

MINI-REVIEW

## Climacteric ripening of apple fruit is regulated by transcriptional circuits stimulated by cross-talks between ethylene and auxin

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### ABSTRACT

Apple is a fleshy fruit distinguished by a climacteric type of ripening, since most of the relevant physiological changes are triggered and governed by the action of ethylene. After its production, this hormone is perceived by a series of receptors to regulate, through a signaling cascade, downstream ethylene related genes. The possibility to control the effect of ethylene opened new horizons to the improvement of the postharvest fruit quality. To this end, 1-methylcyclopropene (1-MCP), an ethylene antagonist, is routinely used to modulate the ripening progression increasing storage life. In a recent work published in *The Plant Journal*, the whole transcriptome variation throughout fruit development and ripening, with the adjunct comparison between normal and impaired postharvest ripening, has been illustrated. In particular, besides the expected downregulation of ethylene-regulated genes, we shed light on a regulatory circuit leading to de-repressing the expression of a specific set of genes following 1-MCP treatment, such as *AUX/IAA*, *NAC* and *MADS*. These findings suggested the existence of a possible ethylene/auxin cross-talk in apple, regulated by a transcriptional circuit stimulated by the interference at the ethylene receptor level.

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Fleshy fruit ripening is a physiological process ongoing at the end of the fruits' life cycle, and comprehends a series of modifications leading to the establishment of important quality properties.<sup>1</sup> Depending to their type of maturation and ripening, fruits can be classified either as climacteric and non-climacteric.<sup>2,3,4</sup> In climacteric fruits (such as tomato, banana, apple and peach) the ripening progression is controlled by ethylene, a plant hormone highly synthesized during the late ripening stage. This burst is moreover accompanied by a rise in respiration rate.<sup>5</sup> On the contrary, non-climacteric fruits are distinguished by a continuous and basal production of this hormone and a linear decrease in the respiration rate.

