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学位の種類	博士（生命科学）
学位記番号	生博第 449 号
学位授与年月日	令和４年９月２６日
学位授与の要件	学位規則第４条第１項該当
研究科，専攻	東北大学大学院生命科学研究科 （博士課程）分子化学生物学専攻
論文題目	Mechanism of drought tolerance associated with epigenomic changes by grafting in tomato plants（接ぎ木によるエピジェネティックな変化を介した乾燥耐性の獲得機構）
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論文内容の要旨

ntroduction

Tomato (*Solanum lycopersicum*) is a fruit crop mostly cultivated worldwide due to its high consumption. It is also a model plant for research due to its genomic information availability, easy cultivation, and aptness for experimental purposes. However, the tomato fruit can easily change its form, flavor, and texture when applied abiotic stress, such as drought, to the plant. Since drought stress will increase with climate change, agriculture will be most affected by low crop yield and economic losses. Grafting is a method used in agriculture to improve crop production and tolerance to biotic and abiotic stress. Plant grafting is the technique of primarily connecting two plant sections, the scion and the rootstock. This technique is widely used in tomato; however, the effects of grafting on changes in gene expression and epigenetic modifications associated with stress tolerance in shoot apical meristem (SAMs) cells are still under-discovered. I used 'Momotaro peace' variety as scion, it is cultivated in Japan and its fruit is known for its sweetness, hardiness, and flavor.

Material and Methods

In the present study, I exposed non-grafted F1 hybrid rootstock varieties, TTM-079 (TD1), green guard (GS), and green force (GF), and Momotaro peace (Momo), together with three grafted combinations (Momo/TD1, Momo/GF, and Momo/Momo) to 12 days drought stress treatment. Moreover, I tested two groups of 'pre-drought' treatment in non-grafted Momo for 4 and 7 days before the 12 days of treatment. To clarify the effect of grafting on drought tolerance, I performed a transcriptomic analysis through RNA-sequencing in the SAMs between non-grafted and grafted tomato plants, before and during 3- days drought stress. Also, I studied the effects of self-grafting tomato plants on epigenetic modification like histone H3 modifications (H3K4me3 and H3K27me3) by ChIP-sequencing, and cytosine-5 DNA methylation by bisulfite-sequencing, in Momo-scion SAMs.

Results and Discussion

Drought tolerance was significantly improved by a combination of compatible resistant rootstock TD1 and self-grafted compared to non-grafted lines and pre-drought stress-treated plants. Next, I found the differences in gene expression between grafted and non-grafted plants before and during drought stress treatment. These altered genes are involved in the regulation of plant hormones, stress response, and cell proliferation. Furthermore, when

comparing compatible (Momo/TD1 and Momo/Momo) and incompatible (Momo/GF) grafted lines, the incompatible line reduced gene expression associated with phytohormones but increased wounding and starvation-stress responsive genes. These results showed that grafting generates drought stress tolerance through several gene expression changes in the apical meristem. Furthermore, self-grafting also alters specific gene expression and epigenetic changes involved in nitrogen compound metabolic process, cellular component organization or biogenesis, response to stimulus, chromosome organization, cell cycle-related genes, and regulation of hormone levels in SAMs. These results indicate that drought stress tolerance in SAMs with epigenetic modifications is acquired during the wound-graft healing process in tomato plants.

Conclusion

This study presented transcriptional and epigenetic modifications in drought tolerance using grafted tomato plants. First, I assessed whether a vigorous rootstock and self-grafted Momo could improve drought tolerance by transcriptomic analysis between grafted and non-grafted lines. In addition, the epigenetic changes due to histone H3K4me3, H3K27me3, and DNA cytosine methylation were compared between self-grafted and non-grafted Momo. My data further support that grafting alters epigenetic marks in the apical meristem tissue of the scion, resulting in increased drought stress tolerance through stress memory within specific genes involved in pathways related with hormone and stress-response.

論文審査結果の要旨

地球規模での温暖化現象は土壌の砂漠化も進行させ干ばつ被害も深刻な問題となり、作物の乾燥ストレス耐性開発が強く求められている。品種改良に加えて接ぎ木は古くからの栽培手法の 1 つで、根が強い台木に穂木を接ぐことで乾燥ストレス耐性や病害抵抗性など様々な耐性を付与できる。接ぎ木の手法は果樹はもとより、主にナス科やウリ科の作物においても広く用いられている。これまで、強い台木を用いることが重要であるとされてきたが、シロイヌナズナなどの近年の研究では、切断‐再結合にともなう接合部位における wounding-healing 過程が、台木‐穂木間に影響しストレス耐性付与に関わることも報告されてきた。

MARIA ISABEL FUENTES MERLOS さんは、トマト品種「桃太郎ピース」を穂木に異なるトマト品種の台木、さらに、自身を一度、切断後再結合させた Self 接ぎ木などを作出し、接ぎ木をしない対照区との乾燥耐性について比較検討を行った。その結果、Self 接ぎ木においても、有意に乾燥ストレス耐性が付与されること、その際に、ABA をはじめとする様々な植物ホルモン関連遺伝子の発現変動、熱ショックシャペロン、酸化ストレス耐性に関わる酵素群などの遺伝子も接ぎ木刺激によって誘導されることを見出した。さらに、これら遺伝子の発現変動には、ヒストン H3K4me3 や H3K27me3、DNA のメチル化など、エピジェネティックな変化が穂木の分裂組織を含む茎頂組織のゲノム遺伝子において生じることを発見した。これらの研究成果の網羅的な遺伝子発現変動に関する解析部分は国際誌 *Plants* に筆頭著者兼責任著者として受理され、エピゲノム変化に関する解析部分は異なる国際専門誌に同じく筆頭著者兼責任著者として投稿中で、いずれも高い評価を受けています。

これらの成果は、接ぎ木においてなぜ乾燥耐性が獲得されるのか、さらにそれらが長期植物体にメモリーされるのかという接ぎ木によるストレス耐性獲得の新たな原理の 1 つとなる知見を見出しました。

以上のことから、本研究成果は、自立して研究活動を行うに必要な高度の研究能力と学識を有することを示していると判断し、 MARIA ISABEL FUENTES MERLOS さん提出の論文は、博士（生命科学）の博士論文として合格と認める。