論文内容の要旨

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Xylem vessels are important tissues that function in water conduction in vascular plants. To function, xylem vessel cells must form a strong lignified secondary wall cell (SCW) and undergo programmed cell death (PCD) during cell differentiation. These two events are known to be initiated by specific group of NAC transcription factors named *VASCULAR-RELATED NAC DOMAIN1* (*VND1*) to *VND7* in Arabidopsis, which can upregulate the gene sets required for SCW formation and PCD. Because of relatively strong activities of VND6 and VND7 to induce ectopic xylem vessel cell differentiation, the functional analysis has been extensively focused on VND6 and VND7, and physiological roles for VND1 to 5 have not fully understood. It has been reported that VND1 to 5 have the potential to upregulate VND7 expression, and each *VND* gene have distinct gene expression patterns. These data suggested functional diversity among VND proteins; however, no critical evidence has been shown yet.

In vitro induction systems for xylem vessel cells have proven to be powerful tools to understand molecular mechanisms of xylem cell differentiation. To investigate potential functional diversity of VND proteins, I established a novel *in vitro* system named the KDB system, in which xylem vessel cells can be induced ectopically in cotyledons by the application of three phytohormones, cytokinin (Kinetin), auxin (2,4-D) and brassinosteroid (Brassinolide). The advantages of KDB system are 1) simple and repeatable material preparation, as excised cotyledons are readily available 6-days after germination of Arabidopsis seedlings and 2) the ease of application of this system to the rich Arabidopsis resources including mutant lines and reporter marker lines.

Using this KDB system, time-course mRNA-seq analysis was performed to obtain global transcriptome profiling during xylem vessel cell differentiation. All of the *VND* genes were upregulated in the KDB system, however, comparing their gene expression with known vascular-related genes, each *VND* gene was separated into different gene clusters, suggesting differential responses to the KDB treatment by *VND* gene. Mutant analysis of *VND* family genes showed that ectopic xylem vessel cell differentiation in the KDB system is largely dependent on VND2 activity, and that *vnd1 vnd2* double and *vnd1 vnd2 vnd3* triple mutants showed additive reductions of cell differentiation efficiencies. Therefore, VND1 and VND3 also contribute with VND2 to the cell differentiation in this system. In contrast, ectopic xylem vessel cells phenotypes of *vnd6 vnd7* mutants was less dramatic. Taken together, VND1 to 3, but not VND6 and VND7, would be main contributors to the differentiation of ectopic xylem vessel cells in cotyledons induced by the KDB system.

The KDB system also showed that the cell differentiation was not observed under continuous dark conditions, indicating the importance of light to induce xylem vessel cell formation in cotyledons. Base on this observation, I checked the vein formation in cotyledons under continuous light or dark conditions in *vnd1*, *vnd2*, *vnd3*, *vnd6* and *vnd7* mutants, as well as double and triple mutant of them. The results demonstrated that VND1 to 3, but not VND6 and VND7, are required for xylem vessel cell differentiation in secondary vein of cotyledons grown under continuous dark condition. Interestingly, secondary venation was not inhibited in *vnd1 vnd2 vnd3* cotyledons under continuous light condition, although *VND* expressions in *vnd1 vnd2 vnd3* cotyledons were not different by the light conditions. These suggested that distinct roles for VND1 to 3 proteins in vein formation in cotyledon under dark condition.

Put all into together, I successfully disclosed specific roles for VND1, VND2 and VND3, in xylem vessel cell differentiation of cotyledons. My results also showed the possibility that the light signals can replace such specific roles for VND1 to 3 in xylem vessel cell differentiation, further suggesting novel connection between environmental signals and VND functions.

論文審査結果の要旨

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道管は被子植物にとって生存に必須の通水組織であり、その分化メカニズムの分子的理解は生物学的にも農学的にも非常に重要な課題である。道管細胞分化の特徴として、強固な二次細胞壁の形成とプログラム細胞死による内容物の消失が挙げられ、これらによって中空の堅い壁をもつ管状構造体となり、高い通水機能を実現していると考えられている。これまでの研究から、道管細胞分化マスター制御因子として、NAC転写因子群に属するシロイヌナズナVASCULAR-RELATED NAC DOMAIN (VND)ファミリー遺伝子が同定され、機能解析が進められてきた。VNDファミリーは二次細胞壁形成とプログラム細胞死に必要な遺伝子セットの発現を誘導し、道管細胞分化を誘導することが分かっている。シロイヌナズナでは7つのVNDファミリー遺伝子が存在しているが、しかしながら、これまでの解析は、過剰発現時の道管細胞形成能が高いVND6およびVND7を中心に行われており、VND1~5の生理的役割については未解明の点が多かった。

申請者はこの点に切り込むため、新たに確立した道管細胞分化誘導系と変異体解析を組み合わせ、以下の成果を得た。

1．　シロイヌナズナ子葉を材料とした植物ホルモン(オーキシン、サイトカイニン、ブラシノステロイド)による道管細胞分化誘導系、KDBシステムを確立し、VNDファミリー遺伝子の発現パターンが異なることを示した。

2．　さらにKDBシステムにおける道管細胞分化誘導はVND1、VND2、VND3に依存しており、VND6およびVND7の役割は限定的であることを示した。

3．　KDBシステムの知見を元に*vnd*変異体の子葉の葉脈形成の解析を行い、暗黒条件下での子葉の二次葉脈形成が、VND6あるいはVND7ではなく、VND1、VND2、VND3に大きく依存していることを示した。

4．　種子給水後～発芽7日目までの芽生えにおけるVNDファミリー遺伝子の発現解析を通して、光条件によってVNDファミリー遺伝子の発現パターンは変化しないものの、連続光条件下では、暗黒条件下に比べて、VNDファミリー遺伝子の発現レベルが上昇することを示した。

これらは、子葉の二次葉脈の道管細胞分化におけるVND1、VND2、VND3特異的な役割を示唆する結果であり、VNDファミリー遺伝子間の生理的機能分化を初めて明らかとする成果であった。

以上のように、本論文は、VNDファミリー遺伝子間の生理的機能分化を明らかにし、さらには光といった環境条件に応答した道管形成制御が、特定のVNDを通して行われているという新たな可能性を示すことに成功した。これらの成果は、学術上、応用上貢献するところが少なくない。よって審査委員一同は、本論文が博士（バイオサイエンス）の学位論文として価値あるものと認めた。