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学 位 論 文 要 旨
(Summary of the Doctoral Dissertation)

学位論文題目 (Dissertation Title)	Functional Analyses of Abiotic Stress-Induced Adaptive Responses and Metabolite Changes in Japanese Bunching Onion (*Allium fistulosum* L.) (ネギにおける非生物的ストレス誘発適応応答および代謝物変化の機能解析)
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Abiotic stresses, which have been increasing due to global warming and drought caused by climate change, have made the development of heat- and drought-tolerant varieties of bunching onion (*Allium fistulosum* L., genomes FF) a critical challenge. Leaf tipburn, a physiological disorder caused by abiotic stresses, significantly reduces the commercial value of the bunching onion. On the other hand, a dark green color is considered a trait that enhances commercial value and is also in demand from producers. In this study, the characteristics of heat- and drought-tolerant bunching onion varieties were elucidated to facilitate their breeding. For this purpose, a series of bunching onion–shallot addition lines (FF+1A to FF+8A), each containing a single alien chromosome derived from a stress-tolerant shallot (*A. cepa* L. Aggregatum group, AA), and heat-tolerant varieties were used. Functional analyses were conducted to investigate adaptive responses to abiotic stress and changes in metabolites, as well as their relationship to the expression of various agronomic traits.

(1) Effects of Drought Stress on Abscisic Acid Content and Its Related Transcripts in *Allium fistulosum–A. cepa* Monosomic Addition Lines

A series of bunching onion–shallot addition lines were subjected to drought treatment, and the contents of abscisic acid (ABA), a key hormone related to drought stress, and its precursor, β-carotene, were measured. Additionally, gene expression analyses were conducted on genes involved in ABA biosynthesis, catabolism, and signaling responses to examine the effects of adding alien chromosomes on the drought-stress response in the bunching onion. As a result, in FF+1A under drought treatment, the contents of β-carotene and ABA were significantly higher at the 5% level than those in FF under the same conditions. Multiple genes involved in ABA biosynthesis were also markedly upregulated. In contrast, the gene expression profile of FF+6A, which had ABA and β-carotene contents comparable to those of FF, differed significantly from that of FF+1A. Notably, *zeaxanthin epoxidase* (*ABA1*) exhibited high expression in FF+1A, while showing low expression in FF+6A. *ABA1* is known to directly participate in the rate-limiting step of ABA biosynthesis. The introduction of the first chromosome from shallot into the bunching onion was suggested to enhance the expression of this gene, leading to an increase in ABA content. Consequently, stomatal closure may be promoted, potentially improving the drought tolerance of the plant.

(2) Metabolite Profiling and Association Analysis of Leaf Tipburn in Heat-Tolerant Bunching Onion Varieties

As plant materials, leaf blades from 8 heat-tolerant varieties and lines of bunching onion, obtained through six patterns of summer cultivation with different sowing and harvesting dates (6 experimental groups × 8 varieties/lines × 3 biological replicates = 144 samples) were used. For all samples, absolute quantification data of six pigment compounds and three functional components, relative quantification data of 267 metabolites, and leaf tipburn rates were obtained.

Statistical analyses using the absolute quantification data revealed that varieties and lines with notably darker leaf

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blade colors generally had higher contents of chlorophyll and carotenoids. In particular, 'Yamakou01,' 'Yamakou03,' and 'YSG1' accumulated higher amounts of these pigment compounds in their leaf blades, suggesting that these compounds are the main contributors to dark green leaf blade coloration. Additionally, machine learning was used to analyze the relationship between the relative quantification data of metabolites from all samples and the same phenotypic traits. As a result, gamma-Glu PRENCSO, an organosulfur compound, and several flavonoids were identified as metabolites associated with leaf tipburn. These metabolites are suggested to suppress leaf tipburn by utilizing the redox reactions of glutathione, which plays a crucial role in the synthesis of sulfur compounds, as well as their antioxidant activities.

