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Intra-organ regulation of gene expression responses for the shade avoidance

Department of Botany, Graduate School of Sciences, Kyoto University
Plant physiology Laboratory, Kim Sujung

Abstract

Sessile plants adjust their patterns of growth and development according to the surrounding environment. Since light is one of the most important environmental factors for plants, they recognize light quality and respond to it properly. Plants under canopy can perceive the shade by detecting the drop in the red/far-red ratio with phytochrome (phy). Perception of the shade triggers responses to compete with surrounding plants seeking for a better light environment. This plant reaction is known as the “shade avoidance response”, which promotes various physiological processes such as hypocotyl/petiole elongation, hyponasty, early flowering, and leaf senescence. To elucidate the spatial structure of the response, different organs have been compared with respect to their responses to the stimulus. Spotlight irradiation has been employed to investigate the inter-organ communications in the response. Among other things, these studies have revealed that auxin is an important mediator of the inter-organ communications in the shade avoidance response.

Although cotyledons have been found to be an important organ for shade perception, little is known about how the shade signal is spatially processed within the cotyledons. Therefore, in this study, we attempted to establish a method to isolate micro-samples from different positions in the cotyledon, which would enable us to compare the gene expression responses in different tissue/position in the cotyledon (Chapter 1). We then conducted the transcriptome analysis using those micro-samples to characterize the responses observed in different tissues/positions within the cotyledons (Chapter 2). Finally, a spotlight experiment was conducted to investigate how the auxin response was spatially regulated by its synthesis and transport in response to the shade stimulus (Chapter 3).

Chapter 1 Isolation of Micro-Samples from Distinct Positions in the Cotyledon

In order to investigate the shade response in different tissues/positions within the cotyledon, we prepared 3 kinds of micro samples collected with a hand-made needle-based sampling device (mesophyll-enriched/prepared with a needle; MN, vasculature-inclusive/prepared with a needle; VN) or by the enzyme/ultra-sonication treatment (vasculature/prepared by sonication; VS). The tissue marker gene analysis demonstrated that vasculature was excluded form MN and highly concentrated in VS. Mesophyll was the main component of both MN and VN although VN additively contained vasculature. A preliminary analysis of the shade response indicated that the *Arabidopsis thaliana* homeobox gene (*ATHB2;*

AT4G16780*)* (homeodomain-leucine zipper; HD-Zip) and long hypocotyls in far-red (*HFR1*; AT1G02340) gene preferentially responded to the shade in mesophyll whereas the auxin-responsive genes responded mainly in vasculature. Hence, the micro-samples prepared were proven to be good materials for the investigation of the spatial structure of the shade avoidance response.

Chapter 2 The Transcriptome Analysis of the Gene Expression Responses in Different Tissues

The RNA sequencing (RNA-seq) analysis was performed on the above 3 micro sample types in order to investigate the overall patterns of gene expression responses in different tissues/positions in the cotyledon. The genes up-regulated by the shade were clustered into several groups according to their expression patterns across the different tissue types. The result indicated that much more genes responded to the shade in VS than in MN or VN. Hence, the vasculature emerged as an important site of the shade response within the cotyledon. Importantly, many of those genes were novel shade-responsive genes whose functions should be revealed in the future study. We also found that many auxin-responsive genes responded preferentially in vasculature, whereas the PIF target genes were enriched in the mesophyll/epidermis group. Hence, the genes responding to the shade in different tissues/positions were found to be functionally different.

Chapter 3 The Spatial Regulation of the Shade Avoidance Response within the Cotyledon

The result of chapter 2 clearly demonstrated that auxin plays an important role in the shade avoidance response in the cotyledon. In order to investigate the site of auxin synthesis in the cotyledon, expression patterns of the auxin synthesis gene, yucca (*YUC)*s, were examined. The result indicated that they were up-regulated by shade mainly in mesophyll/epidermis of the periphery of the distal part of the cotyledon. This pattern is in contrast with those of the up-regulation of auxin-responsive genes in response to the shade. They become more intense towards the basal region of the cotyledon. Hence, auxin was suspected to be transported from the site of auxin synthesis to the site of the auxin response. To test this possibility, spotlight irradiation experiment was conducted. In accordance with the above view, irradiation of a wider area of the cotyledon was required to induce the local auxin response in the basal region of the cotyledon. Hence, the auxin responses in the cotyledons were shown to be the consequence of auxin synthesis and transport within the cotyledon.