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Root Research

Japanese Society for Root Research

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20th JSRR Anniversary Since 1992

根の研究

根研究会(JSRR)

根研究会創立 20 周年シンポジウム
主催者からのメッセージ

During the 20-year history of the JSRR, many studies have been performed on plant roots, contributing new evidence to root science and technology. The JSRR has played an important role in enlarging the international network of root studies as a member of the ISRR in Asia. I look forward to celebrating the JSRR's 20th Anniversary Symposium in the coming November. I hope that the root research network will continue to expand on an international scale, particularly in Asia, and produce many useful results.

Jiro Tatsumi
President of Japanese Society for Root Research
Professor, Kyoto Institute of Technology

The Japanese Society for Root Research (JSRR) was founded in 1992, and it has operated for two decades. We are holding an international symposium to develop a network of root researchers in Asia in order to advance efforts to secure healthy food, a safe environment, and peace. Come join us!

Shigenori Morita
Chair of Organizing Committee
Professor, The University of Tokyo

このメッセージは，シンポジウムの開催案内から再掲しました．

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組織委員会より

本号は，2011 年 11 月 5 日・6 日に東京大学農学部で開催した第 35 回根研究集会（特別講演，根研究会賞授賞式・受賞講演，ポスター発表）と創立 20 周年の記念事業（記念式典，祝賀会，記念シンポジウム）での講演・ポスターの要旨を掲載しています．当日は，下記の冊子として要旨を配布しましたが，この号に掲載の要旨が，一部修正を加えた最終確定版となります．

JSRR ed. 2011. Abstracts for JSRR's 20th Anniversary Symposium, “The Latest Frontiers of Root Research in Asia” and 35th JSRR Biannual Meeting (The University of Tokyo, Tokyo, Japan; November 5-6, 2011). Japanese Society for Root Research, Tokyo.　ISBN978-4 931358 09 6
（根研究会編 2011. 根研究会創立 20 周年記念シンポジウム「アジアの風－アジアにおける根研究の最前線—」及び第 35 回根研究集会要旨集．根研究会，東京.）

なお，開催の趣旨や全体のプログラム，当日の様子などについては，本誌の第 20 巻第 4 号に報告を掲載しましたので，そちらをご参照下さい．

組織委員会（五十音順）
阿部　淳・大橋瑞江・小柳敦史・大門弘幸・村上敏文・森田茂紀（委員長）・山内　章

特別講演　Special Lectures

福島県いわき市における東日本大震災の影響と対策

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１．はじめに

2011 年３月１１日（金）に発生した東日本大震災では、福島県いわき市は、地震、津波及び福島第一原発事故により甚大な被害を受け、農業への影響は深刻なものとなった。本講演では、東日本大震災の影響とその対策について報告する。

２．地震及び津波被害と対策

いわき市の地震による農業被害は、3 月 11 日の本震と 4 月 11 日・12 日の余震で発生した。主な被害は、断層による水田の亀裂や陥没、液状化現象による水田の噴砂及び津波による塩害であり、被害が甚大なものは、今後、大型土木工事等による水田修復が必要になってくる。

津波による塩害は、海岸部を中心にいわき市内の全水田（H22 現在　約 4,610ha）の約 8%に当たる水田で被害を受けた。このうち、約半数以上の水田が塩害対策（除塩対策）を施すことで、今年度の作付けが可能となった。しかし、一部で除塩対策が不完全な水田や堤防、用水路破損等による高潮被害水田では塩害による生育不良が見られた。そのため、今後、それらの水田では収穫後、次年度作付けまでの除塩対策が必要となってくる。

３．東京電力福島第一原子力発電所事故による被害と対策

福島第一原子力発電所事故では、放射能汚染による「直接被害」や「風評被害」が発生した。「直接被害」への対策は、作付け前にいわき市内で水田の空間放射線量と土壌放射能を測定し、後者の値が基準値（5,000 ベクレル/kg）以下であったことを確認し、今年度の水稲作付けを行った。その後、秋の収穫後に玄米の放射能を測定（サンプル数は、いわき市の旧 37 市町村で 80 点）し、多くの地点で放射能は未検出であったが、一部で暫定基準値（500 ベクレル/kg）以下だが微量の放射能が検出された。そのため、今後の対策は、作付け前の水田の空間放射線量測定と土壌放射能測定を行い、その結果を基に放射性物質の吸収抑制対策に取り組むなど集落全体で放射能対策を徹底することで、放射能が検出されない米作りを行うことである。また、同時に「農産物の長期モニタリング検査」と「農用地等の除染」も行うことも重要になってくる。

「風評被害」対策は、農産物の放射線モニタリング検査を通して農産物の安全性を確認し、その情報を逐次、県やいわき市の HP、テレビ CM 等を通して公開することで、消費者目線での「安全・安心」に関する情報を提供することである。また、その対策を通して、いわき産農産物の販売促進につなげていくことが重要になってくる。

４．今後の復興に向けて

この度の東日本大震災ではいわき市の水田農業は、甚大な被害を受けた。今後、風評被害等により米の販売は、極めて厳しい状況になることが予想される。そのため、今後、飼料用米、稲 WCS 等の新規需要米の作付けを増やすことで生産調整を達成し、農業者戸別所得補償制度等の政策を活用しながら、水田農業の復興を図っていくことが重要になってくる。

特別講演　Special Lectures

東京大学農学部における震災復興支援研究

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本年３月に発生した東日本大震災および福島原発事故によって我が国は未曾有の被害を受けたが、東京大学大学院農学生命科学研究科では３月末より、被災地の農林水産・畜産・漁業の復興支援に向けた組織的な研究活動（座長：放射性同位元素施設・中西友子教授）を進めてきた。活動内容は、以下のように放射線汚染関連とそれ以外の復興支援の２つに大別されるが、いずれも本研究科の多様な専門分野の教員が単独あるいは共同して、現場を重視した研究を展開してきている。

（１）高放射能の農畜水産業への影響についての研究開発（影響調査ならびに回復研究）

関係各専攻・附属施設の有志の教員が附属施設内あるいは福島県農業総合センターとの共同でそれぞれの研究を進めており、放射能の測定は本研究科の放射性同位元素施設で行っている。以下のそれぞれの分野について、①獣医・畜産学、②作物生産・土壌学、③水産学、④放射線測定・放射化学、⑤フィールドモニタリング科学・科学コミュニケーション、等の専門的立場から研究開発を行っている。現在、研究結果が 5 つの速報として Radioisotopes 誌の 8 月号に掲載されている。うち三編が農学生命科学研究科と福島県農業総合センターとの共同研究、残り二編は農学生命科学研究科附属牧場および農学生命科学研究科附属生態調和農学機構での研究である。

（２）被災地農業回復についての研究開発

大震災によって食料生産が不可能となった農地を活用したバイオマス資源作物の栽培とバイオマスエネルギーの生産による農業の再生支援、および東北地域の森林で生産された木質バイオマスを利用した木材供給・加工とバイオマスエネルギー生産による林業の再生支援を目指す。

本講演ではこれらの活動を、とくに演者の所属する作物生産・土壌学グループに焦点を当てて紹介したい。

樹木の微細根形態からみた森林炭素動態に関する研究

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はじめに

現在、二酸化炭素(CO_2)濃度の上昇に伴い、大規模な気候変動が危惧されている(IPCC, 2007)。これを緩和するため、森林のもつ炭素固定蓄積機能に注目が集まり、森林生態系における炭素動態の解明が重要な課題となっている。炭素資源の吸収は、樹木が光合成によって大気中の CO_2 を固定するという、植物の最も基本的な営みとして行われ、幹・枝・葉・根などの有機物として樹体に蓄積される。一方で、樹木に利用可能な形でエネルギーを作り出す呼吸代謝機能として CO_2 は大気へ放出される。すなわち、森林生態系における炭素動態を正確に評価するためには、樹木がどれぐらいの炭素を吸収・固定・放出にしているかを定量的・定性的に調査する必要がある。

私は研究室に配属されて以降、樹木の根系に魅せられ、気づけば博士課程に誤って迷い込むまで夢中になっていた。私の研究テーマは、どのような機能の根っこがどのように土壌中に分布しているのか？その分布が、樹木自体にそして森林生態系にどのようなインパクトを与えるのか？ということを解明することである。樹木根は、樹体を支持する太い根（粗根）と水や栄養塩類の吸収を担う細根(一般に直径 2 mm 以下)に分けられて研究されることが多い。このうち細根は、粗根に比べて非常に呼吸速度が高く、また回転速度(成長－枯死－分解サイクル)も早い。しかし、最近の樹木細根の研究では、直径 2 mm 以下の根を同じ生理特性や生活史戦略を持つものとしてひとくくりに扱うのでは、根の機能を正確に捉えることはできないことが分かってきた。例えば、直径 2 mm 以下の根の分枝位置や直径の違いによって、化学組成、あるいは枯死率や分解速度は大きく異なることが明らかにされてきた。したがって、森林生態系における根の役割を正確に評価するためには、直径 2 mm 以下の形態と機能の関係をより詳細に見ていく必要がある。そこで私は、広葉樹二次林(山城水文試験地)のコナラとソヨゴの直径 2 mm 以下の根をより詳細に分類し、①形態と呼吸速度の関係を明らかにした。また、②根現存量・形態・生理機能を土壌深度ごとに調査した。ここでは、その成果を紹介したいとおもう。

材料と方法

調査は、京都府南部のコナラとソヨゴが優占する里山広葉樹林（山城水文試験地）で行った。

①根呼吸と形態の関係

成木の樹冠下で傷つけないように採取した細根系(末端根を含む手のひらサイズの細根ひとかたまり)を丁寧に水で洗い、現場ですぐに、小型閉鎖型容器と CO_2 ガス変換器を組み合わせた測定システムで根呼吸を測定した。その後、サンプル根を実験室に持ち帰り、根形態測定システム WinRHIZO Pro 2007a を用いて細根系の形態（根直径・根長）を測定し、乾燥重量、比根長(SRL:$m\ g^{-1}$)をもとめた。

②根現存量・形態・生理機能の垂直分布

根現存量をもとめるため、対象成木の樹冠下で、深さ 10cm 間隔の合計 5 層(50cm まで)、ブロックサンプリングを行った。その後、土壌ブロックを実験室に持ち帰り、土壌から根系を取り出し、すべてのサンプル根系を 4 つの直径階級（<0.5、0.5－1.0、1.0－2.0、>2.0 mm)に分類した。次にそれらの

根直径・根長を、根形態測定システムを用いて測定した。形態測定が終わったサンプル根は、70℃で48 時間、乾燥させて、乾燥重量、SRL (m g^{-1})、根窒素含有量をもとめた。

結果と考察

①根呼吸と形態の関係

根直径が小さくなるほど根呼吸速度は高く、特に直径 0.5ｍｍ以下の根系における根呼吸量は、直径2㎜以下の細根の中でも非常に大きい値を示した（図 A)。また、SRL の値が大きくなるにつれて、呼吸量が大きくなるという正の相関が明らかとなった（図 B)。これらの結果は、根直径・SRL といった形態特性の違いが、細根系内の呼吸量変動の変動を規定していることを示している。さらに、これまでの根直径２㎜以下という便宜的なサイズによる細根の分類法ではなく、例えば、コナラ根では直径0.５㎜以下を境に根呼吸速度が高くなることから、根呼吸速度を基にした細根の新たな機能的な分類法を提案できることを示唆した。 (Makita et al. 2009 Tree Physiology)。

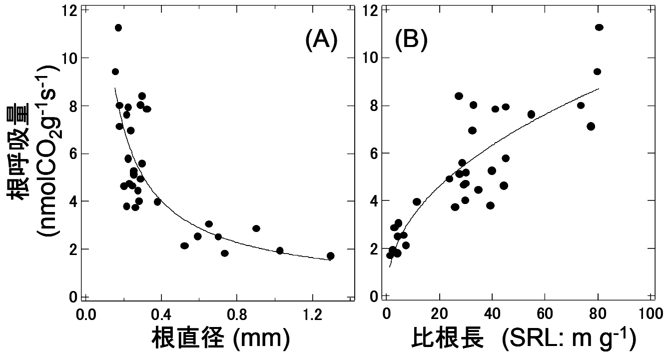

図　里山広葉樹林の山城試験地で採取されたコナラ細根系の根呼吸量と(A)根直径および(B) SRLの関係 (n = 32)

②根現存量・形態・生理機能の垂直分布

直径2㎜以下の根現存量では，直径階級の違いにより、土壌深度に対する減少割合が異なった。コナラとソヨゴにおける直径0.5 ㎜以下(微細根)の現存量は、それぞれ339.4、413.7 g m^{-2} であり、そのうち、およそ半分が表層に存在していた。さらに微細根の現存量は、表層から次層へと土壌が深くなるにつれて、他の直径階級の根と比べて、大幅に減少することが明らかとなった。

形態・生理特性の土壌深度に対する変化は、0.5㎜以上の根では確認されず、微細根のみで確認された。形態特性において、コナラ微細根のSRLは土壌深度が深くなるにつれて低くなり、一方、ソヨゴ根では土壌深度の違いによる変化はなかった。生理特性において、両樹種の微細根の窒素含有量は、土壌深度が深くなるにつれて低くなった。つまりコナラ微細根は、土壌深度ごとの栄養分布に対して、生理・形態的可塑性を発揮し、一方、ソヨゴ微細根は、生理的可塑性のみを発揮することが明らかとなった。微細根は、土壌資源をより効率的に獲得するために、形態や生理機能を変化させながら樹種特有の最適な戦略をとっていると示唆された(Makita et al. 2011 Ecological Research)。

樹木根に関する知見は未だに限られており、森林生態系の構造と機能および炭素循環を理解する上での大きな空白を生み出している。本研究の微細根の生理・形態特性およびその分布を考慮した評価は、これまで量的な研究が主流であった物質循環の研究と生理的機能の研究の架け橋となり、森林炭素動態の解明に重要な示唆を与えると考えられる。

最後になりましたが、推薦してくださった檀浦正子さん、および共同研究者・神戸大学森林資源学研究室・京都大学森林水文学研究室・森林総研関西支所のみなさんに深く感謝の意を申し上げます。

コムギ種子根の誘導的通気組織形成の形態・生化学的解析

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Morphological and Biochemical Analyses of Aerenchyma Formation in Seminal Roots of Wheat

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Introduction: Plants can adapt to waterlogging condition(s) through the multifaceted alterations in cellular and organ structure. One such response is the formation of aerenchyma in the root cortex, the tissue containing enlarged gas spaces, which thought to provide not only an internal pathway for oxygen diffusion, but also simultaneously reduces the number of oxygen consuming cells. The later feature is thought to assist in metabolic adjustment during waterlogging. Here we developed hypoxic pot-culture conditions, and used a combination of morphological and biochemical approaches to understand the mechanism of aerenchyma formation with a view to overcome wheat constraints in the waterlogged field in Japan.

Results:
1) We grew Bobwhite cv. SH 98 26 in conditions, a well-drained control, a water depth 15 cm below (T-15) and 3 cm above (T+3) the soil surface. The root growth was reduced in T+3 plants while the shoot growth did not change significantly during 72 h waterlogging. Root anatomy study showed that wheat formed no aerenchyma under our control condition, but formed aerenchyma initially at 2 to 5 cm behind the root tip after 72 h in T-15 and 48 h in T+3. The aerenchyma in T+3 plants then extended by an additional 5 cm towards root base during the next 24 h; they extended approximately 8 cm long in T-15 and 14 cm long in T+3 from 2 cm behind the root tip after 7d waterlogging. Evans blue staining indicated that wheat aerenchyma was lysigenous which resulted from degradation of cortical cells. We also studied aerenchyma formation in Japanese cultivars.

2) To check the biochemical responses of root to waterlogging we used lysigenous aerenchymatous seminal roots (discussed above). Roots of cv. Bobwhite were excised from T-15 and T+3 plants and subjected to proteomics. 2-D electrophoresis showed that 29 spots changed in the expression levels under waterlogging and 10 of them exhibited a reproducible up- or down regulated fluctuation. The up-regulated proteins were involved in alteration in energy and redox status, defense responses and cell wall turnover. Down-regulated proteins were related to the glycolysis and respiration. Multiple candidates for single spot were found by MASCOT search after mass spectrometry. Four candidate proteins were found for the highest up-regulated protein spot #5. Further confirmation and functional characterization of the candidates of spot #5 are now going.

Conclusion: Our results provide information on the variation in aerenchyma formation in non-Japanese cv. Bobwhite and Japanese wheat cultivars. Correlation data on aerenchyma % with root dry mass in Japanese cultivars implied that wheat aerenchyma might be immature or underdeveloped, i.e., wheat aerenchyma is not formed constitutively. We have not yet found a good germplasm for aerenchyma formation in *T. aestivum* and its relatives. In addition to the waterlogging responsive candidate genes from non-Japanese cv. Bobwhite, it may well be preferable to find the key gene(s) for aerenchyma formation in distant species with a high capacity to form well-developed aerenchyma (e.g., constitutive aerenchyma of rice) that can be introduced into a useful host wheat such as Bobwhite followed by gene introgression into elite Japanese cultivars by crossing.

Acknowledgement: This work was supported by the Program for the Bio-oriented Technology Research Advancement Institution (Promotion of Basic Research Activities for Innovative Biosciences, No. H20/seeds-01-01), Japan.

記念講演

農耕地の持続的生産機能と作物の根

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今から 20 年前の 1992 年 5 月 16 日土曜日に，第 1 回根研究集会が東京大学農学部で開催された．私の古い手帳には，13 時～20 時と記してある．当日は生憎の雨だった．懇親会では初代会長の森田さんとお話させて頂きとても緊張したのを覚えている．この年は作物学会と育種学会が松戸にある千葉大で連続して開催され，作物学会に来られていた小柳さんから研究集会へのお誘いを受けた．育種学会で発表した「線虫対抗植物ルドベキアの根系改良」のスライドがあり，根にも関係あるので発表させてもらうことにした．これが私のこの世界への第一歩であった．毛根病菌の *Agrobacterium rhizogenes*（当時の呼称）の Ri プラスミドが保有する *rol* 遺伝子をキク科のルドベキアに導入して，殺線虫物質の α-ターテニールを根で産生させて線虫防除に応用しようという発想であった．*A. rhizogenes* は千葉県のメロン圃場から分離し，国内野生株 A-5,A13 と名付けた．ルドベキアの形質転換体は，分枝根が多く根毛も旺盛に発生し，ターテニール生産量も増えた．一方，地上部の生育量は著しく少なく，S/R 比の重要性をあらためて感じた．ラッカセイでは，主根の表皮が剥離するので一般に根毛が生じないが，*rol* 遺伝子を導入すると旺盛な根毛発生が認められた．ラッカセイの細胞壁がもつリンの溶解機能との関係やクラッキング感染という独特の根粒菌感染過程にも興味をもって研究を継続している．

これらの研究で根と向かい合うことになったが，私の専門分野である作物生産科学という領域では，当然のことながら栽培という出口を意識して研究を進めなければならない．農耕地の地力維持，休耕地管理，水田転換畑での畑作物生産といった視点で，根の研究の成果を活かせればと思う．マメ科緑肥作物であるクロタラリアやセスバニア，被覆作物のヘアリーベッチ，水田転換畑に導入されるアズキやダイズといった作物の根を見ながらいつもそう思うのである．モンスーンアジアの特徴である雨期とその後の急激な乾燥は，この国で作物研究を進めていると必ず気になる問題である．二次通気組織形成と胚軸根形成のいずれで湛水に適応するかはセスバニアの種によって異なった．梅雨時に播種される大納言アズキでは大粒の品種ほど不定根の発生が多かった．大規模農地だけでなく小規模農地とりわけ中山間地の地力管理はこの国の重要な課題である．ヘアリーベッチの早期刈取りは地下部蓄積窒素を刈取り直後に土壌に放出し，休閑緑肥のイネ科作物の窒素栄養を補った．リン鉱石が自由に取引できなくなると農耕地のリンの再利用を考えなければならない．フィチンや難溶性無機リンの溶解能力をもつ根粒菌を見つけ，そのマメ科作物の根面での挙動とリンと窒素両者の供給力を評価したいと研究を進めている．過剰施肥やハウス土壌で集積した硝酸塩は湛水流去でなく，クリーニング作物で回収して有機物として再利用すべきである．ソルガムのようなイネ科も有用だが，根量が多く C/N 比が低いマメ科緑肥は，速効性有機物として野菜畑に組み込めると生産現場で試してもらっている．

雑多なことを並べたが，本講演ではその一部をご紹介させて頂く．低投入型の作物生産技術と農耕地の持続的管理技術の普及は，まさに「待ったなし」である．根の研究に頭か首か半身か全身かを突っ込み 20 年が経つがなかなか成果は出ない．このところ，土俵際に追い込まれることもしばしばだが，徳俵に足がかかってももう少し踏ん張ってみようと思っている．

The JSRR's 20th Anniversary Symposium

The Latest Frontiers of Root Research in Asia

Abstracts of Invited Lectures

根研究会創立 20 周年記念シンポジウム
「アジアの風－アジアにおける根研究の最前線－」
招待講演要旨


Akira. Yamauchi
(Moderator)


Jun Abe
(Lecturer)


Yowhan Son
(Lecturer)


Xiaojing Liu
(Lecturer)


Xiyin Zhang
(Lecturer)


Amelia Henry
(Lecturer)


Anan Polthanee
(Lecturer)


Roel Suralta
(Commentator)

The necessity of root research in Asia

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The plant root is the organ that develops in the soil to anchor the plant body and absorb water and nutrients. Roots are naked to the soil environments with being subjected to the ceaseless changes of biotic and abiotic conditions. Moreover, many of our managements, such as tillage, fertilizer application, and irrigation, approach plants through soil managements. In addition, the widely increasing consciousness towards environmental problems such as global warming, nitrogen oxide gas, and eutrophication enhances the necessity of studies on the root that is the interface between the plant and the earth and also between the plant and the man. There were not a few pioneers in root research in Asian countries who studied for example on rice, woody plants and desert plants. But the whole activity and international communication among root scientists in Asian countries have been limited in comparison with those in the West.