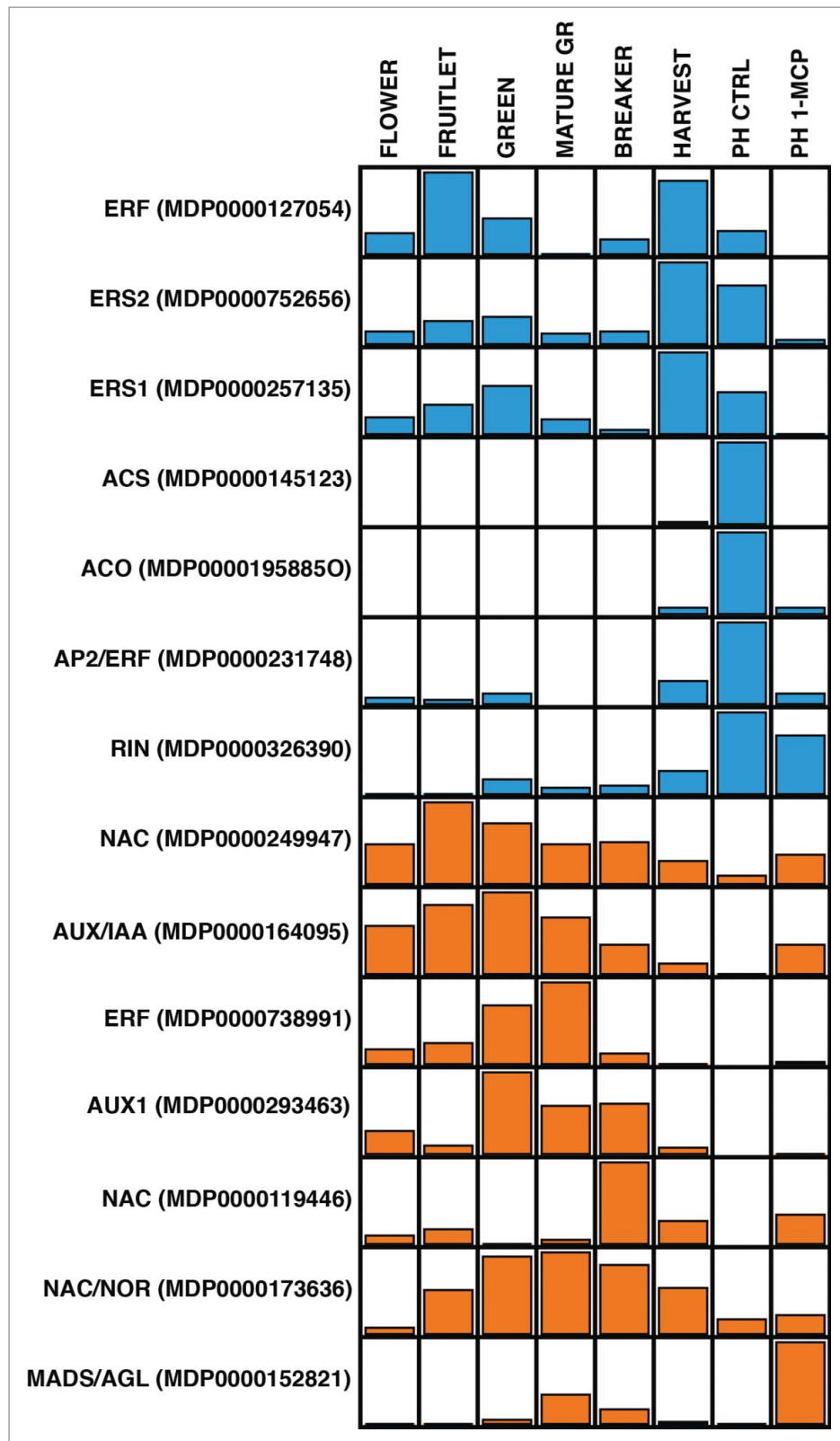
### The ethylene pathway

Ethylene is synthesized by the Yang's cycle with the involvement of two fundamental enzymes, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO<sup>6,7</sup>). The binding of ethylene to the receptors (ERSs and ETRs) turns off the active suppression exerted on CTR1, allowing the ethylene responses to occur.<sup>8,9</sup> The ethylene signaling, due to a MAPKKKs system, modulates a downstream cascade of ethylene-related genes, including elements involved in the control of fundamental processes, such as fruit texture.<sup>10,11,12</sup> In tomato, the starting signal of this pathway is however caused by the upstream action of *RIN*,<sup>13</sup> a *MADSbox* gene triggering the transition from the autoinhibitory production of ethylene

(system 1) to the autocatalytic phase (system 2<sup>14</sup>). The role of this transcription factor, together with that of *NOR*, has been comprehensively elucidated in tomato, a model species for fruit ripening investigation.<sup>15</sup> Taking into account the impact of these genes, the possibility to control the ripening process has represented a major goal, especially to improve the quality of fruits through postharvest storage. Although specific and characterized apple ripening mutants are unknown yet, the ripening process governed by ethylene can be regulated by the exogenous application of 1-methylcyclopropene (1-MCP), an ethylene inhibitor competing at the level of the receptor binding site.<sup>16</sup>

### Auxin pathway

Another important hormone in the fruit physiology is represented by auxin. Initially proposed as antagonistically inhibiting ethylene, auxin is primarily accumulated during the initial growing and developmental phases.<sup>17,18,19</sup> The regulatory pathway of auxin is substantially short, since the hormone action within the cell depends on the regulation of ARF transcription factors through the binding by Aux/IAA transcription modulators. In addition to this mechanism and the well-known polar transport, the auxin homeostasis is also regulated by a conjugation/degradation process. In particular, GH3 proteins can conjugate auxin with amino acids and sugars mainly.<sup>20</sup>



**Figure 1.** Expression profile of 14 candidate genes throughout the apple fruit development and ripening. Stages are reported on the top of the figure. The last stage PH (postharvest) is further distinguished in control (CTRL) and treated with 1-methylcyclopropene (1-MCP). For each gene the name and the ID (according to the GDR database) are reported. The pattern of gene expression is moreover characterized by different colors. While in blue are highlighted genes induced by ethylene and repressed by 1-MCP, in orange are instead depicted the elements with an opposite regulation, thus stimulated by the ethylene competitor 1-MCP.

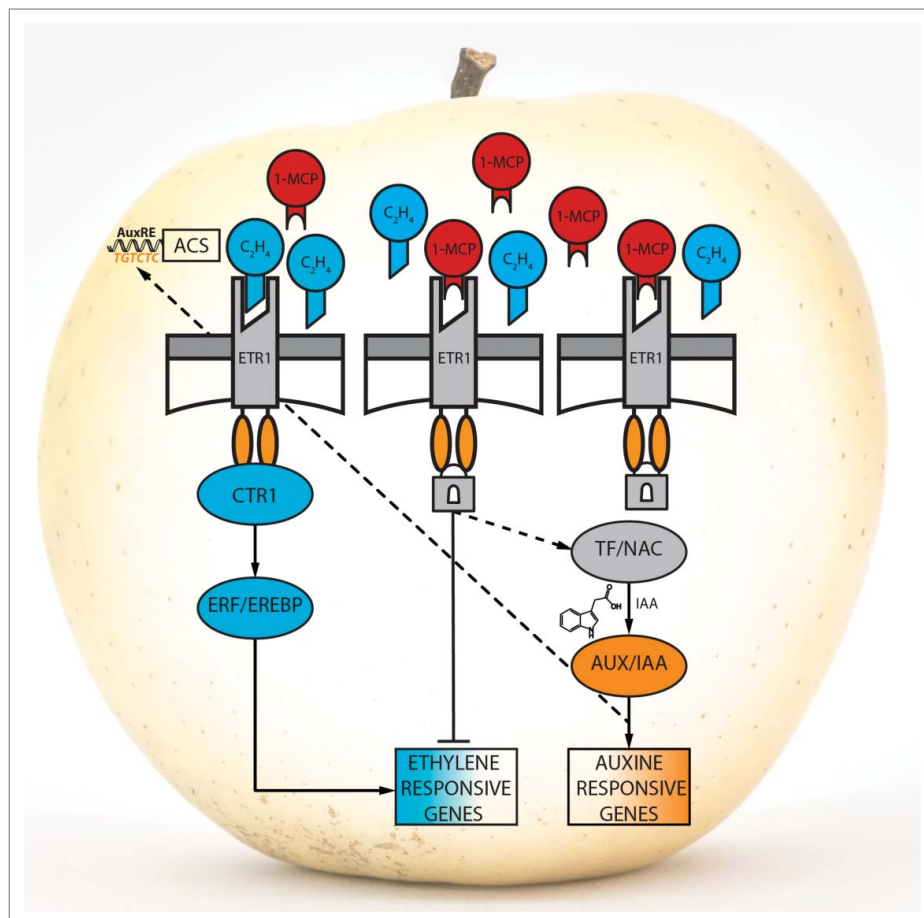
### Hormonal interplay and transcription factor regulatory circuits

