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New perspective of jasmonate function in leaf senescence

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Jasmonates (JAs) induce leaf senescence in many plant species. The Arabidopsis F-box protein coronatine insensitive 1 (COI1) is required for various JA-regulated plant responses including plant fertility, defense responses and leaf senescence. However, the molecular basis for COI1-dependent JA-induced leaf senescence remains unknown. In our *Plant Physiology* paper, we identified a COI1-dependent JA-repressed protein, Rubisco activase (RCA) in Arabidopsis. Further genetic and physiological analyses showed that the COI1-dependent JA repression of RCA correlated with JA-induced leaf senescence, and that loss of RCA led to typical senescence-associated features. Therefore, we suggested that the COI1-dependent JA repression of RCA played an important role in JA-induced leaf senescence. In this addendum, we made a relatively deep discussion on RCA function in JA-induced leaf senescence and JA-mediated defense responses. We also discussed the possible role of JA in plant natural senescence.

Key words: arabidopsis, COI1, jasmonate, leaf senescence, RCA

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Jasmonates (JAs), as a plant signal, function in induction of leaf senescence. Exogenous application of JA has been shown to stimulate leaf senescence[1-5] and to control a serious of senescence-related genes expression.[6,7] The Arabidopsis F-box protein coronatine insensitive 1 (COI1),[8] as a JA receptor,[9] is essential for JA-induced leaf senescence. Upon JA treatment, the senescence phenotype was observed in the leaves of wild type (WT) but not in the *coi1* mutants.[3,5]

We recently identified a COI1-dependent JA-repressed protein, Rubisco activase (RCA) in Arabidopsis[5] (**Fig. 1A and B**). The transcript level of *RCA* was also decreased under JA treatment in a COI1-dependent manner, which preceded the reduction of RCA protein[5] (**Fig. 1A and C**). In addition, we found that the levels of *RCA* transcript and protein were unchanged in the WT leaves treated with synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) (**Fig. 1D**), confirming that the repressed *RCA*/RCA expression is rather a specific response to the JA signal than general response to hormone overexposure.

We further observed that the COI1-dependent JA-repression of RCA correlated with JA-induced leaf senescence. Upon JA treatment for 5 days, severe senescence-associated features were induced in the WT leaves compared to that in the *coi1-1* and *coi1-2* mutants[5] (**Fig. 2A**). Simultaneously, the RCA protein level was dramatically reduced in the WT leaves, but not in the *coi1* mutants[5] (**Fig. 2B**). Furthermore, we isolated the null mutant *rca-1* and the leaky mutant *rca-2*, and found that these mutants showed typical senescence-related symptoms such as yellowing leaf, lower chlorophyll content, increased expression of senescence-induced genes and decreased expression of senescence-reduced genes at different degrees.[5] Thus, we suggested that the COI1-dependent JA repression of RCA played an important role in JA-induced leaf senescence.

It has been reported that the RCA-deficient plants had a lower CO_2 assimilation rate correlated with their defects in growth and photosynthesis, and that exogenous application of high CO_2 could restore these deficiency.[10,11] It remains to be elucidated that whether the senescence symptoms in these *rca*