Asia includes wide and diverse areas that belong to different climatic divisions that ranges from arid lands to tropical rainforests. In plant production industries like agriculture, forestry, herbal plant production and greening, soil drought and accompanying soil hardness and nutrient deficiency are often big problems not only in arid areas but in monsoon areas that may have severe dry season and unstable precipitation in rainy season. In some of those areas, use of broad saline and alkali lands is also an important task, as fertile farming lands are decreasing due to modernization and human population is increasing in many of Asian countries. An agricultural specificity of Asia is that rice is the major staple diet, which is sensitive to drought stress. Improvement of rice production in rainfed lowand rice as well as in upland rice has been required for adequate food supply and increase of household income of small farmers. In rainfed lowand rice, the problem is difficult to solve, because the root system is exposed to both drought and water saturation irregularly and repeatedly. Studies on the tolerance of crop plants against drought are needed in the view of water saving, too. On the other hand, waterlogging and excessive soil water often disturb plant growth and decline yield and quality of agricultural products. They are rather complicated problem; not only the oxygen deficiency in soil but toxicity of chemical components caused by soil reduction and spread of soil pests also inhibit functions of roots. The problem of waterlogging and excessive soil water is serious particularly in East, Southeast, and South Asian countries. Even in dry season, excessive soil water problem occurs commonly in subsidiary upland crops cultivated in drained paddy fields. In Japan, as the government encourages the conversion of paddy fields to upland fields, scientists have struggled to improve the tolerance of wheat, soybean and maize against the excessive soil water, and been focusing root morphology and function. Studying plant anaerobiosis can also contribute to better understanding of important hydrophytes in Asia such as paddy rice and mangrove trees.

Forest and farm lands are important research object when we discuss the global environmental problems, relating to carbon and nitrogen cycles in the ecosystems. While European forestry scientists have developed good community via international activities like COST action, communication among Asian scientists seems still limited. In addition, communication between forestry and agricultural scientist should be important because Asia also holds large areas of paddy fields as the source of methane generation.

This symposium can be the start point of close communication among root scientists in Asia.

Root biomass and respiration for Korean forests

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Investigating the response of terrestrial ecosystems to global climate change has become a priority for the scientific communities. However, the accuracy of nutrient and carbon (C) dynamics for forest ecosystems has been limited by the difficulties in obtaining reliable estimates of belowground components compared with numerous estimates of aboveground components. Root biomass and its ratio to aboveground biomass for Korean forests were investigated using the national forest C inventory data in Korea. Also studies on fine root biomass, production, turnover rate and root respiration were reviewed. Average root biomass (Mg ha^{-1}) of 40 to 50-year-old broad-leaved and coniferous forests in Korea was 54.0 (24.1-135.8) and 43.6 (13.9-94.8), respectively. The average ratio of root to aboveground biomass for Korean forests (0.31±0.01 for broad-leaved forests and 0.28±0.03 for coniferous forests) was higher than those of other regions from the literature (0.25 for deciduous forests and 0.26 for coniferous forests) (Figure 1). Fine root ratio to aboveground biomass, production (kg ha^{-1} yr^{-1}) and turnover rates (yr^{-1}) for broad-leaved and coniferous forests in Korea were 0.016 and 0.017, and 2.58 and 2.53, and 0.73 and 1.33, respectively (Table 1). Based on a trenching study it was reported that root respiration accounted for approximately 31% and 34% of soil respiration for a *Quercus*-dominated forest and a *Abies holophylla* forest, respectively. The intact root respiration rate of a mature *Pinus densiflora* forest using the CO_2 sensors with a newly designed small root chamber was estimated to be 6.28 nmol CO_2 g^{-1} s^{-1}. More studies on investigating and quantifying forest belowground components in the East-Asian countries would be needed to understand the global terrestrial ecosystem C dynamics.

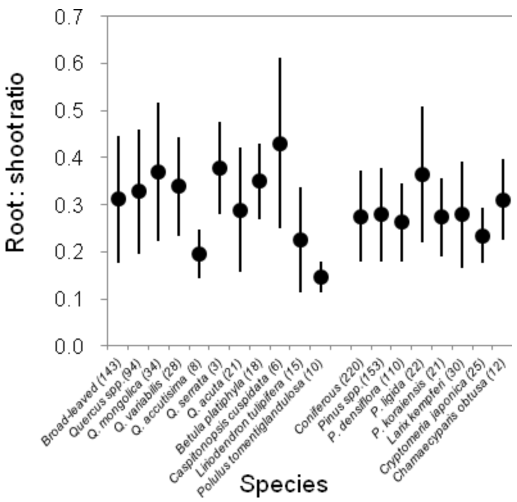

Figure 1. Root:shoot ratio of major tree species in Korea. Vertical bars indicate the standard deviations of the means and numbers in parenthesis indicate the numbers of study sites.

Table 1. Above and belowground biomass (Mg ha^{-1}), fine root biomass (Mg ha^{-1}), fine root ratio to above- and belowground biomass, fine root production (kg ha^{-1} yr^{-1}), and turnover rate (yr^{-1}) for broad-leaved and coniferous forests in Korea.

	Broad-leaved	Coniferous
Mean age (yr)	40 (39-42)	45 (36-75)
Aboveground biomass	135.1 (35.7)	127.7 (8.9)
Belowground biomass	43.0 (2.3)	32.6 (3.9)
Fine root	2.0 (0.3)	2.1 (0.3)
Fine root: aboveground	0.016 (0.002)	0.017 (0.003)
Fine root: belowground	0.048 (0.007)	0.067 (0.012)
Fine root production	2.577 (0.352)	2.529 (0.602)
Turnover rate	0.730 (0.150)	1.325 (0.334)
N	3	5
References	Son and Hwang (2003) Park et al. (2004, 2010) Noh et al. (2011)	

A primary study on the adapting mechanisms of cotton roots to saline soils

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Cotton is one of the most salt tolerant crops and growing large area in saline soils in the world. The mechanisms of salt tolerance in cotton plant have been studies extensively in physiological and molecular aspects. However, less study has being conducted on the mechanisms of salt tolerance in cotton roots despite the inhibitory effects of salinity on root length and the number of lateral roots. Based on our field investigation, we found that in coastal saline soil land, when surface soil salt leached to deeper soil layer by irrigation, the cotton can be grown and cotton roots are mainly distributed in desalinized soil layer. This indicates that cotton roots may have some mechanisms to adapt to non-uniform saline conditions. Here we show that non-uniform salt stress modulates root growth orientation by reducing the gravity response.

We design a double-layer Murashige and Skoog (MS) medium experiment in which extreme high salt (400mM NaCl) MS was on the bottom and plain MS medium on the top. The roots of cotton seedlings penetrated the interface of the layers and grew straight downwards when both layers were plain MS media. In contrast, changes in the root growth orientation of the seedlings were observed in response to salt stress from the bottom medium. The primary root did not pass the interface between normal MS and salt medium, and they grew along the interface between two medium.

To better understand this tropistic response of cotton roots to salt stress, a time course amyloplasts in columella cells of primary roots were visualized by staining with iodine-potassium iodide solution. We found that the amount and distribution of amyloplasts in columella cells of roots was altered when root grow along the interface, but not degradation completely.

Abscisic acid (ABA), as a critical regulator in gravitropism growth of root, increased markedly about 3 times of control plants at the early stage of root cap reaching to the interface, and then, it severely decreased. Abscisic acid concentration in root tap was recovered near to normal level after root grew along with interface between two layers of MS medium without regard to gravity. The changes of reactive oxygen species (ROS) were similar as ABA. The result suggested that salt degree modulates cotton root system architecture, and the amyloplasts in columella cells, ABA and ROS may play critical role on the mechanism of salt avoidance in cotton roots.

Long-term monitoring root growth and soil water uptake by winter wheat for efficient water use in the North China Plain

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Intensive irrigated cropping systems in the North China Plain (NCP) resulted in not only depletion of groundwater resources, but also nitrogen leaching. Efficient management of water in crop root-zone profile is important for reducing irrigation water use as well as nitrogen leaching. This study was undertaken to investigate root growth and distribution, root water uptake by winter wheat under different irrigation treatments, and possible ways to improve soil water use efficiency from 1990 to the present. Root sampling results showed that winter wheat had a prolific root system with an average depth of 2 m. Most of the root system was concentrated in the upper 40 cm of soil. Root length density (RLD) in the top layer of soil (0-20 cm) was very high with values around 3 to 5 cm/cm^3. The distribution of water uptake from the soil profile under good soil moisture conditions was closely related to the distribution of RLD. When RLD was less than 0.8 cm/cm^3, the root was the main factor limiting the complete utilization of soil water by crops. At maturity, over 100 mm of available water remained in the root-zone for the rain-fed treatment, although the upper layers had already entered water deficit, since the scarcity of roots in the deep soil layers restricted the full utilization of soil water. For irrigated wheat, from 40% to 50% of crop water use was from the stored soil water, and for the rain-fed wheat up to 80% of the water use was from the stored soil water in a dry season. Available stored soil water played an important role in the higher production of wheat crops in the NCP.

The results also showed that the yield and evapotranspiration (ET) of winter wheat gradually increased from 1990 to the present. There was no consistent change in total root length (TRL) over time. The difference in root size among seasons and irrigation treatments mainly occurred in the upper soil profile, where RLD was greater. No direct relationship was found between root size and soil water use. Thus, TRL was not a factor that indicated the water extracting capacity of crops. rather, the distribution of RLD along the soil profile plays more important role in soil water utilization. Smaller root size in the upper soil layer did not affect soil water uptake, and might be more economical in terms of production efficiency.

Effective measures to increase the utilization of stored soil water could improve crop performance under conditions of limited water supply in the NCP. Recent measurements showed that the bulky density was increased under the tillage layer and soil pan is becoming thick and moving upwards with the application of minimum tillage practices in the NCP. The increase both in bulk density and thickness of the soil pan significantly affected the distribution of RLD, resulting in greater RLD in the tillage layer and smaller RLD in deep soil profile. Results showed that changing cultivation practices were possible ways to break the soil pan to improve root growth in the deeper soil layers that will benefit soil water use.

IRRI's drought stress research in rice with emphasis on roots: Accomplishments over the last 50 years

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A look back over IRRI's 50-year history reveals that early research insights were key for our current progress in root research for drought. Early recognition of the importance of roots for drought resistance, and the characterization of diversity in rice root architecture, provided a strong foundation for drought research at IRRI. After direct selection for yield during the past decade that is now approaching impact at the farm level, we are seeing that root traits are indeed involved in improved yield under drought. The first mention of drought in the IRRI Annual Reports was in 1970, and by the mid 1970s large efforts were going into research on root growth in response to drought. Deep root growth and formation of large-diameter nodal roots was emphasized. Attempts to identify novel indirect selection methods for deep root growth were initiated, including relationships between plant height and root depth, and evaluation of root pulling force. In the 1980s, an emphasis was on aeroponic studies for root morphology and anatomy, and line-source sprinkler studies were commonly conducted in the field. The use of crosses to better understand the genetics of root traits was initiated in the 1980s, and further efforts towards characterization of the genetics behind root traits were conducted in the 1990s, specifically the use of molecular markers to select for root trait QTLs. A shift towards rainfed lowland systems in addition to upland studies was initiated in the 1990s, with increased recognition of the different types of drought stress, GxE analysis, and characterization of water uptake by roots. In the 2000s, drought breeding efforts moved from selection of root traits to direct selection for yield under drought. Physiological studies on root function were strengthened, including experiments on root hydraulic conductivity and xylem vessel cavitation. Today, the links between genetics and physiology for roots and drought continue to move forward: we have identified traits associated with two major drought-yield QTLs to be root traits, and we are characterizing association panels that have been mapped/sequenced in order to pinpoint genes associated with specific root traits and functional parameters. Detailed characterization of the target drought-prone environments is the current root/drought research need at IRRI, in order to link drought-resistance traits and genotypes with the most appropriate types of drought stress occurring in farmers' fields.

Agricultural problems by waterlogging in Northeast Thailand

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Although the Northeast region is known to have prevailing drought, waterlogging also has serious economic consequences for productivity of much arable farmland. Severity and frequency of waterlogging in Northeast Thailand vary over areas, within a year and between years. However, its potential has been projected to increase in the future. Considerable transient and more persistent waterlogging of the soil and deeper submergence of crops could occur not only in irrigated farmlands but also rainfed areas due to intensity of rainfall, undulating land pattern and change in land use. A short-term waterlogging for a few days may retard plant growth. Prolong waterlogging for 2-3 months due to flash flood, overflow of water from the rivers or extended wet season could result in submergence of the whole plant or some parts of it, which will severe increase in yield loss of crops. In 2010, paddy field, occupied 66% the arable land of the region, is most affected by flooding resulting in about US$ 540 million loss. Impact of waterlogging on crop growth and yield depend on crop species, cultivar, duration of waterlogging and growth stage of plant at which waterlogging occurs. Waterlogging initiated at early vegetative growth stage and long-term waterlogging duration severely reduced growth and yield of fiber crop, castorbean, and sweet sorghum Long-term waterlogging for thirty days at 9.5 months old sugarcane immediately reduced tiller number, youngest leaf expansion rate, leaf number per plant and stem diameter as compared to the control. However, stalk yield and yield-related traits were not affected by waterlogging at harvest (12 months). Roots developed under soil surface die under waterlogging conditions and adventitious roots were developed in water above ground level to replace functions of the damaged roots. After water was drained, suppression of root growth was persisted, particularly when waterlogging was occurred at early vegetative stage. Aerenchyma tissues were formed in cortex layers of the newly developed roots of investigated upland crops, fiber crop, sweet sorghum and sugarcane. Among studied fiber crops, kenaf is high waterlogging tolerant species with its ability to form casparian strip in endodermis and exodermis cells both under control and waterlogging conditions. In terms of sweet sorghum and sugarcane, tolerance to waterlogging may be due to the interconnection of gas spaces like of roots below soil, roots in water, belowground stem and stalk basal above water level.

Importance of rice root research in Asian situations: A commentary

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World crop production must be increased to keep up with the demand of ever increasing population. Asia accounts for more than half of the world's population but the sustainability of its crop production is affected by water scarcity problem brought about by climate change and industrialization. Water stress is associated with salinity ingress, drought and nutrient deficiency (or toxicity) which negatively affects crop production. In rainfed agriculture such as in rainfed lowland rice, fluctuating soil moisture stress is also a problem due to severe waterlogging brought about by heavy rainfall in between periods of drought. A need to increase crop productivity amid water scarcity to sustain crop production is inevitable.

Roots being the plant part directly in contact with the soil, indeed play key roles for crop adaptation under stress to maintain crop productivity. Thus, root research is an important component for improving crop productivity under water stress-prone environments. It is very timely that the issue on the necessity of root research in Asia has been raised. Each Asian country may have unique adverse soil problems affecting crop production but common points for establishing and/or strengthening root research collaborations among countries is crucial. This could maximize sharing of expertise, experience, and resources which will expectedly improve the efficiency of conducting root research.

Rice being one of the common crops grown among Asian countries may serve as the common platform for creating root research collaborations for identifying the ideal root ideotype specifically tailored under various stress prone rice environments. The ideotype of the root system is the key for stable/increased rice production under such environments. A typical example of root research collaboration is the ongoing joint research done by Nagoya University, PhilRice, University of Tokyo, IRRI and JIRCAS. Since root ideotype is dynamic, detailed characterization of each target rainfed lowland sites is a must to identify the ideal root ideotype required for each site. Once detailed characterization has been done, it is only then that the G (or QTL) x E studies will become more relevant and substantial. This type of research would be more efficient when expanded and conducted by different networks of researchers from different ecosystems in Asia, who share common understanding and interests on this concept. This type of root research collaborations among Asian countries can be also replicated to other soil stress factors and crops.

Poster presentations (Titles and presenters)
ポスター発表　表題・発表者一覧

第 35 回根研究集会 35th JSRR Biannual Meeting

20th Anniversary Symposium (The Latest Frontiers of Root Research in Asia)

P01　ポスター発表　要旨

2 層栽培・根系分割灌水による高糖度トマトの栽培法の開発（第 2 報）上下層の土壌容積の違いがトマトの生育に及ぼす影響

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高糖度トマトは、根域制限下での少量灌水や高濃度養液を与え、根系に水分ストレスを付加させる栽培法によって経済生産されている。地床栽培では、トマトは上層土が乾燥すると下層根に吸水を依存するが、下層根が少ない条件では長期間水分ストレスが付加され糖度が高まることがある。そこで、毛管水の影響の少ない上下 2 層の容器を用い、上下層の土壌容積を変えた条件でトマトを栽培して、上層土の乾燥後に付加される水分ストレス強度や果実の糖度、1 果重への影響を検討した。

試験は、2008 年に農業試験場内のガラス温室で実施した。栽培は前報（引用文献）と同一の上下 2 層のアルミ製容器を用い、3 月 6 日に中玉トマト品種‘ルイ 60’を定植し、5 段果房上の本葉 2 枚を残して主枝を摘心した。試験区は、上下層の土壌容積によって (1) 上層 1 区（上層 19L：下層 38L）、(2) 上層 2 区（上層 38L：下層 19 L）、(3) 対照区（上層 38 L：下層 19 L）、の 3 処理を設け、4 反復で実施した。灌水は、定植後から 4 月 8 日までは上層容器に適宜灌水し、4 月 9 日以降は点滴チューブ（Stream Line、Netafim 社）により上下層の各容器に 1 日 2 L を灌水した。4 月 22 日以降、対照区を除く区で上層容器の灌水を停止し乾燥処理を開始した。葉柄水ポテンシャルは、プレッシャーチャンバー法により 3 段果房下の側枝の小葉 2 枚で計測した。果実調査は、5 段果房までの全ての良果で、果実糖度と 1 果重について測定した。

葉柄水ポテンシャルは、対照区では調査期間を通して－1.1 MPa から－0.9 MPa まで緩やかに上昇した。上層 1 区では乾燥処理直後に－1.4 MPa まで低下した後－1.2 MPa まで上昇し、下層根からの吸水が増えることにより水分ストレスが緩和されたとみられた。上層 2 区では、対照区と同程度の－1.2 MPa から－1.6 MPa まで低下し、水分ストレスが長期間付加されていることが示された。

果実糖度は、上層 1 区では 2 段果房果実から糖度が 9 度(Brix%)を超えたが、4 段から 5 段果房果実の糖度は対照区と有意な差がみられず、平均糖度は対照区と同等であった。上層 2 区では、2 段から 5 段果房果実の糖度が対照区より高く、平均糖度は対照区より高い 9.3 度であった。 1 果重は、上層 1 区では、2 段から 4 段果房で対照区に比べて小さく、上層 2 区では 2 段から 5 段果房で対照区に比べ小さかった。平均 1 果重は、いずれの区でも対照区に比べて小さかった(表 1)。

前報の結果とあわせて葉柄水ポテンシャルと果実糖度及び 1 果重の関係を検討した。糖度 8 度となる葉柄水ポテンシャルは－1.1 MPa に相当し、1 果重が 40g となる葉柄水ポテンシャルは、－1.0MPa に相当した。葉柄水ポテンシャルが－1.3 MPa 以下では、果実糖度と 1 果重の変化は認められなかった。

これらの結果から、2 層容器を用い根系を分割し灌水管理したトマトでは、上層根が乾燥処理された直後は下層根からの吸水が少なく、地上部の水ポテンシャルが急激に低下して果実糖度が高まるとみられた。しかし、下層の根域が大きい条件では、下層の根量の増加により再び水分ストレスが緩和されることから、高糖度トマトの生産には下層の土壌容積を上層の 1/2 以下として水分ストレスを持続させる必要があることが示唆された。また、高糖度果実の生産に適する水分ストレスは、乾燥処理後の葉柄水ポテンシャルを指標とすることができ、適当な水ポテンシャルは－1.0 MPa から－1.3MPa の範囲にあると推定された。

表1　土壌容積の異なる2層容器で栽培したトマトの果実糖度と1果重

区 名	1段 1果重 (g)	1段 糖度 (Brix%)	2段 1果重 (g)	2段 糖度 (Brix%)	3段 1果重 (g)	3段 糖度 (Brix%)	4段 1果重 (g)	4段 糖度 (Brix%)	5段 1果重 (g)	5段 糖度 (Brix%)	平均 1果重 (g)	平均 糖度 (Brix%)
上 層 1	38.8a	8.3a	29.0a	9.1a	27.8ab	9.2a	23.2a	9.3ab	28.7ab	9.0ab	29.9a	9.0ab
上 層 2	41.9a	8.2a	30.3a	9.1a	25.3a	9.4a	23.9a	10.1a	22.1a	10.0a	29.5a	9.3a
対　照	44.3a	7.8a	36.7a	7.9b	34.7b	7.9b	40.6b	7.9b	44.5b	7.4b	39.9b	7.8b

注）同一英小文字を付した区間には、5%水準でTurkey検定による有意差がないことを示す。

（引用文献）
林浩之・小川敦史・豊福恭子・田口多喜子・高橋善則 (2010)　第 33 回根研究集会研究発表要旨集

P02　ポスター発表　要旨

根径からみたバスケット法によるテンサイ品種根系の簡易判別法について

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1.はじめに

網走市における土壌タイプ間の収量格差を解決するために筆者ら（2011）は小柳ら(2001）のバスケット（ザル）法を応用したテンサイ根系分布の初期判別の可能性について検討したところ，伊藤ら(2008）によって根系分布の差が明らかにされている「アセンド」(浅根型),「カブトマル」(深根型）および「スタウト」(中間型）を用いた実験にて，根数および発根角度に明確な差を認めている．しかしながら，他の遺伝資源を評価してみると，ここまでの検定では圃場での現象と適合しない場面も観察された．一方，吉田ら（2011）は，田中ら(1994)や有馬ら(1995)が提案したルートモデルを用いて土壌タイプ間での根系発達を「アセンド」を用いて解析したところ，淡色黒ボク土では生育の後期になると根系の骨格である 1 次側根や 2 次側根の発達が停止し，養水分吸収を行う 3 次側根の生育が進むのに対し，土壌物理性に劣る褐色森林土では生育後期になっても 1 次側根は発達を続け，2 次側根や 3 次側根の親根として機能していたことを観察しており，土壌物理性に応じた太さの根を発達させていることを明らかにした．この結果は根系の初期判別には根径の発達も加味することで，より正確に判別できる可能性も示しており，本報告では先の 3 品種を用いて，根径も観察し，バスケット法による簡易判別について再検討した.

