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学 位 論 文 題 名

Molecular genetic study of carotenoid-mediated root development
(カロテノイドを介した根の発達の分子遺伝学的研究)

The plant root system plays a pivotal role in supporting plant development in various aspects, including anchoring, water and nutrient absorption, and signaling in root–shoot interactions. The root system architecture (RSA) comprises primary root (PR) and secondarily developed lateral roots (LRs). The elongation and branching of roots, as one of the main strategies for plant survival, are under the control of metabolism of endogenous metabolites (e.g., carotenoids, apocarotenoids). Under *in vitro* conditions with sucrose application, carotenoid deficiency does not lead to plant death but reduces root elongation. The phytohormones abscisic acid (ABA) and strigolactones (SLs) which are derived from carotenoids, have fundamental roles in root growth regulation (Zhang et al., 2010; Ruyter-Spira et al., 2011; Thole et al., 2014). In recent years, the discovery of bioactive apocarotenoids has increased (Dickinson et al., 2019; Jia et al., 2019; Dickinson et al., 2021). Among them, retinaldehyde (retinal), a precursor of vitamin A required for vision in animals, is identified as a regulator in periodic LR patterning (Dickinson et al., 2021). Although the metabolism pathway and roles of retinal in animals are well documented (Lidén and Eriksson, 2006; Raghuvanshi et al., 2015), the roles of this compound in plants are still largely unknown. On the other hand, carotenoids and apocarotenoids have been reported to be involved in reactive oxygen species (ROS) and respond to various stress (e.g., high light, drought and salt tolerance) (Ramel et al., 2012; D'Alessandro et al., 2019). Plants also show capable ability of organ regeneration for the adaptation to the environmental conditions (e.g., physical damage). The injury of PR encourages the elongation and branching of LRs, which is broadly applied in agricultural and horticultural fields as a technique called “root pruning”. The induction of LR branching by physical damage of PR has been partially clarified (Xu et al., 2017); however, the acceleration of its growth remains elusive, and the involvement of apocarotenoids in this regulation is unclear. In this study, I aim to determine the roles of apocarotenoids in the regulation of PR growth, and the LR growth induced by PR damage.

In the first chapter of this thesis, the regulation mechanism of auxin-mediated root growth regulation via the apocarotenoid retinal was investigated. I found 1) Short-term treatment of carotenoid biosynthesis inhibitor fluridone suppressed PR growth and induced LR density. 2) Fluridone-mediated suppression of PR growth is dependent on phytoene desaturase (PDS) activity. 3) Fluridone treatment elevated endogenous auxin levels and activated auxin signaling in roots. 4) Retinal restored fluridone-induced PR growth suppression and negatively regulated auxin levels possibly via auxin catabolism. These results suggest a regulation mechanism of PR growth by retinal, in which retinal may positively control auxin catabolism to reduce auxin levels and response.

In the second chapter of this thesis, I partially revealed the mechanism of root-cut-induced LR growth (RCG)

regulation. In this study, I found 1) Fluridone suppressed RCG possibly via the auxin pathway. 2) β -cyclocitral and β -ionone are responsible for fluridone-mediated RCG regulation. 3) Root-cut reduced endogenous cytokinin levels. 4) Cytokinins negatively regulate RCG. 5) Chromatin remodeling factor PICKLE is necessary for RCG possibly via the cytokinin signaling pathway. These results indicate that the carotenoid and apocarotenoid biosynthesis pathways positively contribute to RCG. Besides, cytokinins may be a key regulator in RCG, though the interaction between the cytokinin and carotenoid pathways is yet to be revealed in the future.

The apocarotenoids have a large diversity of functions in plants and animals. In animals, the precursor to vitamin A, retinal, is essential for visual phototransduction, which is the initial step in the light-detection process of vision. (Wong, 1999; Lidén and Eriksson, 2006). However, the functions of this important apocarotenoid in plants, especially in root development, have been recently documented (Dickinson et al., 2021), indicating that there is still much to explore in this research field. Besides, there are other bioactive apocarotenoids reported to participate in RSA regulation (e.g., β -cyclocitral, anchorene) (Dickinson et al., 2019; Jia et al., 2019), suggesting a deep interaction between apocarotenoids and root development. In this study, I investigated that the carotenoids and apocarotenoids are important regulators for PR growth and root-cut-induced LR growth. I showed that carotenoid metabolism negatively controls endogenous auxin levels via apocarotenoid retinal to regulate PR growth. I observed β -cyclocitral and β -ionone are responsible for fluridone-mediated LR growth induced by physical damage. I also revealed that the LR growth induced by physical damage is negatively regulated by the phytohormone cytokinins. This study will help elucidate the roles of apocarotenoids in regulating RSA development, which is distinct from their roles in animals.