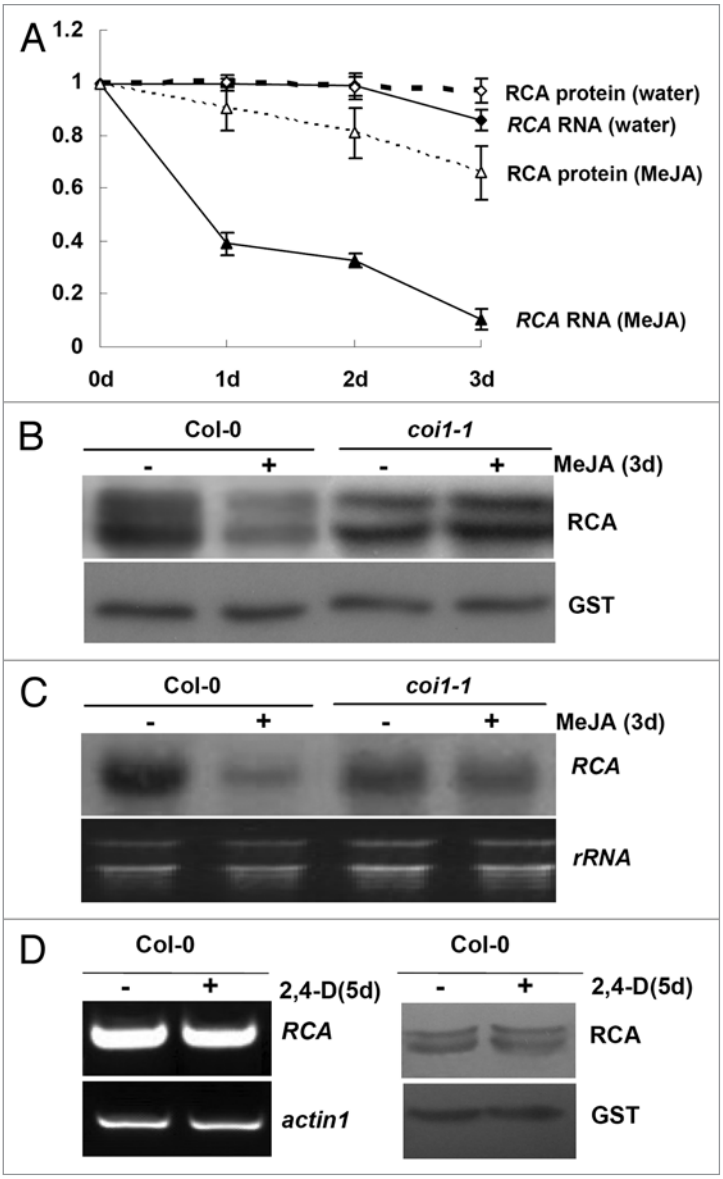

Figure 1. RCA was downregulated at the levels of transcript and protein abundance by JA in a COI1 dependent manner. (A) Quantitative analysis of *RCA* RNA levels and RCA protein levels in 6-week-old WT and *coi1-1* mutant leaves treated with methl jasmonate (MeJA) or water for indicated time-periods. The *RCA* RNA level and RCA protein level in WT upon water-treatment for 0 days were set to 1 respectively, and the relative *RCA* RNA levels and RCA protein levels in other samples were calculated accordingly. This figure was the modification to the Figures 3A and 4B in reference 5. (B) Western blot for RCA in 6-week-old WT and *coi1-1* mutant leaves treated with MeJA (+) or water (-) for 3 days. The immunoblot was detected with GST antibody as a protein loading control. This figure was the supplementation to the Figure 3A in reference 5. (C) Northern blot for *RCA* in 6-week-old WT and *coi1-1* mutant leaves treated with MeJA (+) or water (-) for 3 days. The EB staining of *rRNA* was used as loading control. This figure was the supplementation to the Figure 4B in reference 5. (D) Semi-quantitative RT-PCR (left) and western blot for RCA (right) in 6-week-old WT leaves treated with 2,4-D (+) or water (-) for 5 days. The amplified *actin1* was shown as an internal control (left). The immunoblot was detected with GST antibody as a protein loading control (right).

mutants could be rescued by growth at high CO_2.

Many types of senescence usually associate with upregulation of JA content and JA biosynthetic genes expression.[3,12] We examined the expression pattern of JA biosynthetic gene *lipoxygenase 2* (*LOX2*) in the *rca-1* and *rca-2* mutants. RT-PCR analysis showed that the expression levels of *LOX2* were not induced in these *rca* mutants (**Fig. 3**), indicating that the leaf senescence in these *rca* mutants might not associate with upregulation of JA content.