2.材料および方法

実験は 2011 年に研究会で報告した方法に準じて行った．供試材料には「アセンド」「スタウト」および「カブトマル」のそれぞれ 7 個体を用いた．根は催芽日を 0 日として 30 日間，毎日の 10 時にザルの表面から 5 ㎜以上突き抜けた根をハサミで切り取り保管し，またザル上表面中央から切り取った根とのなす角度を記載した．根径は 30 日間に採取した「アセンド」66 本,「カブトマル」129 本,「スタウト」99 本について解析した．ルートスキャナー（EPSON-Perfection49902.68）と解析ソフト(win, RHIZO）により最大根径を計測し，その根の根径とした．そして根径の仮想円盤を算出してルートマップを作成した.

3.結果および考察

催芽後 30 日目のルートモデルをみると,「カブトマル」では太い根から細い根まで確認されたのに対し,「アセンド」では主に細い根のみ,「スタウト」では両品種の中間の傾向が認められた．次数が異なるとみられる側根数は品種で異なり，3 つの「カブトマル」が最も多く，次いで「スタウト」が 2 つ，最も少ない「アセンド」が 1 つであった．本研究では，このルートモデルを詳細にみて「カブトマル」は根径の太い方の 9 ㎜のピークを 1 次側根，0.7 ㎜のピークを 2 次側根，以下 3 次側根として,「スタウト」は 0.8 ㎜を 1 次側根，0.7 ㎜を 2 次側根，以下を 3 次側根として，ピークの判別が困難であった「アセンド」では最大根径の 0.6 ㎜を 1 次側根とし，以下を 2～3 側根とした．さらにザルの表面から突き抜けた角度によって垂直根(90～70°）と水平根(70°～)に区別した．10 日ごとに作成したルートモデルから，その成長量を算出したところ,「カブトマル」と「スタウト」では 1 次側根の成長量の増加パターンに違いがあった．すなわち,「カブトマル」では 0～10 日目の垂直根に限って成長した．「スタウト」の垂直根では 10～20 日目に，水平根でも 20～30 日目に成長が認められた．2 次および 3 次側根の成長では両品種間に大きな違いが認められなかった．アセンド」の 1 次側根の成長は垂直根をみると，10～20 日目にかけてやや増加し，20 日目以降に大きく増加した．2 次側根の垂直根では 10～20 日目に大きく増加し，20 日目以降には頭打ちとなった．水平根では 20 日目以降に大きく増加した.

以上の結果から，深根型の品種は生育の早い段階から 1～3 次側根まで並行して成長するのに対して，浅根型の品種は 1 次側根を十分に成長させてから 2 次側根,3 次側根と順次成長していくものと推察された．また，実験によって根径に品種間差が認められたことから，根系の簡易判定法に根径の測定の必要性が示された.

P03　ポスター発表　要旨

ジベレリンによるシロイヌナズナの根における鉄吸収関連遺伝子の発現誘導

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植物の微量栄養素の一つとして鉄が存在する。鉄は植物においてクロロフィルの生合成やシトクロムなどの補因子として必要であり、鉄が欠乏すると葉に白化が生じることが知られている。土壌中の鉄は主に三価鉄の状態で存在しており、水への溶解度が低いために獲得することが困難である。これに対して植物は土壌からの鉄の吸収の仕組みを備えている。双子葉植物においては、土壌中に存在する三価鉄-キレート複合体を細胞膜上の三価鉄還元酵素(FRO2)により二価鉄に還元し、根の表皮に局在する二価鉄トランスポーター(IRT1)により植物内へと鉄を取り込んでいる。*FRO2* と *IRT1* の発現は根における短距離シグナルと地上部の鉄含量によって制御される長距離シグナルの両方により調節されている。シグナルの候補として植物ホルモンが考えられ、オーキシンやエチレン、サイトカイニンなどの様々な植物ホルモンが *IRT1* と *FRO2* の発現を調節していることが報告されている。

我々は植物ホルモンの一つであるジベレリン(GA)に着目している。GA は発芽や細胞の分裂や促進、花芽形成の促進に関わっているが、GA による IRT1 と FRO2 の発現への調節作用についてはまだ明らかにされていない。MS 培地で生育したシロイヌナズナの GA 欠損体(*ga3ox1 ga3ox2*)の地上部に活性型の GA である GA_4 を与えて 3 日後の根における *IRT1* と *FRO2* の遺伝子の発現量を RT-PCR で調べた結果、GA 投与により顕著に増加することが示された。また、IRT1 と FRO2 の発現調節は鉄欠乏条件における応答の研究から bHLH 型の転写因子により直接制御されることがわかっている。これらの転写因子の発現についても GA 投与による誘導を調べてみたところ IRT1、FRO2 と同様に促進されることがわかった。このことから GA 投与も鉄欠乏と同様に bHLH 型の転写因子を介して IRT1、FRO2 の発現誘導に関わっていると思われる。さらに、遺伝子発現の誘導だけではなく酵素活性においても上昇が見られるのかどうかを調べるために、三価鉄還元活性と鉄の蓄積量の解析を試みている。GA が鉄欠乏のシグナルとして bHLH 型転写因子の上流で機能しているかを明らかにするために、鉄欠乏条件下での野生型(WT)と GA 欠損体において、IRT1 と FRO2 の発現の誘導に違いがあるのかどうか検証する。また、鉄欠乏応答の研究から示されているシグナル伝達における一酸化窒素(NO)の関与が地上部 GA 処理によっても見られるのかを調査することで GA の作用が既知のシグナル経路を介しているのかどうかを考察する。

P04　ポスター発表　要旨

浸透圧ストレス環境下におけるイネ科作物の水利用機能研究

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近年、イオン毒性回避機構に関与するイオン輸送体等の分子機構が明らかになってきているが、それに比べて浸透圧ストレスに際して根から脱水が起こる分子メカニズムは、大部分が未知である。細胞内外への水輸送は主に水チャネルであるアクアポリンによって行われている。細胞内への水輸送においてはPIP（Plasma membrane Intrinsic Protein）が主要な役割を果たしていることがこれまでの研究で分かっているが、水ストレス時の脱水防御機構を司る分子種の同定やメカニズムの解明には至っていない。本研究では、浸透圧ストレス環境下におけるオオムギ（*Hordeum vulgure*）、イネ（*Oryza sativa*）の根の水輸送に関して分子生物学的、植物生理学的観点から研究し、水ストレス時の脱水防御メカニズムを総合的に解明することを目的としている。

播種後 4 日目のオオムギ（はるな二条）を基準とし、マンニトール 0mM、180mM、360mM の下、24 時間後の root、shoot の成長を測定した。180mM では 0mM と比べて若干 shoot の成長度が下がったが、ほとんど変化がなかった。一方、360mM では、0mM と比べて shoot で 85.3%、root で 45.0%の成長度の減少が見られた。

同じ濃度の浸透圧環境下で根におけるオオムギ *PIP* 遺伝子の転写制御について調査した。発芽後 4 日齢のオオムギの根においては *HvPIP1;3* の転写レベルが最も多く、*HvPIP2;3* の転写レベルが最も低かった。浸透圧ストレスとして 180mM マンニトールを与えた場合、いずれの *PIP* 遺伝子の転写レベルにもコントロール（0mM マンニトール）と比較して顕著な差は見られなかった。一方 360mM マンニトールを与えた場合、*HvPIP1;2*、*HvPIP1;4*、*HvPIP2;1*、*HvPIP2;2* の転写レベルが処理後 2 時間以降で減少した。これは高濃度の浸透圧ストレスに対し原形質膜における水の透過性を減少させ、細胞外への脱水を防ぐため発現が抑制されると考えられる。塩ストレス下でもほぼ同様のアクアポリンの発現量が大幅に減少することがすでに分かっており、つまりは、塩ストレスによる *HvPIP* の減少は塩によるイオンストレスの影響ではなく浸透圧ストレスによるものであることが分かった。

一方、根水透過性（Lp_r）においては浸透圧ストレスによる著しい Lp_r の抑制が見られタンパク質リン酸化、細胞内輸送等による制御であることが当研究室で明らかにされてきている。

今後は強時ストレス時における発現制御が脱水回避にどのような影響をもたらすのかを解明することが今後の課題となっている。

イネにおいても明暗条件下の根水透過性（Lp_r）を測定した。明条件下、暗条件下開始 2 時間目において Lp_r が高くなった。180mM マンニトールによる浸透圧ストレスを付与したところ一時的にわずかな Lp_r 抑制が見られたもののオオムギ程の著しい根水透過性の制御は見られなかった。イネはオオムギのように水ストレスに強くなく、Lp_r の制御による脱水防御応答はほとんどないことが示唆される。

上記のように根水透過性は浸透圧ストレスだけでなく光によって大きく変動する。故に現在は恒明条件下においても調査中である。今後は、水ストレス時 *OsPIP2* 群の発現が制御されるように設計された RNAi 系統を用いて水透過性の変動を観察する予定である。

P05 ポスター発表 要旨

World Rice Collection を用いた塩ストレス条件下での初期生育反応の品種間差異

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［背景］世界の開発途上地域では干ばつや塩害等により，毎年イネの栽培面積の約7,500万haに相当する作物の生育がきわめて不安定である (飯山，2008). 塩類集積の影響を受けた耕地は，最大で毎年200万haずつ増加していると推定もされており，これによる生産量の減少は灌漑施設の整備による生産量増加に匹敵すると報告されている (Postel，1999). 本研究では，世界各地のイネの対立遺伝子の多様性を90%カバーするWorld Rice Collectionを用いて塩ストレス条件下での地上部･根の初期生育反応を調査し，品種間差異を明らかにすることを目的とした.

［材料と方法］供試材料として，World Rice Collectionのうち48品種，および他の３品種 (Azucena, Dular, Akita-komachi)を用いた．次亜塩素酸で殺菌処理した種子を暗黒条件下で３日間催芽した後，1/2 Hoagland水耕液の入った1Lビーカー上のネットに移植し，人工気象器内 (28℃，湿度70%，日長12時間)で栽培した．移植７日後に，水耕液の濃度が 50 mMになるよう塩化ナトリウムを添加し，塩ストレス処理を行った (以下，Stress区)．対照として塩ストレス無処理区を設けた (以下，Ctrl区)．移植14日後にサンプリングし，地上部および根乾物重，根の活性の指標であるα-ナフチルアミン酸化活性，根数，総根長，根の表面積および体積を測定した．

［結果と考察］塩ストレス条件での地上部乾物重は，Ctrl区とStress区を比較した相対値 (Stress/Ctrl)が１以上の塩ストレス耐性を示す品種が９品種あった．一方，相対値が0.4以下の塩ストレス感受性を示す品種が６品種あった．根乾物重は，４品種で相対値が１以上であった．一方，根乾物重の相対値が0.4以下であった品種が６品種あった．また，供試した全品種の地上部乾物重と根乾物重および総根長の間では５%水準，根の表面積の間では１％水準で有意な相関を示した．一方，地上部乾物重とα-ナフチルアミン酸化活性の間では，有意な相関は示さなかった．これらの結果から塩ストレス条件下では地上部の生育と根の生育の間で有意な相関を示し，根の生育が維持される品種が地上部の生育も維持される品種であることが明らかになった．

［引用文献］

1. 飯山賢治 (2008) 国際貢献のための農林水産技術開発，第８回農林水産技術会議資料.
2. Sandra Postel (1999) Pillar of Sand: Can the Irrigation Miracle Last? Worldwatch Environmental Alert Series，W W Norton & Co Inc.

Periderm formation in roots of monocotyledonous medicinal plant *Merwilla plumbea* (Lindl.) Speta. under stress conditions

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RESULTS

In the present contribution two types of cultivation of *Merwilla* plants, highly traded medicinal plant in South Africa, were tested for the evaluation of uptake and translocation of Cd. Anatomical study was performed to investigate the possible effect of Cd treatment on the root structure.

It was found that hydroponical cultivation is not convenient for this species. The growth of plants cultivated in Perlite in control conditions was adequate and after Cd application the growth of *M. plumbea* plants was retarded. Roots accumulated considerably higher amounts of Cd when compared with the bulbs and leaves. Compared to control plants, the concentration of Cd in the roots treated with 1 mg Cd L-1 was significantly higher and increased when higher Cd stress (5 mg Cd L-1) was applied.The adventitious roots of *M. plumbea* seedlings show the typical structure of a monocotyledonous root when grown in control conditions or in nature. The oldest part of *M. plumbea* root, close to the bulb, is characterized by the presence of a contractile zone.

Cadmium application at higher concentration (5 mg Cd L-1) induced the formation of cork cambium in the periphery of cortical tissues, adjacent to the exodermis. Cells divided periclinally from approx. 5 mm from the root apex and the derivatives impregnated their cell walls with suberin. Periderm is only rarely developed in roots of monocotyledonous plants. This is the first observation of periderm formation in monocotyledonous species in reaction to abiotic stress induced by toxic metals.

ACKNOWLEDGEMENTS

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REFERENCE

Lux A, Vaculík M, Martinka M, Lišková D, Kulkarni MG, Stirk WA, Van Staden J. 2011. Cadmium induces hypodermal periderm formation in the roots of the monocotyledonous medicinal plant *Merwilla plumbea* (Lindl.) Speta. Annals of Botany 107: 285–292.

Field investigation of root and ground conditions for native licorice at Mongolia

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Desertification which is caused by climate change, over fishing, and overgrazing is remarkable recently. Regarding with greening, this research paid attention to the medicinal plant licorice (Glycyrrhiza uralensis:Kanzo). Licorice lives wildly in semi-arid lands especially located in China and Mongolia, and its root which contains active ingredient glycyrrhizin (GC) is highly demanded as herbal medicine. However, amount of licorice decreases recently due to over fishing and overgrazing and desertification.
To grasp the environment of semi-arid land where licorice lives, root and ground conditions were investigated in Mongolia in May and September 2010.
Distributions of root in the ground and root nodule of licorice are confirmed.

Photo 1 Excavation of ground at semi-arid land in Mongolia

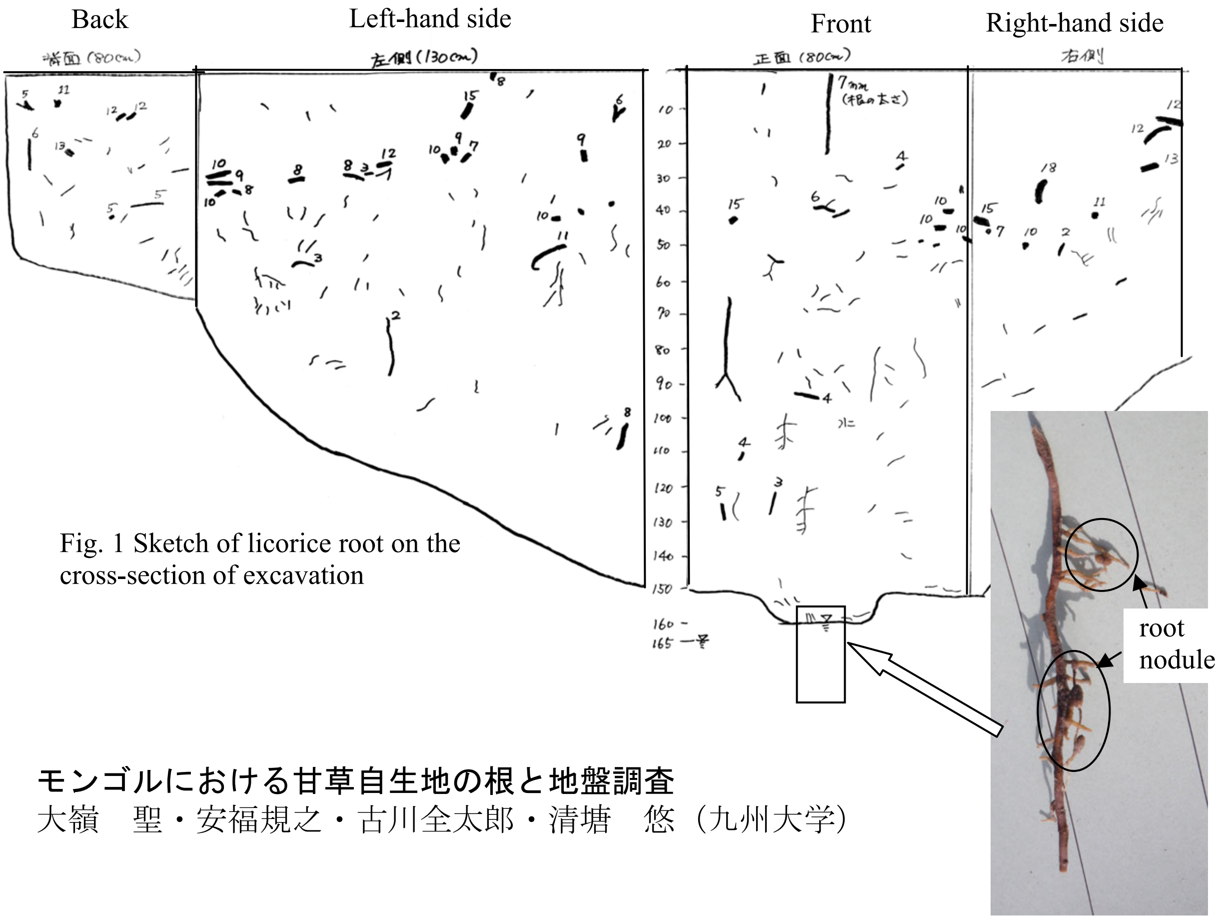

Fig. 1 Sketch of licorice root on the cross-section of excavation

モンゴルにおける甘草自生地の根と地盤調査
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Seasonal changes of starch grain accumulation in roots of *Erianthus*

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Erianthus species, perennial C4 grasses, perform huge biomass in abandoned lands and high tolerance to environmental stresses. Therefore, *Erianthus* may serve as a cellulosic biomass crop for bioethanol production. So far, we noted that *Erianthus* roots have tolerance to environmental stresses (Shiotsu et al. 2010b, Shiotsu et al. 2011), and found that the nodal root stele deposited starch densely (Shiotsu et al. 2010a).
In preparation for commercialization, we must establish a low input sustainable cultivating system of *Erianthus* to supply its biomass as stable fuel resources all through the year. For this purpose, we need to examine the planting or cutting time of *Erianthus*, which could be related with the accumulation and translocation of carbohydrate within the *Erianthus* plants. The aim of this study is to show the seasonal changes of starch grain accumulation in roots of *Erianthus* compared with napier grass and sugarcane. In addition, we investigated the relationship between cutting time and regrowth of the aboveground parts, and discuss results in relation to the starch accumulation in roots.
Material and Methods The field experiment was conducted at the Institute for Sustainable Agro-ecosystem Services of The University of Tokyo. After applying the chemical fertilizer, stocks of the *Erianthus*, napier grass, sugarcane were transplanted in a field on May 31, 2010. The three crop plants with basal part of roots were harvested using a shovel at 42 (July 12), 126 (October 4), 158 (November 5) and 187 (December 24) days after planting (DAP). The nodal roots were randomly chosen from three plants of each species for anatomical observation and their hand cross sections at ca. 5 cm from the base were observed using a light microscope. The nodal roots were stained with potassium iodide solutions to detect starch.
Results and Discussion The accumulation of starch grains was observed in the stele of *Erianthus* roots but varied with the seasons. Meanwhile no or limited starch grains were observed at 42 and 126 DAP, the deposited starch grains increased remarkably toward winter season; dense accumulation of starch grains was observed in all the examined roots at 158 and 187 DAP. Napiergrass also accumulated starch grains in root stele but not as much as *Erianthus*. Sugarcane did not accumulate starch grains at any stage of the growth.
In another field experiment, in which *Erianthus* plants were harvested in either of August, October, December or February, the plants once harvested in October resulted in the poorest regrowth in the second year. Because, *Erianthus* plants reserve carbohydrate into the roots in October, the cutting of leaves (i.e., photosynthetic organ) in October could decline the regrowth in spring season due to the shortage of starch stock in roots.

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Reference Shiotsu et al. 2010a. Jpn. J. Crop Sci. 79 (Extra 1):152-153.; Shiotsu et al. 2010b. Jpn. J. Crop Sci. 79 (Extra 2):212-213.; Shiotsu et al. 2011. Jpn. J. Crop Sci. 80 (Extra 1):172-173.

