

**Factors inducing the chloroplast movement in C₄ plants
under high light-stress conditions and effects of the
response on photosynthesis**

-Abstract version-

2020

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Abstract

In the natural environment, plants experience various stresses such as high-intensity light and drought, which leads to a decrease in crop production. Thus, it is important for stable crop production to understand how stress-tolerant plants respond to changes in their environment. C₄ plants are more stress-tolerant and show higher biomass production than C₃ plants (Brown, 1978; Hatch, 1992). Therefore, C₄ plants are considered to be beneficial and attractive plant resources for agriculture, especially in stressful environments. A better understanding of the C₄ plant-specific adaptive response will help improve stress tolerance of general C₃ crops such as rice, wheat, and soybean. A recent study reported that C₄ plants show a unique chloroplast movement under stress conditions, which is called ‘the aggregative movement’ of mesophyll (M) chloroplasts (Yamada et al., 2009), but little had been known about the detailed mechanism or the significance of the response.

This study was designed to elucidate the factors responsible for the aggregative movement of M chloroplasts in C₄ plants and the effect of the chloroplast response on their photosynthesis.

In Chapter 1, I first introduced ‘the index of aggregative arrangement of M chloroplasts’ as a novel method for quantitative analyses of the chloroplast aggregative movement in C₄ plants. This quantitative analytical method enabled us to statistically evaluate the extent of the aggregative movement of M chloroplasts.

In Chapter 2, I demonstrated that not red light but blue light (BL) induces the aggregative movement as well as the avoidance movement of M chloroplasts in C₄ plants. It was reported that BL induces the avoidance movement of chloroplasts in C₃ plants (Sakai et al., 2001), whereas red light induces the chloroplast response in some alga, moss, and fern (Suetsugu & Wada, 2007). Since C₄ plants evolved from ancestral C₃ plants (Sage et al., 2011), it is possible that the mechanism of the BL-induced chloroplast movement may have been conserved in C₄ evolution. On the contrary, this study revealed some different characteristics in the chloroplast movement in C₄ plants from that in C₃ plants. Although reactive oxygen species (ROS) are reported to promote the chloroplast avoidance movement in *Arabidopsis* (Wen et al., 2008), the chloroplast movement in C₄ plants is not influenced by ROS. In addition, I showed that abscisic acid (ABA) can shift

the avoidance movement to the aggregative movement under BL. This aggregative movement of chloroplasts in response to BL and ABA has not been observed in C₃ plants but in some C₃-C₄ intermediate species. Therefore, it is possible that C₄ plants may have acquired the mechanism of ABA involved in the aggregative movement of M chloroplasts during the C₃ to C₄ evolution. Under stress conditions, ABA is synthesized and it participates in the signal transduction for plant responses to environmental stresses (Shinozaki & Yamaguchi, 1997). Stomatal closure is one major response caused by ABA (Mittelheuser & van Steveninok, 1969; Zhang et al., 2006), but it is not crucial to trigger the aggregative movement of M chloroplasts (Chapter 3). This suggests that the aggregative movement of M chloroplasts does not behave as a secondary response associated with stomatal closure, and ABA is possible to be directly involved in a signaling pathway of the chloroplast response. Because the accumulation of ABA triggers ROS generation as well, ABA is thought to act upstream of ROS (Jiang & Zhang, 2002). The involvement of ABA in the aggregative movement of C₄-M chloroplasts suggests that C₄ plants may withstand stressful conditions more rationally and hastily than C₃ plants, by using ABA for their specific chloroplast movement as well as for other stress responses.