To investigate whether the decrease in RCA is a specific JA-related effect or a common feature of other senescence types promoted by various developmental signals and environmental stresses,[13,14] we detected the RCA expression pattern in dark-induced senescent WT plants, and found that downregulation of RCA was also involved in dark-induced senescence.[5]

In conclusion, we set up a model for JA-induced leaf senescence: JA signal is perceived by COI1, subsequently triggers the COI1-dependent degradation of jasmonate ZIM-domain proteins (JAZs), then releases the JAZs-interacting proteins to activate (or repress) the JA-responsive transcription repressors (or activators) essential for the expression of *RCA*, which thereby downregulates RCA resulting in JA-induced leaf senescence. It is possible that other types of senescence including dark-induced senescence might also accompanied by the reduction of *RCA* RNA and RCA protein.

As a member of the ATPases associated with a variety of cellular activities (AAA^+) protein family, RCA functions in diverse stress-related processes including UV-B exposure, ozone, heat stress, drought and herbivore resistance in different plant systems.[15-21] The *rca-1* and *rca-2* mutants displayed obviously decrease in the JA-induced expression of two defense-responsive genes *plant defensin 1.2* (*PDF1.2*) and *thionin 2.1* (*Thi2.1*),[5,22,23] suggesting that RCA may also play a role in JA-mediated defense responses. The multi-function of RCA in defense responses indicates that there might be some common elements in these processes, which is worthy to be identified.

There was no sufficient evidence to support the possible role of JA in natural senescence except that the JA-signal deficient *coi1* mutant plants exhibited relatively delayed natural senescence phenotypes including elongated flowering time and higher chlorophyll content.[24] It deserves a more thorough analysis to study the role of JA in plant natural senescence: do the natural senescence-associated phenotypes also occur in the JA-biosynthesis

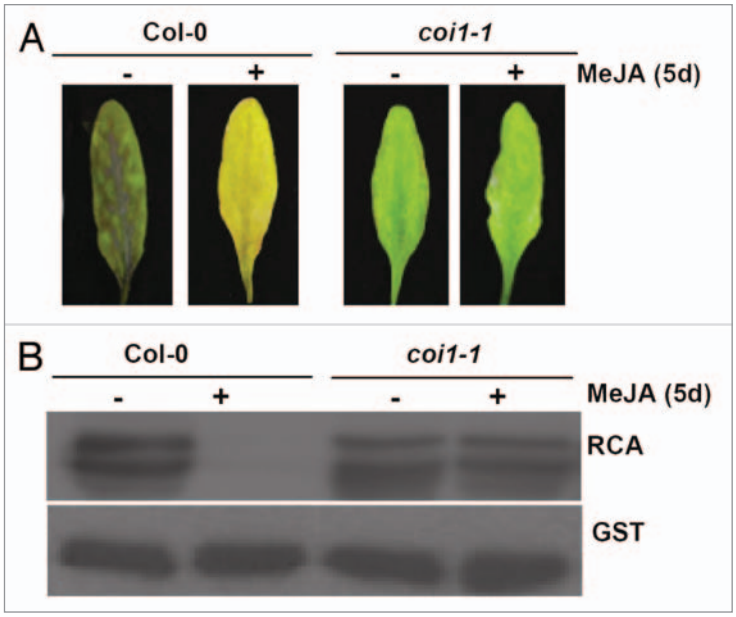

Figure 2. The COI1-dependent JA-repression of RCA correlated with JA-induced leaf senescence. (A) Phenotype of 6-week-old WT and *coi1-1* mutant leaves treated with MeJA (+) or water (-) for 5 days. (B) Western blot for RCA in 6-week-old WT and *coi1-1* mutant leaves treated with MeJA (+) or water (-) for 5 days. The immunoblot was detected with GST antibody as a protein loading control.

deficient mutants *fad3-2 fad7-2 fad8*, *dad1*, *aos*, *dde1* and *opr3*?[25-29]

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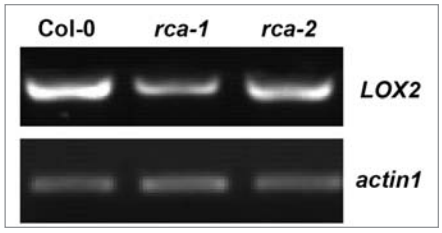

Figure 3. Semi-quantitative RT-PCR for *LOX2* in 3-week-old WT, *rca-1* and *rca-2* mutant plants. The amplified *actin1* was shown as an internal control.

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