The accumulation of cadmium is suppressed by apoplasmic barriers of roots

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The aim of this study was to elucidate the development and the function of apoplastic barriers in endodermal cells of *Arabidopsis thaliana* primary roots based on comparison of 7-days old plants of the wild type L*er* and genotypes with modified endodermal development - *shr*, *scr3*, and *scz*. The anatomy of primary roots and endodermal development on cytological level were analyzed and their relationship to the biomass production, growth rate, cadmium uptake and translocation were compared after 0μM (control), 10μM and 100μM $Cd(NO_3)_2 \cdot 4\,H_2O$ respectively.

The main results are:

♦ Endodermal cells of primary roots in wild type L*er* develop in two stages. In the first stage Casparian bands develop on anticlinal cell walls closer than 2 mm from the root apex. Thereafter suberin lamellae develop on the inner surface of primary cell walls with position effect starting opposite to the phloem poles in the distance of 7 mm from the root apex.

♦ Primary roots of *shr* genotype form neither endodermal cells nor Casparian bands and suberin lamellae. However, electrondense Casparian band-like material is deposited ectopically to the middle lamellae between the cell walls of almost all root tissues.

♦ Primary roots of *scr3* genotype form unstable number of endodermal cell files (3 – 6 on cross section). Separately developed endodermal cell or non-differentiated/non-divided cortical cells deposit the Casparian band-like material to the connection of its anticlinal cell wall and cell wall of neighbouring pericycle cell. In case of two developed neighbouring endodermal cells the typical Casparian band is formed in their conjunct anticlinal cell walls.

♦ The endodermal cells of *scz* genotype are atypically multiplicated. The primary roots form one or more additional endodermal cells or even the whole additional endodermal layer.

♦ Cadmium nitrate at 100 μM concentration inhibits fresh and dry biomass production and has xeromorphic effect (decreases the amount of water) in the roots, shoots and the whole plants of *Arabidopsis thaliana*.

♦ Endodermal cells with apoplastic barriers help to maintain the water in the plants and suppress the xeromorphic effects of cadmium. Genotype *shr*, characteristic by the absence of typical endodermis, accumulates the highest amount of cadmium compared to the other investigated genotypes. Under the influence of cadmium nitrate the *shr* genotype has the lowest ability to maintain the water compared to the other investigated genotypes.

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Identification of an ATP-binding cassette (ABC) transporter that is required for formation of suberin lamellae and the apoplastic barrier at the hypodermis in rice (*Oryza sativa*)

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Suberin is a complex of aliphatic polymers modified by phenolic compounds that is a constituent of apoplastic plant interfaces. In many plant species, cells in the hypodermis form a suberized cell wall in the form of the Casparian strip and suberin lamellae. These structures inhibit apoplastic transport of water and ions and protect the cell against pathogen infection. Suberin is accumulated in response to environmental stresses, including waterlogging. However, no mutants defective in suberin biosynthesis have been studied in rice, and so there is no direct genetic evidence that suberin forms an apoplastic barrier at the hypodermis. Here, we show that a rice mutant of an ATP-binding cassette (ABC) transporter, *reduced culm number 1* (*rcn1*), does not form suberin lamellae at the hypodermis under waterlogged conditions. RCN1 is located at the plasma membrane of hypodermis cells and is involved in the synthesis of long chains of two of suberin's major monomers (fatty acids and ω-OH fatty acid). The *rcn1* mutant has a defective apoplastic barrier at the hypodermis and develops short and frangible roots under waterlogged conditions. These results show that the apoplastic barrier helps rice to tolerate waterlogged environments.

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The Shift of Isoelectric Point of Beta-1,3-glucanases Possibly Involved in Aluminum Toxicity in soybean root

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On the acid soils, which comprise 30–40% of the world's arable soils, crop yields are reduced because of the existence of Al^{3+} ion. Aluminum is the most abundant metal in the earth's crust, however, under acidic condition, Al is solubilized to its ionic form, which shows severe toxicity to plants. Especially inhibition of root elongation is the most critical matter for plants and is considered as a result of direct interaction between Al^{3+} ion and cell wall components including protein. To identify the protein affected by Al^{3+} ion, we focused on the protein weakly bound to cell wall and specifically extracted from the apoplast of root elongation zone.

At first, extracted proteins by 20 mM $MgCl_2$ from the apoplast of elongation zone was compared with those of adjacent non-elongation zone with SDS-PAGE. The protein profile indicated that the band of 27 kDa protein was observed only at the elongation zone. To identify this elongation-zone-specific protein, mass spectrometry analysis was performed with MALDI-TOF-MASS. The result of PMF identification indicated that the protein was supposed to be one of the β-1,3-glucanases and no other candidate protein was identified. For verifying this identification, immunoblotting with tobacco β-1,3-glucanase antibody was carried out, and the bands indicating 33 and 27 kDa were detected. These results indicate that the β-1,3-glucanase is abundant in the apoplast of root elongation zone and it interacts weakly with other cell wall components.

The relationship between β-1,3-glucanases and Al toxicity was investigated with 2D-PAGE and immunoblotting. The extracts from the elongation zone prior treated with and without 20 μM Al for 2 h were compared. The result showed isoelectric point of β-1,3-glucanases were shifted in Al-treated root, suggesting β-1,3-glucanases located at the apoplast might be involved in Al toxicity because of the structural change inducing the shift of isoelectric point after Al treatment. Based on these results and β-1,3-glucanase functions, the involvement of β-1,3-glucanase in Al-induced inhibition of root elongation will be discussed.

Effect of PolySilicate-Iron sludge on rice roots at organic farming

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Organic rice farming, without chemical fertilizer and agrochemicals, is expected as one of environmentally-friendly farming systems. However, it has a number of agronomical problems. One of them is lower nutrient uptake than conventional farming, which causes to lower yield. We focused on enhancing root activity for solving this problem using an application of PolySilicate-Iron (PSI) Sludge. PSI is flocculant for water purification and is composed of $FeCl_3$ and poly-silicic acid. The sludge from water purification plants using PSI contains ferric iron (Fe) and silicon (Si). Fe is often applied for the mitigation of the reduction condition on paddy fields. Si is a very important nutrient for a rice plant and enhances suberization and lignification of rice roots in relation to the root activity. In previous studies, PSI sludge has the possibility of effective amendment for paddy soils. However, there is little information about the effects on roots. In this study, we investigated the effect of PSI sludge application on rice root at organic farming using the ingrowth core method and monitoring the bleeding sap for the indicator of root activity.
Materials and methods: A field experiment was conducted at the Field Science Center, Graduate School of Agricultural Science, Tohoku University, Kawatabi, Miyagi. Rice (*Oryza sativa* L.) plants (cv. Hitomebore) were grown in paddy fields. The 18 square frames (27.5 x 27.5 x 15 cm depth) were placed in the paddy field. Soil in the frame was exchanged for the equivalent amount of soil with organic fertilizer (ORG treatment: N, P_2O_5, K_2O = 7, 7, 5 g/m^2, suzuka yuki) or organic fertilizer + PSI sludge (PSI treatment). PSI sludge was applied at rate of about 1.5 kg m^2. Two hills (three plants per a hill) were transplanted into each frame and two mesh bags were placed in each frame estimating the production of roots using the ingrowth core method. Three frames of each treatment were taken three times: 48, 63 and 78 days after planting (DAP). At each time above ground plant parts were harvested, dried and weighted. After the aboveground parts were removed, the bleeding saps were collected from two hills of the frame and the soil monolith with roots was taken from the frame. The soils were washed carefully on a 1-mm mesh screen. After removing debris, the root were dried and weighted. Between 63 DAP and 78 DAP, two mesh bags were taken from the frame. The dry weights were measured as the production of roots.
Results and discussion: The differences between ORG and PSI treatments were not significant in the number of tillers and shoot dry weight at three sampling dates. The difference in total root dry weight was also not significant but the root dry weight of PSI was higher than of ORG at 63 DAP. Similarly in total root dry weight, the rate of bleeding sap per a tiller of PSI was higher than of ORG. In the production of roots per a plant and a tiller estimating with the ingrowth core method, PSI treatment was significantly higher than ORG treatment from 48 to 63 DAP. On the other hands, two treatments were almost same in mortality of roots estimating as the difference between total roots and production of roots. In this study, the effect of PSI sludge as Fe amendments for mitigating reduction condition is unclear in the dynamics of roots. However, the effect of PSI sludge as Si fertilizer must be effective at the organic farming because the production of roots become higher, which causes to keep the higher root activity during early growth stage.
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Physiological performance of direct seeding using iron-coated rice seeds under submerged and drained conditions

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Introduction: Recently, direct seeding cultivation using iron-coated rice seeds has been paid much attention in Japan because this technique has some merits such as prevention of flow seedling and bird eaten. Re-dried rice seeds after soaking in tap water (seed priming technique) are usually used in the production of iron-coated rice seeds because a rapid seedling emergence and establishment are essential for successful direct seeding cultivation. However, physiological performance of direct seeding using iron-coated rice seeds primed has not been characterized well. We thus examined physiological characteristics of iron-coated rice seeds under submerged and drained conditions.

Materials and Method: Rice seeds (*Oryza sativa* L. cv. Koshihikari) were soaking in tap water at 20℃ for 3d and its seeds were dried at 50℃ for 2d. These seeds were coated with a mixture of iron powder and calcium gypsum. Three hundred grams of dry paddy field soils were put in pot and water was submerged in the pot. Twenty iron-coated rice seeds per each pot were placed at soil surface. Seedling emergence, establishment and roots length were examined under submerged and drained conditions for 14 d. Root length was determined by a ruler or a root scanner. To examine coleoptile elongation under anoxia condition, a stream of N_2 (99.9%) was passed continuously through plastic container at 200 ml min^{-1} for 4d. After 4d, coleoptile length was determined by a ruler.

Results and Discussion: Seeds germination rate was higher in rice seeds priming treatment(20℃-3d) than in control. Seeds germination rate of rice seeds without iron coating was slightly higher than that with iron coating. Coleoptile elongation in rice seeds priming treatment(20℃-3d) was approximately 2.7-fold higher than that in control under anoxia condition for 4d. Seedling emergence and establishment were higher in iron-coated seeds of rice seeds priming treatment(20℃-3d) than in iron-coated rice seeds of control. In addition, roots growth of iron-coated rice seeds of priming treatment was higher than that of control under drained condition. These results indicated that in direct seedling cultivation using iron-coated rice seeds, use of primed rice seeds is advantageous to germination, seedling emergence and establishment, assuming that iron-coated rice seeds using seed priming technique are able to start acquisition of nutrition and photosynthesis at early growth stage.

The effects of compost and phosphorus supply on onion growth of root and shoot.

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Onion plants are transplanted at a density of 300,000 plants/ha in commercial production Hokkaido Japan. The competitions of labor time and green house space for raising seedling between onion and other crops will be severe. Direct seeding can avoid these competitions, however, seedling establishment and early growth in the field are retarded by stresses such as excess or deficit soil water and low temperature in spring season. We aim to improve the onion growth in early growth stage to establish onion direct seeding production system. The objective of this study was to evaluate the influence which fused magnesium phosphate and compost on onion growth in early growth stage.

Field experiments were conducted at the Memuro station of Hokkaido Agricultural Research Center in 2011. Experimental onion variety was `Kitamomiji 2000' which is most popular variety in Japan, and these were seeded on 21 April 2011. The seeding distance was kept 30 x 10 cm and plot size was 2.4 x 6m. Each treatment was replicated three times in a split-plot design, consisting of nine phosphate treatment plots (applied 0, 1 and 6 Mg/ha fused magnesium phosphate‘0P, 1P, 6P’ x 3 replications), with two subplots (applied 0 and 20Mg/ha bark compost ‘0C, 20C’) in each. And in all plots, compound fertilizer (200N - 300P_2O_5 - 200K_2O kg/ha) was incorporated. Onion growth was investigated on 17 June (57 days after seeding) and 2 August (103 days after seeding). Root cutting was done at 13 September and yield parameters were measured at 6 October.

Root length and shoot dry weight at 57 DAS were improved by compost supply. Fused magnesium phosphate application effect on onion growth was not obviously. Relationship between the root length and the dry weight of shoot was closely correlated (R^2=0.90, n=18).

Leaf number, leaf diameter, plant height and dry weight of shoot at 103 DAS were increased in compost applied (20C) plots than in 0 compost (0C), and maximum compost benefits on onion growth was noticed in the 0 P. Relationship between the root length at 57DAS and shoot dry weight at103DAS in 0P treatments was closer (r=0.96, n=6) than that in P input treatments.

The date of the bulb maturity in 20C plots was 9 September, and this date in 0C plots was later than root cutting. Bulb yield in 20C was significantly higher than in 0C.
In conclusion, the compost input improved onion growth and yield, and this improvement was expressed clearly in lower P input treatment.

Influence of different maturation periods of hairy vetch incorporated as green manure on growth of maize inoculated with *Gigaspora margarita*

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Green manure legumes have recently been recognized as valuable constituents for low input cropping systems. As hairy vetch (*Vicia villosa* Roth), a winter legume, contains polyphenolic compounds and organic acids which suppress weed growth, the incorporation of this legume species often inhibits the growth of the following summer crop. To prevent the inhibition, it is recommended to plant the following crops after several weeks of incorporation. Enough days for maturation of the green manure should be kept, but the seeding date would need to be delayed in this case. In order for alleviating the growth inhibition immediately after incorporation, the possibility of inoculation with arbuscular mycorrhizal (AM) fungi known as stress alleviating microorganisms was examined on the following maize crop.

Wagner pots (1/5000 a) were filled with soil of 'Akadamatsuchi' supplied with chemical fertilizer (N, P and K). Shoots of hairy vetch were incorporated into the pots at the rate of 0, 8 and 25 g/pot (-GM, +GM8 and +GM25). Water soaked seeds of maize cv. 'Gold dent KD850' were planted into the pots with (+AM) or without (-AM) inoculation of '*Gigaspora margarita* (Becker & Hall)'. To compare the different periods of maturation of green manure, the seeds were planted immediately after incorporation (-MRN) and 34 days after incorporation (+MRN). Those pots were kept in a greenhouse, and supplied phosphorus solution as needed. Plant height, the SPAD value and number of leaves of maize plants were measured weekly. Five uniform plants were sampled from each treatment at 20 and 40 days after seeding. Root length was analyzed using the Win-RHIZO software. Small parts of roots were also taken to investigate colonization rate of AM fungi.

At 20 DAS, dry weights of shoot and root of maize plant in +GM25 pot were greater than those in -GM pot in +MRN. In addition, the SPAD value was higher in +AM pot than in –AM pot. At 40 DAS, dry weight in +AM pot showed double to seven times higher than that in –AM pot in both GM treatments (+GM8 and +GM25). On the other hand, growth inhibition was definitely observed in +GM/-MRN pots. An alleviating effect of AM fungi on the maize growth was found in those pots. Based on these findings, it appears that inoculation technique of AM fungi might lead to shorten planting dates after hairy vetch incorporation. We have been analyzing the fatty acid profiles of the soil amended with AM fungi to understand how soil microbial biomass changed during maturation of hairy vetch green manure.

Growth and N_2 fixation of soybean plants grown under different soil moisture contents in FRP pots

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Wet injury of soybean is a major problem in an upland field converted from paddy farming system in Japan. This is because soybean is sown in the rainy season, e.g. between late June and early July. Some researchers have reported on the effect of soil moisture condition on growth and yield through field trial. If we can more easily control the soil moisture conditions, we can more clearly define the relationship between soil moisture and growth for each growing stage. Fiber Reinforced Plastics (FRP) pot was developed as the experimental instrument for the paddy crop by Kumiai Chemical Industry Co. Ltd.. Water leakage level at flooding condition is controlled using this system. As a drain plug at the bottom was attached to adjust the levels of water table in FRP pot, it would be also possible to control soil moisture contents under upland condition. In the present study, 1) we constructed a system to control soil moisture contents in a FRP pot experiment for field crops, 2) we investigated the growth and nitrogen fixation of soybean plants grown under different soil moisture contents from the cotyledon stage to before flowering stage under these experimental conditions.

Materials and Methods

Experiment was conducted outside with a roof made of ultraviolet transmitting film at Kikugawa, Shizuoka, between June and July, 2011. Gravels were added above 15 cm from the bottom of FRP pot (inside dimension W500×H500×D500 mm), and the paddy soils (clay loam soil) were filled to 10 cm from top of the pot. TDR sensors were set at 5-10 cm below the soil surface (BSS). At 17 June, 2011, 16 seeds (cv. Fukuyutaka) inoculated with rhizobia were sown in each pot. The pots were irrigated with 1.25 L of water, and then covered with cheesecloth to retain moisture. Seven days after sowing (DAS), the seedlings were thinned to 9 plants per pot, and the pots were subjected to two different soil moisture contents using a slanted pipe adjusted at 0 cm and 15 cm BSS. Three plants were selected from each plot, and stem length, number of stem node and SPAD value were measured. At 17, 27 and 41 DAS, shoot dry weight was measured from two plants per pot. Xylem sap was also sampled and analyzed for NO_3-N, amino acids-N and ureide-N contents. This experiment was conducted with four replications.

Results and Discussion

Soil moisture contents were remained approximately 0.21 and 0.40 m^3/m^3 in irrigation at 15 cm and 0 cm BSS, respectively. Wet injury of shoot growth was markedly found in irrigation at 0 cm BSS. Length and number of the main stem and dry weight of shoot in irrigation at 0 cm BSS were lower than in 15 cm. At 17 DAS, total N contents of leaves and stems were also lower in 0 cm BSS than in 15 cm. The differences in N content between in 0 cm and 15 cm BSS increased at 28 and 41 DAS. Dependency on N_2 fixation (Ureide-N/Total-N) of shoot was lower in 0 cm BSS than in 15 cm. At 41 DAS, it increased in both water levels. The FRP pot system would be available for wet injury experiment in soybean.

Using technology of shoot and residue for green manure and forage production by leguminous crops and barley mixtures at paddy

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The competition between green manure and forage crops frequently occurred at agricultural field because of soil fertility and livestock feeding selection. These experiments were carried out to evaluate the effects shoot and residue for green manure and forage production by leguminous crops and barley mixtures at paddy. Field experiments were conducted at paddy soil (fine loamy, mixed, nonacid, mesic family of Aeric Fluvaquentic Endoquepts) in 2008 to 2009 at the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Suwon, Gyeonggi province, Korea. Treatments consisted of mixture and interseeding of barley and leguminous crops (hairy vetch and crimson clover). These treatments were divided into cutting height of 8 and 25 cm for using of green manure and forage at once. The shoots biomass of hairy vetch and crimson clover mixtures were higher than intercropping seeding. The shoot biomass of hairy vetch and barley mixture was the highest at 8 cm cutting height. The biomass of hairy vetch with barley mixture was higher than that of crimson clover with barley at all of the cutting heights. The shoots and roots biomass of residues showed slightly high at barley and crimson clover mixture of 25cm cutting height. The residues biomass of same cutting height were no significantly difference. The mixture of hairy vetch and barley showed the best biomass of shoot and residue for green manure and forage using at 25 cm of cutting height. Also this treatment could be possible to rice cultivation by no fertilization. Therefore, we suggested that 25 cm cutting of hairy vetch and barley mixture could be used for green manure and forage at the same time under rice-based cropping system.

Effect of root-deposited N of hairy vetch on the growth and N uptake of mixed-cropped oat

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The legume-cereal combination is often used in a mixed-cropping system. Generally, cereals in mixed-cropping with legumes uptake more N than in sole cropping, because of transfer from rhizodeposit-N of legume to the associated cereal crop. In mixed-cropping, cutting the aboveground parts of legume may increase N transferred from legume through turnover of roots and root nodules. We previously showed that cutting the aboveground parts of hairy vetch (*Vicia villosa* Roth) before flowering markedly increased the stem number of mixed-cropped oat (*Avena sativa*). In the present study, we investigated the effects of N released from belowground parts of hairy vetch on the growth and N content of mixed-cropped oat plant.

The experiment was carried out in 2010/2011 at the experimental farm of Osaka Prefecture University, Japan. The treatments were as the follow; sole cropping of oat (A); sole cropping of hairy vetch (V); mixed-cropping (M); mixed-cropping with cutting hairy vetch at blooming stage (MC); sole cropping with cutting the half of oat (ACH). Each plot size was 1.8 x 2.6 m. The seeds were sown on 29 November 2010 with seeding rates of 4.3 kg/10 a (A and ACH) and 2.9 kg/10 a (V). In mixed-cropping treatments (M and MC), the seeding rate of each component crop was half of the sole cropping rate. Ammonium sulfate was applied at the rate of 3 kg N /10 a. The cutting treatment for MC and ACH was conducted on 21 April 2011 (20 weeks after sowing) and the shoots were removed immediately after cutting. To know the aspects of light competition between two crop species in mixed-cropping, relative amounts of accumulated solar radiation at interrow space was measured. Shoots of the two plants were harvested on 22 May 2011, dried for 48 h, weighed and then total N contents were determined.

In spite of one-half seeding density of oat in MC, the shoot dry weight of oat in MC was 68% in A. The number of stems of oat increased in M, MC and ACH compared to A. Maximum stem number was obtained in MC. Cutting the hairy vetch markedly increased relative amounts of accumulated solar radiation at interrow space. Total N content of shoot of oat considerably increased after cutting of hairy vetch. Cutting of oat in ACH, on the other hand, had no effect on total N content of the residual half of oat. These results suggest that cutting hairy vetch at blooming stage results in higher stem number and total N content of mixed-cropped oat, indicating that large amounts of N derived from root deposition of hairy vetch were transferred to the associated oat.

