様　式　C－19、F－19－1、Z－19　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Crosstalk between strigolactone and cytokini signaling pathways in plant growth and stress adaptation

研究課題名（英文）Crosstalk between strigolactone and cytokini signaling pathways in plant growth and stress adaptation

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交付決定額（研究期間全体）：（直接経費）　　3,800,000円

研究成果の概要（和文）：(1)シロイヌナズナにおいて、エチレンがオーキシン、CK、ABA、SA、GB、BRとSLの生合成の律速段階に関与する遺伝子発現に影響し、各ホルモンの量に影響を与える事を証明した。さらに、一次根の成長、胚軸伸長及びシュート分岐において、CKシグナル伝達とMAX2（MORE AXILLARY GROWTH 2、SLとカリキン経路両方の構成因子）シグナル伝達経路の相互作用を解明した。

(2)SL受容体DWARF14経路とカリキン受容体KARRIKIN INSENSITIVE 2経路は、様々な生理学的及び生化学的プロセスに重複、独立して影響を及ぼし、シロイヌナズナの乾燥適応を正に制御することがわかった。

研究成果の学術的意義や社会的意義
(1)We gained more insights into the importance of hormones and their crosstalk in plant growth and drought adaptation.

(2)We might manipulate hormone pathways for improvement of plant performance under normal and stress conditions, thereby contributing to sustainable agriculture and green innovation.

研究成果の概要（英文）：(1) We showed that in Arabidopsis thaliana plants ethylene affects the levels of auxin, cytokinins (CKs), abscisic acid (ABA), salicylic acid and gibberellins, and potentially brassinosteroids and strigolactone (SL), by influencing the expression of genes involved in the rate-limiting steps of their biosynthesis. Furthermore, we reported interactive effects of CK signaling and MAX2 (MORE AXILLARY GROWTH 2, a member of both SL and karrikin pathway) signaling pathways on primary root growth, hypocotyl elongation and shoot branching in Arabidopsis.

(2) We found that the signaling pathways mediated by the SL receptor DWARF14 and the karrikin receptor KARRIKIN INSENSITIVE 2 show both overlapping and specific responses in affecting various physiological and biochemical processes to positively modulate the adaptation of Arabidopsis plants to drought.

研究分野：Biology

キーワード：crosstalk　hormone homeostasis　plant growth　stress adaptation　transcriptome

１．Scientific background for the research　(研究開始当初の背景)

Cytokinin (CK) is an important phytohormone involved in many aspects of plant growth and development. In *Arabidopsis thaliana*, three families of proteins, namely the AHKs (ARABIDOPSIS HISTIDINE KINASES), AHPs (ARABIDOPSIS HISTIDINE CONTAINING PHOSPHOTRANSFERS) and ARRs (ARABIDOPSIS RESPONSE REGULATORS), were identified to conduct CK phosphorelay, which begins with the binding of CK to receptor kinases AHK2, AHK3 and AHK4 (Ha et al. 2012; Li et al. 2019a). Loss-of-function of AHK2 and AHK3 genes results in typical phenotypes of impairment in CK signaling, such as more developed root system (e.g., longer primary roots and longer root hairs), less developed shoot system (eg., shorter plant height and smaller leaf size), enhanced seed size, and reduced callus formation in the presence of CK (Riefler et al., 2006; Ha et al., 2012). Recently, MAX2 (MORE AXILLARY GROWTH 2), an F-box protein, was identified as a member of both SL and karrikin (KAR) signaling pathways and shown to be implicated in regulating various processes of plant growth and development (Mostofa et al., 2018). Accumulating data demonstrated that MAX2 is involved in controlling root development, shoot branching and leaf senescence through SL signaling, and plays a role in regulating seed germination, hypocotyl elongation and root hair development through KAR signaling (Li et al., 2016; Morffy et al., 2016; Mostofa et al., 2018). However, convincing genetic evidence has not been yet available for the interactions of the two signaling pathways in any developmental processes like shoot branching and root development still remain elusive (Li et al., 2019b).

