potentials are able to induce Pin gene expression in tomato plants [113,114]. Hydraulic signals produced by the release of xylem tension at sites of damage in tomato leaves are also candidates to carry the systemic signal. These hydraulic signals are easily detectable throughout the plant following relatively minor mechanical wounding and even insect

feeding in tomato leaves [115]. The authors have shown a correlation between hydraulic signals and wound-induced gene expression.

Genetic analysis of the wound response pathway in tomato (*Lycopersicon esculentum*) indicates that prosystemin and systemin are upstream components of a defensive signaling pathway that involves complex regulation of JA biosynthesis and the ability of cells to perceive and respond to JA. Systemin, an 18 amino acid peptide, has been implicated in systemic signaling [reviewed by [83]. Although no definitive conclusion has been reported that systemin is mobile, it is required for the expression of wound-induced genes and is necessary for systemic signaling in tomato plants. In tomato plants, systemin comprises the C-terminal region of a much larger precursor, known as prosystemin [116]. The prosystemin gene is expressed primarily in the vascular regions of the aerial parts of the plant and is wound inducible. Transgenic tomato plants in which prosystemin gene expression is suppressed via antisense RNA expression display significantly reduced systemic Pin gene expression in response to wounding [116]. Experiments with synthetic systemin analogues containing amino acid deletions and substitutions indicated that the entire peptide is essential for full biological activity and that the four C-terminal residues are the most important in inducing wound gene expression [20]. While the C-terminal region of systemin is essential for its activity, the N-terminal region appears to be necessary for the interaction with its receptor [117]. Studies provide evidence that reactive oxygen species function downstream of JA to amplify wound- and systemin induced responses [118]. It has been suggested that wound signaling pathways independent of JA may exist in tomato [26]. The presence of separate wound signaling pathways dependent and independent of JA has been well established in Arabidopsis [37,82]. How the wound signals are transmitted, and the roles played by all the wound signaling pathway genes in plants has not been established yet and requires further study.

3. CONCLUSİONS

Wounding of fruit tissue induces a cascade of downstream responses leading to the expression of wound responsive degradative and defensive proteins. The studies of wounded tissues has suggested a possible involvement of membrane damage in the rapid softening and deterioration of wounded tissue due possibly to the induction of lipid-hydrolyzing enzymes or proteins involved in the lipid degradation.

Various signals are generated in response to wounding and they are involved in the induction of various wound-responsive genes. A role for systemin, ethylene, abscissic acid, JA and MeJA, volatile compounds, radical oxygen species, oligosaccharides, PA, DAG and other lipids have been implicated in wound metabolism and their contribution to rapid tissue softening and deterioration in response to

wounding has not been established conclusively. The ability of plants to raise local and systemic responses to mechanical damage by the activation or suppression of various sets of genes may have important applications for future work designed for engineering plants with improved responses to various stresses. Unraveling the Arabidopsis genome has had a strong positive effect on the identification of presumed wound signaling genes. Together with the increasing number of ESTs that are being deposited in databases, important information is being generated on gene expression patterns, which will lead to greater knowledge about how signaling events take place.

Analysis of membrane lipids contributed significantly to the understanding of wound metabolism and wound signaling pathway. The significance of some phospholipids such as phosphatidic acid, diacylglycerol diphosphate, phosphatidyl inositol di phosphate, phosphatidyl inositol 5-phosphate, and sphingosine-1-phosphate have already been established, reflecting their significance in plant signaling. Although the contribution of LOX, PLD and PLA to wound metabolism has been studied extensively, the roles of other lipases remains to be established.

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