Effect of rhizobia primed with naringenin on root development and nodulation in *Pisum sativum* L.

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Root nodule formation and N_2-fixation in legume-rhizobia symbiosis are often limited by various environmental factors, such as low temperature and excess nitrate in medium. One of such causes is related to decrease of flavonoid compounds which are released from legume roots and lead to *nod* gene activation. Previously, pre-incubating method of rhizobia with flavonoid (priming method) has been proposed, but the effective priming technique has not been established since the effect of priming is different among plant species. This work is focused on whether pre-incubation of rhizobia with naringenin affects nodulation and root development of pea crop, which is often grown under low temperature condition in Kinki region, Japan.

Seeds of pea cv. Usui, leading varity in Kinki, were surface sterilized in 5% sodium hypochlorite solution and then rinsed several times with sterilized water. They were sown in plant boxes containing 0.8% ager medium. Six-day-old seedlings were transplanted into sterilized plastic box containing Broughton & Dilworth medium supplemented with potassium nitrate at 10 mg N/L and incubated in a growth chamber. Seedlings were inoculated with *Rhizobium leguminosarum* bv. *Viciae* isolate (OD_{620} 0.2) on the next day. For preparation of *R. leguminosarum* primed with naringenin, 10 ml of a bacterial suspension from 3-day-old subculture were aseptically added to 50 ml of sterile 10 mg/L naringenin solution in a 200 ml flask and incubated at 30 °C for 48 h without shaking. The suspensions were filtered with 0.45 μm in pore size paper and centrifuged at 7000 g for 10 min, and then used for the following inoculation. In order to remove flavonoid sufficiently, these processes were repeated twice.

Nodules were found at 7 days after inoculation (dai) in both priming treatment (PA) and the control. Although nodule numbers in PA were higher than those in the control at 14 dai, acetylene-reducing activity (ARA) was not significantly different between PA and the control. At 28 dai, PA increased nodule numbers, ARA and flesh weight of shoot and root. Plants inoculated with primed rhizobia showed alterative root structure such as higher rate of length of the first order lateral roots to total root length and the continuous nodule formation along the root axis. Priming treatment with naringenin before rhizobial inoculation might promote the growth of pea plant grown under unsuitable environmental conditions.

Morphological changes of *Sesbania rostrata* root under different soil properties

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Sesbania rostrata has shown great promise as a green manure due to its fast growth rate and high biomass production, high N_2-fixing activity, dual nodulation (both on stems and roots), and tolerance of flooding. For cultivation of *S. rostrata*, an understanding of root biomass and root morphology is important. The studies reported here have two overriding objectives: 1. To show the root growth under several soil conditions, and 2. To investigate specific roots formed under granular soil conditions.

Seeds of *S. rostrata* were sown in lowland paddy soil, volcanic ash soil (Kuroboku) or granular clay soil (Akadamatsuchi) in 1/5,000 a Wagner pots. Plants were grown in a glasshouse and watered as needed. After 90 days, plants were harvested. Roots were observed with a microscope and were analyzed using the WinRhizo software. To investigate specific roots observed under granular clay soil conditions, small parts of root were also taken at the same time for quantification of rhizoplane and rhizosphere microbials.

There was significant media effect on the growth of *S. rostrata* and root structure. *S. rostrata* increased root biomass in granular soil compared to lowland paddy soil and volcanic soil. Total root length was also highest in granular soil following to lowland paddy soil and volcanic soil. The significant changes in root structure indicated the heavily branching root like cluster roots (Fig. 1). Microscope observation showed as if soil particles were surrounded by heavily branched fine roots. These structures were formed randomly along the axis of primary or secondary lateral roots. A pH of soils surrounded by roots was clearly lower, and mineralized P concentration was higher than the other parts of roots. The rhizoplane and rhizosphere microbial population was also higher in the site of specific roots. Although we could not reveal why this structure was formed only under granular soil, the formation of the specific root structure would be one of the adaptation of *S. rostrata* roots to nutrient acquisition especially P. The specific roots would chemically modify the surrounding soil by exuding compounds such as carboxylate organic acids, acid phosphatases and mucilages.

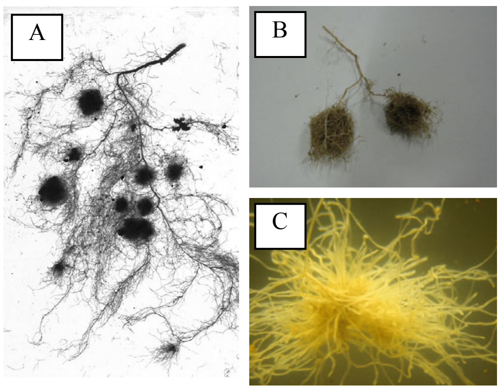

Fig. 1 Specific structure of *S. rostrata* roots formed under granular clay soil conditions.

Nitrogen fixation by endophytic bacteria isolated from sweet potato

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Sweet potato (*Ipomoea batatas*) is known for its ability to grow under nitrogen-limited condition. It is also known that the total amount of N in sweet potato exceeds the amount of N applied as chemical fertilizer. Recently, nitrogen fixation by endophytic diazotrophs has been observed in a wide variety of plants. The sweet potato may also have special mechanisms for enhancing soil N mineralization and/or acquiring N derived from atmosphere. To clarify the possible contribution of endophytic nitrogen fixation, we isolated and identified diazotrophic endophytes associated with sweet potato grown in Japan. The isolates which possess *nifH*, a gene encoding one of the subunits of nitrogenase were identified as strains of *Bradyrhizobium* sp. AT1, *Paenibacillus* sp. T16 *and Pseudomonas* sp. AS2 based on their 16S rRNA gene sequences. These isolated are nitrogen-fixing bacteria as demonstrated by the acetylene reduction method in a semi-solid malate medium. Moreover, *B.* sp. AT1 showed acetylene reduction activity in liquid as well as in semi-solid culture medium containing sweet potato tuber extracts, although addition of 3mM glutamine to the medium inhibited acetylene reduction activity. We also examined the infection of isolated *B.* sp. AT1 in sweet potato and their influence on the growth and N_2-fixation as assessed by acetylene reduction method. After 55 days of inoculation, inoculation of *B.* sp. AT1 resulted in an increase in the shoot and root fresh weight compared to uninoculated control. The acetylene reduction activity was also detected in the stems of inoculated plants. These results suggested that the isolated *B.* sp. AT1 may contribute to the N input in sweet potatoes.

Influence of excess soil water during early growth stage on soil microbial community structure in soybean field

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Soybean plants are mainly cultivated in upland field converted from paddy in Japan. They often suffer wet injury, especially during early growth stage. Excess soil water causes loss of soil oxygen, and it suppresses root growth, nutrient absorption, and yield of the plants. Soil microbial organisms are playing an important role in nutrient absorption of plants. The objective of this study is to investigate the influence of excess soil water in the paddy converted upland field on soybean growth and soil microbial community structure.

A field experiment was carried out on the Experiment Farm at Osaka Prefecture University in Sakai, Osaka, Japan in 2011. Both wet plot and the control plot were established prior to seeding of soybean. Seeds of cv. Fukuyutaka were sown on 15 June. Fertilizer was applied at the rate of N : P_2O_5 : K_2O = 3 : 10 : 10 g m^{-2}. TDR sensors were set at 5-10 cm below the soil surface. Soil and roots were sampled by core sampling methods (5 cm in diameter×20 cm in depth) on 21 July (vegetative stage) and 2 September (reproductive stage). Soil cores were divided into two layers by soil depth (0-10, 10-20 cm). To evaluate microbial biomass and community structure, phospholipid fatty acids (PLFAs) from each soil layer were extracted as indicated by Sakamoto *et al*. (2005). The extraction was analyzed with an Agilent 6890 Plus Chromatograph and the Sherlock system software (MIDI Inc, Delaware, USA). PLFAs were identified by retention time according to the MIDI method.

During early growth stage, soil moisture contents were approximately 0.30 and 0.20 m^3/m^3 in wet plot and the control plot, respectively, and thereafter they were approximately 0.20 m^3/m^3 in both plots. Shoot growth of soybean in wet plot was significantly less than in the control plot. Distribution of the roots in 0-20 cm soil layer was markedly different between the wet and control plots, and biomass and lateral branching of the roots in 0-10 cm layer was remarkable in wet plot. Basically, the total microbial biomass increased along with the growth of soybean. At vegetative stage, the biomass in both soil layers showed higher abundance in the control plot than in wet plot. At reproductive stage, gram-positive and gram-negative bacterial biomass in 0-10 cm soil layer showed higher abundance in wet plot than in the control plot. In 10-20 cm layer, on the other hand, it was greater in the control plot than in wet plot. Actinomycetes, fungi and AM fungi biomass also affected by soil moisture condition, they showed lower abundance in wet plot than in the control plot. Further study on changes in microbial community structure is now in progress.

Respiration rate and sugar concentration of wheat root affected by waterlogging in field and by root zone hypoxia in hydroponic culture

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Introduction

Root growth and respiration can be damaged by waterlogging or root zone hypoxia. In our previous pot experiment, wheat roots temporally waterlogged around anthesis exhibited abnormally higher CO_2 evolution rate until maturity stage. In this study, root oxidization activity, as an indicator of oxidization respiration, was examined for drained and waterlogged wheat at anthesis to test if the roots show abnormal respiratory activity. In the hydroponic experiments, a relationship between sugar concentration and respiration rate or oxidization activity was also examined in hypoxic and aerated wheat roots.

Materials and Methods

The field experiment was carried out at an experimental field of Yamaguchi University in 2010 winter to 2011 spring. Wheat cultivars, Chikugoizumi and Kinuiroha, were grown. The plants were exposed to waterlogging for 21 d in jointing stage, for 11 d in post-anthesis or double. During the treatments, furrows beside 1.2 m wide ridges were flooded so that the roots 10 cm below surface were submerged. Roots 10 cm below soil surface were sampled at pre-anthesis, 14 d post-anthesis and 21 d post-anthesis. Oxidization activity of the roots was determined with the 1-naphtylamine method. In the hydroponic experiments, roots of wheat and rice seedlings around 3rd to 5th leaf stages were imposed root zone hypoxia. Growth, CO_2 evaluation, O_2 consumption, sugar concentration and oxidization activity of the roots were investigated.

Results and Discussion

In the field experiment, the oxidization activity of roots that were waterlogged during jointing stage was significantly high until 14 d post-anthesis, indicating that the abnormal oxidization lasted for more than a month. In the hydroponic experiment, root dry weight and R/S ratio were significantly reduced by hypoxia in both wheat and rice. The oxidization activity, CO_2 evaluation rate, O_2 consumption and sugar concentration of hypoxic roots were significantly high in wheat, but not in rice. There was a significant correlation between sugar concentration and CO_2 evaluation in hypoxic and aerated roots. The high sugar concentration, probably resulted from loss of root growth activity, may account for the high respiration activity in the hydroponic and field experiments.

Are roots involved in poor grain filling of wheat in Western Japan?

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Background

In Western Japan, grain filling of wheat is sometimes poor because of early senescence of leaves, stems and spikes. In some cases, the poor grain filling can be attributed to temporal waterlogging during grain filling stages. This was the case in 2011 spring when heavy rain lasted for 2 days at a week after anthesis. Field soil structure aggravates the damage from waterlogging since wheat is cultivated in upland fields that are temporally converted from paddy fields. In some cases, the grain filling goes poor without waterlogging during grain filling. Locally, the abnormally early ripening (AER) is called “Kareure” and happens to commercially important cultivars in Western Japan. At the moment, there are no measures to reduce AER except replacing cultivars, since causes of AER are not known.

Leaf senescence and grain filling of waterlogged wheat

We carried out field trials in which wheat was imposed waterlogging during jointing stage, post-anthesis and double. Before anthesis, the waterlogging during jointing stage did not affect leaf greenness and photosynthesis rate. The waterlogging during jointing stage and post-anthesis induced quick leaf senescence during 2nd to 3rd weeks after anthesis regardless of cultivars and years. It should be noted that the effect of waterlogging during jointing stage became manifest beyond a latent period for more than one month. Leaf water content and nitrogen content in leaves also became low as the leaves senesced. Remobilization of stored carbohydrate in straw to grain was inactivated.

Is AER induced by latent damage of roots?

AER wheat shows very similar symptoms, i.e. early leaf senescence, low carbohydrate remobilization and poor grain filling, to the ones of waterlogged wheat. Therefore, we assumed that latent damage of roots, as found in waterlogged wheat, may be involved in AER. We investigated distribution of AER in local farmer’s fields in Yamaguchi city. AER fields were concentrated on the area where the fields became readily waterlogged after rainfall. However, AER happened without growth restriction by waterlogging, indicating that waterlogging may partly contribute to AER, but not be the necessary condition for AER.

Root researches can contribute to the poor grain filling caused by waterlogging and AER.

Waterlogging tolerant teosinte (*Zea nicaraguensis*) has a large volume of aerenchyma and a barrier to radial oxygen loss in root

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Combination with a large volume of aerenchyma and barrier to prevent radial oxygen loss (ROL) along the roots is one of important traits for waterlogging tolerance in plants. These traits enhance transportation of oxygen from shoot to root tip under waterlogged condition. Here, we report the formation of aerenchyma, barrier to ROL and barrier properties in roots of teosinte (*Z. nicaraguensis*), one of wild relatives of maize, and maize (inbred Mi29). When grown in stagnant condition, these formations of aerenchyma near the root tip and ROL barrier at the basal part of roots was observed in *Z. nicaraguensis*, but not in maize. The root of *Z. nicaraguensis* grown under stagnant condition showed Casparian strip and the suberin lamella and lignin deposition started to develop from near the tip. By contrast, root of maize showed retarded development of Casparian strip and suberin lamellae and lignin was not detected along the root. These dates indicate that the abilities to form aerenchyma and barrier to ROL of *Z. nicaraguensis* are superior to that of maize in stagnant condition. Higher tolerant to waterlogged condition of *Z. nicaraguensis* would be attributed to large aerenchyma formation and tight barrier to ROL.

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Variation for the capacity to form root aerenchyma and selection for highly aerenchyma forming lines in the rare teosinte *Zea nicaraguensis*

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Wild relatives have been used to improve tolerance to abiotic stress in many plant species. The teosinte *Zea nicaraguensis*, a wild relative of maize, is considered to be a valuable germplasm resource for the development of flooding-tolerant maize because this species is adapted to flooded lowlands in Nicaragua.

Zea nicaraguensis has the capacity to form constitutive root aerenchyma under well-drained conditions. A previous study suggested that the degree of constitutive aerenchyma formation varied within a single accession of *Z. nicaraguensis* (PI 615697). The objectives of this study were to search for variation in the capacity to form aerenchyma in several accessions of *Z. nicaraguensis* and to obtain progenies with higher and lower degrees of aerenchyma formation by using phenotypic selection.

The degree of aerenchyma formation in the root cortex was evaluated in six-leaf-stage seedlings of eight accessions and several S_0–S_3 populations in *Z. nicaraguensis*. Seedlings of eight *Z. nicaraguensis* accessions showed an extremely wide and continuous range of variation in the degree of aerenchyma formation within accessions, suggesting the presence of genetic variation. By phenotypic selection within two accessions, lines with extensive aerenchyma formation (derived from CIMMYT 13451) and lines with little aerenchyma formation (derived from PI 615697) were successfully obtained (Fig. 1).

In conclusion, the capacity to form constitutive aerenchyma was shown to be heritable in the rare teosinte *Z. nicaraguensis*. The contrasting lines with higher and lower degrees of aerenchyma formation will be useful to reveal the effect of constitutive root aerenchyma on flooding tolerance in *Z. nicaraguensis* and to conduct physiological analyses of root aerenchyma formation.

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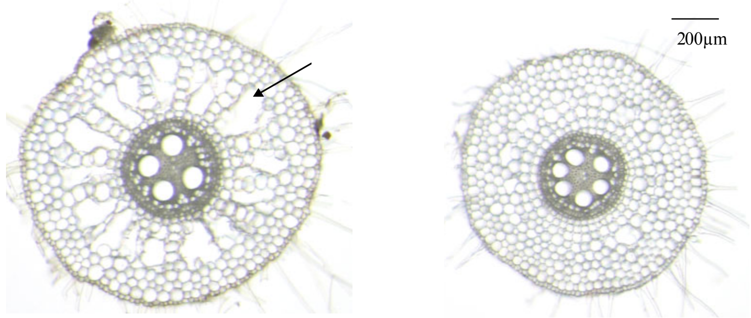

(a) CIMMYT 13451 (b) PI 615697

Fig. 1 Cross sections of adventitious roots that emerged from the second node of six-leaf-stage seedlings of *Z. nicaraguensis* grown in non-flooded conditions. Sections were taken 10–15 cm from the root tip. Arrow indicates an example of constitutive root aerenchyma. (a) Radial formation extended toward epidermis and (b) slight formation. The same scale was used; bar = 200 μm

Root system development and hydraulic conductance in soybean plant grown under waterlogged conditions

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Yield reduction of soybean is often caused by excess water. Mochizuki (2000) pointed out that secondary aerenchyma formation in root is an important trait for soybean plants to maintain root system development under waterlogged soil. Araki (2006) showed the elevated CO_2 significantly reduced hydraulic conductance of soybean roots. These findings indicate that both the root system development and its hydraulic conductance under waterlogged condition need to be examined to evaluate the role of roots in waterlogging tolerance. We therefore hypothesize that waterlogging tolerant varieties may have an ability to maintain the root development and hydraulic conductance under excess soil moisture conditions. The aim of this study was to examine the functional roles of root function in waterlogging tolerance in terms of root development and hydraulic conductance by comparing cultivars that differ in waterlogging tolerance.

Material and Methods: Two soybean *(Glycine max* (L.) Merr.*)* cultivars were used; one was 'Tamahomare' which is less tolerant to excess soil moisture conditions and the other was 'Fukuyutaka' which is tolerant to the same conditions (Koumura et al. 1983). An experiment was carried out in a green house at Nagoya University, Japan. 4.0 kg of air-dried sandy loam soil was filled up in a plastic pot (190 mm high and 158 mm in diameter). Chemical fertilizer was mixed with the soil at a rate of 0.25g kg^{-1} for P_2O_5, 0.25g kg^{-1} for KCl and 0.5g kg^{-1} for magnesium lime. Three seeds were sown on July 5, 2011 per pot. Seedlings were thinned to one plant 14 days after sowing. After thinning, two water levels were applied; control (23% w/w) and waterlogged (33 % w/w). Each pot was weighed daily and the amount of water loss replenished, which was recorded as evapotranspiration. Three pots were prepared in each treatment. Root hydraulic conductance was measured on August 27, 29 and 31, 2011 by using pressure chamber method (Araki et al.2006). Then, shoot and root were sampled. Total root length was determined by using WinRHIZO (Bouma et al. 2000).

Results and discussion: The reduction ratio of shoot dry matter under waterlogged condition relative to control was 39% in Tamahomare, but Fukuyutaka maintain shoot dry matter production only 19% reduction in the same condition. Significant reduction in water uptake was observed under waterlogged condition as compared with control in Tamahomare. But in Fukuyutaka, there was no significant difference between the two treatments. Furthermore, the hydraulic conductance under waterlogged condition was significantly lower by 31 % than that under control in Tamahomare while Fukuyutaka showed significantly higher hydraulic conductance under waterlogged condition. The total root length was significantly greater in waterlogged condition than control in Fukuyutaka. But in Tamahomare, the opposite trend was evident. These results indicate that the ability to maintain root system development and hydraulic conductance under waterlogged condition enables Fukuyutaka to uptake more water and produce more biomass than Tamahomare under the same conditions.

Reference: Mochizuki et al., 2000. Jpn. J.Crop Sci. 69(1): 69-73; Araki., 2006. Field Crops Research 96: 98-105; Koumura et al.,1983. Research bulletin of the Aichi-Ken Agricultural Research Center. 15: 93-104; Bouma et al., 2000. Plant and Soil 218: 185-196;

Dynamic regulation of the root hydraulic conductivity of barely plants under salinity/osmotic stress

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The root hydraulic conductivity (Lp_r) is significantly reduced by salinity stress in several plant species including *Arabidopsis*. maize, and barley (*Hordeum vulgare*). Authors characterized changes in the Lp_r of barley plants in response to salinity/osmotic stress in detail using a pressure chamber. Although salt-sensitive I743 variety did not show any significant change of Lp_r during salt/osmotic stresses, a characteristic time-dependent change of Lp_r was induced by 100 mM NaCl in cultivars Haruna-nijyo and K305. Rapidly repression of Lp_r within 1 h after exposure was observed, but Lp_r transiently recovered peaking at around 4 h, followed with a long-lasting re-repression. Further examination using cv. Haruna-nijyo plants in combination with a pharmacological approach revealed that the same response was evoked by an iso-osmotic 177 mM sorbitol. It was also suggested that the involvement of cellular processes such as protein phosphorylation/dephosphorylation, endocytotic protein recycling, de novo protein synthesis and protein degradation appear. Biochemical analyses using a peptide antibody to HvPIP2;1 protein (a plasma membrane-localized water channel in barley) indicated that HvPIP2;1 channels were subjected to a time-dependent translocation within cellular membranes after the exposure to 100 mM NaCl. Shutting down of the water transport attributed to the *L*p reduction should be essential to minimize water loss at the initial phase of severe salt/osmotic stress for survival. In addition, *L*p reductions may be a sign of conversion of the growth status of plant cells from the rapid growth mode with high water absorption to the protect/tolerant one with less water uptake as a strategy for the survival under salinity stress (Horie et al. PCP 52: 663, 2011).

