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The Senescence-Induced Staygreen Protein Regulates Chlorophyll Degradation

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Abstract

Loss of green color in leaves results from chlorophyll (Chl) degradation in chloroplasts, but little is known about how Chl catabolism is regulated throughout leaf development. Using the *staygreen* (*sgr*) mutant in rice (*Oryza sativa*), we identified *Sgr*, a senescence-associated gene encoding a novel chloroplast protein. Transgenic rice overexpressing *Sgr* produces yellowish-brown leaves, and *Arabidopsis thaliana* pheophorbide *a* oxygenase–impaired mutants exhibiting a stay-green phenotype during dark-induced senescence have reduced expression of *Sgr* homologs, indicating that *Sgr* regulates Chl degradation at the transcriptional level. We show that the leaf stay-greenness of the *sgr* mutant is associated with a failure in the destabilization of the light-harvesting chlorophyll binding protein (LHCP) complexes of the thylakoid membranes, which is a prerequisite event for the degradation of Chls and LHCPs during senescence. Transient overexpression of *Sgr* in *Nicotiana benthamiana* and an in vivo pull-down assay show that Sgr interacts with LHCPII. Thus, we propose that in senescing leaves, Sgr regulates Chl degradation by inducing LHCPII disassembly through direct interaction, leading to the degradation of Chls and Chl-free LHCPII by catabolic enzymes and proteases, respectively.

Media summary

Understanding of Chlorophyll degradation pathway in chloroplast and LHCPII disassembly

Key Words

Staygreen, Chlorophyll degradation, LHCPs

Introduction

In autumn, plant leaves generally change in color from green to yellow or red as a result of the breakdown of the green pigment chlorophyll (Chl) combined with carotenoid retention or anthocyanin accumulation. The color change takes place during leaf senescence or accelerated cell death caused by various biotic or abiotic stresses (Matile et al., 1999). Leaf senescence, the final stage of leaf development, is not due to passive destruction but rather is regulated by genetic programs controlling the transition from nutrient assimilation to nutrient remobilization (Hörtensteiner and Feller, 2002; Lim and Nam, 2005). Hence, leaf degreening is regarded as a visible marker for plant programmed cell death processes, although a series of other degenerative metabolisms also occur in senescing leaf cells (Noodén et al., 1997). In this study, we report the identification of *Sgr* in rice and characterize its regulatory role in Chl degradation during leaf senescence.

Methods

The induction and isolation of *sgr* from the parental rice (*Oryza sativa*) *japonica* cv, Hwacheong-*wx*, the preparation of an F2 population by crossing *sgr* with the *indica-japonica* hybrid cv Milyang23 as a mapping parent. The transformation of Pro_{35S}*:Sgr-GFP* in the pCAMLA vector into the mature embryos of *sgr* seeds was performed by the *Agrobacterium tumefaciens*–mediated cocultivation method as described previously (Jeon et al., 2000). The level of each protein was examined by immunoblot analysis and for the in vitro and in vivo binding assay, proteins were purified from *E. coli*, young leaf tissues of rice and *N. benthamiana.*

Results

During dark-induced, detached leaf senescence, the wild-type *japonica* rice Hwacheong-*wx* turns yellowish-brown, whereas *sgr* maintains leaf greenness much longer due to the slower degradation of Chls (Figures 1A). Interestingly, the transgenic rice plants that regenerated from the transformed calli of *sgr* exhibited three typical color variations in the developing leaves: green, mosaic, and yellowish-brown (Figure 1B). And immunoblot analysis revealed that LHCPI and LHCPII subunits were retained in the senescent leaves of *sgr*, while other thylakoid-bound photosynthetic proteins, D1 and cytochrome *f*, were degraded progressively, as in the wild-type leaves (Figure 2A). To verify this interaction, we performed an in vivo pull-down assay with the leaves of *N. benthamiana* transiently overexpressing the Sgr-GST fusions (Figure 2B). The GST pull-down results revealed that the Sgr-GST fusion was copurified with LHCPII (Figure 2C)