SLs are a group of carotenoid-derived terpenoid lactones that have been identified as a plant hormone affecting various processes of plant growth and development and responses to environmental stresses (Al-Babili and Bouwmeester, 2015; Mostofa et al., 2018; Li et al. 2020). Genes related to SL biosynthesis and signaling have been identified in *Arabidopsis* and rice (*Oryza sativa*) through the analyses of the *max* and *dwarf* (*d*) mutants, respectively (Al-Babili and Bouwmeester, 2015; Mostofa et al., 2018). SL signal transduction begins with the binding of SLs to the ‘open state’ pocket of the *Arabidopsis* D14 protein, which belongs to an α/β-hydrolase superfamily and possesses both enzyme and receptor activities (Al-Babili and Bouwmeester, 2015; Mostofa et al., 2018; Li et al 2019b). Consequently, the D14 protein undergoes conformational changes in a ‘closed state’ and recruits the F-box MAX2 protein. The newly formed MAX2-based Skp1-Cullin-F-box (SCF) complex can polyubiquitinate the transcriptional repressors SUPPRESSOR OF MAX2 1 (SMAX1)-LIKE 6, 7 and 8 (SMXL6/7/8) and trigger the degradation of SMXL6/7/8, thereby releasing the repressed SL-responsive genes (Al-Babili and Bouwmeester, 2015; Mostofa et al., 2018; Li et al 2019b). Similar to SL signaling, a close homolog of D14, named KARRIKIN INSENSITIVE 2 (KAI2), mediates signals from exogenously applied KARs or an unknown endogenous KAI2-ligand (KL), as reported by intensive studies of the *Arabidopsis kai2* mutants in response to KARs during plant growth and development, such as seed germination, hypocotyl elongation and root hair development (Morffy et al., 2016). KARs were purified from wildfire smoke and are capable of promoting seed germination in many plant species. Genetic evidence has shown that MAX2 is also involved in KAI2-mediated signal transduction of KARs in a similar manner as the D14-mediated signal transduction of SLs, suggesting that MAX2 acts as a common protein of both the KAI2- and D14-mediated signaling pathways (Li and Tran, 2015; Morffy et al., 2016; Mostofa et al., 2018).

In *Arabidopsis*, MAX2 has been reported to act as a positive regulator in response to drought through abscisic acid (ABA)-dependent and -independent pathways (Bu et al., 2014; Ha et al., 2014). As MAX2 functions in both D14- and KAI2-mediated signaling pathways, it was hypothesized that both D14 and KAI2 might be functionally involved as important positive regulators of drought responses in plants (Li and Tran, 2015). Indeed, SL-specific *d14* and KAR-specific *kai2* mutants both exhibited drought-sensitive and ABA-insensitive phenotypes

(Li et al., 2017; Lv et al., 2018; Zhang et al., 2018), suggesting the involvement of the two paralogous proteins in plant adaptation to drought in connection with the ABA response. Additionally, a recent in-depth analysis of the *kai2* mutants revealed that KAI2 played a critical positive role in the drought responses of *Arabidopsis* plants, by promoting stomatal closure and by increasing cell membrane integrity, cuticle formation, anthocyanin biosynthesis and ABA responsiveness (Li et al., 2017). However, the detailed mechanisms underlying the functions of D14 in regulating plant responses to drought remain elusive, particularly in comparison with KAI2. Furthermore, D14 evolved from its ancestral KAI2 by duplication and speciation during the evolution process in the plant lineage (Morffy et al., 2016; Waters et al., 2017). Thus, in terms of evolution, it is also interesting to investigate which receptor (and its associated signaling) would play a more critical role, as well as the functional relationship of the two receptors in plant adaptation to drought.

Ethylene (ET), as a gaseous hormone in plants, is known to regulate various biological processes, including plant growth and development (e.g., seed germination, shoot and root growth and development, and leaf senescence), as well as plant responses to various environmental stresses (Thao et al., 2015; Abdelrahman et al., 2017). In the ET biosynthetic process, methionine is first converted to S-adenosyl methionine (SAM) by SAM synthase. SAM is then catalyzed to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS), which is known as the rate-limiting step in the ET production process. Finally, ACC is oxidized to ET by ACC oxidase (Thao et al., 2015; Li et al., 2018). In *Arabidopsis*, *ethylene-overproducer 1* (*eto1-1*) mutant, mutation in the *AT3G51770* gene, releases its inhibition on the ACS5 activity, and thus increasing ET level (Wang et al., 2004). It is well-established that ET interacts with other hormones to regulate plant growth and developmental processes. However, the effects of ET on the biosynthesis of other hormones, including, auxin, CKs, ABA, gibberellin, salicylic acid, jasmonic acid, brassinosteroids and SLs at seedlings stage still remain elusive.

