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Proteomics profiling of ethylene-induced tomato flower pedicel abscission

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ABSTRACT

The control of abscission is an important agricultural concern because of its substantial effect on crop yield and quality. Changes in gene expression are correlated with the ethylene-mediated execution of abscission. However, only few large-scale proteomic studies focused on tomato pedicel abscission. Isobaric tag for relative and absolute quantification labeling was used to examine the protein and phosphoprotein changes in the tomato pedicel AZ (AZ) treated with ethylene or 1-methylcyclopropene. Among the 1429 quantified proteins, 383 unique peptides corresponding to 166 proteins showed higher than 1.5-fold change in abundance. A total of 450 phosphopeptides were detected, among which 85 phosphopeptides corresponding to 73 phosphoproteins were significantly regulated (>1.5-fold abundance change) in response to ethylene. Protein and phosphoprotein sets showed 26 similar proteins. Six phosphorylation motifs were extracted from the 138 phosphorylation sites. By analyzing translational and modification levels, we found that the modification level was not due to the translational changes. Comparison between the protein and phosphoprotein functions revealed that the proteins acted mainly in the metabolic process and showed catalytic activity, whereas most of the phosphoproteins showed signaling and transporting activities. Data revealed the unique features of the AZ phosphoproteomics, thereby suggesting the involvement of a complex network of kinase–substrate and phosphatase–substrate interactions in response to ethylene. Some phosphorylation sites from calcium-dependent protein kinase (CDPK5^{S523}), CDPK5^{S527}, and SRL3^{S329} were also found to perform protective functions for AZ and to be helpful in ethylene signal transduction.

Biological significance

Organ abscission has both positive and negative roles. Abscission is conducive for the fall of ripe fruits and the release and dispersion of seeds, but abscission has been a major limiting factor for crop productivity. Hence, more details about the process may aid in the regulation of organ abscission. However, at present, the detailed mechanism of abscission is still unclear. In tomato, several transcriptome analyses were performed using pedicels as materials. Yet, no large-scale proteomics and phosphoproteomic studies of abscission zone have been reported so far. Hence, in this present study, we determined the

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ethylene-induced changes in the proteomics and phosphoproteomics of tomato flower AZ tissue using the isobaric tag for relative and absolute quantification (iTRAQ). Proteomics data from both data sets revealed the differentially expressed proteins that are associated with the translational and modification levels relevant to abscission mechanism. Two key proteins (CDPK (CDPK5^{S523} and CDPK5^{S527}) and SRL3^{S329}) among ethylene signal transduction and defense-related proteins were obtained from the phosphoproteins. The set of tomato phosphorylation sites presented in this work is useful in at least two ways. First, as a database resource, the data would facilitate research on the identified phosphoproteins. Second, the identified sites of the related proteins could provide enough knowledge for further experiments. Hence, our results contribute to the understanding of the mechanism of abscission in plants.

1. Introduction

The ubiquitous physiological process known as organ abscission occurs in the abscission zone (AZ) of plants. Organ abscission has both positive and negative roles. Abscission is conducive for the fall of ripe fruits and the release and dispersion of seeds, but abscission has been a major limiting factor for crop productivity. Hence, more details about the process may aid in the regulation of organ abscission. However, at present, the detailed mechanism of abscission is still unclear.

