様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）From nutrients to root shape: How nutrients alter root mechanics and shape for an efficient uptake

研究課題名（英文）From nutrients to root shape: How nutrients alter root mechanics and shape for an efficient uptake

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研究成果の概要（和文）：植物の根は養分の有無に応じて形態を変化させ吸収率を高める。本研究では栄養供給変化に応じた根の形態変化と根の機械的特性との関連を解析した。イネで報告されている窒素依存性の根の伸長応答はシロイヌナズナでは容易に観察でなかったため、栄養条件を窒素からホウ素に切替え、本研究で新たに開発したマイクロピラー装置を使用してヤング率を測定した。その結果、異なるホウ素条件での根の機械的特性の測定に成功し、外部からのホウ素供給と機械的特性間の相関関係を明らかにした。さらに根の代謝バランスと機械的特性の関連性を分析し、スクロースの供給が根の直径の変化と機械的な硬さの変化につながることを明らかにした。

研究成果の学術的意義や社会的意義
While nutrients change root morphology, their direct involvement in changes of root mechanical properties is not well understood. This study demonstrated that nutrients (boron) and the metabolic state (sucrose) play a role in root stiffness and form.

研究成果の概要（英文）：Plant roots change their morphology in response to nutrient availability for an improved uptake. These morphological changes are known and the involvement of phytohormone distribution patterns is well established. This project dealt with the measurement of root mechanical properties in response to altered nutrient supply. The nitrogen dependent root growth alteration reported in rice cannot easily be observed in Arabidopsis thaliana. Since the use of the micropillar device for the measurement of the Young`s modulus is not feasible under this condition, the focus was switched from nitrogen to boron. We established the measurement of mechanical properties under different boron conditions and show a correlation between the external boron supply and the mechanical stiffness. Furthermore, a link between the metabolic balance of the root and the mechanical properties was analyzed. We were able to link sucrose supply to an altered root diameter and a changed mechanical stiffness of the root.

研究分野：plant nutrition

キーワード：plant nutrition　mechanics　boron

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様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Plant roots change their morphology in response to nutrient availability for an improved uptake. These morphological changes are known and the involvement of phytohormone distribution patterns is well established. This project dealt with the measurement of root mechanical properties in response to altered nutrient supply. The nitrogen dependent root growth alteration reported in rice cannot easily be observed in Arabidopsis thaliana. Since the use of the micropillar device for the measurement of the Young`s modulus is not feasible under this condition, the focus was switched from nitrogen to boron. We established the measurement of mechanical properties under different boron conditions and show a correlation between the external boron supply and the mechanical stiffness. Furthermore, a link between the metabolic balance of the root and the mechanical properties was analyzed. We were able to link sucrose supply to an altered root diameter and a changed mechanical stiffness of the root.

While nutrients change root morphology, their direct involvement in changes of root mechanical properties is not well understood. This study demonstrated that nutrients (boron) and the metabolic state (sucrose) play a role in root stiffness and form.

２．研究の目的

Topic 1 Transporters and channels: Analyze if the reported nutritropic response in rice to ammonium (Yamazaki, Ohmori and Fujiwara, 2019) is conserved between A. thaliana and rice. Identify underlying transporters or channels to explain the morphological response.
Topic 2 Mechanical response mechanisms: Analyze how an altered nutrient supply leads to a mechanical response.
Topic 3 Metabolic balance in plant mechanical growth: Analyze how metabolic changes of the primary carbon metabolism influence the mechanical properties of roots.

３．研究の方法

Topic 1: To analyze a potential nutritropic response in several ecotypes of Arabidopsis thaliana were used in a plate assay with nutrient filled tips. Since it is known that some tropic response can influence each other, a further analysis of the plate assay was carried out under a rotation treatment to alleviate a potential dominant gravitropic response. To identify underlying genes a RNA-seq analysis was carried out, in combination with
samples for Topic 3.
Topic 2: Mechanical changes of Arabidopsis roots in response to an altered nutrient supply were directly measured with a novel Micropillar device. The Micropillar device allowed the position dependent measurement based on the pillar deformation caused by the intruding root. These measurements were done based on micrograph captured with a stereo- and a confocal-microscope. For a different nutrient supply Boron was used. To explain the phenotype caused by boron, the measurements of the stiffness were combined with observations of phenotypical parameters, like root length, diameter and ionome measurements.
Topic 3: As a means of altering the metabolic balance, Arabidopsis plants were grown under different supply of sucrose to the media. Mechanical changes were observed by the novel micropillar device as described in topic 2. In addition to the mechanical measurements, phenotypical parameters of the plants were measured. To identify underlying genes, RNA-seq was conducted. The RNA-seq results were used in combination with the phenotypical data for a correlation-based network analysis.