２．Purpose of the Research　(研究の目的)

This proposed project will enable us to understand how hormone homeostasis involving the hormones like SLs, KARs, CKs and ET are crucial for plant growth and development as well as for plant stress responses. Findings will allow us to design precise technology for manipulation of hormonal levels and signal transduction to improve plant performance and reduce the negative impacts of environmental stresses on plant productivity, thereby contributing to sustainable agriculture and green innovation.

３．Research Method　(研究の方法)

(1) Characterization of the collected and generated *Arabidopsis* mutant defected in the CK and/or MAX2 signaling pathway(s), and wild-type (WT) plants under normal growth conditions. The root and shoot-related traits of the mutant plants were compared as described previously (Li et al., 2019b) to examine the effect of interactions between CK and MAX2 signaling pathways on plant growth and development.

(2) Comparative analysis of hormone contents and expression of associated genes in the 10 day-old seedling *eto1-1* mutant and WT plants were conducted as described in Li et al., (2018).

(3) A series of physiological, biochemical and molecular experiments, including transcriptome analysis was designed to examine the phenotypes and various responses of the single mutants *d14* and *kai2*, and the double mutant *d14 kai2*, to drought treatments in order to: (i) characterize the detailed functions of D14 in drought responses; (ii) compare the contributions of the two paralogs D14 and KAI2 to *Arabidopsis* drought resistance; and (iii) provide evidence that the additive relationship of D14 and KAI2 modulates some physiological and biochemical processes in the plant response to drought (Li et al., 2017; Li et al., 2020).

(4) Examination of stress-tolerant phenotype of the *Arabidopsis* mutant and WT plants under drought was performed as described in Li et al., (2017; 2020).

(5) Various physiological and biochemical assays, including ion leakage assay, cuticular permeability assay, leaf temperature assay, stomatal closure assay, determination of relative water content, measurement of anthocyanin contents and ABA contents, etc. of the *Arabidopsis* mutant and WT plants under normal and abiotic stress conditions were carried out as described in Li et al., (2017; 2020).

(6) Comparative expression analyses of the *Arabidopsis* mutant and WT plants under normal and stress conditions using qRT-PCR and/or transcriptome approaches were performed as described in Li et al., (2018; 2020).

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４. Research Achievements （研究成果）

(1) Four research articles

Li W, Nguyen KH, Chu HD, Ha CV, Watanabe Y, Osakabe Y, Leyva-González MA, Sato M, Toyooka K, Voges L, Tanaka M, Mostofa MG, Seki M, Seo M, Yamaguchi S, Nelson DC, Herrera-Estrella L, Tran LS (2017). The karrikin receptor KAI2 promotes drought resistance in *Arabidopsis thaliana*. PLoS Genet 13:e1007076

Li W, Nishiyama R, Watanabe Y, Ha CV, Kojima M, An P, Tian C, Sakakibara H, Tran LS (2018). Effects of overproduced ethylene on the contents of other phytohormones and expression of their key biosynthetic genes. Plant Physiol Biochem 128:170-7.

Li W, Nguyen KH, Ha CV, Watanabe Y, Tran LS (2019). Crosstalk between the cytokinin and MAX2 signaling pathways in growth and callus formation of *Arabidopsis thaliana*. Biochem Biophys Res Commun 511:300-6.

Li W, Nguyen KH, Chu HD, Watanabe W, Osakabe Y, Sato M, Toyooka K, Seo M, Tian L, Tian C, Yamaguchi S, Tanaka M, Seki M, Tran LS (2020). Comparative functional analyses of DWARF14 and KARRIKIN INSENSITIVE2 in drought adaptation of *Arabidopsis thaliana*. Plant J (doi: 10.1111/tpj.14712).