The currently accepted model of the abscission process includes four major stages as follows: (a) differentiation of the AZ; (b) acquisition of abscission signals; (c) activation of the abscission process; and (d) and differentiation of a protective layer [1]. Information is lacking on the mechanism underlying AZ differentiation. The most studied gene related to tomato AZ development is *JOINTLESS*, which encodes a MADS box transcription factor [2]. Other genes contributing to AZ development include *Blade On Petiole 1/2* (*BOP1/2*) [3], *SEPALLATA* (*SEP*) [4], *AGAMOUS* (*AG*) [5], *qSH1* [3], *OsCPL1* [3], *SH4* [3], *MACROCALYX* [6], *SEEDSTICK* (*STK*) [5], *Knotted1-like homeobox* (*KNOX*) [7] and *REVOLUTA* (*REV*) [8]. When the distal organ perceives stress, it will generate abscission signals that are transported to the AZ, thereby triggering abscission. Hormones and their metabolic precursors could trigger responses that affect AZ activity. Generally, several hormones, such as ethylene [9–17], auxin [18–20], abscisic acid [1,21,22], cytokinins [23], and jasmonic acid [24–27], act as abscission signals. Activation of AZs involves a high number of gene families, including cell wall remodeling enzymes [11,22,28–34], transcription factors [35–38], and signal peptide [3,39–47]. Ultimately, a defense program becomes activated, thereby preventing pathogen damage in those organs [17,31,48–50].

Evidence from many different abscission model systems supports the hypothesis the promotion and inhibitory roles of ethylene and auxin, respectively, in the regulation of abscission in dicotyledonous plants. In tomato, exposure to exogenous ethylene accelerates the abscission process. However, the mechanism that leads to the increased sensitivity to ethylene prior to abscission is unknown. The generally accepted model is that a basipetal indole-3-acetic acid flux through the AZ prevents abscission by rendering AZ insensitive to ethylene [17]. In abscission, ethylene is the consistent main factor. *JOINTLESS* and *MACROCALYX* may control the timing of pedicel abscission by influencing the auxin/ethylene relationship [6]. The ethylene-responsive factor 52 (*SlERF52*)-suppressed plants showed a significant delay in flower abscission compared with the wild type. *SlERF52* expression is suppressed in plants with the impaired function of *MACROCALYX* and *JOINTLESS* [51]. Ethylene-dependent and -independent processes are associated with organ abscission. In *Arabidopsis*, ethylene was found to accelerate abscission under different conditions by comparing several delayed abscission mutants (*dab*) with the ethylene-insensitive mutants *etr1-1* and *ein2-1*; the plants showed normal response to ethylene [14]. In tomato, the ethylene-insensitive mutant *nr* (*never-ripe*) exhibited indefinite flower attachment and showed no sign of floral abscission [12]. Two *Sletr1* mutant alleles (*Sletr1-1* and *Sletr1-2*) with reduced ethylene responses were identified. Delayed fruit ripening and prolonged fruit shelf life were observed in these mutants [13]. Specific members of the *β*-1,4-glucanase gene family (cellulose) are up-regulated by ethylene during organ abscission in different species [32,34]. The ethylene-induced expansin gene, *RbEXPA1* is associated with petal abscission in *Rosa bourboniana* [52]. The *FYF* transcription factors showed inhibitory effects on genes by blocking the ethylene signaling pathway, which regulates senescence and abscission [38]. Tomato *LX ribonuclease* is a T2/S-like ribonuclease whose expression is known to be associated with phosphate starvation, ethylene responses, senescence, and programmed cell death [53,54].

Recent studies showed that a variety of regulatory genes are involved in abscission. Microarray analyses provided large volumes of data on the expression level of abscission-related genes [6,17,55,56]. The protein coded by the gene and its expression and regulation determined the genes' functions. No large-scale proteomics study (particularly phosphoproteomics) of tomato flower pedicels has been reported as of this writing. Phosphorylation is an important protein chemical modification that plays vital roles in the completion and change of protein function. By screening for mutations that restore organ separation in *nevershed* flowers, Leslie et al. identified two leucine-rich repeat receptor-like kinases, namely, evershed (EVR) and somatic embryogenesis receptor-like kinase 1, which function as inhibitors of abscission; EVR is a functionally specific kinase that autophosphorylates serine, threonine, and tyrosine residues in vitro [45,46]. The inflorescence deficient in abscission (IDA) and IDA-like proteins act through the receptor-like kinases, haesa (HAE), and haesa-like 2 (HSL2) to regulate abscission [57]. However, Burr et al. detected interactions of