

( 続紙 1 )

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論文題目	Intra-organ regulation of gene expression responses for the shade avoidance		
(論文内容の要旨)			
<p>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.</p> <p>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).</p> <p>Chapter 1 Isolation of micro-samples from distinct positions in the cotyledon</p> <p>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 from 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 protein (ATHB2; AT4G16780) (homeodomain-leucine zipper; HD-Zip) and long hypocotyls in far-red (HFR1; AT1G02340) genes 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.</p> <p>Chapter 2 The transcriptome analysis of the gene expression responses in different tissues</p> <p>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</p>			

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.

(続紙 2 )

(論文審査の結果の要旨)

本論文は、植物の子葉が避陰応答する際に、その応答が器官内でどのように制御されるかを解析したものである。他の植物の陰に入った植物は、植物の光受容体であるフィトクロムによってこれを感知し、陰環境から抜け出すために茎の伸長促進を含む様々な生理応答を起こす。これらは総称として避陰応答と呼ばれる。これまでの研究により、個体レベルの避陰応答において、子葉が陰刺激を認識する器官として重要な役割を果たすことが示されてきた。しかしながら、子葉内で陰シグナルがどのように処理されるのかについては知見は乏しかった。この点を明らかにすることで、避陰応答に対する理解が大いに深まると期待される。

本論文において申請者はまず、子葉の微細領域から組織組成の異なる試料を調製し遺伝子発現解析を行うための手法を確立した。申請者は、注射針をベースとする組織片採取器具を駆使し、主に表皮と葉肉組織よりなる組織片と、これに加えて維管束を含む組織片を採取し、組織特異的マーカーを含む幾つかの遺伝子の発現をqRT-PCR法により測定することによって、これが有効な実験手法であることを示した。また、これを補完するために、短時間で維管束を単離する実験手法も確立した。以上によって、上記の課題に取り組むための実験的な基盤が確立された。

次に申請者は、上記で確立した実験手法を用いて、陰刺激を与えた子葉から試料を採取し、遺伝子発現を網羅的に捉えるトランスクリプトーム解析を行った。得られた網羅データをもとに、陰刺激に応答した遺伝子を選抜しクラスター分析によって応答の組織特異性を調べた。その結果、1) 維管束でより多くの遺伝子発現応答が見られ、これまでの注目度が低かった維管束が実は重要な光応答部位であること示された。また、2) 避陰応答においてフィトクロムの下流で働く植物ホルモンであるオーキシシンに対する応答は維管束でより顕著であることを示した。以上の成果は、子葉内の異なる組織の応答を網羅的に比較した初めての例であり、ここで得られた結果の新奇性は高い。

さらに申請者は、上で述べたオーキシシン応答に注目し、陰刺激によってオーキシシン合成が誘導される場所と、合成されたオーキシシンに対する応答が起こる場所がどのように異なるかを調べた。まず、オーキシシン合成の場を調べたところ、子葉の先端側の葉肉組織であることが確認された。一方、オーキシシンに対する応答は子葉の基部側でより高くなる傾向が見られた。次に子葉の部分照射を行い、維管束周辺でオーキシシン応答を引き起こすには、子葉の部分照射では不十分であり、子葉全体の照射が必要なことを示した。以上により、子葉内の避陰応答において先端から基部へ向けてのオーキシシンの移動が重要であることが明確に示された。これも植物の避陰応答の制御機構理解に向けた重要な成果である。

以上により、本論文は博士(理学)の学位論文として価値あるものと認める。また、平成30年4月17日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。

要旨公表可能日： 年 月 日以降