A similar pattern of the chloroplast movement in response to BL and ABA is conserved in different subtypes of C₄ plant species (Chapter 2), but there is a difference in the chloroplast attitude and the photosynthetic response under a natural light regime between C₄ species (Chapters 3 and 4); M chloroplasts in finger millet, an NAD-malic enzyme (ME) type C₄ plant, show the apparent aggregative movement whereas those in sorghum, an NADP-ME type C₄ plant, preferentially show the avoidance movement as well as the aggregative movement. Yamada et al. (2009) have found the preference of the chloroplast avoidance movement in an NADP-ME type C₄ plant, maize. These results suggest that the chloroplast response may depend on C₄ subtypes. There are some differences in the morphological and physiological characteristics between C₄ subtypes, such as a presence/absence of suberized lamella in bundle sheath cell walls (Hattersley & Browning, 1981; Ohsugi et al., 1988). The suberized lamella is advantageous in preventing CO₂ leakage from bundle sheath cells. It has been supposed that the aggregation of M chloroplasts to the bundle sheath side shortens the diffusion pathway of metabolites (Yamada et al., 2009). For a better understanding of the significance of

the aggregative movement of M chloroplasts, further study would be needed on chloroplast attitudes among C₄ species and factors underlying a difference in the response.

In Chapter 3, I demonstrated that the aggregative movement of M chloroplasts in finger millet is induced under natural regimes of light whether light intensity frequently fluctuated or not, and the speed and the extent of the response may vary with light conditions. In addition, I also demonstrated that the avoidance movement of M chloroplasts in sorghum is induced under a natural regime of light (Chapter 4). These chloroplast movements were observed during midday depression of photosynthesis. In the natural environment, midday depression of photosynthesis has been commonly observed in various crops including rice (Hirasawa et al., 1992) and maize (Hirasawa & Hsiao, 1999), resulting in reduced plant growth, yield, and quality. Although shading could alleviate midday depression of photosynthesis (Cartechini & Palliotti, 1995; Mohotti & Lawlor, 2002), shading decreases net photosynthetic rate (P_n) when light intensity is low (i.e., in the early morning and late afternoon) and leads to reduced biomass productivity (Stampar et al., 2001). Importantly, this study proposed a possibility that the aggregative movement of M chloroplasts contributes to increasing photosynthetic performance in the morning (Chapter 3). On the contrary, this effect on photosynthesis seems not applicable in the avoidance movement of M chloroplasts (Chapter 4). These suggest that controlling M chloroplasts to ‘the aggregative position’ rather than to the avoidance position may help minimize a decrease in the total P_n during the daytime. In addition, based on these results, I would emphasize the potential of the aggregative movement of M chloroplasts as a valuable trait for C₄ plant breeding and crop improvement. Further investigation of the relationship between the aggregative movement of M chloroplasts, photosynthesis and environmental conditions will provide a guide to the development of C₄ crops that show higher P_n under high light-stress conditions than conventional C₄ crops.

In recent years, many studies have attempted to introduce a C₄ pathway and the associated features in C₃ crop plants to improve stress tolerance and yield (Taniguchi et al., 2008; Kajala et al., 2012; Williams et al., 2016). Taniguchi et al. (2008) developed rice that expresses genes for enzymes involved in C₄ photosynthesis, but this trial has failed to increase P_n . Importantly, Wang et al. (2017) pointed out that anatomical changes

to the leaf are necessary for C₄ engineering and that partitioning of photosynthetic reaction between bundle sheath and M cells is a decisive factor controlling photosynthetic efficiency in an engineered C₃-C₄ leaf. These studies showed that a single-cell approach limits the energy efficiency of C₄ photosynthesis, and suggested that structural differentiation of the cells including their chloroplast arrangements would be a more successful strategy for the efficient operation of C₄ photosynthesis. This study provides basic information about the attitude of differentiated C₄-M chloroplasts and its relation to C₄ photosynthesis. Elucidation of the whole mechanism of the chloroplast movement exclusive to C₄ plants as well as the relationship between the chloroplast positioning and C₄ photosynthesis will be conducive for improving the ability of photosynthesis in C₃ crop plants through C₄ engineering. I believe that the findings of this study should provide useful information in plant physiology and crop production control science against current and future stressful environments.