４．研究成果

Topic 1: A nutritropic response as observed in rice was not clearly observable in the

tested Arabidopsis ecotypes. Due to existing literature on the interaction between hydrotropism and gravitropism, the same set-up was tested under rotation, which leads to a reduction in the gravitropic response. A circularization of the root, similar to the nutrient dependent response in rice was observed. However, this response was independent of additional supplied tips containing specific nutrients. Due to the confined experimental space on the rotator, the intended screening of the ami-RNA library was changed to a RNA-seq analysis, which compares the mRNA accumulation under rotation (reduced gravity) and normal growth conditions. We were able to identify some transporters and some other likely involved genes. Respective mutant analyses are still ongoing. These results will be presented and published in the future.

Topic 2: To measure the mechanical response to nutrients it was necessary to switch from the nitrogen dependent response (Topic 1) to another nutrient, since the root under rotation grows not into the micropillar device. Since the only known biological function of boron is its role in Rhamnogalacturonan-II (RG-II) crosslinking, the link to changes in the mechanical properties of the root is straight forwards. We were able to measure changes in the Young`s modulus of Arabidopsis roots with the novel micropillar device. An increase in the external boron concentration from limited boron supply to normal concentrations in the range of 100 µM lead to a steady increase in the Young`s modulus, which is reflected by an increased root length. The cell wall boron concentration as a proxy for the RG-II crosslinking rate under these conditions is correlating with both, the Young`s modulus and the root length. This suggests that an increased external boron supply leads to an increased RGII crosslinking rate, which increases the Young`s modulus (the stiffness) of the root, allowing for an extended growth. While this was hypothesized before by Miwa et al. 2013, the actual measurement of the mechanical properties is novel. These results were already presented in several scientific meetings, and a peer-reviewed article is to be expected.

Topic 3: The use of sucrose as an additional carbohydrate source to mimic an altered supply of photosynthate to Arabidopsis roots lead to a clear morphological change in the root structure. The diameter drastically increases with an increased sucrose supply. This is accompanied by a more mild but significant increase in the root stiffness. The combination of the mechanical and phenotypical analysis with rna-seq under different supply of sucrose lead to the identification of several involved gene clusters. The analysis of respective mutant lines is still ongoing.

These results were already presented in several scientific meetings. A publication will follow in the future.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　0件）

１．著者名 Marcel Pascal Beier, Satoru Tsugawa, Taku Demura, Toru Fujiwara	４．巻 37
２．論文標題 Root shape adaptation to mechanical stress derived from unidirectional vibrations in Populus nigra	５．発行年 2020年
３．雑誌名 Plant Biotechnology	６．最初と最後の頁 423 - 428
掲載論文のDOI（デジタルオブジェクト識別子） 10.5511/plantbiotechnology.20.0813a	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Marcel P. Beier, Soichi Kojima	４．巻 171
２．論文標題 The function of high‐affinity urea transporters in nitrogen‐deficient conditions	５．発行年 2020年
３．雑誌名 Physiologia Plantarum	６．最初と最後の頁 802-808
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/ppl.13303	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Marcel Pascal Beier, Shumpei Hayashi, Kyoko Miwa, Hirotaka Hida, Toru Fujiwara
２．発表標題 The influence of sucrose on Arabidopsis root diameter and mechanics
３．学会等名 The 61th Annual Meeting of the Japanese Society of Plant Physiologists
４．発表年 2020年

１．発表者名 Yunshu Wang, Marcel Pascal Beier, Shumpei Hayashi, Kyoko Miwa, Hirotaka Hida, Toru Fujiwara
２．発表標題 Boron induced stiffness changes in Arabidopsis roots
３．学会等名 The 62th Annual Meeting of the Japanese Society of Plant Physiology
４．発表年 2021年

１．発表者名
Marcel Pascal Beier, Shumpei Hayashi, Hirotaka Hida, Kyoko Miwa, Toru Fujiwara

２．発表標題
Sucrose alters Arabidopsis thaliana root diameter and mechanics

３．学会等名
The 62th Annual Meeting of the Japanese Society of Plant Physiology

４．発表年
2021年

１．発表者名
Beier MP, Hayashi S, Miwa K, Hida H, Fujiwara T

２．発表標題
Arabidopsis root adaption to sucrose

３．学会等名
Soil Science and Plant Nutrition meeting 2020

４．発表年
2020年

〔図書〕　計1件

１．著者名	４．発行年
Soichi Kojima, Keiki Ishiyama, Marcel Pascal Beier, Toshihiko Hayakawa	2020年
２．出版社	５．総ページ数
Springer Nature Switzerland AG	20
３．書名	
Progress in Botany Vol. 82, Chapter: Ammonium Assimilation and Metabolism in Rice	

〔産業財産権〕

〔その他〕
All presentations are directly linked to the grant. The article, the review and the book are on related topics. In addition to the results in the report a further analysis of root structure in Poplar was conducted. While a structural observation was observed under stress (which lead to the publication), a direct link to nutrient flux was hard to establish. The review and the book are dealing with urea and nitrogen assimilation. Some of the materials mentioned in the review and the book were used in the analysis of mechanical responses and nutritropism, however, the results are not conclusive so far.
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6．研究組織

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研究協力者	王　韻書 (Wang Yunshu)	東京大学	
研究協力者	林　駿平 (Hayashi Shumpei)	神戸大学	

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関