Chloroplasts are a significant site for reactive oxygen species production under illumination and, thus, possess a well-organized antioxidant system involving ascorbate. Ascorbate recycling occurs in different manners in this system, including a dehydroascorbate reductase (DHAR) reaction. We herein investigated the physiological significance of DHAR3 in photo-oxidative stress tolerance in *Arabidopsis*. GFP-fused DHAR3 protein was targeted to chloroplasts in *Arabidopsis* leaves. A DHAR3 knockout mutant exhibited sensitivity to high light (HL). Under HL, the ascorbate redox states were similar in mutant and wild-type plants, while total ascorbate content was significantly lower in the mutant, suggesting that DHAR3 contributes, at least to some extent, to ascorbate recycling. Activation of monodehydroascorbate reductase occurred in *dhar3* mutant, which might compensate for the lack of DHAR3. Interestingly, glutathione oxidation was consistently inhibited in *dhar3* mutant. These findings indicate that DHAR3 regulates both ascorbate and glutathione redox states to acclimate to HL.

The redox regulation of ascorbate and glutathione by a chloroplastic dehydroascorbate reductase (DHAR3) is essential for photo-oxidative stress tolerance in *Arabidopsis*.