In a recent work<sup>21</sup> a transcriptional deviation occurring after 1-MCP application, with regards to the normal condition, was shown. Interestingly, the artificial interference at the ethylene receptor level by 1-MCP, besides down-regulating a set of known genes, also induced the de-repression of genes usually expressed in pre-mature stages rather than in the full-ripening phase. As illustrated in Fig. 1, the genes repressed by the ethylene inhibitor are mainly related to the ethylene pathway. Among them, genes involved in the ethylene biosynthesis, as well as in the responsive pathway (such as *ACS*, *ACO* and *AP2-ERF*) are highly expressed at harvest and after 1 week of shelf-life ripening ( $PH_{CTRL}$ ), coincident with the climacteric ethylene burst. In addition to these, other genes, related to ethylene receptors (*ERS1* and *ERS2*) and one *ERF*, show, instead, an expression pattern characterized by two phases, with a first peak at the beginning of fruit development and a second at the beginning of the climacteric accumulation of ethylene. Within the genes positively correlated with the ethylene increase, it is also worth noting the expression of the apple homologous of the tomato *RIN* (MDP0000326390). Although its expression follows the transcription dynamics observed for the full-climacteric genes, the application of 1-MCP only slightly decreases its expression level. The second group of genes, induced by 1-MCP, is interestingly represented by transcription

factors and auxin related genes (*Aux/IAA* and *AUX1*). Within the class of transcription factors, *NAC* are the most abundant. Among them a specific element (MDP0000173636) is supposed to be homologous of the tomato *NOR*, whose mutation induces a non-ripening phenotype similar to what was observed for *RIN*.<sup>4</sup> In tomato, both *rin* and *nor* mutants fail to produce climacteric ethylene. For all these genes the expression pattern suggests a functional de-repression mechanism. While their activity is stimulated during the initial developmental pre-climacteric phase, their expressions tend to show a decrease at the onset of the climacteric ripening, till a complete repression during the ethylene burst. Application of 1-MCP re-establishes the gene transcription at late ripening stage, in some cases at a higher level compared with the control stage, such as for the *MADSbox* gene (MDP0000152821).

### Model of action

Our recent results, together with those of other Authors,<sup>22,23,24,25</sup> shed light on the possible ethylene/auxin cross-talk in rosaceae species triggered by the interference at the receptor level (depicted in Fig. 2). As already documented,<sup>10,26</sup> 1-MCP efficiently delays the general fruit ripening in apple, especially due to the hampered accumulation of ethylene and the reduced dismantling of the cell-wall polysaccharide architecture, a process leading to fruit softening.



**Figure 2.** Tentative model of action of the ethylene/auxin cross-talk. In blue and orange are indicated the ethylene and auxin pathway, respectively.

However, it needs to be taken into account that fruits are organs originally programmed to disperse seeds. To this end, fruits with an artificially induced impaired ethylene perception machinery attempt to restore the normal progression of physiological ripening. Since the entire ethylene perception system is compromised by the binding of 1-MCP to the receptors, a parallel pathway seems to be triggered. This alternative pathway toward the production of ethylene involves the initial de-repression of *NAC*, encoding transcription factors. In other species (*Arabidopsis*), the connection between *NAC* and auxin-related genes has been already suggested.<sup>27</sup> In this scenario, a set of *NAC* transcription factors can be re-activated to stimulate a late synthesis of auxin, with the final purpose to induce the expression of ACS genes<sup>21,28</sup> and the final accumulation of the hormone ethylene.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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