(3) Metabolome Profiling and Predictive Modeling of the Dark Green Trait in Heat-Tolerant Bunching Onions

Seven heat-tolerant varieties and lines of bunching onion were cultivated during the summer season to measure pigment compounds, metabolome, and spectral reflectance in the 400–700 nm range at 20 nm intervals. Based on these measurements, a system of classification of dark green types and predictive models for pigment compound contents were developed. Principal component analysis (PCA) of the spectral reflectance data revealed that the green coloration of heat-tolerant varieties could be categorized into three groups: "green" (high reflectance at 540–560 nm), "dark green" (low reflectance at 540–560 nm), and "gray green" (high reflectance across all wavelengths). Metabolome analysis using representative varieties and lines from these three groups revealed an increase in cyanidin derivatives in the "dark green" group and an increase in sinapinic acid in the "gray green" group. Furthermore, using absolute pigment compound data and spectral reflectance values, a predictive model was constructed via random forest regression. For total chlorophyll content, the regression model achieved high accuracy with an R^2 value of 0.87 in leave-one-out cross-validation.

This study elucidated the effects of adding shallot chromosomes on the drought stress response in the bunching onion, as well as the metabolites involved in leaf tipburn and dark green coloration. In addition, for the first time in the *Allium* genus, a novel method was successfully developed to construct a highly accurate regression model for predicting the total chlorophyll content using spectral reflectance data. By integrating genetic and physiological–ecological approaches, a method combining metabolite analysis with simplified pigment measurement was developed, providing foundational knowledge for the development of cultivation techniques for drought-tolerant bunching onions. In subsequent research, the insights gained from this research are expected to contribute to the development of bunching onion varieties suitable for cultivation under adverse environmental conditions.

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学位論文審査の結果及び最終試験の結果報告書

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【論文審査の結果及び最終試験の結果】

近年の気候変動に伴い、葉ネギの耐乾性・耐暑性向上が求められている。本研究では、シャロット由来の異種染色体を添加したネギ系統（FF+1A～FF+8A）および耐暑性品種・系統を用い、非生物的ストレス耐性に関する機能解析を実施した。乾燥ストレスに対する影響を調査した結果、FF+1A はβ-カロテンおよびアブシシン酸の含量が増加し、乾燥耐性向上の可能性が示唆された。耐暑性ネギにおける葉先枯れの代謝プロファイリングでは、同形質の関連代謝物として数種の硫黄化合物とフラボノイド類を特定し、特に gamma-Glu PRENCSO が葉先枯れ区で顕著に増加していた。一方、対照区では抗酸化作用を持つ代謝物が増加し、葉先枯れ抑制に寄与する可能性が示された。濃緑色形質に関する分析では、耐暑性品種の葉身部緑色が「green」、「dark green」、「gray green」の３種類に分類され、メタボローム解析の結果、dark green では赤色色素化合物、gray green ではポリフェノール類が増加していた。さらに、色素化合物と分光反射率を基にランダムフォレスト回帰モデルを構築し、総クロロフィルやβ-カロテンの量を高精度に予測可能なモデルを得た。本研究で得られた研究成果により，葉ネギの非生物的ストレス耐性向上と品質評価に貢献する新たな知見が得られた。また、供試した品種・系統の中には非生物的ストレスに対する抵抗性をもつ系統や機能性成分の生産能が高い品種が含まれることも判明し、栽培品種の新たな育種戦略の策定に役立つ知見も得られた．以上より，審査委員会は本論文の内容を評価し、学位論文として十分価値を有するものと判断した．

本審査委員会（主査・副査の５名）は，令和７年２月４日に学位論文審査会を開催し，学位申請者本人に対して論文の内容説明を求め，それに対する質疑応答を行った．さらに，関連事項についても設問を行った．その結果，いずれも満足すべき回答が得られた。よって，本審査委員会は同人を大学院創成科学研究科博士課程の修了者としての学力および識見をもつものとして認め，博士（生命科学）の学位を与えるに十分の資格を有するものと判定した．