Water permeability of protoplasts derived from different portion of seminal root system in rice

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A root system of an individual plant consists of various types of component roots that have different hydraulic properties, and therefore the hydraulic architecture of a whole root system is complex. Ichikawa (2003) intensively examined the hydraulic architecture of sorghum seminal root system and found out that the hydraulic architecture can not be fully explained with only apoplastic architecture such as xylem vessel maturity, casparian band development, and lateral root development, and pointed out the need to evaluate the contribution of symplatic pathway to the entire water uptake and conductance. In this study, we estimated root water uptake by measuring absorbance of sulphorhodamine G (SRG) that is a apoplastic tracer, by plant grown in hydroponics, while symplastic pathway was determined by measuring water permeability (P_{os}) of protoplasts that were taken from roots, which were then calculated from the swelling rate in hypotonic solution. Rice plants were grown in hydroponic culture for 10 days, and then transferred in SRG solution for 4h. SRG was extracted from the seminal roots by water, and its absorbance was measured with spectrometry. To determine water permeability of protoplast, firstly, we examined 4 different concentrations of the macerate solutions to find the most efficient one to take protoplast from different parts of the seminal root. Secondly, root segments from the tip to the base of the seminal roots were collected 10 days after sowing, and incubated with the appropriate macerate solutions. The composition of macerate solution (Cellulase RS: Macerozyme R-10: Pectolyase Y-23) are the following: 4%: 0.2%: 0.03% for the basal, 4%: 0.3%: 0.03% for the middle and 2%: 0.1%: 0.03% for the tip of the seminal root. Lastly, the protoplast was transferred from hypertonic to hypotonic solutions and the swelling rate was calculated based on the images that were captured on the video monitor that is equipped with an inverted light microscope (OLYMPUS, IX70). The water permeability was calculated using the equation of Suga et al., (2003): $dV/dt = P_{os}SV_w(C-C_0)$. The following are legend of the equation: V, protoplast volume; t, time; P_{os}, Osmotic water peameability (ms^{-1}); S, surface area of the plotoplast; V_w, partial molar volume of water (18×10^{-6} m^3 mol^{-1}); C, solute concentration inside (mol m^{-3}); C_0, solute concentration outside (mol m^{-3}). The accumulations of SRG were different at various types of roots for seminal root with 169.0 nmol mm^{-2}, S-type lateral root with 108.3 nmol mm^{-2} and L-type lateral root with 189.4 nmol mm^{-2}.
In addition, the rate of water uptake was found to differ along the root axis depending on the distance from the root tip. The largest SRG accumulation was observed at the basal portion of the seminal root, which was 225.0 nmol mm^{-2} of SRG. The P_{os} of protoplasts along the seminal root axis (from the basal to root tip) ranges from 28.5-35.8 μm s^{-1}(basal), 1.7-13.5 μm s^{-1} (middle) and 1.2-11.6 μm s^{-1} (tip).Our results concluded that the water uptake ability of 10-day-old rice was higher in the older (basal) portion than in the younger (root tip) portion along the seminal root axis. This higher water uptake ability was contributed in larger P_{os} and larger amount of SRG at the basal part on the seminal root in rice plants.

Reference: Ichikawa R. 2003. Master thesis. Graduate school of Bioagriculture Science, Nagoya University, 107pp. Suga S, et al., 2003. Plant Cell Physiol. 44: 277-286.

Promotion of aerenchyma formation in rice primary roots by treatment with 1-Aminocyclopropane-1-carboxylic acid

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Wetland plants like rice adapt themselves to low-oxygen conditions by developing aerenchyma in their roots. Induction of lysigenous aerenchyma formation and its mechanism have been well investigated in maize root. When maize roots were exposed to low-oxygen environment due to stagnant conditions, activities of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and of ACC oxidase increased (He et al. 1997) and, as a result, enhanced ethylene production induced cell death in root cortex and induced lysigenous aerenchyma formation (He et al. 1996). In rice roots, aerenchyma is formed constitutively and, therefore, it has long been discussed whether ethylene is involved in its formation (Webb and Jackson 1986, Jackson et al. 1985, Justin and Armstrong 1991). Recently, it has been shown that aerenchyma formation is enhanced by stagnant conditions (Colmer et al. 2006, Shiono et al. 2010) and indicated that ethylene is involved in this process (Colmer et al. 2006). To elucidate this issue, our group recently established a unique “sandwich method” which enables to detect differences in development of tissues sensitively under different environmental conditions. In this method, roots are sandwiched between two agar media containing different constituents and, thereby, a comparison of development of tissues between two different conditions, i.e., the control side and the treatment side, is possible in each root. In this study, we have tested whether ethylene is involved in aerenchyma formation in rice roots using this method.

Sterilized and imbibed rice (*Oryza sativa* ssp. *japonica* cv. Nipponbare) caryopses were sandwiched between 2% (w/v) agar plates containing 1/10 strength Hoagland medium, only one of which contained ACC and the roots were grown for 4 days. Cross sections of 100 μm thickness were cut using a microslicer at 5 mm intervals from root tip and were observed under a light microscope. Development of aerenchyma was monitored by quantifying areas of aerenchyma observed on cross sections. As a result, aerenchyma formation was enhanced significantly in the ACC treatment side compared to the control side. Next, rice caryopses sandwiched between two agar plates containing ACC only on one side as described above were placed in an airtight container for 4 days in the presence of an ethylene action inhibitor, 1-methylcyclopropene (1-MCP), so that its final concentration was 0.1 ppm. In the presence of 1-MCP, growth inhibition of the roots by ACC was recovered and the significant enhancement of aerenchyma formation in the ACC-treated side of the roots disappeared. These results indicated that ethylene signaling is involved in aerenchyma formation in rice roots.

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Recovery and compensation of nitrogen uptake by change of root morphology and gene expressions for ammonium transport under the osmotic stress condition in rice seedlings

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Since soil nutrient availability of roots is high heterogeneity, and nutrient uptake conditions are easily influenced by various environmental factors, plants encounter nutrient deficiencies frequently during their life cycle. Therefore, plants require nutrient transport systems with high flexibility to manage morphological and physiological responses to nutrient deficiencies. Rice plants grown in paddy fields predominantly utilize ammonium, which is the major form of inorganic nitrogen in hypoxic and anaerobic soils. Furthermore, ammonium is the preferential form of nitrogen uptake when plants are subjected to nitrogen deficiency, since ammonium assimilation requires less energy than that of nitrate. Plants have the ability to change developmentally and functionally their form to survive and grow under various environmental conditions. This phenotypic plasticity in root system morphology is an important key for crop stress tolerance.

We studied sequential changes of rice root morphology and the gene expressions for ammonium transport under the osmotic stress condition. The aim of this study is to evaluate how rice root recover from and adapt the osmotic stress after the dissolving of nitrogen deficit.

Rice (*Oryza sativa* L. cv Akita-komachi) seedlings were grown hydroponically in distilled water for seven days and then transferred to nutrient solution (modified Hoagland medium) in the absence of a nitrogen source for ten days to consume their endosperms completely. Seedlings were then transferred to three different solutions; nitrogen-free nutrient solution, nutrient solution containing 0.15 mM $(NH_4)_2SO_4$, and nutrient solution containing 0.15 mM $(NH_4)_2SO_4$ and 10% polyethylene glycol 6000 (PEG). Plants were grown under 12 h light/12 h dark condition, at 70% relative humidity, and a temperature of 28℃. Roots were sampled at 0, 1, 3, 24, 48, 96 and 168 h after the treatments to evaluate root system morphology and nitrogen content and to analyze gene expressions for ammonium transport.

The number of crown and lateral root increased after the treatment with the $(NH_4)_2SO_4$ supplemented solution, and with this, total root length increased at 168 h after the dissolving of nitrogen deficit. In contrast, total root length in the treatment with nitrogen-free nutrient solution was compensated by extending lateral roots without increasing the number. In the treatment combined with PEG and $(NH_4)_2SO_4$, total root length did not increase due to the short and small number of lateral roots. However, the number of relatively long lateral roots (L-type lateral root) increased. These suggested that the rice root attempted to compensate the root expansion in a manner to increase root surface area under the unfavorable nitrogen uptake condition.

Although the extension of root under the treatment combined with PEG and $(NH_4)_2SO_4$ was stopped during 96 hours after the dissolving of nitrogen deficit, the ammonium transporter genes were expressed quickly in the duration, and consequently the nitrogen content in seedlings was maintained similar to that of the PEG-free $(NH_4)_2SO_4$ supplemented treatment. Further experiments will be needed to evaluate the expression of root plasticity when plants were subjected to multiple stresses.

Cultivar differences in root development under water deficit condition and its association with water uptake capacity in rice

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Genetic improvement in water uptake capacity in rice cultivars is an option to enhance productivity under water-limited condition, however, root function and systems to improve water uptake under water deficit are still remain unknown. The objective of the present study was to examine cultivar differences in root development and its association with water uptake capacity among three rice cultivars, Azucena (upland japonica), Puluik Arang (lowland indica) and Akitakomachi (lowland japonica). Azucena and Puluik Arang were observed its great water uptake capacity under water deficit condition in our previous study that examined genotypic variation of water uptake capacity among 70 rice cultivars (Matsunami et al., accepted).

Plants were grown in an environmentally regulated growth chamber: 12 h light period, 28 ℃ of temperature, and 70% relative humidity. 10 germinated seeds per each pot (240 mm height, 90 mm diameter) were grown on a plastic net placed on the surface of a 1.2 L solution of 1/2 strength Hoagland nutrient solution for three days. Then polyethylene glycol 6000 was dissolved in water at concentrations of 0 (control) and 100 g per litter (PEG) to attain water deficit. The water potentials of the PEG treatment solution was -0.17 MPa. 7 days after treatments, the amount of water uptake was measured by the reduction of pot weight, and then plants were sampled and separated into shoot and root. Roots were scanned for image analysis of total root length, surface area and lateral root number by using WinRHIZO. Dry matter weight of shoot and root were measured after dying at 80℃ for more than three days. The expression for six aquaporin genes (*OsPIP1;3*, *OsPIP2;3*, *OsPIP2;4*, *OsPIP2;5*, *OsTIP2;1*, *OsNIP2;1*) in seminal root at 7 days after treatment were investigated by reverse transcription-PCR. These six aquaporin genes showed specific expressions in roots among 33 rice aquaporin genes that were identified by Sakurai et al. (2005).

Puluik Arang exhibited higher dry matter production and maintained water uptake under the PEG treatment. Root length and surface area were similar in the both regimes in Puluik Arang, thus it was suggested that the maintaining of water uptake under the PEG treatment was partly due to the root development. On the other hand, the root length and surface area decreased in the PEG treatment in Azucena, however the amount of water uptake did not decrease in Azucena. Thus, we suggested that the water uptake capacity per unit root system in Azucena increased under the PEG treatment, hence maintained water uptake under water deficit condition. Puluik Arang showed slightly higher expression of aquaporin genes than the other two cultivars, but no clear differences between treatments were observed regardless of cultivars. The expression of these genes seemed to have no relation with the water uptake capacity. The participation of other aquaporin genes and/or other factors such as osmotic adjustment to water uptake capacity was remained to be elucidated.

Matsunami et al. *Plant Prod. Sci.* accepted.
Sakurai et al. 2005. *Plant Cell Physiol.* 46: 1568-1577

qSOR1, a major rice QTL involved in soil-surface rooting in paddy fields

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The efficient acquisition of water and nutrients by plant root systems is very important, because the availability of these soil resources is a limiting factor for plant growth. Therefore, the architecture of the root system determines the ability of a plant to exploit those resources that are unevenly distributed in soil. Some of Indonesian lowland rice (*Oryza sativa* L.) cultivars elongate thick primary roots on the soil surface of paddy fields. To clarify the genetic factors controlling soil-surface rooting, we performed quantitative trait locus (QTL) analyses using 124 recombinant inbred lines (RILs) derived from a cross between Gemdjah Beton, an Indonesian lowland rice cultivar with soil-surface roots, and Sasanishiki, a Japanese lowland rice cultivar without soil-surface roots. These cultivars and the RILs were tested for soil-surface rooting in a paddy field. We identified four regions of chromosomes 3, 4, 6, and 7 that were associated with soil-surface rooting in the field. Among them, one major QTL was located on the long arm of chromosome 7. This QTL explained 32.5% to 53.6% of the total phenotypic variance across three field evaluations. To perform fine mapping of this QTL, we measured the basal root growth angle of crown roots at the seedling stage in seven BC_2F_3 recombinant lines grown in small cups in a greenhouse. The QTL was mapped between markers RM21941 and RM21976, which delimit an 812-kb interval in the reference cultivar Nipponbare. We have designated this QTL *qSOR1* (*quantitative trait locus for SOIL SURFACE ROOTING 1*). We used marker-assisted selection to develop a near-isogenic line, designated qSOR1-NIL, in which small chromosomal segments of Gemdjah Beton including *qSOR1* was substituted into the genetic background of Sasanishiki. The qSOR1-NIL showed soil-surface rooting in the paddy field while Sasanishiki did not show that. This indicates that *qSOR1* plays a key role in soil-surface rooting in the paddy field.

QTL for the plasticity in root system development triggered by soil moisture fluctuation stress in rice

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Fluctuating soil moisture can adversely affect rice growth. To cope with that negative effect, plasticity in root system development is the key trait that plays an important adaptive role against moisture stresses. Utilization of chromosome segment substitution lines enabled us to demonstrate the significant roles of root plasticity, as expressed by higher lateral root production, in adaptation to soil moisture fluctuation stress. Moreover, our previous study, we identified QTL at the short-arm of chromosome 12 regions that is associated with root plasticity (Niones *et al.,* 2010). This QTL controls the productions of L-type lateral roots with the increase effect from Kasalath allele. In this study, we quantified the expression of QTL at chromosome 12 regions (referred as *qLLRn-12*) in relation to the total root length under various water stresses. The CSSL genotype with introgressed segment of Kasalath allele only on chromosome 12 (referred as **+KAS** genotype) and Nipponbare were evaluated and grown using root box method at the Nagoya University vinyl house for 38 days. Four water regimes were applied in the experiment; well-watered (WW), transient drought (21d) to waterlogged (17d) (D_{20}-W), transient waterlogged (17d) to drought (21d) (W-D_{20}) and constant drought (38d) (C-D_{20}) conditions. In the drought conditions, soil moisture content (SMC) was maintained at 20%. Two coleoptile nodal roots were collected for root traits measurement related to root plasticity. Tiller number and shoot dry weight were also measured. To further demonstrate the effect and expression of *qLLRn12*, **+KAS** genotype was evaluated for plastic root system development under various water stresses. The **+KAS** genotype showed significantly greater root system with the increase of total root length by 10% in W-D_{20} and 17% in D_{20}-W compared to Nipponbare. On the other hand, these parameters were not significantly different between **+KAS** genotype and Nipponbare parent under non-stress condition (WW) and C-D_{20}. However in C-D, **+KAS** genotype root system development significantly reduced by more than 45% compared to control +KAS. The **+KAS** genotype (with *qLLRn-12)* demonstrates better root system development based on higher total root length in response to moisture fluctuation stresses (D_{20}-W, W-D_{20}). Longer total root length was attributed to higher production of L-type lateral roots, which can effectively maintain the uptake of water and nutrient in the soil. Such plastic root responses resulted to increased stomatal conductance and photosynthesis, and eventually increase of shoot dry matter production with an average of 36% compared to Nipponbare parent. These facts clearly suggest that the unique function of QTL at short-arm of chromosome 12 region (*qLLRn12*) which regulates the production and branching of lateral roots (*i.e.*, L-type lateral root) are expressed only under fluctuating soil moisture stress. There have been reports on QTLs that are associated with root length, root number, root dry weight and branching index traits (Price *et al*., 2002; Horii *et al*., 2006; Gowda *et al*., 2011). However, with the best of our knowledge, this is first report on QTLs at chromosome 12, which are related to lateral root production under soil moisture fluctuation stress. Fine mapping is necessary to narrow down the distance of the target trait.

Reference: Niones *et al.,* 2010. Jpn. J. Crop Sci. 79 (Extra 1) 268-269; Price *et al*., 2002. Field Crop Res. 76:25-43; Horii *et al*., 2006. Plant Breed. 125:198-200; Gowda *et al*., 2011. Field Crops Res. 122:1-13.

Identification of QTLs on lateral root development and the importance under soil moisture fluctuation stress conditions in rice

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Rice (*Oryza sativa* L.) is an essential crop for global food security, but its productivity is restricted to various factors. Especially, soil moisture fluctuation causes the yield decrease in rainfed lowland that occupies about 30% of rice cropped area in the world. We previously reported that increased root length density is important in order to adapt to drought stress conditions and that KDML105, a rainfed lowland *indica* cultivar in Thailand, had much higher root length density compared to other investigated cultivars (Kanou *et al*., 2007; Kano *et al*., 2011). Here, we report the identification of quantitative trait loci (QTLs) on lateral root development and the importance under soil moisture fluctuation stress conditions.

First, we compared the responses of KDML105 to water stress with a lowland *japonica* cultivar, Nipponbare. The result was that KDML105 clearly developed more vigorous lateral roots so much under water stress conditions than those in Nipponbare. Second, we conducted QTL analysis using 59 DNA markers and 96 F_2 lines derived from cross between Nipponbare and KDML105 to detect the QTLs for lateral root responses to water stress. In consequence, the QTLs were detected on chromosomes 4 and 6, 7.

To evaluate the contribution to water stress of those QTLs, we produced BC_3F_3 lines through the repeated backcrosses with Nipponbare and self-pollination of the backcrossed lines. We selected one of the BC_3F_3 lines, G1-1, substituted for three QTL regions from KDML105 into the Nipponbare genetic background, and compared the growth of this line with that of Nipponbare under drought stress conditions. The result was that G1-1 line showed more developed lateral root system than that in Nipponbare under stress conditions. Compared with Nipponbare, G1-1 line also tended to maintain the tiller number and the shoot dry weight under stress conditions, suggesting that those QTLs are important for adaptation to soil moisture fluctuation stress conditions. Now, we are going to perform fine-mapping the loci to make the useful DNA markers for Marker Assisted Selection (MAS).

<Reference>

Kanou *et al*., 2007. Proc. of The 2nd Int’l Conf. on Rice for the Fut., 216-220.
Kano *et al*., 2011. Plant and Soil 342: 117-128.

Role of plasticity in lateral root development triggered by mild drought stress in dry matter production using OryzaSNP panel of rice

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Roots play an important role in rice adaptation to drought stress. Several studies suggested that rice cultivar with deep root could enhance drought resistance, however such deep root trait may not be expressed when soils are hard or hardpan exists. In this study, therefore, we aim to evaluate the functional roles of root plasticity that contribute to dry matter production under various intensities of drought stress in the field when roots are confined in relatively shallow soil layer. We used five varieties of OryzaSNP panel (Cypress, Tainung67, LTH, FR13A and Nippanbare) and two varieties (Rexmont and IRAT109). Seeds were provided by International Rice Research Institute, Philippines. The experiment was conducted at the experimental farm of Nagoya University, Nagoya, Japan during the summer in 2010. The soil depth of this field was about 20cm. Thirty-days-old seedlings were transplanted and grown in the field up to maturing under soil moisture gradients ranging from 9 % to 34 % v/v of soil moisture contents by using line source sprinkler system. Shoot were dried at 70°C for 72 hours and then shoot dry weights and panicle dry weights were measured. The root system was sampled with the monolith method (Kang et al., 1994). Number of nodal root and nodal root length were manually measured. Total root length and total lateral root length were measured using NIH image analysis software (Kimura and Yamasaki. 2001). Most of rice genotypes reduced shoot dry matter production as drought stress intensified, especially, Nipponbare and FR13A severely reduced in dry matter production among all the genotypes. Under mild drought stress condition (19-27 % v/v of soil moisture contents), Cypress, Tainung67, LTH, IRAT109 and Rexmont increased total root length compared to Nipponbare and FR13A, while all the varieties reduced number and length of nodal roots. Increase of total root length of five varieties was attributed to higher total lateral root length, which increased by 20–80% under mild drought stress. These results clearly indicate that promoted lateral root development due to plasticity, which was triggered by mild drought stress played important roles in relatively highly maintaining dry matter production.

References: Kang et al., 1994. Jpn. J. Crop Sci. 63: 118-124; Kimura and Yamasaki. 2001. Plant Soil. 234: 37-46

Investigation into molecular mechanisms of crown root formation and its application for improvements in root architecture in rice.

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Monocot plants produce numerous adventitious (crown) roots from nodes and form fibrous root system. Roots play essential roles in plant development by absorbing water and nutrient, supporting the plant body and so on. Rice is one of the most important crops because it is a staple food for more than half of the global population. Therefore, it is necessary to improve rice yield to attain a stable food supply worldwide, however, the yield of rice is easily influenced by environmental stress, especially, drought. To overcome the drought stress, improvement the roothold of rice is one of the most effective strategies.

Recent progress in the understanding of the molecular mechanisms of root formation has revealed the elaborate regulations by plant hormones and their interactions. To investigate the molecular mechanisms of crown root formation in rice, we have been identified some *crown rootless* (*crl*) mutants. One of them, *crl5*, produced fewer crown roots and displayed impaired initiation of crown root primordia. *CRL5* gene encodes a member of AP2/ERF transcription factor family protein. We found that *CRL5* functions in downstream of AUX/IAA and ARF-mediated auxin signaling pathways involved in crown root initiation. Further analysis revealed that *CRL5* up-regulates a type-A response regulator of cytokinin signaling, *OsRR1*. These results indicated that auxin-induced *CRL5* functions as a positive regulator of crown root initiation through the repression of cytokinin signaling.

