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論文題目	Comparative study of the regulatory mechanisms for hydrotropism in rice and lotus roots （イネとミヤコグサの根の水分屈性制御機構の比較解析）
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論文内容の要旨

Plant roots exhibit tropisms such as hydrotropism and gravitropism and thereby avoid environmental stresses in their terrestrial life. It has been shown that a plant hormone, auxin, plays an important role in gravitropism. In gravistimulated roots, auxin transport changes so that more auxin accumulates in the lower side of the horizontally reoriented roots. It has been shown that columella cells in the root cap perceive gravistimulation, which causes a change in auxin transport and asymmetric auxin distribution. Following graviperception in Arabidopsis roots, for instance, PIN3/PIN7 efflux carriers localized in columella cells play an important role in transporting auxin to the lower side of the gravistimulated roots. This redistribution of auxin causes differential growth of the roots and their downward bending; namely, relative extension growth becomes less in the lower side than in the upper side of the elongation zone. The involvement of auxin in root gravitropism in this manner is considered to be mutual among plant species. In root hydrotropism, however, it has been suggested that the dependency on auxin transport differs among plant species. For example, application of inhibitors of auxin efflux and influx reduces hydrotropic response in cucumber roots but not in Arabidopsis roots. In contrast, these chemicals are inhibitory to gravitropism in roots of both plant species.

To extend our knowledge on differences in the regulatory mechanisms of root hydrotropism among plant species and to understand how auxin regulates root hydrotropism, this study focused on *Oryza sativa* (rice; monocots) and *Lotus japonicus* (lotus; dicots), because these plants are useful for molecular dissection of hydrotropism as model plants and for the application of hydrotropism to agricultural purpose. In this study, I investigated the effects of various inhibitors of auxin efflux, auxin influx, auxin response, and auxin biosynthesis on hydrotropic responses, and compared them with the effects of those inhibitors on gravitropism in rice and lotus roots. Also, I analyzed the role of the root cap in hydrotropism of the two plant species, with a special emphasis on auxin regulation of hydrotropism.

Auxin transport in plants is composed of its cell-to-cell influx and efflux. 3-chloro-4-hydroxyphenylacetic acid (CHPAA) and 2,3,5-triiodobenzonic acid (TIBA) are known to inhibit the activities of auxin influx carrier AUX1 and auxin efflux carrier PINs, respectively. It has been shown that *p*-chlorophenoxy isobutyric acid (PCIB) inhibits auxin-inducible degradation of Aux/IAAs that are negative regulators of auxin inducible gene expression and are degraded by binding of auxin to TIR1-AFBs. In the biosynthesis pathway of auxin, IAA, TAA1/TAR catalyzes a step from tryptophan to indole-3-pyruvuc acid. L-kynurenine (Kyn) inhibits IAA biosynthesis by blocking the TAA1/TAR activity. When rice roots were treated with CHPAA, TIBA, PCIB, and Kyn, their hydrotropic response was significantly reduced in all of these treatments. Rice roots treated with these inhibitors reduced their gravitropic response as well. Thus, rice roots require auxin transport, auxin action and auxin biosynthesis for the induction of both hydrotropism and gravitropism. On the other hand, these

chemicals, except for Kyn, did not reduce the hydrotropic response of lotus roots. All of these chemicals inhibited gravitropism of lotus roots. These results suggest that neither auxin transporters (AUX1, PINs) nor auxin receptor (TIR1/AFBs) are involved in the regulation of hydrotropism of lotus roots. Because auxin binding proteins other than TIR1/AFBs have been reported, and because hydrotropism is inhibited by Kyn but not by PCIB, it is likely that hydrotropism of lotus roots is mediated by an auxin-signalling mechanism other than TIR1/AFBs pathway.

Next, I surgically removed the root cap to examine the role of the root cap in the regulation of auxin transport for hydrotropism of rice. The removal of the root cap resulted in the reduction of gravitropism in both rice and lotus roots, and placement of an IAA-containing agar block to the cut surface failed in recovering gravitropic bending. On the other hand, removal of the root cap did not decreased root hydrotropism in rice. In rice, further more, I extended the length of de-tipping and confirmed whether hydrotropism can express or not. As a result, 0.7 mm de-tipping totally repressed root hydrotropism, and this repression did not rescue by IAA application. These results suggest that the root cap of both plant species has an apparatus for sensing gravity but not moisture gradients.

In conclusion, the results of this study demonstrated that the mode of auxin involvement in hydrotropism differs depending on plant species. In rice roots, auxin transport is essentially required for the induction of hydrotropism, and thus the root cap may be not required for the bending response. However, lotus roots might possess a novel regulatory mechanism, independent of auxin transport and TIR1/AFBs pathway, for hydrotropic response.

論文審査結果の要旨

植物の根は、重力屈性および水分屈性を発現させて、高水分側に伸長し、乾燥ストレスを軽減・回避する能力を有する。しかし、重力屈性に比較して、水分屈性に関する研究は極めて少ない。根の重力屈性では、根冠のコルメラ細胞で重力刺激を感受し、植物ホルモンであるオーキシンの輸送体の細胞内局在が変化し、オーキシンが横になった根の下側を、根冠から伸長領域へ求基的に輸送され、そのオーキシンの不均等分布が屈曲成長を誘導する。これに類似したオーキシンの輸送・分布が水分屈性にも重要であることが報告されているが、最近、それは植物種によって異なることが示唆されている。そこで中島佑介氏は、水分屈性発現機構の植物種間差を明らかにするために、植物の分子遺伝学のモデルとして確立されているイネとミヤコグサに着目し、根の水分屈性におけるオーキシンの関与を生理・薬理学的に比較解析した。

その結果、イネ根の水分屈性はオーキシン輸送に依存し、それが重力屈性の場合に類似するものであるが、ミヤコグサ根の水分屈性は、このようなオーキシン輸送やオーキシン応答とは独立して誘導されることがわかった。すなわち、オーキシン輸送、オーキシン応答の阻害剤の処理は、イネでは重力屈性だけでなく水分屈性の発現も顕著に抑制するが、ミヤコグサでは重力屈性を抑制するものの、水分屈性を抑制しないことを明らかにした。しかし、オーキシン生合成阻害剤の処理は、イネとミヤコグサのいずれでも重力屈性と水分屈性を抑制したことから、ミヤコグサでは水分屈性を制御する新規のオーキシン応答機構の存在が示唆された。さらに、イネ根では、根端 0.2 mm（根冠）を除去すると重力屈性は阻害されるが、水分屈性は阻害されず、根端 0.7 mm 以上を除去すると水分屈性も阻害されることを明らかにした。

以上の結果は、根の水分屈性の発現機構が植物種によって異なり、イネの水分屈性は重力屈性の場合に類似してオーキシン輸送システムを必要とするが、それが根冠非依存的であり、ミヤコグサの水分屈性はオーキシン輸送非依存的な機構、且つ、新規のオーキシン応答を介することを明らかにした。これらの成果は、これまで未解明であった根の水分屈性の発現機構の包括的な理解および今後の分子機構の解明に貢献するもので、中島佑介氏が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、中島佑介氏によって提出された論文は、博士（生命科学）の博士論文として合格と認める。