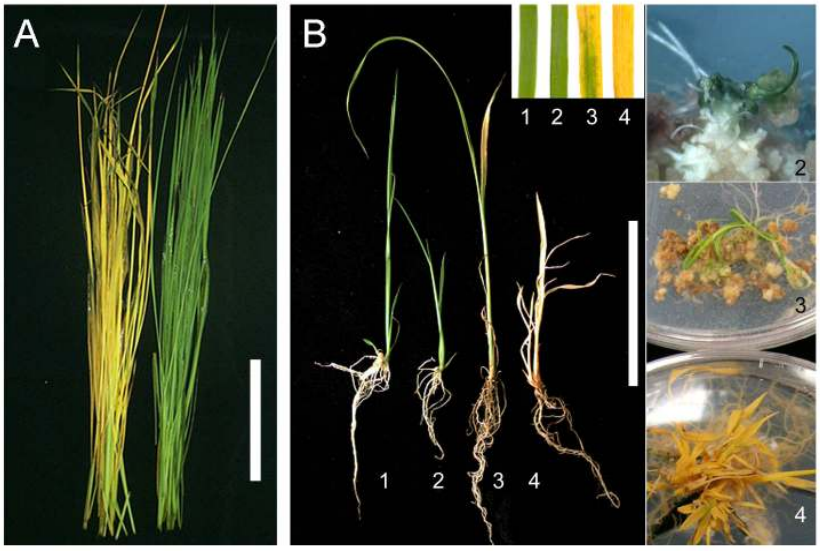

Figure 1. Phenotypic Characterization of *sgr* and Overexpression of *Sgr-GFP*.

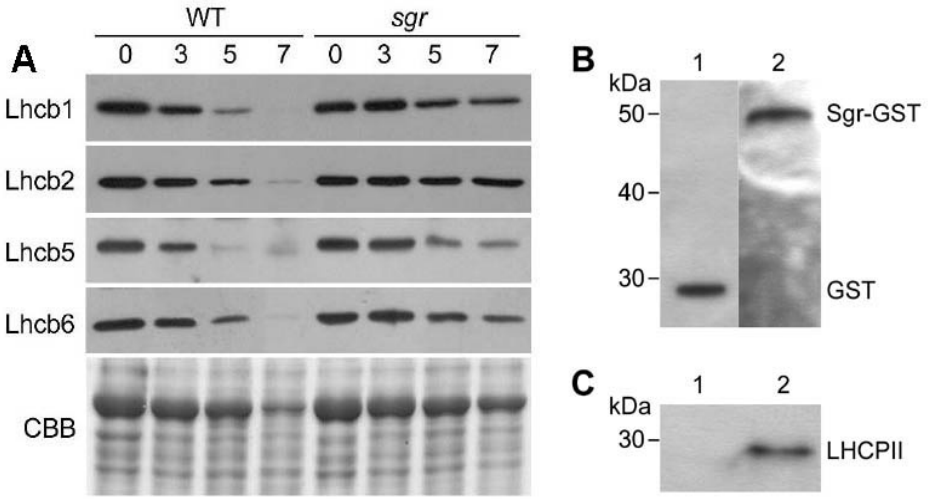

Figure 2. Immunoblot of *sgr* and Sgr Interacts with LHCPII

Conclusion

The *sgr* mutation is caused by a single-base change (G295A) in the coding region of *Sgr*. *Sgr* is highly

senescence-inducible and encodes a previously uncharacterized chloroplast protein. The overexpression of *Sgr* in transgenic rice demonstrates that Chl degradation is regulated by *Sgr* at the transcriptional level. We show that the *sgr* mutant exhibits high stability of LHCPI and LHCPII in senescing leaf cells, while other components decay normally. And an in vitro pull-down assay shows that Sgr has specific affinity to LHCPI and LHCPII. Through a transient overexpression assay in *Nicotiana benthamiana* and an in vivo pull-down assay, we confirm that Sgr interacts with LHCPII in vivo. Thus, we speculate that an amino acid substitution in sgr (V99M) may disrupt either an enzymatic activity or a binding activity to other regulatory factor(s) that is essential for LHCPII disassembly.

References

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