We observed that *CRL5*-overexpressing callus could regenerate numerous roots, including not only adventitious roots from callus, but also lateral roots from adventitious roots. This result indicated the ability of *CRL5* for *de novo* root induction, expecting that *CRL5* is useful gene to improve the root architecture of rice. Now, we are investigating other factors essential for crown root initiation acting downstream of *CRL5* in addition to *OsRR1*. We have found three candidate genes whose expressions are suspected to be directly regulated by CRL5 protein, by using DNA beads display system. We hope to provide useful information not only for investigation into molecular mechanism of root initiation but also for breeding through our research that grasp more genetic information involved in root formation.

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Inhibition of auxin transport changes the morphology of root system and distribution of auxin in root system of rice seedlings

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Auxin, a key plant growth hormone, has important roles in root system formation. In this study, we investigated the change of distribution of the endogenous indole-3-acetic acid (IAA) by the inhibition auxin transport in some portions of root system of rice seedlings using LC/MS/MS to clarify the relationship between the root system formation and the IAA distribution. Additionally, the localization of endogenous auxin in the root tissue was detected in detail by the immunocytochemistry method. We also showed the change of lignification in seminal root axis accompanied with the change of IAA distribution.

Oryza sativa L. (cv. Akita-komachi) seedlings were grown hydroponically in half strength of Hoagland solution. The nutrient solution was supplied with 0, 0.1, 1 and 10 μM N-1-naphthylphthalamic acid (NPA) to detect morphological changes and the changes of distribution of auxin in the root system by inhibiting auxin transport. After seven days, roots were sampled and total root length and the number of lateral root were measured. The apical portion (0–1 cm from the root tip including the cell division cell elongating zones), the middle portion (3–4 cm from the root tip where lateral roots had begun to emerge) and the basal portion (0–1 cm from the basal portion of the seminal root) were collected. The endogenous IAA contents in each portion were measured by the LC/MS/MS. IAA localization and lignification in apical and basal portion were detected by the immunocytochemistry using anti-IAA monoclonal antibody and by the Mäule colour reaction methods, respectively.

Growth of seedlings was inhibited by the NPA treatments. The formation of crown roots was also inhibited at higher NPA concentrations. Gravitropism in root system was diminished with increasing NPA concentrations. In the 1 and 10 μM NPA treatments, brown pigment was deposited in the root axis. Total root length and lateral root number decreased with NPA concentration. In the 10 μM NPA treatment, growth of lateral roots was hardly initiated. Endogenous IAA contents were highest in the apical portion in all treatments and were gradually dropped from the apical portion to the basal portion, except for the 10 μM NPA treatment. In each portion, endogenous IAA contents were increased with increase in NPA concentration to the 1 μM NPA treatment. In the 10 μM NPA treatment, the endogenous IAA contents were lower than that in the 1 μM NPA treatment in the all portions. The distal elongation zone (DEZ) transferred to the basal part of the cell under NPA treatment accompanied by the increase of endogenous IAA content in the apical portion. The seminal roots in the NPA treatments, especially 1 μM NPA treatment, were enlarged in the middle portion. IAA localization was detected around the distal elongation zone, in the cortex and in the stele in all treatments. IAA localization was also detected in the primordia of lateral roots. IAA localization was detected more strongly in the higher content portion of endogenous IAA. In the all NPA treatments, it was observed that the initiated primordia of the lateral root turned into the seminal root in basal portion, and lateral root tissue was elongated in the cortex of the seminal root. In this portion, lignification was detected at the hypodermis and stele in all NPA treatments. From these results, we discussed the role of auxin for root system formation.

Identification and characterization of reduced biomass production mutant of rice accompanied with reduced root elongation

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Biomass production at mature stage is one of important agronomical traits in rice. It is known that biomass production of rice is controlled by many genes and its interaction at various physiological and anatomical traits. For example, root is a sole organ to uptake water and nutrients from surrounding soils. We isolated a spontaneous rice mutant with reduced biomass production of top portion at mature stage in the genetic background of ‘Koshihikari’, Japonica-type variety. The dry weight of the mutant grown in paddy field at mature stage showed 64% or 23% reduction in top portion or root portion, respectively, compared with wild-type ‘Koshihikari’. Furthermore, the mutant showed significant reduction of maximum length of top or root, respectively. Also, these reductions were observed at vegetative stage. The biomass production in the mutant was controlled by one recessive gene demonstrating with clear segregation of top dry weight in backcrossed progeny. Interestingly, tight linkage between reduced biomass production at mature stage and maximum root length at seedling stage was observed in BC_1F_2/ BC_1F_3 families. These data indicate a possibility that reduced biomass production in the mutant would be caused by reduced root elongation. Toward better understanding physiological and molecular mechanisms of the relationship between biomass production and root elongation, gene responsible for root elongation were finely mapped within a 330 kb region in the ‘Nipponbare’ genome by a mean of positional cloning strategy in F_2 plants developed from a cross between the mutant and ‘Koshihikari’.

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Non-destructive imaging of aerenchyma development in the primary root of rice using X-ray Computed Tomography – A trial for time-course observation –

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Wetland plants, such as rice, develop intercellular space which are called aerenchyma in their roots as pathways for diffusion of oxygen. To elucidate how these plants develop aerenchyma is essential to understand the resistance mechanism of these plants to waterlogging stress as well as to improve capacity of crop plants to adapt themselves to water logging stress. Synchrotron X-ray computed tomography (CT) has been shown to be useful for nondestructive observation of aerenchyma in pome fruits (Verboven et al. 2008). We aimed to perform *in situ* visualization of aerenchyma and quantitative morphological analysis of its development by using this technique. In this study, we have tried to establish an experimental system to visualize aerenchyma in rice roots *in situ* and perform time-course observation of its development in the same roots.

Rice (*Oryza sativa* ssp. *japonica* cv. Nipponbare) caryopses were sterilized in 2.5% (w/v) sodium hypochlorite solution for 5 min, washed with water, imbibed for the first 4 days at 4ºC and then for 1 day at 30ºC for germination. Imbibed rice caryopses were placed on plastic tubes filled with agar containing Hogland medium. Roots were allowed to grow at 25ºC in darkness for four days. X-ray CT was performed at the experimental hutch No.1 of a bending magnet beamline BL20B2 of SPring-8 in Hyogo, Japan (http://www.spring8.or.jp/). Rice roots has been shown to develop traditionally classified two types of aerenchyma, i.e., schizogenous aerenchyma and lysigenous aerenchyma. Both types of aerenchyma were clealy visualized using this experimental system. The same roots were further allowed to grow for 3h or 8h, and then X-ray tilt series were obtained again. A comparison between the tomographic images, corresponding to the same position of one root, sliced from the tomograms obtained at different time points showed the development of lysigenous aerenchyma. Three-dimensional models of aerenchyma were reconstructed using IMOD software package (http://bio3d.colorad.edu/imod/) and its quantitative morphometrical analysis is currently in progress.

Different gene expression of the creeping-rooted type of a kind of Alfalfa (*Medicago varia*)

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Alfalfa has the highest value of legume forages for cultivation as well as ecosystem rehabilitation using in the world. The creeping-rootedness of alfalfa has important significance to improving the ability of stress resistance and nurture new cultivar.

In this research we used the creeping-rooted alfalfa "BL-101" for the experimental material and constructed the transcription library of creeping-rooted (CR) and non-creeping-rooted (NCR) separately. Analyzing the library with next generation sequences (RNA-seq) and the differential gene expression through cDNA-SRAP technology in order to find the molecular mechanism of creeping-rootedness in alfalfa and also to find the excellent gene relevant. The results are as follow:

1. Separate the RNA from CR and NCR root tissue using the Trizol method and construct the library. Through the analysis of library which sequenced with RNA-seq technology that we obtain 15978 differently expressed genes totally. The highest multiples of differentially expressed genes were GA3 related proteins, so we determined that the occurrence of creeping-rootedness may be induced by GA3 preliminary. According to the FDR method, we got 996 up-regulated unigenes and 970 down-regulated unigenes. The result of GO analysis was that we could classify the unigenes from three aspects, there were 237 kinds of gene in molecular function, 114 kinds of gene in cellular component and 584 kinds of gene in biological process.
2. Find 5 unigenes were related with the root development from the biological process, two of the five genes were up regulated unigene. Making sequence alignment through NCBI, we concluded that one gene was *RAV* subtribe of AP2/EREBP transcription factor family and the other was transferrin receptor protein.
3. Using cDNA-SRAP technology screen the differently expressed genes, and spraying GA3 outside as induction. We chose 15 primers for PCR augmentation got 134 bands, 76 of them were diversity. 42 bands were recycled which were stable repeatability and difference to sequencing. The result of sequence alignment for 42 recycled genes showed that 9 genes were unknown genes, 26 were functional genes. Most of these functional genes were zinc finger protein、thioredoxin h7、*MYB* transcription factor、AP-2 complex subunit beta-1、and xyloglucan galactosyltransferase.
4. We determined that the occurrence of creeping-rootedness may be induced by GA3, through signal transduction makes the *RAV* transcription factor expressed, protein kinases and enzymes participate in root metabolism, acetyl-CoA and pyruvate dehydrogenase participate in root respiration, than the root node developed. Golgi membrane secreted the signal substances, and ion exchange proteins on plasma membrane control the material exchange, *WRKY* transcription factors and ubiquitin ligases promoted the performance of creeping-rooted organization.

Variation of ^{13}C natural abundance in CO_2 released from the roots of rice and maize plants

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A large amount of photo-assimilated carbon is allocated to the roots and utilized for growth, respiration, nutrient uptake and other physiological processes. Recently much attention has been focused on the distribution of ^{13}C natural abundance ($\delta^{13}C$) in plant parts in relation to carbon metabolism. The signature of ^{13}C in CO_2 released by dark respiration depends partly upon the $\delta^{13}C$ content of the carbon pool available for respiration, and partly upon the isotopic fractionation occurred in enzymatic reactions involved in respiration chain. The size and turnover rate of carbon pool for respiration may change with plant parts, growth stage, stress conditions and plant species, and the ^{13}C isotopic signature of respiration may shift considerably. However, little is known on the ^{13}C signature in root respiration. In the present experiment we tried to analyze the $\delta^{13}C$ value of CO_2 released from roots by respiration of rice and maize in both vegetative and reproductive stages.

Materials and Methods:

Rice (var. Nippon-bare) and maize (var. Yellow Pop) were grown hydroponically under outdoor conditions from May to Aug. 2011. Plant were harvested and separated into the roots and shoots (vegetative stage) or the roots, leaves and ears (reproductive stage), then the separated organ placed in the airtight plastic chamber. The CO_2 free air was supplied to the chamber from inlet tube by an air pump at constant flow rate (500mL/min) and the gas flowing out the camber from outlet tube was introduced to the CRDS isotopic CO_2 gas analyzer (G2101-i, PICARRO). Then concentration of CO_2 and $\delta^{13}C$ values of the sample gas was analyzed.

Results:

Respiration rate of the roots and shoot organs was significantly higher in maize than rice at both vegetative and reproductive stages. The $\delta^{13}C$ in CO_2 respired by all the plant parts was always higher in maize than rice plants, due to the difference of photosynthetic system between C_4 and C_3 plants. In maize $\delta^{13}C$ of the root respiration is not significantly different from the shoots, leaves and ears in both vegetative and reproductive stages. On the contrary, in rice plants $\delta^{13}C$ of the root respiration is significantly higher than the shoots (vegetative stage) as well as higher than the leaves and ear (reproductive stage). The results suggest that in rice roots the size and turnover rate of carbon pool available for respiration is different from shoot organs, as compared to maize.

Stable carbon isotopic ratio ($\delta^{13}C$) of fine roots in tropical forests

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We have been studying the decomposition rate of soil organic carbon (SOC) in Thailand by using stable isotope analysis. To this end, we analyzed the carbon and nitrogen contents and the stable carbon isotopic ratio in leaves and roots, which are the main sources of SOC. In this study, we report the stable isotopic ratio of fine roots (<2-mm diameter) from 6 plots—a natural forest, 4 plantations, and a grassland.

The study sites were located at Sakaerat Silvicultural Research Station (14°28′06.1″ N, 101°54′15.0″E; altitude, 420 m), Nakhon Ratchasima Province, Northeast Thailand. The meteorological conditions were as follows: annual mean air temperature, 26°C and annual precipitation (including the dry (November–April) and wet (May–October) seasons), 1,100 mm. Six adjacent plots were chosen: a natural forest (Fo), an *Eucalyptus camaldulensis* (Ec) plot, an *Acacia auriculiformis* (Aa) plot, an *Acacia mangium* (Am) plot, a *Dalbergia cochinchinensis* (DcII) plot, and a grassland (inhabited by *Saccharum spontaneum*,Yp).

The soil-root samples at each soil profile until 100 cm of depth were suspended in water, and poured over fine-mesh sieves to retain the roots. After drying, the fine roots were selected to analyze the stable carbon isotopic ratio. The $\delta^{13}C$ values of the samples were analyzed with a continuous flow isotopic ratio mass spectrometry system (Finnigan MAT 252 and Conflo II).

Fig.1 shows the vertical distribution of the $\delta^{13}C$ values of fine roots. There was a large difference between the $\delta^{13}C$ values of grass roots and tree roots, with the average $\delta^{13}C$ values being -28.4 ‰ and -17.2 ‰, ‰ respectively. This difference is thought to be reflected in the $\delta^{13}C$ values of tree leaves (C3 plants) and grass leaves (C4 plants). Among the tree species, the $\delta^{13}C$ values of the Aa and Am plots were lesser than those of the DcII, Ec, and Fo plots. The changes in the vertical distribution of $\delta^{13}C$ values of fine roots from the natural forest and plantation plots were less compared to that of the grassland fine roots

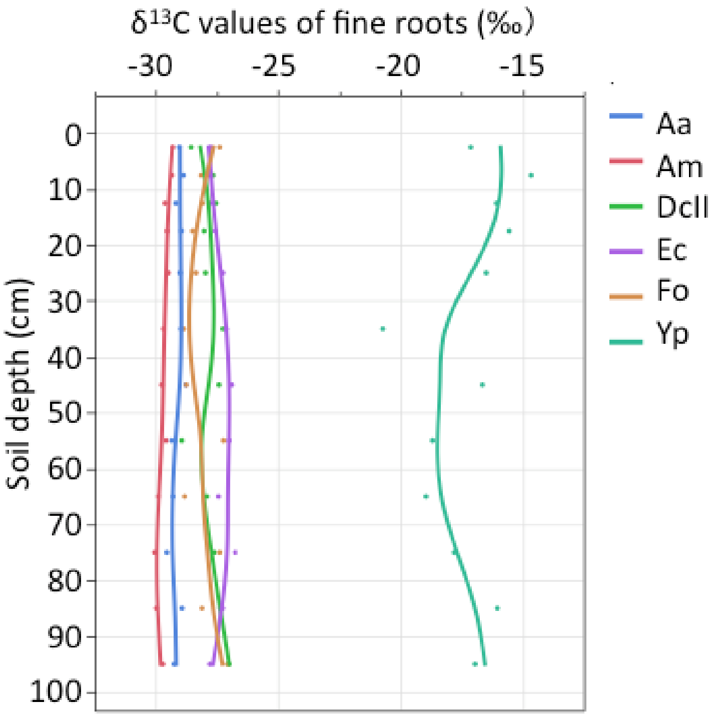

Fig.-1 Vertical distribution of $\delta^{13}C$ values of fine roots

Growth and development of birch (*Betula* sp.) roots grown under elevated CO_2 in FACE (A preliminary result)

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Atmospheric CO_2 has been increasing at the rate of 2.0ppm/yr for recent decade. We strongly expect the CO_2 fixation capacity of forest ecosystems for moderating atmospheric CO_2. Fine root production is estimated to be 1/3 of net primary productivity in terrestrial ecosystems. In this sense, below-ground activities have an essential role in forest productivity. Therefore, we should pay attention to fine root productivity as affected by elevated CO_2. It is also considered the turnover rate of fine root may be accelerated by elevated CO_2 and consequently it affects carbon cycling in a ecosystem. Needless to say, as fine root acts very important role of water and nutrient physiology, we have been monitoring the root growth of birch species for predicting forest productivity.

Betulaceae plants are the representative component in cool temperate and boreal forest and compose of about 40 species. Among them, mountain birch, Monarch birch and white birch are common species in northern Japan and grow together. These species have strong preference to sunny places after disturbance, and produce high quality timber and several commercial products. In this report, we present tentative results of monitoring of root growth with use of rhizotoron in these birch raised under elevated CO_2.

We set the tube of rhizotoron of 30cm depth in May 2010, for monitoring root growth and death of birch seedlings from April 2011in FCAE (Free Air CO2 Enrichment: 500ppm) of Hokkaido University Forest in Sapporo. With use of the photos, we estimated the root productivity based on the allometric relationship between RML (Root Mass per unit Length) and root diameter. For obtaining this relation, we sampled soil core (diameter 5cm depth 30cm) from the bottom part of each species in July 2011 when the root activity of birches is largest.

After water-washing root was scanned, the sample root was oven dried at 60^oC for 48 hrs. We analyzed the root length and diameter with use of software of WhinRHIZO in the photos obtained by a scanner (Epson). We estimated the root dry mass by way of root diameter (n=322).

Here we showed an example of allometric relationship between root diameter and RML in white birch. As no difference in white birch was found in CO_2 treatments, we pooled data obtained two CO_2 levels (Fig. 1). We noticed the critical level of fine root diameter because number of roots was decreased sharply at the point of 1.5mm in root diameter.

Financial support in part by the JSPS innovation research is gratefully acknowledged.

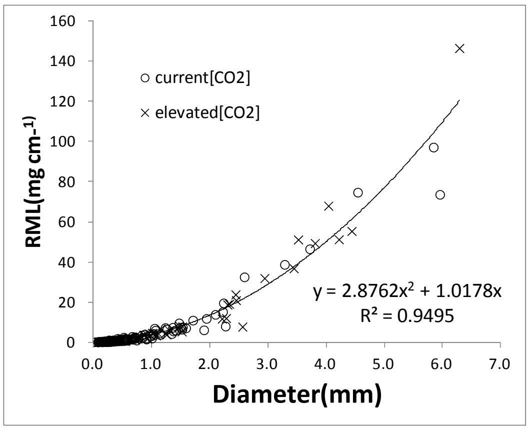

Fig1. Allometric relationship between root diameter and RML in white birch

Can microbial respiration explain mass loss, morphology, and chemical properties of dead fine root?

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Fine roots of forest trees have a rapid turnover and contribute considerably to carbon (C) and nitrogen (N) dynamics of ecosystems in the belowground. In previous studies, fine root decomposition has mostly been characterized by microbial activity and dead root properties such as mass loss and chemical compounds. Although we understand the change of dead root properties occurs through catabolism by microorganisms, less is known about the direct quantitative relationship between microorganisms and dead root properties. Therefore, estimating root properties based on microbial activity can reveal mechanisms of decomposition processes. Recent studies report that the process of root decomposition differs even within 2mm diameter due to different chemical and morphological properties. To clarify responses of microbial activities to changes in dead root properties with decay time, we examined specific microbial respiration and its relationships to mass loss and root chemical composition (C and N) of fine roots across two diameter classes (<0.5mm, 0.5-2mm) of *Quercus serrate* and *Ilex pedunculosa* in a broad-leaved temperate forest at “Ryukoku Forest” in Shiga prefecture, Japan.

One-hundred and twenty root litterbags (2 species×2 diameter class×6 replicates×5 times) were buried in the forest soil on 29 June 2010, and were sequentially collected at each 1 or 3 month-intervals. After collection, microbial respiration rate from root litter samples (n=3) was measured using a closed dynamic chamber system in the field. For the remaining of litter samples (n=3), microbial respiration rate was measured in the laboratory at fixed temperatures of 10, 15, 20 and 25 °C to estimate fixed $Q10$ temperature coefficient. Then, mass loss and concentration of C and N were analyzed using the same samples as used for the respiration measurement.

Microbial respiration rate at 20 °C of all the roots increased with decay time. In both species, <0.5mm root tended to higher respiration rate than 0.5-2mm root. When comparing decomposition rates of mass loss between diameter classes, the <0.5mm root in both species decomposed more slowly than 0.5-2mm root. The C/N dynamics of <0.5mm root in both species were constant with the decay time, whereas that of 0.5-2mm root were not. For both species, microbial respiration, mass loss, and chemical properties differed between diameter classes. The respiration rate was correlated positively with mass loss and N concentration and negatively with C/N within and among diameters and species. These results indicate that microbial respiration reflects mass loss and chemical properties of dead fine root. We found that the relationship between microbial respiration and root properties enables us to predict the process in the decomposition of dead roots, with consequences of accurate and consistent estimates of belowground C and N dynamics.

Mycorrhizal Hyphae Respiration

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In forest ecosystems, researchers have been challenged in recent years to measure carbon storage and whether forest soils will be a sink or a source for carbon. One obstacle is that CO_2 respiration from the soil to the atmosphere is a complex flux of roots, mycorrhiza and root associated microbe (autotrophic respiration) as well as free-living soil microbes and animals get carbon from decomposition of plant debris (heterotrophic respiration). The few works that measured the part of soil respiration from mycorrhizal hyphae showed that it ranged between 3% in beech, 8% in spruce (Moyano et al. 2008) and 25% in pine forests (Heinemeyer et al. 2007). In order to verify this, measurements over a larger range of forest biomes and under different climatic gradients need to be carried out. Ideally, this calls for comprehensive studies that include pool sizes, turnover of the pools and metabolic activities.