(2) Four review articles

Mostofa MG, Ghosh A, Fujita M, Tran LS (2018). Methylglyoxal – a signaling molecule in plant abiotic stress responses. Free Radic Biol Med 122:96-109.

Abdelrahman M, Jogaiah S, Burritt DJ, Tran LS (2018). Legume genetic resources and transcriptome dynamics under abiotic stress conditions. Plant Cell Environ 41:1972-83.

Mostofa MG, Weiqiang L, Nguyen KH, Fujita M, Tran LS (2018). Strigolactones in plant adaptation to abiotic stresses: an emerging avenue of plant research. Plant Cell Environ 41:2227-43.

Li W, Herrera-Estrella L, Tran LS (2019). Do cytokinins and strigolactones crosstalk during drought adaptation?. Trends Plant Sci 24:669-72.

5．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　1件）

1．著者名 Weiqiang Li, Kien Huu Nguyen, Chien Van Ha, Yasuko Watanabe, Lam-Son Phan Tran	4．巻 511
2．論文標題 Crosstalk between the cytokinin and MAX2 signaling pathways in growth and callus formation of Arabidopsis thaliana	5．発行年 2019年
3．雑誌名 Biochemical and Biophysical Research Communications	6．最初と最後の頁 300-306
掲載論文のDOI（デジタルオブジェクト識別子） https://doi.org/10.1016/j.bbrc.2019.02.038	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Mohammad Golam Mostofa, Weiqiang Li, Kien Huu Nguyen, Masayuki Fujita, Lam-Son Phan Tran	4．巻 41
2．論文標題 Strigolactones in plant adaptation to abiotic stresses: An emerging avenue of plant research	5．発行年 2018年
3．雑誌名 Plant, Cell & Environment	6．最初と最後の頁 2227-2243
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/pce.13364	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Mostafa Abdelrahman, Sudisha Jogaiah, David J. Burritt, Lam-Son Phan Tran	4．巻 41
2．論文標題 Legume genetic resources and transcriptome dynamics under abiotic stress conditions	5．発行年 2018年
3．雑誌名 Plant, Cell & Environment	6．最初と最後の頁 1972-1983
掲載論文のDOI（デジタルオブジェクト識別子） DOI: 10.1111/pce.13123	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Li W, Nguyen KH, Chu HD, Ha CV, Watanabe Y, Osakabe Y, Leyva-Gonzalez MA, Sato M, Toyooka K, Voges L, Tanaka M, Mostofa MG, Seki M, Seo M, Yamaguchi S, Nelson DC, Herrera-Estrella L, Tran LSran	4．巻 -
2．論文標題 The karrikin receptor KAI2 promotes drought resistance in Arabidopsis thaliana	5．発行年 2017年
3．雑誌名 PLoS Genetics	6．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） https://doi.org/10.1371/journal.pgen.1007076	査読の有無 有
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〔学会発表〕　計4件（うち招待講演　4件／うち国際学会　4件）

1．発表者名 Tran LS	
2．発表標題 Hormones and their crosstalk in plant adaptation to drought: an insight into the functions of strigolactone and karrikin	
3．学会等名 International Symposium “Plant stress science: What can we do for future agriculture?”（招待講演）（国際学会）	
4．発表年 2019年	

1．発表者名 Tran LS	
2．発表標題 Cytokinin, strigolactone and karrikin, and their crosstalk in plant adaptation to drought	
3．学会等名 Invited seminar by Henan University, Kaifeng, PR China（招待講演）（国際学会）	
4．発表年 2019年	

1．発表者名 Tran LS	
2．発表標題 Cytokinin, strigolactone and karrikin, and their crosstalk in plant adaptation to drought	
3．学会等名 Invited seminar by Nanjing Agricultural University, Nanjing, PR China（招待講演）（国際学会）	
4．発表年 2019年	

1．発表者名 Tran LS	
2．発表標題 Hormones and their crosstalk in plant adaptation to drought: an insight into the functions of cytokinin, strigolactone and karrikin	
3．学会等名 Invited seminar by Prince of Songkla university, Hat Yai, Thailand（招待講演）（国際学会）	
4．発表年 2018年	

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考