This project aims at investigating belowground carbon allocation in a temperate mixed forest dominated by the ectomycorrhizal forming *Quercus serrata*, in particular contribution of respiration and production by external mycorrhizal mycelium.

To this end, an ingrowth bag approach was used. In March 2011, 253 nylon mesh bags with pore size of 41 µm filled with 60g dry sterile granite soil was installed in the top 5 cm of the soil profile. 55, 1 µm pore sizes nylon bags were installed as well. The area for ingrowth was 64 cm^2 per bag. The pore size of 41 µm allows ingrowth of fungal hyphae but exclude roots. The sterile granite sand filling of the bags makes it possible for mycorrhizal hyphae to enter the bag as their host tree supply C while hyphae from saprophytic fungi will not enter to the same extent. For studies of seasonal variation and to get a gradient of hyphae biomass 20 ingrowth bags was collected after 2 (May), 4 (July) and 6 (September) months. Direct after sampling the content of the ingrowth bag was emptied into a small respiration chamber (305 cm^3) and the CO_2 flux measured with an infrared gas analyzer (GMP343, Vaisala, Finland). Preliminary results from these measurements will be presented on the poster.

This project is ongoing and sampling will be executed every 2nd month until March 2012. Biomass, morphological and genetic analyses of the hyphae will start in winter 2011. In the future this project will give an important insight in the relationship of carbon storage and the metabolic activity of mycorrhizal hyphae as well as seasonal variation contributing to a better understanding of the carbon cycling in the temperate forest.

Heinemeyer A., Hartley I.P., Evans S.P., Carreira De La Fuentes J.A. and Ineson P. Forest soil CO_2 flux : uncovering the contribution and environmental responses of ectomycorrhizas. 2007. Global Change Biology 13, 1786-1797.

Moyano F.E., Kutsch W.L. and Rebmann C. Soil respiration fluxes in relation to photosynthetic activity in broad-leaf and needle-leaf forest stands. 2008. Agricultural and Forest Meteorology 148, 135-143.

Relationships between morphology and respiration of fine roots

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【Introduction】

Fine roots (< 2 mm in diameter) release a lot of carbon by their respiration, which has important role in the carbon cycling of the ecosystem. Fine root respiration could have also close relationships with root physiological functions such as nutrient and water uptake from the soil. Understanding of fine root variation is important to know those mechanisms. Makita et al. (2008) reported that fine root respiration can change with their diameter and specific root length (SRL). This study suggests that morphology is one of the factors explaining the variation. However, there are still many root morphological indexes, which are not examined how they correlate to the respiration. In this research, therefore, we aimed to clarify the relationships between various morphology indexes and respiration rate of fine roots.

【Materials and Methods】

We used fine roots of mature *Cryptomeria japonica* and *Zelkova serrata* trees grown in Shisou city in Hyogo prefecture, Japan. We sampled 60 fine roots from 12 mature trees of each species (120 samples in total). Respiration of fine roots (μmol h^{-1} g^{-1}) was calculated from the increase of CO_2 concentration (ppm) in a respiration chamber with a fine root sample, and dry mass of the sample root (g) was determined thereafter. Then, we took image of each fine root by scanner. Morphorogy analysis of the fine root images was conducted using morphorogy analysis software (WinRhizo Reg, Regent). Here, we obtained data of mean diameter (mm), length (cm), surface area (cm^2), volume (cm^3) and SRL (mg^{-1}) of each fine root for the morphorogy analysis using the software.

【Result and Discussion】

Respiration of fine roots related to many of the morphological indexes, such as diameter, length, surface area, volume and SRL of the samples. Only the length of *C.japonica* did not have correlation to the respiration (Table. 1). Surface area, volume and SRL had higher correlation with respiration than others. We conclude that morphology of fine roots is strong controller of fine root respiration.

Table.1 Relationship between morphorogy and respiration of fine root

morphorogy index	Total(n=120)	C.japonica(n=60)	Z.serrata(n=60)
mean diameter(mm)	−0.40**	−0.54**	−0.29*
length(cm)	−0.20*	−0.18	−0.36**
surface area(cm^2)	−0.52**	−0.61**	−0.47**
volume(cm^3)	−0.49**	−0.59**	−0.52**
SRL(mg^{-1})	0.70**	0.62**	0.74**

significant **($p < 0.01$) *($p < 0.05$)

Tea roots changed respiratory activity in response to irradiated light information

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We have examined the effects of LED light irradiation on the growth of tea plants in order to use them as a kind of indoor plants, and got the results that the growth and function of tea roots were changed by the irradiated LED light information[1)]. In the present study, we reported the effects of different wavelength and light intensity of irradiated LED light on respiratory activity of tea roots.

Respiratory activity (RA: quantity of oxygen consumption) of roots excised from 1-year-old (only white roots) or 2 years-old (white and lignified roots) rooted cuttings of *Camelia sinensis* L. 'Yabukita' species was measured by O_2 uptester (Taitec, 5B) with continuous irradiation of LED light (Stanley Electric Co., Ltd.) during the measurement. As a condition of irradiated LED light, wavelength was blue, (main peak wavelength: 465 nm), green (502 nm), red (660 nm) or white (470 and 570 nm), and light intensity (photon flux density) at each wavelength was adjusted to ~5, ~15, or ~30 μmol/m^2/s, respectively. As a control, RA was measured under shade condition.

RA excised from 2-years-old rooted cuttings measured on each LED irradiation condition was shown in Fig.1. By light irradiation, RA became smaller than the control except the cases irradiated light intensity was weak at red and green LED lights (R5 and G5 in the figure). RA changed variously depended on the irradiated light wavelength and intensity. Similar results (differences of RA depended on the irradiated light condition) could be obtained from the excised roots of 1-year-old rooted cuttings. The results suggest that tea roots may recognize the irradiated light information. Generally, roots should be shaded, but they may become able to utilize light information for their growth and production effectively.

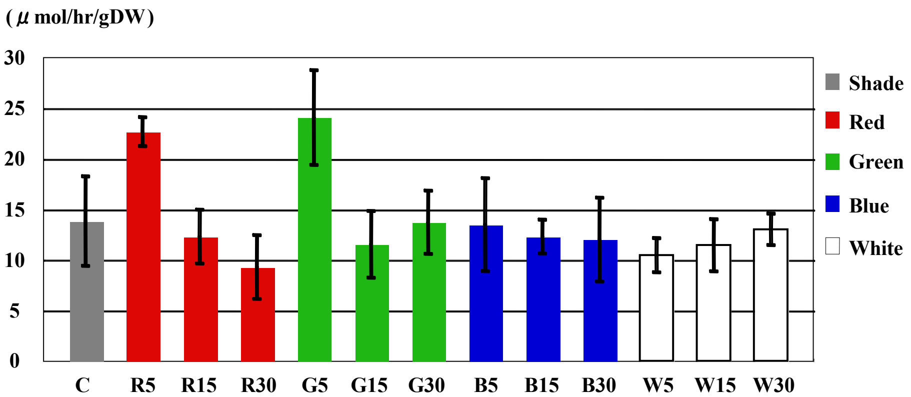

Fig.1 RA obtained from excised roots of 2-years-old rooted cuttings.

1) T.Homma, *et al.*, *Proc.The 4th ICOS,* Pr-P-15 (2010).

Coarse root respiration measurement using automatic chamber system

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Root respiration is an important fraction of soil respiration that contributes to the forest carbon cycle. However the difficulties lie ahead because root is living in rhizosphere with strongly connecting with mixture of such as mycorrhiza, hyphae, and organic matter. In spite of these difficulties, trials to separate root respiration from soil respiration have been done using several methods. One of these methods is destructive method, where the root is removed from the soil and the root sample measures directly. This method is well suited to detect characteristics of root respiration according to root morphology (e.g. diameter) or root chemistry (e.g.CN ratio). (ex, Dannoura et al., 2006, Marsden et al., 2008, Makita et al., 2009) However this method has the shortage that measurement cannot be done continuously thus it is not possible to estimate long term value considering response to environmental factors and seasonal variations. Thus, this study aims measuring root respiration directly and continuously.
This study was conducted in a pine (*Pinus pinaster*) plantation at the INRA domain of Pierroton (44° 45' N, 0° 42' W, elevation 60 m) in France. The top soil on part of a coarse root segment was removed and replace with sterilized sand. Respiration chambers were set on the root segment and CO_2 efflux was measured with a trace gas analyzer (TGA 100A; Campbell Scientific) coupled to flow-through chambers during over one month. The target roots were selected from 3 to 5 trees at each season (June, August, October 2009, and February 2010). Finally, 27 roots which have 7.1 to 90 mm in the diameter were measured. After CO_2 efflux measurements, root samples were dug out and diameter, length and biomass of the root segment inside the chamber were measured.
Figure 1 shows CO_2 efflux from each root sample. Root respiration showed seasonal variation, and Q10 value also change seasonally.

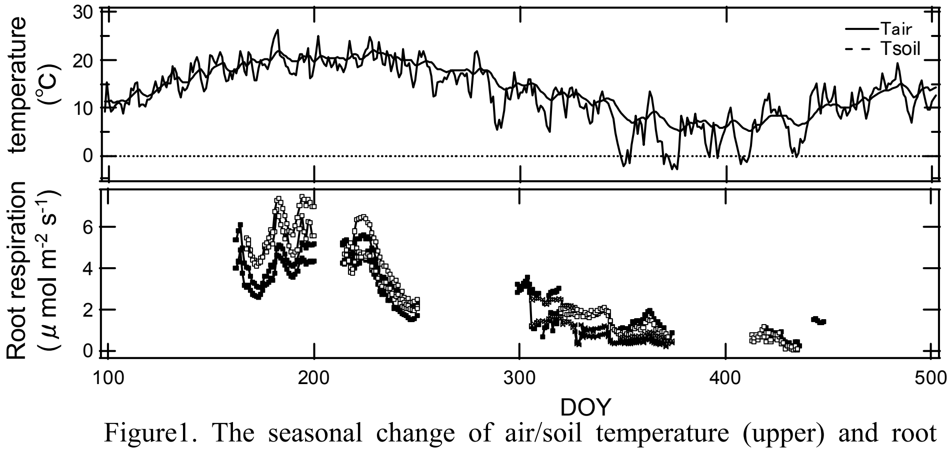

Figure1. The seasonal change of air/soil temperature (upper) and root respiration (daily average) per soil surface area (under)

Fine root respiration for *Pinus densiflora* forests in Mt. Jukyeob of Korea and Mt. Fuji of Japan

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This study was conducted to investigate the fine root and litter respiration rates for two mature *Pinus densiflora* forests with different environmental conditions. The study stands were located in Gwangneung region of central Korea (GNP, 37° 47′ N 127° 10′ E, 425 m a.s.l.) and Fujiyoshida city of central Japan (FYP, 35° 27′ N, 138° 46′ E, 1,030 m a.s.l.). GNP was naturally regenerated following harvesting in Gwangneung Experimental Forests of Mt. Jukyeob, whereas FYP was established on a lava surface of Mt. Fuji. Annual mean temperature and precipitation were 11.3°C and 1,518 mm for GNP, and 10.9°C and 1,561 mm for FYP, respectively. Stand density and basal area were 650 tree ha^{-1} and 54.3 m^2 ha^{-1} for GNP and 842 tree ha^{-1} and 46.8 m^2 ha^{-1} for FYP, respectively. Soil respiration was measured using the portable infrared gas analyzer (EGM-4, PP-systems Inc., UK). Fine root and litter samples were collected using a core method (ø=10cm, n=9 or 10); fine root samples were taken up to 10 cm soil depth after collecting litter samples in GNP whereas fine root and litter samples were taken up to 10 cm depth in FYP. Fine root (< 2 mm diameter) and litter (plus humus) respiration were measured using the portable CO_2 sensors (GMP343, Vaisala CARBOCAP, Finland) with small root chambers on August 4, 2011 in FYP and on August 11, 2011 in GNP. Fine root respiration rates for both GNP and FYP were approximately four-fold higher than litter respiration rates. Fine root and litter respiration rates were higher in GNP than those in FYP while fine root mass was higher in FYP than in GNP (p<0.01) (Table 1). Soil respiration rates (0.63 g CO_2 m^{-2} h^{-1} for GNP and 0.68 g CO_2 m^{-2} h^{-1} for FYP) might be influenced by the differences in fine root and litter respiration rates between two study sites with the soil microbial respiration. These measurements and quantifications of the components respiration rates (fine root, litter and soil) can provide useful information to understand the contribution of roots to belowground carbon dynamics in forest ecosystems.

Table 1. Fine root and litter mass (Mg ha^{-1}), soil depth (cm), and respiration rates of root, litter (nmol CO_2 g^{-1} s^{-1}) and soil (g CO_2 m^{-2} h^{-1}) for *Pinus densiflora* forests in Gwangneung region of central Korea (GNP) and Fujiyoshida city of central Japan (FYP). Numbers in the parenthesis are the standard errors of means and the different letters indicate the significant differences between GNP and FYP (p=0.05).

		GNP	FYP
Fine root mass (Mg ha^{-1})		1.12 (0.12)b	3.48 (0.35)a
Litter mass (Mg ha^{-1})		74.85 (6.19)a	75.76 (4.45)a
Soil depth (cm)		< 100	< 20
Respiration rate	Fine root (nmol CO_2 g^{-1} s^{-1})	8.23 (1.19)a	4.03 (0.60)b
	Litter (nmol CO_2 g^{-1} s^{-1})	2.21 (0.25)a	1.11 (0.15)b
	Soil (g CO_2 m^{-2} h^{-1})	0.63 (0.06)b	0.68 (0.05)a

Linkage between under- and above-ground of three kinds of larch species grown under different levels of nitrogen and phosphorous

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The rhizosphere is a complex environment where roots interact with physical, chemical and biological properties of the soil. Root system senses and adapts to changes in the nutrient status of soils and supports canopy to accumulate bio-energy. Nitrogen (N) is one of the most common limiting elements and the next is phosphorous (p), especially in Hokkaido where most soils are originated from immature volcanic ash or allophane. Larch is a deciduous conifer, has fast growing characteristics and good tolerance to severe environment. It distributes widely through northern hemisphere. Recently we developed a new larch hybrid (*Larix gmelinii* × *L. kaempferi*) crossing between Dahurian larch (*L. gmelinii*) and Japanese larch (*L. kaempferi*).

On the other hand, nitrogen deposition is reported to be increasing yearly and reached to be about 11 kg N $ha^{-1}yr^{-1}$ in central Hokkaido. For edaphic condition of Hokkaido Island, volcanic ash soil is very common, which also is a phosphorous poor soil. How about larch species response to the increasing nitrogen with limited amount of phosphorous loading? For approaching this phenomenon, the linkage between above- and bellow-ground is necessary to be clarified. We hypothesized that N and P loading may enhance three kinds of larch growth and their canopy photosynthesis capacity via root development with symbiotic micro-organisms.

Three years old seedlings, Dahurian larch, Japanese larch and the hybrid larch were planted in 15L pots in June 2010. Nitrogen (3 levels, 0, 50 and 100 N ha^{-1} $year^{-1}$) and P (2 levels, 0 and 50 kg ha^{-1} $year^{-1}$) were supplied every two weeks until October 2011. Diameter and height were monitored for 2 years. Gas exchange rate of needles was conducted in every late summer. In-growth core root samples were collected every 40 days interval from June to October 2011 because of the small size of seedlings in the first year.

According to our observation, mycorrhizal infection was found in roots of three kinds of larch. With invading of mycorrhizal fungi, larches can grow better. Fine roots at surface area of hybrid larch were increased by 25.4% with high N and P loading, while the coarse roots at surface area was decreased by 23.2% compared to control (i.e. no N and P application).

Based on these findings, we will discuss the essential role of N and P application on the growth and development of root-shoot in three kinds of larch species with particular references to the ectomycorrhiza infection. In our presentation, we would like to emphasis on the responses of hybrid larch because it is economically important species in Hokkaido. Moreover we also discuss problems in the in-growth method for applying to three kinds of larch species.

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Do trees growing on the steep slope have different root to shoot ratios from those on the flat site?

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It is well known that the trees planted on the steep slope have deformed root systems, especially in snowy regions. Every winter those trees at juvenile stages are subject to pressure from the snow slide on the plant body (especially stem), forming basal bending and buried stems. It is reported that the root to shoot (R/S) ratios of conifers get close to 0.25 (Fukuda et al. 2003) as the trees get larger, but to date no one has detected the effect of slopes on the R/S ratios of conifers in Japan, where the greater part of forests are located on slopes. The object of the study is to determine if there is a change in R/S ratios derived from growth conditions on slope. It is important to clarify the effect of slope on the R/S ratios in terms of measuring precisely carbon storage in root biomass in the forest ecosystem.

The root samples were taken in two region of central Japan, The one with maximum snow depth more than 2 m and the other with much less. The plant species was Japanese red cedar (*Cryptomeria japonica* D. Don), the most common conifer in Japan. The average tree size was 21 and 26 cm in trunk diameter at breast height in snowy region (S) and non-snowy region (NS), respectively. The gradient of the slope was 37 and 10 degrees. Annual ring widths were measured of the coarse roots and stems at breast height and at the base (ground level). The eccentricity of radial growth was investigated as follows. The distances were measured from the center to the outer rings in four directions on the same wood cross section. The eccentricity was evaluated by the indices indicating variability between the four directions.

The mass proportions of stump to total belowground were 0.69 and 0.59 for S and NS, respectively. This may indicate the effect of buried stems in S. The yearly changes of annual ring widths were similar between stems at breast height and ground base both in S and NS. The eccentricity of the roots became smaller as it got further away from the tree center, reflecting that the eccentric growth was an adaptation for tree stability. It was suggested that the R/S ratios were larger on the steep slope in the snowy region owing to basal bending and buried stems.

Key words: root to shoot ratio, root biomass, snowfall, slope, basal bending, buried stem, deformed root system, eccentricity of radial growth, annual ring analysis

Inter-specific robustness and intra-specific flexibility of whole-plant metabolic scaling from seedlings to giant trees

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The scaling of respiratory metabolism with body mass is one of the most pervasive phenomena in biology. Using a single allometric equation to characterize empirical scaling relationships and to evaluate alternative hypotheses about mechanisms has been controversial. We developed a method to directly measure respiration whole plants, spanning eleven orders of magnitude in body mass, from small seedlings to large trees, and from tropical to boreal ecosystems. **Our measurements include the roots, which have often been ignored.**

For evaluation of whole-tree respiration, we selected variously-sized trees spanning from the smallest to the largest trees in each forest. In that way, we were able to show the maximal variation of individual respiration rates in the general plant metabolic scaling. However, it must be noted that in any forest community the smallest trees are not always the depressed trees. Therefore, some of the smallest trees have much of adventitious branches and roots adapted with the environments under a canopy gap, and relatively high specific respiration rates per individual weight in contrast to dominant trees. Such smaller trees may also play an important role in keeping the sustainability of a natural forest community. Thus, the intra-specific flexibility of individual metabolism gathers into the inter-specific robustness of metabolic scaling of plant communities.

Figure　Quick excavation of roots (a) and measurement (b).

(References) **:** 1) Mori et al. *PNAS* **1073** (2010). 2) 森、山路 (2011) *農業および園芸* **86**. 3) Mulder & Mori et al. (in press) *Advances in Eol. Res. invited chapter*. 4) 森　(印刷中) *生物科学*.

Comparison of the mesh material in a root mesh method

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1. Introduction

Accurate estimate of the fine root production is important for understanding the mechanisms of carbon cycling in ecosystems. However, it's hard to figure out the quantity of the fine root production in the soil, so, various techniques have been developed so far.

The mesh method is one of the most recent techniques for measuring the root production (Godbold et al. 2003). This technique inserts a mesh sheet into the soil for a certain period, and measures the number and weight of roots that grow through the mesh. This technique is innovative because of the simplicity with a few labors and little disturbance of soil. However, the methodological protocol has still uncertainty: for example the effect of mesh quality on the results is unknown. In this study, therefore, we aimed to clarify the influence of different material of a mesh sheet on the results of fine root production.

2. Materials and methods

Six different kinds of mesh sheets were prepared (n=8) (Table 1). The size of a mesh sheet was 10 cm × 20 cm, and the mesh size was 2 mm. We conducted field experiment in a *Cryptomeria japonica* plantation in Kamikawa-cho in Hyogo prefecture in Japan. We placed the meshes vertically into forest soil in November 2008 and uptake them in December 2010. After taking the sample, soil was removed carefully. Then, the fine root grown-up through the mesh was collected, and the number and dry weight (g) were measured for calculated of the fine root production (g m^{-2} $year^{-1}$) (Hirano et al, 2009).

Table 1. Mesh list

Mesh No.	Material	Hardness
1	Polyamide	hard
2	Polyamide	soft
3	Polyethylene	hard
4	Polyethylene	soft
5	Stainless	hard
6	Polyester	soft

3. Result

Average number of the fine root passed through the mesh and the calculated fine root production (g m^{-2} $year^{-1}$) was highest Mesh 4 and lowest Mesh 3 (Table 2). In a previous study, fine root production has been estimated 157-320 g m^{-2} $year^{-1}$ in other *Cryptomeria japonica* plantation (Noguchi et al. 2007). The values of fine root production were 40 times smaller than those values in this study.

Table 2. Average number of the fine root passed through the mesh and the calculated fine root production

Mesh No.	Number	Production
1	7 ± 11	5 ± 7
2	9 ± 5	8 ± 6
3	6 ± 7	5 ± 5
4	14 ± 19	11 ± 11
5	8 ± 6	9 ± 10
6	7 ± 6	7 ± 6
Average	9 ± 3	8 ± 2

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